CHARACTERIZATION OF FREE RADICALS IN CIGARETTE SMOKE AND E-CIGARETTE AEROSOLS BY SPIN-TRAPPING EPR SPECTROSCOPY

Clayton Probert[1], Michael F. Davis[2], G. L. Prasad[3], Tatyana Smirnova[1], Alex Smirnov[1]

[1]Department of Chemistry, North Carolina State University, Raleigh, NC, [2]R.J. Reynolds Tobacco Company, Winston-Salem, NC, [3]RAI Services Company, Winston-Salem, NC

INTRODUCTION

Formation of free radicals in cigarette smoke was first demonstrated over 60 years ago.[1,2] Free radicals are detected both in gas phase and particulate matter of cigarette smoke. In addition to being highly reactive, free radicals have been implicated in inducing oxidative damage and triggering dysregulation of redox processes in biological tissues; both key mechanisms that lead to smoking-related diseases. Since the introduction to the market, e-cigarettes have gained popularity; however, only limited data about free radical content of e-cigarette aerosols and the nature of the radicals formed are available in the literature.[3-5] The overall goal of this pilot project was to characterize and quantify short-lived radicals produced in e-cigarette aerosol condensate and compare them with radicals produced in smoke of combustible cigarettes. To achieve this goal, we employed a spin-trapping method in combination with Electronic Paramagnetic Resonance (EPR) detection. Many radicals are short-lived and cannot be directly detected by EPR. The spin-trapping method is based on reactions between a diamagnetic molecule (called a spin-trap) and radicals which results in the formation of relatively stable radicals (spin-adduct) that accumulate to sufficiently high concentration in a solution to be detected by EPR. The EPR spectrum contains information about the nature of the trapped radical and the signal intensity can be used to quantify the amount of radical trapped (**Figs 1 and 2**).
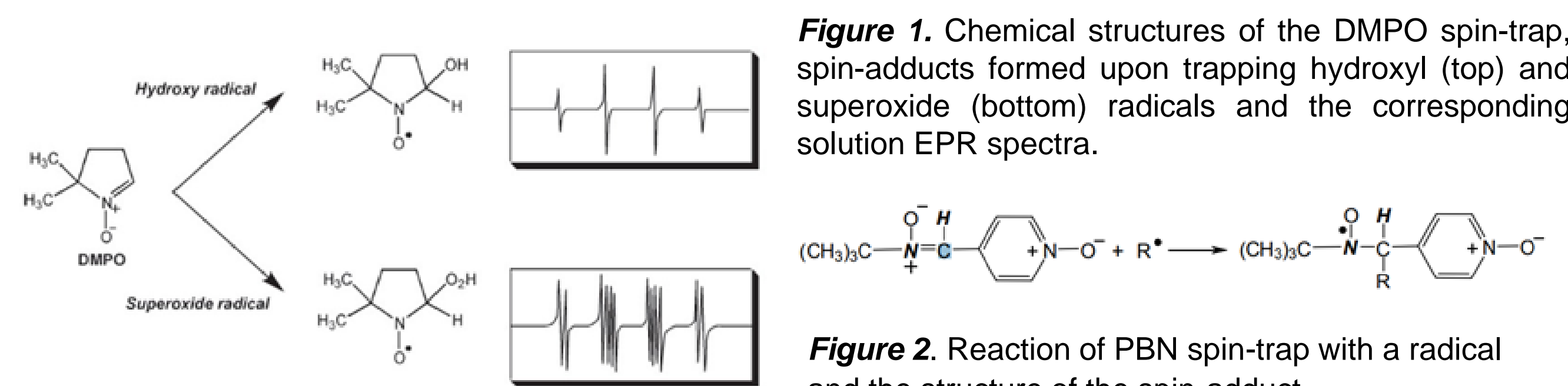

