Functionalized graphene oxide as nanocatalyst in dephosphorylation reactions: pursuing artificial enzymes

*Elisa S. Orth *, Jéssica E. S. Fonsaca, Thomas Golin Almeida, Sergio H. Domingues, José G. L. Fereira, Aldo J. G. Zarbin**

Department of Chemistry, Universidade Federal do Paraná (UFPR), CP 19081, CEP 81531-990, Curitiba, PR, Brazil.

SUPPLEMENTARY INFORMATION

1. Experimental details

Graphene oxide (GO) was obtained by the modified Hummers method and functionalization with cysteamine was carried as previously described, leading to a solid catalyst: GOSH.[1] The nanocatalyst GOSH was not dispersible in any solvents tested: water, ethanol, methanol, benzene, toluene and acetonitrile.The phosphate ester DEDNPP was prepared by standard methods from $POCl_3$, as described previously.[2] Inorganic salts were of analytical grade and used without further purification.

Infra-red spectra were recorded on a FTIR Bio-Rad spectrophotometer over the range of 4000-400 cm^{-1}, using the KBr pellet method, and the graphs obtained are shown in the article. Further detailed band assignments were described previously.[1]

2. Kinetics

Reactions of DEDNPP were followed spectrophotometrically (UV-Vis Spectrophotometry UV-2450) by monitoring the appearance of 2,4-dinitrophenolate

(DNP) at 400 nm. Reactions were started by adding 10 μL of 5 mM stock solutions of the substrate in acetonitrile to 3 mL of aqueous solutions to give a final concentration of the substrate of 1.67×10^{-5} mol L^{-1}. Solid GOSH was added to 3 mL of a buffered solution (KHCO3, 0.01 mol L-1) at different pH values (pH 8-10) and kept under controlled temperature (20°C) and constant magnetic stirring. The temperatures of reaction solutions in quartz cuvettes were controlled at 20°C. Absorbance versus time data were stored directly on a microcomputer, and observed first-order rate constants, k_{obs}, were calculated, using an iterative least-squares program; correlation coefficients were > 0.999 for all kinetic runs. The pH was maintained with 0.01 M buffers of $KHCO_3$ (pH 8-10). The rate constants for the spontaneous reaction of DEDNPP (k_{H2O}) was obtained from a previous report (at 25°C)[2] and extrapolated to 20°C by using the commonly known Arrhenius equation. Thus, at pH 8, 9 and 10, the corresponding k_{H2O} values are 5.93×10^{-8}, 1.02×10^{-7} and 3.60×10^{-7} $M^{-1}s^{-1}$, respectively.

2.1 Reaction of GOSH with DEDNPP at different pH values

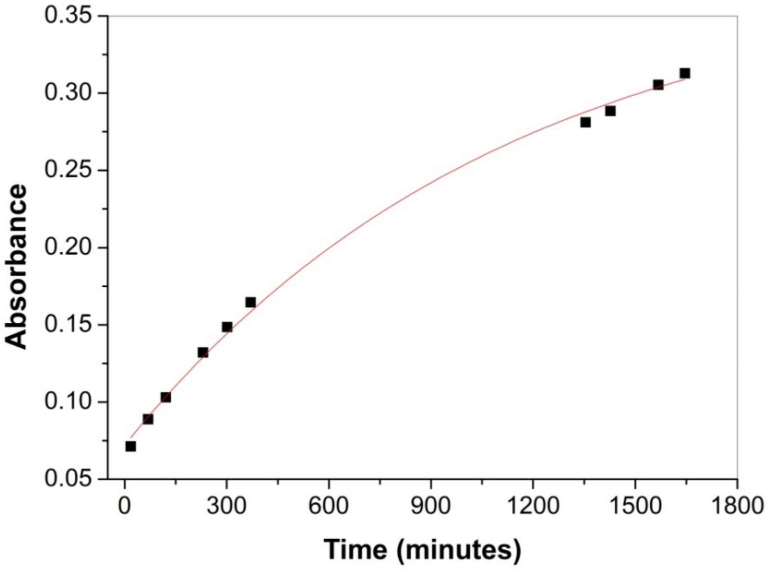

Figure S1. Kinetic profile obtained at 400 nm for the reaction of GOSH with DEDNPP at pH 8. Solid line is the pseudo-first order fit.

