Published in final edited form as:

Energy Fuels. 2013 September 19; 27(9): . doi:10.1021/ef4010253.

Role of the Filters in the Formation and Stabilization of Semiquinone Radicals Collected from Cigarette Smoke

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Abstract

The fractional pyrolysis of Bright tobacco was performed in nitrogen atmosphere over the temperature range of 240 – 510 °C in a specially constructed, high temperature flow reactor system. Electron paramagnetic resonance (EPR) spectroscopy was used to analyze the free radicals in the initially produced total particular matter (TPM) and in TPM after exposure to ambient air (aging). Different filters have been used to collect TPM from tobacco smoke: cellulosic, cellulose nitrate, cellulose acetate, nylon, Teflon and Cambridge. The collection of the primary radicals (measured immediately after collection of TPM on filters), the formation and stabilization of the secondary radicals (defined as radicals formed during aging of TPM samples on the filters) depend significantly on the material of the filter. A mechanistic explanation about different binding capability of the filters decreasing in the order: cellulosic < cellulose nitrate < cellulose acetate < nylon ~ teflon is presented. Different properties were observed for the Cambridge filter. Specific care must be taken using the filters for identification of radicals from tobacco smoke to avoid artifacts in each case.

Keywords

Cambridge; cellulose; Teflon; and nylon filters; hydroquinone; catechol

1. Introduction

Since 1970 more than three decades of experiments in detecting, identifying, isolating gas phase as well as TPM (Total particular matter) radicals during tobacco smoking have been performed ^{1–6}. TPM was defined as a portion of a tobacco smoke that can be collected on a fiberglass Cambridge filter pad ⁷. The Cambridge filter collects aerosol particles $d < 0.1 \mu\text{m}$ in diameter with an efficiency of 99.9%. The radicals collected on a Cambridge filter as a result of tobacco smoking (burning of tobacco material in presence of oxygen) were identified as semiquinone (SQ) type of radicals that is associated with quinone and hydroquinone groups (Q/QH₂) bound in a polymeric matrix ². However, the origin of radicals produced in pyrolysis of tobacco, i.e. during conversion of tobacco in zones of oxygen deficiency, was not investigated thoroughly. This question was addressed in series of publications concerning fractional pyrolysis of tobacco without presence of oxygen ^{8–10}.

Two types of primary radicals were observed in the initially produced TPM during fractional pyrolysis: (1) the Type I radicals characterized by the 5-line EPR spectrum with an apparent g-value of ~2.0064 and assigned to a constrained tyrosyl radicals transferred at lower

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Supporting Information Available: [Primary and secondary TPM radicals; Comparison of filters. Specific behavior of Cambridge filters, SFig. 1 – Sfig.3] This material is available free of charge via the Internet at <http://pubs.acs.org>.

temperatures of pyrolysis from the specific components of cell wall biopolymers¹⁰, and (2) the Type II radicals with g-factor of 2.0035–2.0040 consistent with surface-associated, carbon-centered radicals where unpaired electron were vicinal to an oxygen-containing functional group².

When the TPM samples were exposed to aging, the concentration of radicals in TPM increased due to production of secondary radicals with a g-factor 2.0053 ± 0.0003 and their concentration achieved a maximum for TPM samples produced at a pyrolysis temperature of 280 °C^{8,9}. The EPR spectra of these radicals were consistent with many aspects of oxygen-centered, semiquinone-type radicals (SQ) formed from hydroxylated aromatic molecules including hydroquinones (HQ) and catechols (CT), chemisorbed to metals or other electron-acceptor sites in the TPM¹⁰.

All these pyrolysis experiments were performed in a commercial pyroprobe using a continuous flow-reactor system and the TPM were collected on the cellulose filter supported by glass fiber Cambridge filter. The substances with a molecular weight below ~ 60 amu tend to be predominantly in the gas phase, and substances with a molecular weight above 200 amu tend to be in the particulate phase¹¹. The compounds with a molecular weight between 60 and 200 amu, so called semi-volatiles, can be retained by the filter but can be volatilized from the filter at a defined temperature without appreciable decomposition¹². This partition on the filter can be a function of a number of factors, including the nature and the amount of material being collected; the flow through the filter, the temperature and moisture level of the filter¹³. For instance, it was found that the volatile phenols are selectively removed from mainstream smoke by passage through a plasticized cellulose acetate filter^{12,14}.

