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Mendeleev Commun., 2020, **30**, 22–24

Mendeleev Communications

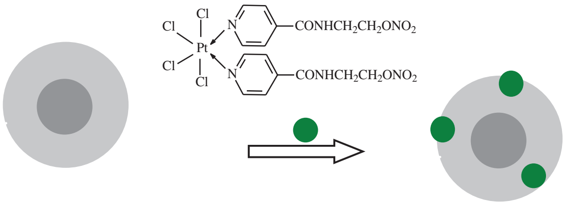
New antitumor hybrid materials based on Pt^{IV} organic complex and polymer nanoparticles consisting of *N*-vinylpyrrolidone and (di)methacrylates

Svetlana V. Kurmaz,*[a] Natalya V. Fadeeva,[a] Boris S. Fedorov,[a†] Galina I. Kozub,[a,b] Nina S. Emel'yanova,[a,c] Vladimir A. Kurmaz,[a] Roman A. Manzhos,[a] Anastasya A. Balakina[a,b] and Alexei A. Terentyev[a,b]

[a] *Institute of Problems of Chemical Physics, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. Fax: +7 496 522 3507; e-mail: skurmaz@icp.ac.ru*
[b] *Research and Education Center, Moscow Region State University, 141014 Mytishchi, Moscow Region, Russian Federation*
[c] *Department of Physical and Chemical Engineering, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation*

DOI: 10.1016/j.mencom.2020.01.007

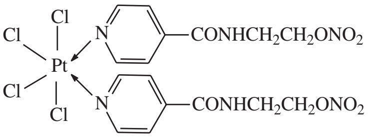
New *cis*-bis[*N*-(2-nitroxyethyl)isonicotinamide-*N*]platinum(IV) tetrachloride hybrid materials were obtained *via* its solubilization by nanoparticles of *N*-vinylpyrrolidone copolymer with dimethacrylates and characterized. The materials containing 2% of Pt^{IV} complex cause a cytotoxic effect on the tumor A-172 cells; IC$_{50}$ values were *ca*. 106 μM after incubation for 48 h.



Keywords: *N*-vinylpyrrolidone, (di)methacrylates, amphiphilic copolymers, polymer particles, platinum(IV) organic complex, hybrid materials, cytotoxicity, antitumor activity.

Various Pt^{II} complexes such as cisplatin, carboplatin, oxaliplatin, *etc.* have well proven themselves in modern medical practice as the drugs possessing a high antimetastatic activity.[1] However, their applicability is limited because of their high overall toxicity and a consequent rather narrow range of the therapeutic doses, as well as a low selectivity. Therefore, much efforts were applied to obtain platinum tetrachloride complexes with biogenic low-toxic compounds such as nicotinic and isonicotinic acids and their derivatives.[2,3] To control the properties of platinum complexes (solubility, bioavailability, prolonged action, distribution in the body, duration of circulation in blood, accumulation in tumors and foci of inflammation, toxicity, *etc.*), they are introduced into various carriers incorporated *via* encapsulation, covalent attachment, or complexation/coordination binding.[4–11] We have previously proposed an encapsulation of organic Pt^{IV} complexes in amphiphilic copolymers of *N*-vinylpyrrolidone (VP) with triethylene glycol dimethacrylate (TEGDM),[12] the interest in which was based on modern advances in the application of VP polymers as carriers of hydrophobic biologically active (including anticancer) substances.[13–18] We have earlier demonstrated the prospects of using VP-TEGDM branched copolymers as the carriers of binuclear tetranitrosyl iron complexes with antitumor activity,[19] fullerene C$_{60}$,[20] and zinc tetraphenylporphyrinate.[21,22]

This work was aimed at obtaining and characterization of hybrid materials based on *cis*-bis[*N*-(2-nitroxyethyl)-isonicotinamide-*N*]platinum(IV) tetrachloride, Pt^{IV}C, known for its antitumor activity[2,3] and capable of releasing nitrogen monoxide during biotransformation, and on *N*-vinylpyrrolidone polymer nanoparticles. Their cytotoxicity against A-172 tumor cells was also estimated. As a polymer carrier, the VP copolymers with TEGDM of various composition and molecular weights, and VP–TEGDM copolymer modified with oligoethylene glycol methacrylate (OEGM) of 500 g mol^{-1} molecular weight were used[‡]. Complex Pt^{IV}C was synthesized from potassium salt of platinum tetrachloride and fully characterized by elemental analysis, IR and NMR spectroscopy.[2] Figure 1(*a*) shows its optimized geometry.



