Micelle Directed Chemical Polymerization of Polypyrrole Particles for the Electrically Triggered Release of Dexamethasone Base and Dexamethasone Phosphate

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Abstract

Conducting polymers such as polypyrrole (PPy) can be used as electrically responsive drug delivery systems typically prepared by electrochemical polymerisation, however, the amount of drug that can be delivered is typically low. To increase drug delivery capacity and prepare larger amounts of polymer, PPy nanoparticles were produced by chemical polymerisation over drug-loaded micelles. Two forms of dexamethasone were included to increase total drug loading and to explore the mechanisms of loading and release. The particles produced were approximately 50 nm in size and their conductivity and reversible redox activity were demonstrated. ~~Encapsulation~~ Loading of the hydrophobic dexamethasone base was more efficient than for the more hydrophilic phosphate salt. After pressing the particles into the desired form, electrically-responsive drug release was achieved with a pulsed potential signal being the most effective way to trigger release. Notably, the anionic phosphate salt of the drug was more sensitive to electrically stimulated release than the uncharged base of dexamethasone, highlighting the role of electrostatic forces in driving drug release. This system has potential to be loaded with different drugs widening the scope of application of these smart particles to treat a range of disease states.

Keywords

Electrically responsive; drug delivery; controlled release; drug release mechanism; tunable release

1. Introduction

Responsive drug delivery systems can provide release of drugs upon application of a range of stimuli including light (Alvarez-Lorenzo et al., 2009), pH (Gupta et al., 2002), temperature (Bromberg and Ron, 1998), redox (Li and Zhang, 2016; Tian et al., 2016) and electricity (Svirskis et al., 2010a). Electrical stimulation is an attractive approach to tune drug release in the body as it relies on simple setups, and requires typically less than 1 V (Santini et al., 1999; Svirskis et al., 2010a; Uppalapati et al., 2016). Conducting polymers are electroactive materials which can be loaded with drug. Electrical stimulation can be used to alter drug delivery rates from conducting polymers (Abidian et al., 2006; Luo and Cui, 2009) attributed to changes in the charge of the polymer backbone, volume of the polymer bulk, molecular permeability and hydrophilic/hydrophobic balance of the polymer (George et al., 2005). Amongst conducting polymers, polypyrrole (PPy) has been widely used as a platform material for tuneable drug delivery (Geetha et al., 2006; George et al., 2006; Svirskis et al., 2010a). This is due to its inherent electrical conductivity, ease of preparation, stability and excellent biocompatibility (George et al., 2005; Wang et al., 2004). PPy is synthesised through the oxidation of pyrrole monomer units which can be initiated electrochemically using electrical stimulation (Diaz et al., 1979; Li et al., 2005) or chemically using chemical oxidants such as iron (III) chloride ($FeCl_3$) (Armes et al., 1987; Jang and Yoon, 2005), iron (III) perchlorate (Nishio et al., 1996), iron (III) sulphate (Kudoh, 1996; Stejskal et al., 2003) and ammonium persulfate (APS) (Oh et al., 2001), in both aqueous and non-aqueous media.

A range of conducting polymer systems have been reported in the literature for the release of anionic (Kontturi et al., 1998; Zinger and Miller, 1984), cationic (Miller and Zhou, 1987) and neutral drugs (Bidan et al., 1995). These systems can be used to modify drug delivery rates over time, depending on patient needs. However, the amount of drug that can be delivered is low (Richardson et al., 2009). To enable wider application of conducting polymer delivery systems the amount of drug delivered should be increased while the ability to electrically tune drug release rates should be maintained. While electrochemical polymerisation of these systems is widely reported, polymerisation is limited to the size of the conductive substrate on which it is polymerised with thin films, typically less than a few microns, produced (Svirskis et al., 2010b). Chemical polymerisation overcomes these limitations as a conductive substrate is not required, with polymerisation resulting in insoluble polymer precipitates forming in solution. The large amounts of conducting polymer that can be rapidly produced makes

chemical polymerisation suitable for scale-up (Ramanavičius et al., 2005), and attractive for drug delivery applications.