Figure 1. Chemical structures of the DMPO spin-trap, spin-adducts formed upon trapping hydroxyl (top) and superoxide (bottom) radicals and the corresponding solution EPR spectra.
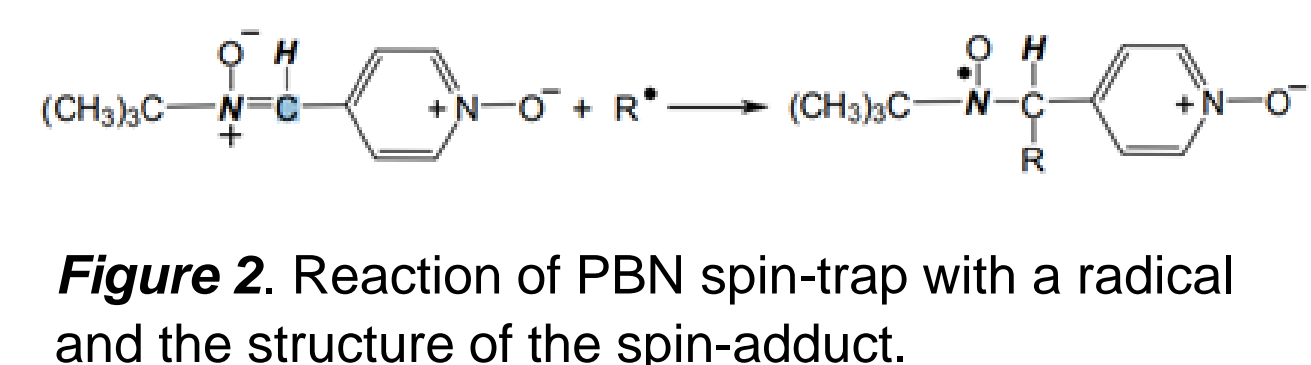
Figure 2. Reaction of PBN spin-trap with a radical and the structure of the spin-adduct.

MATERIALS AND METHODS

A commercially available e-cigarette was used in all the experiments. Pods of four different flavors were tested and are referred in the report as EC I through IV. E-cigarette aerosol was passed directly through one impinger (50 mL volume, approximately 15 cm downstream) containing 4 mL of spin-trap solution and glass beads to improve dispersion of gas (**Figure 3**). For each measurement, aerosol was collected from 40 puffs (80 mL puff volume, 5 s puff and 15 s intervals between puff cycles). The control samples were obtained by drawing air through an e-cig with a discharged battery. 1R6F research cigarettes were purchased from the Center for Tobacco Reference Products, University of Kentucky, KY. Combustible cigarette smoking was performed under the following puffing protocol: 35 mL puff volume, 2 s puff, and 60 s puff interval, 6 puffs per experiment. Particulate and gas phase were separated via the use of a single downstream 44 mm diameter Cambridge filter pad. Gas phase was directed through a glass tube (3 mm id, 4 mm od) outfitted with a porous fritted glass tip to the bottom of a glass container filled with 3 mL of spin-trap solution. The control samples were obtained by drawing air using the puff machine airflow with the same conditions as smoking, but without a cigarette. Both PBN and DMPO spin-traps (**Figs 1 and 2**) were purchased from Enzo Life Sciences, Inc., Farmingdale, NY. EPR spectra were recorded using an X-band Bruker BioSpin E500 (Billerica, MA).

RESULTS

Spin-adduct signal was detected in all four flavors of the EC product studied using PBN and DMPO spin-traps, thereby indicating production of free radicals in e-cigarette aerosols. By comparing the intensity of the detected EPR spectra with one from a radical solution with a known concentration, the total amounts of trapped radicals were calculated (**Table 1**). The total amount of trapped radicals per combustible cigarette was estimated to be 27- to 45-fold higher than e-cigs. **Figure 4** shows the experimental EPR spectra from a PBN spin-trap for research combustible cigarette 1R6F, EC I, II, III and IV products and the signal from control (air drawn through an e-cig with a discharged battery). All spectra show a typical PBN adduct signal of a triplet of a doublet. The EPR signals can be simulated (**Figure 5**) assuming two spin-adducts, one having hyperfine couplings typical for oxygen-centered radicals $A_N = 13.58–13.65$ G, $A_H = 1.8–2.0$G, g=2.0062 and another much weaker one with $A_N = 14.2–14.3$ G, $A_H = 3.0–3.2$ G, g=2.0061. The former radical adduct has parameters in agreement with those reported for alkoxy radicals, and the second, minor component, can be assigned to a carbon-centered alkyl radical •C-R.

Table 1. Measurement of free radical levels produced from different e-cigarette products and a 1R6F reference cigarette using 50 mM PBN spin-trap solution in toluene.

