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Arsenic Speciation in Tobacco and Cigarette Smoke *

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SUMMARY

Arsenic is one of the metals found in cured tobacco and mainstream cigarette smoke. Levels of arsenic in modern filtered cigarette smoke range from sub-ppm to a few tens of ppms. To enable accurate smoke toxicity assessment on arsenic in cigarette smoke, it is desirable to establish its chemical forms in addition to total quantities because different arsenic compounds possess different toxicological potentials.
Progress has been made on measuring the arsenic speciation in tobacco and mainstream cigarette smoke by using a combination of synchrotron-based X-ray absorption spectroscopy and high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS). In this paper, we describe the experimental procedures developed together with the main findings. A transient redox transformation between As(V) and As(III) was confirmed in freshly generated mainstream smoke. Potential areas for future research are highlighted in order to further our understanding of the speciation mechanism for arsenic in tobacco products. [Beitr. Tabakforsch. Int. 25 (2012) 375–380]

KEY WORDS: cigarette, smoke, synchrotron, arsenic, As(III), As(V), HPLC-ICP-MS, tobacco

RESUME

L'arsenic est l'un des métaux généralement mesurés dans les tabacs séchés à l'air chaud et dans la fumée principale de cigarette. Les concentrations d'arsenic dans la fumée de cigarette à filtration moderne sont comprises entre un niveau inférieur à 1 ppm à quelques dizaines de ppm. Afin de permettre une évaluation précise de la toxicité de la fumée relative à la présence d'arsenic dans la fumée de cigarette, il est souhaitable de déterminer également ses formes chimiques en plus des quantités totales, étant donné que différents composants de l'arsenic possèdent différents potentiels toxicologiques.
Des progrès dans le domaine de la mesure de la spéciation de l'arsenic dans le tabac et dans la fumée principale de cigarette ont été obtenus en utilisant une combinaison de la spectroscopie d'absorption des rayons X par rayonnement synchrotron et la spectrométrie de masse par plasma à couplage inductif couplé avec la chromatographie en phase liquide (HPLC-ICP-MS). Dans cette étude, nous décrivons les procédures expérimentales développées et les conclusions principales établies. Une modification de l'état rédox en conditions transitoires de As(V) à As(III) a été confirmée dans l'aérosol de fumée de cigarette fraîchement émis. Les domaines potentiels pour les recherches futures sont soulignés dans le but d'approfondir notre compréhension des mécanismes de spéciation de l'arsenic dans les produits à base de tabac. [Beitr. Tabakforsch. Int. 25 (2012) 375–380]

ZUSAMMENFASSUNG

Arsen ist eines der Metalle, die routinemäßig in getrocknetem Tabak und im Hauptstromrauch von Zigaretten gemessen werden. Die Arsenkonzentration im Rauch moderner Filterzigaretten liegt zwischen unter 1 ppm und wenigen Dutzend ppm. Für eine exakte Bewertung der Rauchtoxizität in Bezug auf Arsen in Zigarettenrauch ist es wünschenswert, zusätzlich zur Gesamtmenge dessen chemische Formen festzustellen, da verschiedene Arsenverbindungen ein unterschiedliches toxisches Potenzial besitzen. Durch den Einsatz einer Kombination von synchrotronbasierter Röntgenabsorptionsspektroskopie und Hochleistungsflüssigchromatographie - induktiv gekoppelter Plasma-Massenspektrometrie (HPLC-ICP-MS) wurden Fortschritte bei der Messung der Arsenspeziierung in Tabak und im Hauptstromrauch von Zigaretten erreicht. In diesem Artikel werden die entwickelten Versuchsverfahren

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sowie die wichtigsten Erkenntnisse daraus beschrieben. Eine vorübergehende Redoxtransformation von As(V) zu As(III) wurde in frisch erzeugtem Hauptstromrauch-Aerosol bestätigt. Mögliche zukünftige Forschungsgebiete werden aufgezeigt, um unsere Kenntnisse vom Speziierungsmechanismus von Arsen in Tabakerzeugnissen weiter auszubauen. [Beitr. Tabakforsch. Int. 25 (2012) 375–380]

