



Electrohydrodynamic encapsulation of cisplatin in poly (lactic-co-glycolic acid) nanoparticles for controlled drug delivery

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Abstract

Targeted delivery of potent, toxic chemotherapy drugs, such as cisplatin, is a significant area of research in cancer treatment. In this study, cisplatin was successfully encapsulated with high efficiency (>70%) in poly (lactic-co-glycolic acid) polymeric nanoparticles by using electrohydrodynamic atomization (EHDA) where applied voltage and solution flow rate as well as the concentration of cisplatin and polymer were varied to control the size of the particles. Thus, nanoparticles were produced with three different drug:polymer ratios (2.5, 5 and 10 wt% cisplatin). It was shown that smaller nanoparticles were produced with 10 wt% cisplatin. Furthermore, these demonstrated the best sustained release (smallest burst release). By fitting the experimental data with various kinetic models it was concluded that the release is dependent upon the particle morphology and the drug concentration. Thus, these particles have significant potential for cisplatin delivery with controlled dosage and release period that are crucial chemotherapy parameters.

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Key words: Cisplatin delivery; Cancer chemotherapy; Nanoparticle; Electrohydrodynamic atomization; Controlled release

Cisplatin (cis-diamminedichloroplatinum) is a widely used anticancer agent^{1,2} that has been shown to be highly effective in the treatment of testicular, ovarian, breast, bladder, lung and head and neck cancer. However, due to its significant toxicity, the maximum dose and hence therapeutic effect in many applications is restricted.³ Controlled release drug delivery systems offer a potential means of overcoming the challenges with the administration and release of anticancer drugs with the optimum clinical response.⁴ Numerous controlled release formulations, e.g. liposomes⁵ and polymeric particles,^{6,7} have been proposed to reduce systemic toxicity.⁸ Encapsulation within a lipidic or polymeric particle protects the drug from degradation in the

biological environment and, in particular, nanoscale formulations have shown promise for cellular targeting and prolonged circulation times.^{9,10}

Poly (lactic-co-glycolic acid) (PLGA) is a biodegradable polymer that is extensively used in drug delivery systems, due to its safety profile.¹¹ However, the physico-chemical properties of cisplatin, in particular its poor solubility in organic solvents,¹² pose a considerable challenge in generating a nano-encapsulated formulation.¹³ This limits the range of techniques that can be used for particle fabrication. However, electrohydrodynamic atomization (EHDA) has been shown recently to facilitate encapsulation of both hydrophobic and hydrophilic drugs with

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high encapsulation efficiency and offers excellent control over particle size and size distribution in both the nano and micro length scales^{14–16} Controlling the size of the particles results in adjusting the surface area to volume ratio of particles, enabling them to penetrate tissue structures.¹⁷ Size distribution is also a crucial factor that provides control over the release profile and bioavailability of the loaded drug in the body.¹⁸ Unlike other conventional methods that require more than one step to achieve drug-encapsulated particles, in EHDA, the drug is directly encapsulated within the polymeric carrier using a single step procedure.¹⁹ Another advantage of EHDA is that volatile solvents evaporate during particle formation even under ambient conditions, removing the need for heating or subsequent solvent extraction that can lead to damage of the particles and/or degradation of the drug.

In this study, therefore, electrohydrodynamic atomization was utilized in a single step process to fabricate cisplatin-loaded particles with high drug encapsulation efficiencies, which can be used without further treatment. While other drug-encapsulated materials have been made using this technique,²⁰ there are no reports of applying this method in manufacturing cisplatin-loaded biomaterials. A systematic study of variation of the concentration of both cisplatin and PLGA was conducted, with the ultimate aim of controlling the dose and release rate of cisplatin. The influence of nanoparticle morphology, size, and drug concentration was explored *in vitro*, with the underlying delivery mechanism discussed, based on diverse material characterization and kinetic modeling.

Methods

Materials

PLGA (copolymer 50:50, Resomer RG503H, molecular weight of 33,000 Da, inherent viscosity 0.41 dl g⁻¹) was supplied from Boehringer Ingelheim (Ingelheim, Germany). Dimethylacetamide (DMAc) was obtained from Sigma Aldrich (Poole, UK). Cisplatin (*cis*-Platinum(II)diamine dichloride, molecular weight of 300 g mol⁻¹) was purchased from Enzo Life Sciences (Exeter, UK).

Particle fabrication

PLGA solutions (2 and 4 wt%) were prepared by dissolving the polymer in DMAc and mechanically stirring for 400 s. Cisplatin (either 1 or 2 mg/ml) was then added to the solutions followed by stirring for a further 500 s in ambient temperature (20 °C) to ensure the total dissolution of both the drug and polymer. In order to investigate the effect of cisplatin and PLGA concentration on the size, morphology and eventually the release profile of the particles, three different solutions with various cisplatin to PLGA ratios (2.5, 5 and 10 wt% cisplatin with respect to PLGA) were prepared. The initial experiments were conducted with the higher concentration of PLGA (4 wt%). While the PLGA concentration was kept constant, the amount of cisplatin added to the solution was increased from 1 to 2 mg/ml (2.5 and 5 wt% cisplatin with respect to PLGA). A third solution was prepared with the lower concentration of PLGA (2 wt%) in

the solution while a higher amount of the drug (2 mg/ml, 10 wt% cisplatin with respect to PLGA) was added to investigate the morphology and size of the particles as well as the effect of lower PLGA concentration on the release profile of cisplatin. Finally, a solution with 4 wt% PLGA without the addition of the drug was prepared to be used as the control system for the process.

The solutions were electrosprayed using a single needle EHDA setup (Figure 1) to produce particles. The solutions were made to flow through a stainless steel needle (18G, ID: 0.84 mm and OD: 1.27 mm) via a syringe pump (PHD 4400, Harvard Apparatus Limited, Edenbridge, UK) at a constant flow rate of 2.5, 3, 4 or 5 µl/min. A high precision voltage generator (Glassman Europe Ltd, Bramley, UK) was used to apply an electric potential difference between the needle and a ground electrode to the solution and was varied from 12 to 20 kV as required to form a stable cone jet. The particles were collected at a working distance of 200 mm below the device exit directly onto glass slides or aluminium foil respectively for characterization and measurement of drug release. Increasing the working distance lead to reduction in particle size due to longer time for evaporation of the solvent and therefore allowing columbic fission to occur even more.¹⁷ The working distance in this study was optimized at 200 mm and it was shown that particles of desired size upon complete drying were deposited at this distance. The jet and particle formation processes were monitored using a Leica DMS300 camera. Experiments were conducted at the ambient temperature of 19–21 °C and relative humidity of 40–50%. Each experiment was conducted at least 3 times to ensure the reproducibility of the EHDA process and consistency of the particles produced.

