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REVIEW ARTICLE

The geochemistry and bioreactivity of fly-ash from coal-burning power stations

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Abstract
Fly-ash is a byproduct of the combustion of coal in power stations for the generation of electricity. The fly-ash forms from the melting of incombustible minerals found naturally in the coal. The very high coal combustion temperatures result in the formation of microscopic glass particles from which minerals such as quartz, haematite and mullite can later recrystallize. In addition to these minerals, the glassy fly-ash contains a number of leachable metals. Mullite is a well-known material in the ceramics industry and a known respiratory hazard. Macroscopically mullite can be found in a large range of morphologies; however microscopic crystals appear to favour a fibrous habit. Fly-ash is a recognized bioreactive material in rat lung, generating hydroxyl radicals, releasing iron, and causing DNA damage. However, the mechanisms of the bioreactivity are still unclear and the relative contributions of the minerals and leachable metals to that toxicity are not well known.

Keywords: *Fly-ash; coal-burning; mullite; quartz; bioreactivity; fibrous minerals; lungs*

Introduction

There is compelling evidence that ambient respirable particulates in urban air present a significant cardiorespiratory risk to the Public Health (COMEAP 2006). The composition of this pollution is highly variable and heterogeneous, however usually contains a significant component of anthropogenic 'technogenic' particles (BéruBé et al. 2006, Moreno et al. 2004); typically as a result of some type of combustion process. Combustion-generated particles can be broadly separated into two groups: carbon based and non-carbon based. Non-carbon based particles are commonly composed of materials from a geologically related source, where the original minerals have been thermally altered or melted as a result of manufacturing or power-generation combustion (Jones & BéruBé 2006, Marrero et al. 2007). For example, in the UK a common type of these particles is fly-ash from coal combustion (Vassilev & Vassileva 2007). Geologically sourced fuels such as oil and gas can also generate carbon-based particles (soot). A number of industries, for example the ceramics industry, already recognize that particles containing the same minerals as coal-burning fly-ash can present an occupational health hazard (Rogers et al. 1997, Walker et al. 2002). It is therefore self-evident that if fly-ash is present in ambient air then it could also present an environmental respiratory health hazard (van Maanen et al. 1999).

Geochemistry

When heated in an incinerator or furnace at sufficiently high temperatures, many minerals will melt and when removed in a liquid form from the heat, then rapidly solidify to form glass, with the composition of that glass determined by the original mineral mixture. In the case of coal combustion the melting of the small fraction of associated minerals, such as clays in the coal, results in

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the formation of glassy fly-ash. Fly-ash tends to form an aluminosilicate glass, with Si (46%), Al (22%) and Fe (5%) the dominant elements; the values in brackets are the percentage of these elements in our UK fly-ash samples. The glass has approximately the same density as quartz (specific gravity ~2.6–2.7), and rather than a melting point has a 'softening' range between 1740 and 1800°C. However, if the 'glass' particles are subject to continued significant submelting temperature heating after formation (*de novo* heating) then the glass will start to recrystallize back to different minerals. This recrystallization occurs within the glass particles as a solid-state reaction. Those recrystallized minerals are predominantly quartz (SiO_2), mullite (Schneider 2004) ($Al_8[(O.OH.F)(Si.Al)O_4]_4$) and haematite ($Fe_2O_3$), as confirmed by X-Ray diffraction (XRD) of fly-ash (Figure 1). The recrystallization can also be seen under the electron microscope in the transformation of glass spheres to spherical networks of fibrous mullite crystals (Figure 2A–D). It is assumed that the degree of recrystallization of the glassy particles is related to the time and temperature of the *de novo* heating.

Mullite is an *ortho*-silicate mineral found naturally where clay minerals have been subjected to high temperatures, typically as a result of volcanic activity. Formally named in 1924 (Bowen et al. 1924) the type location for the mineral is in Port Na Cloidheig on the Isle of Mull, Scotland, where in the geological past sedimentary layers of clay minerals have been heated under flows of tertiary basaltic lava. Macroscopically, as a result of chemical substitutions within the basic formula, mullite can appear in a number of different habits from prismatic, fibrous to more massive. Some investigations (Dyson et al. 1997, Chawla et al. 1995, Kim et al. 2008) suggest that mullite microscopically favours a fibrous morphology.

Fly-ash from UK and Chinese coal-burning power stations has been investigated. PM_{10}-sized ash was separated from the bulk ash by a dry airborne resuspension method. Analyses showed that the relative proportions of quartz, mullite and haematite found in these fly-ashes were different, with the Chinese fly-ash having a greater crystalline mineral component. Numerous worldwide studies have shown fly-ash to be a common component of urban air pollution, as demonstrated by a typical collection taken in central Cardiff in 2006 (Figure 3).

