RSC Advances

ARTICLE

Supplementary information
Supplementary methods

S.M.1 SEM and EDX analysis.

A high resolution Schottky field emission-scanning electron microscope (FE-SEM, Jeol JSM-7800F, Japan), with a base pressure of approximately 2×10-5 Pa coupled to an energy dispersive X-ray spectrometer (EDX) was employed to observe the morphology and to determine the elemental composition of the particles of PEGylated cisplatin.

S.M.1.1 FTIR spectroscopy.

Fourier transform infrared (FTIR-ATR) spectra were recorded on a Spectrum Two™ (Perkin Elmer, USA) spectrophotometer in order to estimate the functional groups present in the PEGylated drug.

S.M.1.2 Drug release study.

We prepared the hydroxyapatite (HAp) scaffold to perform the drug release survey. 1 g of the biomaterial prepared in our laboratory and 0.5 g of sodium acetate (J.T. Baker) used as porogen, were ground to a mesh size of 200 (74 μm). A polyester hydroxylated resin (Reichhold, Mexico) and polyisocyanate were utilized in a proportion of 1:4 (v: v) as an agglutinant. The powder was mixed with the agglutinant to form a dough. The scaffolds were obtained from cylindrical molds of approximately 4.5 mm diameter and 3 mm thickness. The compression tests were performed using a universal testing machine (Instron 5500R) at a constant crosshead speed of 1 mm/s. Second, the synthesized polyurethane was placed in a Soxhlet apparatus to remove the salt and to form the pores. The scaffolds were afterwards loaded with M1G1D2 for 24 hours. Next, the sample was immersed in a sol-gel solution of tetraethyl orthosilicate (Sigma-Aldrich, Germany) to form a silica coat. The coating allows the impregnated drug not to be delivered immediately. The drug release experiment was then carried out by adding the coated sample to 2 mL of Milli-Q water and moving the scaffold to fresh water approximately every seven days for three months.

The PEGylated CDDP activity in the samples was quantified using a microplate spectrophotometer reader (Multiskan GO, Thermo Fisher Scientific Inc., Waltham, MA, USA) in multimode wavelength scans from 200–1000 nm at room temperature and with linear shaking.

Supplementary results

The findings are discussed in detail. We noted that small dispersed insoluble particles were formed during the PEGylation. These particles were separated by sedimentation and analyzed. It was challenging to gather enough particles for examination by SEM and FTIR, because the particles strongly adhered to the Eppendorf tube surface. The analysis was conducted to search for further evidence that the hereafter suggested structures were formed (see Fig. S1 and S2).

S.R.1 Evidence of the synthesis and presence of the carbonyl group

An SEM image of the sample revealed that clusters of small particles of roughly 50 nm in diameter were formed (Fig. S1). The aggregates were surveyed by energy-dispersive X-ray spectroscopy (EDX), captured during SEM, to verify the obtained structure (Fig. S3).

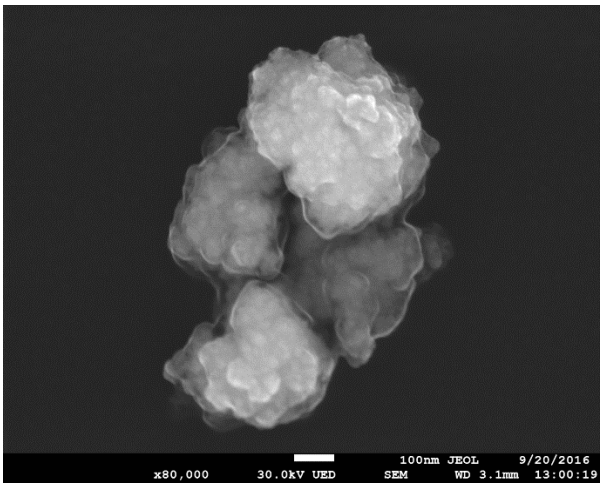

Fig. S1 SEM image of CDDPPEG clusters (bar code 100 nm)