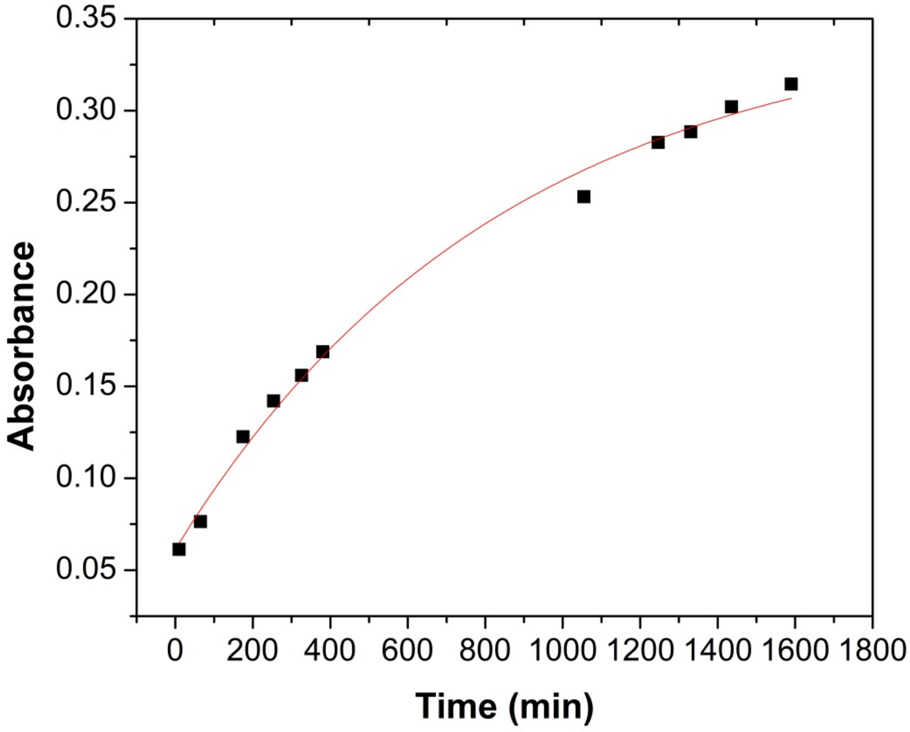

Figure S2. Kinetic profile obtained at 400 nm for the reaction of GOSH with DEDNPP at pH 9. Solid line is the pseudo-first order fit.

