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Kinetics of Aldehyde Flavorant-Acetal Formation in E-Liquids with Different E-Cigarette Solvents and Common Additives Studied by ^{1}H NMR Spectroscopy

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Abstract

Flavorants, nicotine, and organic acids are common additives found in the e-liquid carrier solvent, propylene glycol (PG) and/or glycerol (GL), at various concentrations. Some of the most concentrated and prevalent flavorants in e-liquids include *trans*-cinnamaldehyde, vanillin, and benzaldehyde. Aldehyde flavorants have been shown to react with PG and GL to form flavorant-PG and -GL acetals that have unique toxicity properties in e-liquids before aerosolization. However, there is still much that remains unknown about the effects of different e-cigarette solvents, water, nicotine, and organic acids on the rate of acetalization in e-liquids. We used ^{1}H NMR spectroscopy to determine the first-order initial rate constant, half-life, and % acetal formed at equilibrium for flavorant-acetal formation in simulated e-liquids. Herein, we report that acetalization generally occurs at a faster rate and produces greater yields in e-liquids with higher ratios of GL (relative to PG). *trans*-Cinnamaldehyde acetals formed the fastest in 100% PG-simulated e-liquids, followed by benzaldehyde and vanillin based on their half-lives and rate constants. The acetal yield was greatest for benzaldehyde in PG e-liquids, followed by *trans*-cinnamaldehyde and vanillin. Acetalization in PG e-liquids containing aldehyde flavorants was inhibited by water and nicotine but catalyzed by benzoic acid. Flavorant-PG acetal formation was generally delayed in the presence of nicotine, even if benzoic acid was present at 2-, 4-, or 10-fold the nicotine concentration, as compared to the PG e-liquids with 2.5 mg/mL flavorant. Thus, commercial e-liquids with aldehyde flavorants containing a higher GL ratio (relative to PG), little water, no nicotine, nicotine with excess organic acids, or organic acids without nicotine would undergo acetalization the fastest and with the highest yield. Many commercial e-liquids must therefore contain significant amounts of flavorant acetals.

Graphical Abstract

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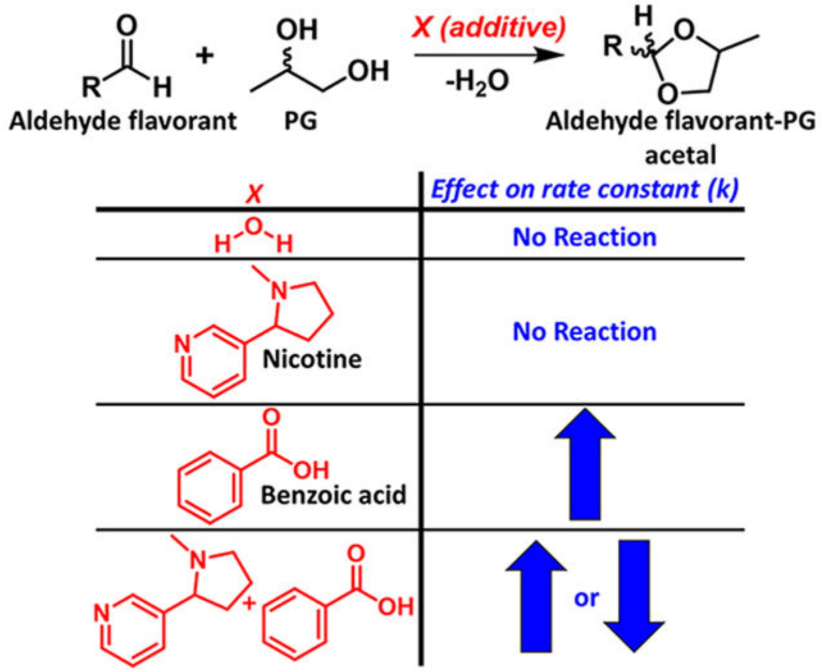
The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.2c00159.

