Synthesis and characterization of GO-hydrogels composites

J Y Pereyra, E A Cuello, R Coneo Rodriguez, C A Barbero, E I Yslas, H J Salavagione, D F Acevedo

1 Departamento de Química, Facultad de Ciencias Exactas, Fisicoquímicas y Naturales, Universidad Nacional de Río Cuarto, 5800, Río Cuarto, Argentina.
2 Dept. Polymer Physics, Elastomers & Energy Applications. CSIC-ICTP, C/ Juan de la Cierva, 3. 28006. Madrid, Spain.
3 Departamento de Biología Molecular, Facultad de Ciencias Exactas, Fisicoquímicas y Naturales, Universidad Nacional de Río Cuarto, 5800, Río Cuarto, Argentina.

Abstract. The preparation of poly(N-isopropylacrylamide) (PNIPAm) hydrogel nanocomposites containing graphene oxide (GO) and GO plus carbon nanotubes (CNT) in the polymer network is communicated. This one-pot preparation methods include the dispersion of GO (or GO plus CNT) in a solution of monomers and the subsequent polymerization. The texture of the nanocomposites was studied using scanning electron microscopy (SEM), where very compact surfaces are observed suggesting good dispersion of GO sheets and CNTs within the polymer matrix. The presence of GO inside the polymer network diminished the equilibrium swelling values and increased the elastic modulus up to 162 % with respect to the pure gel. Similar results were observed for the composite with CNT. Furthermore, the electrical resistivity of PNIPAm-GO diminishes as the applied compression force increases, being 50 % lower than hydrogel without GO. Moreover, the electrochemical properties of the hydrogels, evaluated by cyclic voltammetry, indicate highly reversible electrical charge/discharge response. In order to apply these materials for antibiotic delivery, the absorption of tetracycline (tet) is evaluated and the nanocomposites showed better absorption capability and improved antibiotic delivery. Preliminary results suggest that tet loaded PNIPAm-GO and PNIPAM-GO-CNT display antimicrobial activity against the Pseudomonas aeruginosa turning these materials as potential candidates for biomedical applications.

1. Introduction
Carbonaceous nanomaterials such as graphene, graphene oxide and carbon nanotubes, have been attracting interest due to their properties that make them useful for technological applications, such as catalysis, coatings, and bio-applications [1–5]. On the other hand, responsive hydrogels have been in the focus of scientific attention in the last few decades owing to their numerous properties like high water content and relatively good deformability [6,7]. Among the different kinds of hydrogels, Poly(N-isopropylacrylamide) (PNIPAm) is one of the most popular termo-responsive hydrogels due to its peculiar volume phase transition temperature at c.a. 34 °C [8,9]. Notwithstanding the above
mentioned properties, these hydrogels present some limitations, mainly related to its poor mechanical strength.

In this work, we describe the incorporation of GO and GO-CNT with the goal to improve the properties of the hydrogel. It is observed that the composites with GO and GO-CNT enhance the hydrogel mechanical and electric properties and also increase the capacity to incorporate tetracycline antibiotic molecules (tet) that allow to inhibit bacterial growth. Even though the swelling of these composite materials is smaller than the pure hydrogel, the elastic modulus increases up to 160% with the incorporation of GO. Also, the hydrogel conductivity is enhanced with the introduction of GO and GO-CNT allowing potential technological applications in the resistive sensors areas. Likewise, the composites cyclic voltammetry studies show that these materials could be used as electrochemical electrodes. Furthermore, it is demonstrated that composites allow loading up to 50% more of tetracycline solution than the pristine hydrogel. Accordingly, PNIPAm-GO and PNIPAM-GO-CNT with tetracycline inhibit the bacterial growth as is demonstrated in the inhibition halo experiments.

2. Materials and Methods

2.1. Graphene oxide synthesis
Natural graphite powder (Aldrich) was oxidized using a modification of the Hummers method [10,11]. Briefly, graphite powder was added to concentrated H₂SO₄ (200 mL) immersed in an ice bath. Then, KMnO₄ were added gradually under constant stirring and cooling to keep the suspension temperature below 20 °C. The reaction was kept under stirring during 2 hours. Subsequently, 360 mL of distilled water were added ensuring that the temperature does not exceed 50 °C. Then 40 mL of H₂O₂ (30%) was added until the color of the mixture turned bright yellow. The mixture was washed with bidistilled water until the pH reached a value of 3. The supernatant was discarded and the graphite oxide obtained was dispersed in distilled H₂O and subjected to ultrasound to generate GO sheets during one hour.

