Poly(3–hydroxybutyrate)–chitosan polymer conjugate for the synthesis of safer gold nanoparticles and their applications

Daniele Silvestri,^a Stanisław Wacławek, ^{*a} Bartłomiej Sobel,^b Rafael Torres–Mendieta,^a Vít Novotný,^a Nhung H.A. Nguyen,^a Alena Ševců,^a Vinod T.P. Padil,^a Jana Müllerová,^a Martin Stuchlík,^a Marco Petrangeli Papini,^c Miroslav Černík^{*a} and Rajender S Varma^{*d}

^aInstitute for Nanomaterials, Advanced Technologies and Innovation, Technical University of Liberec, Studentská 1402/2, 461 17 Liberec, Czech Republic. Tel: +420 485 353 006; E–mail: stanislaw.waclawek@tul.cz; miroslav.cernik@tul.cz;

^bInstitute of Engineering Materials and Biomaterials, Faculty of Mechanical Engineering, Silesian University of Technology, Konarskiego 18a St., 44–100 Gliwice, Poland.

^cDepartment of Chemistry, University of Rome "La Sapienza", Piazzale Aldo Moro 5, 00185 Rome, Italy.

^dRegional Centre of Advanced Technologies and Materials, Faculty of Science, Palacký University in Olomouc, Šlechtitelů 27, 783 71 Olomouc, Czech Republic. E–mail: Varma.Rajender@epa.gov;

Supporting Information Content:

- Experimental setup
- 12 Pages (including the cover page)
- 10 Figures

Figure S1 Chromatograms of a blank run of water (blue) and the PHB-chit conjugate (green). Measured on the Macherey-Nagel Nucleosil column. The calculated molecular weight of the conjugate was 260 kDa using the values for the pullulan standards.

Figure S2 Chromatograms of a blank run of tetrahydrofuran (blue) and the PHB (red). Measured on the Phenogle column. The calculated molecular weight of the polymer is 3 kDa using the values for polystyrene standards.

Figure S3 UV-Vis spectra of nAu-PHB-chit determined for different time of NP growth from $HAuCl_4$ at concentration a) 0.25 mM and b) 0.5 mM (insets c) d) show change of absorbance intensity on a wavelength with maximum absorbance at 530nm (PHB-chit concentration - 1.6 g/l).

Figure S4 Evaluation of nAu-PHB-chit size distribution in time, NP growth from HAuCl₄ at concentration a) 0.25 mM and b) 0.5 mM; c) and d) show median size distribution of NPs in time (PHB-chit concentration - 1.6 g/l).

Figure S5 NP characteristics as a function of PHB-chit concentration, a) absorption spectra, b) size distributions, c) maximum absorption and d) median size (HAuCl₄ concentration of 0.5 mM, and synthesis time 45 min).

Figure S6 NP characteristics as a function of $HAuCl_4$ concentration, a) absorption spectra, b) size distributions, c) maximum absorption and d) median size (concentration of PHB-chit conjugate 1.6 g/l, synthesis time 45 min).

Figure S7 FTIR analysis of a) chitosan and b) PHB-chit conjugate. These two areas of spectrum (A and B) were taken from Fig. 3.

Figure S8 ¹H NMR analyses of PHB-chit conjugate (D₂O), chitosan (D₂O) and PHB (CDCl₃).

Figure S9¹³C NMR analyses of PHB-chit conjugate (D₂O), chitosan (D₂O) and PHB (CDCl₃).

Figure S10 a) Catalytic effect of nAu-PHB-chit median diameter on the rate constants of 4-NP reduction, b) pseudo-first-order reaction constant k as a function of the added amount of nAu-PHB-chit and its approximation by a linear relationship (the standard deviation between the measurements is less than <5%).

EXPERIMENTAL SETUP

Gel permeation chromatography: For GPC measurements, the system used was Dionex Ultimate 3000 with a LPG-3400SD quarternary gradient pump, a SR-3000 solvent rack, a WPS-3000TSC autosampler, a TCC-3000SD column compartment and a DAD-3000 detector. Varian 385-LC detector was used for ELSD detection. A single Phenomenex Phenogel 5 µm particle size 104 Å pore size, 300 mm length and 7.8 mm internal diameter column was used for the determination of the molecular weight of PHB. AMacherey-Nagel EC 250/4.6 Nucleosil 1000-7 OH column with 7 µm particle size, 103 Å pore size, 250 mm length and 4.6 mm internal diameter was used for the determination of the determination of the molecular weight of the molecular weight of the PHB-chit conjugate. Standards from a Phenomenex Poly(styrene) Standards Kit (Order No. AL0-2762) were used to calibrate the Phenoglel column. The standards from MW 96000 to 12500 were measured. HPLC grade (ChromaR) Tetrahydrofuran was purchased from (Avantor Performance Materials Poland S.A., Gliwice, Poland). Shodex P-82 pullulan standards of the molecular weight 800, 400 and 200 kDa were used to calibrate the Machery-Nagel column.