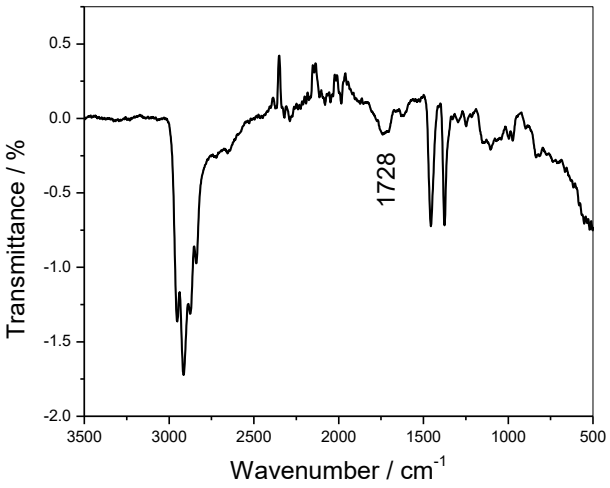

Fig. S2 FTIR spectra of CDDPPEG sample

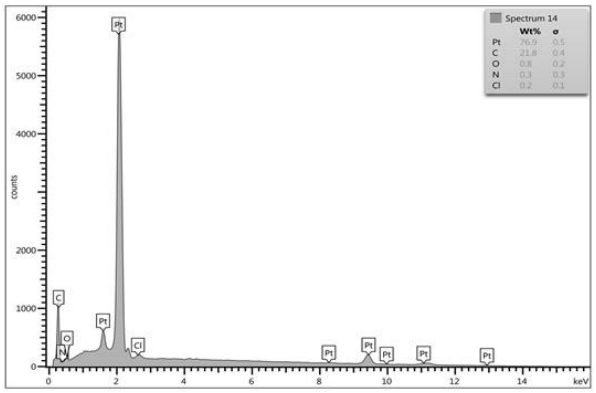

Fig. S3 EDX spectra coupled to SEM

FTIR of the same sample (CDDPPEG) was attempted after determining the impossibility of using Raman spectroscopy, which did not provide good and reliable results for comparison with the spectra shown hereafter (Fig. S2). We noticed that the product displayed bands at 813 and 976 cm^{-1} ascribed to amine out-of-plane bend. The band at 1092 cm^{-1} was recognized as a stretching vibration of the hydroxyl group linked to platinum (ν(Pt–O)), while the band at 1168 cm^{-1} was assigned to symmetrical rocking of the methylene group. We also observed the wagging of N–H at 1249 cm^{-1} and 1296 cm^{-1}. The amine in-plane bend (δ(N–H)) was attributed to the bands 1375 cm^{-1} and 1457 cm^{-1}, and the scissoring of N–H corresponded to the band at 1622 cm^{-1}. The band at 1728 cm^{-1} confirmed the presence of a carbonyl stretching vibration. It was also possible to detect a noisy group of bands within the range 1960–2300 cm^{-1} that were hereafter associated with the contribution of hydrogen bonds C-H•••O=C, N-H•••O=C and Pt-OH•••O=C. Finally, a group of bands were present around 2913 cm^{-1}, which provided evidence for the existence of different modes of methylene stretching vibrations.

Fig. S3 clearly shows the presence of platinum (Pt: 1.67; 2.15; 9.75 keV), carbon (C:0.2777 keV), oxygen (O: 0.525 keV), nitrogen (N: 0.392 keV), and even chlorine (Cl:2.621) peaks. The relative weight of each analyte changed with the area of analysis because PEG molecules of different lengths were attached to CDDP. The multiple peaks of Pt did not allow accurate determination of their relative weight. The weight % of Pt was estimated as roughly 36±5 %, which is consistent with the attachment of PEG oligomers possibly formed by radiolytic degradation.

The presence of the carbonyl group was still doubtful because it was relatively small with respect to cisplatin and PEG suggesting that it was scarce. Hence, we performed additional UV analysis of the samples (Fig. S4). This study was tremendously challenging, first because the literature was controversial and second, because of the weak signal and low concentration of the carbonyl functional group[37–39].

Some researchers have suggested that cisplatin has a low molar absorptivity in the UV region, so that it should be derivatized for observation[51,37], while others have claimed that cisplatin displays absorption peaks at 208 nm and 300 nm respectively[38]. In our case, we found that cisplatin has several absorption peaks, at 229, 242, 247, 260, and 271 nm, which were also seen in PEGylated cisplatin.

Interestingly, a weak band was observed in the CDDPPEG sample. This new band was attributed to carbonyl groups because PEG has no absorption bands in the UV region[39]. This observation strongly suggested the presence of a carbonyl group in the modified molecule.

S.R.1.1 Estimate of mean lifetime of PEGylated cisplatin.

Earlier work on the in vitro release of cisplatin from hydroxyapatite (HAp) scaffolds showed that cisplatin release conforms to an exponential decay profile[31]. We were prompted to study CDDPPEG release under the same experimental conditions in order to compare the release profiles. Nevertheless, PEGylated cisplatin concentration could not be directly determined by inductively coupled plasma (ICP), because the covalent attachment of PEG requires the digestion of the sample to detect Pt and the end of the process. Hence, variations in the absorption intensity of the band at 206 nm versus time were studied by ultraviolet spectroscopy.