2.2. Hydrogel and composites synthesis:
Hydrogels matrix used N-isopropylacrylamide (NIPAm, 0.5 M) as monomer precursor and N,N-methylene bisacrylamide (BIS, molar ratio 0.02:1 monomer based) as crosslinking agent [12]. The free radical polymerization was initiated by a redox initiator system: ammonium persulfate (APS, 1 mg mL⁻¹) and tetramethylenediamine (TEMED, 10 μL mL⁻¹). The Poly-N-isopropylacrylamide (PNIPAm) was synthesized dissolving NIPAm and the crosslinked agent BIS in 3 mL of bidistilled water, the reaction was initiated previous degasification incorporating APS and TEMED. The reaction was carried out at 22 °C during 1 hour. Hydrogels were polymerized in cylindrical molds, cut and washed several times with distilled water and dried for 48 hours at room temperature and moderate vacuum (0.1 atm) for later use. The hydrogels loaded with nanomaterials were synthesized using the same chemical reagents adding GO (5 mg mL⁻¹) to obtain PNIPAm-GO, and adding the same quantity of GO and CNT (0.34 mg mL⁻¹) into the synthesis media to obtain PNIPAm-GO-CNT. The composites were polymerized in cylindrical molds, cut and washed several times with distilled water following the procedure described previously for PNIPAm. The composites were observed by SEM (FE-SEM HITACHI S4800) microscopy.

2.3. Hydrogel and composites characterization:
The hydrogels ability of absorb/desorb water it is known as swelling capacity [13]. To analyze the swelling kinetics, PNIPAm, PNIPAm-GO and PNIPAm-GO-CNT dry gels were weighed and then immersed in bidistilled water at room temperature. Then, the materials were removed, dried, weighed and placed back in the solution at regular time intervals. The mass was measured after each interval
time. The procedure was repeated until the materials achieved a constant weight. The swelling ratio was calculated according to Equation 1, as a function of time.

\[
\%S_w = \frac{w(t) - w_0}{w_0} \cdot 100
\]  

(1)

Where: \(w(t)\) represents the weight of hydrogel in swollen state at time \(t\) and \(w_0\) is the weight of the dry hydrogel. All the measurements were performance by triplicate.

2.4. Mechanical assays: Elastic-viscoelastic behaviour by uniaxial compression assay

Uniaxial compression measurements were performed on individual discoidal hydrogels and composites in swollen state at room temperature. For these measurements, a homemade device was built to measure the Young module, under compression. The stress (\(\sigma\), KPa) versus strain (\(\varepsilon\)) curve was built following the procedure described by Martinez et al. [7], and from the slope the Young module was calculated [14].

2.5. Measurements of electric resistance

All the measurements were conducted at 25 ± 3°C. A cylindrical gel of 10 mm in diameter and 15 mm in height was placed on an analytic digital balance (OHAUS Pioneer). On the extremes of the cylinder two electrodes were placed, and then different loads were imposed vertically to the gel. The electrodes were connected to a Siemens multimeter and the electrical resistance was measured for each compression state [15].

2.6. Electrochemical measurements

Cyclic voltammetry experiments were carried out in a conventional three electrode cell, controlled by a computerized potentiostat (Autolab). All potentials were quoted against the saturated Ag/AgCl electrode and platinum wire as contraelectrode, in all the measures the materials were cut in cylindrical pieces (10 mm diameter and 2 mm of height) and were contacted using glassy carbon and at scan rate 50 mV s\(^{-1}\). The support electrolyte used was \(\text{H}_2\text{SO}_4\) (1 M).

2.7. Partition coefficient measurements by UV-vis spectroscopy

The CP value determines the distribution of tet between two phases, hydrogel and water and it is defined by the Equation 2.

\[
CP = \frac{C_{\text{material}}}{C_{\text{solution}}}
\]  

(2)

Where \(C_{\text{material}}\) and \(C_{\text{solution}}\) is the tetracycline molal concentration in the gels and tet molal concentration in the solution, respectively.