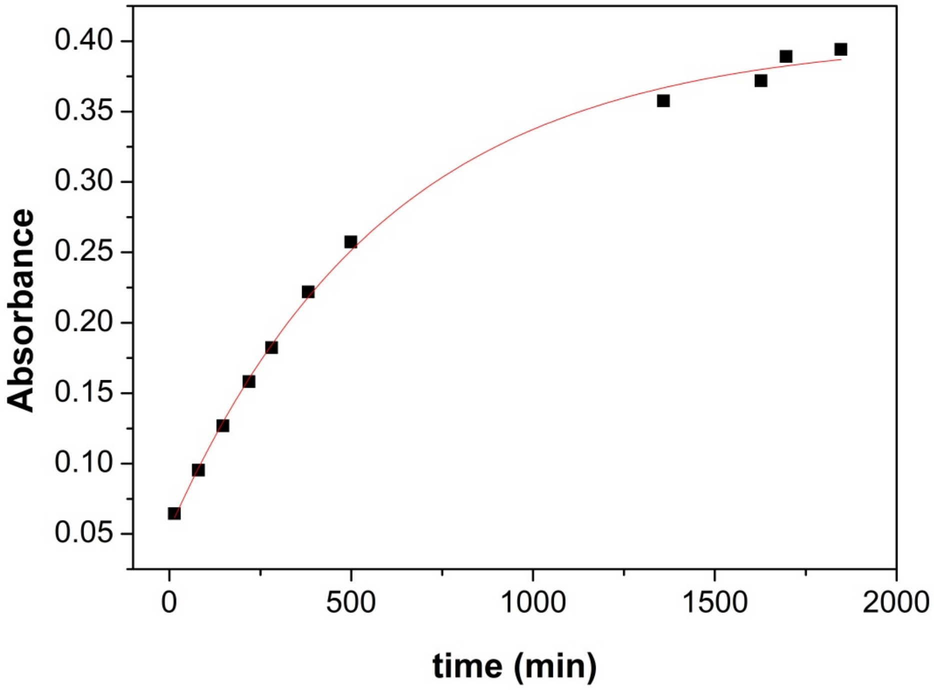

Figure S3. Kinetic profile obtained at 400 nm for the reaction of GOSH with DEDNPP at pH 10. Solid line is the pseudo-first order fit.

3. Potentiometric titration

The pK_a values for GOSH were determined using a digital pH meter and a combined glass electrode. Titrations were performed in a 150 mL thermostatted cell, at 25.0 °C. The solution was titrated with small increments of $5x10^{-3}$ M CO_2-free KOH. All precautions were taken to eliminate carbonate and CO_2 during the titration. The program BEST7 was used to calculate the dissociation constants, using the value -13.78 for pK_w.[3] Three pK_a´s values were determined for GOSH, which refer to (i) carboxylic acid (~4.5); (ii) thiol group (~6.5) and (iii) alcohol groups (~9.0). These pK_a´s are expected, since the values for carboxylic acid and alcohol moieties have been reported for GO samples.[4] Values for thiol groups are low, but also expected for complex samples where neighboring groups can lead to more acidic thiol groups, such as in proteins (cysteine residues).[5] This approach has been successfully used by other authors in complex systems[6,7] such as the presented here, thus validating the present considerations.

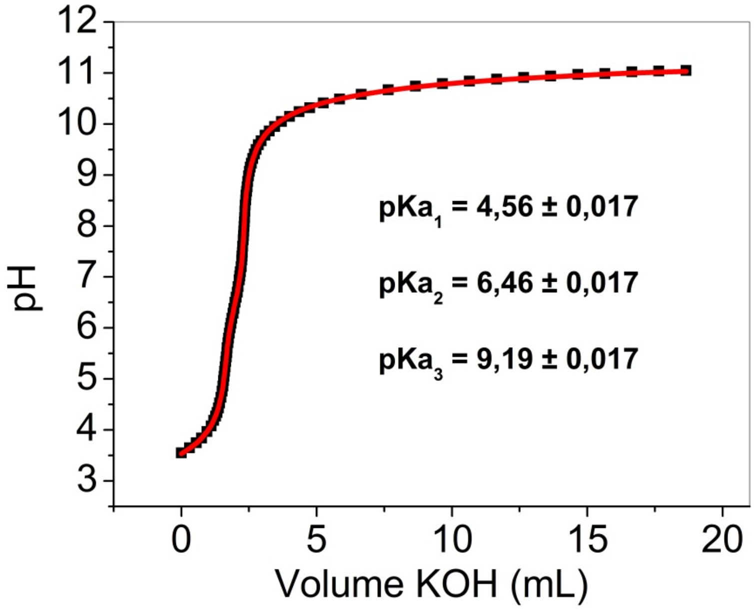

Figure S4. Potentiometric titration curve for GOSH (1mg) using KOH 0.1008 M with pK_a´s values determined. Solid line is the fit calculated by the program BEST7.