Characterization of solutions

The viscosity, surface tension, density and electrical conductivity of all the prepared solutions were measured. Density was measured using a standard density bottle (DIN ISO 3507 - Gay-Lussac). Viscosity measurements were conducted using a U-tube viscometer (size E, VWR, Lutterworth, UK). Prior to measurements the viscometer was calibrated with ethanol. A Kruss tensiometer (Model DSA100, Kruss GmbH, Hamburg, Germany) was used to measure the surface tension (Wilhelmy's plate method). Electrical conductivity of each solution prepared was estimated using a conductivity probe (Jenway 3540 pH/conductivity meter). All the measurements, presented in Table 1, were conducted at the ambient temperature (21 °C) and relative humidity of 40–50% after calibrating the equipment with ethanol or distilled water.

Particle characterization

Optical microscopy and scanning electron microscopy

Samples of particles were collected on glass slides. These were analysed initially under an optical microscope (Nikon Eclipse ME 600) fitted with a camera (Micropublisher 3.3 RTV, 3.3 megapixel CCD Color-Bayer Mosaic, Real Time Viewing camera, Media Cybernetics, Marlow, UK). Further analysis of particle size and morphology was carried out using the scanning electron microscope (SEM, XL30 FEG, Philips). Both optical (bright field) and scanning electron micrographs were analysed

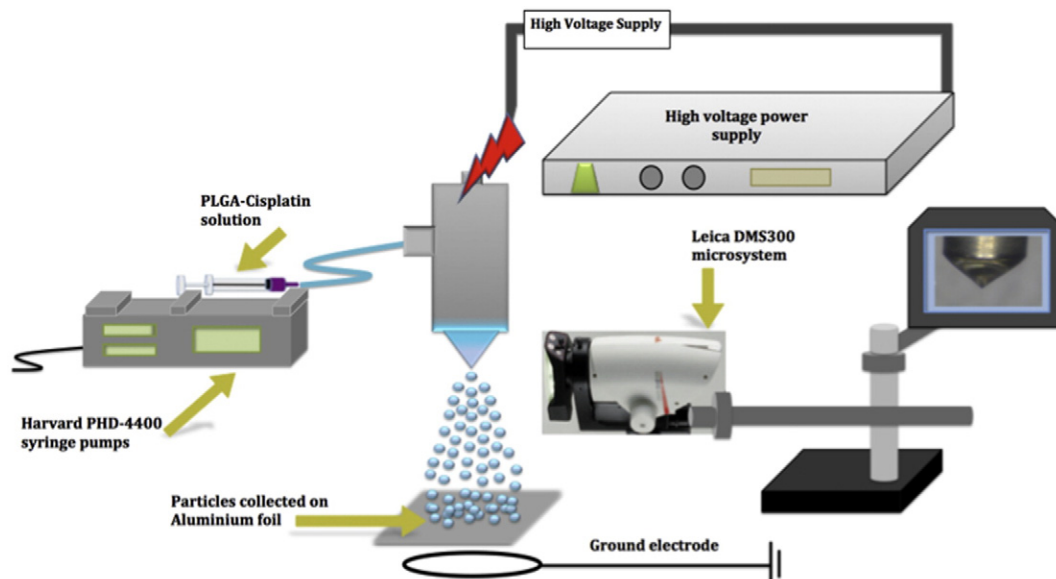


Figure 1. Schematic of the experimental set-up used to prepare drug-loaded particles.

using Image J to determine the average diameter and standard deviation of the population of particles (300 particles were measured from each sample). An INCA X-sight EDAX system (Oxford Instruments) was used with the XL30 microscope for EDX (Energy dispersive X-ray) spectroscopy analysis to identify the presence of cisplatin in the nanoparticles.

Fourier transform infrared spectroscopy

The infrared spectra of cisplatin, PLGA nanoparticles, and drug loaded nanoparticles were recorded using a fourier transform infrared (FTIR-ATR-Perkin Elmer 2000) spectrophotometer. Spectra of all materials were recorded using a frequency range of $400\text{--}4000\text{ cm}^{-1}$, and averaged over 4 runs. Powdered samples were placed on the attenuated total reflectance (ATR) crystal, and then compressed using an axial screw.

Table 1
Physiochemical characteristics of the solutions used to prepare particles.

Solution	Cisplatin concentration with respect to PLGA	Electrical conductivity ($\mu\text{S}/\text{cm}$)	Surface tension (mN/m)	Viscosity (mPa s)
2 w/w% PLGA in DMAc	0	3.65	27	1.62
4 w/w% PLGA in DMAc	0	3.75	27.5	2.49
0.1 w/w% cisplatin, 4 w/w% PLGA in DMAc	2.5 wt%	4.45	27.5	2.73
0.2 w/w% cisplatin, 4 w/w% PLGA in DMAc	5 wt%	4.82	29	2.77
0.2 w/w% cisplatin, 2 w/w% PLGA in DMAc	10 wt%	13.52	33.5	1.87

Transmission electron microscopy

The structural characteristics and cisplatin distribution of the nanoparticle formulations were examined using a transmission electron microscope (TEM, CM12, Philips), and EDX analysis (JEM-2100- Jeol TEM fitted with X-max EDAX system-Oxford Instruments). For this part of the work, particles were sprayed directly onto carbon coated copper grids and analysed without additional contrast.

Differential scanning calorimetry

The endothermic peaks of pure cisplatin, PLGA, and cisplatin loaded PLGA nanoparticles were recorded using differential scanning calorimetry (Pyris Diamond DSC, Perkin Elmer Instruments). 5 mg of the samples were sealed in standard aluminium pans with lids and analysed over a temperature range of $0\text{--}200\text{ }^{\circ}\text{C}$.

