Supporting Information

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

*Federico Figueredo [ξ], Paulo T. Garcia [ε], Eduardo Cortón [ξ], and Wendell K. T. Coltro [ε] **

[ε] Instituto de Química, Universidade Federal de Goiás, Goiânia, GO, 74690-900, Brazil

[ξ] Laboratorio de Biosensores y Bioanálisis (LABB), Departamento de Química Biológica e IQUIBICEN−CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (UBA). Pabellón 2, Ciudad Universitaria, Ciudad Autónoma de Buenos Aires, Argentina.

AUTHOR INFORMATION

Corresponding Author

*E-mail: wendell@ufg.br

1. MATERIALS AND METHODS

Chemicals and materials

Glucose oxidase (GOx) (from *Aspergillus niger*, 181 $U.mg^{-1}$), horseradish peroxidase (HRP) (73 $U.mg^{-1}$), D-glucose, 3,3',5,5'-tetramethylbenzidine (TMB), methanol, sodium monohydrogen phosphate, sodium dihydrogen phosphate, multi walled carbon nanotubes and graphene oxide were acquired from Sigma Aldrich Co. (Saint Louis, MO, USA). Fe_3O_4 nanoparticles were synthetized by co-precipitation method. Briefly, 1.17 g of ferric chloride and 0.68 g of ferrous sulfate were dissolved in 50 mL of deoxygenated deionized water with nitrogen gas being bubbled to prevent ferrous ion oxidation. After 60 min of agitation at 70 °C, 5 mL of 32% ammonium hydroxide was added to the mixture and stirred for 120 minutes at 70 °C. The precipitated product was separated by magnet and washed several times with deoxygenated deionized water. Particles were dried in N_2 atmosphere at 25 °C for 48 h. MWCNT (100 mg) were immersed in 50 mL of 65% HNO_3 and heated in a reflux apparatus for 6 h. Afterwards, the mixture was filtrated with ultrapure water until neutral pH. The oxidized MWCNT were dried in an oven at 80 °C for 24 h. Filter paper (grade 1 CHR) was obtained from Whatman (Maidstone, Kent, UK). A scanner (model Scanjet G4050) was acquired from Hewlett-Packard (Palo Alto, CA, USA). All reagents were analytical grade and used as received. All solutions were prepared in ultrapure water.

Fabrication of µPADs

The fabrication of µPADs was performed using a CO_2 laser ablation system. The laser cut the paper, thus creating microfluidic channels and detection zones that conduct the sample and perform the color development, respectively. µPADs were designed in a geometry containing three circular detection zones interconnected by microfluidic channels. All channels were fabricated with 8 mm length and 1 mm width. The diameter values for detection zones were 5 mm.

Colorimetric detection

Colorimetric measurements were performed with an office scanner (Hewlett-Packard, model G4050) using 600-dpi resolution. The images were captured 15 min after sample addition. The recorded images were analyzed in a 24 bits color scale (RGB channel) using Corel Photo-Paint™

software. The arithmetic mean of the pixels intensity within each detection zones was used to quantify the glucose concentration.

The limit of detection (LOD) values were estimated taking into account the ratio between three times the standard deviation for the blank (SD) and the angular coefficient (b) of the respective analytical curve (LOD= (3SD)/b).

2.RESULTS

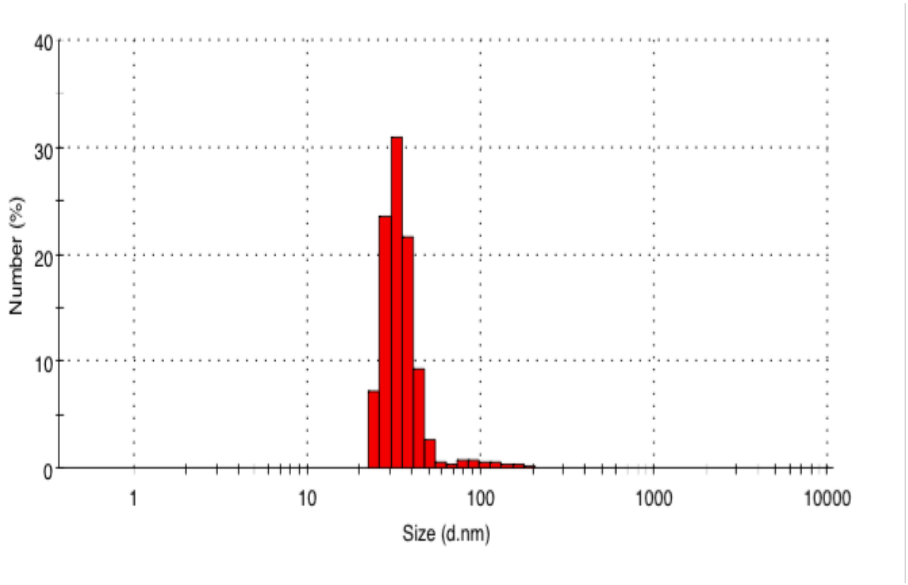

Fig S1. Dynamic light scattering analysis of Fe_3O_4 NPs suspended in 0.15 M $NaNO_3$ (pH 6).

