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

Recently, reduced graphene oxide (rGO) has attracted attention for food packaging applications owing to its ability to provide enhanced mechanical and barrier properties<sup>1</sup>. The commercial rGO (Graphitene, Ltd), prepared by thermal reduction procedure, was assessed by structural studies and its potential toxic effects on a human colorectal adenocarcinoma cell line (CaCo-2).

The commercial rGO was characterized by Fourier-Transform Infrared Spectroscopy (FTIR), Zeta Potential ( $\zeta$  potential), transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD). By FTIR the main functional groups still remaining onto the surface of GO after its thermal reduction process was determined. The  $\zeta$  potential shows negative values in cell culture medium ( $-15.8 \pm 2.5$ ) and Milli-Q water ( $-17.4 \pm 0.4$ ). TEM and SEM images revealed wrinkled and scrolled structures in rGO samples. The atomic content, measured by XPS, showed oxygen content (13.6 At %), carbon content (86.3 At %) and traces of chlorine (0.1 At %). The diffraction peak, analyzed by XRD, was detected at  $2\theta = 21.5^\circ$ .

The cytotoxicity of rGO was investigated by mitochondrial activity (MTS) and protein content (PC) on CaCo-2 cells at  $0-250 \mu\text{g ml}^{-1}$  after 24-48h of exposure. MTS reduction shows a significant reduction in cell viability from  $62.5 \mu\text{g ml}^{-1}$  for 24 h and at  $250 \mu\text{g ml}^{-1}$  after 48h of exposure. Nevertheless, CaCo-2 cells exposed to rGO showed no significant changes in PC after both exposure times at any concentration assayed.

In conclusion, rGO was characterized and the toxicity observed should be further evaluated before its potential application of rGO as food contact material.

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### References

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