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Enhanced Analytical Performance of Paper Microfluidic Devices by Using Fe_3O_4 Nanoparticles, MWCNT, and Graphene Oxide

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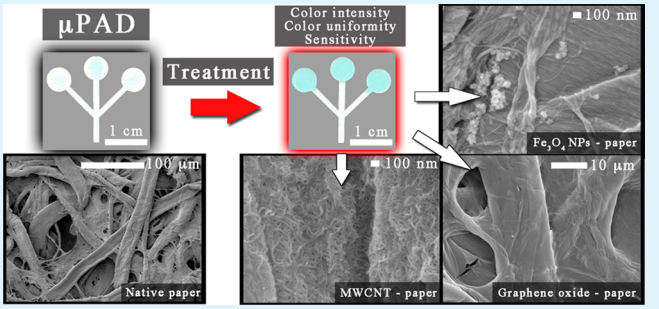
Supporting Information

ABSTRACT: Spheres, tubes, and planar-shaped nanomaterials as Fe_3O_4 nanoparticles (MNPs), multiwalled carbon nanotubes (MWCNT), and graphene oxide (GO) were used for the first time to treat microfluidic paper-based analytical devices (μPADs) and create a biocompatible layer with high catalytic surface. Once glucose measurements are critical for diabetes or glycosuria detection and monitoring, the analytical performance of the proposed devices was studied by using bienzymatic colorimetric detection of this carbohydrate. The limit of detection values achieved for glucose with μPADs treated with MNPs, MWCNT, and GO were 43, 62, and 18 μM, respectively. The paper surface modification solves problems associated with the lack of homogeneity on color measurements that compromise the sensitivity and detectability levels in clinical diagnosis.



KEYWORDS: *carbon nanotubes, cellulose, clinical diagnostics, colorimetric biosensors, digital image analysis, magnetic nanoparticles, paper microfluidics*

In the last years, nanomaterials have gained considerable attention because of their high surface and unique mechanical, electrical, optical, and magnetic properties. Several interfaces between different kind of nanomaterials with well-known properties (e.g., composition, shape, size) and biomolecules (e.g., enzymes, DNA, antibodies) have been used for bioassays and biosensing applications, molecular biology and molecular medicine.[1−4] On the other hand, cellulosic substrate is globally recognized as one of the most used tools in chemistry and it was recently rediscovered for the development of microfluidic paper−based analytical devices (μPADs).[5] Paper microfluidic devices have emerged as a new class of disposable microfluidic systems with the capability to be used at point-of-care (POC) applications.[6] In addition, cellulose fiber treatment and further modifications with Ag nanoparticles[6,7] (NPs), ceria NPs,[8] Au NPs,[6,9−12] curcumin NPs,[13] and SiO_2 NPs[14] for analytical applications have been reported in the literature in order to improve the analytical performance and minimize washing effects often observed in lateral flow assays associated with colorimetric detection.[14] However, the study of analytical applications of Fe_3O_4 magnetic NPs (MNPs), multiwalled carbon nanotubes (MWCNT) and graphene oxide (GO) to modify μPADs, based on colorimetric measurements, has not been reported. MWCNT and GO can increase the linkage of aromatic molecules through $\pi-\pi$ stacking or van der Waals forces.[15] Furthermore, biomolecules can be adsorbed into the surface of oxidized carbon-based nanomaterials by electrostatic interaction[1,16−18] or hydrogen bonding.[19] On the other hand, some studies reported reversible noncovalent interaction between MNPs and biomolecules.[20,21] Moreover, a peroxidase mimetic activity of MNPs with a Michaelis−Menten kinetics was found to have high affinity for TMB in comparison to HRP.[22,23] With this background, we describe for the first time a treatment procedure of μPADs by using spheres, tubes and planar shaped nanomaterials as MNPs, MWCNT, and GO. These nanomaterials differ from each other on their surface-to-volume ratio and shape. Moreover, we propose that they can act as support for GOx and HRP immobilization. If the kinetics of the reaction is governed by diffusion, sphere-shaped nanomaterial among others[24] could achieve higher sensitivities. The hypothesis of this study is that the used nanomaterials will enhance the analytical signal for glucose colorimetric detection by improving the available superficial area of the μPADs.