PRODUCT	RELATIVE INTENSITY	NUMBER OF SPINS
1R6F	1	5.1×10^{15}
EC-I	0.037	1.9×10^{14}
EC-II	0.022	1.1×10^{14}
EC-III	0.027	1.4×10^{14}

Figure 3. Experimental setup for passing e-cigarette aerosol through an impinger filled with a spin-trapping solution.
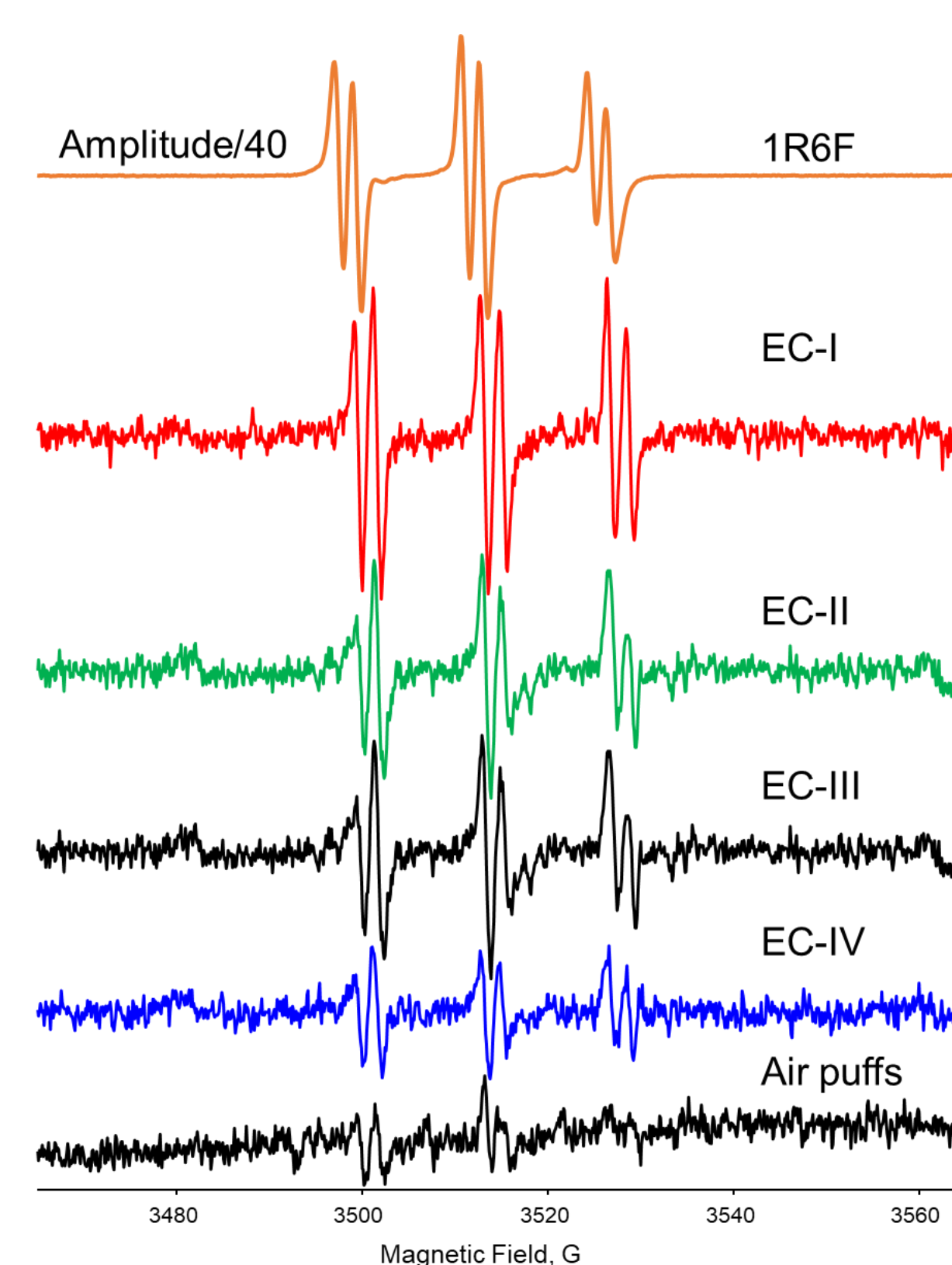