Experimentally, a piece of known mass dry materials was immersed in 3 mL of tet solution. After 8 days of immersion, the concentration of the remaining tet in the solution was determined by UV-visible spectroscopy (Hewlett-Packard- 8453 UV-visible Spectrophotometer). Previously, a calibration curve of tet solution was carried out fixing the wavelength at 360 nm. The volume of the hydrogel was evaluated by gravimetric measures, considering the solution density same to the water density and at 25 °C [16].

2.8. Antibacterial activity studies

The materials antimicrobial activity against \textit{Pseudomonas aeruginosa} (ATCC) was tested according to agar diffusion test [17]. For agar diffusion method, the samples were exposed to bacteria in solid
media (nutrient agar), and the inhibition zone around each sample was measured and recorded. This method was performed in Luria Bertani (LB) medium solid agar Petri dish. Luria Bertani medium was used as a growing medium of *Pseudomonas aeruginosa* bacteria in overnight inoculums. Then, 100µL of bacterial solution inoculum was placed over the agar into sterilized Petri dishes. The materials were placed on the *Pseudomonas aeruginosa* cultured agar plate. The agar plates were incubated for 24 h at 37 ºC and the inhibition zone was monitored. After incubation, the presence of a bacterial growth inhibition halo around the samples was observed and their diameter in millimetres was measured. The assays were performed in triplicate.

3. Results and Discussion

Figure 1 shows digital images of PNIPAm hydrogel and its composites with GO and GO-CNT, where the black color, imparted by the fillers is clearly observed, confirming the presence of GO and CNT in the hydrogel network (Fig. 1 b and c.). SEM image of the pure PNIPAm hydrogel depicts a homogeneous polymer surface (Fig. 1d). However, in the case of PNIPAm-GO the surface presents a layered structure due to the GO sheets. The PNIPAm-GO-CNT displays a similar structure as PNIPAm-GO with additional filamentous structures owing to the presence of the CNT (Fig. 1f).

*Figure 1:* Digital photography of a) PNIPAm, b) PNIPAm-GO and c) PNIPAm-GO-CNT and SEM images of d) PNIPAm, e) PNIPAm-GO and f) PNIPAm-GO-CNT.

Figure 2 shows a comparison of the swelling kinetics between the hydrogel and the composites materials. The equilibrium swelling of PNIPAm is 3300 % and for the composites, the swelling percentage reaches lower values c.a. 1850 % for PNIPAm-GO and 2100 % for PNIPAm-GO-CNT. Considering that the composites equilibrium swellings are lower than that of the pure polymer it is reasonable to propose that their matrices are less elastic.

Regarding the mechanical performance, an increase the Young modulus has been observed when nanomaterials are incorporated: the PNIPAm-GO sample presented the highest value, 9.7 KPa, an enhancement of 160 % with respect to PNIPAm (3.7 KPa), resembling the case of other hydrogel nanocomposites [7]. This behavior could be explained considering two factors: i) the high rigidity of GO material (\(E = 290-430\) GPa) [18], and ii) the GO homogeneous distribution within the hydrogel. Moreover, the groups -COOH, -OH and -C=O present in the GO surface are able to generate hydrogen bonding with the polymer groups, leading to greater rigidity. The composite with GO-CNT present a similar change in the equilibrium swelling that PNIPAm-GO, however the Young module value is slightly altered. These results could be explained considering that the GO-CNT in the matrix could produces a less pronounced decrease in the matrix elasticity due to the hydrophobic character of the CNT or a not a perfect homogeneous distribution of the CNT in the matrix.
Figure 2: Swelling kinetic of (■) PNIPAm, (●) composite of PNIPAm with graphene oxide and (▲) PNIPAm with GO and CNT.

