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Tobacco smoke particulate matter chemistry by NMR

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The submicron liquid droplets constituting the particulate matter of mainstream tobacco smoke (PM_{MTS}) are viscous and of a composition that is complex and poorly understood. PM_{MTS} is often ~80% w/w 'tar' where 'tar' = total PM_{MTS} − (nicotine + water). Many of the chemical agents in MTS responsible for smoking-related cancers are found at least partially in the PM_{MTS} portion of MTS. The properties of PM_{MTS} vary with brand and with puffing patterns. The chemical forms and total levels of nicotine, the identities/levels of other compositionally dominant compounds, and the identities/levels of carcinogens are of interest. Most studies of the composition of PM_{MTS} have involved extraction then chromatography. Such methods allow the determination of low-level constituents, but alter the samples such that direct information regarding chemical conditions within the PM_{MTS} cannot be obtained. Here, we utilize nuclear magnetic resonance spectroscopy (NMR) to examine native PM_{MTS} in conventional cigarettes, including measurements of the brand-dependent fraction of PM_{MTS} nicotine that is in the free-base form (increasing this fraction in inhaled tobacco smoke affects the rates of the processes governing nicotine deposition in the respiratory tract, and so has implications for smoking behavior and addiction). We also demonstrate the use of NMR for characterizing the composition of PM_{MTS} (including the levels of selected cigarette additives) when the cosolvent DMSO-d_6 is added to improve spectral resolution. The native and solvent-assisted results open the door to a range of future studies. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H; ^{13}C; tar; nicotine; additives; free-base; tobacco additives; composition analysis

INTRODUCTION

The submicron liquid droplets constituting the particulate matter of mainstream tobacco smoke (PM_{MTS}) are viscous and of a composition that is complex and poorly understood. The 'tar' fraction of PM_{MTS} is operationally defined according to 'tar' = total PM_{MTS} − (nicotine + water), and is often ~80% (w/w) of the PM_{MTS}. Many of the chemical agents responsible for smoking-related cancers are found at least partially in the PM_{MTS} portion of MTS.[1] The properties of PM_{MTS} vary with brand and with puffing patterns. The chemical forms and total levels of nicotine,[2] the identities/levels of other compositionally dominant compounds, and the identities/levels of carcinogens are of interest. 'Tar' properties affect its behavior in the respiratory tract, and this is of particular interest when clearance by cilia becomes impaired.[3]

Most studies of the composition of tobacco smoke PM_{MTS} have involved extraction then chromatography. Such methods allow the determination of low-level constituents, but alter the samples such that direct information regarding chemical conditions within the PM_{MTS} can no longer be obtained. In a previous study,[4] we have described the use of nuclear magnetic resonance spectroscopy (NMR) in the examination of PM_{MTS} from a new type of 'cigarette' in which tobacco materials are heated but not burned. Here, we describe the first published use of NMR in the study of native PM_{MTS} from conventional cigarettes, including measurements of α_{fb}, the fraction of PM_{MTS} nicotine that is in the free-base (fb) form (Scheme 1). (Increasing α_{fb} in inhaled PM_{MTS} affects the rates of the processes governing nicotine deposition in the respiratory tract,[2] and so has implications for smoking behavior and addiction.) We also use NMR to examine the composition of PM_{MTS} (including the levels of selected cigarette additives) when the solvent DMSO-d_6 is added to improve spectral resolution. The native and solvent-assisted results open the door to a range of future studies.

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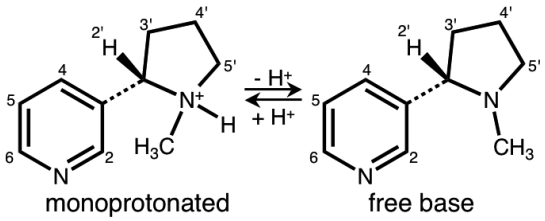
EXPERIMENTAL PROCEDURES

Sample collection

Each 30–50 μL sample of PM_{MTS} was collected as an average over ~8 cigarettes. Collection occurred first through an 18 × 450 mm collection tube (to allow initial particle coagulation), then through a jet (to allow particle impaction) onto the upper inside wall of a Wilmad Stem Coaxial NMR Tube Insert (part number WGS-5BL). Cigarettes were smoked according to the 'Massachusetts' smoking protocol:



Scheme 1. The physiologically relevant acid/base equilibrium of nicotine in tobacco smoke PM_{MTS}.