Template directed chemical polymerisation of PPy can be utilized to increase drug loading and release efficiency (Ge et al., 2011; Uppalapati et al., 2016). Ge, et al. have demonstrated the release of fluorescein or daunorubicin from PPY nanoparticles prepared by polymerisation over micelles formed from dodecyl trimethylammonium bromide and decyl alcohol. These particles were dispersed in a temperature sensitive hydrogel, which was injected directly into mice with release of the loaded drugs triggered upon application of an electrical potential (Ge et al., 2011). The present work used sodium dodecyl benzene sulfonate (SDBS) as an anionic surfactant which readily forms micelles in water with a hydrophobic core (Palazzesi et al., 2011). These micelles, with the capacity to solubilise drug, act as a template to guide PPy polymerisation. It was hypothesised that drug will be entrapped following polymerization of PPy over the micelles. In this study, we investigated the loading and release of two drugs, the hydrophobic and neutral dexamethasone (Dex) and its more hydrophilic anionic salt, dexamethasone sodium phosphate (DexP). Dexamethasone is a synthetic steroid that has anti-inflammatory and immunosuppressant effects. DexP is the sodium salt of dexamethasone and a prodrug which is converted into dexamethasone in the body (Dimopoulos et al., 2001; Richardson et al., 2005; Webber et al., 2012).

In this study, drug-loaded PPy particles were prepared utilizing micelles as soft templates capable of enhanced drug encapsulation. Electrochemical and morphological characterisation of the formed particles are described in detail. The formed particles could be compressed into a desired shape and size, as dictated by clinical requirements. Release of both Dex and DexP was examined under different forms of electrical stimulation, while the toxicity of PBS extracts prepared from electrical stimulation of the nanoparticles was tested on human retinal pigment epithelium-19 (ARPE-19) cells.

2. Materials and Methods

2.1. Materials

Pyrrole was purchased from Sigma Aldrich (Australia) and was distilled and stored at -20°C under nitrogen until use. Sodium dodecyl benzene sulfonate (SDBS), ammonium per sulphate (APS) and phosphate buffered saline (PBS) tablets were obtained from Sigma Aldrich (Australia) and used without further purification. Dexamethasone sodium phosphate (DexP)

was purchased from Jai Radhe Sales (India), dexamethasone base (Dex) was purchased from Cfm Oskar Tropitzsch (Germany). ITO coated glass slides (resistance 70-100 Ω) were purchased from Delta Technologies (USA) and silver conductive epoxy (four hour working time) was obtained from MG Chemicals (Canada). Milli-Q water was from a Millipore/Millipak system with a filter size of 0.22 µm and a resistivity of 18.2 MΩ.cm. All other reagents were of analytical grade. ARPE-19 cells (a human retinal pigment epithelial cell line) were obtained from American Type Culture Collection (USA) and were used within 20 passages from the time of purchase. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide (MTT) was purchased from Thermo Fischer Scientific (USA). DMEM/F12, GlutaMAX™ and TrypLE™ Express media were purchased from Thermo Fischer Scientific (USA).

2.2. Determination of the critical micelle concentration (CMC) of SDBS

A series of SDBS solutions at increasing concentrations up to 25 mM were prepared in Milli-Q water. The surface tension of the prepared solutions was measured using a Du Nouy ring tensiometer (White Electrical Instruments Co Ltd, UK.) in order to determine the CMC of SDBS. Briefly, the platinum ring of the tensiometer was immersed in SDBS solution at each specific concentration. The ring was slowly lifted from the liquid. The surface tension, γ, was determined by the force required to separate the ring from the surface of the liquid at 25°C. Triplicate measurements of surface tension for each concentration were obtained.

To further confirm the formation of micelles and CMC of SDBS, dynamic light scattering (DLS) measurements were made. A series of dilutions of SDBS were prepared from the 25 mM SDBS solution. The average size of the micelles (Z-average) was determined using a Zetasizer Nano ZS (Malvern Instruments Ltd, UK). All measurements were conducted in triplicate at 25 °C. The size distribution was measured at a scattering angle of 90°, a wavelength of 633 nm and a viscosity 0.8872 mPa.s.

2.3. The ability of SDBS to solubilise Dex

Dex is lipophilic (logP = 2.03) and sparingly soluble in water (0.035 gL^{-1}; 0.089 mM) . SDBS micelles have a hydrophobic core which can solubilise hydrophobic drugs such as Dex. A series of concentrations of SDBS were prepared into which excess Dex was added. The solutions were stirred continuously for 48 h until the solution was saturated with Dex. The solutions were

filtered (0.22 µm) and analysed for drug content by high performance liquid chromatography (HPLC).

