Gas-Phase Radicals in Cigarette Smoke: A Re-evaluation of the Steady-State Model and the Cambridge Filter Pad

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Abstract: Cigarette smoke free radicals have been the subject of many years of investigation. Recently, individual acyl and carbamoyl radicals in fresh whole cigarette smoke were isolated, identified, and quantified by HPLC-mass spectrometry for the first time. These carbon-centered radicals do not conform to the established "steady-state model" for radical formation in gas-phase cigarette smoke. In separate studies, NO_2 in fresh whole mainstream smoke has been measured on a puff-to-puff basis by highly precise and rapid tunable infrared diode laser spectroscopy (TILDAS). Only the first puff contained a significant amount of NO_2 and its overall yield was substantially lower than previously reported. Nevertheless, when the smoke was passed through a Cambridge filter pad, NO_2 appeared in the filtered gas-phase smoke during every puff, suggesting that NO_2 forms directly on the pad. For fresh smoke, these results also challenge the applicability of the steady-state mechanism, which postulates that NO_2 in the smoke itself is an essential intermediate for gas-phase radical formation. In this review, the historical investigations of cigarette smoke radicals are re-examined and the implications of the new spectroscopic and radical trapping data are discussed. Possible alternative mechanisms for radical formation in cigarette smoke are proposed. When combined with data from previous corroborating reports, these recent results raise serious issues about the use of the Cambridge pad, which is conventionally used to separate gas smoke constituents from whole smoke, but may introduce more artifacts of measurement for certain reactive smoke constituents than previously appreciated.

Keywords: Cigarette smoke, Gas-phase radicals, Steady-state model.

INTRODUCTION

The long-standing effort to understand the nature of the harmful effects of cigarette smoking has been a tremendously daunting and complex task. To grasp the scope of the problem, one only has to look at the complexity of cigarette smoke, which, like the smoke from the combustion of any plant material, contains thousands of individual chemical constituents [1]. The number of chemical species in cigarette smoke that have been identified with certainty continues to grow [2]. Many studies have focused on the routine quantification of specific smoke constituents because they are subject to regulatory and public scrutiny due to their known toxic effects (see, for example, Counts *et al.* [3, 4] and Gregg *et al.* [5]). Other studies are more fundamental in nature and aim to understand the mechanisms of how individual or classes of constituents form in cigarette smoke, and how the construction of a cigarette affects their yield [6]. Among the most widely studied classes of potentially harmful constituents in cigarette smoke are free radicals, including the gaseous smoke constituents NO and NO_2.

Eminent chemist Prof. W.A. Pryor and his associates captured the overall picture of cigarette smoke radicals beginning in 1976 [7-14], making many fundamental investigations and studying the physiological effects of radicals [7, 15-22]. Pryor showed early on that there are distinctly different types of radicals in the gas phase of cigarette smoke compared to radicals found in the particulate phase or smoke condensate. For smoke analysis, a standard cigarette smoking protocol (FTC) has been used in the majority of studies [23]. This smoking method entails the use of a smoking machine (or equivalent device) to draw a 35 ml volume puff of smoke from a lit cigarette for 2 seconds every 60 sec until the coal reaches the cigarette butt. The smoke is drawn through a fiberglass "Cambridge" filter pad that has a pore size of 0.1 μm that collects 99.9 % of the smoke particulate matter contained in the smoke stream. "Gas-phase smoke" is defined as the mostly volatile smoke constituents that can pass through this filter. Radicals in gas-phase smoke were found to be reactive, short-lived, and oxidizing. The smoke condensate captured on the Cambridge pad is referred to as the "total particulate matter" (TPM) or simply "particulate-phase smoke" or smoke condensate. Radicals in the TPM were observed to persist on the pad for days and were found to be reducing towards many substances; of particular import, the particulate-phase radicals were found to reduce molecular oxygen to the superoxide radical anion [7].

Until recently, most analyses of cigarette smoke radicals have involved the separation of the phases using a Cambridge filter pad [1]. Analyses typically employed EPR spectroscopy in combination with nitrone-type spin-trapping agents, sometimes dissolved in solution and sometimes deposited on a solid-phase support [8]. The separation of phases has always been regarded as necessary since the analysis of radicals in whole mainstream smoke, i.e., the unaltered smoke emitted from the lit end of a cigarette, is obfuscated by the simultaneous presence of both gas-phase and particulate-phase constituents, which have very different redox and chemical properties. Recently developed methods avoid the necessity of separating the gas-phase and particulate-phase constituents, permitting improved characterization of the relevant smoke chemistry and identification of specific radicals in whole, minimally aged cigarette smoke. These methods include new solid phase trapping methods, the application of alternative spin-trapping agents, alternatives to EPR spectroscopy for radical detection, the use of high precision mass spectrometry for structure identification, and sophisticated spectroscopic techniques for measuring NO and NO_2 [6, 24-37]. The importance of indentifying specific radicals by mass spectrometry has long been appreciated [27, 38], but these measurements have only recently been realized [28-30, 34, 35, 39-41].

That the Cambridge filter introduces artifacts of measurement for a variety of smoke constituents has long been known [1, 42]. Recent measurements revealed that the Cambridge filter significantly reduces the amount of carbon-centered radicals measured in filtered gas-phase smoke compared to whole smoke, while actually causing the measured amount of NO_2 to increase [37, 40, 41]. These results are significant, since the formation of carbon-centered radicals (and their oxidation products) has long been linked to the formation of NO_2 in smoke *via* oxidation of NO and aging processes. Church and Pryor proposed a mechanistic model for radical formation in smoke, now commonly referred to as the "steady-state mechanism," that postulates that most of the radicals are created in the smoke plume, rather than by combustion in the cigarette coal, in a cyclic process involving reactions of NO_2 that forms by the oxidation of NO generated by the burning tobacco [11]. The steady-state mechanism has been frequently investigated, cited and reviewed

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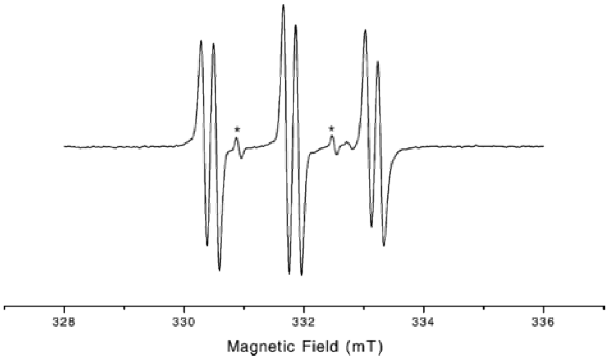


Fig. (1). EPR spectrum from the mainstream filtered gas-phase smoke of a single 1R4F cigarette using PBN (0.01 M) in benzene. The peaks associated with the oxidized product PBNOX are marked with the * symbol. Baum *et al.* 2003 [46].

[11, 15-17, 26, 27, 38, 42-47]. Here, we give the historical background of the model, and then focus on how recent developments have challenged the conventional view of the formation of radicals in smoke, the role played by NO_2, and the obfuscating effects of the Cambridge filter pad. Since the gas-phase radicals are both constituents of the whole mainstream smoke aerosol and constituents in the "gas-phase smoke" obtained by passing whole cigarette smoke through a Cambridge filter pad, to avoid an ambiguous discussion, we use the expression "filtered gas-phase smoke" to refer expressly to smoke that has passed through the Cambridge filter (not the filter attached to the cigarette). Other references to the gas-phase constituents in whole smoke will be explicitly stated.

EARLY MEASUREMENTS ON FILTERED GAS-PHASE SMOKE

Based on previous evidence that suggested that the gas-phase of cigarette smoke contained abundant radicals [48], Pryor *et al.* initiated a systematic study of radicals in filtered gas-phase cigarette smoke utilizing EPR spin-trapping techniques [8]. At the time, the state-of-the-art for analyzing short-lived radicals was to stabilize the reactive species with a radical-specific reagent, for example, a nitrone such as N-tert-butyl-α-phenylnitrone (PBN) or 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), that form relatively stable radical adducts that have life-times suitable for EPR analysis [38]. A variety of nitrone spin traps have been employed in many subsequent investigations [34, 35, 43, 44, 46, 47]. Using the PBN trapping agent and measuring the EPR spectra of its radical adducts with smoke radicals, both carbon and oxygen-centered radicals were distinguished in filtered gas-phase smoke [7, 11]. Solid-phase radical trapping with the PBN trapping agent adsorbed on silica gel was found to be particularly efficient for trapping both alkoxyl and alkyl free radicals. However, few additional details about chemical structures could be deduced by EPR since the precision of the g-values and resolution of the hyperfine coupling constants were insufficient in the majority of cases to identify specific radical species. Moreover, the EPR spectra of spin-trapped smoke radicals sometimes displayed artifacts, such as oxidation products of the spin-adducts or side-products from reactions with the solvent, and quantification was in general prone to systematic errors [38, 46].

Although the nitrone spin-adducts are more stable than the smoke radicals, the radical adducts themselves slowly decay [8, 11, 42, 46, 49], which necessitates carefully executed and timed measurements following the trapping step to obtain consistent and accurate results. Pryor *et al.* studied the decay of PBN adducts of radicals from filtered gas-phase smoke in their 1976 report [8]. Reszka *et al.* showed that NO, an abundant cigarette smoke constituent, destabilizes the spin-adducts of the commonly used DMPO trapping agent [50], while Gosh *et al.* observed a decay of cigarette smoke radical adducts of DMPO within two minutes [43]. Despite these limitations, EPR spectroscopy has yielded very fundamental information about the dynamics and origin of radicals in cigarette smoke.

Fig. (**1B**) shows the solution EPR spectrum of the spin-adduct obtained from bubbling the mainstream smoke of a single 1R4F cigarette into a 0.01M solution of PBN in benzene [46]. The structure of the spectrum is a triplet of doublets; the triplet corresponds to the hyperfine coupling between the unpaired electron of the radical adduct and the ^{14}N nucleus (spin = 1) of PBN. The doublet results from the hyperfine coupling between the unpaired electron and a single hydrogen nucleus (spin = 1/2). In a benzene solution, the hyperfine couplings for the PBN adduct with a small alkoxy radical is a_N =1.359-1.36 and a_H = 0.18-0.20 mT. For the comparable alkyl radical adducts, the values are a_N = 1.42-1.43 and a_H = 0.30–0.34 mT [38]. These values are somewhat dependent on the trapping solvent [7, 11]. Frequently, spectral spin-simulation is required to resolve the alkyl and alkoxy adducts in complex spectra where there are multiple species and the EPR resonances tend to be broad and not fully resolved [8]. Quantification is accomplished by double integration of the EPR second-derivative signal, which is then compared to a spectrum of a reference standard, typically a weighed amount of the stable radical such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) [8], or 2,2,6,6-tetramethyl-1-piperinyoxyl (TEMPO) [46].

