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# Free-Base Nicotine Is Nearly Absent in Aerosol from IQOS Heat-Not-Burn Devices, As Determined by $^1$H NMR Spectroscopy

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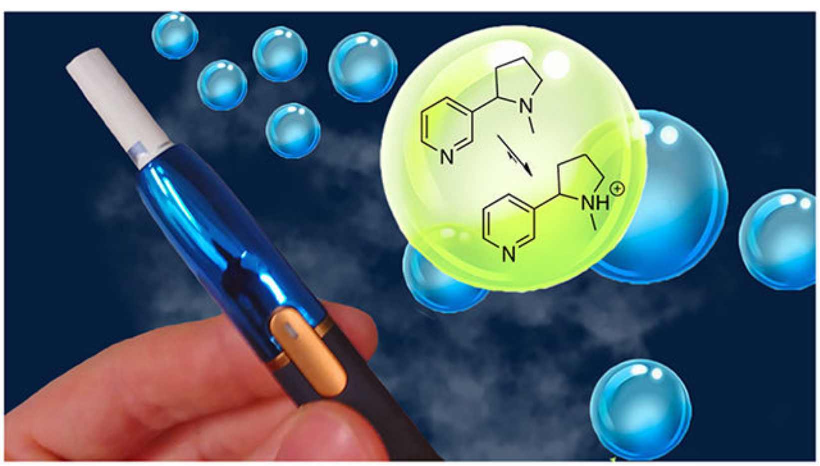
## Abstract

Heat-not-burn products, eg, I quit ordinary smoking (IQOS), are becoming popular alternative tobacco products. The nicotine aerosol protonation state has addiction implications due to differences in absorption kinetics and harshness. Nicotine free-base fraction ($\alpha_{fb}$) ranges from 0 to 1. Herein, we report $\alpha_{fb}$ for IQOS aerosols by exchange-averaged $^1$H NMR chemical shifts of the nicotine methyl protons in bulk aerosol and verified by headspace-solid phase micro-extraction-gas chromatography-mass spectrometry. The $\alpha_{fb} \approx 0$ for products tested; likely a result of proton transfer from acetic acid and/or other additives in the largely aqueous aerosol. Others reported higher $\alpha_{fb}$ for these products, however, their methods were subject to error due to solvent perturbation.

## Graphical Abstract



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Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.9b00076.
Materials and methods, statistics, error analysis, and experimental challenges (PDF)

The authors declare no competing financial interest.

Heat-not-burn (HNB) tobacco products, which originally received poor commercial reception when first introduced in 1988[1], are experiencing a rapid rise in popularity.[2] As of 2017 in Japan, 4.7% of the population ages 15–69 in a cross-sectional survey panel used HNB products, and 3.6% used Phillip Morris International's HNB product "I quit ordinary smoking" (IQOS).[3] IQOS devices consist of three main components: a tobacco "heatstick," a holder, and a charger. The heatstick, which resembles a normal cigarette, contains a "tobacco plug" of ~320 mg of reconstituted tobacco[4] treated with glycerin humectant.[4–6] The heatstick has two separate filter components: a polymer film and a cellulose acetate mouthpiece similar to traditional cigarettes.[4,5] The heatstick inserts into the holder, which contains a small blade or flange which provides heating.[4,5] In an attempt to limit formation of pyrolytic products, the IQOS operating temperature does not exceed 350 °C, which is significantly lower than the 600–900 °C combustion temperature in traditional cigarettes.[4] Heating tobacco at this temperature creates an aqueous aerosol with a water content of ~57% by mass of the particulate matter (PM),[7] which inspired Gasparyan et al. to dub this unique aerosol a "distillate."[7]

