Characterisation of the Borgwaldt RM20S whole smoke exposure system using an electrical mobility spectrometer

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Summary

British American Tobacco has developed a whole smoke exposure system to investigate and assess toxicity of cigarette smoke to human lung epithelial cells exposed at the air-liquid interface. Cigarette smoke is generated and diluted using a Borgwaldt RM20S smoking machine and delivered to a small (188 cm³), clear Perspex™ exposure chamber, which houses cells supported on commercially available inserts. In this study we have assessed smoke loss/deposition within the tubing of our whole smoke exposure system. An electrical mobility spectrometer was used to measure cigarette smoke particulates at three points in the system: after smoke generation, at delivery to the chamber and at the exhaust from the chamber. We conducted a three-way comparison of different tubing types in the RM20S including polyurethane (conditioned and new) and Viton®. Results indicated that 76-90% of particulates reached the chamber with 18-39% being deposited in the chamber. Minimal losses were observed using conditioned polyurethane tubing.

Introduction

Whole smoke exposure systems introduce in vitro cellular culture systems to both the particulate and gas phases of cigarette smoke for toxicological assessment. There are many systems available, each incorporating a means of generating and diluting freshly generated mainstream cigarette smoke to be delivered to a cellular exposure chamber or well plate. Such smoke generators include the Borgwaldt RM20S (1,2), the Burghart Mimic Smoker-01 (3) and the Vitrocell Smoking Machine VC10 (4). There are also several aerosol cell exposure systems described in the literature including the CULTEX system (5), the rocking platform system (6) and an electrostatic system (7). British American Tobacco have designed and developed a whole smoke exposure chamber (patent publication number: WO 03/100417 A1) (1) to use in combination with a Borgwaldt RM20S smoke engine (Figure 1).

The Borgwaldt RM20S smoking engine comprises 8 syringes which are used to generate and dilute whole smoke at the ISO smoking regime (35ml, 2 second puff, once a minute) and are capable of performing up to 8 different smoke dilutions from 1:1 to 1:685 000 (smoke:air, v:v) which are subsequently delivered to 8 independent exposure chambers connected to the smoke engine. The exposure chamber houses cells cultured on inserts. These provide a porous support, and allow cellular cultures to be exposed at the air-liquid interface with cigarette smoke apically and warmed cell culture media basally. This whole smoke exposure system may also be used to deliver only the gas phase of whole smoke to the cells by placing a Cambridge filter pad in-line pre-chamber.

Deposition studies traditionally use chemical methods to extract particulates followed by absorbance measurements which can be converted into concentrations using a standard curve. Scian et al., 2009 (3)
described the characterisation of the Burghart Mimic Smoker-01, using particulate deposition studies which quantified smoke losses by inserting Cambridge filter pads to trap particulates at several points along the system. The group reported mainstream cigarette smoke losses of up to 50% prior to cell delivery. Preceding this study, there was no information as to the smoke losses experienced in our exposure system and the relative performance of the current tubing type. Therefore, the aim of this study was to determine the smoke loss/deposition through our system, testing three different types of tubing using an electrical mobility spectrometer (DMS-500, Cambustion, UK) to quantify cigarette smoke particle concentration in-line.

Materials and Methods

A comparison of different tubing types was conducted between conditioned polyurethane, unused polyurethane (Farnell, Leeds, UK) and Viton® (Altec Products Ltd, Cornwall, UK), a PTFE-like fluoroelastomer. Conditioned polyurethane was the default tubing installed in the RM20S prior to this study having a visible build-up of tar (used over 20+ smoke runs). The experiment was designed to assess the performance of each tubing type and see if there was a significant reduction in smoke loss. Comparing new, unused polyurethane tubing with used, conditioned polyurethane will give an indication of how deposition is affected as tar builds up over time, if at all, and potential ‘hot spots’ of deposition. A 3.4m length of tubing (4mm internal diameter) was used for each run irrespective of tubing type.