The overall yield of radicals, as for all other smoke constituents, depends upon the design of the cigarette, which includes such parameters as the cigarette rod density and the amount of tobacco burned, filter type (including charcoal or not), the blend of tobacco varieties in the cigarette filler, paper porosity, filter ventilation, smoking protocol (volume, duration and number of puffs) etc [51]. Industry-wide reference cigarettes such as the 1R4F and 2R4F cigarettes have traditionally been employed for many investigations [52]. Pryor *et al.* reported ca. 10^{14} spin/puff (ca. 0.2 nmoles) for filtered gas-phase smoke from the 1R1 research cigarettes available at the time [8]. When commercial cigarettes having different construction designs are compared, it is customary to normalize the yield of radicals to the yield of cigarette "tar" (the TPM, less water and nicotine) [46, 51].

Because of the uncertainties presented by multiple experimental variables involved in quantitative EPR spin-trapping measurements, accurate quantification of gas-phase cigarette smoke radicals by EPR is problematic [38]. Baum *et al.* proposed a standard experimental protocol that can potentially yield more consistent results between various investigators [46]. Their analysis protocol is rooted in the established methods previously reported by Pryor and others, including the separation of the gas-phase from the particulate-phase with the Cambridge filter pad [7, 8, 10, 11]. Historically, the radical abundance reported from EPR measurements is typically reported in terms number of spins, i.e., unpaired electrons. This practice has at times created a kind of innumeracy and confusion in the literature regarding the abundance of radicals relative to other smoke constituents; most constituents are reported as nanograms or nanomoles. As alternative methods for quantifying radicals become available, it is preferable to report radical yields in nmoles so that comparisons can be made with other smoke constituents.

SMOKE AGING EFFECTS

In the initial studies by Pryor *et al.* on the spin trapping of filtered gas-phase smoke radicals, they reported a surprising and fundamental property of the filtered gas-phase radicals [8]. By varying the length of the delivery tubing between the Cambridge filter pad and the vessel containing the PBN trapping solution, they found that the maximum radical concentration was not measured for the shortest path length, but for a longer 60 cm path of tubing. Similar results were obtained with the DMPO trapping agent. In either case,

Table 1. Some Example First-Order (Except as Noted) Gas-phase Reactions and Rate Constants Related to Gas-phase Cigarette Smoke Reactions

Reaction			k (298 °K)	Reference[a]
$2NO$•	+ O_2	→ $2NO_2$•	$7.25x10^{3\ b}$	Atkinson *et al*., 2004
CH_3•	+ O_2	→ CH_3OO•	$1.08x10^{9}$	Atkinson *et al*, 1997
CO	+ CH_3•	→ CH_3CO	$4.96x10^{3}$	Baulch, *et al*., 1994
CH_3•	+ NO•	→ CH_3NO	$5.36x10^{9}$	Davies *et al*., 1994
HOO•	+ NO•	→ HO• + NO_2•	$5.3x10^{9}$	Atkinson *et al*., 2004
HO•	+ NO•	→ HNO_2	$1.99x10^{10}$	Atkinson *et al*. 2004
CH_3O•	+ NO•	→ CH_3ONO	$2.17x10^{10}$	Atkinson *et al*., 1997
CH_3OO•	+ NO•	→ CH_3O• + NO_2•	$4.39x10^{9}$	Atkinson, *et al*, 2001
CH_3O•	+ NO_2•	→ CH_2O + HONO	$1.81x10^{8}$	Atkinson *et al*, 1989
isoprene	+ NO_2•	→ products	$1.08x10^{2}$	Ohta, *et al*., 1986
1,3 butadiene	+ NO_2•	→ products	1.53x10	Jenkin *et al*., 2003
isoprene	+ HO•	→ products	$6.23x10^{10}$	Singh & Li, 2007
CH_4	+ HO•	→ CH_3• +H_2O	$3.78x10^{6}$	Atkinson *et al*., 1997
CH_3CH_3	+ HO•	→ CH_3CH_2• + H_2O	$1.45x10^{8}$	Atkinson *et al*., 2001
CH_3CHO	+ HO•	→CH_3CO• + H_2O	$9.02x10^{9}$	Atkinson *et al*., 2001
CH_3CHO	+ NO•	→ products	$5.6x10^{8}$	McDade *et al*., 1982
CH_3CHO	+ CH_3•	→ CH_3CO• +CH_4	$2.98x10^{3}$	Baulch *et al*., 1992 (300°)
CH_3CHO	+ CH_3CH_2•	→ $C_2H_5CH(CH_3)O$	$6.78x10^{2}$	Curran, 2006
CH_3CHO	+ CH_3CH_2•	→ C_2H_6 + CH_3CH_2CO•	$5.9x10^{3}$	Chatgilialoglu *et al*. [86].
CH_3CH_2•	+ O_2	→ CH_3CH_2OO•	$4.7x10^{9}$	Atkinson *et al*., 1997
CH_3CO•	+ O_2	→ $CH_3C(O)OO$(•)	$3.01x10^{9}$	Atkinson *et al*., 1997
CH_3CO•	+ CH_3CH_3	→ CH_3CHO + CH_3CH_2•	$1.15x10^{8}$	Tsang & Hampson, 1986
$CH_3C(O)OO$(•)	+ NO	→ $CH_3C(O)O$• + NO_2	$1.29x10^{10}$	Atkinson *et al*., 2001
$CH_3C(O)OO$(•)	+ NO_2	→ $CH_3C(O)OONO_2$	$7.23x10^{9}$	Atkinson *et al*., 1997

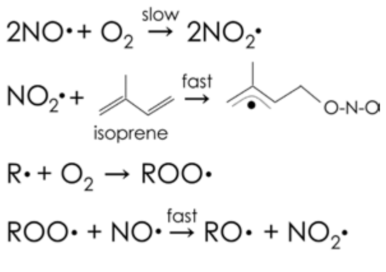
a. Kinetic rate constants are from the NIST Chemical Kinetics Database, http://kinetics.nist.gov/ (accessed January 2010) unless otherwise indicated. b. Third-order reaction.

predominantly oxy type radicals were detected. Later it was shown that the radical concentration in filtered gas-phase smoke continued to increase for aging periods up to one minute to five minutes! This result clearly presented a dilemma. The common consensus was that the carbon-centered radicals, created in abundance by combustion or pyrolysis inside the cigarette, were too reactive and short-lived to survive their passage through the cigarette rod. Either they oxidized to some type of oxy radicals or they quenched in terminating reactions with other smoke constituents not observable by EPR. Some oxy type radicals are more stable, but they are also reactive species that are expected to decrease in quantity and disappear within a few seconds. Thus, the observed plethora of radicals in aged smoke could not be accounted by combustion, but were apparently created primarily within the smoke plume by some continuous process of formation consistent with their improbably long lifetimes.

To explain the unlikely long-lived radicals in filtered gas-phase smoke, considerable chemical intuition, experimentation, and persistent investigations were required. The result was a mechanistic model, proposed by Pryor *et al.* in 1984 [7, 11], commonly referred to as the "steady-state mechanism," that is well familiar to smoke chemists worldwide. We provide a short summary of this work, which represents more than two decades of research. We shall argue, however, that modern analytical methods have raised serious questions regarding the applicability of the model to the fresh mainstream smoke inhaled by the smoker.

THE STEADY-STATE MECHANISM

To account for the apparently long-lived radicals in filtered gas-phase smoke, Pryor *et al.* postulated that the radicals were continuously created and destroyed by chemical reactions in the smoke plume [7, 11]. Thus, some oxidative species must combine with other smoke constituents to form radicals. Nitric oxide (NO), itself a radical, is a well-known oxidation product derived from the combustion of plant biomass and fossil fuels. Although it is not particularly reactive towards the organic constituents of cigarette smoke, it slowly oxidizes to the much more reactive species nitrogen dioxide (NO_2). The concentration of dienes, such as isoprene and butadiene, was known to be comparatively high in cigarette smoke [2, 4, 5, 42], and the reactivity of NO_2 towards such species is well known [9]. Pryor *et al.* proposed a mechanism whereby NO_2 forms in the smoke by the slow oxidation of NO and reacts rapidly with dienes such as isoprene, creating nitro-alkyl radicals as shown in Scheme **I**. Citing existing data that showed that NO_2 reacts rapidly with isoprene in the gas phase by addition to the double bond [53], the authors adopted NO_2 addition into their model, despite that they previously showed that NO_2 can react with olefins by allylic proton abstraction in very dilute solution (< 100 ppm) [9, 54]. In point of fact, allylic addition of NO_2 to isoprene in the gas phase is consistent with the observations of others [55-57].



Scheme I.

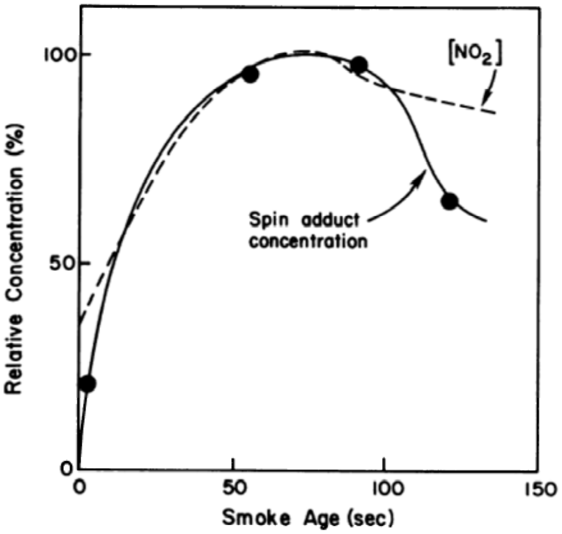


Fig. (2). Comparison of the time evolution of NO_2 in filtered-gas phase smoke and radical adduct formation in smoke bubble solutions containing the spin-trapping agent PBN. Church and Pryor 1985 [7].

In the third step, the nitro-alkyl radicals react rapidly with oxygen to form peroxyl radicals, consistent with well-known reaction kinetics (see Table **1**). In the final step, the peroxyl radicals, a class of highly reactive radicals, were postulated to react with the remaining NO to form additional NO_2, and to give the alkoxy radicals observed by EPR in the spin-trapping experiments. The NO_2 created in the final step becomes available to react with more smoke dienes, thereby creating a cycle that can persist until the supply of NO is depleted. Thus, the yield of alkoxy radicals should increase over a period of many seconds while the concentration of NO_2 remains relatively high.

Since the third step in the steady-state mechanism is simply the reaction of a carbon-centered radical with oxygen to form a peroxyl radical, whether the radical was formed by NO_2 addition or hydrogen abstraction might not affect the overall validity of the model, even if the product distribution changed, so long as the rate-limiting step was the formation of NO_2. Such mechanisms were considered. However, the requirement of very low NO_2 concentration for hydrogen abstraction to occur would likely diminish the importance of the steady-state mechanism relative to alternative pathways, which Pryor acknowledged were possible [7, 11, 54].