MNPs were synthesized by the co-precipitation method.[25] Hydrodynamic diameter of 35 nm was determined by dynamic light scattering (DLS) (Figure S1). Fourier transform infrared spectroscopy (FT-IR) analysis of particles showed the

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characteristic Fe−O bond of Fe_3O_4 at 590 cm^{-1} (Figure S2). MWCNT (Sigma−Aldrich, ≥ 98%, O.D × I.D. × L 10 nm ± 1 nm × 4.5 nm ± 0.5 nm × 3−6 μm) were oxidized by acid treatment to improve dispersion in water. GO (Sigma−Aldrich) was prepared from a 2 mg mL^{-1} stock suspension in water. Nanomaterials were dispersed in ultrapure water and vigorously stirred prior to use. μPADs were fabricated by CO_2 laser using 20 × 20 cm filter paper Whatman #1. Additional information about the fabrication of μPADs is available in Supporting Information. Paper chips were soaked in a colloidal and stable MNPs, MWCNT and GO nanomaterial solution during 30 s to obtain MNP-μPADs, MWCNT-μPADs, and GO-μPADs, respectively. The μPADs were dried over a hydrophobic plastic film at room temperature for 40 min and then laminated on one side with a poly(methyl methacrylate)-coated thermosensitive polyester film at 130 °C. After the manufacturing process, reagents for glucose colorimetric assay were spotted in the detection zones. Briefly, 1 μL of chromogenic substrate (15 mM TMB) and enzymatic solutions (GOx:HRP, 120:30 U mL^{-1}) were added to the detection zones in two independent steps and dried for 20 min at room temperature. In all cases, 10 μL of sample solutions were disposed at the bottom region of the μPAD main channel. After 20 min at 25 °C, the devices were digitalized with a desktop scanner and analyzed using the arithmetic mean of the pixel intensity within each detection zone through Corel Photo−Paint graphical software. The fabrication process of μPADs and signal analysis procedure is detailed in Figure 1.

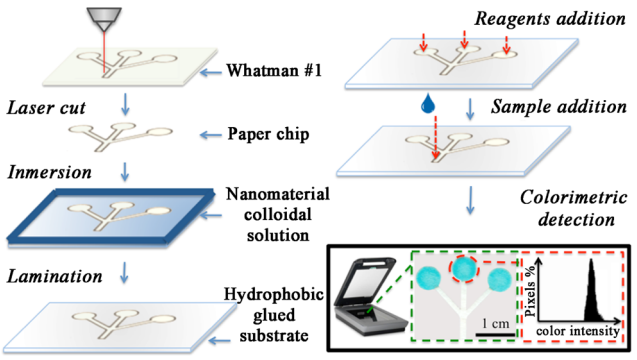

Figure 1. Schematic representation of the construction procedure of the μPADs showing the treatment step with MNPs, MWCNT, and GO as well as the procedure required for colorimetric detection involving scanner and pixel intensity analysis.

An optimization process was performed in order to select the conditions (nanomaterial type and colloidal solution concentration) that would produce high color intensity in the glucose microfluidic assay. Figure 2 shows the intensity signal values of native μPADs and those treated with nanomaterials after the addition of 1 mM glucose. In all cases, the analytical signal was determined by subtracting the background color intensity. Paper soaked with nanomaterial colloidal solutions have exhibited enhanced color signal and uniformity. Optimal concentration with high color intensity was determined to be 100, 10, and 100 μg mL^{-1} for MNPs, MWCNT, and GO respectively. When higher concentrations of nanomaterials were used to treat native paper, strong background color intensity was detected for MWCNT-μPADs and GO-μPADs (Figure 2). For this reason, the color intensity was low for high concentration of MWCNT and GO colloidal solutions.

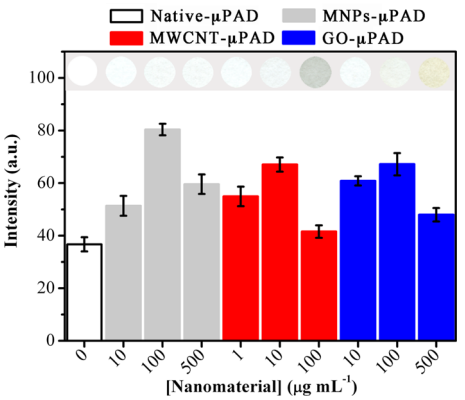

Figure 2. Color intensity of native and treated μPADs with different concentrations of MNPs, MWCNT, and GO colloidal solutions. Color intensity corresponds to the glucose assay at concentration of 1 mM. The inset optical micrographs show the background color developed in the paper according to the proposed treatment. Error bars indicate the standard deviation value (n = 6).

Additionally, only for higher concentrations of GO (1000 μg mL^{-1}) the μPAD becomes hydrophobic and therefore lateral flow velocity decreases (data not shown). On the other hand, the low signal response of μPADs treated with 500 μg mL^{-1} of MNPs could be due to nanoparticles aggregation (high magnetic forces) over the paper fibers, resulting in a decrease in surface area to volume ratio of the nanoparticles.