Pt^{IV}C

Copolymers **1** and **3** were obtained *via* radical copolymerization of VP–TEGDM and VP–OEGM–TEGDM at 100 : 5 and 95 : 5 : 5 molar ratios in toluene, respectively. Copolymer **2** precipitated from the reaction mixture under the conditions of VP–TEGDM copolymerization. After copolymerization of active (di)methacrylates, a structure with side double bonds is formed; these bonds attach predominantly PVP chains. As a result, 3D structure is formed from dimethacrylates core and a shell consisting of VP polymer chains. Copolymers **1** and **2** differed with VP (n) and TEGDM (k) unites (Table 1), and copolymer **3** contained OEGM unites, which was confirmed by their IR and ^{1}H NMR spectra (Figure S1 in Online Supplementary Materials).

[†] Deceased.

[‡] See Online Supplementary Materials for experimental procedures and characteristics of obtained compounds, and for quantum chemical simulation details.

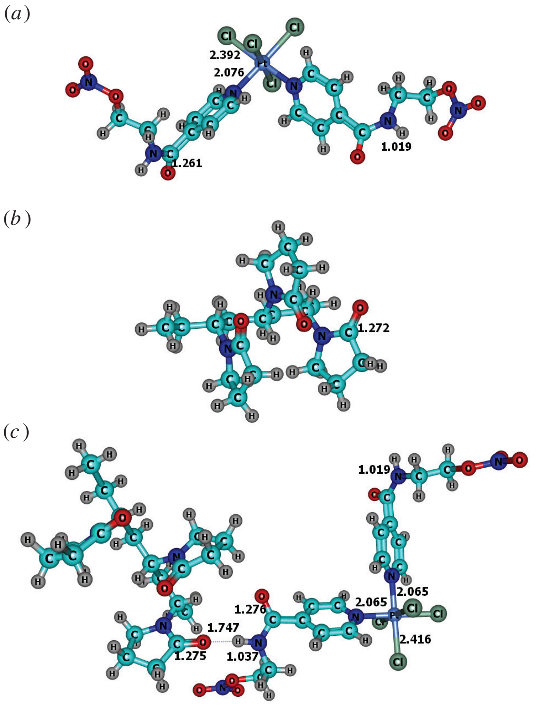
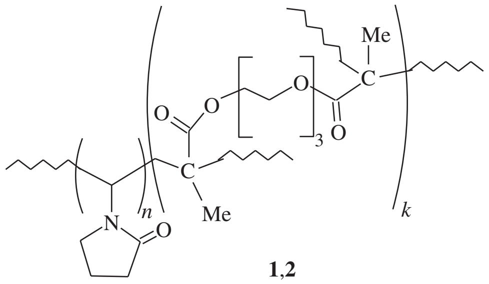


Figure 1 Optimized geometries of (*a*) $Pt^{IV}C$, (*b*) the site of copolymer consisting of three VP moieties and (*c*) the proposed adduct. The distances are given in Å.



1,**2**

Characteristic absorption bands of stretching vibrations of the C=O bond in dimethacrylates and VP units and C–O bond vibrations in the OEGM substituent were observed in IR spectra at ~1720, ~1660, and 1100 cm^{-1}, respectively. The average molecular weight (M_W) of copolymer **2** enriched with TEGDM units was significantly higher as compared to copolymer **1** due to a globular nature of its branched macromolecules (see Table 1). The M_W of copolymer **3** with OEGM units was more than an order of magnitude higher than that of copolymer **1**. Thus, copolymers **1**–**3** differed significantly in monomer composition, M_W, and topological structure (Figure S2).

$Pt^{IV}C$ was introduced into the polymer particles, and yellowish solutions of Pt^{IV} complex encapsulated in polymer particles, *i.e.* $Pt^{IV}C1$, $Pt^{IV}C2$, and $Pt^{IV}C3$, were obtained. We have assumed that the 'guest–host' nanostructures are formed *via* solubilization of Pt^{IV} complex by copolymer particles, where the 'guest' molecules are retained through non-valent interactions.

Table 1 The CHN content in VP–(di)methacrylate copolymers, their average monomer composition, and the absolute values of molecular weight M_W.