Table 1. Mechanical properties of the materials, Young modulus and percentage of Young modulus increase

| Materials          | Young Modulus (KPa) | % of increase |
|--------------------|---------------------|---------------|
| PNIPAm             | 3.7 ± 0.1           | 0             |
| PNIPAm-GO          | 9.7 ± 0.3           | 161.83        |
| PNIPAm-GO-CNT      | 6.8 ± 0.3           | 82.96         |

The changes of the resistance at different loadings imposed vertically to the gel relative to the initial unloaded resistance are shown in Figure 3a. The PNIPAm hydrogels present higher values of resistance than the composites, as expected. In all cases the materials increase the conductivity as the applied load increase. The initial PNIPAm resistance at $t = 0$ (c.a 50 MΩ) is higher than PNIPAm-GO-CNT (45 MΩ) and NIPAm-GO (25 MΩ). It is possible to observe that the resistance diminishes when the vertical load applied increases, this is an expected behaviour, since the application of stress changes the spacing between conductive filler particles resulting in resistivity changes. The composites present lower resistivity values for all the load applicated, changing the value up to 0.6 for PNIPAm-GO. These results indicate that these composite materials are more sensitive to external pressure than the pure hydrogel. Beside the composite loaded with CNT present a higher change (0.7) this could be attributed to the best conductivity of the CNT.

The cyclic voltammetry at a scan rate of 50 mV/s is shown in Figure 3b. The profile revealed a rectangular shape of the curves, which indicate highly reversible electrical charge/discharge response. All results indicate that the conductive behavior of these materials has been improved with the introduction of GO and with GO and CNT allow potentials technological application.

In order to apply these materials as inhibitors of bacterial growth, the partition coefficient (CP) of solutions of tet, an antibiotic used to treat several bacterial infections, was studied. Clearly, the composite materials present higher CP values (PNIPAm-GO 119; PNIPAm-GO-NTC 137) than PNIPAm (83), indicating that the GO and CNT improve the adsorption of the antibacterial agent (Figure 4a). This behaviour is in agreement with other studies that shown the GO ability to sorbe organic molecules [1,19]. To improve its use as antimicrobiane, the kinetics of liberation of tet from the hydrogels was evaluated (Figure 4b). As it can be seen in Figure 4b, the composites show enhanced delivery of the antibiotic in comparison with the pure hydrogel.
Figure 3: a) resistance variation of the materials versus stress (■) PNIPAM, (●) composite of PNIPAm with graphene oxide and (▲) PNIPAm with GO and CNT. b) Cyclic voltammetry of PNIPAm (black line), composite of PNIPAm with graphene oxide (dark gray) and PNIPAm with GO and CNT (light gray).

Figure 4: a) Partition coefficient of Tetracycline in PNIPAm hydrogels and PNIPAm-GO PNIPAm-GO-CNT composites and b) Kinetic of tet liberation of i) PNIPAm (light gray line, ●), ii) PNIPAm-GO (gray line ▲) and iii) PNIPAm-GO-CNT (black line ■).

The in vitro antibacterial properties of composite hydrogels were investigated by agar diffusion tests against gram-negative Pseudomonas aeruginosa bacteria. The inhibition zones are presented in Figure 5. The results clearly show that the inhibition zone around each loaded composite PNIPAm-GO-tet (1.60 ±0.2 cm) and PNIPAm-GO-CNT-tet (1.75±0.3 cm) are higher than the PNIPAm-GO and NIPAm-GO-CNT without the antibiotic which not present inhibition zone. The results suggest that the tet loaded composite hydrogels revealed a toxic effect on bacteria than hydrogel without the antibiotic under similar conditions as evidenced by higher inhibition zone due to the more efficiency delivery of the tet.
Figure 5: Photographs of the inhibition zones of nanocomposite hydrogels against *Pseudomonas aeruginosa*. a) composite PNIPAm with graphene oxide, b) composite PNIPAm with graphene oxide and tet, c) PNIPAm with GO and CNT and d) PNIPAm with GO and CNT and tet. The white line represents the hydrogel diameter and the yellow line shows the inhibition diameter.

4. Conclusion
Novel materials composed of a hydrogel (PNIPAm) matrix and GO or GO-CNT has been synthesized using a facile one-pot procedure. The nanomaterials incorporated in the matrix improve its mechanical properties. Even though the swelling degree of these composite materials is smaller than the pristine hydrogel, the elastic modulus is clearly enhanced. Furthermore, the hydrogel conductivity is enhanced with the introduction of GO and GO-CNT allowing potentials technological applications in resistive sensors. The electrochemistry behavior also opens a new application for these materials as e.g. selective electrodes. Moreover, the loading capacity to incorporate an antibiotic is around 50 % higher for the composites than for the pristine hydrogel. Besides, the agar diffusion test demonstrates the composite antimicrobial activity against the *Pseudomonas aeruginosa* of tet loaded composite hydrogels (PNIPAm with graphene oxide and tet and PNIPAm with GO and CNT and tet). These results indicate potential of tet loaded composite hydrogels for biomedical applications.