Drug encapsulation efficiency

In order to determine the encapsulation efficiency of cisplatin, 10 mg of cisplatin-loaded nano/micro particles was mixed with DMAc followed by addition of PBS. The solution was then passed through a $0.22\text{ }\mu\text{m}$ filter and analysed by reverse-phase HPLC method (see *in vitro* drug release). Encapsulation efficiency (percentage of the amount of drug added initially that was entrapped in the nanoparticles) was calculated using below formula:

$$\text{Encapsulation efficiency (EE \%)} = (W_t/W_i) \times 100\%$$

Where W_t is the actual drug loading and W_i is the weight of drug used in particle synthesis.

In vitro drug release

Following a previously published protocol,²¹ 20 mg of cisplatin loaded nanoparticles were dispersed in 1.5 ml of PBS (pH 7.4) and incubated at $37\text{ }^{\circ}\text{C}$. At predetermined time intervals, 0.5 ml aliquots of solution were removed for the

purpose of measurement, and replaced with fresh buffer solution. Aliquots of the supernatant were centrifuged and analysed using high performance liquid chromatography (HPLC). Liquid chromatography was optimised on a Hypersil GOLD SEX column (Thermo Scientific) under isocratic conditions. A mobile phase of NaCl soln. (0.9% w/v) and methanol (95:5) was used at a flow rate of 0.7 ml/min. A calibration plot to calculate the cisplatin concentrations of unknown measurements was produced by measuring the absorbance of different concentrations of cisplatin solution (0.9% w/v saline) from 1 to 50 µg/ml.

The mechanism of drug release from the particles was further investigated by fitting the kinetic release data to previously published theoretical models; zero order, first order, Hixson Crowell, and Ritger-Peppas. These are expressed mathematically in Eq. (1). Zeroth and first order release kinetics model processes are independent and directly proportional to the drug concentration, respectively. First order kinetics could describe release from a capsule-like particle, in which the drug diffuses through a thin layer. Another mechanistic-based model, that of Hixson and Crowell, is used for processes in which dissolution takes place at the surface of a particle, thereby reducing the size of the particle, which results in a 2/3-order model.²² Based on the assumption that the release rate is determined by the diffusion of solute through the particle, Ritger and Peppas proposed that the release fraction $f(t)$ up to 60% may be represented by a power law, where the release exponent is typically slightly less than 0.5: their expression is a best fit to an exact mathematical result which depends on the particle radius and the bulk diffusion constant.

Zero order : $f(t) = A_0 t$

First order : $f(t) = 1 - e^{-A_1 t}$

$$\text{Hixson and Crowell : } f(t) = \begin{cases} 1 - (1 - A_{HC} t)^3 & \text{if } t < \frac{1}{A_{HC}} \\ 1 & \text{if } t \geq \frac{1}{A_{HC}} \end{cases}$$

Ritger and Peppas : $f(t) = A_{RP} t^n$

(1)

where n is the release exponent, A_i are fitting parameters, and t is time. It is worth noting that at short times the zero order, first order and Hixson and Crowell models all show a release fraction directly proportional to time, whereas the Ritger and Peppas release varies as t^n .

The *NonlinearModelFit* function within Mathematica (Wolfram Research) was used to fit the release rates of 2.5, 5, and 10 wt% cisplatin loaded PLGA particles, using the various models, accounting for the measurement uncertainties by weighting each data point with the inverse of its associated standard deviation. Quality of fit was assessed using the adjusted- R^2 parameter (the adjustment allows for different numbers of fitting parameters).

Results

EHDA of cisplatin dissolved in PLGA solution

In EHDA, the flow of liquid under the influence of an electric field undergoes different modes. This is a function of the operating parameters, primarily: the applied electrical potential

difference (voltage), the distance between the needle outlet and the ground electrode as well as the liquid flow rate, needle diameter and the properties of the flowing liquid.²³ At a constant liquid flow rate and with no electrical field voltage, liquid droplets will form at the tip of the needle and detach once they reach a certain volume. Applying a relatively small electrical potential difference will reduce the diameter of the liquid droplets formed at the tip of the needle but dripping will continue. As the applied voltage is increased however, the atomization mode changes from dripping to jetting.²⁴ The stable cone jet mode is normally the most desirable atomization mode as it can produce uniform size particles (Figure A1 in supporting information). When the applied voltage is slightly higher or lower than that when a single permanent cone jet is emitted from the needle tip, the cone jet pulsates.²⁵ While the pulsed cone jet emits at perfectly timed intervals, the diameter of the cone jet varies and as a result droplets with different diameters are formed leading to generation of polydispersed particles (video camera images and corresponding SEM images are provided in supplementary information).

In this work, PLGA particles were generated both with and without cisplatin. Initially, the 4 wt% PLGA solution was processed to produce drug free particles as the control system to investigate the effect of cisplatin on the size and morphology of particles. At a constant flow rate of 5 µl/min, the voltage required to form a stable cone jet was between 12 and 15 kV (videos of the cone jet mode are presented in the supplementary information). Addition of cisplatin influenced the properties of the solution by increasing the surface tension, viscosity, density and electrical conductivity. The higher electrical conductivity led to a higher applied voltage being required at the same flow rate (5 µl/min) to form a stable cone jet (13-19 kV). Higher or lower voltages produced an unstable jet and thus non-uniform particles with a large size distribution.