The semi-volatile fraction in general consists of about 300 smoke components, including phenol, hydroquinone, catechol and their derivatives. The hydroquinone, catechol and their derivatives are the main precursors as molecular species which undergo oxidation to semiquinone radicals during aging^{12 15}.

There are many examples of artifacts occurring in collecting smoke condensate^{16 17}. For instance, mainstream smoke semi-volatile composition can be strongly affected by filters, their materials and diameter of pores. Some compounds due to their chemical nature interact stronger with a filter material than others, thus being retained to a greater extent. This phenomenon depends on the affinity between the filter material and the chemical compound to be retained and the number of active sites within the filters.

A main interest of this work was to study the effect of different filter materials and the diameter of pores on collection of the primary radicals trapped on the filter. Cellulose filters (cellulosic, cellulose acetate or cellulose nitrate) have a polar structure which will possess a high affinity not only for polar smoke constituents, but also for radicals. As alternative filter materials, Cambridge, Teflon and Nylon were chosen. In contrast to cellulose filters, they have a non-polar, completely hydrophobic structure.

2. Experimental Procedure

Materials

Samples of Bright (flue-cured) tobacco were provided by Philips Morris USA (Richmond, VA) and used without further treatment.

Cellulosic filters (o.d. 47 mm, 0.8 micron pore size) were obtained from Osmonics Inc., Cellulose nitrate (o.d. 47 mm, 0.45 micron pore size) from Whatman, and Acetate Plus,

Nylon, and Teflon filter (o.d. 47 mm, 0.8 micron pore size) from GE Water & Process Technologies. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (free radical, 98%) was obtained from Aldrich Chemical Co. Catechol, hydroquinone, deuterium oxide were obtained from Sigma Chemical Co. The EPR tubes were standard X-band Suprasil highpurity quartz tubes (4 mm inner diameter) and were supplied by the Wilmad/Labglas Co.

Filters

Cellulose ($C_6H_{10}O_5$)_n is a long-chain polymeric polysaccharide carbohydrate derived from -glucose. The hydroxyl groups of cellulose can be partially or fully reacted with acetic acid to form cellulose acetate, (where more than 92 % of the hydroxyl groups are acetylated.) or with nitric acid to form cellulose nitrate, Table 1. Teflon is a polytetrafluoroethylene polymer, and nylon is a semicrystalline polyamide.

3. Pyrolysis

The samples were pyrolyzed in a commercial Pyroprobe 1000 (CDS Analytical, Inc.), using a continuous flow-reactor system. The experimental details are given elsewhere⁸. Samples (80 ± 2 mg) of Bright tobacco were placed in a quartz tube (i.d. = 6 mm), and inserted inside the pyroprobe coil. Both sides of the tube insert were plugged with quartz wool to prevent sample loss. Before the experiments samples were flushed with nitrogen for 24 hours to remove traces of oxygen. The pyrolysis was induced by rapidly heating the reaction tube with a rate of 100°C/s . The temperature in the center of tobacco bed was measured using a retractable chromel-alumel (K type) thermocouple (o.d. = 0.25 mm). The temperature gradient along the layer of tobacco in the reaction tube did not exceed $\pm 5^\circ \text{C}$. Nitrogen containing no more than 5 ppm of oxygen was used as a carrier gas in the pyrolysis experiments. The pyrolyzing gas was introduced at a flow rate in the range $100 - 200 \text{ cm}^3/\text{min}$, which corresponded to residence time of 0.05–0.1 s at 20°C . The flow rate was controlled by a mass controller (McMillan Co., Model 80D).

Fractional Pyrolysis

The sample was heated for a reaction time of 60 s at each temperature, and TPM was collected on the filter. The filter with TPM was flushed for 5 minutes with the carrier gas, then removed and replaced with a new filter. The entire experiment was performed with the same bed of tobacco and the temperature varied from 200 to 550°C in $30\text{--}60^\circ \text{C}$ increments.

Collection of TPM

The smoke from the heated tobacco was passed through a designated filter (supported by conventional glass fiber Cambridge filter), and the TPM was collected and weighed. All filters had an active surface area of 0.5 cm^2 and were efficient for particles larger than $0.8 \mu\text{m}$ in diameter. After collecting the smoke sample, the filter was cut into 2-mm wide strips, and the strips inserted directly into an EPR tube. The sample was stored at 77K until it was analyzed by EPR at room temperature.