In their 1985 report, Church and Pryor showed that the amount of radicals sampled periodically from aging cigarette smoke approximately follows the time evolution of NO_2 appearing from the oxidation of NO in air, as depicted in the well known diagram shown in Fig. (**2**) [7]. The authors did not state how they estimated the NO_2 level, but it may have been based upon a kinetic model utilizing the known rate constants for the oxidation of NO in air. Subsequent investigations, however, showed directly by FTIR that the evolution of NO_2 with time in filtered gas-phase smoke follows a similar profile to the amount of radicals trapped in PBN spin-trap solutions [13, 14]. However, Baker [42], citing the results of Borland *et al.* [58], noted that the rate of oxidation of NO to NO_2 in whole smoke is slower than in filtered gas-phase smoke, and that the observed build up of radicals in whole smoke should be significantly slower than that depicted in Fig. (**2**). Nevertheless, the initial yield for NO (327 µg) reported by Cueto and Pryor in the whole smoke from a single 1R1 cigarette [13, 14] is close to the result reported by others for 1R4F cigarettes smoked under FTC conditions: 283-317 µg measured by chemiluminescence [3, 4, 59], 310 ± 0.02 µg by FTIR, and 290 ± 0.03 µg by tunable infrared laser differential absorption spectroscopy (TILDAS) [36]. For NO in the gas phase of unfiltered mainstream smoke, 240 ± 0.05 µg /cig was reported for 2R4F cigarettes measured by TILDAS [24] and 191 ± 9.3 µg /cig for 3R4F cigarettes measured by quadrupole mass spectrometer with combined chemical and electron ionization [6].

MODEL GAS SYSTEMS

Without the modern analytical instrumentation that is capable of isolating and identifying specific radical species, and thereby verifying and validating the model, Pryor and his colleagues performed additional EPR spin-trapping experiments on model gas mixtures that mimic the chemistry of reactions in filtered-gas phase smoke suggested by the steady-state mechanism. Two important related papers appeared. The first was a study of radical formation in a mixture of NO in air containing isoprene or other olefins [11]. Several radical spin-trapping strategies were employed: spin-trapping by bubbling the gas mixture or cigarette smoke into solutions of PBN in either benzene or CCl_4, and, for cigarette smoke, spin-trapping with PBN either coated on a solid silica-gel support or impregnated in a fiber-glass Cambridge filter pad. Of particular significance for the discussion below, in every case when cigarettes were smoked, the Cambridge pad was employed. The overall result of this study was, consistent with the steady-state model, that the NO/air/isoprene model system and filtered gas-phase smoke gave very similar types of radicals as found in filtered gas-phase smoke.

By each sampling strategy, high concentrations of both oxygen- and carbon-centered free radicals were detected. The relative proportion of carbon to oxygen-centered radicals was reported for each trapping method. Absolute quantities were not measured because quantification is time consuming and subject to errors for the reasons discussed by Church [38] and by Baum *et al.* [46]. The highest percentages of carbon-centered radicals were measured for the reference 1R1 cigarette gas-phase smoke trapped by PBN in benzene solution (30 %) or coated on silica gel (25%), and from the model NO/isoprene gas mixture when the gases were bubbled directly into a benzene/PBN solution (55 %). The NO_2/isoprene/air mixture gave a comparatively lower yield (5 %) trapped directly in a benzene/PBN solution. In accordance with the steady-state model, for a NO_2/air mixture without isoprene, the PBN/benzene trapping solution yielded a very low amount of radicals. No peroxyl spin adducts were observed, but, in any case, peroxyl radicals are highly transient in the gas phase (see Table **1**) and the trapping efficiency of PBN is inadequate for these radicals to be detected at low concentration.

A second report in 1994 by Cueto and Pryor [14] also supported the validity of the steady-state model. In this investigation, the relative gas-phase concentration was measured by Fourier transform infra-red spectroscopy (FTIR) as a function of aging time in cigarette smoke and in various model gas mixtures containing combinations of NO in air with isoprene, methanol, acrolein, and acetaldehyde, all abundant constituents in cigarette smoke [3-5, 42]. The FTIR method provided a method to monitor each of the gases in a mixture or in filtered gas-phase smoke simultaneously. With gas concentrations typical of those measured in whole cigarette smoke, a 20 cm single-pass gas cell, and a spectroscopic resolution of 4 cm^{-1}, their overall sampling rate was 7.5 sec. This sampling rate was sufficient to permit kinetic measurements and the calculation of rate constants. When cigarette smoke was compared to the NO-air system, the disappearance of NO in cigarette smoke was shown to be faster than in the NO-air system. However, the disappearance of NO and the appearance of NO_2 in a mixture of NO in air containing other smoke constituents, such as methanol and isoprene, followed time courses that more closely resembled cigarette smoke. Moreover, the time course for the production of NO_2 was similar to the rate of the development of organic free radicals in whole cigarette smoke, and gas phase IR absorption frequencies corresponding to nitro-alkyl groups were observed, supporting the formation of radicals by the steady-state mechanism.

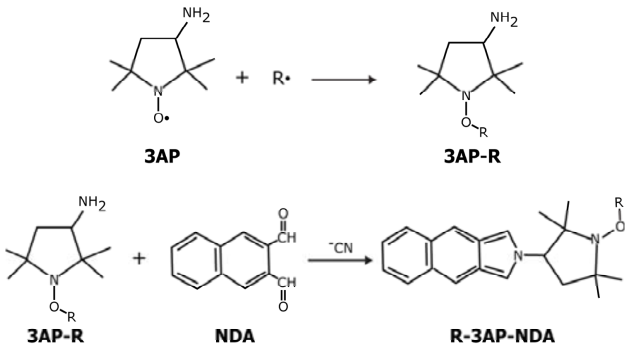


Fig. (3). 3-aminoproxyl (3AP) reagent for trapping of carbon-centered radicals in whole smoke followed by derivatization with a fluorophore (NDA) for HPLC/FLD detection. Flicker and Green, 2001 [27].

Interestingly, the authors reported that other combinations of gases, in particular those containing acetaldehyde and acrolein, did not lead to any significant improvement in their kinetic comparison with cigarette smoke. They analyzed the differential spectral kinetic profiles from the spectral region corresponding to the carbonyl absorption and found very low reactivity for any carbonyl-containing components found in gas phase cigarette smoke. The implications of the absence of a kinetic affect due to acetaldehyde and acrolein in the study by Cueto and Pryor are uncertain, but their results are worth noting since acetyl radicals have recently been identified and measured in abundance, and radicals from acrolein, also abundant in smoke, have also been detected [28, 40, 41].

Cueto and Pryor reported that the maximum yield of NO_2 measured by FTIR was ca. 50-60 μg/1R1 cigarette [14]. They employed a continuous flow smoking protocol, performing calculations to adjust their data to that of the conventional per puff procedure. The smoke sample was pulled from the cigarette through a Cambridge filter pad and into a gas cell at 250 mL/min and the smoke was trapped in the cell 90 sec after the cigarette was lit. The scanning was begun when the cell was closed and required 7.5 sec for collecting 5 scans at 4 cm^{-1} resolution. A correction factor was applied to the final NO_2 yield to account for the difference between the continuous flow and the puff protocols. Not withstanding this correction, within the cigarette industry where standard smoking protocols are used [23], such a sampling procedure is never employed because this mode of sampling disrupts the normal partitioning of smoke constituents between mainstream and sidestream smoke, and alters the usual pathways for NO formation as well as the overall smoke chemistry. Recent measurements of NO_2 using more sensitive and rapid spectroscopic methods [37] have shown that the amount of NO_2 in fresh smoke is significantly less than the amount of NO_2 in smoke aged many seconds as measured by Cueto and Pryor.

RADICAL SCAVENGING BY NITROXIDES

While the many investigations by Prof. Pryor are notable for their depth and breadth, others have also developed methods for detection and quantification of cigarette smoke radicals. In 1998, Flicker and Green reported a novel solid-phase trapping method designed to specifically trap and quantify carbon-centered radicals in fresh whole smoke, the presumed intermediate reaction product between NO_2 and smoke dienes in the steady-state model [26, 27]. Their method introduced a new solid-phase trapping method that avoids the partitioning of phases with the Cambridge filter pad, and an alternative radical trapping agent [31] that offers a solution-free approach to radical trapping. Using FTC smoking conditions, whole smoke from a commercial cigarette was allowed to pass through a tube packed with 3-mm glass beads coated with 3-aminoproxyl (3AP), a nitroxide radical that forms a stable, non-radical adduct with carbon-centered radicals, as shown in Fig. (**3**). 3AP selectively reacts at a diffusion-limited rate with carbon-centered radicals to form stable diamagnetic adducts. The scavenged radical adducts were derivatized with a fluorophore, naphthalene dicarboxaldehyde (NDA). The 3AP-NDA radical adducts were separated by HPLC and detected and quantified with high sensitivity with a fluorescence detector (FLC). Separate smoking experiments were required using appropriate blanks without 3AP or NDA to eliminate false positive artifacts. At least 8-10 carbon-centered radical candidates were resolved and quantified in the HPLC chromatogram from the whole smoke. However, neither this approach, nor the established EPR methods, afforded actual identification of specific radicals, a limitation acknowledged by all previous investigators.

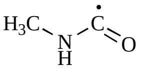
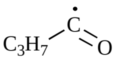
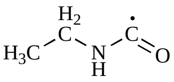
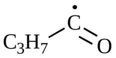
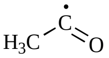
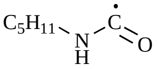
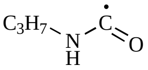
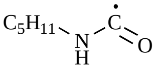
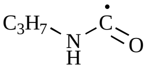
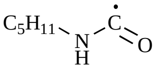
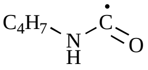
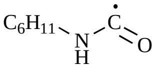
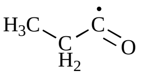
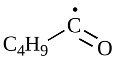
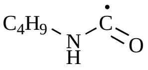
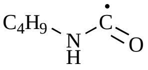
After adjusting for potential artifacts, Flicker and Green reported 54-66 nmoles of carbon-centered radicals per cigarette for two commercial brands. This amount compares with a yield of radicals of 17 nmol from one 1R1 research cigarette, using PBN in benzene reported by Pryor *et al.* [8]. Their estimate employed the entire fluorescence chromatogram, which may not have fully eliminated all the false positive radical candidates. Since the authors did not employ a standard research cigarette, such as the 1R1 or 2R4F cigarettes, the two sets of results are not immediately comparable, even allowing for differences in trapping methods. As noted earlier, the yield of NO and radicals from different commercial cigarettes depends on a variety of design factors [51]. The yield of NO varies significantly between different commercial brands (ca. 30-300 μg/cig) under the same machine smoking conditions, depending on the cigarette design [4]. If the results for commercial cigarettes are normalized to tar delivery, comparisons between brands become more objective. Despite the issues and concerns that we have raised, our own measurements [40, 41] of radicals in fresh mainstream smoke from 2R4F research cigarettes were higher than the earlier estimates of either Flicker and Green [26, 27] or Pryor *et al.* [10, 11], but, as noted by Flicker and Green [26], comparisons of data obtained by different analytical methodologies are often problematic in any case.

