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# Pemetrexed conjugated with gold nanoparticles – Synthesis, characterization and a study of noncovalent interactions

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ABSTRACT

Gold nanoparticles (AuNPs) have been widely used as nanocarriers in drug delivery application. However, the binding mechanism between AuNPs and drug bases still remains a puzzle. Our study included: (i) optimization of three synthesis of the AuNPs-pemetrexed (PE) nanocomposites formation which was monitored by UV–Vis spectroscopy, (ii) identification of PE in gold nanocomposites and mechanism of PE interaction with gold nanoparticles by electrochemistry, NMR and Raman measurements, (iii) characterization of the three nanocomposites by TEM, DSL, ESL, zeta potential, XRPD and TGA analysis. The obtained nanocomposites are homogeneously shaped and have a maximum diameter of around 14 nm and 88 nm, as measured by the TEM and DSL techniques, respectively. The zeta potential of the nanocomposites is − 43 mV and suggests a high stability of the nanoparticles and lower toxicity for the normal cells. Quantum chemical calculations were also performed on model systems to estimate the strength of the AuNPs-PE interaction. Taking into account the experimental and theoretical data a mechanism of the nanocomposites' formation has been proposed in which PE interacts with the gold surface by the COOH/$COO^-$ group.

## 1. Introduction

The interactions between drugs and AuNPs are one of the most interesting subjects of modern nanotechnology, giving rise to plenty of applications (Stolarczyk et al., 2004). In particular AuNPs are attractive drug carriers because of their high tissue permeability, small size and high colloidal stability (Coelho et al., 2015; Harrison et al., 2016; Soanpet et al., 2016). They can also be helpful in resolving of the problem of the lack of specificity in many anticancer drugs, such as capecitabine, cisplatin, paclitaxel, tamoxifen, doxorubicin, 5-fluorouracil, methotrexate. Drugs can be conjugated with nanoparticles via physical adsorption, ionic or covalent bonding. For example, doxorubicin (DOX), methotrexate (MTX) and 6-mercaptopurine (MP) were successfully loaded into the AuNPs by ionic interaction (DOX and MTX) and disulphide-covalent bond formation (MP) (Ghorbania and Hamishehkarc, 2017). In order to maximize the targeting ability of the AuNPs, the loaded drugs should be released in a controllable and rapid manner at the site of the tumor. For this reason it is important to understand the character of the interactions between the drug and AuNPs.

Continuing our experimental research (Stolarczyk et al., 2017) that addressed the basic knowledge of chemical and structural properties of organic-inorganic hybrid systems, we investigated the chemistry of the pemetrexed as a ligands for colloidal gold nanoparticles (AuNPs). Pemetrexed disodium, chemically known as *N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid, disodium salt (PE, Scheme 1) primarily inhibits thymidylate synthase resulting in a decreased thymidine availability for the DNA synthesis. PE also inhibits dihydrofolate reductase and glycinamide ribonucleotide formyltransferase which are key enzymes required for the de novo bio-synthesis of thymidine and purine nucleotides (Paz-Ares et al., 2003; Eli Lilly, Canada Inc, 2004; Hanauske et al., 2001). The therapeutic effect of PE is known to be limited, because it leads to toxic dose-related side effects, such as anemia, nausea, fatigue, diarrhea and stomatitis, due to the drug resistance of the tumor cells (Lu et al., 2016). To avoid toxicity Lu et al. (Lu et al., 2016) established pemetrexed-loaded PEG-Peptide-PCL nanoparticles that revealed anticancer and antimetastatic effects. According to our knowledge the synthesis of AuNPs-PE nanocomposites has not been described yet. Bessar et al. described AuNPs functionalized by sodium 3-mercapto-1-propansulfonate (Au-3MPS) as a promising carrier for methotrexate (MTX) (Bessar

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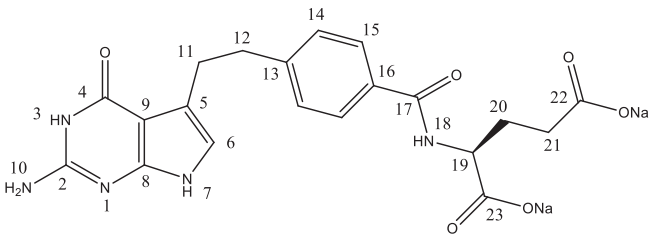

**Scheme 1.** Pemetrexed (PE) disodium salt.

et al., 2016). Some other works (Tran et al., 2013; Chen et al., 2007) pointed at AuNPs-MTX as an alternative drug formulation of MTX in cancer therapy. MTX, like PE, belongs to the group of folic acid antagonists.