Particle size and morphology

Experiments were conducted initially with the higher concentration of PLGA (4 wt%) in the solution and two different concentrations (2.5 and 5 wt%) of cisplatin with respect to PLGA. The optical microscope images of the particles are shown in supplementary Figure A2 and it was noted that larger particles were produced with the higher concentration of cisplatin (5 wt%). Detailed SEM images (Figure 2) confirmed that all of the particles were spherical and with a smooth outer surface. The concentration of both the polymer and drug was found to influence the diameter of the cisplatin-loaded particles. Thus, in order to further reduce the size of the particles, the concentration of PLGA was reduced to 2 wt% whilst a higher concentration of cisplatin (2 mg/ml) was added to the solution to giving a drug concentration of 10 wt% with respect to PLGA. As shown in Figure 2, at the same flow rate of 5 µl/min, reduced the mean particle diameter from 1.2 µm to 550 nm (Table 2). It was also noted that, while the polymer concentration was decreased, the outer surface of the particles stayed smooth and no pores were observed in the SEM images. The decrease in the particle size can be attributed to the lower surface tension, lower viscosity and higher electrical conductivity of the 10 wt% cisplatin solution

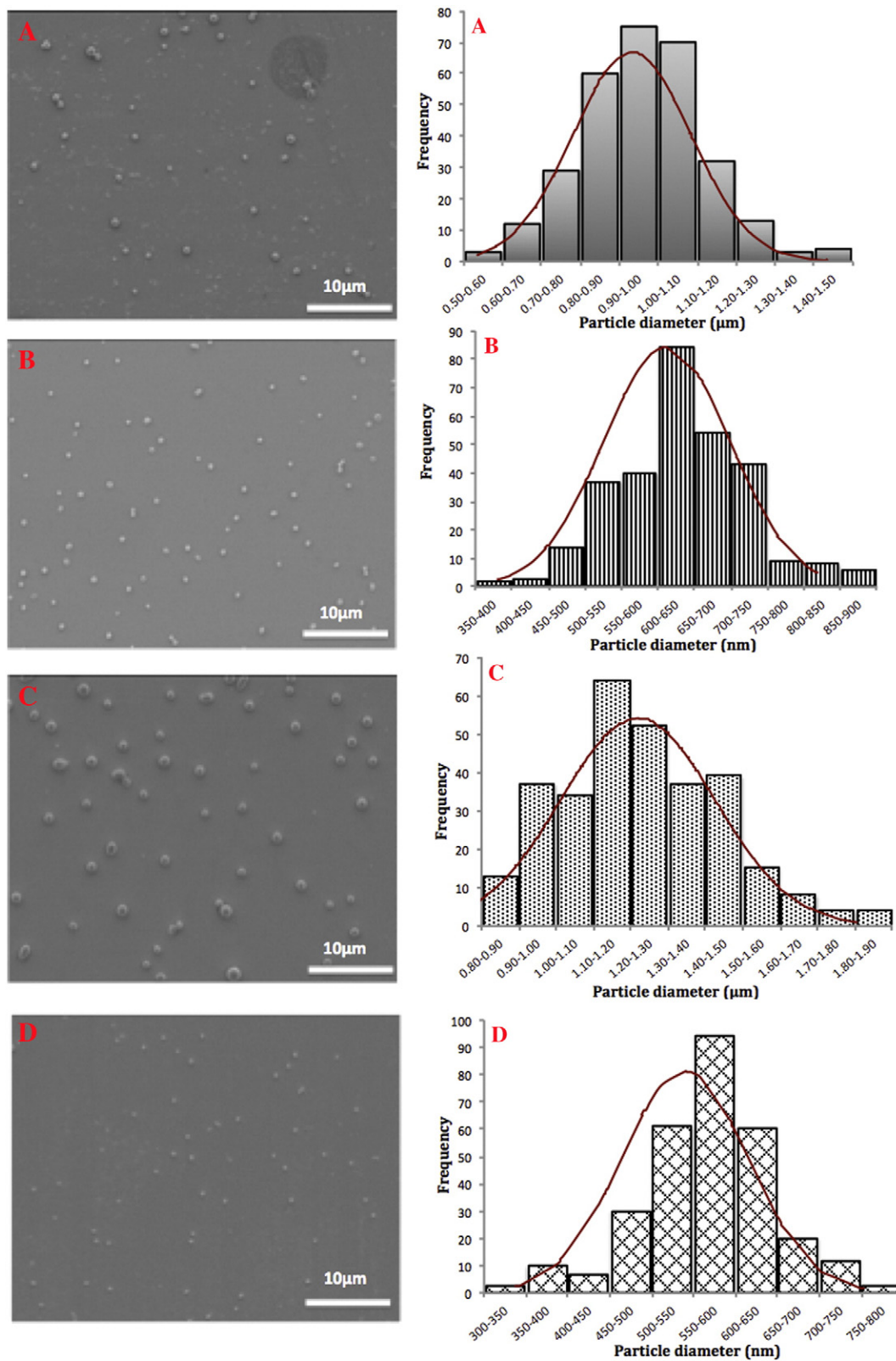


Figure 2. Scanning electron micrographs of particles (A) without cisplatin, (B) 2.5 wt% (C) 5 wt% and (D) 10 wt% cisplatin with respect to PLGA. Size distribution of particles produced (A) no cisplatin, (B) 2.5 wt% (C) 5 wt% and (D) 10 wt% cisplatin with respect to PLGA.

Table 2
Operating conditions tested and corresponding size characteristics of particles formed.

Solution	Flow rate ($\mu\text{l}/\text{min}$)	Voltage (kV)	Particle	PDI	Yield	Encapsulation efficiency
			Mean size	%		
4 w/w% PLGA in DMAc	5	13	950 \pm 150 nm	2.8	75%	-
0.1 w/w% cisplatin, 4 w/w% PLGA in DMAc	5	14	630 \pm 95 nm	2.2	85%	75%
0.2 w/w% cisplatin, 4 w/w% PLGA in DMAc	5	13	1.2 \pm 0.2 μm	2.7	75%	70%
0.2 w/w% cisplatin, 2 w/w% PLGA in DMAc	5	16	550 \pm 80 nm	2.1	80%	72%

(values are shown in Table 1). The size distribution of unloaded and drug loaded polymer particles was characterised by measuring the diameter of 300 particles randomly chosen from each sample and the polydispersity indices (PDI) were calculated (Table 2). Generally, size distribution graphs (Figure 2) demonstrated a normal size distribution for all the particles produced. At a constant flow rate of 5 $\mu\text{l}/\text{min}$, particles obtained from the 10 wt% cisplatin solution also exhibited a lower PDI of 2.1%.

