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Headspace stir bar sorptive extraction–gas chromatography/mass spectrometry characterization of the diluted vapor phase of cigarette smoke delivered to an *in vitro* cell exposure chamber

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ABSTRACT

Advanced smoke generation systems, such as the Borgwaldt RM20S® smoking machine used in combination with the BAT exposure chamber, allow for the generation, dilution and delivery of fresh cigarette smoke to cell or tissue cultures for *in vitro* cell culture analyses. Recently, our group confirmed that the Borgwaldt RM20S® is a reliable tool to generate and deliver repeatable and reproducible exposure concentrations of whole smoke to *in vitro* cultures [1]. However, the relationship between dose and diluted smoke components found within the exposure chamber has not been characterized. The current study focused on the development of a headspace stir bar sorptive extraction (HSSE) method to chemically characterize some of the vapor phase components of cigarette smoke generated by the Borgwaldt RM20S® and collected within a cell culture exposure chamber. The method was based on passive sampling within the chamber by HSSE using a Twister™ stir bar. Following exposure, sorbed analytes were recovered using a thermal desorption unit and a cooled injection system coupled to gas chromatograph/mass spectrometry for identification and quantification. Using the HSSE method, sixteen compounds were identified. The desorption parameters were assessed using ten reference compounds and the following conditions led to the maximal response: desorption temperature of 200 °C for 2 min with cryofocussing temperature of −75 °C. During transfer of the stir bars to the thermal desorption system, significant losses of analytes were observed as a function of time; therefore, the exposure-to-desorption time interval was kept at the minimum of 10 ± 0.5 min. Repeatability of the HSSE method was assessed by monitoring five reference compounds present in the vapor phase (10.1–12.9% RSD) and n-butyl acetate, the internal standard (18.5% RSD). The smoke dilution precision was found to be 17.2, 6.2 and 11.7% RSD for exposure concentrations of 1, 2 and 5% (v/v) cigarette vapor phase in air, respectively. A linear response of analyte abundance was observed as a function of dilution. Extrapolation to 100% (v/v) cigarette vapor phase, *i.e.*, undiluted smoke, gave yields for the five compounds ranging from 6 to 450 ng for 10 min exposure.

1. Introduction

Recent advancements have led to the development of several smoke generation systems such as the Borgwaldt RM20S® [2], the Burghart Mimic Smoker-01® [3,4] and the Vitrocell Smoking Robot VC 10® [5]. Also, this has led to the development of novel *in vitro* exposure systems such as British American Tobacco's (BAT) exposure chamber [2] and the CULTEX system [5]. These systems generate fresh cigarette smoke over a wide range of dilutions (*i.e.*, exposure concentrations) required for *in vitro* cell culture investigations.

The Borgwaldt RM20S® in combination with BAT's exposure chamber using Transwell® inserts enables direct exposure of *in vitro* cellular cultures to whole cigarette smoke at the air–liquid interface (Fig. 1) [2,6,7]. This smoking machine, first commercialized in 2005, can smoke up to four cigarettes simultaneously with the smoke collected into four independent syringes. Each syringe can dilute the cigarette smoke with air in ratios ranging from 1:1.14 to 1:4000 (smoke volume:air volume), which corresponds to a range of 87–0.025% (v/v) cigarette smoke in air. For biological exposures, doses tend to be in the range of 0.4–5% (v/v) cigarette smoke in air [2]. The dose at this range of whole smoke dilution has only been correlated to the mass of total particulate matter (TPM) deposited on a Cambridge filter pad (CFP) placed either before the exposure chamber or within it, on a Transwell® insert [2,6].