Impregnation Experiment

Filters were placed in the aqueous solution of hydroquinone or catechol for one hour. The concentration was varied from 0.0005 to 0.1 M and pH values from 6.0 to 8.0. The filters were dried 24 hours in the stream of nitrogen, cut into 2-mm wide strips and the strips inserted directly into an EPR tube.

Electron Paramagnetic Resonance

All EPR spectra were recorded at room temperature on a Bruker EMX-10/2.7 EPR spectrometer (Bruker Instruments, Billerica MA) with dual cavities, X-band, and microwave frequency (9.72 GHz). The typical parameters were as follows: (i): sweep width, 100 G; power, 2 mW; receiver gain 3.56×10^4 ; modulation amplitude, 4 G; and time constant 1.28 ms. Values of g-factor were calculated using Bruker's WINEPR program. The concentration of free radicals in the samples was calculated using double integration of the first derivative signal and comparison with a sample of DPPH.

4. Results and Discussion

4.1. Primary and secondary TPM radicals

The fractional pyrolysis of Bright tobacco was performed over the temperature range of 240 – 510°C. The EPR spectra of the “primary” and the “primary + secondary” radicals were presented in Figure 1 for the two most characteristic temperature of pyrolysis, 280°C and 450°C. The “primary” radicals (Fig. 1, **spectra 1**) display the signals of the radicals that were produced during the pyrolysis and analyzed directly after the experiments. The five broad lines in the EPR spectrum with the apparent g value of 2.0064 was suggested to be constrained or immobilized tyrosyl radicals formed from protein components of the cell-wall biopolymer of tobacco⁸. The primary radicals at 450°C were consistent with surface-associated, carbon-centered radicals, where the unpaired electron is vicinal to an oxygen-containing functional group⁸. After these first EPR measurements all TPM samples were stored in open EPR tubes at room temperature. Periodically, the concentration of radicals was determined and the maximum values were chosen. The signals “primary + secondary” (Fig. 1, **spectra 2**) presents the total signals of the radicals; the primary ones formed during pyrolysis and the secondary that were generated after exposure of TPM to ambient air. The comparison of these two sets of results revealed the dramatic increase in the concentration of secondary radicals over the entire range of temperature studied (SFig. 1 in supporting information). The EPR spectra of these radicals (Fig. 1, **spectra 2**) were consistent with many aspects of oxygen-centered, semiquinone-type radicals formed from hydroxylated aromatic molecules including hydroquinones and catechols, chemisorbed to metals or other electronacceptor sites in the TPM or filters¹⁰. Note that the hydroquinone/catechol precursors are the most abundant phenolic compounds in tobacco smoke^{18–20}. The concentration of hydroquinone and catechol in the mainstream of tobacco smoke was found to be 110–300 µg/g and 100–360 µg/g of tobacco, respectively, and they can be selectively collected, especially on cellulosic filter¹². Importantly, the maximum yields of CT/HQ and their derivatives peaked at ~300°C²¹; this is solid evidence that the yields of secondary radicals (likely p-SQ from HQ and o-SQ from CT) produced from those compounds during aging pass a maximum at around 280 – 300 °C (rf. SFig.1 in supporting information).

4.2. Effect of pore diameter of the filter on the collection/formation of TPM radicals

The effect of pore diameter on accumulation of TPM radicals was studied and the results are presented in Fig. 2. The maximal yield of TPM radicals were detected for the filter with pore size 0.65 – 0.8 µm. At higher than 0.8 µm pores the concentration of TPM radicals drops significantly (~ 2 times) which might be due to the fact that less TPM is collected. For instance, the filters with pore diameter of 1.2 and 5 µm were permeable only for about 40% of radicals in comparison to the radicals trapped on the filter with 0.65 µm pore size. It was surprising that the concentration of radicals diminishes also at small pores with less than i.d. = 0.65 – 0.8 µm; for instance the concentration drops almost in the same amount (~ 2 times) at pores with i.d. = 0.1 µm. At this small pore size high quantities of TPM (or high concentration of TPM radicals) may be collected and the total radical yield can be decreased

due to high possibility of radical-radical recombination. Therefore the filters with pore sizes of ~ 0.6–0.8 μm were chosen as optimal.