45-mL puffs of 2 s each with a 30-s interval. Centrifugation was used to bring the material into the capillary portion of the NMR tube. The tube was then placed inside a standard 5-mm NMR tube containing D_2O as the lock solvent. By analogy with previously reported measurements,[4] parallel samples involving addition of appropriate amounts of base (*tert*-butylamine) or acid (acetic acid) permitted determination of the limiting chemical shifts for the nicotine resonances when all of the nicotine was in the fb form ($\alpha_{fb} = 1$) and when all of the nicotine was in the conjugate-acid form ($\alpha_{fb} = 0$). (To minimize sample perturbation, a chemical shift reference compound was not added.) The 2′H line of nicotine changed by more than 1 ppm on going from the acid to the fb form, while the pyridine ring protons (e.g. 5H) had <0.2 ppm chemical shift changes. Thus, α_{fb} was deduced on the basis of the changes in the chemical shift difference between the 2′H and 5H nicotine resonances. The geraniol, triacetin, and solanesol used to prepare 'spiked' samples in DMSO-d_6 were from Sigma-Aldrich.

NMR spectroscopy

NMR spectra were acquired on a Bruker AMX-400 NMR spectrometer, using a 5-mm 'quad' (QNP) probe, with 90° pulse widths of 11 and 5.5 μs for ^1H and ^{13}C respectively; the 90° ^{13}C GARP decoupling pulse was 70 μs. The residual water resonance from the outer tube was suppressed by application of a presaturation pulse during the 2–4 s relaxation delay. Two-dimensional spectra were performed in the usual way, often using water suppression by presaturation.[5,6] The ^1H frequency and spectral width were 400.14 MHz and 4032.3 Hz, whereas the ^{13}C frequency and spectral width were 100.62 MHz and 20124.4 Hz. Adequate signal-to-noise ratios were obtained in about 0.5 h for most samples (4 scans per t_1-increment; 256 such increments), with all of the spectra obtained within 4 h each (16 scans per t_1-increment). Two-dimensional TOCSY spectra were obtained with an MLEV-17 mixing cycle of ~40 ms, at 400.14 MHz and a spectral width of 4032.3 Hz. The one-dimensional ^1H NMR spectra were recorded with the same spectral width and frequency as already stated for the two-dimensional spectra, often with preNOESY solvent suppression.[5,6]

GC/MS

Specific constituents in the DMSO-d_6 solutions of smoke PM_{MTS} were determined using a Perkin Elmer (Shelton, CT, USA) TurboMass Gold gas chromatograph/mass spectrometer (GC/MS). In order to avoid the difficulties of DMSO as a solvent in GC, each DMSO-d_6 solution was first diluted by a factor of 200 with isopropanol. Acenaphthene-d_{10} was added as internal standard. Splitless injection (0.5 μL) followed using an injector temperature of 225 °C, and a 30 m, 0.32 mm i.d., 1.0 μm film thickness DB-5 capillary column (Agilent Technologies, Palo Alto, CA, USA). The GC temperature program used was as follows: hold at 135 °C for 1 min; ramp 300 °C at 10 °C min^{-1}; then hold at 300 °C for 5 min. MS ionization was by electron impact. Compound identification and internal-standard-based quantitation using authentic standards were carried out using an MS mode that allowed alternation between full mass scanning (m/z = 40–350) and selected ion monitoring for specific quantitation ions.

RESULTS AND DISCUSSION

Although PM_{MTS} sample viscosities impede acquisition of interpretable NMR spectra from native smoke PM_{MTS} at room temperature, highly interpretable spectra (Fig. 1) can be obtained after warming to 40 °C (*cf* human body temperature = 37 °C). At 5–10% of PM_{MTS} by weight, nicotine is readily apparent. The nicotine resonances obtained for base-amended samples (so that $\alpha_{fb} = 1$) and for acid-amended samples (so that $\alpha_{fb} = 0$) allowed estimation of