Bioreactivity

Method 1: haemolysis

The haemolysis assay is an *in vitro* technique that measures the oxidative capacity of samples on their ability to cause erythrocyte cell membrane lysis. Haemolysis can occur as a result of oxidative stress exerted by reactive oxygen species (ROS); therefore the observation of haemolysis in the presence of a particular suspension or leachate sample indicates its capability to generate ROS (Duffin et al. 2001).

Method 2: 2',7'-dichlorodihydrofluorescin assay

The 2',7'-dichlorodihydrofluorescin (DCFH) assay is an *in vitro* assay used to determine the oxidative capacity of samples relative to H_2O_2. The assay is based on the knowledge that any ROS present are capable of performing the oxidation reaction of DCFH to 2',7'-dichlorofluorescin (DCF), the strongly fluorescent moiety (Bonini et al. 2006). The fluorescence is then detected at the correct wavelength range, giving an equivalent H_2O_2 (ROS) concentration in μM, when compared with the H_2O_2 standards calibration curve (Hung & Wang 2001).

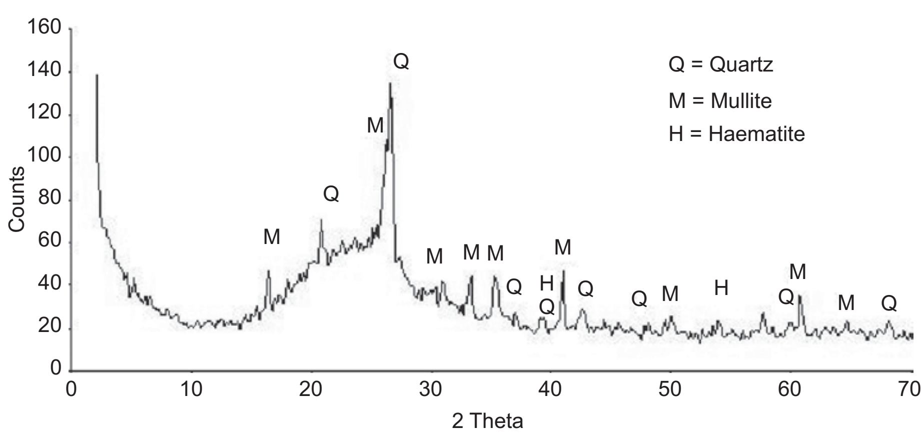

Figure 1. X-ray diffraction pattern for UK coal-burning fly-ash, with mineral peaks for quartz, mullite and haematite. The distorted baseline is caused by the high non-crystalline glass content.

Method 3: human-based lung model

Tissue engineering of healthy human lung cells, from human heart/lung transplant donors, was employed to develop three-dimensional (3D) cell cultures (BéruBé et al. 2006). The cultured cells form 3D tissue closely resembling the epithelia of the human respiratory tract, with distinct cell types functioning as they would in the lung. Conventional toxicological analysis was used, in the first instance, to establish the dose of fly-ash needed to achieve the key epithelial response(s) denoted by changes in permeability (i.e. epithelial resistance), cellular damage (i.e. secreted surface protein) and release of inflammatory markers (e.g. cytokines) secreted into the culture medium. In the future, the lung tissue will be processed through genomic experiments designed to identify early molecular biomarkers for events in pulmonary injury.

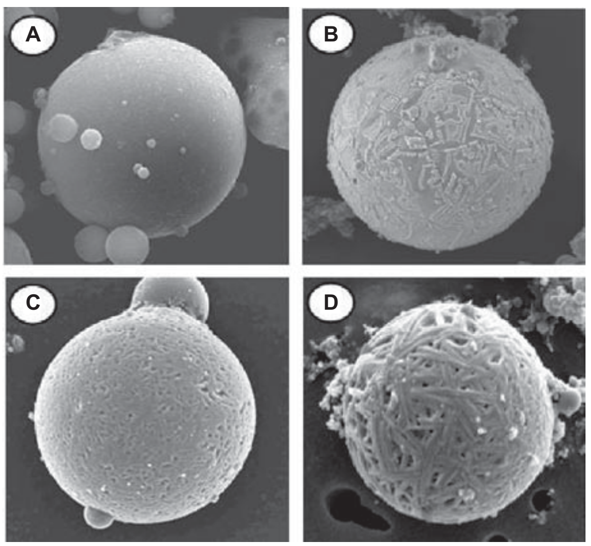

Figure 2. Scanning electron micrographs of coal-burning fly-ash particles all approximately 2–3 μm in diameter. (A) Smooth-surfaced round sphere of glass; (B) a surface pattern can be seen on the sphere; (C) the surface now has a texture of small evenly distributed holes; (D) the sphere is now a 'pseudomorph' of the original glass sphere and composed of fibrous mullite crystals.