INTRODUCTION

Arsenic is one of the metals (Cr, Cd, Ni, Pb and Se being the rest) among the so-called Hoffmann toxicants in cigarette smoke (1). This work deals with arsenic speciation exclusively. The level of arsenic in mainstream smoke under the ISO smoking condition is ca. 10.4 ng per cigarette for 2R4F Kentucky reference cigarette (2). International Agency for Research on Cancer (IARC) classifies arsenic as Class 1 carcinogen (3). Different forms of arsenic species (either by arsenic valence or by associated anions) are known to have different toxicities (4). For example, the most toxic arsenic species are the inorganic species, i.e., arsenite ($As(III)O_3^{3-}$) and arsenate ($As(V)O_4^{3-}$). In contrast, organic arsenicals in food and plants (arsenobetaine, monomethyl arsenic acids, dimethylarsonic acids, arsenochline and arsenosugars, etc.) have little or no toxicity (4, 5). It is therefore desirable to know both the total arsenic level and the arsenic species in tobacco and any transformation upon pyrolysis and combustion during cigarette smoking for accurate risk assessment.

Based on the knowledge gained from thermal transformation of arsenic species during food cooking (6), the combustion temperature during smoking (from 800 to 950 °C) is sufficiently high to induce thermal transformation of arsenic species presented in the cut leaf. Hence it is important to track and to preserve the native redox environment during the cigarette smoking and trapping processes.

Various options were considered for an effective measurement before choosing synchrotron based X-ray absorption spectroscopy (XANES) as our preferred technique, chiefly for its ability to uncover speciation information at ppm levels *in situ*. The main challenge was to design a metal-free method to trap mainstream smoke and also to preserve the smoke samples during storage and analysis. The preliminary results indicated possible the complex redox behaviour between two dominant arsenic valencies (7), the tri- and penta-valent arsenic during smoke formation. However, the XANES signal intensity was too low to be used to identify the arsenic species within. More recently, we developed a hyphenated HPLC-ICP-MS method that was able to study the arsenic compounds in water-soluble fractions of 3R4F cut tobacco and its mainstream smoke (8). Up to six inorganic and organic arsenic species have been identified. The results obtained from the chemical and physical methods complement each other and confirm the presence of an arsenic redox reaction.

BRIEF EXPERIMENTAL CONSIDERATION

Detailed experimental protocols are published elsewhere (7, 8).

Cigarette & smoke samples

The cigarettes used were Kentucky 3R4F reference cigarettes (University of Kentucky, Kentucky Tobacco Research and Development Centre). Cut tobacco samples were taken from ten 3R4F cigarettes and milled using a titanium coated grinder to fine powder (size distribution not measured). To avoid metal contamination and to preserve species to be detected, commercial Cambridge filter pads (a glass-fibre substrate) were not used. During machine smoking (a RM20 rotary smoking machine and a single-port smoking machine were used under 35 mL puff volume, 2-sec puff duration and once every 60 seconds), the smoke passage before impaction trapping was minimised to reduce dead volume and hence smoke ageing. Smoke particulate matter was trapped onto a metal-free plastic substrate (Kapton Tape from Fisher Scientific) with its surrounding glass chamber immersed in solid CO_2 (−78 °C).

Because this trapping method is different from the room-temperature trapping by Cambridge filter pads as normally carried out, the smoke particulate matter collected may contain a higher percentage of semi-volatile species; however the net effect of this procedure is unknown. The smoke samples were kept in clean glass containers under the solid CO_2 during the storage and transport. The storage period prior to analysis was kept below one week.

X-ray Absorption Near Edge Structure (XANES) analysis

This experiment was conducted at either Station 16.5 (Daresbury Synchrotron Radiation Source, UK) or Beamline X1 (HASYLAB, Hamburg, Germany). Further experimental details and data processing protocols are available elsewhere (7).

HPLC-ICP-MS measurements

These were performed using an Agilent 1200 HPLC system in combination with an Agilent 7500ce ICP-MS for element-specific detection.

Size-exclusion separation of arsenic compounds was carried out on a Supelco TSK gel 3000XL column (250 mm x 4.6 mm id x 6 µm). Anion-exchange HPLC was performed on a Hamilton PRP-X100 column (250 mm × 4.1 mm id × 10 µm). The size-exclusion column was calibrated with a gel filtration mixed standard solution containing albumin (66 kDa), SOD (32 kDa), MT1 (~10 kDa), vitamin B12 (1.35 kDa) and glutathione (307 Da). The HPLC column was connected directly to a 100 µL min^{-1} PFA microflow concentric nebulizer of the ICP-MS *via* PEEK tubing (30 cm × 0.1 mm id).

