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Silica nanoparticles as vectors for Pt(IV) prodrug

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Introduction

The platinum(II) complexes are the most important drugs in anticancer chemotherapy but their low selectivity of action represents a critical aspect for their clinical application because it can lead to serious side effects. In recent years, therefore, the research has moved towards Pt(IV) compounds and it is generally accepted the hypothesis that the reduction to their parental Pt(II) complexes is the basis of their antitumour activity (*Figure 1*).

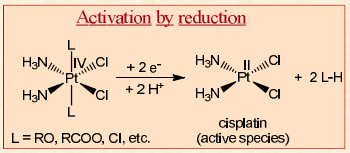
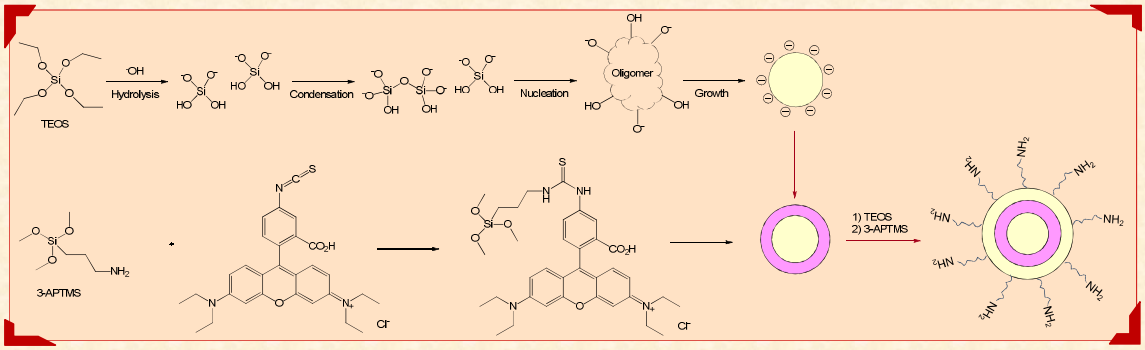


Figure 1. Pt(IV) complexes mechanism of action: the so-called "activation by reduction"

Figure 2. Scheme of Pt(IV) complex with one vector linked through a spacer

Moreover, in order to send drugs selectively to the tumor site, a strategy of *drug targeting and delivery* (DTD) can be exploited using vectors able to lead cytotoxic agents to tumor cells, thus not damaging healthy cells. In particular, in this work we have decided to pursue a passive DTD method (*Figure 2*), which exploits the so-called "*enhanced permeability and retention* (EPR) effect", according to which the solid tumors tissue, being in rapid and uncontrolled growth, shows a high permeability of blood vessels and an inefficient lymphatic drainage from the cell interstices. The combination of these effects makes the tumor iperpermeable to circulating macromolecules (e.g. proteins, nanoparticles, liposomes, etc.), which extravasate and are retained into the tumor mass. Therefore, such macromolecules can be used as vectors for the selective accumulation of the drug.

Synthesis and characterization of the *core-shell* silica nanoparticles



NPs	SEM diameter (nm)	fluorophore
a	262 ± 16	rhodamine B
b	116 ± 17	fluorescein
c	96 ± 13	rhodamine B
d	48 ± 5	fluorescein

Table 1. Features of the synthesized silica nanoparticles

Figure 3. Synthetic pathway of silica NPs

Synthesis of the Pt(IV) complexes

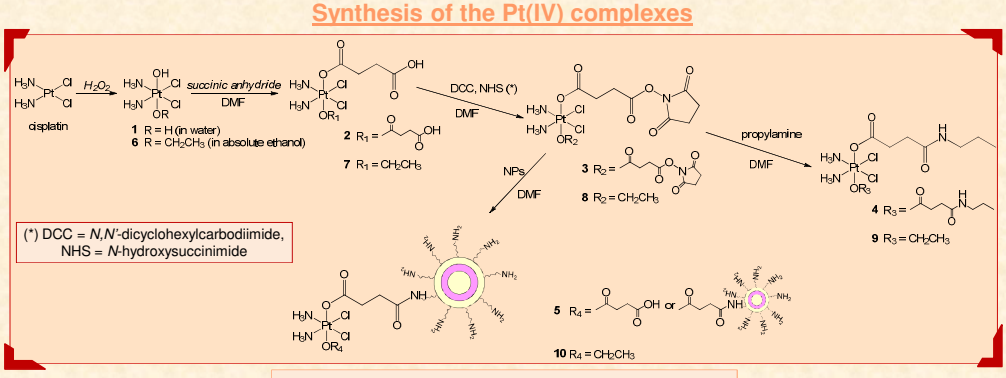
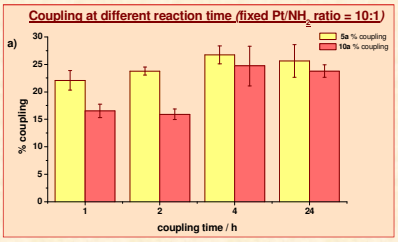