Copolymer	C (%)	H (%)	N (%)	The molar content of VP and dimethacrylate units in the copolymers (%)	M_W/ 10^{-3} g mol^{-1}
1	60.8	8.9	10.9	94.6/5.4	26.0
2	60.9	8.4	7.7	80.4/19.6	196.0
3	59.6	8.5	8.6	88.2/11.8	351.0

The platinum and chlorine contents in the encapsulated complex (11% per copolymer **1**), determined using Pregl chemical analysis and by mercurymetric titration, respectively,[23] were *ca*. 4%, which was in satisfactory agreement with the calculated data. This indicates indirectly its chemical stability. According to TEM, $Pt^{IV}C3$ is spherical particles with small $Pt^{IV}C$ aggregates of ~5 nm in size (Figure S3).

Ordinarily, a polymodal size distribution of particles was obtained during the analysis of changes in the intensity of light scattered from $Pt^{IV}C1$–$Pt^{IV}C3$ solutions. There are scattering centers in solutions with a hydrodynamic radius R_h of ~4–7 and ~50–60 nm, and the latter ones make the main contribution to the light scattering. With increasing the temperature, the distribution becomes narrower in all the cases, and unimodal for $Pt^{IV}C2$ solution at 38 °C (Figure 2). The maximum of peak R_h value of aggregates is ~49 nm.

$Pt^{IV}C1$ aggregates with the temperature rise, and the sizes of polymer particles are increased. $Pt^{IV}C2$ begins to aggregate at lower temperature, while $Pt^{IV}C3$ is resistant to aggregation, and its size is weakly dependent on temperature (Figure S4). The reason for polymer particles aggregation is apparently a collapse of their polar shell due to the destruction of a hydrogen bond between water molecules and free or hydrated C=O groups of the lactam cycle of VP units, resulting in their solubility decrease. The OEGM chains in copolymer **3** apparently ensure the stability of its polymer particles and nanostructures. According to TGA data (Figure S5), the weight loss of initial $Pt^{IV}C$ begins only at ~100 °C, and temperature scanning does not affect its thermal stability.

IR spectroscopy of $Pt^{IV}C3$ (18% of the Pt^{IV} complex per the copolymer) revealed that the characteristic absorption of water involved in the formation of a hydrogen bond with the copolymer in the region of 3600–3000 cm^{-1} was significantly diminished with respect to copolymer **3** (Figure S6). At the same time, the frequency of stretching vibrations of C=O groups of the lactam cycle is shifted to higher values from 1655 to 1665 cm^{-1}. It indicates a formation of hydrogen bond between the C=O group of VP moiety of the copolymer and the N–H group of $Pt^{IV}C$ ligand. The optimized geometries of site of the copolymer consisting of three VP moieties and the proposed adduct are shown in Figure 1(*b*), (*c*). The adduct structure was confirmed by results of the theoretical spectra modeling for the reagents and adduct (Figure S7). The length of formed H-bond is 1.747 Å, and the energy of its formation calculated from the difference of energies of the optimized geometries of adduct and reagents is equal to 8.3 kcal mol^{-1}. The water molecules present in the original copolymer are replaced by guest $Pt^{IV}C$ molecules according to the IR spectroscopy data. According to the calculation, this process in adduct of the VPVPVP copolymer part with one water molecule has a small energy gain (0.16 kcal mol^{-1}).

Electrochemical data acquired by cyclic voltammetry (CVA) indicate the inclusion of $Pt^{IV}C$ in polymer particles as well. CVA curves of the polymer particles loaded with $Pt^{IV}C$ in an aqueous phosphate buffer solution and DMSO are quite different. Thus, only two overlapped peaks are observed in the

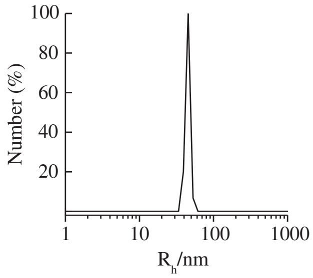


Figure 2 DLS curve for $Pt^{IV}C2$ in aqueous buffer solution (pH 6.8) at 38 °C.