Extraction of water-soluble arsenic from smoke condensates

5 mL of deionised water were added to each smoke condensate collected on the metal-free plastic substrate to ensure full immersion by water. Extraction was performed by sonication in a water bath for 2 h. Water extracts were separated by decantation and filtered before analysis.

Arsenic speciation by HPLC-ICP-MS

The separation and elution of the arsenic compounds was achieved using 10 mM ammonium acetate (pH 8.5) at 0.5 mL min^{-1}. Arsenic-specific detection of the chromatographic fractions with different hydrodynamic volume was performed using on-line ICP-MS detection. For anion-exchange HPLC, 100 mL of the digest was injected and elution was achieved by using 20 mM ammonium hydrogencarbonate (pH 9.0) in 1% (v/v) methanol at 1.0 mL min^{-1}. Quantification of water-soluble arsenic compounds in the extracts (relative concentrations only) was performed by anion-exchange HPLC coupled to ICP-MS and external calibration with arsenic species standards using the peak area response for ^{75}As.

MAIN EXPERIMENTAL RESULTS

It was relatively straightforward for XANES spectra to reveal the dominant arsenic species as As(V) in both ground 3R4F tobacco and the cigarette ash samples, based on the K-edge position of the As(III) and As(V) standards (7). Grinding and pelleting the cut tobacco and ash samples were useful to improve the signal-to-noise ratio. However, in both cases the signal-to-noise ratio was not sufficient for the extended absorption fine structure spectra to be carried out for speciation detection.

When the smoke particulate matter was analysed by XANES, initial samples collected using Cambridge filter pads showed inconsistent trends in signal intensities, suggesting possible presence of arsenic in the filter material. Therefore, Cambridge filter pads were not used to trap smoke particulate matter for the remaining study. After 20 cigarettes were smoked using a rotary smoking machine under ISO puffing parameters, a visible layer of particulate matter was seen to build-up under the impaction trap. Varying the number of cigarettes smoked (1 to 20) did not significantly affect the shape or the intensity of the subsequent X-ray absorbance detected.

An example of the normalised arsenic XANES spectra from four different smoke particulate matter samples together with those from sodium arsenite and arsenate standards are shown in Figure 1. The XANES spectra from the smoke samples displayed a mixed character of As(III) and As(V). Using standard spectrum modelling approach based on a linear combination of As(III) and As(V), the two synchrotron facilities estimated that relative percentages of the two arsenic valence status (not species) were approximately 50% (Table 1). In this case, the As concentration range in the smoke particulate matter is in the range of a few ppm for 3R4F cigarettes. Without knowing the exact chemical species present, the shape of the XANES spectra of the combined models can only match the experimental arsenic XANES by edge positions. Even with identical species, differences in homogeneity, particle size or degree of crystallinity of the compounds could affect the fine degree of matching. These are one of the inherent limitations of the XANES technique.

Spectra-fitting of Figure 1 shows that the "fresh" particulate matter samples collected under solid CO_2 contained a higher percentage of As(III) than the other two samples