The protonation state of nicotine in an aerosol depends on pH of the medium[8,9] and has important toxicological implications, which will be briefly described.[10] Of the three protonation states of nicotine (free-base [Nic], monoprotonated [$NicH^+$], and diprotonated [$NicH_2^{2+}$]), only Nic and $NicH^+$ exist in significant amounts in tobacco smoke PM, because conditions therein are not sufficiently acidic to generate significant $NicH_2^{2+}$. In order to compare relative amounts of Nic to $NicH^+$ in an aerosol PM, free-base fraction ($\alpha_{fb}$) can thus be calculated:

$$\alpha_{fb} \equiv \frac{[\mathrm{Nic}]}{[\mathrm{Nic}] + \left[\mathrm{NicH}^+\right]} \quad (1)$$

with values ranging from 0 to 1.[9–11] Nic can exist in both PM and gas phase, while $NicH^+$ is nonvolatile and exists exclusively in PM. Nicotine phase differences may affect respiratory tract deposition as well as nicotine absorption kinetics.[10] A tobacco product with greater $\alpha_{fb}$ could result in a faster physiological response if this leads to a greater spike in blood nicotine concentration, implying that $\alpha_{fb}$ could have implications for addiction potential.[11–13] Furthermore, nociception in the posterior pharynx triggered by Nic upon inhalation[14] leads to a perception of harshness, whereas a lower $\alpha_{fb}$ value may be linked with a less harsh sensation upon inhalation.[15,16]

The $\alpha_{fb}$ values for IQOS products were reported by Salman et al.,[17] who used aqueous solvent extraction to quantify free-base nicotine of total PM captured on filter pads. As described by Duell et al.,[11] issues related to solvent extraction can lead to significant perturbation of $\alpha_{fb}$. The novel method used herein to directly measure $\alpha_{fb}$ of aerosols from IQOS products is based on one previously used by us,[9,11,18] which is the only method described in the literature that uses NMR to measure $\alpha_{fb}$ of e-cigarette e-liquids without perturbing the sample with a solvent.[11] Results herein were cross-validated with a novel headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-

GCMS) method from Luo, Motti, McWhirter, Pankow (2019, in preparation; see SI). Total nicotine delivery was quantified by HPLC-UV based on previous methods.[19–21]

Previously, $\alpha_{fb}$ determination by NMR was done using a concentric NMR tube insert containing pure e-cigarette e-liquid,[11] condensed aerosol, or cigarette smoke,[18] which was inturn surrounded by lock solvent, DMSO-$d_6$. Duell et al. calculated $\alpha_{fb}$ by comparing *relative* chemical shift differences between methyl and aromatic protons of Nic and $NicH^+$ standards in glycerol/propylene glycol with commercial e-liquids.[11] However, $\Delta\delta$ values calculated for IQOS bulk aerosol using this method resulted in inaccurate values due to inconsistencies in $\delta$ for nicotine aromatic protons, which might arise from formation of complexes between acetic acid and nicotine's pyridine nitrogen.[23] Therefore, calculation of $\alpha_{fb}$ for IQOS products required use of the absolute chemical shift of nicotine methyl protons, referenced relative to 4,4-dimethyl-4-silapentane-1-sulfonic acid using eq 2:[9]

$$\alpha_{fb} \equiv \frac{\left[\left(\delta_{\text{monoprotonated standard}}\right) - \left(\delta_{\text{commercial sample}}\right)\right]}{\left[\left(\delta_{\text{monoprotonated standard}}\right) - \left(\delta_{\text{free base standard}}\right)\right]}$$

Puff topography was studied to assess its influence on $\alpha_{fb}$. The Health Canada Intense (HCI) puffing regime (55 mL puff volume, 2 s puff period, 30 s puff interval, with bell-shaped puffs) has been suggested as the most appropriate for these products,[24] however, a variety of puffing parameters have been used in literature.[25,26] Given this disagreement, two puffing parameters were used: a modified HCI (mHCI; HCI with a square-shaped puff) and that specified for e-cigarettes by the Cooperation Center for Scientific Research Relative to Tobacco (CORESTA, 55 mL puff volume, 3 s puff period, 30 s puff interval, with square-shaped puffs).[27]

The $\alpha_{fb}$ values determined by NMR and nicotine delivery by HPLC-UV for three IQOS brands under mHCI and CORESTA are reported in Table 1. A larger assortment of HNB product $\alpha_{fb}$ values are shown in Table 2. The $\alpha_{fb}$ and nicotine aerosol concentration values were found to be consistent across all heatsticks tested. The $\alpha_{fb}$ values consistently suggested that the majority of aerosol nicotine from these products is $NicH^+$, with very little Nic. The $\alpha_{fb}$ values determined by NMR were cross-validated using the HS-SPME-GCMS method, which found no significant difference between them for the brand tested. The puffing parameter did not significantly affect $\alpha_{fb}$ or nicotine delivery. A comprehensive description of error analysis and statistical tests is presented in the SI.