Figure 4. Room temperature CW EPR spectra from PBN spin-trap solution in toluene after bubbling with aerosols produced by research combustible cigarette (top) and the EC with four different e-liquids and control air puffs.
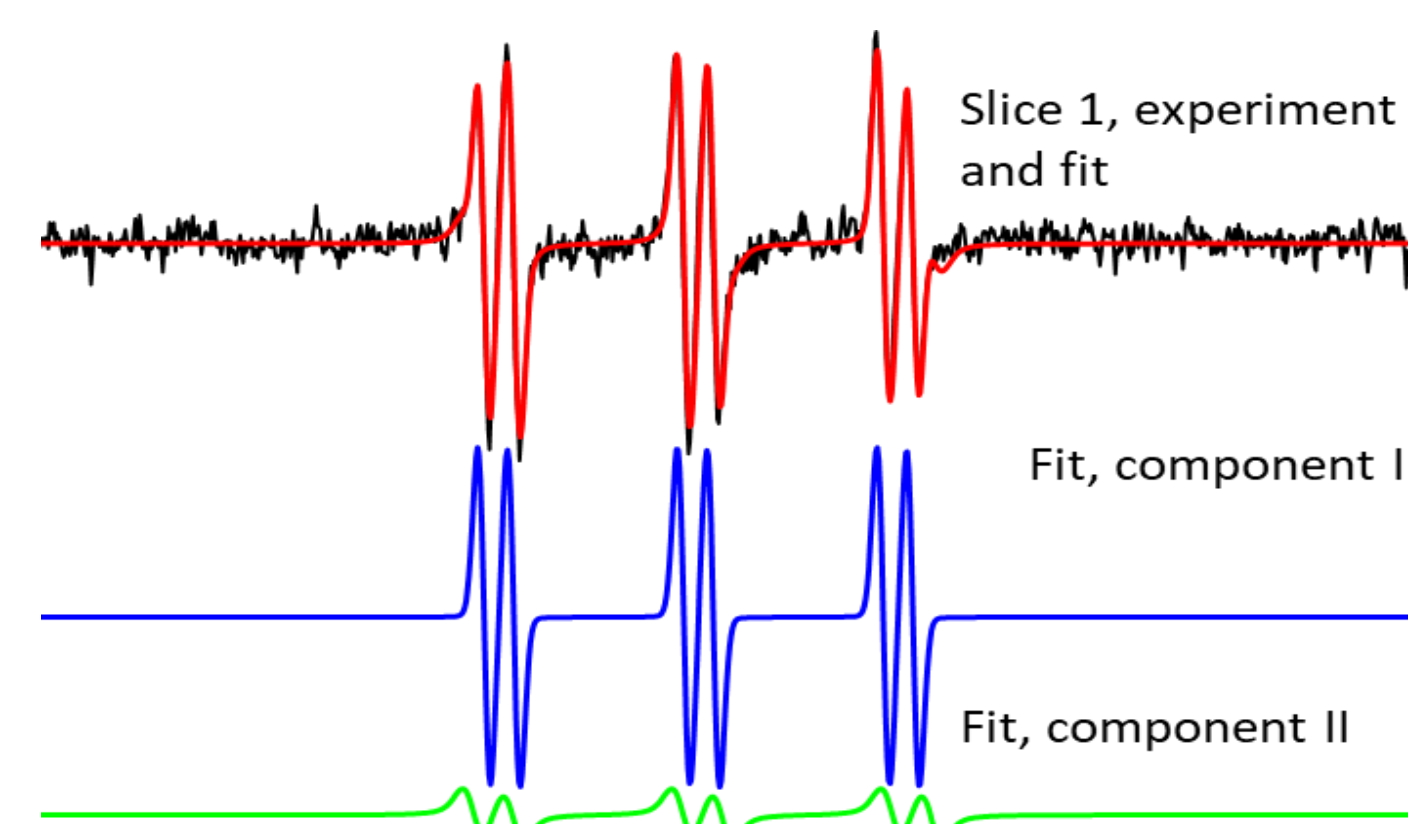

Figure 5. Room temperature experimental CW EPR spectrum from 50 mM PBN spin-trap solution after bubbling with aerosol produced by EC-I and the simulation with two components.

The DMPO spin-trap has certain advantages over PBN. In particular, DMPO adducts are mostly redox inactive and allow for more certainty in the precursor assignments. We have observed formation of DMPO spin-adducts in aerosols formed by four flavors of EC. **Figure 6** shows illustrative continuous wave (CW) EPR spectra from EC-I, EC-II and EC-III as well as a control spectrum from air drawn through an e-cig with a discharged battery. EPR spectra from DMPO spin-adducts are more complex but can be well described by 2 main components (**Figure 7**).