Flicker and Green illuminated their discussion on the formation of peroxy and alkoxy radicals by describing the chain reactions that are likely to additional carbon-centered radicals by either radical addition or proton abstraction reactions [27]. Based on these considerations and kinetic arguments, they suggested that the actual overall yield of radicals (carbon or oxygen-centered) is likely to be significantly larger than the amounts trapped by 3AP because the carbon-centered radicals rapidly react with oxygen and other smoke constituents in competition with 3AP to form oxygen-centered radicals and non-radical products [25, 27]. Though their estimate of trapping efficiency and yield has not been experimentally confirmed, the high reactivity of carbon-centered radicals towards molecular oxygen is well known (see Table **1** for a variety of relevant gas-phase rate constants).

IDENTIFICATION AND QUANTIFICATION BY MASS SPECTROMETRY

In 2005, Bartalis *et al.* modified the original Flicker and Green method with the goal to obtain the long missing mass spectrometric data to identify individual radical species in whole smoke [40, 41]. HPLC separation was combined with high-resolution mass spectrometric methods to identify for the first time specific whole smoke radicals and to quantify the carbon-centered radicals in the gas-phase of whole smoke [30]. One important innovation was to employ another nitroxide trapping agent, 3-cyanoproxyl (3CP). Like 3AP, 3CP reacts with a favorable rate constant specifically with carbon-centered radicals to form stable diamagnetic adducts, and it eliminates the requirement for derivatization with a fluorescence agent, which is not required for detection by mass spectrometry. Also, 3CP naturally avoids the potential artifacts of 3AP, which can react with carbonyl or carboxyl compounds in smoke, and NDA, which can react with amines; eliminating fluorescence detec-

Table 2. Precise Mass, Chemical Structure and Number of Exchangeable Hydrogen for Carbon-Centered Radicals in Smoke from 2R4F Cigarettes

No.	Formula R·	H[a]	Delta[b] 3AP-R (ppm)	Delta 3CP-R (ppm)	Mass[c] R· (amu)	No.	Formula R·	H	Delta 3AP-R (ppm)	Delta 3CP-R (ppm)	Mass[c] R· (amu)
1		1	0.370	0.398	58.029	8		0	0.087	0.293	71.049
2		1	0.348	0.375	72.044	13		-	n.d.	0.334	71.049
3		0	0.398	0.284	43.018	9		1	0.404	0.390	114.091
4		1	0.491	0.236	86.060	10			0.147	0.248	114.091
11			n.d.	0.275	86.060	14			n.d.	0.213	114.091
12		1	n.d.	0.225	98.060	15		-	n.d.	0.306	126.091
5		0	0.279	0.266	57.033	16-18		0	n.d.	0.711	85.065
6		1	0.465	0.298	100.076						
7			0.426	0.224	100.076						

a. Number of active hydrogens determined by mass spectrometry by deuterium labeling. b. Relative deviation in ppm between the experimentally determined and theoretical molecular masses of radical adducts. c. Mass of radical calculated from the theoretical mass of radical adducts determined by FTICR.

tion avoids interference from naturally fluorescent constituents in smoke. Two types of radicals were identified: acyl and alkylaminocarbonyl (carbamoyl) radicals (Table **2**). The results are surprising because these radicals cannot easily be attributed to the long-accepted steady-state mechanism, which otherwise appears to describe the radicals in aged filtered gas-phase smoke adequately.

Beginning with a similar glass bead trap, 3AP trapping agent, and NDA derivatization as Flicker and Green, but smoking the reference 2R4F cigarette under FTC conditions, Bartalis *et al.* used HPLC/FLD analysis of the 3AP-NDA radical adducts in smoke condensate recovered from the glass beads to identify and quantify six radicals. The unambiguous detection of the three most abundant NDA-3AP radical adducts by HPLC/FLD-MS afforded the mass units of these individual radicals. Additional radicals could not be detected because of interferences from smoke 3AP and NDA related impurities. However, with the knowledge of these specific masses, subsequent analyses by HPLC-MS/MS of the 3AP-R adducts without NDA derivatization and duplicate analysis using 3CP permitted the detection and quantification of 18 radical species directly by mass spectrometry (Fig. **4**).

For radical identification, electrospray ionization tandem mass spectrometry (ESI(+)-MS/MS) was used to screen derivatized smoke samples for radical adducts. Precursor ion monitoring (PIM) allowed rapid monitoring of radical-related ions in the cigarette smoke samples by direct infusion into the ESI(+) source [30]. Highly selective spectra were obtained for 3AP-R (m/z 98) and 3CP-R (m/z 151) samples, respectively. Smoke samples were infused in either normal or deuterated 5% aqueous methanol (5% CH_3OD in D_2O) and 0.1% formic acid. The deuterated solvent was employed to determine number of active hydrogens for each MH^+ and product ion by isotopic exchange. Large numbers of carbon-centered radicals were discovered: the m/z of the most intense molecular ions for radicals were 43(0), 57(0), 58(1), 72(1), 100(1), and 114(1), the number in parentheses is the number of active hydrogens detected. The 3CP-R samples confirmed the nature of each 3AP-adduct as radical-related, and not products of the 3AP-amine group with smoke constituents.

The 18 carbon-centered radicals identified by HPLC-MS/MS in mainstream whole smoke are listed in Table **2**. The structures of the acetyl and methylaminocarbonyl radicals were confirmed by chemical synthesis, *ab initio* calculations, and NMR spectroscopy. The 3CP adducts with methylaminocarbonyl, 3CP-C(O)$NHCH_3$, were synthesized and found to match the LC-MS/MS characteristics of the most abundant carbamoyl radical, 3CP-1 in Table **1**. For quantitative analysis by mass spectrometry, the response factors for alkylaminocarbonyl and acyl radicals relative to an internal standard were based on the concentration of either the methylaminocarbonyl or the acetyl radical, determined in appropriate smoke samples by HPLC/FLD. HPLC-MS/MS allowed the relative measurement of radical concentration in the whole vs. filtered gas-phase

Table 3. Relative Yield of Acyl Radicals Compared to their Parent Aldehyde Molecules from in Filtered Gas-Phase Smoke from 1R4f and 2R4F Research Cigarettes

	Radicals	**Aldehydes**		
Parent Species	2R4F[a]	2R4F[b]	1R4F[c]	2R4F[c]
acetaldehyde	1.00	1.00	1.00	1.00
propionaldehyde	0.06	0.074	0.06	0.078
butyraldehyde	0.03	0.041	0.03	0.053

[a] Bartalis *et al.* 2007
[b] Counts *et al.* 2005
[c] Chen and Moldoveanu, 2003

smoke. Precise mass measurement were made by linear ion trap Fourier transform ion cyclotron resonance mass spectrometry with electrospray ionization ESI(+)-LTQ-FTICR-MS. The individual HPLC fractions of 3AP-R and 3CP-R smoke samples (Fig. **4**) were subsequently evaluated by direct infusion into the mass spectrometer.

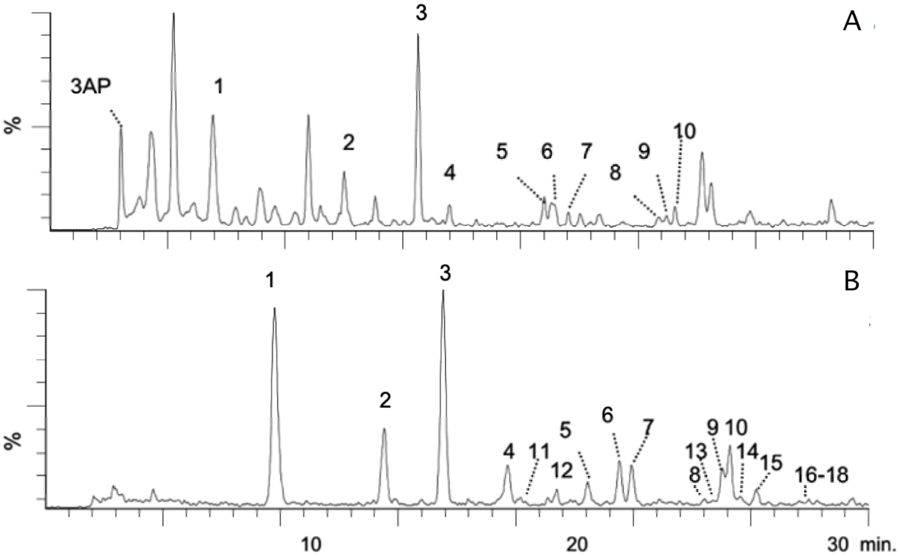


Fig. (4). Representative chromatograms of radicals detected by MS/MS by precursor ion monitoring in fresh mainstream smoke of 2R4F cigarettes trapped with the nitroxides (A) 3AP or (B) 3CP. Numbers correspond to radicals in Table **2**. Bartalis *et al.* 2009 [41].

FACTORS AFFECTING RADICAL YIELDS

The yields of the ten most abundant radicals from 2R4F cigarettes and cigarettes from single tobacco types are shown in Fig. (**5A**) for whole smoke. Radicals 1-3 were the most abundant, representing 87% of the total yield. Cigarettes containing more cellulose such as 100% Oriental or baseweb (tobacco extracted with hot water) cigarettes exhibited the largest amounts of acyl radicals. Pryor *et al.* also previously measured significant amounts of both carbon and oxygen-centered radicals from 100 % cellulose cigarettes using PBN/EPR spin-trapping [21]. The yield of the acyl radicals dropped 80-90% in filtered gas-phase smoke in comparison to whole smoke, while the relative yield of carbamoyl radicals dropped by 50-60%, with the exception of radicals from oriental tobacco, for which the abundance dropped by only 27% (Fig. **5B**). When smoke samples were collected in inert Kynar tubing and aged for 1 min before collection, the yield of carbon-centered radicals was dramatically reduced, comparable to the reduction effected by the Cambridge pad [41].

OTHER RECENT RADICAL MEASUREMENTS

Others have also contributed to the broader understanding of radicals in cigarette smoke. Two research groups have shown the importance of using different spin-trapping agents for trapping cigarette filtered-gas phase radicals. Gosh *et al.* used a variety of spin traps including PBN, DMPO, DEPMPO, and DPPH-PBN [43, 44], and others have employed POBN, BMPO, and DIPPMPO, among other EPR spin-trap reagents [34, 39, 60]. Gosh *et al.* made the interesting and significant observation that, as the TPM accumulates from puff-to-puff on the Cambridge pad, the type of radicals trapped by PBN tends to shift from carbon-centered to oxygen-centered radicals [43]. Although the authors make the plausible suggestion that this shift results from the changes in smoke chemistry that occurs as the cigarette is consumed, we make an alternative proposal below that chemistry occurring on the Cambridge pad can influence the type of radicals detected in filtered gas-phase smoke. Robinson and Dyakonov [34], using the nitrone spin-trap BMPO, showed by EPR that the amount radicals per cigarette decreases as the interval between puff is increased while the g-values of the radicals shift, indicating that smoking conditions affect the radical yield and type. Both Robinson and Dyakonov [35] and Gosh *et al.* [44] found only small variations in radical yield for individual puffs from a single cigarette. Interestingly, both groups found that PBN, the most widely employed reagent used for trapping smoke radicals, was the least efficient at trapping oxygen-centered radicals compared to several alternative nitrone type trapping agents.