4. Recycling of nanocatalyst

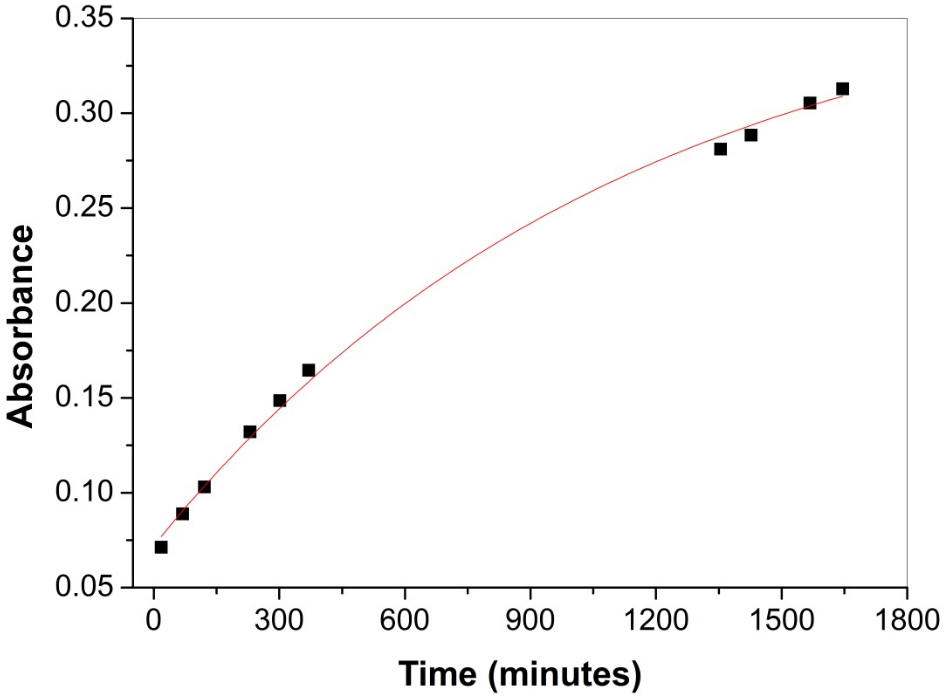

Figure S5. Kinetic profile of the first reaction cycle obtained at 400 nm for the reaction of GOSH with DEDNPP at pH 8. Solid line is the pseudo-first order fit.