Two important parameters that control the morphology and size of the electrosprayed particles are voltage and flow rate.²⁶ In order to overcome the surface tension of the droplet at the tip of the EHDA needle, high voltage is required to form a stable cone jet and produce charged droplets. Further experiments were conducted to demonstrate the ability of EHDA technique to enable control over the size of particles by adjusting the processing parameters. In these experiments, the solution with 10 wt% cisplatin was initially infused at a constant liquid flow rate of 5 $\mu\text{l}/\text{min}$. Once the stable jet is formed, the voltage was increased from 16 to 19 kV. As shown in size distribution graphs and microscopic images in Figure 3, the particle mean diameter

decreased from 550 nm to 360 nm by increasing the voltage to 19 kV. From size distribution graphs shown in Figure 3, particles produced with the higher voltage had a narrower size distribution, while 65% of particles produced had diameters between 300 and 400 nm.

In a separate study, while the applied voltage was kept constant at 16 kV, the liquid flow rate was reduced to 3 $\mu\text{l}/\text{min}$ (Table 3). This led to a reduction in the mean particle size to 506 nm. As shown in Figure 3 more than 60% of the particles produced at the lower liquid flow rate had diameter (D_p) in the range of 400 < D_p < 500 nm. However, decreasing the liquid flow rate also reduces the rate of particle production. Thus increasing the applied voltage is likely to be preferable as a practical means of reducing the particle size.

Characterisation of cisplatin loading

Fourier transform infrared spectroscopy

FTIR spectra were recorded to investigate whether there was any interaction between PLGA and cisplatin during encapsulation. FTIR spectra of cisplatin, PLGA nanoparticles and PLGA

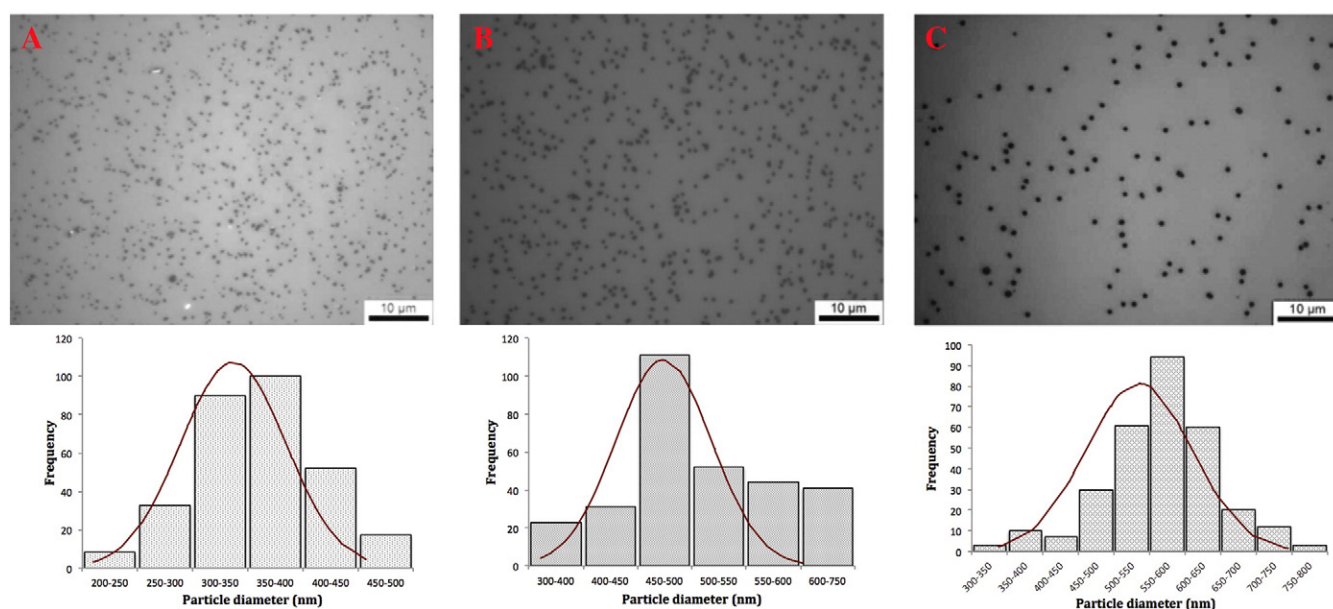


Figure 3. Optical micrographs and corresponding size distribution graphs for 10 wt% cisplatin with respect to PLGA solution at different operating conditions stated in Table 3.

Table 3

Operating conditions tested and corresponding size characteristics of particles formed for 10 wt% cisplatin with respect to PLGA.

10 wt% cisplatin with respect to PLGA	Flow rate ($\mu\text{l}/\text{min}$)	Voltage (kV)	Particle mean size (nm)
A)	5	19	360 ± 55
B)	3	16	505 ± 70
C)	5	16	550 ± 80

nanoparticles loaded with different concentrations of cisplatin are shown in Figure 4. The pure cisplatin sample exhibited characteristic peaks including amine stretching (3271 cm^{-1}), symmetric amine bending (1292 cm^{-1}) and chloride stretching (780 cm^{-1}). PLGA nanoparticles showed characteristic peaks attributable to C=O stretching (1754 cm^{-1}) and C-O stretching ($1050\text{--}1250\text{ cm}^{-1}$). Additionally, the 10 wt% cisplatin loaded nanoparticles also showed a weak peak for amine stretching (3294 cm^{-1}), indicating the presence of intact drug (cisplatin). Thereby the FTIR data indicate that there were no chemical interactions between PLGA nanoparticles and cisplatin molecules. Furthermore, no peaks corresponding to cisplatin were observed in the other drug loaded materials, confirming the reduced quantity of cisplatin in the particles loaded with lower concentrations of drug.