4.3. Effect of filter material on the collection of TPM radicals

The collection of primary radicals and the formation and stabilization of secondary radicals on different filters was studied at 280°C. Only cellulose nitrate and cellulosic filters, which is a mixture of cellulose nitrate and cellulose acetate, were able to collect the primary (black lines) and stabilize secondary radicals (red lines), Fig. 3A. Noticeable amounts of TPM primary (or secondary) radicals were not detected for teflon, nylon and cellulose acetate filters. Teflon and nylon filters similar to polypropylene²² have a non-polar, hydrophobic structure (Table 1), and probably, do not collect or stabilize the radicals. The Cambridge filter binds the radicals (or precursors of radicals) but its behavior is different from that of nitrate or cellulose filters: the concentration and shape of the EPR signal does not change after aging, Fig. 3A, B. In fact, there are sizeable differences in binding (collecting) ability of the filters toward formation/stabilization of radicals from tobacco smoke depending on the material of the filters, Fig. 3A. The behavior of p-SQ radicals on the filters produced from HQ as precursor (Fig. 3B) were similar to that of secondary radicals from tobacco smoke (Fig. 3A) which, as it was established elsewhere, formed because of further oxidation of adsorbed dihydroxybenzenes^{10,23}.

The effectiveness of the filters to collect semiquinone-type radicals were decreased in the order cellulosic < cellulose nitrate < cellulose acetate < Teflon ~ nylon (Cambridge filter behaves differently, *vide infra*). For instance, the cellulosic filter collects 6 times more radicals than nitrate cellulose (cf. SFig. 2 in supporting information).

The molecular theory of the adhesion of polymers is the concept of acid-base interaction on a polymer interface²⁴. As expected from the chemical composition of cellulose nitrate (Table 1), its surface has acid properties: NO₂ group acts as a strong electron-acceptor substituent. The presence of such substituents as nitro groups reduces the basic capacity, shifting electron density towards them and causing a decrease in the bond order and an increase in polarity.

Polarization of the C – H bond in cellulosic ring results in a rise of acid dissociation, thus imparting acidity to the surface²⁴. Consequently, the more acidity / basicity of the interface layer of polymer the more adhesive / binding capacity of the polymer. An increase in the content of polar groups in cellulose nitrate is accompanied by an increase of the free surface energy²⁴. As a result the surface acidity of cellulose nitrates increases as the substitution degree increases.

Therefore, it can be concluded that the collecting capability of cellulosic filters, which are a mixture of cellulosic nitrate and acetate groups, is higher than that for cellulose nitrate filters, Fig. 3A and B. Naturally the binding capability of cellulosic acetate is less than that for cellulose nitrate if the Hammett constants will be compared for two groups, NO₂ and COCH₃ in benzoic acid²⁵: the electron-acceptor behavior of nitro group is higher ($\sigma_m = 0.71$ for NO₂) in comparison with the group COCH₃ in cellulose acetate filter, Table 1 ($\sigma_m = 0.38$ for COCH₃). As a result, the EPR signal intensity on cellulose acetate filter is always negligible (Fig. 3A) or small (Fig. 3B) in comparison with cellulose nitrate filters.

Similar binding between neutral molecules, for instance proteinaceous and cellulose filters was described in an earlier work²⁶, which lists the following hierarchy of adsorption to filter materials: cellulose nitrate < mixed cellulose esters < cellulose diacetate < cellulose triacetate. The study with IgG (immunoglobulin) G, A and M, albumin, transferrin and other proteins adsorption²⁷ confirm the fact that cellulose nitrate adsorbs the most strongly. One

explanation is that IgG has a configuration that allows binding to the chemical structure of the filter material.

The binding proteins to cellulose nitrate filter have been described by many authors. The quantitative adsorption of protein on cellulose nitrate filter has been used in the assay of total protein²⁸. Bovine serum albumin²⁹, antibody and antigen/antibody complexes³⁰, and specific binding and receptor proteins have all been shown to adsorb to cellulose nitrate containing filters.

4.4. pH driven formation of semiquinone radicals on cellulosic filters

Since the semiquinone radicals are major radicals during aging as well as usually observed in aqueous media¹⁰, the stabilization behavior on cellulosic filter, Fig. 3 becomes important to evaluate. One of the reasons why the cellulosic filter was chosen is the high free surface energy and therefore the high ability of cellulosic filter to bind radicals on the surface, Fig. 3B. However, due to the high acidity behavior of the cellulosic filter, as described above, one may expect some influence of the filter on formation of semiquinone radicals; it is known that the formation of SQ radicals is pH driven and usually observable in alkaline solutions^{6,31,32}.