The dominant component, characterized by $A_N = 12.86–12.88$ G, $A_{H1} = 6.45–6.46$ G, $A_{H2} = 1.72–1.76$ G and g=2.0061, is assigned to an oxygen-centered alkoxy radical, confirming the assignment of PBN adduct. The minor component with $A_N = 13.6 –13.8$ G, $A_H = 11.1–11.2$ G and g=2.0061 is close to parameters for peroxy radical in ethylene glycol.
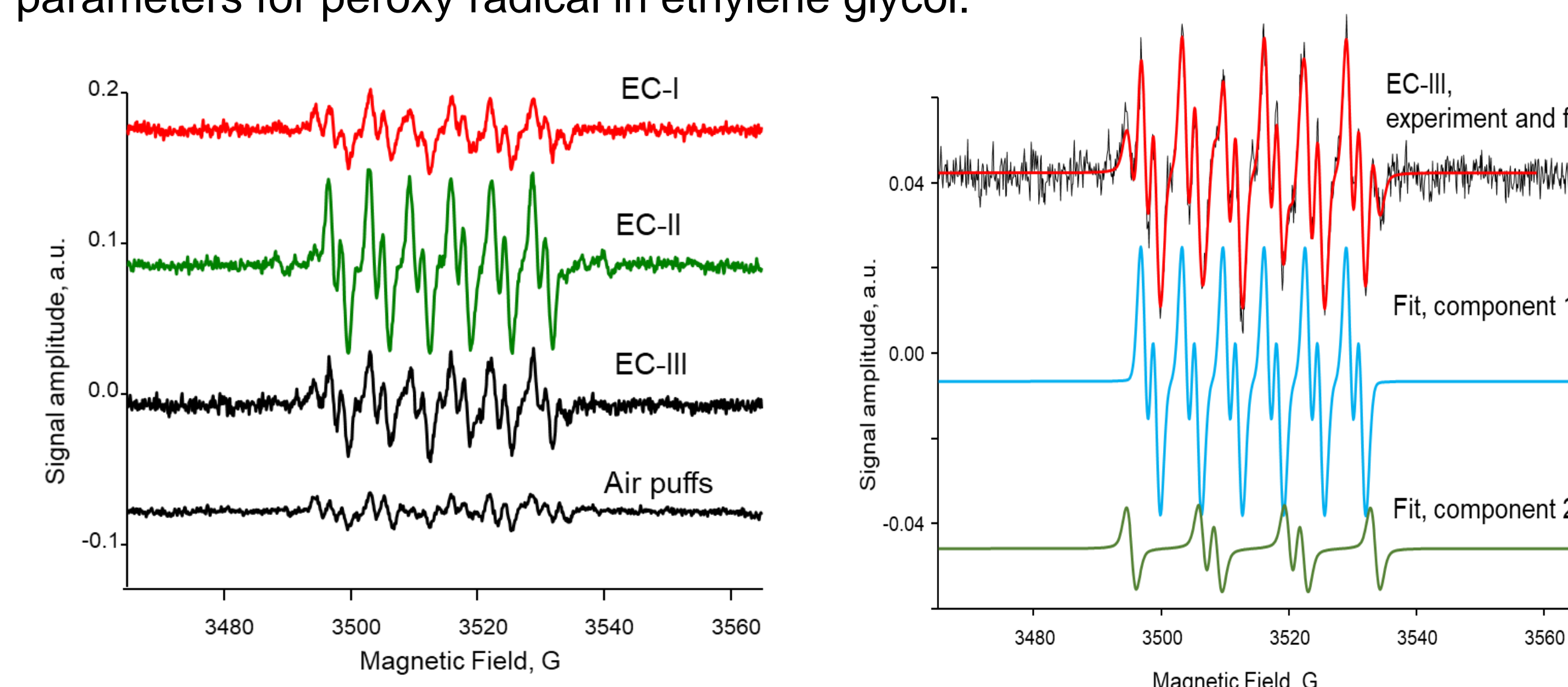