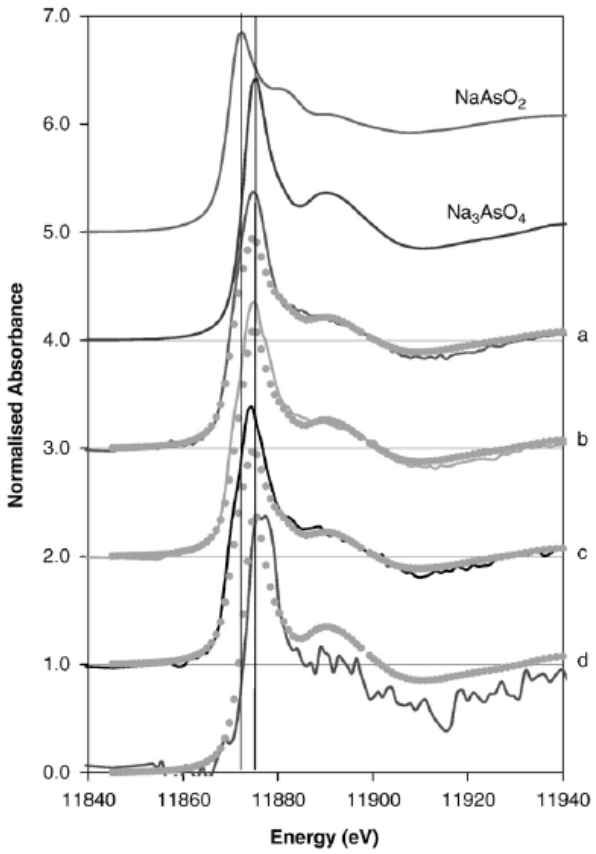

Figure 1. Normalised arsenic XANES spectra from the four different smoke particulate matter samples together with arsenite and arsenate standards. (a) 10 cigarettes on 1 filter (room temperature), (b) 20 cigarettes on 1 filter (solid CO_2), (c) 20 cigarettes on 1 filter (room temp.) and (d) ash pellet.

that were stored at room temperature. In other words, the reducing capacity during the tobacco combustion/pyrolysis converted a significant part of As(V) into As(III), and the cold trapping condition helped to stabilise the As(III) species which would otherwise be oxidised during ageing. Alternatively, when the cold-trapped smoke particulate matter was allowed to return to the room temperature, the As(III) feature was weakened (not shown). The dynamic balance between As(V) and As(III) in "fresh" smoke encouraged us to pursue the subject further, not only because it would be ideal to have an independent experimental verification of the transformation but also to gain some

Table 1. The estimated levels of arsenic species and their distributions in three types of samples from 3R4F cigarettes. The ratios of As(III)/As(V) from the XANES experiments were modelled based on linear combinations of the two As standards: $NaAs(III)O_2$ and $Na_3As(V)O_4$.

Sample	XANES Fitting		
	As(V) %	As(III) %	R* (%)
Cut tobacco	94	6	15
Room-temperature smoke condensate	53	47	—
Cryo-trapped smoke condensate	40	60	28
Cigarette ash	0	100	24

* Uncertainty values given by the modelling software.

insights into the chemical species involved. For these reasons, we pursued the chemical speciation analyses using HPLC-ICP-MS.

First we experimented using Ge as an internal standard for the accurate determination of mono-isotopic arsenic at ng g^{-1} levels in cut tobacco (8). Using this method, the cut tobacco from 3R4F cigarette was found to contain 318 ± 9 ng g^{-1} of arsenic (on a dry weight basis). The recovery of total arsenic from the NIST pine needles (1575) and rice flour (1568a) reference materials (certified reference materials for arsenic speciation analysis) was 102% and 100%, respectively. Various optimisation steps were also carried out to improve the extraction efficiency (8).

Speciation analysis of the water extract was undertaken using anion-exchange HPLC-ICP-MS. The results suggest the presence of arsenate (As(V)) (as a major species) and arsenobetaine, arsenite (As(III)) and DMA (dimethylarsenic acid, as minor species).

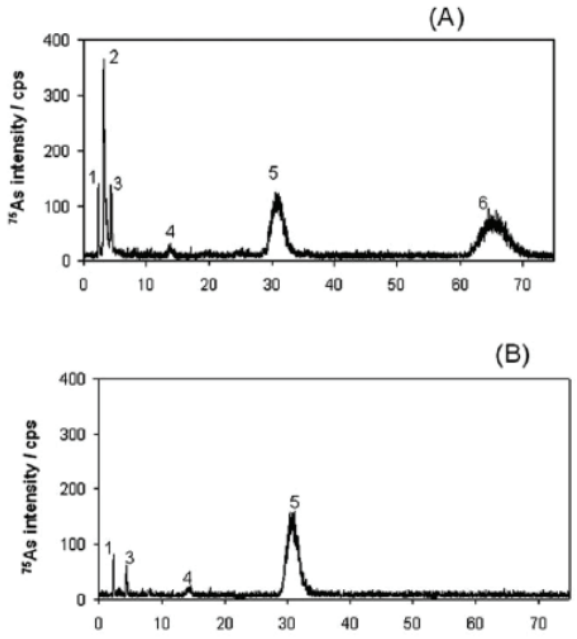

Figure 2. Arsenic speciation in the mainstream smoke particulate matter: anion-exchange HPLC-ICP-MS chromatograms of water extracts stored under (a) dry ice; and (b) room temperature.