Differential scanning calorimetry

DSC was performed to assess the physical state of cisplatin within the PLGA particles (Figure 5). In the thermogram of PLGA, a glass transition peak was observed at $51\text{ }^\circ\text{C}$; this peak was not observed in the drug loaded nanoparticle formulations. An exothermic peak for the drug alone was observed at $281\text{ }^\circ\text{C}$. The absence of this peak in the cisplatin loaded particles suggests that the drug is molecularly dispersed in an amorphous form.²⁷

Electron microscopy

The EDX spectra captured during SEM showed the presence of Cl and Pt peaks for both the 5 and 10 wt% drug loaded particles, indicating the presence of intact cisplatin (supporting information Figure A3). This correlates with the FTIR spectra of the 10 wt% cisplatin materials which indicated the presence of cisplatin (Figure 4). To further examine the encapsulation and distribution of cisplatin in the PLGA nanoparticles TEM analysis was carried out. Micrographs of the nanoparticles loaded with different cisplatin concentrations (Figure 6, A–C) showed areas of darker coloration within the particles, indicating regions of higher density or atomic number (using bright field imaging mode). In contrast, the particles that were not loaded with cisplatin (Figure 6, D) appeared even in image intensity, suggesting that these darker areas in Figures 6, A–C are regions of cisplatin. TEM-EDX analyses of the cisplatin loaded (10 wt%) and not loaded PLGA (0 wt%) particles are shown in Figure 7, it can be seen that peaks for Cl and Pt are present only in the drug loaded material, correlating with the EDX analysis described above and confirming the presence of cisplatin. Furthermore, when comparing EDX spectra from regions of apparent higher and lower density as indicated on the micrograph (Figure 7, A), the relative intensity of the peaks corresponding to Pt and Cl is greatly reduced, and there was a ca.

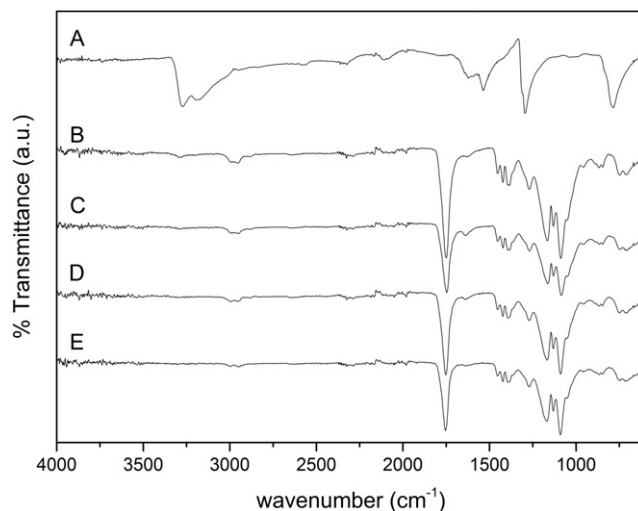


Figure 4. FTIR spectra of: (A) cisplatin, (B) 10 wt% cisplatin loaded PLGA, (C) 5 wt% cisplatin loaded PLGA, (D) 2.5 wt% cisplatin loaded PLGA, and (E) PLGA.

50% reduction in the wt% values calculated for these elements. It can be concluded that cisplatin was effectively encapsulated inside the PLGA nanoparticles, and importantly was well distributed throughout the particle core.

Drug in-vitro release kinetics

To examine the influence of the different cisplatin formulation on the release kinetics, *in vitro* release of cisplatin encapsulated in PLGA nanoparticles was observed in PBS at physiological temperature ($37\text{ }^\circ\text{C}$) and pH (7.4). All three formulations showed a biphasic release, consisting of an initial burst release (<4 h), followed by a sustained release period due to loss from drug localised in the PLGA matrix. Generally, burst release behavior in drug-loaded particles is explained in two ways: first, initial burst release can occur due to heterogeneous

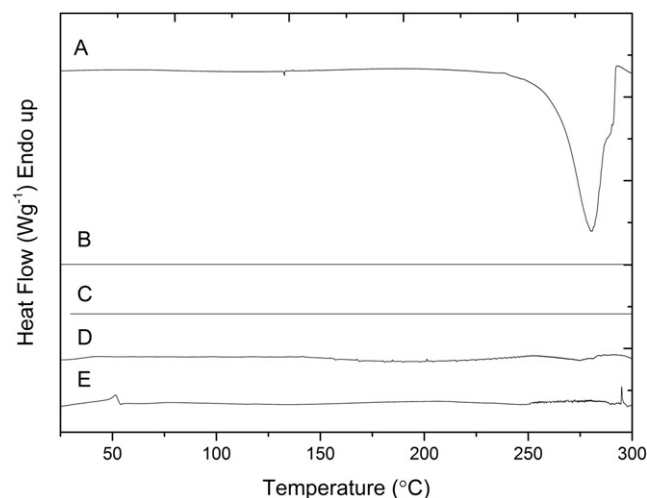


Figure 5. DSC thermogram of (A) cisplatin, (B) 2.5 wt% cisplatin loaded PLGA, (C) 5 wt% cisplatin loaded PLGA, (D) 10 wt% cisplatin loaded PLGA, and (E) PLGA (endotherms are indicated as peaks).