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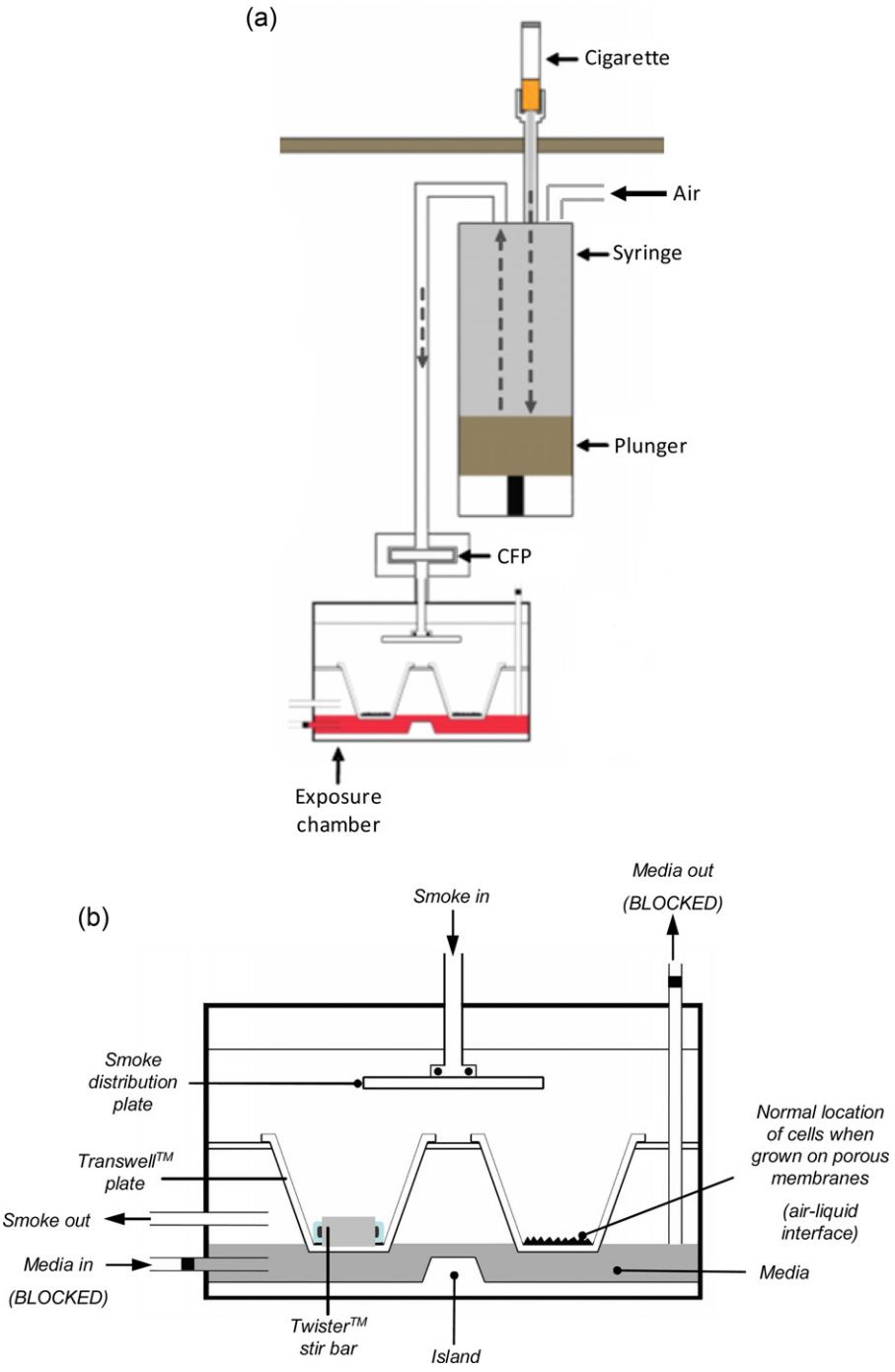

Fig. 1. (a) Schematic of the Borgwaldt RM20S® in combination with the BAT exposure chamber showing one of the four smoking ports connected to a dilution syringe. The machine smokes the cigarette, dilutes the smoke and delivers it to the exposure chamber [7]. Insertion of a Cambridge filter pad (CFP) to trap particulate matter downstream of the syringe allows exposure of cell or tissue culture to the diluted vapor phase only. (b) Cross-section of the exposure chamber [7] showing the location for the Twister™ stir bar for HSSE experiments. For *in vitro* cell culture assays, medium flows in and out of the chamber. In this work, no cells and no culture medium were used.

Our group recently carried out a study to determine the precision and accuracy of dilution of the smoke dose generated by the Borgwaldt RM20S® and delivered to the exposure chamber by measuring two reference standard gases (CH_4 and CO) introduced at the smoking port and a cigarette particulate phase marker (solanesol) from whole smoke [1]. The repeatability of vapor phase dilution was ≤4.5% RSD for dilutions of 0.1–0.52% (v/v) CH_4 in air and was ≤3.7% RSD for dilutions in air of 1–10% (v/v) CO. The accuracy of CO measurements was 5.8–6.4% error for the dilution range studied. The repeatability of dilution of the particulate phase in air ranged from 8.8 to 12% RSD when quantifying solanesol. Overall, the findings suggested that the Borgwaldt RM20S® is a reliable tool to generate and deliver repeatable and reproducible doses of whole smoke to *in vitro* cultures [1]. Scian et al. [4] measured in detail the chemical constituents of the particulate phase and reported recoveries at the exposure chamber of <40% in the Burghart smoking system for most of the compounds monitored, with repeatability of the measurements reaching over 35% RSD for smoke diluted to 50% (v/v) in air. To date, no studies have reported the chemical characterization of the vapor phase smoke components within the exposure chamber