Dosimetric assessment of whole smoke particulate deposition *in vitro*: A proposed common approach using quartz crystal microbalance technology

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Abstract

There are a number of different smoking machines and exposure chamber combinations used by our industry to assess the toxicological impact of cigarette smoke *in vitro*. The amount of smoke delivered to cells within an *in vitro* exposure system can be presented in many ways: ratios of smoke to air, mixing airflow rate, vacuum rate, percentage or fraction. However, dosimetry (the quantifiable amount of smoke cells are directly exposed to) is more relevant and is becoming increasingly important in the field of cigarette smoke *in vitro* assessment. Dosimetry techniques will hopefully bridge the gap between different technologies and allow cross-platform comparisons.

Installed into various exposure chambers, quartz crystal microbalance (QCM) technology has allowed us to quantify cigarette smoke particulate dose *in vitro*. For example, 4 QCMs installed into the Vitrocell 6PT-CF exposure module enabled quantification of whole smoke deposited mass at a range of diluting airflows (0.25 - 4.0 L/min) resulting in 16.66 ± 2.67 µg/cm^2 – 0.72 ± 0.13 µg/cm^2 deposited particulate mass (3R4F cigarettes).

Moreover, QCM tools have enabled the quality control of smoke runs and highlighted limitations/improvements to established whole smoke methodologies. Furthermore, for the first time we are able to perform direct comparisons of whole smoke particulate dose delivered from different *in vitro* whole smoke exposure systems: the Borgwaldt RM20S and Vitrocell VC 10.

We can demonstrate that QCM technology is a reliable, effective and simple tool that can accurately quantify smoke particulate deposition in real-time, *in vitro*. Additionally, QCM data can be used to unify *in vitro* toxicological data irrespective of exposure system.

Introduction

Exposure systems

Exposure systems comprise a smoking machine coupled with an exposure chamber that houses cell cultures. These machines dilute smoke to obtain a cellular dose-response, but the 'dose' can be presented in many different ways. Dosimetry tools that measure dose are key to link biological effects of whole smoke.

BAT employ two set-ups: the RM20S with the BAT exposure chamber and the VC 10 and 6PT-CF module (Figure 1). The RM20S uses a syringe serial dilution technique to achieve the desired dose. The VC 10 dilution principle is based on continuous flow, where diluting air and turbulent flow are used to create and achieve the desired dose.

Fig 1 Exposure systems. [**A**] Borgwaldt RM20S Smoking Machine; [**B**] BAT's exposure chamber [1]; [**C**] Vitrocell VC 10 Smoking Robot; [**D**] Vitrocell 6PT-CF module

QCM

A small, sensitive weighing scale able to measure changes in mass in the nanogram range, the QCM can measure smoke dose in real-time. Historically, it has been used to quantify environmental smoke, pollution and dust, as well as inhalation toxicity assessment of aerosols and engineered nanoparticles *in vitro* [2].

Methods

- Identical QCMs [3] were installed into two different exposure chambers (Figure 2, 3A & 3B) (Vitrocell® Systems GmbH, Germany).
- The QCM read at a resolution of 10 nanogram/cm^2/second and during exposure recorded mass every 2 seconds (Figure 4) [2].
- The BAT QCM chamber (Figure 3A) was connected to the RM20S (Borgwaldt-kc, Germany) and the Vitrocell QCM module (Figure 3B) was connected to the VC 10 (Vitrocell®).
- Before smoke exposure, the QCM devices were sealed and acclimatised to ensure quartz crystal stability (zero point stability of less than 20 ng/cm^2).
- 3R4F cigarettes (University of Kentucky) were smoked according to ISO 4387:2000.
- The RM20S smoked 6 puffs on 5 cigarettes (30 mins) at 5 dilutions programmed as a ratio of smoke to air 1:5 - 1:400 (smoke:air, v/v).
- The VC 10 smoked 8 puffs on 3 cigarettes (24 minutes) at 5 diluting airflows 0.25 - 4.0 L/min (vacuum 5 ml/min).
- Post exposure, QCMs were left to record real-time deposition until mass reached plateau (Figure 4). All experiments were n=5/dose.