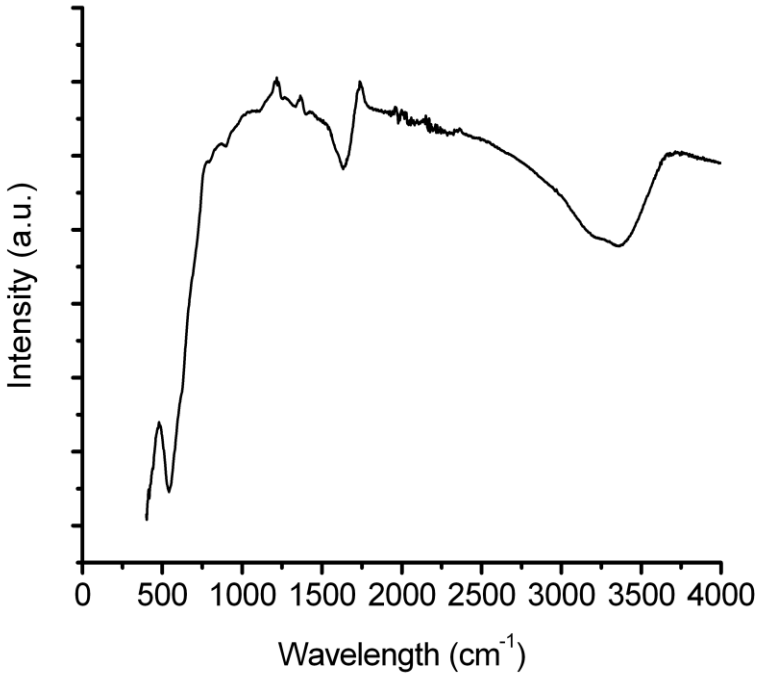

Fig S2. Fourier transform infrared spectroscopy of Fe_3O_4 NPs.

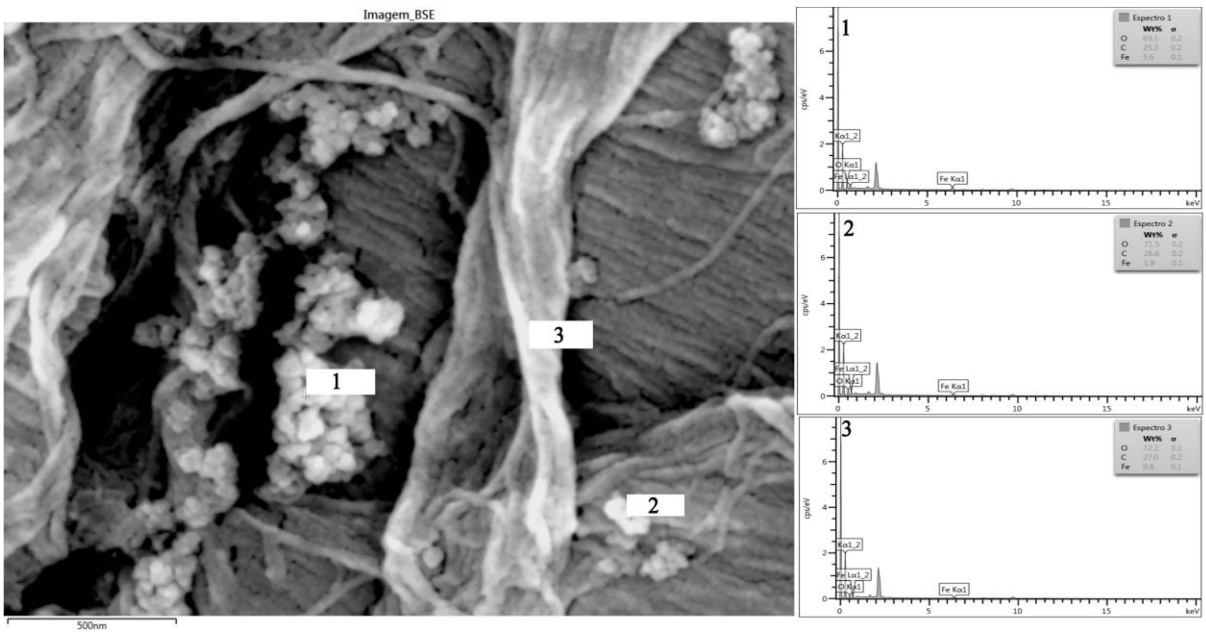

Fig S3. Energy−dispersive X−ray analysis of MNPs−µPAD.