For the determination of PHB, the mobile phase consisted of 100% tetrahydrofuran. The flow rate was 1 ml/min and the column was kept at 35 °C. The diode array detector was operating at 200, 220, 250 and 300 nm wavelengths. The ELSD detector had nebulizer and evaporator temperature set to 80 °C, light intensity to 50% and the nitrogen flow rate was 1.1 l/min. The injection volume was 20 μ l. The chromatograms were recorded for 14 min. For the determination of the conjugate the mobile phase consisted of 5 % methanol in water with 50 mM ammonium acetate. The flow rate was 1 ml/min and the column was kept at 35 °C. The diode array detector was operating at 200, 220, 250 and 300 nm wavelengths. The ELSD detector had nebulizer and evaporator temperature set to 80 °C, light intensity to 50% and the nitrogen flow rate with 50 mM ammonium acetate. The flow rate was 1 ml/min and the column was kept at 35 °C. The diode array detector was operating at 200, 220, 250 and 300 nm wavelengths. The ELSD detector had nebulizer and evaporator temperature set to 80 °C, light intensity to 50% and the nitrogen flow rate was 1.6 l/min. The injection volume was 20 μ l. The chromatograms were recorded for 7

min. The PHB sample was diluted 1:5 v:v in THF. The PHB-chit conjugate was diluted in the same ratio by water. All samples were filtered through 13 mm diameter 0.45 µm pore size PTFE syringe filters before injection into the column.

Nuclear magnetic resonance (NMR): Chitosan, PHB and PHB-chit conjugate were measured on Bruker Ultrashield Avance 400 NMR spectrometer, operating at 400.13 and 100.613 MHz, at room-temperature measurements (293 K).

Ion chromatography: Formic acid was determined on ion chromatograph Thermo Scientific[™] Dionex[™] ICS-2100 with Regent-Free[™] IC (RFIC[™]) system, equipped with conductivity detector and IonPac[™] AS11-HC column.



Figure S1 Chromatograms of a blank run of water (blue) and the PHB-chit conjugate (green). Measured on the Macherey-Nagel Nucleosil column. The calculated molecular weight of the conjugate was 260 kDa using the values for the pullulan standards.



Figure S2 Chromatograms of a blank run of tetrahydrofuran (blue) and the PHB (red). Measured on the Phenogle column. The calculated molecular weight of the polymer is 3 kDa using the values for polystyrene standards.



gure S3 UV-Vis spectra of nAu-PHB determined for different time of NP growth from HAuCl₄ at concentration a) 0.25 mM and b) 0.5 mM (insets c) d) show change of absorbance intensity on a wavelength with maximum absorbance at 530nm (PHB-chit concentration - 1.6 g/l).



Figure S4 Evaluation of nAu-PHB size distribution in time, NP growth from $HAuCl_4$ at concentration a) 0.25 mM and b) 0.5 mM; c) and d) show median size distribution of NPs in time (PHB-chit concentration - 1.6 g/l).



Figure S5 NP characteristics as a function of PHB-chit concentration, a) absorption spectra, b) size distributions, c) maximum absorption and d) median size (HAuCl₄ concentration of 0.5 mM, and synthesis time 45 min).



ure S6 NP characteristics as a function of HAuCl₄ concentration, a) absorption spectra, b) size distributions, c) maximum absorption and d) median size (concentration of PHB-chit conjugate 1.6 g/l, synthesis time 45 min).



Figure S7 FTIR analysis of a) chitosan and b) PHB-chit conjugate. These two areas of spectrum (A and B) were taken from Fig. 3.



Figure S8 ¹H NMR analyses of PHB-chit conjugate (D₂O), chitosan (D₂O) and PHB (CDCl₃).



Figure S9 ¹³C NMR analyses of PHB-chit conjugate (D₂O), chitosan (D₂O) and PHB (CDCl₃).



Figure S10 a) Catalytic effect of nAu-PHB-chit median diameter on the rate constants of 4-NP reduction, b) pseudo-first-order reaction constant k as a function of the concentration of nAu-PHB-chit and its approximation by a linear relationship.