^{1}H NMR spectra showing the formation of *trans*-cinnamaldehyde-PG acetals over time and proton assignments of flavorant-PG and -GL acetals, as well as additional information for assigning the flavorant acetal ^{1}H NMR peaks (PDF)



1. INTRODUCTION

Electronic cigarettes (e-cigarettes) have become increasingly popular since their introduction to the United States market in 2007.[1] In 2021, ~3 and ~11% of adolescents in middle and high school reported e-cigarette use, respectively.[2] Flavorants and nicotine are frequently added to the e-liquid carrier solvent (propylene glycol (PG) and/or glycerol (GL)) and can aerosolize and degrade during vaping. Disposable e-cigarettes (i.e., Puff Bar) replaced JUUL as the most popular e-cigarette device among adolescents after the Food and Drug Administration (FDA) prohibited the sale of prefilled e-cigarette cartridges in any flavor except tobacco or menthol in 2020.[3] Fix et al. reported how regulations concerning flavorant and nicotine concentrations in e-liquids vary by region and that the concentration of nicotine listed on the packaging can be inaccurate.[4] Flavored e-liquids with and without nicotine are widely accessible to consumers despite the limited information on the potential harmfulness of flavorants before and after e-liquid aerosolization.

Behar et al. identified some of the most common flavorants in commercial e-liquids as benzaldehyde (cherry flavor), vanillin (creamy, vanilla flavor), and *trans*-cinnamaldehyde (cinnamon flavor).[5] Aerosolized commercial e-liquids with *trans*-cinnamaldehyde can be cytotoxic,[6] increase respiratory infection by disrupting mitochondrial function and bioenergetic processes,[7] promote oxidative stress on osteoblast-like cells,[8] and impair respiratory immune cell function.[9] Commercial e-liquids that contain benzaldehyde and vanillin have been linked to the impairment of phagocytosis[10] and hepatotoxicity[11] upon aerosolization, respectively. The physiological effects of inhaling aerosolized e-liquids with flavorants require further assessment to minimize the consumers' exposure to harmful and potentially harmful chemicals (HPHCs).

PG and GL can thermally degrade during aerosolization to produce propanal, acetaldehyde, glycolaldehyde, formaldehyde, acrolein, formaldehyde hemiacetals (formaldehyde adducts of PG or GL formed by a reversible reaction), and other HPHCs.[12] Khlystov and Samburova[13] found that aerosols produced from flavored commercial e-liquids contain increased levels of toxic aldehydes compared to aerosolized unflavored e-liquids. The addition of sweeteners (e.g., sucralose) and flavor enhancers (e.g., triacetin) to e-liquids can increase the degradation levels compared to e-liquids that are unsweetened and unflavored upon aerosolization as shown by Duell et al.[14] and Vreeke et al.,[15] respectively. The type of e-cigarette device,[16] heating element,[17] e-liquid composition,[18] and use patterns the consumer employs[16] can enhance the formation of HPHCs upon aerosolization.

Popular disposable e-cigarette brands (e.g., Puff Bar, SEA, Ezzy Oval) mimic aspects of JUUL but do not have a microcontroller to regulate electrical power to the heating coil and consequently can emit higher levels of carbonyls and metals compared to JUUL.[19] Noël et al.[20] showed that aerosolized butter-flavored e-liquids produced under subohm conditions (<1 Ω; increased wattage) leading to higher temperatures contained higher levels of carbonyls and nicotine compared to supraohm (>1 Ω; decreased wattage) at presumably lower temperature conditions using the same e-cigarette with different atomizers. Yogeswaran and Rahman found that disposable e-cigarettes containing tobacco-derived nicotine generated more reactive oxygen species upon aerosolization than disposable e-cigarettes with tobacco-free nicotine.[18] Further studies are necessary to understand how flavorants, nicotine, and organic acids react in e-liquids before and after aerosolization under different conditions.

Aldehyde flavorants can react with PG and GL to form flavorant-PG and -GL acetals in e-liquids before aerosolization. Erythropel et al.[21,22] observed that greater than 40% of vanillin, ethylvanillin, benzaldehyde, citral, and *trans*-cinnamaldehyde were converted to flavorant acetals in PG e-liquids. The aerosol transfer efficiency of the flavorant-PG acetals from e-liquids to aerosols ranged from 50 to 80%. PG-flavorant acetals have similar scents but different toxicological properties compared to the parent flavorant.[23] Jabba et al.[24] showed that benzaldehyde- and vanillin-PG acetals can increase respiratory epithelial cell mortality and be more cytotoxic than their parent flavorants, respectively. The kinetics of acetal formation in e-liquids with water, nicotine, organic acids, and mixtures of nicotine and benzoic acid are unknown. Herein, we used ^{1}H NMR spectroscopy to analyze the rate and yield of aldehyde flavorant-acetal formation in PG, GL, and equimolar PG + GL e-liquids, as well as PG e-liquids with water, nicotine, benzoic acid, and mixtures of nicotine and benzoic acid.