To evaluate the peroxidase mimetic behavior of MNPs in the conditions of the bioassay, we performed an additional experiment using MNP-μPADs prepared without HRP. Results indicated that color development was not evident after 20 min of incubation (data not shown) suggesting that the peroxidase activity is not related to MNPs. Figure 3A shows the improvement of the color intensity and uniformity for images captured on detection zones using devices treated with nanomaterial colloidal solutions after glucose addition. It is important to remark that a noticeable difference in color intensity between treated and nontreated μPADs was found for

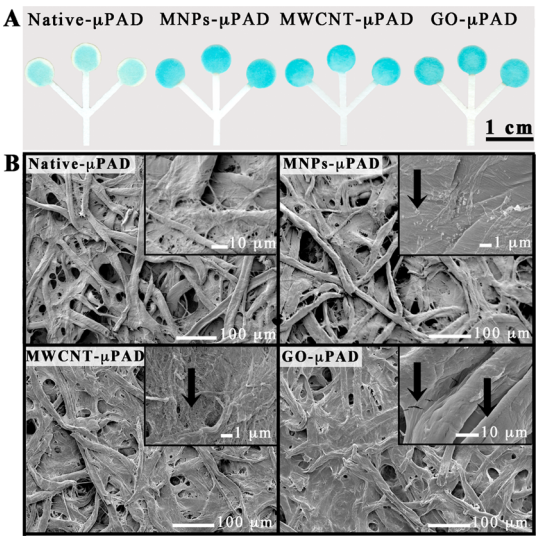

Figure 3. (A) Optical micrograph of μPADs after addition of 1 mM glucose sample and (B) FESEM images of native and treated devices with MNPs, MWCNT, and GO. Arrows indicate the presence of nanomaterials over the paper fibers.

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all treatments. Representative field emission scanning electron microscopy (FESEM) images of μPADs used in this study (Figure 3B) reveal the presence of the nanomaterials over the paper fibers after the treatment procedure. MNPs were confirmed by the presence of Fe detected with energy-dispersive X-ray spectroscopy (EDS) (Figure S3). The MNPs were not evenly distributed over the cellulose material; however, when higher concentrations were used (Figure 2), low analytical signal was obtained suggesting more aggregation and less distribution over the fiber surfaces. The characteristic tubular morphology of MWCNT is also shown in Figure 3B. GO was detected over the surface of paper due to the presence of thin and long edges present on the fibers. In this study, diluted nanomaterial colloidal solutions were used to modify paper cellulose fibers suggesting physical absorption between both materials involving surface forces as hydrogen bonding, electrostatic interactions, and van der Waals forces among others.[26,27]

On the basis of the presented results, the analytical performance of the μPADs treated with MNPs, MWCNT and GO colloidal solutions were investigated using the optimized conditions. For this purpose, analytical curves for glucose concentrations ranging from 0 to 5 mM (Figure 4A) were obtained and they are displayed in Figure 4. It was possible to see that the intensity of the colorimetric signal was enhanced in all concentrations of tested glucose. In addition, a linear relationship for low concentrations of analyte was observed using μPADs treated with nanomaterials (Figure 4B–D). For native μPADs, the colorimetric response offered linear behavior from 0.3 to 1 mM (Figure S4) with a limit of detection (LOD) of 238 μM. Meanwhile for MNP-μPAD and MWCNT-μPAD, the linear range was between 0.05 and 1 mM with a LOD of 43 and 62 μM, respectively. GO-μPAD presented the best results with a linear range between 0 and 1 mM and LOD of 18 μM (Table S1). It has been proposed that the kinetics of the reaction could be controlled by diffusion mechanisms.[24] Higher sensitivities of the analytical method could be achieved with MNP-μPAD followed by MWCNT-μPAD and GO-μPAD, as was experimentally demonstrated in the current study (Table S1).

According to optical micrographs presented in Figure 4E, it can be seen the visual detection associated with the linear increment on the characteristic blue color of TMB in the detection zones corresponding to all tested glucose concentrations. Furthermore, in all cases there is better color intensity and uniformity than in native μPADs. LOD values of paper devices treated with nanomaterials were lower than native μPADs and other μPADs recently reported in the literature (see Tables S1 and S2). In addition, the use of μPADs treated with carbon-based nanomaterials and magnetic nanoparticles provided linear response in a glucose concentration range from 0.05 to 1 mM (MNPs and MWCNT) and from 0 to 1 mM (GO), meanwhile for clinical glucometers the range is between 0.5 and 33 mM.[28] On the basis of these results, the analytical reliability of the μPADs treated with nanomaterials was tested by performing glucose assay in a clinical sample like human urine where the normal concentration levels of glucose are between 0.1 and 0.8 mM.[29] For this purpose, an artificial urine sample was prepared[5] and spiked with different glucose concentration levels to perform the recovery test and therefore demonstrate the accuracy of the proposed device. The results obtained for artificial urine samples tested with μPADs treated with nanomaterial colloid solutions are displayed in Table 1.