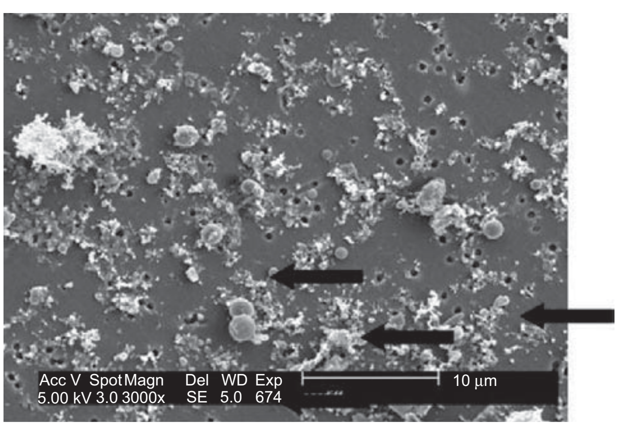

Figure 3. Scanning electron micrograph of Cardiff urban air PM_{10}, with a number of small spherical fly-ash particles (arrows).

Results

Haemolysis (method 1)

Haemolysis showed a toxic dose response with the Chinese material approximately four times as toxic when compared with the UK material. Although the values were different for the different power station Chinese fly-ashes, there did not appear to be any damage at doses of 4 mg ml^{-1} or less, and at doses of 15– 20 mg ml^{-1} or above, the damage was 100% (Figure 4).

2',7'-dichlorodihydrofluorescin assay (method 2)

The results for the DCFH assay were problematic in that the UK material showed a reverse dose–response, whereas the Chinese material showed a positive dose–response. The Chinese PM_{10} fly-ash was approximately three times more bioreactive than the Chinese bulk fly-ash samples. The reverse dose–response of the UK material was interpreted as an artifact where the increased concentrations of fly-ash in suspension clouded the solution and masked the fluorescent signal. This technique therefore has some

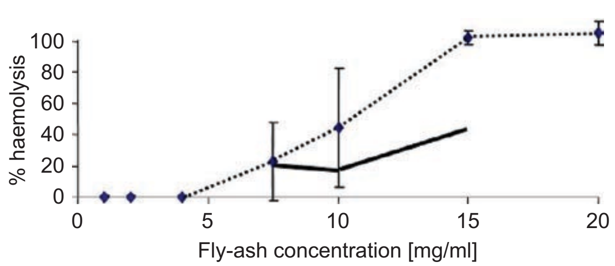

Figure 4. Haemolysis assays for fly-ash from two Chinese coal-burning power stations. Dotted line, power station 1; solid line, power station 2.

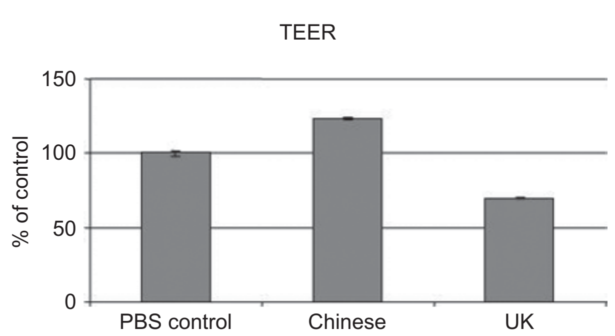

Figure 5. Transepithelial electrical resistance (TEER) analysis for phosphate-buffered saline (PBS) control, Chinese fly-ash and UK fly-ash PM_{10}. The UK fly-ash shows a decrease in electrical resistance; however, the Chinese fly-ash shows an increased resistance that is interpreted as a hormetic response due to hyperplasia.

application with more bioreactive fly-ashes, but not for the less bioreactive material.

Human-based lung model (method 3)

The results for the human-based model conventional assays supported the findings of the two screening assays described above. For example the transepithelial electrical resistance (TEER) (Figure 5) showed increased resistance for the Chinese material and decreased resistance for the UK material. This was interpreted as mild bioreactivity in the UK material resulting in open tight junctions, whereas the greater bioreactivity of the Chinese fly-ashes resulted in hyperplasia and a hormetic response. This interpretation was supported by the ATP assay, which shows the Chinese material being more metabolically active and undergoing hyperplasia (cell proliferation in response to a toxic challenge).

Biomarkers

The biomarkers of exposure and harm following exposure to fly-ash particles include: the generation of ROS; the opening of previously tight junctions in 3D human-based lung models; and increase of ATP and hyperplasic cell proliferation.

Final remarks and conclusions

As fly-ash is ubiquitous in the environment, the effect of this class of PM could have upon the human population, was modelled using three *in vitro* bioassays. Chinese and UK sources of fly-ash were assessed in their bulk and PM_{10} forms. The Chinese PM_{10} was found to be the most reactive in all three ROS-sensitive assays, indicating source-variation within fly-ash toxicity.

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