The two-step dissociation of the hydroxyl protons for hydroquinone in an oxygen free solvent takes place at $pK_1 = 9.91$ and $pK_2 = 11.56$ ^{6,31,32}. Both protons are dissociated before an one-electron oxidation leads to semiquinone radicals in the presence of molecular oxygen, i.e. semiquinones are observed only at elevated pH. However, Pedersen observed that the radical of hydroquinone occurs at a pH slightly below 7, implying the oxidation process may follow a different route³².

The mono anion from HQ dissociation exists at about $1 \mu\text{M}$ concentrations in 1mM hydroquinone solution at $\text{pH} = 7.0$ (buffered solution) and is oxidized to the p-hydroxyphenoxyl radical (or p-semiquinone, p-SQ radical) in the presence of molecular oxygen³², Scheme 1. This radical is unstable, and due to high acidity (pK_a value for the neutral radical is 4.0)³³, is immediately transferred to the semiquinone stage (p-SQ anion radical) since the equilibrium in practice is shifted 100% to the right³², Scheme 1. This transformation presented in Scheme 1 can be seen in Fig. 4A when the intensity of p-SQ anion radicals generated in presence of cellulose filter from the solution of aerated HQ increases with increasing of pH. The spectrum of p-SQ anion radical in oxygen free HQ solution presents typical 5 lines (at high pH, not shown) with intensity distributions of 1:4:6:4:1 and hyperfine splitting constant - $hfsc = 2.5\text{--}3.75 \text{ G}$ ³². In presence of oxygen all these lines are merged and a single, broad line is usually registered, Fig. 4A.

The validity of the Scheme 1 can be deduced from the experimental data presented in Fig. 5, which clearly illustrates the acidic dissociation character of p-SQ radical. If p-SQ radical dissociates similarly to Scheme 1, the pH value at 7.0 (at the half intensity of p-SQ anion radicals) equals to pK_a of the corresponded acid form i.e. for p-SQ radical $pK_a = 7.0$, Fig. 5, (red line) This value significantly differs from the $pK_a = 4$ for p-SQ radical previously reported³³, Table 2. This means p-SQ radical adsorbed on cellulosic filter shows much less acidity i.e. it doesn't easily dissociate. Therefore, the free electron on p-SQ radical, which provides high acidity character, is most probably shared on the surface of cellulosic filter; either the charge of the electron is compensated to some extent by a proton from cellulosic ring (due to high acidity of the cellulosic filter) or p-SQ radical bonds directly to highly polarized NO_2 group, Fig.6.

Oxygen-centered radicals usually exhibit a high g value. For instance, one can see from SFig. 2 (Supporting information) that the g value 2.0052 of p-SQ radicals on cellulosic filter

(50% nitrate and 50 % acetate groups) increases to 2.0058 on cellulose nitrate filter (100% nitrate groups) indicative on transferring of carbon centered p-SQ radical into oxygen centered nitroxide radical, Fig. 6. The p-SQ radical can also be bound to the nitrate group because of hydrogen binding, Fig.6. Therefore, due to these interactions the p-SQ radicals may be stabilized on cellulosic or cellulose nitrate filters. As a consequence the p-SQ radicals lose to some extent the acidic character, and the pKa increases to 7 from 4 when they were free in solution ³³.

Similar to Fig.6, a binding is reported in literature between tyrosyl radical (analogue to p-SQ radical) and an nitroso spin trap compound DNBBS (3,5-dobromo-4-nitrosobenzenesulfonate)³⁴. The carbon centered isomer structure of tyrosyl radical form similar to that in Fig.6 adduct with DNBBS.

Due to high acidity of cellulose nitrate filters, the semiquinone anion radicals can also bind to the surface due to hydrogen binding (between cellulosic ring hydrogen and the anion part of p-SQ anion radical) which is very common for semiquinone radicals in protic solvents ³⁵.