In this paper we have developed a preparation of PE conjugated with AuNPs as three nanocomposites via a one-pot reduction method in an aqueous solution. It is multidisciplinary research for a complete characterization of new products. The obtained nanocomposites were fully characterized by UV–Vis, TEM, DLS, ELS, zeta-potential, XRPD and TGA techniques. The second objective of the present research was to discover what kind of the interaction occurs between the gold surface and PE. The research on the gold-PE binding sites was performed using electrochemistry as well as NMR and Raman techniques supported by theoretical calculations for medium-size models, using the density functional theory (DFT) combined with pseudopotentials for gold atoms.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Pemetrexed (batch No. PRI/2007.02/8/14) was manufactured in Pharmaceutical Research Institute (PRI), Warsaw, Poland. Dimethyl sulfoxide (GC-headspace tested 99.9%) was purchased from Fluka and DMSO-$d_6$ from Sigma-Aldrich, Saint Louis, Missouri. PBS buffer-conc. was obtained from Syngen. $HAuCl_4 \cdot 3H_2O$ and citric acid were purchased from Sigma Aldrich. Water used in this study was purified and deionized using the Polwater System D-300, Cracow, Poland.

### 2.2. UV–Vis spectroscopy

UV–Vis spectroscopic measurements were performed using a UV–Vis spectrophotometer, series Evolution 220 (Thermo Scientific, Waltham, MA) with a 1-cm quartz cell in the wavelength range from 220 to 750 nm.

### 2.3. Nuclear magnetic resonance (NMR) spectroscopy

The $^1$H NMR spectra of three nanocomposites were performed using Varian Gemini-2000 and Bruker Advance 500 (Bruker Corporation, Billerica, Massachusetts) spectrometers with the resonance frequency of 200 ad 500 MHz, respectively. The sample was dissolved in deuterated DMSO-$d_6$ and water-$d_2$, where the residual signal of the solvent was simultaneously the internal standard of the chemical shift (2.49 and 4.67 ppm, respectively). Standard measurement parameters were used.

After NMR studies liquid nanocomposite samples were dried in a drying chamber at 30 °C in aluminum sample holders, and then measured directly in a powder diffractometer and a Raman spectrometer.

### 2.4. Raman spectroscopy

The FT Raman spectra were recorded on a Nicolet NXR 9650 instrument (Thermo Scientific, Waltham, MA) using 1064 nm excitation from the Nd:YVO4 laser in the range from 3700 to 150 $cm^{-1}$ with the spectral resolution of 4 $cm^{-1}$. For one spectrum from 300 to 2000 scans were recorded with the laser power from 0.1 to 0.8 W.

### 2.5. Transmission electron microscopy (TEM)

The TEM images of the AuNPs-PE nanocomposites were obtained using a TEM Zeiss LIBRA 120 (HT = 120 kV, $LaB_6$ cathode) apparatus (Germany). The samples for the TEM imagining were prepared by dropping the water solution of nanocomposites onto a formvare grit mesh 400, where the nanoparticles were left to dry in room temperature.

### 2.6. Dynamic light scattering (DLS) and electrophoretic light scattering (ELS)

The DLS and ELS were performed on a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK). The specimens were diluted with ultrapure distilled water. The angle of the incident light was 173°. Each specimen underwent 5 measurements, then the mean size and zeta potential were calculated.

### 2.7. Electrochemical measurements

The differential pulse voltammograms were recorded in a three-electrode arrangement including Ag/AgCl (KClsat.) as the reference electrode, a platinum foil counter electrode and a glassy carbon electrode (GCE, BASi) as the working electrode. All voltammetry experiments were carried out using an ECO Chemie Autolab potentiostat (Utrecht, The Netherlands) at 22 ± 2 °C and all current densities were calculated using the geometric electrode area (0.071 $cm^2$). The voltammetric experiments were recorded in the deoxygenated PBS buffer solution pH 7.4 containing PE or AuNPs-PE nanocomposites.

### 2.8. Thermogravimetry (TGA) analysis

The TGA measurements were performed by means of a TGA/SDTA851 cell (MettlerToledo GmbH, Schwerzenbach, Switzerland). About 0.2 mg and 0.5 mg of samples, were weighed into aluminum oxide crucibles (70 μL). The samples were heated from 30 to 1000 °C at 10 °C/min in the nitrogen atmosphere (60 mL/min). The measurements were blank curve corrected.

### 2.9. Powder X-ray diffraction (XRPD)

The diffractograms were recorded on a MiniFlex diffractometer (Rigaku Coporation, Tokyo, Japan) using CuKα1 radiation. The nanoparticles conjugated with PE, PE disodium and PE diacid samples were loaded on aluminum sample holders and their surfaces were smoothed by glass plates. The samples were measured at a sample rotation attachment with the spinning speed of 60 rpm. The instrument was operated in the range from 3 to 90° with the scan rate of 0.02°/min. The phase identification was performed by means of PDF-2 Database (PDF-2 Databases sets, Powder Diffraction File, 1997).

### 2.10. Computational methods - density functional theory calculations

The B3LYP/6-31G(d,p) method for H, C, N, O atoms was used together with the LanL2DZ effective core potential for Au atoms (Hay and Wadt, 1985a; Wadt and Hay, 1985; Hay and Wadt, 1985b). All calculations were done with the Gaussian 09 program (Frisch, et al., 2013). The geometry optimizations, harmonic frequencies and Raman activities were done with the codes implemented in the Gaussian 09 program. The Raman intensities were obtained using the formula derived from the intensity theory of Raman scattering (Krishnakumar et al., 2004; Polavarapu, 1990) and the laser beam excitation energy of 1064 nm. As a representative sample of the AuNPs a small size cluster