Robinson and Dyakonov [34, 35] were the first group to report the use of tandem mass spectrometry with parent ion monitoring to identify specific radicals in filtered gas-phase smoke. The radicals detected appear not to be formed by Pryor's steady-state model, primarily because none of the radical adducts contained nitro-alkyl groups and none appear to be derived directly from the abundant dienes in smoke such as isoprene and 1,3-butadiene. However, the

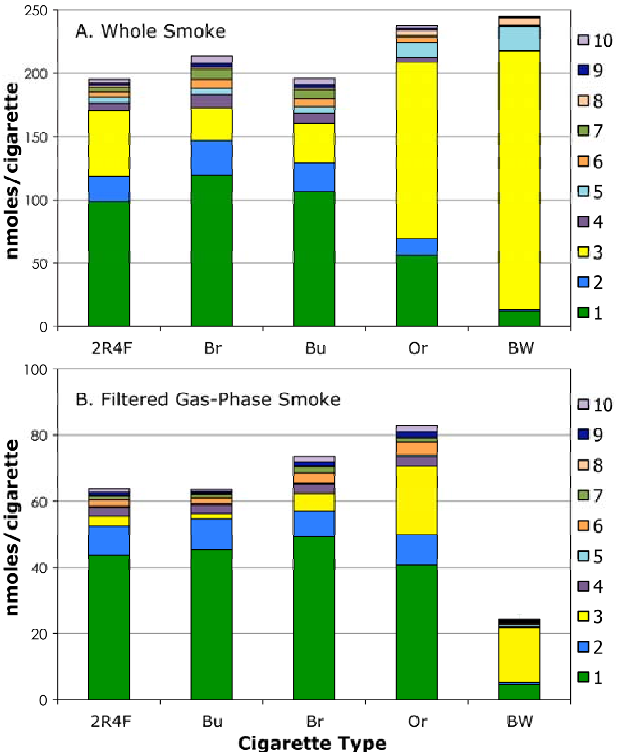


Fig. (5). Radical yields for smoke from single-component and blended cigarette samples in nanomoles per cigarette. (**A**) Radical yields in whole smoke. (**B**) Radical yields in filtered gas-phase smoke. The green bars represent the methylaminocarbonyl radicals and yellow bars represent the acetyl radical, the most abundant of the acyl and carbamoyl radicals, respectively. Numbers correspond to radicals in Table **2**. Bartalis *et al.* [41].

propensity of radicals to undergo rapid re-arrangements, recombination, and termination reactions leaves the origin of these radicals open to debate. Among the radicals indentified were seven alkoxides, one amide, one amine, and two phenoxides. At the current stage of investigations, it is difficult to evaluate the implications for smoke chemistry or the impact for the smoker, but this is an important first step. The results should also help to resolve the issues surrounding potential artifacts of measurement resulting from the use of the Cambridge filter pad, as discussed below.

Gerardi and Coleman [28, 29], also using the nitroxide radical traps 3-AP, and 3-CP, LC-MS/MS or GC-MS/MS with precursor ion monitoring, but with a different solid-phase trapping approach, observed the same acyl and alkylaminocarbonyl radicals in whole smoke reported by Bartalis *et al.* with a very similar overall yield (265 nmoles/2R4F cigarette compared to the previous 168-245 nmol/cigarette). In addition, they tentatively identified new carbon-centered radical species in whole smoke including formyl, crotonyl, acrolein, aminocarbonyl, and anilinocarbonyl radicals, as well as detecting methyl radicals previously identified by others by EPR spin-trapping.

In the interest of efficiency and sample throughput, Gerardi and Coleman continued to use the Cambridge pad, but the nitroxide spin trapping agent was saturated directly in the pad. The coated filter pad was placed in a holder directly aft of the smoking port on the smoking machine. Filtered-gas phase measurements of carbon-centered radicals were made with an unmodified Cambridge filter placed at the smoking port followed by a second pad coated with the nitroxide spin trap. A similar decrease in yield in carbon-centered radicals was reported in the filtered gas-phase smoke compared to whole smoke as previously observed [40, 41]. Currently, the reason for the difference in the types of some of the radicals trapped by the saturated Cambridge pad method compared to the glass bead approach has not been investigated; nevertheless, the unique types of radicals reported by Gerardi and Coleman are significant since they correspond to known unsaturated aldehydes that are abundant in smoke including formaldehyde, acrolein and crotonaldehyde. A third approach by Gerardi and Coleman was to use electrostatic precipitation to remove the particulate phase and pass the gas phase through a series of tandem impingers containing the 3-CP solution, but the 3CP saturated Cambridge pad was regarded as more reliable for quantitative comparison between radical species. This result is notable because it implies that active chemistry on the Cambridge pad did not alter the types of radicals identified.

NO_2 MEASUREMENTS AND SMOKE AGING

Formation of NO_2 is the rate-limiting step in the steady-state model. Before the FTIR report of Cueto and Pryor [14], others had studied the oxidation of NO in air and in cigarette smoke using the methods generally available at the time. The earliest references noted a scarcity of NO_2 in fresh mainstream smoke as well as its high reactivity. In a 1968 review, Stedman [61], citing Newsome *et al.* [62], stated that NO_2 is either absent or present only in trace amounts and that NO_2 can only be detected if a delay occurs between smoke collection and analysis, thus permitting oxidation of NO.

An early *in situ* smoke measurement by FTIR made by Vilcins and Lephardt addressed the question of whether methyl nitrate and NO_2 are present in fresh smoke or form during smoke aging during the period after smoke generation, but prior to the smoke analysis [63]. The "real-time" smoke experiments used the 5th puff simultaneously collected through Cambridge filter pads from four 100% burley cigarettes with 4.1% nitrate added to increase the reaction rates of methyl nitrite. The scan time per spectrum on the FTIR spectrometer, set at an 8 cm^{-1} spectral resolution, was less than 1 sec. This provided spectra every 3.5 sec as the levels of NO, NO_2, CH_3OH, and CH_3NO_2 changed during the smoke aging process for 2 to 3 minutes in the 10 cm path length, 140 cm^3 volume gas cell. With a detection limit for methyl nitrite of ca. 2 µg/cigarette, no methyl nitrite was found in filtered gas-phase smoke less than 50 sec old. This report was among the first to suggest, based on experimental observations, that measurements made on aged smoke are not representative of fresh cigarette smoke. Nevertheless, the known formation of methyl nitrite in aging cigarette smoke apparently led Pryor and Cueto to add methanol to their model system of NO, air, and isoprene to obtain closer agreement between the reaction kinetics in their model gas mixture and filtered gas-phase cigarette smoke. Their first measurement was at 20 sec, which is sufficient time for CH_3NO_2 to form and a greater amount of NO_2 to form than is present in the fresh whole cigarette smoke inhaled by the smoker, where the realistic aging time is at most 1-2 sec depending on the individual's smoking behavior. Flicker and Green also employed the NO/isoprene/methanol mixture in air [27].

In 1983, Norman *et al.* reported an investigations of nitrate levels and NO and NO_2 analysis in filtered gas-phase smoke using a chemiluminescent analyzer [64]. No significant quantity of NO_2 was found in either mainstream or sidestream smoke when the analyzer was used in the NO_x mode. The authors sampling system was designed to minimize both the dead volume and the time between smoke generation and analysis. They estimated that ca. 5% of the combustion generated NO could react with oxygen within 1 minute inside the tobacco column to form NO_2 with the oxygen that diffuses into the tobacco rod through the wrapping paper. The oxygen level was estimated to increase from ca. 1 % immediately behind the burning cone to about 12 % at the exit, especially during the initial puffs. Nevertheless, no NO_2 was detected, but their detection limits were not provided.

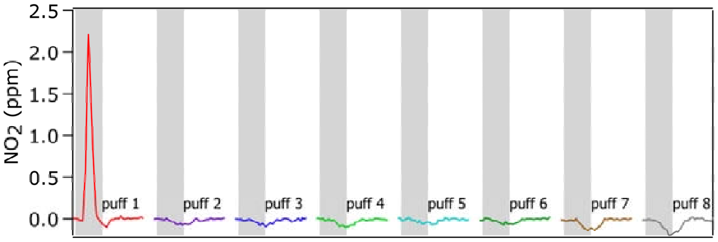


Fig. (6). Puff by puff monitoring of NO_2 in the fresh mainstream whole smoke from a 2R4F cigarette, without a Cambridge filter pad, measured by QC-TILDAS (35 ml puff, 2 s duration, 60 s between puffs, lit using a match). The trace for each puff is 5.2 s with the shaded region reflecting the 2 s puffing period. The lapsed time between puffs (60 s) is not shown. Shorter *et al.* 2006 [37].

TUNABLE INFRARED LASER DIFFERENTIAL ABSORPTION SPECTROSCOPY

From 1980 until 2007, when the program was discontinued, a dedicated effort was made by the cigarette industry to refine and optimize the spectroscopic methods for measuring NO, NO_2 and other gases in whole mainstream smoke and in sidestream smoke. Tunable infrared laser differential absorption spectroscopy (TILDAS) was developed for detecting combustion product molecules having resolvable rotational-vibrational structure in the mid-IR spectral region. Using TILDAS, certain gas-phase constituents in whole cigarette smoke can be measured effectively in real time, with a very substantial improvement in selectivity and sensitivity compared to conventional Fourier transform infrared (FTIR) spectroscopy.

TILDAS using conventional lead-salt tunable diode infrared lasers provides high sensitivity, selectivity, and a fast time response. For example, TILDAS can achieve 0.001 cm^{-1} spectral resolution, ppb detection limits, and millisecond temporal resolution using a collimated lead-salt diode laser and a multiple-pass gas cell with an effective path length as great as 58 m, and 300 ml volume. Previously, conventional FTIR spectrometers typically employed measured the entire mid-infrared smoke spectrum simultaneously at 16 cm^{-1} spectral resolution in the millisecond time domain using a single-pass gas cell of 0.08 m path length and 0.25 ml volume. Modern FTIR spectrometers are capable of higher spectral and temporal resolution, but still do not afford equivalent resolution or sensitivity to TIDLAS. The latest TILDAS instruments employ sophisticated quantum cascade lasers with improved stability and equipped with 36 M multiple pass gas cells. The concentration of multiple gasses such as ammonia, ethylene, NO, and carbon dioxide can be quantified simultaneously at a data-sampling rate of 20 Hz, while a dual gas cell arrangement allows the mainstream and sidestream smoke to be sampled simultaneously. This extraordinary capability provides sufficient time resolution to provide concentration profiles with multiple sampling during each 2 sec puff [24, 36]. Mantz has summarized the history of the development of QC-TILDAS by Aerodyne Research Inc. in collaboration with cigarette industry scientists (see [32] and references cited therein).