The same single syringe (150mL volume) of the RM20S and the same exposure chamber were used in combination for all readings of all tubing types to reduce inter-syringe variability and syringe-chamber pairing bias. Particle size and concentration were measured by the DMS at three points along its length: (α) at the syringe exhaust line, to assess smoke at the point of generation, (β) before the exposure chamber to assess the amount of smoke from generation of exposure chamber and (γ) just after the exposure chamber to indicate the amount of smoke deposition within the chamber (Figure 2). Readings at these three key points were used to calculate smoke losses within the system. The chamber was filled with 30mL Dulbecco’s Modified Eagles Medium (DMEM) (Fisher Scientific UK Ltd, Loughborough, UK) for γ readings to represent usual exposure conditions and provide the correct internal volume (139cm³); media-in and media-out ports were also blocked.

Conditioned 3R4F-reference cigarettes (University of Kentucky, Kentucky, USA) were smoked on a single syringe for 8 minutes per experiment at a programmed dilution of 1:60 (smoke:air, v:v) to ISO standards (35ml puff over 2 seconds, once a minute). Two cigarettes were smoked in triplicate for each tubing type and region, with a total of four puffs taken from each cigarette.

Results

The 100% (α) valve is the Total Number (concentration) of smoke particles calculated using the following formula: Average N/cc x Flow (cc/s) x Time period (s). The total number (concentration) of smoke particles at (α) differed depending on tubing type: 9.01x10¹⁰ (old PU), 1.06x10¹¹ (new PU) and 9.24x10¹⁰ (Viton®). Pre-chamber losses were calculated by the difference in smoke concentration between the point of smoke generation (α) and the chamber (β), and chamber deposition calculated by the difference in smoke concentration before (β) and after (γ) the chamber. All tubing types show a similar trend with average smoke losses between α and β of 10-24% (total smoke penetration of 76-90%) and chamber deposition between β and γ of 18-39% (Figure 3 and Table 1). Although results for the tubing types are similar, conditioned polyurethane gives the best ratio of minimum smoke loss to maximum chamber deposition. Smoke losses within conditioned polyurethane are lowest of the three tubing types at 10%, and chamber deposition is highest of the three at 39%.

Table 1. Summary of average smoke losses in the different tubing types.

<table>
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<th>Old PU</th>
<th>New PU</th>
<th>Viton</th>
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<tbody>
<tr>
<td>Loss between source and chamber (α-β) (%)</td>
<td>10 %</td>
<td>24 %</td>
<td>12 %</td>
</tr>
<tr>
<td>Smoke penetrating from source to chamber (%)</td>
<td>90 %</td>
<td>76 %</td>
<td>88 %</td>
</tr>
<tr>
<td>Deposition in chamber (β-γ) (%)</td>
<td>39 %</td>
<td>18 %</td>
<td>27 %</td>
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The aim of this study was to determine cigarette smoke losses/deposition through the system with different types of tubing installed using a DMS to quantify smoke particle concentration at different regions along the system. The study was used to give information about the current tubing used within the RM20S to potentially understand the affect of visible smoke build up over time by comparing it with new/unconditioned tubing of the same type. The information from this study was also to be used to investigate a novel tubing type, Viton®, as a potential upgrade to the system.

For whole smoke exposure systems it is important to minimise smoke loss between the point of generation and the exposure chamber and to maximise smoke deposition within the chamber itself. Our results show that conditioned polyurethane (PU) (visibly brown from smoke build up/deposition) gives the best ratio of loss to deposition of the three tubing types: 10% smoke loss between point of generation and chamber, compared to up to 50% smoke losses in the Burghart system (3), and 39% smoke deposition in the chamber (Table 1).

Using this study, it has been decided that conditioned polyurethane tubing will continue to be used instead of converting to an alternative, Viton®. Not only does it give the best ratio of loss to deposition and smoke deposition in the chamber of the three tubing types; it is less flexible and therefore less likely to kink and potentially get blocked but also the relative cost is considerably less. Prior to this study, the build up of tar, creating a visible browning of the tubing was thought to be disadvantageous as it indicated a significant amount of smoke loss through the system. However, the results from this study suggest this build up actually improves particulate smoke transit through the system and is a characteristic that should be favoured over new polyurethane tubing. It has therefore been decided that any newly installed polyurethane tubing should undergo conditioning, smoking 25 cigarettes per line before routine analysis. However, this will require further verification for vapour phase species.

References


Figure 3. Representative data showing the total average number of cigarette smoke particles in different tubing types (Conditioned Polyurethane (PU) (orange), New PU (blue), Viton® (green)) at different regions based on a Borgwaldt standard (n=3). Results expressed as percentage of mainstream smoke (α value).