Both DexP and Dex concentrations were determined by HPLC. Briefly, HPLC analysis was conducted on an Agilent 1200 series HPLC (Agilent Technologies, USA) equipped with an auto sampler (injection volume: 10 µL), a vacuum solvent degasser, a quaternary pump and an online diode array detector. Results were analysed using ChemStation® software (Agilent Technologies, Germany). A reverse phase C18 Phenomenex column (250 x 4.6 mm, particle size 5 µm) and a mobile phase consisting of 50% phosphate buffer, 27.3% acetonitrile and 22.7% methanol at 40°C and a flow rate of 1 $mL.min^{-1}$ was used. Absorbance due to both DexP and Dex was determined at 254 nm over a range of 5 $\mu g.\ mL^{-1}$ to 1000 $\mu g.mL^{-1}$ with satisfactory linear regression achieved (R^2=0.999) for both the drugs.

2.4. Fabrication of drug loaded PPy particles

SDBS (25 mM) was dissolved in Milli-Q water to prepare the micelles into which Dex (1.25 mM) and DexP (1 mM) were then added with continuous stirring until a clear solution was obtained. Pyrrole was added dropwise into the stirred drug-loaded micelle solution to achieve a concentration of 0.05 M and equilibrated for 30 min. The solution was transferred into an ice bath and stirred for another hour. The oxidant APS (0.15 M) was dissolved in 2 mL of Milli-Q water and added to the pyrrole/SDBS/drug solution to initiate polymerization. The reaction was carried out for 4 h at 4 °C. The reaction was terminated by adding an excess amount of water and the precipitated PPy nanoparticles were washed with excess Milli-Q water to remove unreacted monomer, SDBS and APS. The resulting PPy particles were dried overnight at room temperature (Figure 1~~Figure 1~~A).

2.5. Morphological Characterisation

2.5.1. Size distribution and zeta potential

The size distribution and zeta potential of the formed PPy particles were determined by DLS measurements as described earlier. A dispersion of PPy particles was prepared in Milli-Q water at a concentration of 0.1 $\mu g.mL^{-1}$.

2.5.2. Scanning Electron Microscopy (SEM)

The surface morphology of the PPy particles was investigated using a Philips XL30S field emission gun scanning electron microscope (Netherlands) at an accelerating voltage of 5 kV.

The samples for SEM were mounted on aluminium studs using adhesive graphite tape and lightly sputter-coated with platinum using a Quorum Q150RS Sputter Coater.

2.5.3. Transmission Electron Microscopy (TEM)

Characterisation using TEM was carried out on a Tecnai™ G² Spirit Twin transmission electron microscope (USA), operated with Xplore 3D software. The fine PPy powder was mixed with Milli-Q water to form a dispersion and 5 µL was added to the coated grid. The coated grid was left under a hot lamp to evaporate water, leaving a thin layer of particles on the grid.

2.6. Electrochemical Characterisation

To support electrochemical characterisation, particles (*c.a.* 25 mg) were compressed into a pellet (Figure 1~~Figure 1~~A), 13 mm in diameter (around 2 mm in thickness) using a hydraulic pellet making machine which applied 8 tonnes of pressure. The pellet was firmly adhered to an ITO covered glass slide working electrode by means of silver epoxy. The exposed ITO was masked by kapton tape leaving only the pellet in contact with the surrounding environment for all the samples (Figure 1~~Figure 1~~B). The ITO substrate with attached pellet was fixed into the custom built horizontal setup (Figure 1~~Figure 1~~C).

2.6.1. Cyclic Voltammetry (CV)

The electroactivity of the PPy particles was characterized using CV. A three-electrode, electrochemical setup was utilized for recording the CV of the particles. The PPy particle pellet adhered to an ITO covered glass slide was used as a working electrode. Platinum mesh was used as a counter electrode and Ag/AgCl as a reference electrode (Figure 1~~Figure 1~~C). The particle pellets were cycled between -0.7 V to +0.7 V at a rate of 100 mV s^{-1} in PBS for 100 cycles using a Bio-Logic potentiostat (USA) and data was analysed using the EC-Lab® software.