Another important observation is existence of SQ neutral radicals at lower pH (Figures 4, 5) as was discussed in previous work ^{6,31,32}. That both p-SQ radical and p-SQ anion radical (Scheme 1) may exist in the solution has been clearly shown ³³. The occurrence of Rxns 1–4 below may explain this phenomenon. In the presence of oxygen p-SQ anion radicals may reduce oxygen to superoxide anion radical, converting into benzoquinone, rxn. 1

Rxn.1 is a well-known source for formation of superoxide radicals in the biological environment ³⁶. Further dismutation of superoxide anion-radicals forms hydrogen peroxide in presence of protons, rxn. 2

This reaction has been proven to occur over a very broad pH range from 3 to 10. The overall rate of superoxide dismutation, a second order reaction is usually slow, $k_2 \sim 10^5 \text{ M}^{-1}.\text{s}^{-1}$ and pH dependence and reaches its maximal value in aqueous media at pH = 7.4 ^{36,37}. Therefore at close to neutral conditions the reaction known as Haber-Weiss reaction, rxn. 3 may occur to some extent to generate hydroxyl radicals ³⁸.

Rxn.3 can be catalyzed by trace amounts of transition metals ³⁸, and the p-SQ radicals may form due to the unimolecular elimination of water from an OH adduct on hydroquinone at room temperature ³³, rxn. 4

Rxn. 4

The non-catalyzed rate constant for the reaction 4 (formation of p-SQ radical) is small, $4.6 \times 10^{-4} \text{ s}^{-1}$, but it still may be a source of detectable amounts of p-SQ radicals in neutral and even acidic environment ³³.

Almost the same physico-chemical phenomena have been demonstrated with ortho-SQ radicals generated on cellulose filter, Fig. 4B, Fig.5 (**blue line**) and Table 2. While p-SQ radical EPR spectra were close to singlet, symmetrical lines at all pH values (Fig. 4A), the EPR spectrum of o-SQ radical /o-SQ anion radicals mixture have shown clear curvature at $g \sim 2.0063$ at almost all pH values (Fig. 4 B). The same difference in EPR spectra between p-SQ (symmetric singlet line) and o-SQ radicals (an split in singlet spectrum) generated and accumulated at 77 K from gas phase pyrolysis of HQ and CT, respectively has been established in ^{39,40}. A split in the EPR spectrum of o-SQ radical was explained by formation of strong intermolecular H-bonded o-SQ dimer (radical pairs of o-SQ radicals) when the hydrogen bonding splits the spectrum due to hyperfine spin coupling between hydroxyl hydrogen and the unpaired electron on the vicinal oxygen ⁴⁰. Similar behavior of semiquinone radicals in protic/aprotic solutions is not without literature presence: the hydrogen bonding and its influence on EPR spectra have been mentioned in earlier works ^{35,41}.

4.5. The specific behavior of Cambridge filters

The recent studies ⁴² 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. The Cambridge filter introduces artifacts of measurements for a variety of smoke constituents has long been known ⁷¹⁶. Recent measurements revealed that the Cambridge filter significantly reduces the amount of carbon-centered radicals measured in filtered gas-phase smoke compared to the whole smoke, while actually causing the measured amounts of NO₂ to increase ⁴²⁻⁴⁵. When the smoke was passed through a Cambridge filter NO₂ appeared in the filtered gas-smoke during every puff, suggesting that NO₂ forms directly on the pad ⁴². While the Cambridge filter pad acts on tobacco smoke as a highly pro-oxidant reactor ⁴² some publications stated the active chemistry on the Cambridge filter did not alter the types of radicals identified ⁴⁶.

These as well the findings in this work confirm that the Cambridge filter is more reliable for collection of stable intermediate radicals from tobacco smoke. Surprisingly, no differences were found in radical EPR signal shape and intensity between fresh or aged TPM from tobacco smoke on Cambridge filter, in contrast to the data on different cellulose filters, Fig. 3A. The same phenomenon was found for p-SQ radicals produced from aerated HQ solution in the presence of Cambridge filter: the concentration of p-SQ radicals generated on Cambridge filter was not changed during aging, Fig. 3B.

This phenomenon can be understood, if the formation of p-SQ radicals on the filter (as secondary radicals) can occur in “dead zone” – it is a dead volume in Cambridge filter which may be as high as 14 ml⁴⁷. The high amounts of HQ/CT (and other phenolic compounds)³⁷ may be trapped in these pores and condensed in water as one of the reaction products. Further auto oxidation chemistry of HQ/CT (aging) may occur in presence of oxygen only (Scheme 1 and rxns 1–4). However, due to limited access of oxygen through the pores of Cambridge filter, partly blocked also by TPM layer, the formation of p-SQ anion radical from HQ is not been observed.