Figure 4. Synthetic pathway leading to Pt(IV)-NPs conjugates

Coupling results

The resulting Pt loading of conjugates **5a-5d** and **10a-10d** is determined, after microwave mineralization, by means of *Inductively Coupled Plasma - Mass Spectrometry* (ICP-MS)



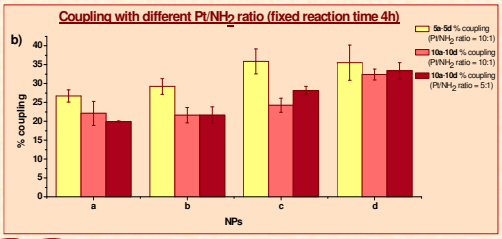


Figure 5. % coupling of the conjugates a) at different coupling time; b) at fixed reaction time and with different Pt/NH_2 ratio

Conjugates characterization - *Scanning Electron Microscopy* (SEM)

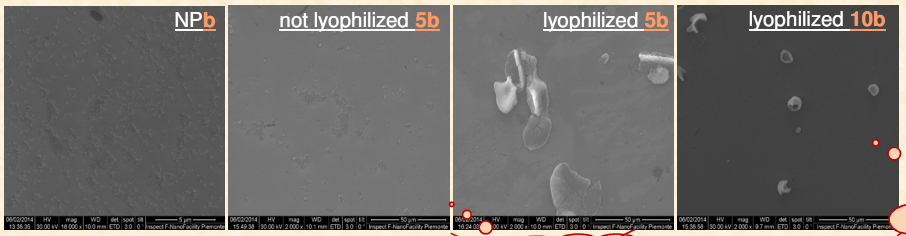


Figure 6. SEM micrographs of conjugates

bis-functionalized Pt(IV) complex

monofunctionalized Pt(IV) complex is chosen

Antiproliferative activity - IC_{50} (nM)

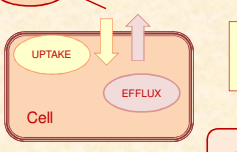
Compound	IC_{50} (nM) A2780
cisplatin	460 ± 110
7	5070 ± 380
b	>> 5000
c	>> 5000
d	>> 5000
10b	11 ± 3
10c	70 ± 12
10d	40 ± 34

IC_{50} (*half inhibiting concentration*), or potency = drug concentration capable of reducing by 50% cell vitality

The synthesized conjugates, in particular **10b**, show a higher activity respect to **7** and cisplatin itself

Table 2. IC_{50} values for a 72h continuous treatment (CT) on A2780 cell line (ovarian carcinoma). Data are means ± standard deviation of at least 3 independent replicates.

Accumulation Ratio (AR)



$$AR = \frac{[Pt]_{intracellular}}{[Pt]_{extracellular}}$$

10b ≈ 10c > 10d >> cisplatin ≈ 7

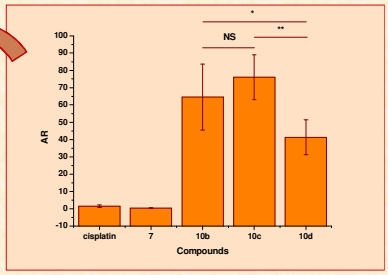


Figure 7. AR values on A2780 cell line treated for 4h CT. Data are means ± standard deviation of at least 3 replicates. The three conjugates were compared with each other by means of the paired t-test (* $p < 0.5$; ** $p < 0.01$; *** $p < 0.001$).

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