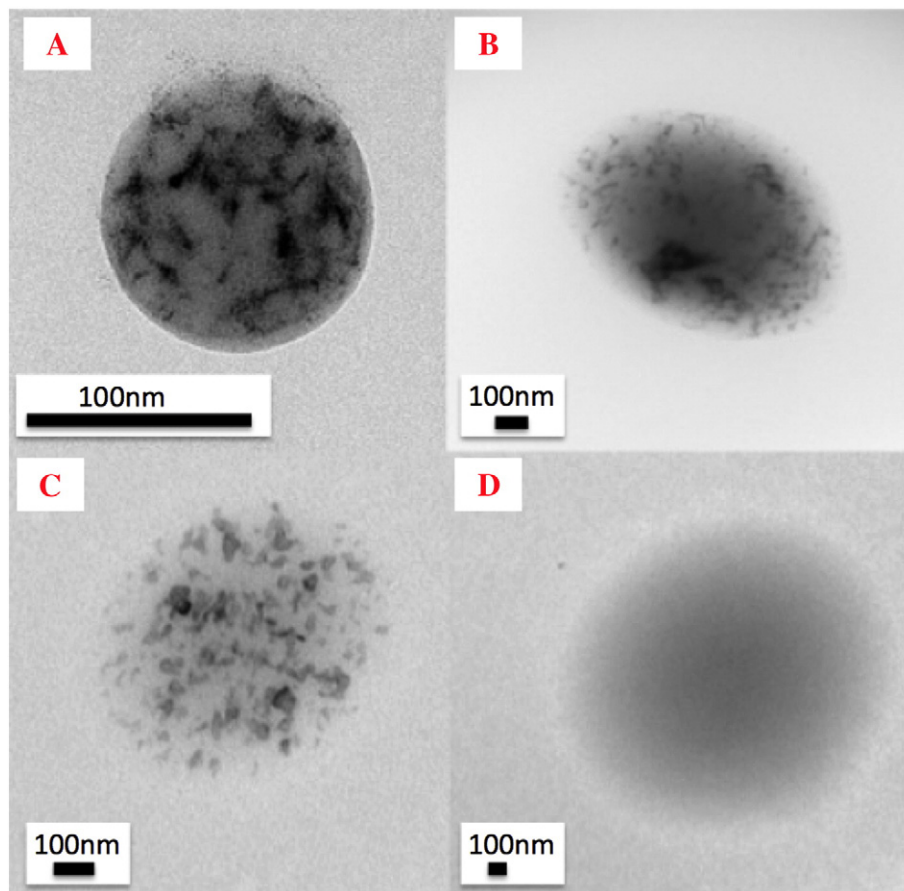


Figure 6. TEM micrographs of (A) 10 wt%, (B) 5 wt%, (C) 2.5 wt% cisplatin loaded particles, and (D) pure PLGA particle.

drug distribution; for example therapeutic molecules that are either loosely associated with the surface or embedded in the surface layer²⁸ and second, release of drug molecules through the pores and cracks, associated with variation in particle morphology. Electron micrographs of the drug-loaded particles indicate that cisplatin was capsulated within PLGA particles by EHDA (Figure 6), in keeping with the nature of this technique. Therefore, it can be inferred that the initial burst may have been caused by the diffusion of embedded cisplatin close to the surface or through water-filled pores in the PLGA matrix rather than by surface-bound drug molecules.

An initial sharp release can be desirable for certain delivery systems, for example targeted delivery or when rapid treatment is required.²⁹ However, for the most part, avoiding burst release behavior is desirable to reduce side effects associated with premature delivery, particularly for highly cytotoxic drugs such as cisplatin.³⁰ The highest initial release rate was exhibited by the 5 wt% particles (Figure 8); after 4 h ca. 45% of the initial cisplatin loaded had been release from the 5 wt% sample, compared to only 25 and 14% from the 2.5 wt% and 10 wt% particles, respectively (Figure 8). Therefore, this suggests control over the burst release may be achieved by controlling the formulation using EHDA, which is highly important for cytotoxic drugs as the dosage must be carefully tailored to each patient to minimize side-effects. Additionally, at the end of

the time period of the experiment the cumulative cisplatin release from the PLGA particles correlated with loading amount, also suggesting control over this property. For example, a total of 329 and 688 μg of cisplatin were released after 144 h from 20 mg of 2.5 and 10 wt% loaded materials, respectively.

To further analyse the mechanism of cisplatin release, the release data were fitted to the Ritger-Peppas, zeroth, first order, and Hixson Crowell models. The values of the coefficient of determination (R^2) for the models applied to each formulation are listed in Table 4. The Ritger-Peppas model provided the best fit to the release data, with an $R^2 > 0.99$ for all the formulations tested. This suggests that at early times the cisplatin release from the PLGA particles has a non-linear dependence on time, which is consistent with diffusive release from distribution throughout the bulk of the particles. Accordingly, using the approximation by Ritger-Peppas et al at short times (total release $< 60\%$) the fractional release depends only on one parameter $(Dt)^{1/2}/a$ (where D is the diffusion constant, t represents time, and a is the sphere radius).³¹ Therefore, assuming the diffusion constant does not vary between materials and drug movement is via matrix permeation, the rate of release should decrease with radius. However, this is not in agreement with the cisplatin release data (Figure 8), from which it can be seen that for cumulative release $< 60\%$ the release rate followed the trend 5 wt% particles ($1.2 \pm 0.20 \mu\text{m}$) $>$ 2.5 wt% particles ($630 \pm$