Another specificity of fiberglass Cambridge filter is almost complete (99.9 %) collection of the smoke particular matter contained in the smoke stream due to its pore size of 0.1 μm ⁷. However, the small pore size was not optimal in sense of collecting high amounts of radicals from tobacco smoke, as in case of cellulosic filter, Fig.2: the collected radicals may disappear by self-recombination reactions. The SQ radicals attached to the TPM matrix⁸ may exist in oxygen-centered or carbon-centered resonance forms. The high recombination efficiency of carbon centered phenoxyl radicals has been shown in number of gas-phase, surface experimental and theoretical calculations and modeling works^{48–52}. Due to that the carbon centered SQ radicals may recombine much easily in TPM in comparison with oxygen-centered SQ radicals which have less mobility on the surface⁵¹. Because of that the relative ratio of o-SQ oxygen-centered radicals will increase in total radical yield on TPM and as a consequence, the g value of EPR spectra will be higher, Fig. 3A, B. Therefore, a care should be taken in identification of TPM radicals due to their selective annihilation on Cambridge filter pad.

In summary, from the study of nature and origin of cigarette smoke radicals collected on different filters (cellulosic, cellulose nitrate, cellulose acetate, nylon, teflon and Cambridge) the Cambridge filter pad is recommended as an appropriate filter pad (while not ideal) for identification of TPM radicals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was partially supported from Philip Morris, USA (under Grant No. 14358). We acknowledge partial support of this work through NIEHS SRP at LSU 2 P42 ES013648-03.

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Fig. 1.

The EPR spectra of TPM collected on cellulosic filter during pyrolysis of Bright tobacco at 280 and 450 °C; fresh collection (“primary” radicals – spectra 1) and after exposure to environmental air (“primary + secondary” radicals – spectra 2). $g = 2.0064$ for “primary” radicals and 2.0053 for “primary + secondary” radicals at 280 °C and $g = 2.0036$ for primary radicals and 2.0045 for “primary + secondary” radicals at 450 °C

Fig. 2.
The effect of pore diameter of cellulosic filter on the collection and formation of radicals (“primary + secondary”) from Bright tobacco at 320 °C. The g values for majority EPR spectra were between 2.0054 – 2.0059, and H_{p-p} between 8.6 – 9.7 G.

Fig. 3. The effect of filter material on the collection/formation of primary radicals (black lines) and stabilization of secondary (semiquinone) radicals formed; **A.** During aging of TPM (red lines) produced from pyrolysis of Bright tobacco at 280 °C, **B.** From HQ (the filters were impregnated in 0.05 M hydroquinone solution at pH 8.0 and then dried in N₂ flow for 24 hours). The peak assigned by asterisk is background signal from Cambridge filter (rf. supporting information, SFig.3).

Fig. 4.
The effect of pH on the formation and stabilization of p-SQ (A) and o-SQ (B) radicals on the cellulosic filter derived from impregnation of the filter into 0.05 M HQ and 0.05 M CT solutions, respectively.

Fig. 5.
The effect of acidity on the formation and stabilization of p-SQ (red line) and o-SQ (blue line) radicals. DI-double integration value of EPR spectra

Fig. 6. Schematic bonding between nitrate group of cellulosic nitrate filter and carbon centered isomer structure of p-SQ radical as well as hydrogen binding between another nitrate group and p-SQ radical.

Scheme 1.
(from ³²)

Table 1

The structural formulas of the filters

Filter	Formula
Cellulose acetate	<p>R = H or $\text{CH}_3\text{C}(=\text{O})\text{—}$</p>
Cellulose nitrate
Cellulosic	A mixed esters of cellulose nitrate and acetate
Cambridge	Glass fiber
Teflon	$\left(\text{—CF}_2\text{—CF}_2\text{—} \right)_n$
Nylon

Table 2

pKa literature values for HQ/CT in oxygen free solutions and for the corresponding radicals in the presence of oxygen (this work)

	HQ	HQ	CT	p-SQ radical [*]	p-SQ radical ^{**}	o-SQ radical ^{**}
pK _{a1}	9.85	9.91	9.34	4	7.0	7.2
pK _{a2}	11.4	11.56	12.6			
Citation	[34]	[33]	[34]	[33]	This work	This work

^{*} Oxygen free water solution

^{**} on cellulosic filter in the presence of oxygen