

Synthesis, characterization and cytotoxicity of graphene oxide

Óscar Cebadero¹, Soraya Sánchez-Ballester², María Jorda-Beneyto², María Puerto Rodríguez¹,
Ana María Cameán Fernández¹, Ángeles Jos Gallego¹.

1.Universidad de Sevilla. Dpto. de Nutrición, Bromatología, Toxicología y Medicina Legal. Profesor García González 2, 41012 Seville. Spain.
2.Packaging, Transport and Logistic Research Institute. Albert Einstein 1. Paterna, 46980 Valencia. Spain.

RESEARCH
CENTER

ITENE

INTRODUCTION

Graphene oxide (GO) has many properties to be used in improved materials¹, but it is important to know the potential toxic effects derived from its use in this application. In this sense, the European Food Safety Authority had published a Guidance of the risk assessment of nanoscience and nanotechnology applications in the food and feed chain². Appropriate characterization and toxicological studies should be undertaken before the potential application of GO as food contact material.

MATERIALS AND METHODS

GO was synthesized from graphite by using the Modified Hummers Method³. The samples were sonicated for 1 hour and diluted at different concentrations for:

Characterization:

- Fourier-Transform Infrared Spectroscopy
- X-ray photoelectron spectroscopy
- ζ potential
- X-Ray diffraction
- Scanning electron microscopy
- Transmission electron microscopy

Toxicity assays:

- Toxicological effects were evaluated on CaCo-2 cells after 24-48h of exposure by:
- MTS reduction
 - Protein Content (PC)

