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Characterization and cytotoxicity of Reduced Graphene oxide on CaCo-2 cells

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INTRODUCTION

Recently, reduced graphene oxide (rGO) has attracted attention for food packaging applications due to its ability to provide enhanced mechanical and barrier properties[1]. But before its use, the European Food Safety Authority[2] requires a thorough characterization and toxicological evaluation.

MATERIALS AND METHODS

The commercial rGO (Graphitene, Ltd.) was prepared by thermal reduction. The samples were sonicated for 1 hour and diluted at different concentrations for:

Characterization:

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

Cytotoxicity assays:

Toxicological effects were evaluated on CaCo-2 cells at 0-250 µg ml^{-1} after 24-48h of exposure by:

- Mitochondrial activity (MTS)
- Protein content (PC)

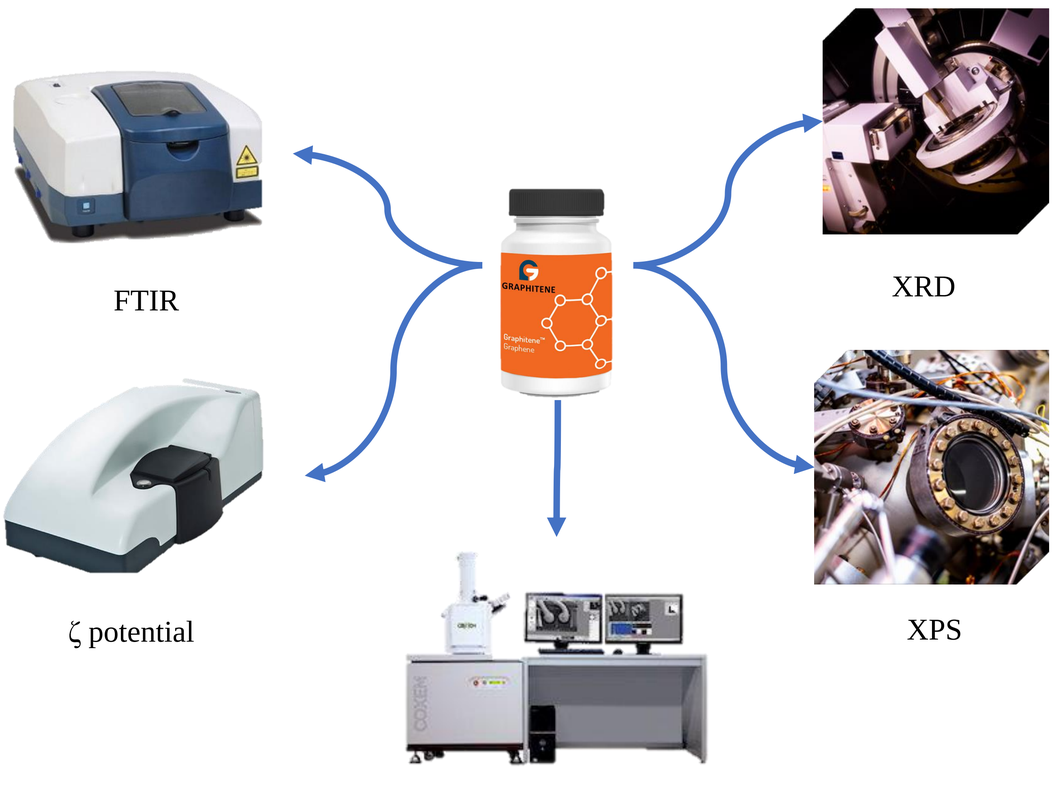

Fig.1: Scheme of characterization techniques applied to rGO.

RESULTS

- FTIR spectrum confirms the successful reduction of GO by disappearing the bands attributed to the oxygen functional groups of GO. (Fig.2)
- The rGO dispersity in cell culture medium (-15.8 ± 2.5) was like that Milli-Q water (-17.4 ± 0.4) according to ζ potential measurement.
- TEM and SEM images revealed wrinkled and scrolled structures in rGO samples (Fig.3)
- The atomic content showed oxygen content (13.6 At %), carbon content (86.3 At %) and traces of chlorine (0.1 At %). The C/O atomic ratio was 6.35.
- The diffraction peak was detected at 2Θ = 21.5º.
- MTS reduction shows a significant reduction in cell viability at the highest concentrations assayed at 24 and 48h of exposure. Nevertheless, Caco-2 cells exposed to rGO showed no significant changes in PC after both exposure times at any concentration assayed (Fig.4)

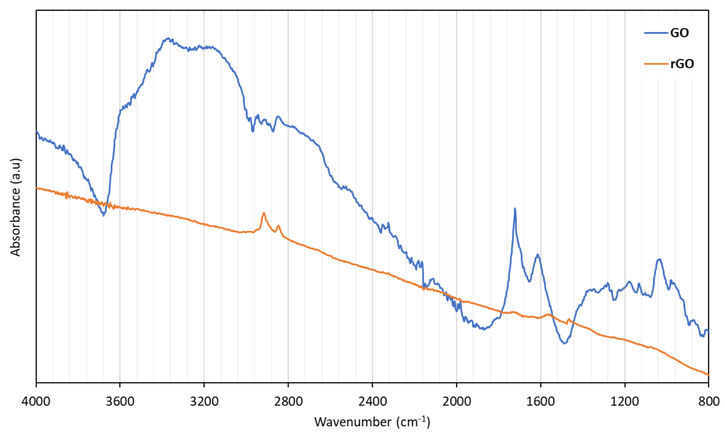

Fig.2 : FTIR spectra of GO and rGO samples.