In 2006, Shorter *et al.*, using TILDAS equipped with a quantum cascade (QC) laser, reported that there is significantly less NO_2 present in whole smoke than reported in any previous measurement [37]. The combination of the direct whole smoke sampling system and the temporal response of the spectral analysis allowed the time of the initial sampling of fresh smoke to be reduced to ca. 0.1 sec. Overall, the fresh whole smoke from a single 2R4F cigarette under the FTC smoking protocol produced 9 ± 3 ng (n=6) in the first puff using an electric lighter. Very surprisingly, no additional NO_2 was detected continuously in the following puffs, as shown in Fig. (**6**). NO_2, however, was detected continuously in the sidestream smoke. The detection limit of this experiment was ca. 1.0 ppbV or 0.2 ng/35 ml puff [36, 37].

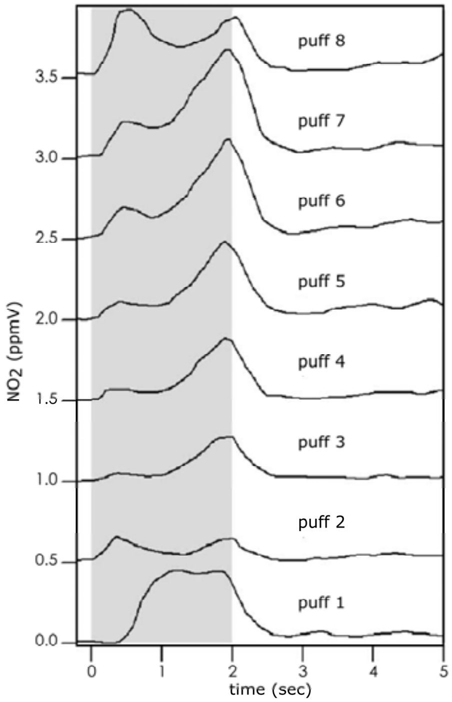


Fig. (7). Concentration of NO_2 vs. time for each puff from a 2R4F Kentucky Reference cigarette using a Cambridge filter pad monitored by QCL-TILDAS. The puff duration was 2 s and the puff interval was 60 s. The shaded region represents the duration of the puff as drawn by a smoking machine. Each puff profile is offset from the previous puff by 0.5 ppmV. The lighting source was a blue flame. Shorter *et al.* 2006 [37].

Much of the NO_2 measured in the first puff of a cigarette was shown to come from the cigarette lighting source rather than the burning tobacco. The amount of NO_2 generated in the lighting puff is greater if a match or blue flame lighter (29±21 ng) is used to light the cigarette than when using an electric lighter (9±3 ng). A simple demonstration of holding the ignition flame in front of the cigarette while continuously monitoring NO_2 showed that NO_2 from the ignition source appears at the filter end of the cigarette during puffing whether the cigarette is lit or not [37].

More surprisingly, and significantly for the current discussion on radicals, the measurements on mainstream smoke during machine smoking were repeated using a Cambridge filter pad and NO_2 was observed in every puff, yielding a total of 200 ± 33 ng/cigarette (n=10) for 8 puffs or 25 ng/puff. Most of the NO_2 was observed during each 2 sec puff rising and falling in a bimodal manner, but dropping to baseline levels in between puffs. This latter observation led the authors to the remarkable conclusion that most of the NO_2 generated in standard machine smoking experiments utilizing the Cambridge filter pad is probably formed on the pad between puffs. Both maxima in the evolution profile of NO_2 increase from puff to puff, as shown in Fig. (**7**), approximately tracking the accumulation of TPM on the pad. This behavior implicates two potential separate or coupled processes occurring on the pad during a puff that can generate NO_2. Unfortunately, no simultaneous measurements of NO and NO_2 together with other species relevant to the formation of organic radicals, such as isoprene or butadiene, were made in cigarette smoking experiments before this research program was discontinued. However, independent measurements of NO in the gas phase of whole smoke on a puff-by-puff basis have been made [6, 24].

Another long-standing issue addressed by real time TILDAS measurements of NO_2 in cigarette smoking experiments was whether NO_2 forms at the cigarette coal, but is either adsorbed or reacts inside the tobacco rod. Norman and Keith suggested in 1965 that the hot, oxygen deficient reducing environment just behind the cigarette coal favors NO formation and that any NO_2 that forms by oxidation of NO inside the coal reacts with the tobacco or water and other smoke constituents before exiting the cigarette rod [62]. This conjecture might be tested in TILDAS experiments by inserting a bypass tube through the center of the cigarette rod. In this sampling arrangement, some of the nascent NO_2 is prevented from coming into contact with the tobacco shreds or the humid environment inside the cigarette rod. Currently, no one has reported NO_2 to be emitted from the filter-tip end of unmodified cigarettes, leaving combustion generated NO_2 unaccounted [37].

RE-THINKING THE MODEL

In their seminal paper of 1985, Church and Pryor elaborated upon their state-state model but observed that "cigarette smoke is such a complex mixture of species that there are almost certainly other reaction pathways that could be responsible for radical production. Nevertheless, the concept of a steady-state and continuous radical production through the reaction of metastable species would have to be similar to that demonstrated for the NO/air/isoprene system" [7]. While an alternative, parsimonious model for the formation of gas-phase radicals in whole smoke may not be forthcoming, and multiple processes may be active, certain inferences and testable conjectures can be made based on the many experimental reports on cigarette smoke radicals published to date.

Two major questions have been evoked: what is the origin of NO_2 in filtered gas-phase smoke, if not by oxidation of NO by air within cigarette smoke, and what chemical process can account for the highly reactive acyl and carbamoyl radicals, since they do not appear to be the product of the reaction of NO_2 and smoke dienes, but are nevertheless found to be abundant in fresh whole smoke. The latter issue is not unlike the dilemma that first prompted the formation of the steady-state model to explain the persistence of the normally short-lived radicals in filtered gas-phase cigarette smoke. A metastable species, however, may not be required to explain the short-lived radicals, since a continuous process of radical chain reactions, re-arrangements and termination reactions involving oxygen, NO, carbon and oxygen centered radicals, and other reactive and nitrogen species likely can account for the current observations.

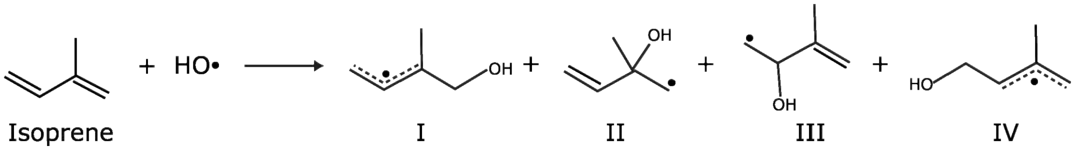
NO_2 FORMATION ON THE CAMBRIDGE FILTER PAD

The TILDAS results of Shorter *et al.* showed that NO_2 forms on the Cambridge pad during machine smoking, apparently by two distinct or coupled processes. Here we propose an example process, based on well-known smoke chemistry, which may account for part of the NO_2. The condensate that collects on the Cambridge pad, as well as the fresh smoke aerosol particles, contain various polyphenols, the two most abundant being catechol and hydroquinone [2-5, 10, 64, 65]. A significant part of the radicals in the particulate phase is well known to originate from these substances and the chemistry has been thoroughly investigated by Pryor and others [7, 10, 66-69].

The mildly reducing properties of cigarette tar have been attributed primarily to the semiquinone radicals that form by the autooxidation (or other mechanisms) of the above dihydroxybenzenes after the nascent smoke condensate is initially exposed to air [7, 10, 68]. The semiquinone radicals in the fresh air-exposed particulate phase can reduce ambient molecular oxygen to the superoxide radical anion ($O_2\bullet^-$), which has been detected in numerous EPR spin-trapping experiments in aqueous solutions of fresh [22, 45, 69], though apparently not in whole smoke aged at least three minutes [70]. The latter result by Tanigawa *et al.* has frequently been over looked, but it may be a key observation. Apparently, the presence of copper ions in the particulate aerosol can act as an SOD mimetic and eliminate the superoxide from the filtered gas-phase smoke [73, 90-92]. Superoxide catalytically undergoes dismutation in the presence of water and metal ions to give hydrogen peroxide [7, 71, 72]. Trace metal ions in smoke such as Fe^{2+} and Cu^{+1} can then catalyze the formation of the highly reactive hydroxyl radical *via* the well known Fenton reaction [12, 71-77].

We suggest that the hydroxyl radicals generated by the above process on the Cambridge pad during machine smoking can promote the formation NO_2 in a continuous process similar to the steady-state mechanism, probably by various pathways. In atmospheric chemistry, hydroxyl radicals are known to react rapidly with isoprene by addition to form four isomeric radical isomers, as shown in Scheme **II** [78]:

The initially formed carbon-centered radicals react rapidly in turn with ambient oxygen to form hydroxyperoxy radicals. In the presence of moderate amounts of NO, these radicals yield new alkoxy radicals and thereby form NO_2. The fate of the resultant alkoxy radicals is predicted to involve a competition between isomerization, decomposition, or reaction with oxygen to yield a variety of radical and other products [79]. Interestingly, the miscellaneous products proposed for this sequence include methylvinyl ketone, methacrolien, formaldehyde and other compounds that may undergo further reactions to produce some of the carbon-centered radicals that have been identified recently [28], but not otherwise observed by Bartalis *et al.* [41]. Notably, however, similar byproducts have been also observed from the oxidation of isoprene by NO_2 [57].



Scheme II.

North *et al.* have proposed that, in the atmosphere, under moderate concentrations of NO, large O_2 concentrations, and products of dienes and hydroxyl radical, NO_2 can form continuously while regenerating hydroxyl radicals in a cyclic process as shown in Fig. (**8**) [80]. A similar process may, in principle, occur in machine smoking experiments as smoke is drawn through the Cambridge filter pad, since all the necessary species are present on the TPM coated pad. Whether hydroxyl recycling occurs or not, the reaction of hydroxyl radicals with isoprene and other dienes is likely be confined to the Cambridge filter pad since hydroxyl radicals are so

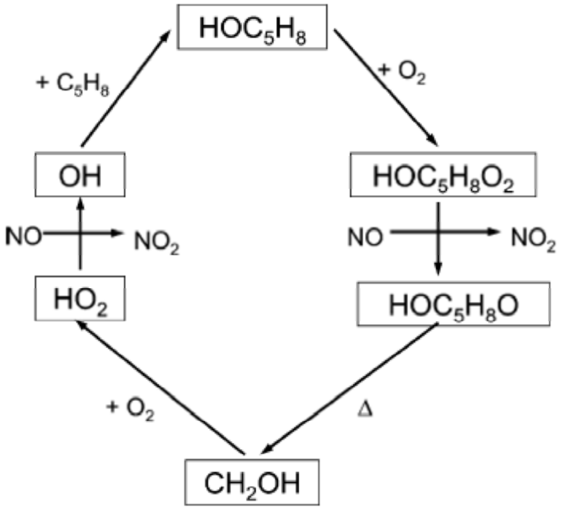


Fig. (8). Hypothetical scheme taken from atmospheric chemistry for NO_2 formation in filtered gas-phase smoke involving oxidation of isoprene by hydroxyl radicals. McGivern *et al.* 2000 [78].

highly reactive that they are exceedingly transient (see Table **1**), available for reaction only in the vicinity of their originating source, i.e., the moist particulate condensate collecting on the pad, or even possibly within the smoke aerosol particles themselves. The NO_2 that is generated on the pad may also in principle participate in the oxidation reactions; however, no nitro-alkyl moieties in trapped radical adducts have yet been identified by LC-MS/MS in filtered gas-phase smoke using a variety of oxygen-radical-specific spin traps, and the fate of the NO_2 is currently not known, although we recall that nitro-alkyl groups were detected by FTIR by Cueto and Pryor [13, 14], which can in principle be radical re-combination products.