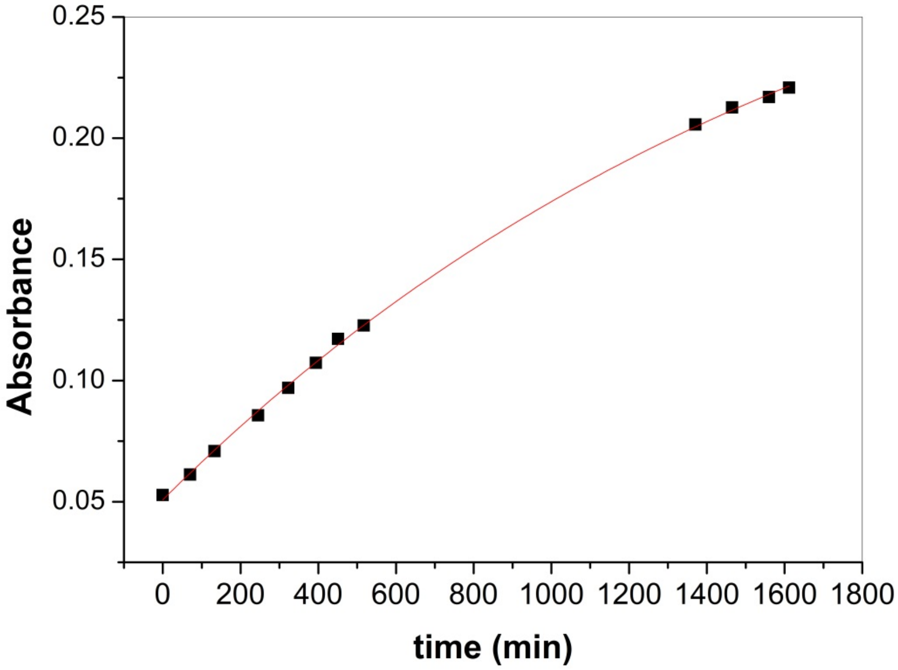

Figure S6. Kinetic profile of the second reaction cycle obtained at 400 nm for the reaction of GOSH with DEDNPP at pH 8. Solid line is the pseudo-first order fit.

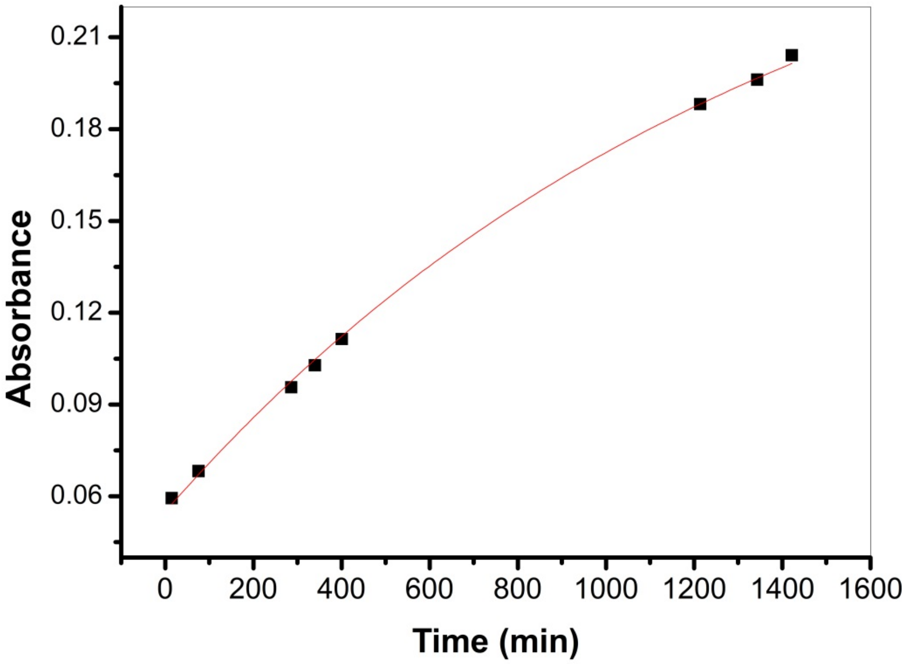

Figure S7. Kinetic profile of the third reaction cycle obtained at 400 nm for the reaction of GOSH with DEDNPP at pH 8. Solid line is the pseudo-first order fit.

5. Thermogravimetric analyses

Simultaneous thermogravimetric analyses (TGA/DSC) were carried out in SDT Q600 equipment (TA Instruments) from TA Instruments under 100 mL min^{-1} Ar at a heating rate of 5 °C min^{-1}. The software OriginPro 8.0 was used to analyze all experimental data and graphs.