2. MATERIALS AND METHODS

2.1. Materials.

USP grade propylene glycol (PG), USP grade glycerol (GL), benzoic acid (>99.5%), and benzaldehyde (>99%) were purchased from Sigma-Aldrich (St. Louis, MO). (*S*)-(−)-Nicotine (99%) and vanillin (>99%) were obtained from Alfa Aesar (Haverhill, MA). *trans*-Cinnamaldehyde (>98%) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Benzaldehyde-PG acetal (>95%) and *trans*-cinnamaldehyde-PG acetal (*trans*-4-methyl-2-(2-phenylvinyl)-1,3-dioxolane) were purchased from Sigma-Aldrich (St. Louis, MO). Vanillin-PG acetal was purchased from Carbosynth Ltd. (Compton, UK). 1,3,5-Trimethoxy benzene (TMB) was purchased from Oakwood Chemical (Estill, SC). DMSO-d_6 (D 99.9%), $CDCl_3$ (D 99.8%), and D_2O (D 99.9%) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA).

2.2. Methods.

The compositions of the simulated equimolar PG + GL, pure GL, and pure PG e-liquids used for the studies comparing the rates and yields of acetal formation in different solvents are shown in Table 1. The internal standard, TMB, was first added to PG + GL, PG, and GL by heating and stirring the mixture. Lastly, the flavorant was added to each e-liquid and stirred for ~5 min. The concentrations of flavorants were within the range observed in commercial e-liquids, based upon values from the literature.[5] PG e-liquids containing 10 mg/mL flavorant without the internal standard TMB were formulated and used as control experiments to demonstrate that TMB had no effect on acetal formation (Table 1). All ratios were verified using ^{1}H NMR spectroscopy before the addition of flavorant (Table 1).

Once the flavorant was dissolved in the e-liquid, aliquots of each sample were placed in NMR tubes precharged with 0.5 mL of DMSO-d_6 at various time points during the monitored reaction period. Each sample was evaluated by ^{1}H NMR spectroscopy, using a Bruker AVANCE III NMR spectrometer using a 30° observation pulse with 16 scans and a 3 s relaxation delay at 25 °C, shortly after generation. The first-order initial rate constant, half-life, and % acetal formed at equilibrium for each e-liquid were determined by integrating the aldehyde flavorant peaks relative to TMB in each sample.

2.3. Experimental Details.

2.3.1. Effects of Water, Nicotine, Benzoic Acid, and Nicotine + Benzoic Acid on PG-Flavorant Acetal Formation.—

The % flavorant acetal formed at equilibrium, first-order initial rate constant, and half-life were determined in PG e-liquids containing 2.5 mg/mL flavorant with water, nicotine, benzoic acid, and nicotine+benzoic acid (at different mol ratios). PG e-liquids with 2.5 mg/mL flavorant were chosen as the standard for these experiments with common e-liquid additives. The compositions of the simulated flavored e-liquids with additives are shown in Table 1. First, the additives were mixed into PG, followed by the addition of flavorant to each e-liquid. The reaction time began after the flavorant was mixed into the e-liquid (after ~5 min).

Some simulated PG e-liquids with flavorants and additives were placed in an oven set at 100 °C for 24 h and then reheated for another 24 h to determine their acetal yield (Tables 2, 3, and 4). Aliquots of the heated e-liquids were analyzed before the first 24 h in the oven and then after each 24 h period (to verify that their compositions were similar) by ^{1}H NMR spectroscopy. We compared the ^{1}H NMR spectra before, after 24 h, and after 48 h of heating—knowing the peak assignments for PG, GL, flavorants, additives, and flavorant acetals—to ensure that other degradants did not form (e.g., presence of unknown peaks in heated e-liquids).

2.3.2. Analysis of Commercial E-Liquids.—Commercial e-liquids (>~5 years old, based on their purchase date) containing vanillin, ethyl vanillin, or *trans*-cinnamaldehyde were analyzed by ^{1}H NMR spectroscopy as above to determine if any flavorant-PG or -GL acetals formed over time. The total age of the commercial e-liquids, including the time they sat on store shelves, was unknown. The brand designation, flavor, and composition of the commercial e-liquids studied are shown in Table 5. The original flavorant and nicotine concentrations in e-liquids were determined using gas chromatography–mass spectrometry (GC-MS) when purchased.