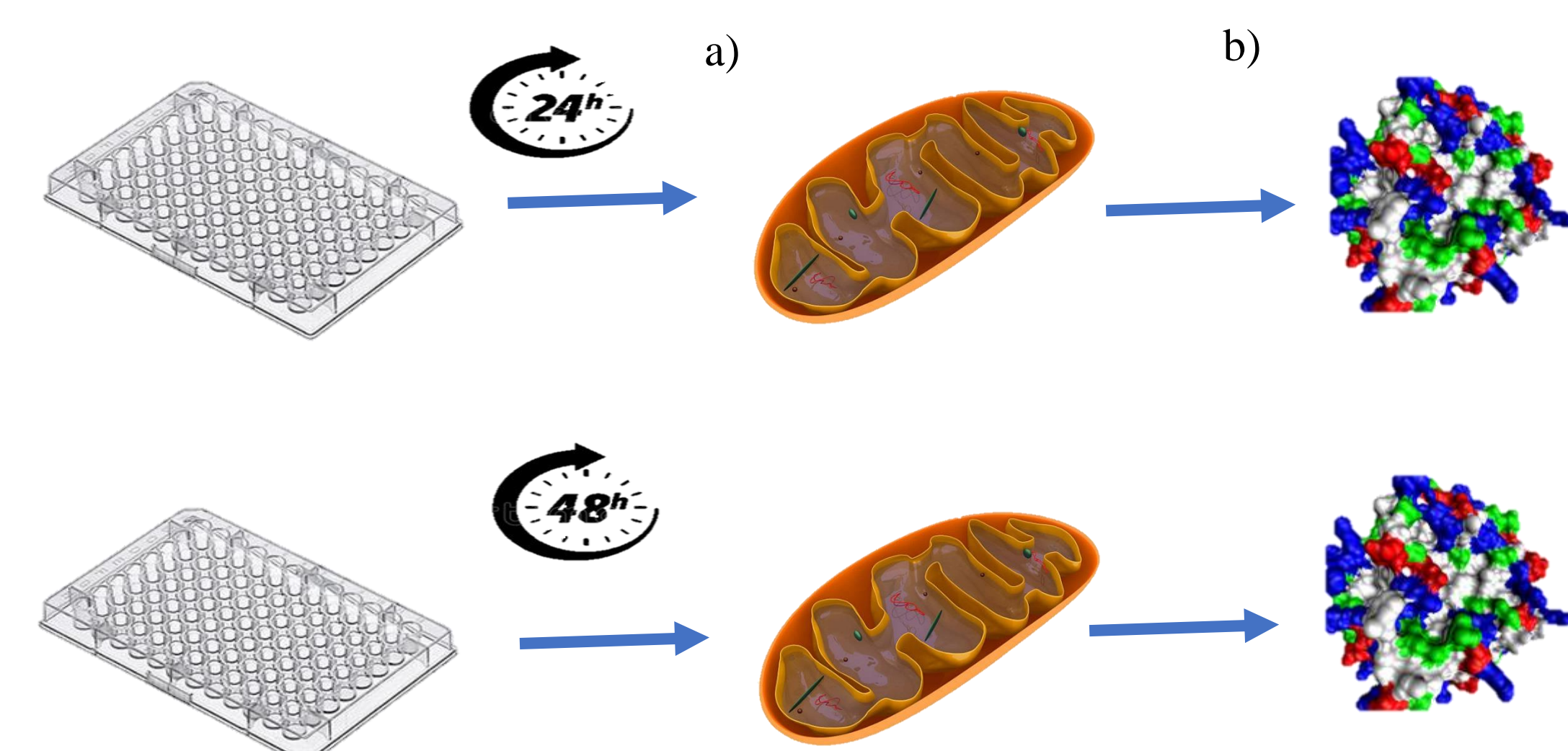


Fig.1: Scheme of MTS (a) and PC (b) assays on CaCo-2 cells

RESULTS

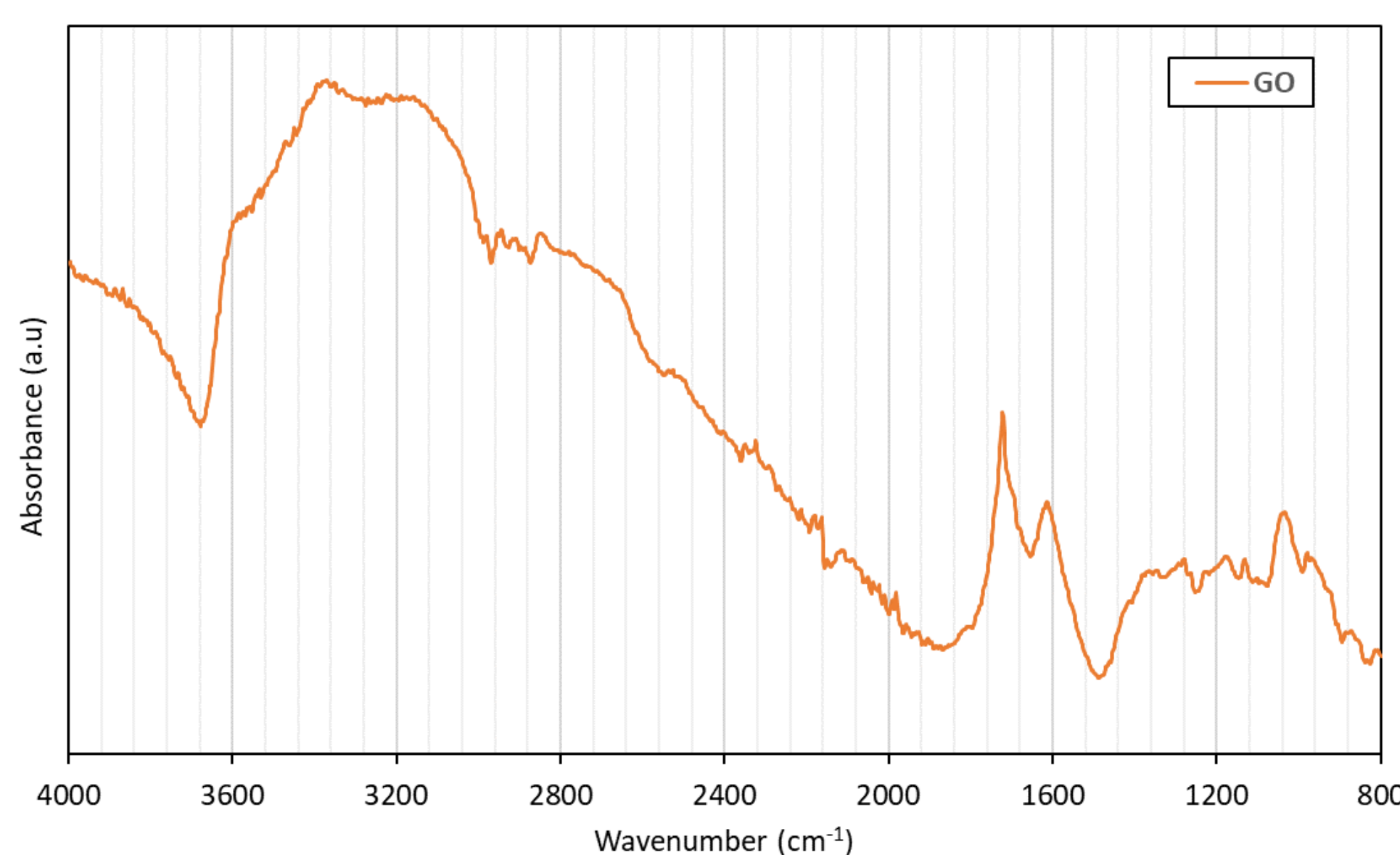


Fig.2: Image of FTIR spectrum of graphene oxide (GO)