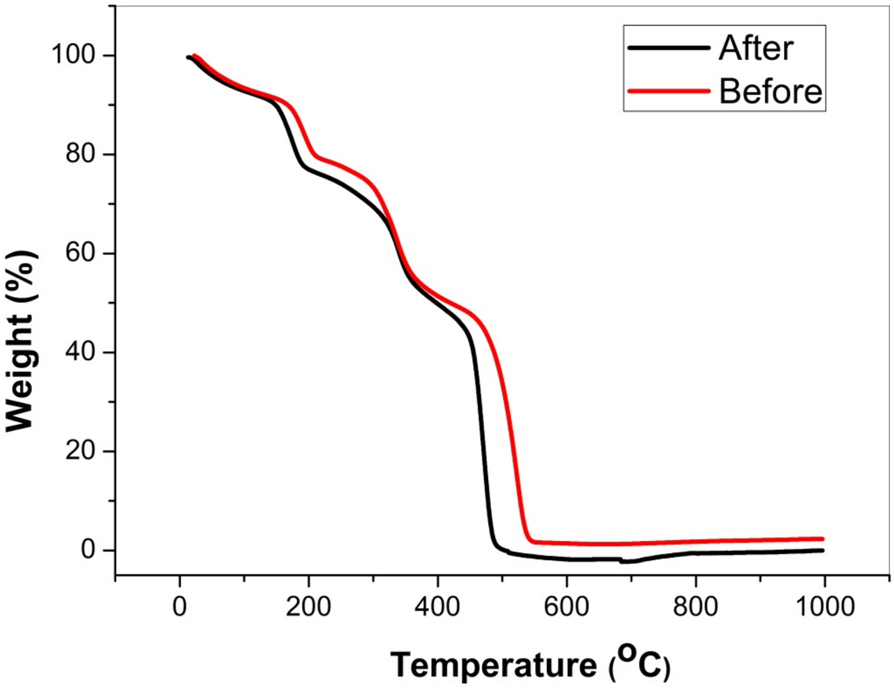

Figure S8 – TGA analyses for all samples before and after reaction with DEDNPP.

Table S1. Most relevant information obtained from TGA and DTA analyses.[1]

	Group assigned to weight loss	**GOSH before reaction**	**GOSH after recycling**
T (°C)/ Weight loss (%)	Oxygenated	140-220 / 12.2	110-230 / 17.0
	Thiol	255-383 / 22.8	240-400/ 28.4
	Carbon backbone	440-550 / 46.0	420-525 / 54.0
% residual		1.5	0

The local concentration of reactive thiol groups on the GO surface was calculated considering the second major weight loss, which is due to the thiol moieties.[1] Therefore, considering molar mass of thiol functionality 104 g/mol, gives a concentration of thiol groups of 8.42×10^{-4} mol L^{-1} /mg of solid GOSH

6. Raman Spectroscopy

The Raman spectra were done by a Renishaw Raman image spectrophotometer using a long range 50 objective lens (Olympus). The wavelength of the excitation laser was 514.5 nm (He-Ne laser) with the laser power fixed under 1.0mW. Raman spectra for GO and GOSH (before and after reaction) are given in Figure S9. The band at ~2930 cm^{-1} is related to the CH$_2$ asymmetric stretch of cysteamine[1] anchored on the GOSH (marked with an arrow in Figure S9). Depiction of the peaks and intensity ratios were obtained by deconvolution using Lorentzian method (Figure S10). The I_D/I_G ratios were calculated by taking the average of at least 7 spectra from each sample. I_D/I_G ratio found for GO is 0,99.[1]