The e-liquids were stored in a freezer when not in use. Similar to Section 2.3.1, the commercial e-liquids were placed in an oven at 100 °C for 24 h and then reheated for another 24 h to simulate naturally aged e-liquids (Table 5). Aliquots of the heated e-liquids were analyzed before the first 24 h in the oven and then after each 24 h period (to verify that their compositions were similar) by ^{1}H NMR spectroscopy. The ^{1}H NMR spectra of the unheated and heated e-liquids were compared, with the aldehyde flavorant and acetal peaks known, to confirm that degradants did not form (e.g., the presence of unknown peaks in the heated e-liquids).

3. RESULTS

3.1. Rate of Flavorant-PG and -GL Acetal Formation.

The first-order initial rate constant and half-life for the formation of *trans*-cinnamaldehyde-, vanillin-, and benzalde-hyde-PG and -GL acetals in simulated e-liquids were determined by analyzing aliquots of the samples over time with ^{1}H NMR spectroscopy. The kinetics were based on the consumption of the flavorant concentration in the e-liquid, assuming the rate constant was pseudo-first-order due to excess PG and GL (relative to the initial flavorant concentration). The initial rates were for the early time data points which gave a linear fit to the first-order rate equation. The flavorant and flavorant-PG and -GL acetal peaks—identified following the procedure in the supplemental information—were integrated relative to the internal standard (1,3,5-trimethoxy benzene; TMB) peak in each ^{1}H NMR spectrum (Figures S1-S3). We compared the ^{1}H NMR spectra from the flavored e-liquids with versus without TMB (at different points in time) and found that TMB did not interact with acetal formation based on (a) their similar acetal yields and (b) the absence of any unknown peaks in e-liquids with TMB (besides the known resonances of TMB). The reaction time was taken as the point when the flavorant was dissolved in the e-liquid (after ~5 min of mixing time).

Behar et al.[5] detected 155, 31, and 2.5 mg/mL as the highest concentrations of *trans*-cinnamaldehyde, vanillin, and benzaldehyde in commercial e-liquids, respectively. The concentration of *trans*-cinnamaldehyde chosen for this study was ~15 times less than the maximum determined by Behar et al. because conversion to the acetal was nearly instantaneous at 155 mg/mL (Table 1). The first-order initial rate constant and half-life were measurable at the maximum concentrations previously detected in commercial e-liquids for vanillin (31 mg/mL) and benzaldehyde (2.5 mg/mL; Table 1).

Acetal formation[25] typically includes an acid catalyst (not included in Figure 1) that protonates the carbonyl oxygen, making the carbonyl carbon more partially positive (e.g., an electrophile), and then the alcohol moiety (e.g., a nucleophile) can attack the carbonyl carbon (Figure 1). Next, the acid catalyst is regenerated with the formation of a hemiacetal intermediate. The acid protonates the −OH group on the hemiacetal, and water is eliminated as a product. Then, the alcohol moiety attacks the carbonyl carbon on the reactive *O*-alkylated intermediate, and the acid is regenerated by removing a proton from the acetal. Acetal formation is possible without an acid catalyst, but the rate of formation is much slower.

The *trans*-cinnamaldehyde, vanillin, and benzaldehyde acetals formed ~2, ~12, and ~35 times faster in GL than their respective flavorant acetals in PG (Tables 2-4). The rate of flavorant-acetal formation was higher in GL than in PG, in part because GL forms two acetals (5- and 6-member rings),[26] but PG forms one acetal (a 5-member ring;[27] Figures S2 and S3). There are additional effects to consider such as relative nucleophilicity and more. The formation of flavorant acetals was slowest in PG compared to GL and PG + GL for the flavorants used in this study (Tables 2-4). *trans*-Cinnamaldehyde acetals formed at a slightly faster rate in PG + GL versus GL, with half-lives of 3.0 and 3.4 h, respectively (Table 2). However, the rates of vanillin and benzaldehyde-acetal formation were faster in GL than in PG + GL (Tables 3 and 4).

The acetal yield was lower with PG as the e-cigarette solvent, compared to GL, for *trans*-cinnamaldehyde and vanillin (Tables 2 and 3). However, the acetal yield was >99% for benzaldehyde in PG, GL, and PG + GL (Table 4). The percentages of *trans*-cinnamaldehyde- and vanillin-GL acetals formed were 5 and 24% greater than PG acetals at equilibrium in GL and PG e-liquids, respectively (Tables 2 and 3). The first-order initial rate constants, half-lives, and acetal yields at equilibrium for flavorant acetals formed in pure GL generally had a faster rate and gave a higher final yield as compared to acetals formed in pure PG for the flavorants used in this study.