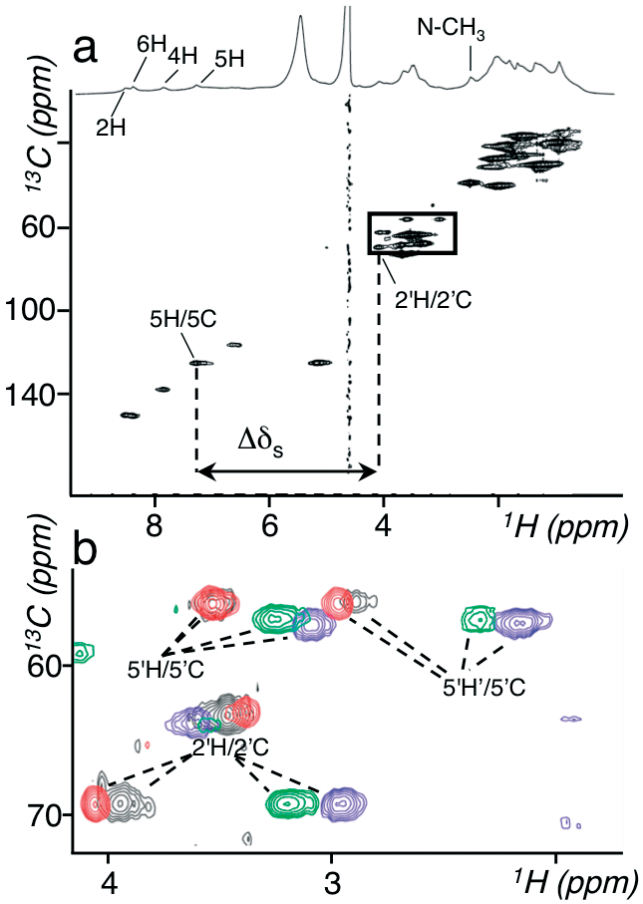


Figure 1. NMR spectra of cigarette PM_{MTS} with selected nicotine peaks labeled. (a) 2D HMQC $^1H/^{13}C$ spectrum of brand B PM_{MTS} recorded at 40 °C; $\Delta\delta_s$ gives $\alpha_{fb} = 0.07$ for nicotine as described in the text; (b) Expansion of the 2′ and 5′ region (boxed in (a)) for brand A (black contours) and brand C (green contours) PM_{MTS}, and the limiting locations amended with acid (red contours) or base (blue contours).

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degree of protonation based on changes in chemical shifts. The nicotine resonances were in sufficiently 'rapid exchange' between the fb and protonated forms so that the peaks were averaged. The chemical shift difference, $\Delta\delta$, between two nicotine ^{1}H resonances (2′H and 5H) was measured for three versions of each sample: $\Delta\delta_s$ for the native sample; $\Delta\delta_a$ for the acid-amended sample for which $\alpha_{fb} \sim 0$; and $\Delta\delta_{fb}$ for the base-amended sample for which $\alpha_{fb} \sim 1$. Then, $\alpha_{fb} = (\Delta\delta_s - \Delta\delta_a)/(\Delta\delta_{fb} - \Delta\delta_a)$.[4]

PM_{MTS} from three subbrands of filtered, hard-pack cigarettes (designated A, B, and C) gave nicotine $\alpha_{fb} = 0.06 \pm 0.01$, 0.07 ± 0.01, and 0.81 ± 0.02 respectively. These direct measurements of α_{fb} by NMR indicate a wide range of α_{fb} for PM_{MTS} from commercial cigarettes at 40 °C. Measurements based on the volatility of the fb form of nicotine carried out at 20 °C have also indicated a range in α_{fb} from different brands.[7] Possible reasons for the wider range observed in the current measurements (by NMR) include differences due to (i) sample temperature; (ii) variability in smoke chemistry due to puff sequence and timing; (iii) pack-to-pack cigarette variability; and (iv) manufacturing changes. Regarding (i), reactions of the type $Am + RCOOH = AmH^+ + RCOO^-$ are known to be exothermic ($\Delta H < 0$), therefore higher temperatures would be expected to increase α_{fb}.[8] (Am = amine (e.g. nicotine); RCOOH = carboxylic acid; and $\alpha_{fb} = [Am]/([Am] + [AmH^+])$.)

Although the above data, as in Fig. 1, allow measurement of α_{fb} in the native PM_{MTS}, adding DMSO-d_6 solvent allows resolution of hundreds of NMR lines from a host of constituents (Fig. 2). Among the constituents identified are alkaloids besides nicotine as well as terpene-derived

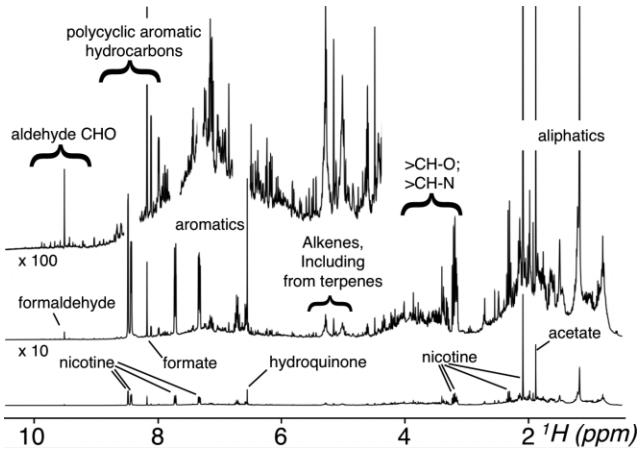

Figure 2. 1D NMR 'preNOESY' spectrum of brand C cigarette PM_{MTS} dissolved in DMSO-d_6, recorded at 40 °C. Some constituents of interest are labeled, as are classes of molecules known to exist in cigarette smoke.