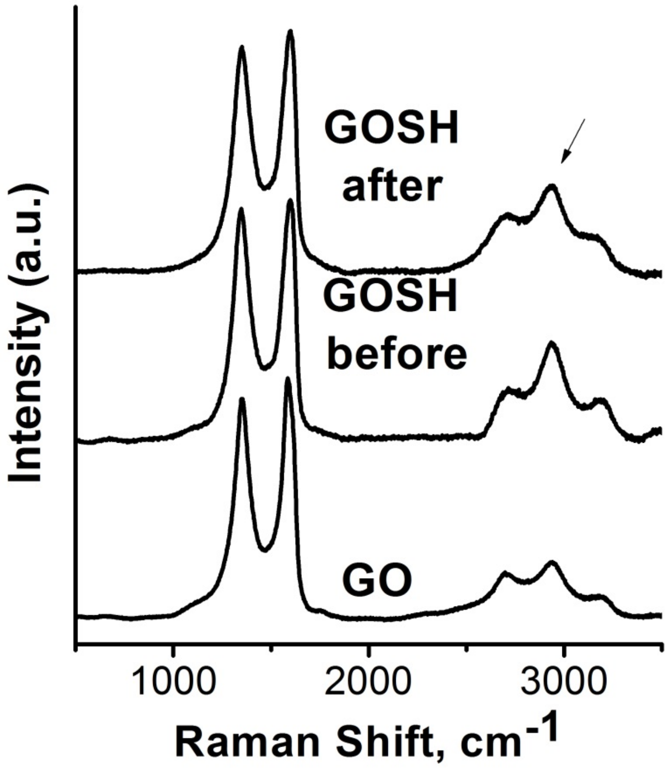

Figure S9. RAMAN spectra for GO and GOSH before and after the reaction with DEDNPP.

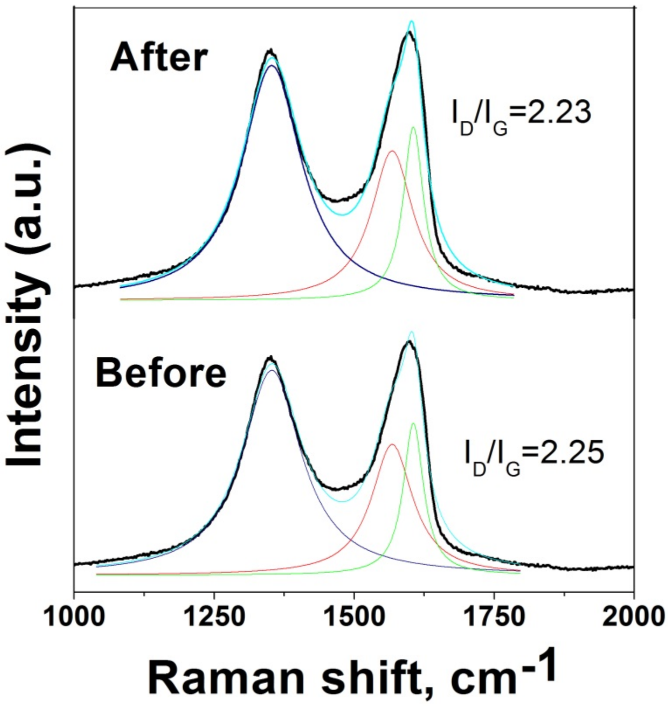

Figure S10. RAMAN spectra with typical deconvolution, using Lorentzian method, for GOSH before and after the reaction with DEDNPP.

REFERENCES

(1) Orth, E. S.; Fonsaca, J. E. S.; Domingues, S. H.; Mehl, H.; Oliveira, M. M.; Zarbin, A. J. G. *Carbon* **2013**, *61*, 543.

(2) Orth, E. S.; Wanderlind, E. H.; Medeiros, M.; Oliveira, P. S. M.; Vaz, B. G.; Eberlin, M. N.; Kirby, A. J.; Nome, F. *J Org Chem* **2011**, *76*, 8003.

(3) MARTELL, A. E.; HANCOCK, R. D. *Metal Complexes in Aqueous Solutions*; Plenum Press: New York, 1996.

(4) Konkena, B.; Vasudevan, S. *J Phys Chem Lett* **2012**, *3*, 867.

(5) Vohnik, S.; Hanson, C.; Tuma, R.; Fuchs, J. A.; Woodward, C.; Thomas, G. J. *Protein Sci* **1998**, *7*, 193.

(6) Fernandes, A. N.; Giacomelli, C.; Giovanela, M.; Vaz, D. O.; Szpoganicz, B.; Sierra, M. M. D. *J Brazil Chem Soc* **2009**, *20*, 1715.

(7) Sierra, M. M. D.; Arend, K.; Fernandes, A. N.; Giovanela, M.; Szpoganicz, B. *Anal Chim Acta* **2001**, *445*, 89.