CV was also used to determine the surface area of the nanoparticle based electrode using the same setup. The particles were cycled between -0.2 V to +0.6 V at a rate of 100 mVs^{-1} in PBS containing 5 mM ferri-ferrocyanide ($Fe(CN)_6^{3-/4-}$; 5 mM ferricyanide and 5 mM ferrocyanide). The charge passed through the PPy pellet is related to the surface area and square root of time according to Equation 1, (Anson, 1964; Kong et al., 2014).

$$\boldsymbol{Q = (2nFAD^{\frac{1}{2}}\pi^{-\frac{1}{2}}C)t^{\frac{1}{2}}} \qquad \textbf{Equation 1}$$

$$Q = Kt^{\frac{1}{2}}$$

where Q is the charge passed, n is the number of electrons used in the reaction (n=1 for ferriferrocyanide, $Fe(CN)_6^{3-/4-}$), F is the Faraday constant (9.65 x 10^4 C mol^{-1}), A is electrode surface area, D is the diffusion coefficient (6.2 x 10^{-6} cm^2 s^{-1}) and C is a mediator concentration (5 x 10^{-6} $mol.cm^{-3}$ $Fe(CN)_6^{3-/4-}$ in PBS is used as a mediator).

2.6.2. Conductivity

Electrical conductivities (S cm^{-1}) of the PPy particles pressed into pellets were measured using a Jandel (RM2 model) Multi Height Probe, Resistivity Test Unit (UK) with a four-point linear probe (1.0 mm tip spacing). The pellets were placed on a glass slide and four replicate measurements were taken for each pellet. The current was set at 500 µA and the voltage was measured. These values were used to calculate the resistivity (ρ in Ω.cm) according to Equation 2.

$$\rho = \frac{4.532 \times V \times t}{I} \qquad \textbf{Equation 2}$$

Where; V = voltage (V), t = sample thickness (cm), I = current (A) and ρ = resistivity (Ω.cm).

2.6.3. Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy was performed using a three-electrode setup with a Bio-Logic electrochemical workstation. Either PPy pellets, bare ITO, or silver epoxy coated ITO was used as a working electrode, with a platinum mesh counter electrode and Ag/AgCl reference electrode. Impedance was measured at open circuit potential in PBS with a 10 mV alternating current component from 10 kHz to 1 Hz. Impedance data was analysed using EC-Lab® software.

2.7. Drug Loading and Electrically Responsive Release

The PPy particles were investigated for drug loading (Equation 3) and encapsulation efficiency (Equation 4) indirectly by determining the amount of drug remaining in the supernatant of the synthesis solution, using~~by~~ HPLC.

$$Drug\ Loading = \frac{Mass\ of\ drug\ added - mass\ of\ drug\ in\ the\ supernatant}{Total\ mass\ of\ drug\ loaded\ particles} * 100\%$$

Equation 3

$$Encapsulation\ Efficiency = \frac{Mass\ of\ drug\ added - mass\ of\ drug\ \ in\ the\ supernatant}{Mass\ of\ drug\ added} * 100\%$$

Equation 4

The same setup of pressed pellets on ITO covered glass slides used for electrochemical characterisation was used for drug release studies with PBS as the medium (Figure 1~~Figure 1~~C). To determine the effect of redox state of PPy on drug release, the release was determined under different conditions. A voltage of +0.6 V was applied to oxidise PPy, -0.6 V was applied to reduce PPy and pulses of ±0.6 V was applied at 2 Hz to alternately oxidise and reduce the PPy pellet for 3 h. Release under these conditions was compared against passive release in the absence of electrical stimulation for 3 h. Sink conditions were maintained at all times by adding fresh PBS each time when a sample was taken. A paired t-test was applied between the pulsed stimulation group and other groups (oxidation, reduction and no stimulation) to determine the statistically significant difference in release after 3 h.

To further determine the ability to alter drug release from the particles, pulses of stimulation in the form of ±0.6 V at 2 Hz were applied for 5 h after 24 h and 48 h. Sink conditions were maintained at all the times. Release samples were analysed by HPLC.