Fig 2 Schematic cross-section of the QCM unit installed into the BAT exposure chamber [2]

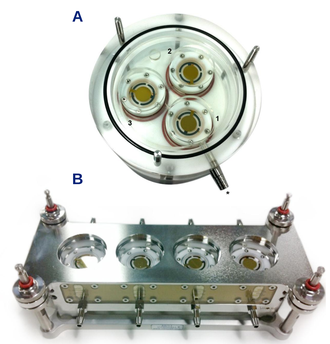

Fig 3 Whole smoke exposure chambers installed with QCMs. [**A**] BAT's *3-in-1* QCM exposure chamber; [**B**] Vitrocell's 6PT-CF module, termed the *4-in-1* QCM

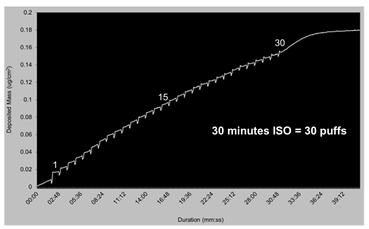

Fig 4 Real-time QCM trace from the *3-in-1* device. 3R4F cigarettes were smoked at a dilution of 1:400 for 30 minutes on the RM20S. Note the distinct and precise puff-by-puff profile recorded by the QCM [2]

Results

Using identical QCM units in different exposure chambers (Figure 3) we were able for the first time to record real-time deposition data (Figure 4). The QCM also enabled a comparison of particulate dose delivered from two different exposure systems.

The QCM was able to quantify a dose range on the RM20S between 1:5 – 1:400 (smoke:air, v/v), with deposition of 34.55 – 0.21 µg/cm^2 (Figure 5).

For the VC 10 0.25 – 4.0 L/min a range of 16.66 – 0.72µg/cm^2 deposition was detected (Figure 5).

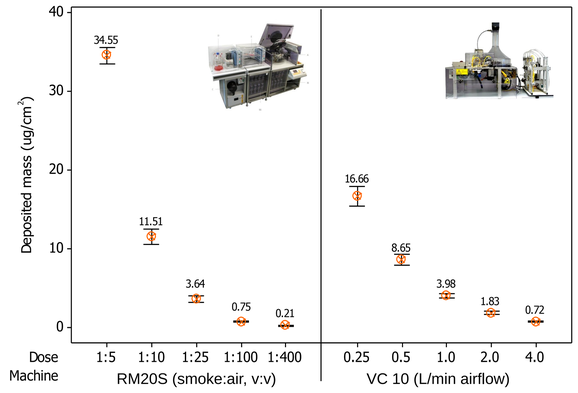

Fig 5 Interval plot for the comparison of dose ranges on the RM20S and VC 10, 95% confidence interval for the mean (15 replicates per dose for RM20S, 20 per dose for VC 10)

These data have allowed us to finally align doses from different machines, such that:

RM20S **1:25** = VC 10 **2.0 L/min** = **~3µg/cm^2**

RM20S **1:100** = VC 10 **4.0 L/min** = **~0.7µg/cm^2**

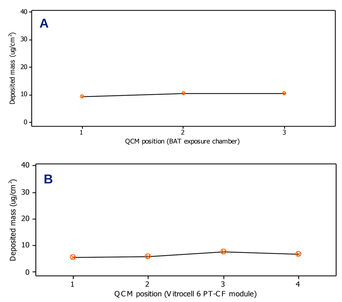

Fig 6 Multi-Vari charts showing mean regional deposition of particulate across the chambers. [**A**] BAT's *3-in-1* QCM exposure chamber; [**B**] Vitrocell's *4-in-1* QCM module; n=25

To assess regional deposition in both exposure devices, Multi-Vari charts were produced. The BAT exposure chamber showed no statistically significant difference between positions at all dilutions tested (Figure 6A).
The Vitrocell module showed a slight ascending pattern of deposition (Figure 6B), reflective of delivery through the dilution bar at all doses tested.

Conclusions

- Real-time
- Sensitive (nanogram range)
- Dose-response
  - Different machines & products
  - Repeatable
- Single person operator
- Requires no analytical resource
- Quick set-up & fast analysis of data
- QC tool
- Easy to use, train & transfer
  - Robust kit withstands transit

References

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Adamson J et al. (2012) Chem. Cent. J. in press 10 Sept 2012 [**2**]
Mülhopt S et al.(2009) J. Phys.: Conf. Ser. 170:-012008 [**3**]