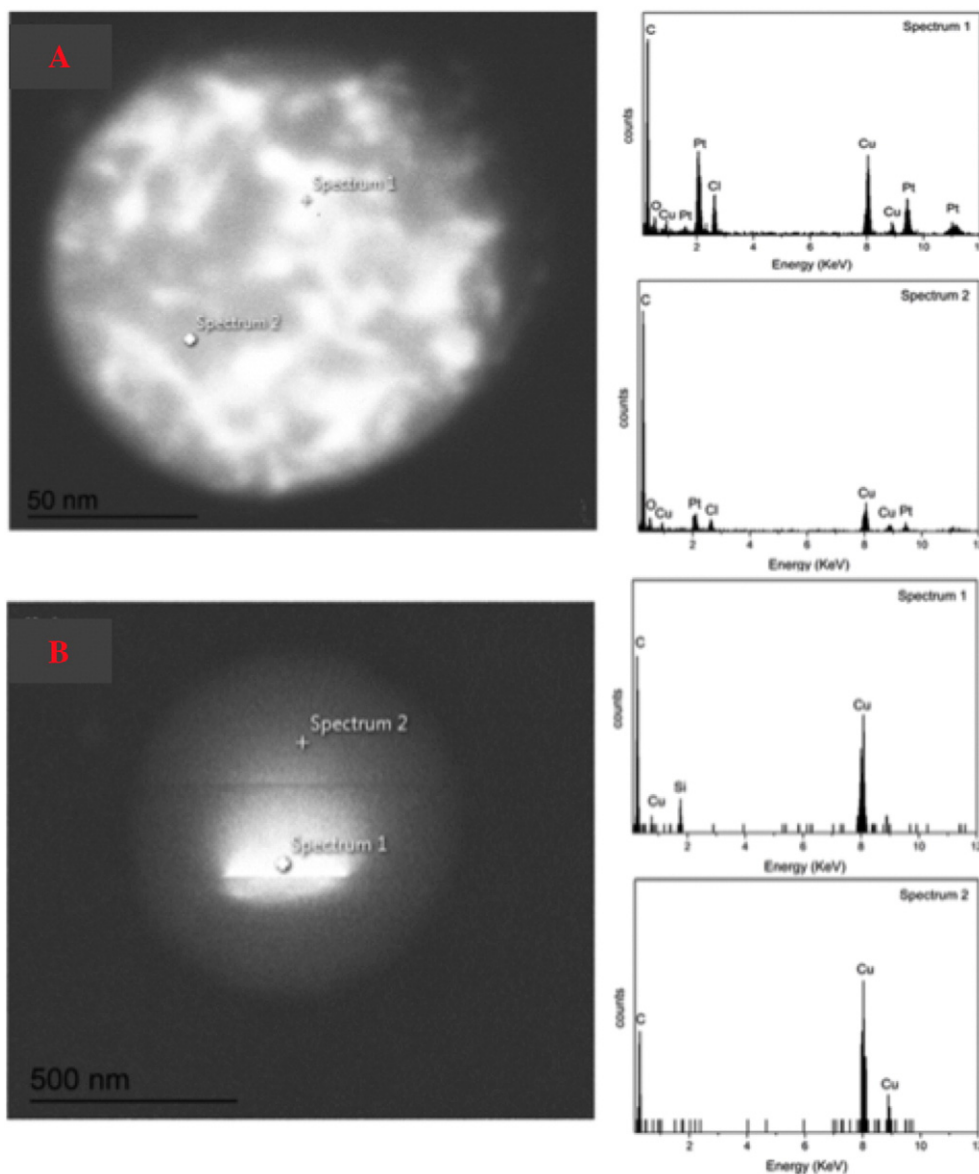


Figure 7. EDX spectra and corresponding dark-field scanning transmission electron micrograph indicating sites of analysis for (A) 10 wt% and (B) 0 wt% loaded PLGA nanoparticles.

95 nm) > 10 wt% particles (550 ± 80 nm). Therefore, it is not possible to attribute the largest difference in initial release rate entirely to size using this model, suggesting that something more complex than a uniform structure with simple permeation diffusion may exist: perhaps the cisplatin distribution is not so uniform throughout the particle, or the PLGA matrix may be slightly different near the surface, leading to a different diffusion constant.

Discussion

The characteristics of polymeric drug delivery systems are important in determining the release profile of the drug. The degradation rate of the polymer and consequently the release of

the drug are affected by the size and shape of the polymeric particles. The size of the particles may also affect the distribution of the drug within the polymeric matrix.³² Many of the properties characterizing the delivery systems are influenced by the manufacturing methods.³³ As demonstrated in Figures 2 and 3, EHDA provides a bottom-up approach in producing cisplatin-encapsulated particles with precise control over the size and polydispersity of particles. In this study, cisplatin loaded PLGA particles of different size have been successfully synthesised using the highly efficient (encapsulation efficiency >70%) and controllable EHDA method. Drug loaded PLGA materials were morphologically controlled by varying the cisplatin and PLGA concentrations in the solution. Particles were produced with mean diameters ranging from 100 nm to 1.80 μ m and with low polydispersity indices of 2.1–2.8%.

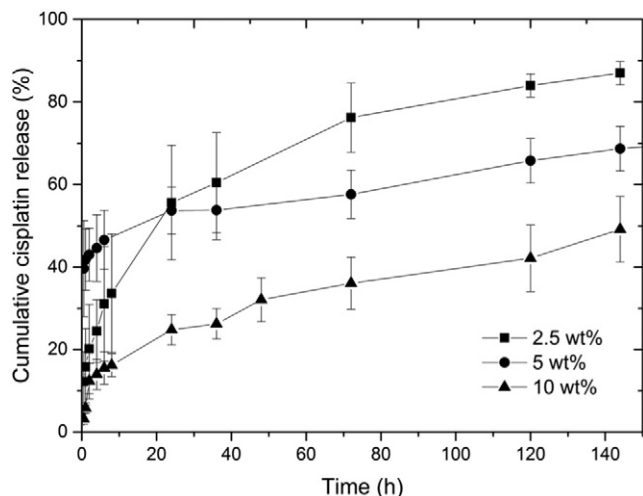


Figure 8. Cumulative release profile of cisplatin from PLGA nanoparticles loaded with different drug concentrations.

Further analysis with TEM/EDX showed effective encapsulation of cisplatin within the polymeric particles. All the formulations exhibited a two stage release profile *in vitro*; an initial burst release <4 h followed by a sustained release period. This initial burst release may be attributed to the surface/near-surface loading of the drug that is generally observed in the drug-loaded polymeric particles produced by single-capillary electrospray method.¹⁶ On the other hand, nanoparticles encapsulated with 10 wt% cisplatin demonstrated the lowest initial burst release of approximately 14%, compared to 45% for the 5 wt% cisplatin microparticles (>4 h), and delivered over double the mass of drug. This enhanced performance cannot be explained by simple permeation diffusion kinetics, implying enhanced functionality due to particle morphology and drug concentration, which enable the potential for high cisplatin encapsulation and tunable release.

Drug release from poly(esters) such as PLGA involves two main mechanisms; diffusion and degradation/erosion.³⁰ Upon initial contact water adsorption leads to pore formation, which over time leads to swelling and pore formation large enough for drug transport.³⁴ Ester bond hydrolysis is an autocatalytic process in PLGA, which can lead to heterogeneous degradation driven by an acid gradient, and therefore accelerated diffusion and erosion processes of the drug and polymer.³² Water adsorption and drug release rate are governed by a number of parameters. Importantly, the rate of release is influenced by the particle size, due to increased pH gradients in larger particles.³⁰ Additionally, the mass of drug encapsulated, and therefore the

difference in remaining space in the matrix can also affect release. Consequently, the results from this work indicate that the drug release from the cisplatin particles is likely a permeation and pore diffusion-based process, which are dependent upon particle morphology and drug concentration. The efficient control of these crucial material properties, afforded by cisplatin capsulation using EHDA, can lead to control over the drug dosage and period of release, both of which are essential in delivery of cytotoxic chemotherapy agents.

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Data supporting this study are provided in the paper and as supplementary information accompanying this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.nano.2016.05.005>.

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Table 4
Summary of fits of different kinetic models using R² values.

Cisplatin loading	R ²			
	Ritger-Peppas	Zeroth-order	First-order	Hixson-Crowell
2.5 wt%	0.99	0.80	0.95	0.93
5 wt%	0.99	0.55	0.72	0.67
10 wt%	0.99	0.84	0.88	0.87

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