Figure 6. Room temperature CW EPR spectra from 20 mM DMPO spin-trap solution in toluene after bubbling with aerosols produced by the EC with different e-liquids and control air puffs
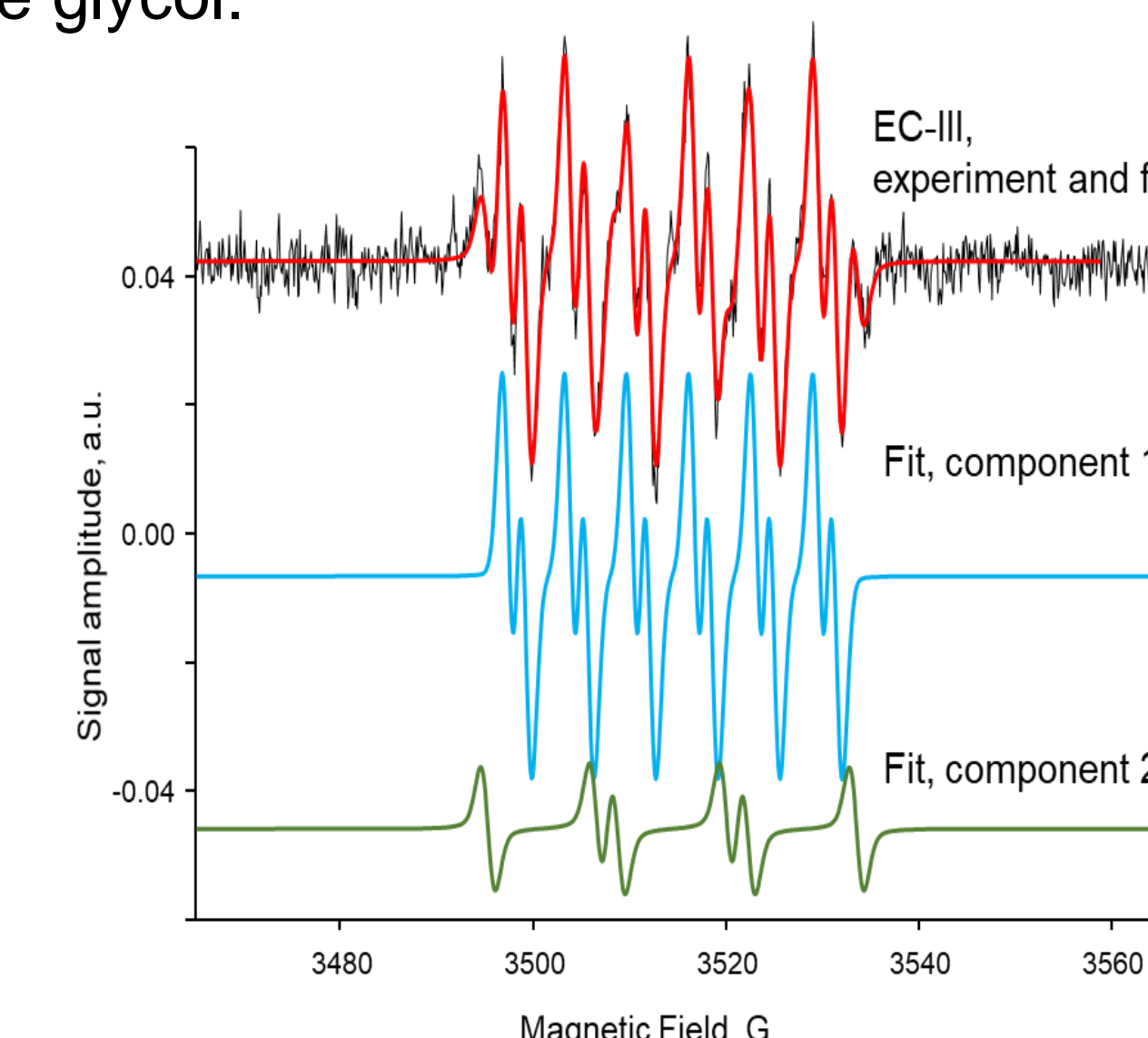
Figure 7. Room temperature experimental CW EPR spectrum from 20 mM DMPO in toluene after bubbling with aerosol produced by the EC-III and the simulation with two components.

We observed significant variations in both signal intensity and the lineshape of the signals detected in experiments with the various EC flavors and DMPO. We hypothesize that the observed variations in the total spin count are due to occasional variations in the heating conditions. Variations in appearance of the spectral components can vary due to different nature of radicals formed or partitioning of the same adduct into two phases – toluene and the more polar glycerol/propylene glycol phase formed from solvents of the e-cigarette aerosols.

CONCLUSIONS

- In this pilot study, we have demonstrated the feasibility of trapping and characterizing radicals formed in EC aerosols by PBN and DMPO spin-traps in toluene.
- We quantified production of short-lived radicals in e-cigarette aerosol condensate and have shown that they are at the level of 2.2% - 3.7% of the radicals trapped from smoke of a reference 1R6F combustible cigarette.
- The major component of the spin-adduct spectra was assigned to an oxygen-centered alkoxy radical.

ACKNOWLEDGEMENT

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