2.8. Cytotoxicity studies

ARPE-19 cells were used to determine the cytotoxicity of the particle pellets with viability determined using the MTT assay. PBS extracts were obtained from the pellets in the presence (± 0.6 V, 2 Hz for 1 h) and absence of electrical stimulation. The influence of the conductive silver epoxy used to adhere PPy pellets to ITO-coated glass slides was also evaluated. Negative control cells were treated with DMEM/F-12, GlutaMAX (100% cell viability) whereas positive control cells were treated with Triton X-100 in DMEM/F-12, GlutaMAX (0% cell viability). ARPE-19 cells were seeded at a density of $2x10^5$ cells/mL in 96-well plates and incubated at 37°C in a 5% CO_2 humidified atmosphere overnight. PBS extracts from each sample (n=6) in DMEM/F-12, GlutaMAX (1:1) were added by replacing the cell culture medium. Plates were then incubated for 1 h at 37°C and 5% CO_2. After the incubation, solutions from each well were removed and replaced with MTT solution (0.5 mg mL^{-1}) and incubated at 37°C for 4 h. The MTT solution was removed from all the wells and replaced with 0.04 M HCl-isopropanol solution to dissolve the formed formazan. The intensity of the purple colour was quantified by

measuring the absorbance at 570 nm with correction of any interference at 650 nm (Bio Tek Synergy HT) and percentage cell viability was calculated.

3. Results and Discussion

3.1. CMC of SDBS

In order to construct micellar templates used to direct the chemical polymerisation of PPy, the CMC of SDBS was determined from a change of surface tension using a Du Nouy ring tensiometer (Lee et al., 2012). The surface tension of water was 72.8 mNm^{-1} which decreased upon addition of SDBS until the CMC was reached, after which the surface tension plateaued (Figure 2). Three linear portions were found in the graph (before 1 mM, between 1 mM to 2 mM and after 2 mM). This indicates that while micelle structures formed at 1 mM there might be a transition between different micelle structures at 2 mM. These data are in line with literature values for the CMC of SDBS between 0.86 and 1.6 mM determined by a variety of methods, these values vary based on method of determination, the presence of counterions, pH and temperature (Jódar-Reyes et al., 2006; Paria et al., 2005; Yu et al., 2012).

The size of the SDBS micelles was determined by DLS. SDBS micelles were confirmed by the presence of a peak between 3-4 nm (Palazzesi et al., 2011). The intensity of this peak corresponding to the micelles decreased as the concentration of SDBS decreased with the peak disappeared when the concentration was diluted below 1 mM (Figure 3~~Figure 3~~), corroborating the surface tension data.

3.2. Solubility of Dex in SDBS

As the concentration of SDBS approached the CMC, a sharp increase in the solubility of hydrophobic Dex base was observed (Figure 4~~Figure 4~~), indicating the ability of the SDBS micelles to solubilise this poorly water soluble drug, presumably in their hydrophobic core. Above the CMC, the solubility of Dex increased out to the highest SDBS concentration tested, 25 mM. For subsequent drug loading and release experiments 25 mM of SDBS was used which could solubilise 1.25 mM (0.5629 mg. mL^{-1}) of Dex.

3.3. Preparation of PPy Particles

PPy particles, loaded with both Dex and DexP were successfully prepared by chemical polymerization of pyrrole using micelles as a soft template. A proposed schematic describing

the synthesis of the drug-loaded particles is presented in Figure 5~~Figure 5~~. Dex with a logP of 2.03 was expected to associate with the hydrophobic core of the micelles while DexP (logP = 0.54) is assumed to preferentially associate near the hydrophilic head groups of the micelles in close proximity to the surrounding media.

These drug-loaded micelles were then used as a template to carry out the polymerization of PPy. Pyrrole ~~(logP = 0.7)~~ was then added to the micellar solution, where, due to the logP of 0.7 it would associate with the ~~hich tends to orient towards the hydrophilic heads of the~~ micelles (CSID:7736, 2013). Subsequently, pyrrole monomer was polymerized by adding the oxidant APS (Figure 5~~Figure 5~~D). The black PPy particles that formed over 4 h were collected by filtration and the unreacted oxidant and monomer were washed away with excess water.

3.4. Morphological Characterisation

SEM demonstrated aggregates of particles around 50 nm in diameter (Figure 6~~Figure 6~~), with individual particles able to be visualised under TEM (Figure 6~~Figure 6~~ inset). DLS measurements ~~confirmed this data with particles~~ were in a similar range with ~~of~~ a hydrodynamic diameter of 50 nm ~~in size present~~ (poly dispersity of 0.362). The zeta potential of the PPy particles was -45.7 mV. The zeta potential remained stable after a week indicating stability of particles in solution (Park et al., 2014; Samanta et al., 2015). A zeta potential value greater than +30 mV or less than -30 mV indicates a high degree of colloidal stability due to repulsion between particles (Clogston and Patri, 2011).