THE ROLE OF HYDROGEN PEROXIDE

Hydrogen peroxide is a non-radical reactive oxygen species that can undergo catalytic disproportionation to form hydroxyl radicals [12]. The presence of hydrogen peroxide in the TPM, which has been measured previously by slower fluorometric assays [71, 72, 81], was recently measured electrochemically in real-time puff-by-puff measurements that compared H_2O_2 yields in either whole smoke and filtered gas-phase smoke bubbled into phosphate buffered saline (PBS) solution [82]. Quantitative measurements were made using an amperometric detector with an electrode designed with a membrane specific for hydrogen peroxide. In Fig. (**9A**) the instantaneous accumulation of hydrogen peroxide measured in PBS solution bubbled with fresh whole smoke from a single cigarette smoked by the conventional 2 sec puff, 60 sec puff interval (FTC protocol). For a hand made cigarette containing only bright tobacco inside a conventional cigarette rod, fresh whole smoke yielded significantly more hydrogen peroxide than filtered gas-phase smoke using the Cambridge filter pad, Fig. (**9B**). Similar results were obtained with other tobacco types and blends. The constant delivery of H_2O_2 from puff-to-puff for whole smoke bubbled into PBS is expected, since the polyphenolic smoke constituents (hydroquinone, catechol, etc.) in the particulate phase undergo rapid auto-oxidation in solution producing semiquinone radicals and hydrogen peroxide initiated by the rapid dismutation of the superoxide. Significantly, the yield of H_2O_2 in the filtered gas-phase smoke bubbled solution decreases after every puff, suggesting that processes occurring on the Cambridge pad reduce the amount of H_2O_2 in the filtered gas-phase smoke as the TPM accumulates. The filtered gas-phase smoke on the other hand lacks the necessary polyphenolic compounds to initiate superoxide formation by semiquinone reduction of ambient oxygen. The wet milieu of the TPM that collects on the Cambridge pad, however, contains abundant semiquinone radicals and is therefore a favorable environment for H_2O_2 generation.

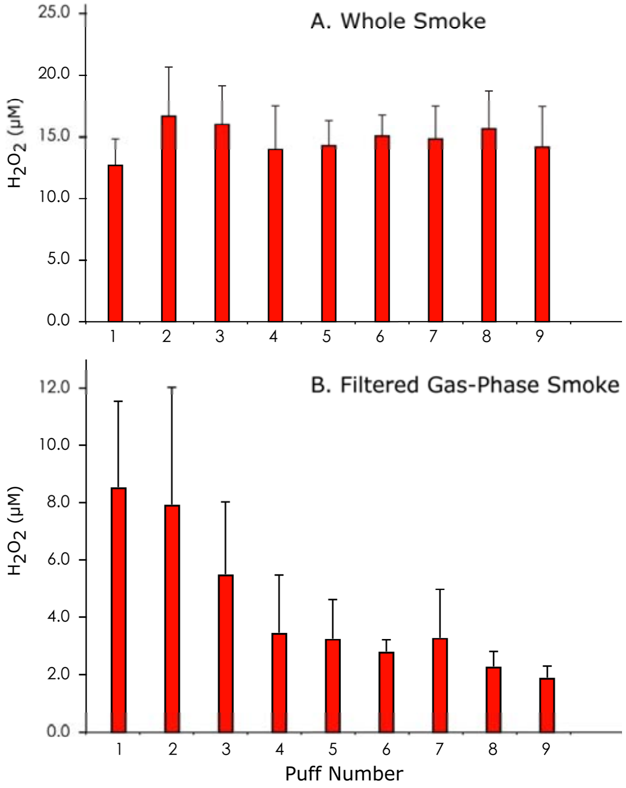


Fig. (9). Hydrogen peroxide measured in solution using electrochemical (amperometric) sensor covered with hydrogen peroxide specific membrane. Smoke was collected from a single 2R4F cigarette and bubbled into a phosphate buffered saline solution. **A**. Whole smoke; **B**. Gas-phase smoke filtered with a Cambridge filter pad.

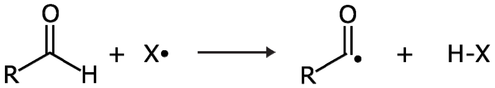
Several familiar coupled processes (albeit, supported by indirect experimental evidence) can be reasonably be postulated to occur on the TPM coated pad during machine smoking or in the particulate phase of whole smoke: the oxidation of quinols to form semiquinone radicals; the reduction of ambient oxygen by semiquinones to superoxide radical anion; the metal ion catalyzed dismutation of superoxide to form H_2O_2, and the catalytic disproportionation of hydrogen peroxide to hydroxyl radicals (and probably hydrogen abstraction from H_2O_2 by radical species to form peroxyl radicals). The scenario is consistent with the EPR spin trapping experiments of Tanigawa *et al.* who showed that PBS solutions of fresh, filtered gas-phase smoke contain appreciable amounts of superoxide (the putative source of H_2O_2), whereas solutions of aged whole smoke do not [70]. The observation of peroxynitrite in solutions of whole smoke by Perini and Robinson also demonstrate the presence of superoxide in TPM solutions, since peroxynitrate forms by the rapid reaction of NO and superoxide [83]. These observations taken together suggest that dismutation of superoxide on the TPM saturated pad is a plausible and sufficient source of hydrogen peroxide and abundant hydroxyl radicals by catalytic disproportion of H_2O_2 by trace catalytic metal ions in the smoke condensate [73].

Thus, the chemically active environment of the TPM coated Cambridge pad appears to be an ideal system to initiate a plethora of reactions initiated by hydroxyl and other radicals. The hydroxyl radicals are sufficiently reactive to transform smoke constituents while passing through the pad, or on the pad during the smoldering phase in between puffs [6], and they can act as progenitors of NO_2 (along with other mechanisms) *via* reaction with isoprene, 1,3-butadiene by the suggested atmospheric mechanism. The hydroxyl radicals can initiate any one of a number of radical chain reactions, e.g., radical additions or H-abstractions that create additional carbon-centered radicals and generate NO_2 as a by-product. While conjecture at the current time, the proposed pathways suggest that NO_2 can reasonably be expected to evolve from the Cambridge pad into the filtered gas-phase smoke.

ACYL RADICALS AND THEIR REACTIVITY

The measurements of Bartalis *et al.* revealed that acyl radicals account for a large proportion of the carbon-centered radicals in fresh whole smoke (Fig. **5**) [41]. Like other carbon-centered radicals (Table **1**), acyl radicals can react rapidly with other surrounding species, particularly oxygen [84]; consequently their abundance is surprising. That the yield of the acyl radicals is reduced 80-90 % by the Cambridge filter pad attests to their reactivity and demonstrates that they must be created in the vapor stream ahead of the Cambridge pad, perhaps created continuously within the smoke, rather like Pryor's steady-state mechanism involving isoprene, but by an unrelated process.

Given the surfeit of radicals in whole smoke together with the abundance of aldehydes such as acetaldehyde, propionaldehyde, and butyraldehyde, and unsaturated aldehydes such as acrolein and crotonaldehyde [4, 5], the abstraction of an aldehydic hydrogen from an aldehyde by a smoke borne agent is likely source of the acyl radicals, as shown:



Scheme III.

The measured relative yields of the three most abundant acyl radicals occur in very similar ratios to their parent aldehydes (Table **3**), suggesting that hydrogen abstraction is the probable formation mechanism [41]. A variety of studies indicate that natural tobacco polysaccharides, including cellulose, are the primary precursors of acetaldehyde in mainstream smoke [85]. This explains the higher relative yield of acetyl radicals from cigarettes made from 100% Oriental tobacco (Fig. **5**), which is processed into cigarette filler without removing the stem because of the small size of the leaves, and therefore has higher proportionally more cellulose. Similarly, cigarettes made from water washed tobacco ("baseweb"), which has a larger percentage of cellulose by weight than unwashed tobacco, also exhibited high acyl radical yields. These results are consistent with the known trend that acetaldehyde in cigarette smoke increases in direct proportion to the percentage cellulose in tobacco [85].

Chatgilialoglu *et al.* have reviewed the chemistry of acyl radicals, primarily from the standpoint of solution chemistry [86]. Electrophilic abstraction by peroxyl or alkoxy radicals was reported to be more efficient than nucleophilic abstraction by an alkyl radical, which is known to be relatively slow in the gas phase. As shown in Table **1**, the second-order kinetic rate constants for gas-phase abstraction of hydrogen from acetaldehyde by either NO or hydroxyl radical are orders of magnitude greater than abstraction by small alkyl radicals. Oxygen-centered radicals in whole cigarette smoke are presumed to be abundant due to the rapid air oxidation of carbon-centered radicals despite that specific oxygen-centered radicals have not yet been identified. The rapid oxidation of acetyl radicals by oxygen to acetylperoxy radical (Table **1**) [84] suggests that peroxyacetylnitrate may also be present, as proposed by Flicker and Green [27]. Lower molecular weight aldehydes are considered to be "semi-volatile," since they are known to partition between the particulate and gas-phase of whole smoke [42]. Consequently, part of the acyl radicals may in principle evolve from the smoke aerosol where the hydrogen abstraction may be promoted by species such as metal ions (as proposed below), peroxyl radicals, or other oxygen-centered radicals emanating from or contained in the aerosol particles. The putative chemical species that abstracts hydrogen from the abundant aldehydes in the gas phase of whole smoke, however, is not currently known, but such an agent would have to form in a continuous process and exhibit rapid gas-phase kinetics.

In filtered gas-phase smoke, the acyl radicals are potentially a source of the alkoxy radicals recently identified by Robinson and Dyakonov [34]. If an acetyl radical formed by a thermolytic process contains sufficient internal energy, the radical can spontaneously dissociate:

$$CH_3CO\bullet \rightarrow CH_3\bullet + CO.$$

Scheme IV.