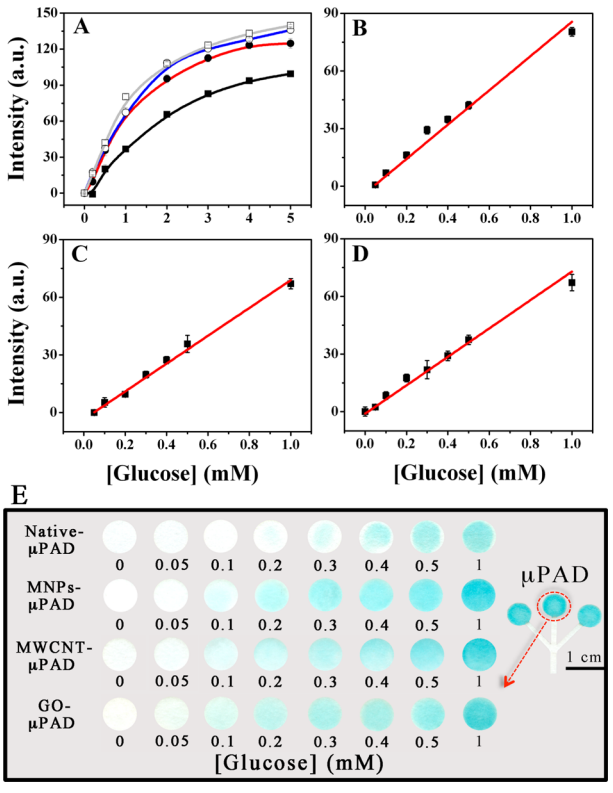

Figure 4. (A) Analytical curves for glucose assay using native μPAD (■, black line) and treated μPADs with 100 μg mL^{-1} of MNPs (□, gray line), 10 μg mL^{-1} of MWCNT (●, red line), and 100 μg mL^{-1} of GO (○, blue line). Linear ranges of the analytical curve for glucose using (B) MNPs-μPAD, (C) MWCNT-μPAD, (D) GO-μPAD. (E) Optical images of the detection zones corresponding to glucose assays in concentration ranging from 0–1 mM for native μPAD, MNP-μPAD, MWCNT-μPAD, and GO-μPAD. Error bars displayed in graphs A–D represent the standard deviation value (n = 6).

Table 1. Glucose Concentration Levels Determined on Artificial Urine Samples Using μPADs Treated with Nanomaterials

known concentration (mM)	found concentration (mM)		
	MNP-μPAD	MWCNT-μPAD	GO-μPAD
0.3	0.16 ± 0.06	0.28 ± 0.08	0.26 ± 0.05
0.4	0.20 ± 0.08	0.37 ± 0.07	0.38 ± 0.04
0.5	0.36 ± 0.11	0.51 ± 0.08	0.56 ± 0.05
0.8	0.61 ± 0.12	0.82 ± 0.08	0.82 ± 0.04

As can be seen in the presented data, we obtained acceptable values of glucose concentration determined with MWCNT-μPADs and GO-μPADs in comparison with MNP-μPADs. Best results were achieved with GO-μPADs with recovery values between 83 and 109% obtained in a considerable glucose concentration range (0.3–0.8 mM) following by MWCNT-μPADs with recovery values between 76 and 96%. On the other hand, the recovery levels for μPADs treated with MNPs were between 40 and 71%. This can be related to the presence of urea in the artificial urine sample. This compound, as a denaturalizing agent, is capable of disrupting enzyme structure and therefore decreasing the catalytic activity.[30] On the basis of the analytical performance of glucose assay in urine samples,

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the carbon-based nanomaterials used in our study seem to protect enzymes from urea. Some physical and chemical characteristics present in carbon-based nanomaterials but not in MNPs improved the analytical signal for glucose detection in the tested artificial urine samples.

Overall, this study described for the first time the modification of μPADs by incorporation of MNPs, MWCNT and GO. The main strategy employed to modify cellulose fibers with nanomaterials previously synthesized was planned without the use of any linker, binder or retention aid. The used procedure solves the drawback of using additives avoiding possible interaction with enzyme activity. On the basis of the presented results, the modified μPADs provided enhanced analytical performance allowing the visual detection of glucose at low concentrations. The improvements reported in this study enable the use of proposed devices for POC diagnosis with many advantages including simple instrumentation, easy operation, and low cost. Lastly, the association of μPADs with carbon-based nanomaterials and magnetic nanoparticles offers great potentiality to be explored in bioanalytical applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b10027.

Detailed information on the materials and reagents used, fabrication of μPADs, and colorimetric detection. Results of DLS and FT-IR analysis of MNPs, EDS analysis of MNP-μPAD and linear range of the analytical curve of glucose using native μPADs (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

TMB, 3,3′,5,5′-tetramethylbenzydine
HRP, horseradish peroxidase
GOx, glucose oxidase

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