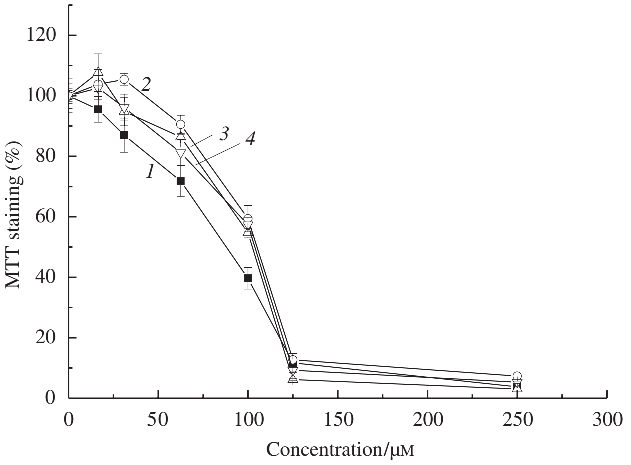


Figure 3 Changes in MTT staining for A-172 cells 48 h after (*1*) $Pt^{IV}C$, (*2*) $Pt^{IV}C1$, (*3*) $Pt^{IV}C2$, and (*4*) $Pt^{IV}C3$ introduction into the incubation media. Concentrations of $Pt^{IV}C$ are indicated according to their content in the copolymers.

first case until $E \sim -1.6$ V (Figure S8). However, CVA curves of polymer particles loaded with this complex in DMSO are complicated and contain 4 to 5 cathodic peaks within the potential range of $E = -(0.2–1.6)$ V like free complex. Evidently, $Pt^{IV}C$-copolymer structures were destroyed in this solution, free complex was released and reduced irreversibly.

Polymer **1–3** particles cause no significant effect on cell viability at all the studied concentrations, which was evidenced by results of their cytotoxicity estimation towards A-172 cells within the concentration range of 7–700 μg ml^{-1} (Table S1). The dependences of MTT staining on the concentration of free and immobilized complex confirm their cytotoxicity against the cells (Figure 3).

Higher cytotoxicity of free $Pt^{IV}C$ (Table 2) may be associated with its greater lipophilicity as compared to the encapsulated one, and as a result, with faster penetration through the intracellular membrane. At the same time, encapsulation of the Pt complex into nanoparticles does not prevent any development of a cytotoxic effect towards the tumor cells. The $Pt^{IV}C$ complex was apparently released from the polymer particles and its intracellular biotransformation occurred.

In conclusion, the stable in aqueous buffer media nanostructures containing 2% of Pt^{IV} organic complex known for its antitumor activity were obtained. They were based on *N*-vinylpyrrolidone copolymers of different composition, molecular weight, and topology. It has been demonstrated that the polymer particles do not significantly affect the cell viability at all the studied concentrations and are biocompatible. The encapsulated complex possesses a lower cytotoxicity towards A-172 cell line than that of the free one at equal concentrations. Platinum(IV) complex encapsulated in different polymer particles demonstrated the similar cytotoxicity. Thus, the biocompatible copolymers of *N*-vinylpyrrolidone are of interest as delivery vehicles of the organic complexes of Pt^{IV}. It is assumed that a Pt^{IV} complex encapsulated in polymer particles will show *in vivo* a decrease in the overall toxicity as compared to the free one.

Table 2 IC_{50} dose values for A-172 cells after incubation for 48 h with $Pt^{IV}C$ and $Pt^{IV}C1$–$Pt^{IV}C3$.[a]

Substance	$IC_{50}{}^{b}$/μM
$Pt^{IV}C$	90.64±6.52
$Pt^{IV}C1$	106.05±4.84
$Pt^{IV}C2$	105.73±5.08
$Pt^{IV}C3$	105.55±8.12

[a] Concentrations of $Pt^{IV}C$ are indicated according to their content in the copolymers. [b] The cytotoxicity index (IC_{50}) was determined on the basis of the dose-dependent curves using a median effect analysis.[24] The criterion of statistical significance was $p < 0.05$.

This work was performed within the framework of State Tasks AAAA-A19-119041090087-4 (no. 0089-2019-0008), AAAA-A19-119032690060-9 (no. 0089-2019-0012), and AAAA-A19-119071890015-6 (no. 0089-2019-0014) using the equipment of the Multi-User Analytical Center at the Institute of Problems of Chemical Physics of the Russian Academy of Sciences.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2020.01.007.

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Received: 24th July 2019; Com. 19/5997