3.5. Electrochemical characterisation

The electroactive nature of the prepared PPy particles was established by CV and conductivity measurements. The PPy particles were cycled at a constant rate of 100 mV s^{-1} between two set of points (-0.7 V and +0.7 V vs Ag/AgCl) in PBS. Cyclic voltammetry over 100 cycles confirmed the reversible electroactive nature of the prepared PPy particles (McCormac et al., 1995). The oxidation peak was found at 0.42 V and the reduction peak was found at -0.18 V (Figure 7~~Figure 7~~).

The geometrical surface area of the exposed ITO was 1.32 cm^2. The electrode surface area of the PPy pellet was calculated from CV and was found to be 2.59 cm^2, whereas the electrode surface area for silver epoxy on ITO was found to be 1.22 cm^2. Hence the roughness factor was calculated to be 2.12. The increase in surface roughness increases the polymer: media interface

which is expected to decrease the impedance and increase the responsiveness of the system (Luo et al., 2011).

The conductivity of the PPy particles pressed into a pellet was 22.9 ±5.49 S cm^{-1}. The conductivity of the PPy particle pellet was consistent with PPy films reported in other studies, which typically ranged between 1 and 60 S cm^{-1} (Kassim et al., 2002; Planche et al., 1994; Reung-U-Rai et al., 2008). This verifies that the PPy particles contain polymer chains that are conductive and that charge can pass between the particles in the pressed pellet (Svirskis et al., 2010b).

Impedance determines the resistance including capacitance and inductance of the polymer offered on application of current as a function of frequency (Fernández-Sánchez et al., 2005; Lasia, 2002). The PPy particle pellet had a reduced impedance modulus (|Z|) compared to bare ITO or ITO coated silver epoxy over all frequencies. A comparison can be made between samples by taking (|Z|) at 2 Hz (frequency used during stimulation of drug release). ITO had an impedance of 2337 Ω, silver epoxy on ITO had an impedance of 1240 Ω while particle pellets stuck on ITO using silver epoxy had an impedance of 205.5 Ω.

The decrease in impedance may be attributed to an increase in surface roughness which results in a higher electrochemical surface area (also indicated by CV results). Decreasing impedance values are favourable for drug releasing systems. A lower impedance value suggests that less current or voltage is required to drive drug release from the polymer systems (Luo et al., 2011).

3.6. Drug Loading and Electrically Responsive Release

Drug loading and entrapment efficiency data for DexP and Dex in PPy particles are shown in Table 1~~Table 1~~. ~~Entrapment efficiency of Dex was higher than for DexP, indicating that Dex, being a poorly water soluble drug, could have been entrapped in the hydrophobic core of the micelles, whereas DexP could have been solubilised between micelles and the surrounding medium. This suggests that PPy was indeed formed over the micelles.~~

The entrapment efficiency of this process was very high (80.5 ± 1.19%) for Dex, compared to DexP (58.3 ± 2.50%), ~~while~~ drug loading was limited by the amount of drug solubilised in the micelles. This finding could be predicted from the logP values as Dex (logP 2.03) would preferentially associate with the micelles compared with DexP (logP 0.54). Since these particles can be compressed into different shapes and sizes, the total quantity of drug delivered

by the final system can be increased depending on the dosing requirements. In the studies below the *c.a.* 25 mg of PPy pellets contained 1.13 mg DexP and 1.56 mg Dex.

Table 1: Entrapment efficiency and loading of DexP and Dex in the PPy particles

	Entrapment efficiency (%) (Mean ± SD) (n=3)	Drug loading (%) (Mean ± SD) (n=3)
DexP	58.3 ± 2.50	4.50 ± 0.26
Dex	80. 5 ± 1.19	6.24 ± 0.04