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References

[1] Zhu Y, Murali S, Cai W, Li X, Suk J W, Potts J R and Ruoff R S 2010 Graphene and graphene oxide: Synthesis, properties, and applications Adv. Mater. 22 3906–24
[2] Hu K, Kulkarni D D, Choi I and Tsukruk V V. 2014 Graphene-polymer nanocomposites for structural and functional applications Prog. Polym. Sci. 39 1934–72
[3] Jeong H Y, Kim J Y, Kim J W, Hwang J O, Kim J E, Lee J Y, Yoon T H, Cho B J, Kim S O, Ruoff R S and Choi S Y 2010 Graphene oxide thin films for flexible nonvolatile memory applications Nano Lett. 10 4381–6
[4] Levi-Polyachenko N, Young C, MacNeill C, Braden A, Argenta L and Reid S 2014 Eradicating group A streptococcus bacteria and biofilms using functionalised multi-wall carbon nanotubes Int. J Hypert. 30 490–501
[5] Zhou F, Xing D, Ou Z, Wu B, Resasco D E and Chen W R 2009 Cancer photothermal therapy in the near-infrared region by using single-walled carbon nanotubes J. Biomed. Opt. 14 21007–9
[6] Singh N K and Lee D S 2014 In situ gelling pH- and temperature-sensitive biodegradable block copolymer hydrogels for drug delivery J. Control. Release 193 214–27
[7] Martínez M V., Bongiovanni Abel S, Rivero R, Miras M C, Rivarola C R and Barbero C A 2015 Polymeric nanocomposites made of a conductive polymer and a thermosensitive hydrogel: Strong effect of the preparation procedure on the properties Polym. (United Kingdom) 78 94–103
[8] Kumar A, Srivastava A, Galaev I Y and Mattiasson B 2007 Smart polymers: Physical forms and bioengineering applications Prog. Polym. Sci. 32 1205–37
[9] Sugiura S, Sumaru K, Ohi K, Hiroki K, Takagi T and Kanamori T 2007 Photoresponsive polymer gel microvalves controlled by local light irradiation Sensors Actuators, A Phys. 140 176–84
[10] Hummers W S and Offeman R E 1958 Preparation of Graphitic Oxide J. Am. Chem. Soc. 80 1339–1339
[11] Chen J, Yao B, Li C and Shi G 2013 An improved Hummers method for eco-friendly synthesis of graphene oxide Carbon N. Y. 64 225–9
[12] Caykara T, Kiper S and Demirel G 2006 Thermosensitive poly(N-isopropylacrylamide-co-acrylamide) hydrogels: Synthesis, swelling and interaction with ionic surfactants Eur. Polym. J. 42 348–55
[13] Baker J P, Stephens D R, Blanch H W and Prausnitz J M 1992 Swelling equilibria for acrylamide-based polyampholyte hydrogels Macromolecules 25 1955–8
[14] Mulko L, Rivarola C R, Barbero C A and Acevedo D F 2016 Bioethanol production by reusable Saccharomyces cerevisiae immobilized in a macroporous monolithic hydrogel matrices J. Biotechnol. 233 56–65
[15] Rivero R E, Molina M A, Rivarola C R and Barbero C A 2014 Pressure and microwave sensors/actuators based on smart hydrogel/conductive polymer nanocomposite Sensors Actuators, B Chem. 190 270–8
[16] Molina M A, Rivarola C R and Barbero C A 2012 Study on partition and release of molecules in superabsorbent thermosensitive nanocomposites Polymer (Guildf). 53 445–53
[17] Bonev B, Hooper J and Parisot J 2008 Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method J. Antimicrob. Chemother. 61 1295–301
[18] Liu L, Zhang J, Zhao J and Liu F 2012 Mechanical properties of graphene oxides Nanoscale 4 5910
[19] Potts J R, Dreyer D R, Bielawski C W and Ruoff R S 2011 Graphene-based polymer nanocomposites Polymer (Guildf). 52 5–25