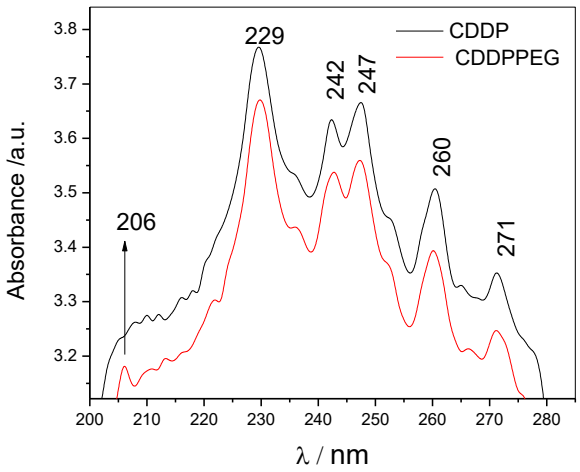

Fig. S4 UV/Vis of CDDP and CDDPPEG

Fig. S5 shows the morphology of the hydroxyapatite polyurethane composite used for the release of the products. The HAp scaffold exhibited nanopores and micropores of different shapes and sizes, with great interconnectivity. Fig. S6 indicates that the scaffold showed suitable mechanical properties, with a compressive modulus and compressive strength of 200 ± 2 and 3.4 ± 0.1 MPa, respectively ($p<0.05$) and an elongation at break of 3.21 %.

The relative concentration of CDDPPEG released by the hydroxyapatite scaffold at different times was determined (Fig. S7). The release was fitted to an exponential decay (see Eq.1):

$$[CDDPPEG] = \alpha + \beta e^{-t/\tau} \quad (1)$$

where [CDDPPEG] is the PEGylated cisplatin concentration, "α" and "β" are fitting parameters and τ is the characteristic release time. The parameters obtained were substituted and compared with those previously obtained for the release of cisplatin (HAP1)[31]

$$[CDDPPEG] = 3.04 + 0.16e^{-t/10.30} \quad (2)$$

$$[CDDP] = 0.0263 + 1.7701e^{-t/4.18} \quad (3)$$

As seen in Equations 2 and 3, a longer-term release was obtained for CDDPPEG, which showed a greater mean lifetime (τ). These results provide evidence that the attachment of PEG to the drug defines a delay in the release process *in vitro*.

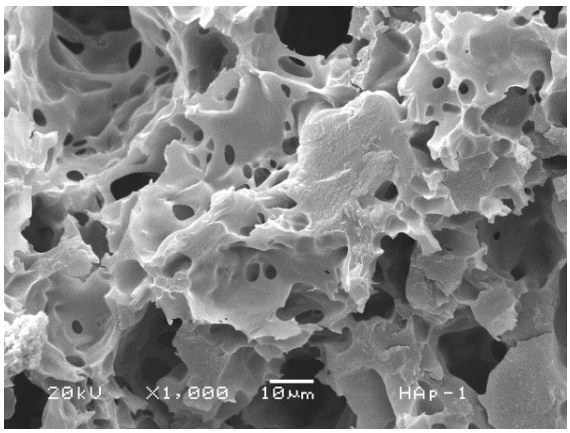

Fig. S5 SEM image of HAP polyurethane scaffold (bar code 10 µm)

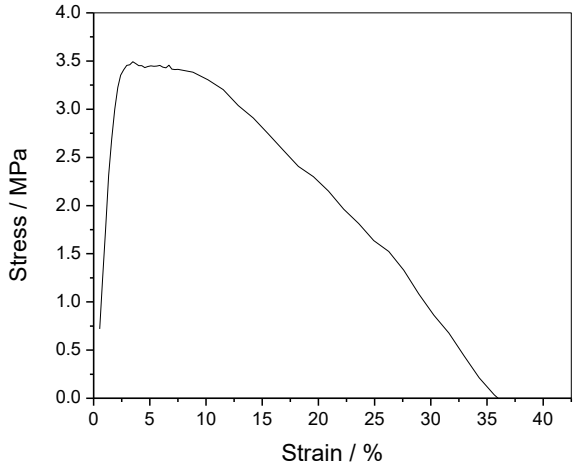

Fig. S6 Tensile stress–strain curve of the scaffold.

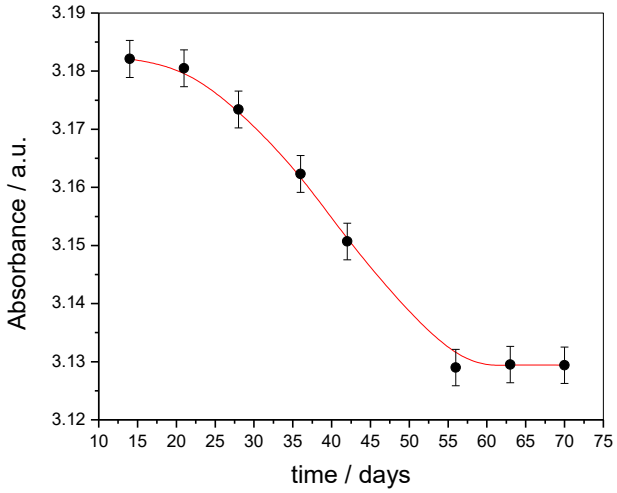

Fig. S7 Graph of relative concentration of CDDPPEG released by the hydroxyapatite scaffold versus time.