1 - Unknown, 2 - Arsenite, 3 - Dimethylarsinic acid (DMA),
4 - Monomethylarsonic acid (MMA), 5 - Arsenate, 6 - Thioarsenate

This result agrees qualitatively with that obtained by XANES. The limits of detection obtained for arsenite and arsenate were found to be 10 and 50 pg g^{-1} As, respectively. The quantitative arsenic speciation data indicated that approximately 89% of the total water-soluble arsenic was present as inorganic arsenic (arsenite + arsenate) with the most abundant inorganic arsenic species being arsenate. The sequential extraction (after water extraction) with driselase and SDS (sodium dodecylsulfate) solutions (an enzyme preparation procedures used to break down plant structure) enabled further extraction of approximately 21% arsenic from the cell wall of the sample. The total arsenic recovery of approximately 64% (of the total arsenic in the solid) was obtained (Table 2). Due to the very low arsenic concentration in the analysed extract and the high matrix complexity, characterisation of the arsenic species detected by HPLC-ICP-MS using organic mass spectrometry was not possible.

The total concentration of water-soluble arsenic extracted from smoke condensates (mass fraction of water-soluble arsenic in smoke condensate) was found to be 0.14 ± 0.03 mg kg^{-1}. The standard deviation (18%) could be attributed to the variability of the non-standard smoke collection process and the fact that only 6 smoke condensates (6 runs of 20 cigarettes per run) were collected. For arsenic speciation in the mainstream smoke particulate matter, anion-exchange HPLC-ICP-MS chromatograms of water extracts stored under two different conditions (room temperature vs. stored at −78 °C) before analysis were compared. Figure 2A shows the profile stored at −78 °C. Retention time matching with the arsenic standards enabled the identification of major arsenite and arsenate, accounting for 51% of the total arsenic in the water extract. Figure 2A also shows the presence of arsenobetaine, DMA (dimethylarsenic acid) and MMA (monomethylarsonic acid) and of a major thioarsenite peak at the retention time of 65 min. Its peak area comprises approximately 41% of the total chromatographic peak area. In Figure 2B, almost all the As(III) species detected in Figure 2A were converted to As(V) for the smoke particulate matter stored at room temperature. This again agrees with the previous XANES results on the smoke condensate. Table 3 summarises the estimated percentages of the arsenic species based on the HPCLC-ICP-MS analysis. The important feature to note is that the peak 2 corresponding to As(III) (Figure 2B) was not seen when the smoke condensate was collected at room-temperature.

Table 2. Fractions of arsenic species in cut 3R4F tobacco.

Total arsenic in tobacco:	318 ng/g	
Water soluble extraction	134 ng/g (42%)	119 ng/g (89% water soluble): mainly inorganic (arsenite + arsenate) – As(V) being the dominant species. The remaining (11%): organic As(V): (DMA, MMA, etc)
Driselase extraction	42 ng/g (13%)	80 ~ 90% to be inorganic (arsenite + arsenate): As(V) being the major species
SDS Extraction	25 ng/g (8%)	~ 80% to be inorganic (arsenite + arsenate): As(V) being the major species

Table 3. Relative concentrations and fractions of arsenic species in mainstream smoke condensate.

Water soluble mainstream smoke condensate	
Cryo-trapped	~ 51%: inorganic (arsenite + arsenate) ~ 41%: unknown As-S species ~ 8%: organic arsenic species
Room-temperature trapped	~ 89%: inorganic (arsenite + arsenate): As(V) being the major species

Many previous publications have shown the dynamic and reactive nature of cigarette smoke (9, 10). The transformation of the arsenic species observed in this work, firstly As(V) → As(III) during the combustion/pyrolysis followed by the reverse As(III) → As(V) upon smoke ageing, adds another aspect to this complex phenomenon.