Drug release from conducting polymers depends on the redox state of the polymer which both determines the electrostatic interactions between polymer and the loaded drug and drives volume changes in the polymer (Uppalapati et al., 2016). The slowest rates of release of the anionic DexP were observed when the PPy particles were maintained in the oxidised state and when they were not stimulated (Figure 8~~Figure 8~~A). Without stimulation, PPy would be expected to remain close to the oxidised state, as the result of polymerisation. In the oxidised state, the positive charges on the polymer backbone would attract the anionic DexP. DexP was released faster on reduction when the polymer backbone is neutralised and the net negative charge in the polymer, due to the presence of anionic DexP, forces drug out of the polymer. DexP release was highest when a pulsed stimulus of ± 0.6 V at 2 Hz was applied. This pulsing causes de-doping of DexP from the polymer in the reduced state and doping of anions from the PBS on oxidation. The non-ionic drug Dex showed a similar release pattern to DexP (Figure 8~~Figure 8~~B). The main point of difference was that reduction did not increase drug release rates beyond no stimulation. As Dex is an uncharged molecule electrostatic charges would not be expected to directly cause release. Interestingly, alternating the redox state of the polymer resulted in increased release rates. This could be attributed to volume changes in the polymer in different redox states altering the rates of drug diffusion from the PPy particles into the surrounding solution (Svirskis et al., 2013). The release of both DexP and Dex was statistically significant on pulsed stimulation compared to oxidation, reduction or no stimulation after 3 h ($p<0.001$ in all cases).

The release of both the anionic DexP and the non-ionic Dex was highest on application of an alternating potential stimulus. To further demonstrate the tunability of the PPy particles, the release of DexP and Dex was determined by intermittent bursts of an alternating potential

stimulus (Figure 9~~Figure 9~~). After an initial 24 h of passive release, application of a 5 h period of ±0.6 V stimulation at 2 Hz caused a surge in release. A similar trend was observed when this stimulation was applied again at 48 h. The experiment was terminated at 72 h as the pellets started delaminating from the substrate. Based on loading levels there was a considerable amount of both DexP and Dex remaining in the particles.

Interestingly, for both DexP and Dex more drug was released during the second period of stimulation than the first. The pellets could be seen visibly swelling during stimulation, eventually resulting in delamination from the underlying substrate. This swelling may be due to electro-actuation of the polymer (Svirskis et al., 2010c) and may have increased the rate of drug release. Future work is required to ensure the stability of the PPy particle based delivery system. During these release experiments a higher percentage of DexP than Dex was released. This indicates electrically driven release of the anionic DexP is far more efficient than release of the uncharged base form of the drug.

3.7. Cytotoxicity studies

The cytotoxicity of PBS extracts from drug loaded PPy particles prepared with and without electrical stimulation was determined on ARPE-19 cells using the MTT assay. There were no significant differences in cell viability among any of the groups compared with the control (Figure 10) indicating a lack of cytotoxicity. The slight increase in cell viability in the group containing DexP (0.5 $mg.mL^{-1}$) and Dex (0.05 $mg.mL^{-1}$) indicates an increase in metabolic activity of ARPE-19 caused by the corticosteroid dexamethasone as previously reported (Du et al., 2009).

4. Conclusion

SDBS micelles loaded with the anionic and hydrophilic drug DexP, and the non-ionic and hydrophobic Dex were prepared. PPy particles were polymerised chemically using drug loaded SDBS micelles as a soft template. Chemical polymerisation can prepare large amounts of polymer and is suitable for scale-up. The formed nanoparticles were around 50 nm in size and negatively charged. The PPy particles were conductive and reversibly electroactive. The lipophilic Dex was more efficiently entrapped than the more hydrophilic DexP in the particles reinforcing the role of micelles in polymerisation. Pressing the particles into a desired form can be achieved with electrically responsive drug release demonstrated. An increase in release of DexP was observed on application of a reducing potential suggesting electrostatic forces

influence the release of the anionic drug. Meanwhile a reducing potential had no effect on the release of the uncharged Dex. An alternating potential was the most efficient stimulation to trigger release of both Dex and DexP. Overall, the electrically driven release of the anionic DexP was more efficient than the electrically driven release of the uncharged Dex. Periods of alternating potential resulted in bursts of release for both drugs. Extracts obtained from the PPy particles indicated no cytotoxic effects on ARPE-19 cells. These particles could be compressed into different shapes and sizes enabling customizability of these systems that could be used for tuneable drug release in a number of conditions including chronic retinal diseases. In addition, the particles could be loaded with drugs of different properties widening the scope of application of these smart particles to a range of disease states.

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