- FTIR showed the characteristic bands of the GO, a strong broad-band at 3430 cm^{-1} due to hydroxyl groups, a band around 1730 cm^{-1} arising from the carboxylic acid groups, the band at 1620 cm^{-1} assigned to the aromatic C-C groups, and about 1044 cm^{-1} , a band corresponding to the C-O groups. (Fig.2)
- XPS revealed carbon content (66.29 At%), oxygen content (33.16 At%) and traces of nitrogen (0.55 At%).
- The ζ potential results demonstrated less aqueous dispersity of GO in cell culture medium (-10.9 ± 0.3) than in Milli-Q water (-30.3 ± 0.6).
- GO showed two diffraction peaks at $2\Theta = 12.6$ and $2\Theta = 42.5$.
- GO are visualized as agglomerated nanosheets in aqueous suspension (Fig.3)
- Caco-2 cells showed no significant changes in the endpoints considered after 24 and 48h of exposure at any concentration assayed (0-250 $\mu\text{g/mL}$). (Fig.4)

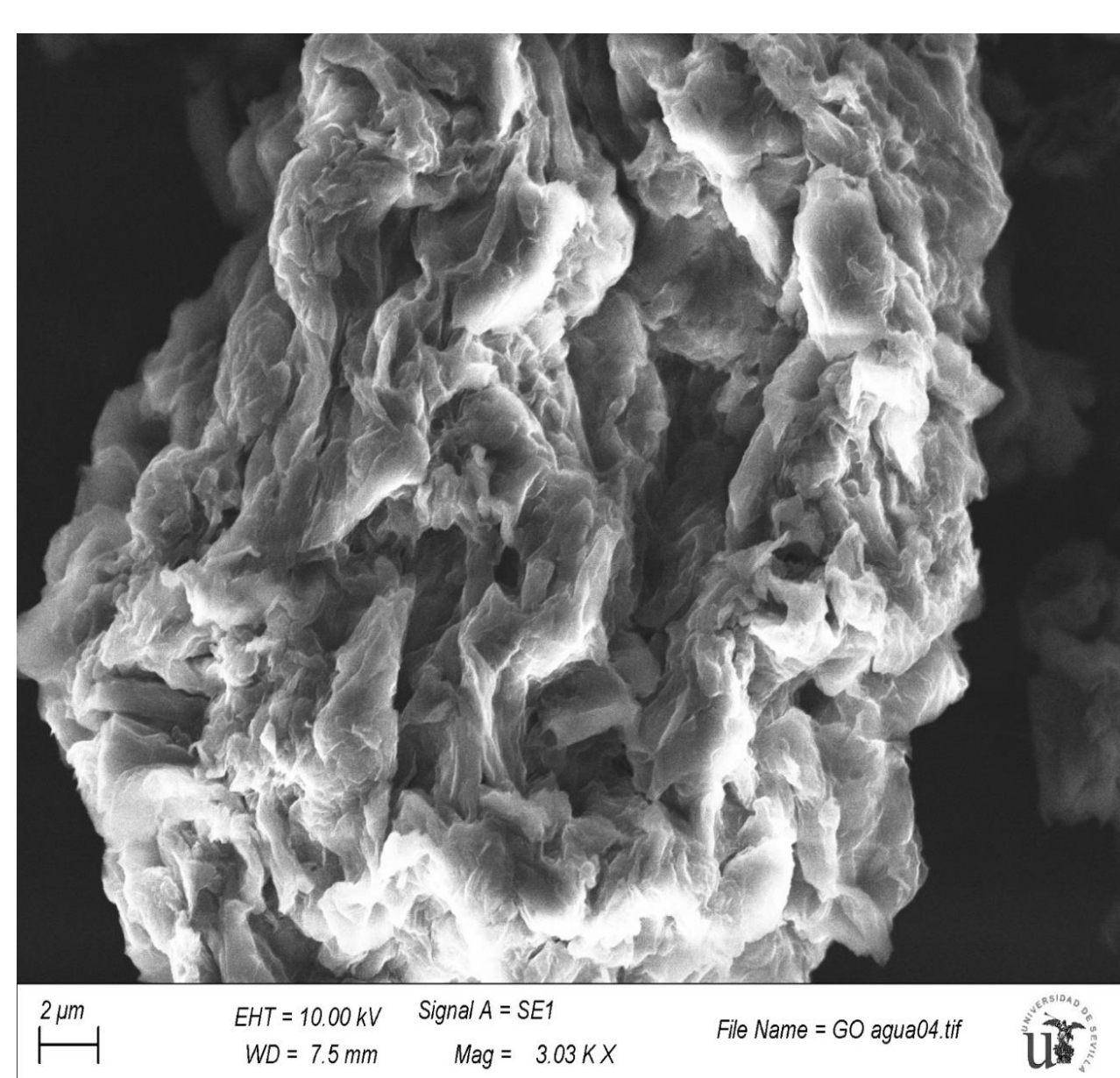
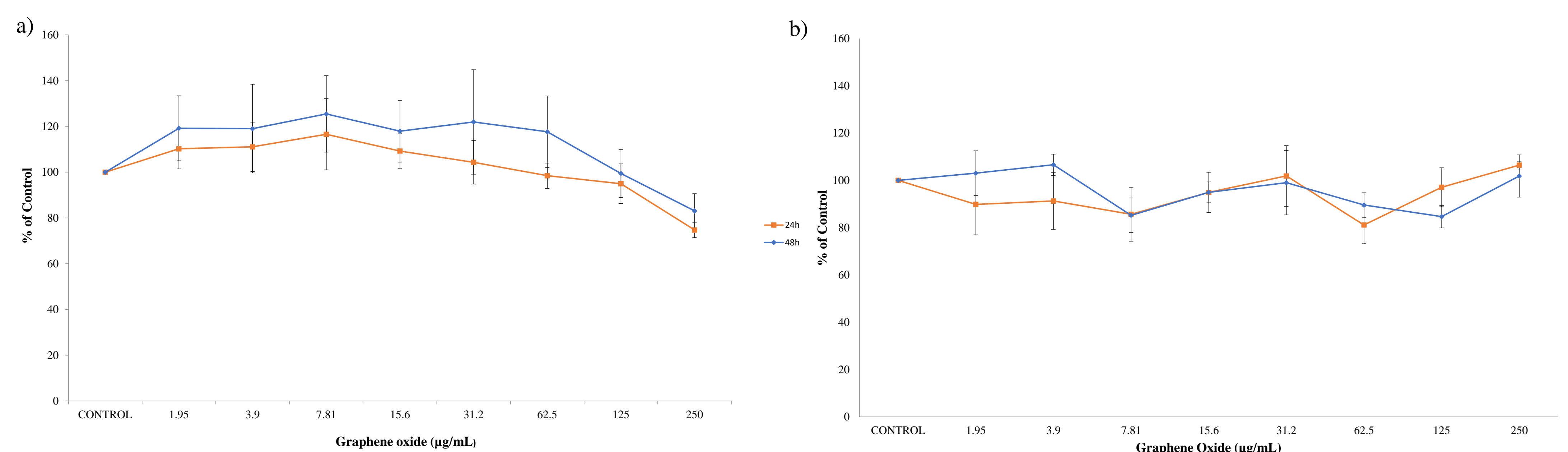