molecules including geraniol (Fig. 3). The latter may be present naturally in tobacco smoke, but is also a well-known tobacco additive used for its flavorant properties.[9] The presence of solanesol, a compositionally important natural tobacco smoke PM_{MTS} compound,[10] is also demonstrated. 2D COSY, TOCSY, and HMQC spectra were used, together with spiking with authentic compounds to verify the assignments. Other smoke constituents suggested by the spectra are styrene, polycyclic aromatic hydrocarbons, hydroquinone, and triacetin; all of these have been found in cigarette smoke by other methods.[11] Triacetin is known to be present in

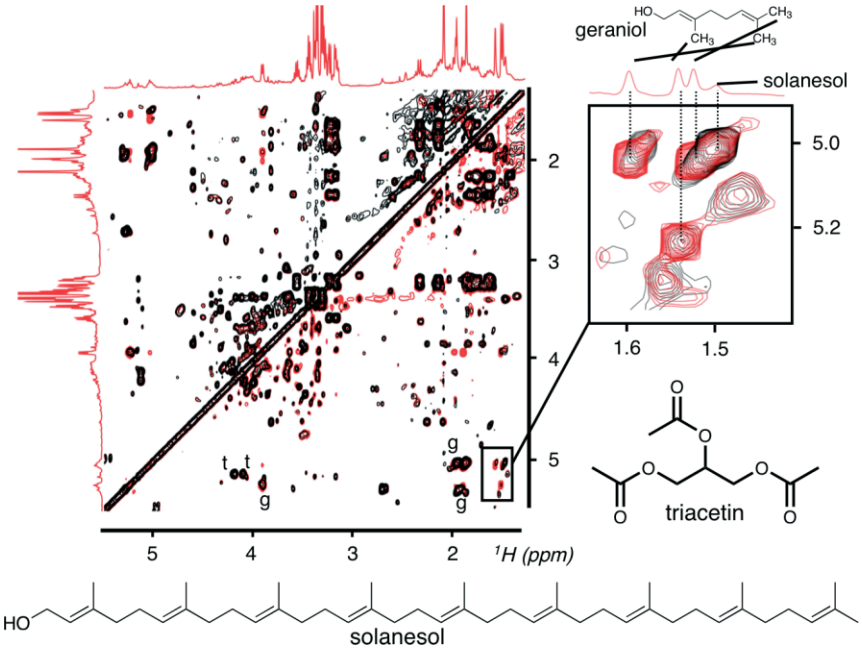

Figure 3. 2D TOCSY spectra of brand D cigarette PM_{MTS} in DMSO-d_6 solvent, illustrating the presence of geraniol, triacetin, and solanesol. Spectra are depicted both without (black) and with 2 μL (red lines and contours) of added geraniol, along with a 1D NMR 'preNOESY' reference spectrum (with added geraniol); all recorded at 40 °C. The TOCSY correlations between the methyls (1.52, 1.53, and 1.59 ppm) and the associated alkene-Hs (5.04 and 5.23 ppm) are shown in the expanded region. Note that there are TOCSY cross-peaks between these resonances, even though there is not sufficient splitting to resolve the multiplets in the 1D NMR spectra. Other geraniol cross-peaks are marked 'g'; triacetin cross-peaks are marked 't'.

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cigarette filters.[12] The NMR line intensities for geraniol and triacetin in one sample of PM_{MTS} from brand D (filtered) cigarettes indicated levels of about 4 and 7 $\mu g\ mg^{-1}$ in the PM_{MTS}, respectively; the presence and approximate levels of the two compounds were confirmed by GC/MS. In addition to providing a means to identify and quantify PM_{MTS} constituents, spectra like those in Figs 2 and 3 can be used to obtain NMR line assignments for chemical species of special interest, e.g. formic acid/formate ion, and acetic acid/acetate ion. By analogy to the measurements described in the preceding text for nicotine, the fractional ionization of the two acids could then be examined in native PM_{MTS} samples. Such measurements could provide valuable insight regarding the acidity/basicity factors affecting α_{fb} values in native PM_{MTS} samples.

CONCLUSIONS

This work demonstrates the significant potential of NMR for characterizing the *in situ* chemistry of native PM_{MTS}. Moreover, emerging developments in the field(s) of metabolomics/metabonomics[13] suggest that important PM_{MTS} constituents may be readily quantifiable and subjected to brand-to-brand comparisons simply by adding an appropriate solvent to allow improved NMR resolution. What is lacking in such analyses is an NMR spectral database of known smoke PM_{MTS} components under defined chemical conditions; we have so far adopted dilution with the solvent DMSO-d_6. Finally, we note that these methods will be useful for analyzing PM from many other combustion sources including forest fires, wood burning, and traditional indoor biomass burning in developing areas of the world.

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