The very low $\alpha_{fb}$ calculated for IQOS heatsticks herein is consistent with the identification and quantification of acetic acid in the aerosol, as confirmed by NMR and GC-MS, as well as the IQOS flavor additive listing (at levels "no higher than 0.01%").[22] Given the aqueous nature of the aerosol,[7] rapid acid-base equilibration occurs. Very low, approximating zero, free-base nicotine in IQOS aerosols differs from findings in Salman et al.,[17] who reported a %free-base of 5.7 ($\alpha_{fb}$ = 0.057), nearly 6 times the average $\alpha_{fb}$ measured in work herein, and beyond the upper bound of the 95% CI for the largest $\alpha_{fb}$ value presented herein. A toluene extract of an aqueous extract of the filter pad used for aerosol collection removes Nic from the Nic $\leftrightharpoons$ $NicH^+$ equilibrium, which, by Le Chatelier's principle, must generate more Nic as it continuously migrates from aqueous to toluene phases, leading to an overextraction of Nic and an over-estimation of $\alpha_{fb}$. Additionally, analysis done by Salman et al.[17] may also suffer from inaccuracies due to dilution effects and atmospheric $CO_2$ incursion.[11]

The $\alpha_{fb} \approx 0$ in the aqueous aerosols of HNB products tested is unprecedented when compared to values observed in cigarettes and e-cigarettes.[11,28] A comprehensive analysis of $\alpha_{fb}$ values for 12 commercial and reference cigarettes performed by Pankow et al.[28] found $\alpha_{fb}$ ranges from 0.010 ± 0.008 to 0.29 ± 0.08, with 9 of 12 < 0.1. Duell et al.[11] reported $\alpha_{fb}$ for 11 e-liquids from various brands and found they ranged from 0.03 to 0.84, with only 3 having a value below 0.1.

The NMR method herein is a direct and accurate technique for determining $\alpha_{fb}$ in the aqueous aerosols seen in HNB products. The extremely low Nic values seen in these products likely stem from occurrence of acetic acid, and perhaps other additives, in an aerosol with significant aqueous character. The $\alpha_{fb} \approx 0$ may translate to low apparent harshness. It is likely that other similarly designed devices will be comparable in $\alpha_{fb}$. Low $\alpha_{fb}$ indicates nicotine will almost exclusively be in the PM. The low level of gaseous nicotine in IQOS aerosols may explain the finding that HNB devices are "less satisfying" than traditional cigarettes.[29]

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGMENTS

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## ABBREVIATIONS

| | |
|---|---|
| **IQOS** | I quit ordinary smoking |
| **HNB** | heat-not-burn |

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**Table 1.**

$\alpha_{fb}$ Determined by $^1$H NMR and Nicotine Delivery for Three Brands of IQOS Heatsticks Under mHCI and CORESTA Puffing Topographies[a]

| brand/flavor | $\alpha_{fb}$ mHCI | $\alpha_{fb}$ CORESTA | Nic, mg, mHCI | Nic, mg, CORESTA |
|---|---|---|---|---|
| Parliament | 0.00 | 0.02 ± 0.03 | 1.08 ± 0.11 | 1.19 ± 0.35 |
| HEETS/Yellow | 0.00 | 0.00 | 1.23 ± 0.24 | 1.22 ± 0.12 |
| Marlboro/SmoothRegular | 0.00 | 0.01 | 1.21 ± 0.15 | 1.11 ± 0.08 |

[a]Uncertainties are estimated to be at the 95% CI.

**Table 2.**

$\alpha_{fb}$ for Select Brands As Determined by $^1$H NMR Using CORESTA Puffing Regime[a]

| Brand/flavor | $\alpha_{fb}$ |
|---|---|
| Parliament | 0.02 ± 0.03 |
| HEETS/Amber | 0.00 |
| HEETS/Yellow | 0.00 |
| HEETS/Turquoise | 0.01 |
| Marlboro/Menthol | 0.01 |
| Marlboro/Smooth Regular | 0.01 |
| Marlboro/Balanced Regular | 0.00 |
| Marlboro | 0.00 |
| Marlboro/Mint | 0.02 |
| Marlboro/Purple Menthol | 0.01 |

[a]Error in the first measurement is assumed to be representative of all measurements.