Fig.3: Image of graphene oxide obtained by scanning electron microscopy

Fig.4: Reduction of the tetrazolium salt (a) and protein content on Caco-2 cells after 24 h and 48 h of exposure to 0-250 $\mu\text{g mL}^{-1}$ Graphene oxide. All values are expressed as mean \pm SD.

CONCLUSION

GO was characterized and cytotoxicity studies showed not relevant changes. However, further toxicological tests are required before the potential application of GO as food contact material.

Acknowledgement: Project US-1259106 cofunded by Programa Operativo FEDER 2014-2020 and Consejería de Economía, Conocimiento, Empresas y Universidad de la Junta de Andalucía. And project P18-RT-1993 (PAIDI-2020, Junta de Andalucía). Functional characterization, microscopy, photoelectron spectroscopy, X-ray diffraction and Biology Services of CITIUS are acknowledged for technical assistance.

Junta de Andalucía
Consejería de Economía,
Conocimiento, Empresas y Universidad

CONTACT PERSON

• Óscar Cebadero
ocebadero@us.es



REFERENCES

1. Arfat, Y. A., Ahmed, J., Ejaz, M. & Mullah, M. Polylactide/graphene oxide nanosheets/clove essential oil composite films for potential food packaging applications. *Int. J. Biol. Macromol.* 107, 194–203 (2018).
2. EFSA Scientific Committee. Hardy et al. Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health. *EFSA Journal* 2018; 16(7):5327, 95 pp
3. Zaaba, N. I. et al. Synthesis of Graphene Oxide using Modified Hummers Method: Solvent Influence. *Procedia Eng.* 184, 469–477 (2017).

GRAPHENE FOR US
International Conference
★ Feb. 23-24, 2021 ★