ROS surface activity in three classes of engineered carbonaceous nanomaterials

Malmborg, V.; Elam, D.A.; Rissler, J.; Vogel, U.; Wohlleben, W.; Jacobsen, N.R.

2024

Document Version:
Publisher's PDF, also known as Version of record

ROS surface activity in three classes of engineered carbonaceous nanomaterials

V. Malmborg[1,3], D.A. Elam[2], J. Rissler[1], U. Vogel[3], W. Wohlleben[2] and N.R. Jacobsen[3]

[1]Ergonomics and Aerosol Technology, Lund University, Lund, SE-22100, Sweden
[2]BASF SE, Dept. Analytical and Materials Science, D-67056 Ludwigshafen, Germany
[3]The National Research Centre for the Working Environment (NFA), 2100 Copenhagen, Denmark
Keywords: ROS, Carbon Black, Graphene, Nanodiamond, EPR
Associated conference topics: 4.3, 5.6, S.S. Oxidative potential of aerosol particles and health risks
Presenting author email: Vilhelm.malmborg@design.lth.se

Carbon Black (CB), graphene, and nanodiamonds represent three classes of manufactured carbonaceous nanoparticles consisting almost exclusively of insoluble material. Particle-related Reactive Oxygen Species (ROS) have been linked to in vivo and in vitro toxicity and determining particle ROS activity is an efficient way of estimating nanomaterial toxicity and assist in the safe-by-design development of new nanomaterials. We used multiple techniques and assays to probe the ROS activity of carbonaceous nanomaterials: 13 CB, 6 graphene, and 3 nanodiamonds. In addition, surface-specific phys-chem properties were probed. The study aimed to cross-validate ROS assays and to explain the observed differences in ROS activity between the tested materials.

Electron Spin Resonance spectroscopy (EPR) at -196°C and 20°C was conducted to assess bulk free electrons. Nanoparticle ROS activity was determined using acellular and cellular $DCFH_2$-DA assays, and EPR with the CPH spin-probe (EPR-CPH). Nanomaterial BET specific surface area (SSA) was determined and surface composition was studied using X-ray photoelectron spectroscopy (XPS). The elemental composition (atomic %) at the particle surface was determined by an overview XPS spectra. To assess the chemical form of major elements, spectra with higher resolution were recorded for C 1s, O 1s, S 1s, and N 1s. In addition, the D-parameter, linearly correlated to the carbon sp^2/sp^3 hybridization, was determined from Auger electrons.

ROS activity was highly correlated between methods, EPR-CPH, acellular and cellular $DCFH_2$-DA [r>0.86]. SSA was found to be the primary predictor for overall ROS activity for all three methods [r = 0.86, 0.86, 0.92]. When assessed by unit mass, ROS activity for the CB and graphene materials increased 100-fold (low-to-high). We define the ROS *surface activity* (Figure 1) as the ROS activity normalized by SSA. For ROS surface activity, the low-to-high was reduced to a 4-fold increase. EPR bulk spin counts showed no apparent correlation to ROS activity or SSA. In fact, the highest EPR spin count was linked to the lowest SSA and weakest ROS activity, indicating that bulk free electrons do not (to any large extent) contribute to particle ROS activity.

The 4-fold range in ROS surface activity was hypothesized to depend on different surface properties. The XPS analysis showed carbonaceous surfaces with minor traces (<5%) of oxygen, sulphur, and nitrogen. Other elements, including metals, were found at levels below or close to the detection limit. Low oxygen did not allow determination of the carbon/oxygen bonding environments in C 1s spectra, however, the S 2p spectra revealed varying contributions from sulphur oxides ($SO_2/SO_3^{x-}/SO_4^{2-}$).

Multiple linear regression analysis of acellular $DCFH_2$-DA ROS activity and XPS surface composition was employed to test the hypothesis that surface composition modifies ROS surface activity. The resulting model (adj. R^2 = 0.95) included a constant term and significant first order terms of C, O, S [atomic %] and the D-parameter. The model was improved significantly after adjusting (removing) the contribution of sulphur oxides to sulphur and oxygen. We speculate that these sulphur oxides are soluble and do not perturb inherent nanomaterial ROS surface activity. Model interpretation: *inherent ROS surface activity increases with carbon sp^2-hybridization and is modified lower by surface oxygen and sulphur (not oxides) functional groups.*

Clarifying mechanisms behind ROS activity can contribute to the description of nanomaterial toxicity and the toxicity of carbonaceous air pollutants in general. Questions remain regarding the model interpretation as, for example, quinone-like moieties on soot can increase ROS activity (Antiñolo et al., 2015) and the presence of more reactive surface groups may enhance toxicity.

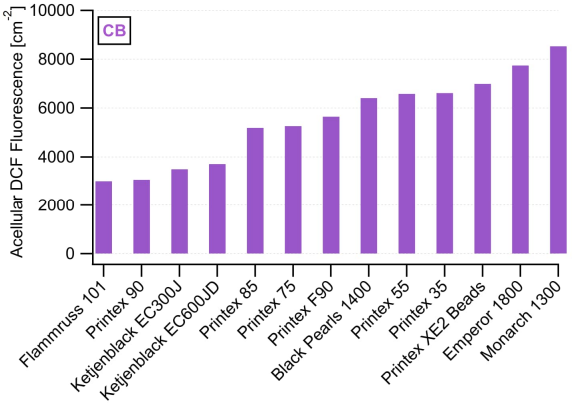

Figure 1. acellular $DCFH_2$-DA ROS surface activity of CBs.

This work was funded by Focused Research Effort on Chemicals in the Working Environment (FFIKA) from the Danish Government and Swedish Formas (2021-02010).

ANTIÑOLO, M., et al. 2015. Nature Communications, 6, 6812.