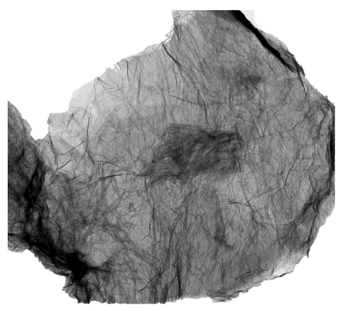

Fig.3 : Image of rGO by TEM.

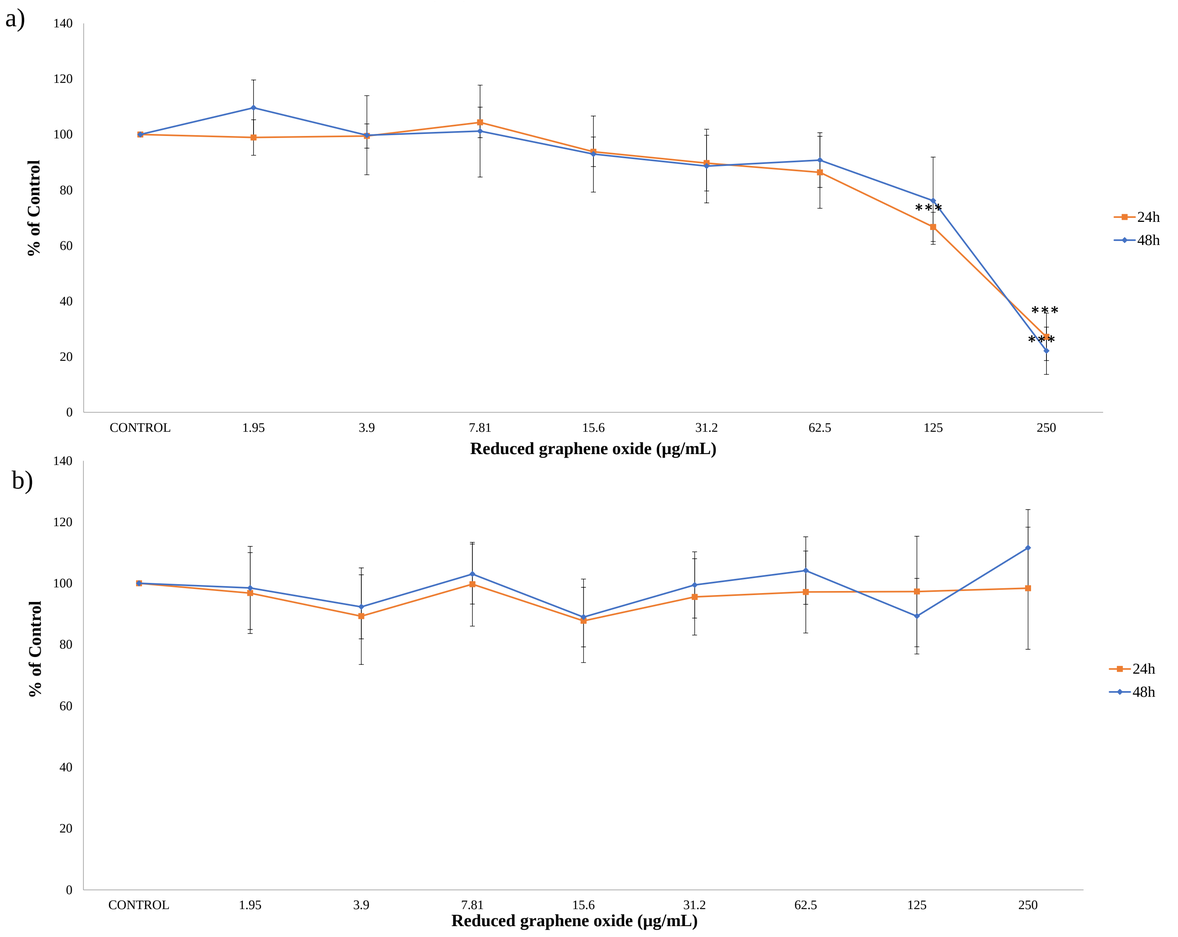

Fig.4 : Reduction of tetrazolium salt (a) and protein content (b) of Caco-2 cells after 24 h and 48 h of exposure to 0–250 µg ml^{-1} reduced graphene oxide. All values are expressed as mean ± SD. *** Significantly different from control ($p < 0.001$)

CONCLUSION

In summary, rGO was characterized and its toxicity evaluated on Caco-2 cells. Results of MTS assays showed that the cell viability was reduced in a concentration-dependent way. However, protein content showed not significant changes. Additional investigations will be required before the potential application of rGO as food contact material.

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