The reduction of As(V) in the cut tobacco to As(III) in smoke aerosol appears to agree with the fact that the cigarette coal is oxygen-deficient and hydrogen-rich (9, 11). This reduction is however incomplete, as a significant amount of As(V) remains to be found in the smoke particulate (Figure 3). The reducing activity is reported to increase from the 2nd puff to the last puff driven by free radical reactions (11). The "electrochemical potential" for the fresh smoke aerosol particles ranges from approx. +0.24 to +0.17 V(S.C.E., Standard Calomel Electrode), equivalent to approx. 0.00 to −0.07 V(S.H.E., Standard Hydrogen Electrode). Using a "smoke pH" value of a typical blended type of cigarette, around 5.5 to 6.5, it is also thermodynamically feasible for the As(V) /As(III) redox couple to change direction depending on the "electrochemical potential" found in cigarette smoke (12). Post smoking, the "electrochemical potential" of the smoke

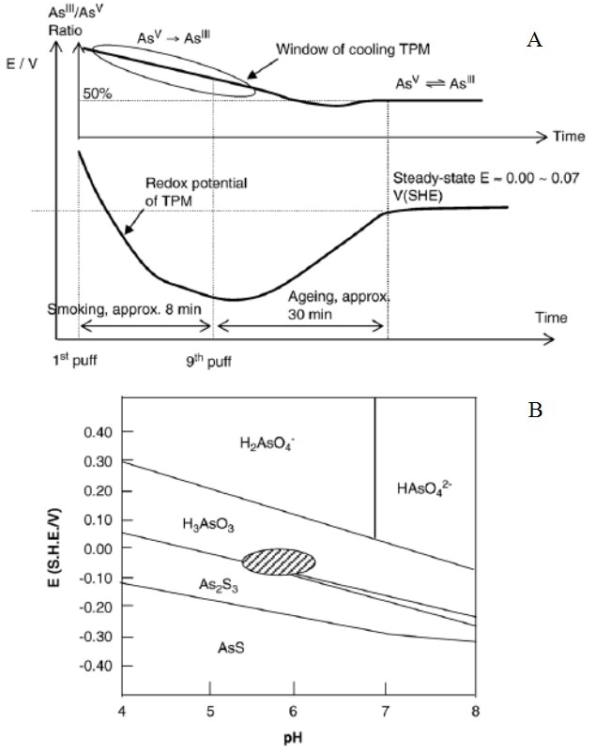

Figure 3. (a) "Electrochemical potential" of mainstream smoke aerosol as a function of puff number; (b) a region of the arsenic Pourbaix diagram.

particulate matter starts to rise gradually, possibly due to self-quenching of the radical species within. This process can be slowed down by storing the smoke sample at lower temperatures.

SUMMARY

The sequential extraction procedure (water, enzymes and surfactants) developed for cut tobacco was efficient but only able to achieve ca. 64% extraction of the total arsenic (approximately 300 ng g^{-1} As). Hence, a more thorough extraction of possibly other arsenic species other than those identified here is needed. Using AE-HPLC-ICP-MS, 89% of the water-soluble arsenic (137 ng g^{-1}) from the mainstream smoke particulate matter was found to be inorganic arsenic: among which approximately 51% was present as arsenite and arsenate, the remaining being thioarsenite species. The two arsenite (As(III)) species were only detected in "fresh" smoke particulate matter that was stored under dry ice and were not stable when the same smoke condensates was stored at room temperature. This As(III) to As(V) redox transformation was detected by both XANES and HPLC-ICP-MS.

The overall scheme of this redox processes appears to agree with the current mechanistic understanding of the combustion/pyrolysis processes responsible for mainstream smoke generation, and also the theoretical thermodynamics of arsenic in an aqueous environment. However, free radical and thermodynamic properties from modern cigarettes such as 3R4F are required to ascertain specific details, which may further enhance our understanding of this complex phenomenon. It would also be interesting to investigate the thermal transformation of endogenous and extragenous arsenic during the smoke formation.

The chemical speciation analyses developed in this work show that synchrotron-based spectroscopy is an appropriate tool to investigate the main redox species involved and any redox reactions during cigarette smoke aerosol formation. The effectiveness of this *in situ* physical detection method however depends on the concentration of the species in the sample materials. For arsenic in 3R4F cigarettes, this has proven to be a challenge. The overall results add a further dimension to the complexity of dynamic changes during cigarette smoke formation.

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