The alkyl radical can then follow the pathway of the steady-state mechanism: rapid peroxidation by reaction with ambient oxygen, followed by reaction with smoke borne NO to form an alkoxy radical and NO_2. Acetyl radicals created by photolysis of acetone were not previously detected using the PBN spin trapping agent, although methyl radical have been identified [26, 27]. Church suggested that acetyl radicals disproportionate to methyl radicals and carbon monoxide too rapidly to be trapped by PBN [38]. Decarbonylation is known to occur significantly more rapidly in solution than in the gas phase [86]. Thus, part of the methyl radicals that have been detected in filtered gas-phase smoke may result from the decarbonylation of the acetyl radicals in the spin-trapping solution.

Trace metals such as Fe and Cu found in cigarette smoke are well known to catalyze the auto-oxidation of aldehydes in solution. Such metal-catalyzed aerobic oxidation of aldehydes is expected to generate acyl radicals as intermediates that subsequently oxidize to acyl peroxy radicals [86]. Such a process in principle may occur either in the aerosol particles themselves or on the TPM saturated Cambridge filter pad. This suggestion is speculative but, as mentioned, the aldehydes are partitioned between smoke gas and particulate phases and the suggested pathway is plausible.

APPLICABILITY OF THE STEADY-STATE MECHANISM

In his 1999 review of smoke chemistry, distinguished industry scientist Richard Baker emphasized that smoke properties change rapidly and that the relevant smoke to smoker is only 1-2 sec old [42]. Given that the aging time of cigarette smoke is at most 2 sec before it is inhaled by the smoker, and that the initial amount of NO_2 in fresh smoke from a 2R4F cigarette lit by blue flame match is only ca. 0.6 nanomole, the critical question relative to the steady-state model becomes whether an aging time of 2 sec is sufficient to account for a significant proportion of the radicals inhaled by the smoker. In principle, the formation of NO_2 can continue *via* the steady-state mechanism after the smoke is inhaled, but the smoke is diluted 50 to 100 fold by the smoker during inhalation, thus significantly slowing the reaction kinetics of NO oxidation to NO_2. Norman and Keith estimated that oxidation of half of NO to NO_2 would take ~8 min in undiluted cigarette smoke and ~83 min in diluted smoke in the human lung [87].

Most reports of aging experiments and kinetic measurements involving cigarette smoke or model gas systems have entailed aging times of many seconds or minutes. Moreover, the limitations of the experimental apparatus and detection device allowed significant aging to occur before the first measurement. Before the advent of QCL-TILDAS, the shortest dead-time reported for detection of radicals or NO_2 in either cigarette smoke or model gas systems appears to be that of Flicker and Green [27]. In an air/NO/isoprene/methanol mixture; some radicals were detected at ca. 3.5 sec, but they were not quantified. In the aging experiments of Cueto and Pryor there was a 13 sec dead time before the first FTIR measurement for the model gas systems and a 90 sec collection time for smoke before the first measurement. No carbon-centered radicals were detected by Bartalis *et al.* in an air/NO/isoprene mixture, even with prolonged aging or elevated isoprene, and the overall carbon-centered radical yield in whole cigarette smoke was found to decrease with aging time, rather than increasing as predicted by the steady-state model [41]. These discrepancies have not yet been resolved; they may depend sensitively on the precise experimental conditions and the formation of carbon-centered radicals in the model gas mixtures is not in question. Nevertheless, the initial 0.6 nmoles of NO_2 measured in fresh smoke from a single 2R4F cigarette by QCL-TILDAS and allowing for 2 sec of additional aging and oxidation of NO to NO_2 is inadequate to account for the total amount of radicals observed from the same cigarette given the slower oxidation rates of NO in whole smoke [58].

Since the rate-limiting step in the steady-state model is the formation of NO_2, the carbon-centered radicals are expected to increase along with the alkoxy radicals. Contrary to the steady-state model, however, the reduction of the acyl and alkylaminocarbonyl radicals by filtration with the Cambridge pad shows that these radicals must form either by combustion inside the cigarette or in the smoke plume before significant aging occurs. It remains to be determined whether the oxidation products of these radicals, or other products of such radicals with other smoke species, can account for the abundant alkoxy type radicals observed in recent the PBN/filtered gas-phase trapping experiments. Currently, none of the carbon-centered radicals detected in solution spin-trapping experiments with PBN have been identified, while Robinson and Dyakonov have identified some of the oxygen-centered radicals [34]. Part of these radicals is consistent with the steady-state model, but, as discussed above, other mechanisms can account for their formation.

We have seen that separating gas-phase smoke from whole smoke using the Cambridge pad changes the profile of radicals dramatically. The NOx chemistry previously proposed as the major source of organic radicals in filtered-gas phase smoke does not account for the acyl radicals alkylaminocarbonyl radicals. The estimated half-lifetime of the CH_3-NH-C(O)· radical was ca. 1 min, significantly longer than that of the acyl radicals, suggesting that most of these carbamoyl radicals would be inhaled by the smoker before their oxidation, while the acyl radicals would mostly oxidize to oxygen-centered radicals or recombine to other products before they could be inhaled.

ARTIFACTS OF THE CAMBRIDGE PAD

Dube and Green have discussed the drawbacks of a number of different methods of smoke collection, including the Cambridge filter pad, and the potential for artifact formation [1]. For example, Good *et al.* [88] found that sampling smoke through a Cambridge filter pad wet with smoke condensate removes most of the water soluble nitrogen-containing volatiles as measured by a nitrogen-specific GC detector. When the Cambridge pad was removed, there were a large number of volatile components detected that are not normally found in the filtered gas-smoke. The pad also reduced the measured yield various aldehydes and ketones. In another example, Caldwell and Conner [89] reported that the N-nitrosamines form on the pad due to the nitrosation of amines as tobacco smoke passes through the pad. The recent measurements of Shorter *et al.* discussed above suggest that these nitrosation reactions may from NO_2 that forms endogenously within the pad as the TPM accumulates during smoking [37].

That is not to say, however, that the nitrosamines reported in smoke are artifacts. While some of the artifacts of measurement are significant, Baker has emphasized that the established procedures for the validation of the analysis of smoke constituents are stringent, and that most of the chemical species reported to be in smoke are actually present [42]. Alternative methods for separating particulate and gas phase constituents are available. In point of fact, Dube and Green compared several alternative methods for trapping particulate-phase smoke such as electrostatic precipitators, cold traps, impaction traps, etc. Some of these methods clearly should be considered for future analyses of radicals in the gas phase of whole smoke. Moreover, the Cambridge pad should be avoided in general for the analysis of smoke substituents that are subject to facile nitrosation or oxidative reactions.

A growing number of reports support a scenario whereby the Cambridge filter pad acts as a highly pro-oxidant reactor. Although the smoke condensate is overall reducing, the chemistry occurring on the pad during machine smoking produces abundant reactive oxygen species that can alter the constituents detected in the filtered gas-phase smoke. This conjecture is supported by the following accumulated evidence: (1) most of the NO_2 in fresh filtered gas-phase smoke appears to form on the pad itself [37]; (2) the Cambridge pad removes or alters ca. 50-60% of the carbamoyl radicals and ca. 80-90 % of the acyl radicals as the volatile smoke constituents pass through the Cambridge pad [41]; (3) the PBN spin-adducts of radicals that pass through the Cambridge pad become more oxygen-centered and less carbon-centered as the TPM builds up on the pad from puff to puff [43]; (4) peroxynitrite has been detected in fresh solutions of whole smoke in the presence of NO and fresh smoke condensate, suggesting that superoxide can form on the pad as well [83]; (5) the amount of hydrogen peroxide that forms in PBS solutions of filtered gas-phase smoke decreases on a puff to puff basis as the amount of NO_2 in each puff increases, implicating a very active chemistry on the pad (Fig. **9**); (6) superoxide radical anion has been detected in solutions of fresh whole smoke containing an iron chelating agent, but apparently not in aged whole smoke by [47, 70]; (7) Cu ions and some Cu complexes are known to act as SOD mimetics, promoting the formation of H_2O_2, and to participate directly in REDOX cycling [90-92], while the presence of smoke-borne Fe^{3+} and Cu^{2+} ions that collect in the wet TPM on the pad are well known to promote the formation of hydroxyl radicals from H_2O_2 *via* Fenton chemistry [73, 74]. Thus, we suggest that continuous oxidations of various smoke constituents can occur on the Cambridge filter pad during puffing over the several minutes required to smoke a cigarette. While the pad is saturated with water and smoke condensate, the release of oxidizing constituents to the vapor phase increases from puff to puff as the TPM accumulates and ages in the presence of ambient oxygen.

Borland *et al.* [58] noted years ago that the "particulate matter 'quenches' highly active oxygen-containing species is significant. Since these latter species are alleged to be toxic, experiments in which animals, enzymes and cell suspensions are exposed to isolated gas phase may yield results not applicable to whole smoke." More recently Baker warned that the use of old smoke condensate in biological assays is a questionable practice [42]. Ironically, it has been shown that the particulate-phase smoke behaves in some measure as an antioxidant towards the gas-phase; for example, the toxicity of filtered gas-phase smoke appears to be reduced by the particulate component in some biological assays [47], and cigarette whole smoke, especially aged smoke, appears to be significantly less active towards lipid peroxidation than filtered gas-phase smoke [93, 94]. These results were presaged by Church and Pryor who observed in 1985 that whole smoke is slightly less oxidizing than filtered gas-phase smoke, "a finding with obvious toxicological implications for smokers" [7].

SUMMARY

New solid phase trapping methods using nitroxide-trapping agents coated on glass beads show that there are plentiful carbon-centered radicals in fresh mainstream cigarette smoke. The radicals were examined by LC-MS/MS techniques and were shown to have acyl and carbamoyl structures. Although these radicals oxidize in air, the carbamoyl radicals have sufficient long lifetimes to be inhaled by the smoker. In contrast to the established steady-state mechanism, the yield of these radicals per cigarette decrease with cigarette smoke aging and they are reduced the Cambridge filter pad commonly used to separate the cigarette gas and particulate phases. These results show that these radicals are already present in smoke before filtration and not formed by NOx chemistry, which requires a minimum of a few seconds of aging of the smoke.

Infrared TILDAS have revealed that NO_2 in filtered gas-phase smoke results predominantly from reactions on the Cambridge pad, and that the yield of NO_2 in fresh whole smoke is very low. Part of this NO_2 comes from the cigarette lighting source. Multiple investigations suggest that the Cambridge pad is a highly pro-oxidant environment (overall reducing, but capable of generation of abundant reactive oxygen species) where hydroxyl radicals form and promote the rapid formation of NO_2 from NO and other non-native smoke products while the smoke gas stream passes through the condensate accumulating on the pad. We suggest that the oxygen-centered radicals recently identified using nitrone spin-trapping agents in filtered gas-phase smoke may originate in part from oxidation reactions involving the acyl radicals. The origin of other identified carbon-centered radicals trapped on the pad by nitroxide agents, as well as other radicals in filtered gas-phase smoke by nitrone trapping agents currently remain unexplained. In this review, the discussion has focused on mainstream smoke and the processes discussed do not necessarily apply to environmental or sidestream cigarette smoke where NO_2 is abundant and aging effects are likely to be significant.

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