Table S1. Analytical parameters obtained for glucose assay performed on μPADs treated with MNPs, MWCNT and GO.

Platform	*LOD (μM)*	*Sensitivity (a.u. mM^{-1})*	*Linear range (mM)*
Native−μPAD	238	52.29	0.3 – 1
MNPs−μPAD	43	89.24	0.05 – 1
MWCNT−μPAD	62	72.40	0.05 – 1
GO−μPAD	18	73.89	0 – 1

Table S2. Analytical parameters of μPADs recently reported in the literature.

Reference	*LOD (mM)*	*Sensitivity (a.u. mM^{-1})*	*Linear range (mM)*
1	0.7	n.r.	0 – 10
2	0.5	8.6	0.5 – 10
3	0.7	2.32	0 – 12
4*	0.5	49.45	2.5 – 100
5	0.3	6.16	1 – 11
6	1.1	n.r.	0.6 – 15

*Log transformation of analytical curve; n.r. = not reported.

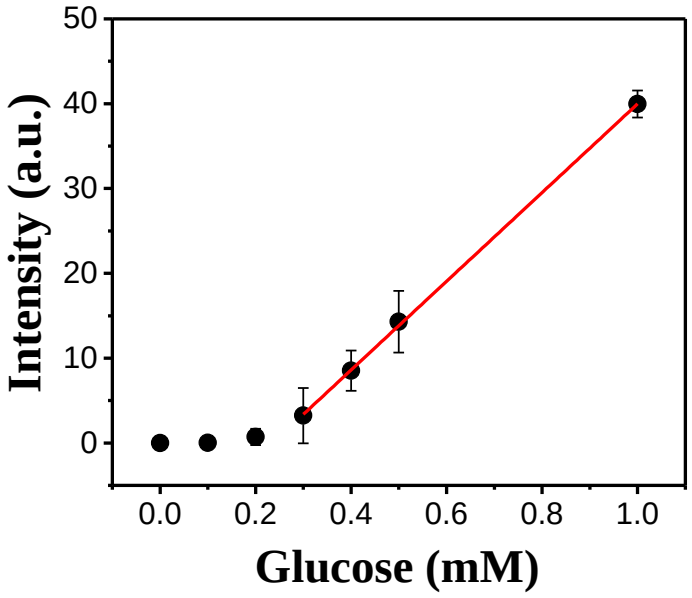

Fig S4. Linear range of the analytical curve of glucose using native−μPADs.

3_ REFERENCES

(1) Chun, H. J.; Park, Y. M.; Han, Y. D.; Jang, Y. H.; Yoon, H. C. Paper-Based Glucose Biosensing System Utilizing a Smartphone as a Signal Reader. *BioChip J.* **2014**, 8, 218–226.

(2) Evans, E.; Gabriel, E. F. M.; Benavidez, T. E.; Coltro, W. K. T.; Garcia, C. D. Modification of Microfluidic Paper-Based Devices with Silica Nanoparticles. *Analyst* **2014**, 139, 5560–5567.

(3) Garcia, P. T.; Cardoso, T. M. G.; Garcia, C. D.; Carrilho, E.; Coltro, W. K. T. A Handheld Stamping Process to Fabricate Microfluidic Paper–Based Analytical Devices With Chemically Modified Surface For Clinical Assays. *RSC Adv.* **2014**, 4, 37637–37644.

(4) Li, B.; Fu, L.; Zhang, W.; Feng, W.; Chen, L. Portable Paper–Based Device For Quantitative Colorimetric Assays Relying on Light Reflectance Principle. *Electrophoresis*, **2014**, 35, 1152 – 1159.

(5) Zhu, W. –J.; Feng, D. –Q.; Chen, M.; Chen, Z. –D.; Zhu, R.; Fang, H. –L.; Wang, W. Bienzyme Colorimetric Detection of Glucose With Self–Calibration Based on Tree–Shaped Paper Strip. *Sens. Actuators B Chem.* **2014**, 190, 414–418.

(6) Yetisen, A. K.; Martinez–Hurtado, J. L.; Garcia–Melendrez, A.; Vasconcellos, F. C.; Lowe, C. R. A Smartphone Algorithm with Inter–Phone Repeatability for the Analysis of Colorimetric Tests. *Sens. Actuators B Chem.* **2014**, 196, 156–160.