3.2. Rate of Flavorant-PG Acetal Formation with the Common Additives Water, Nicotine, and Benzoic Acid.

PG e-liquids containing 2.5 mg/mL flavorant without versus with water, nicotine, benzoic acid, and nicotine+benzoic acid (at varying mol ratios) were compared to determine the additives' effects on the rate constant, half-life, and % acetal formed at equilibrium (Table 1). The addition of 20% water (by wt) to the PG e-liquid with 2.5 mg/mL *trans*-cinnamaldehyde inhibited acetal formation (Table 2). Water is a product of acetal formation, and the addition of excess water to the e-liquid shifted the equilibrium toward the reactants (i.e., parent flavorant + PG; Figure 1). Roldán et al.[28] increased the yield of solketal (the ketal product of acetone and glycerol) using a zeolite membrane batch reactor to remove water from the reaction environment. Half and twice the amount of nicotine relative to *trans*-cinnamaldehyde (by mol) and twice the amount of nicotine relative to vanillin (by mol) also inhibited acetal formation (Tables 2 and 3).

Twice the amount of benzoic acid relative to *trans*-cinnamaldehyde, vanillin, and benzaldehyde (by mol) decreased the half-life by ~4.2, ~458.6, and ~45.2 times compared to PG e-liquids with 2.5 mg/mL flavorant, respectively (Tables 2-4). The % acetal formed at equilibrium decreased by 46% for *trans*-cinnamaldehyde, increased by 10% for vanillin, and was unchanged for benzaldehyde in PG e-liquids containing 2.5 mg/mL flavorant with benzoic acid compared to without (Tables 2-4). Acetalization in e-liquids was inhibited by nicotine (a base) and catalyzed by benzoic acid. The behavior of aldehyde flavorants in e-liquids with nicotine and benzoic acid was consistent with the acid-catalyzed acetal formation[26,29] (Figure 1; also see the textbook *Organic Chemistry*).[25]

Nicotine can exist in the free-base (harsh upon inhalation), monoprotonated (pK_a = 8.0 in water; more palatable than free-base upon inhalation), or diprotonated (pK_a = 3.1 in water) form in e-liquids depending on their acid/base conditions.[30] Duell et al.[31] have also shown that e-cigarette manufactures (i.e., Puff Bar) have recently been using synthetically created tobacco-free nicotine (often (*R*,*S*)-(±)-nicotine) instead of tobacco-derived nicotine ((*S*)-(−)-nicotine) perhaps to avoid FDA regulations. E-liquid manufacturers frequently add organic acids (e.g., benzoic acid, levulinic acid, and malic acid) to protonate nicotine, thus decreasing the harshness and increasing the inhalability of the aerosol.[32] Simulated PG e-liquids with 2.5 mg/mL flavorant and a 1:2, 1:4, and 1:10 nicotine:benzoic acid mol ratio (relative to each flavorant) were formulated to determine the effects of nicotine and benzoic acid mixtures on acetal formation. Two additional PG e-liquids with 2.5 mg/mL *trans*-cinnamaldehyde and vanillin were mixed with a 2:2 mol ratio of nicotine to benzoic acid (relative to each flavorant).

Flavorant-PG acetals formed ~6.1 and ~1.5 times slower when in the presence of nicotine, even if benzoic acid was present at 10-fold the nicotine concentration compared to PG e-liquids with 2.5 mg/mL flavorant for *trans*-cinnamaldehyde and benzaldehyde, respectively (Tables 2 and 4). Vanillin-PG acetals formed ~2.9 times faster in the presence of nicotine when benzoic acid had a concentration 2-fold greater than nicotine compared to PG e-liquids with 2.5 mg/mL vanillin (Table 3). The acetal yields were similar in PG e-liquids containing 2.5 mg/mL flavorant with and without mixtures of nicotine and benzoic acid for *trans*-cinnamaldehyde and benzaldehyde (Tables 2 and 4). However, the acetal yield increased by 10–19% in PG e-liquids containing 2.5 mg/mL vanillin with mixtures of nicotine and benzoic acid versus without (Table 3).

3.3. Analysis of Commercial E-Liquids.

Most commercial e-liquids selected in this study had a greater total flavorant concentration (considering only vanillin + ethyl vanillin+*trans*-cinnamaldehyde) than nicotine by mol. Flavorant-PG and -GL acetals were present in every e-liquid, as shown in Table 5. Two of the seven commercial e-liquids evaluated, “Winter’s Bite” and “Aries,” did not contain nicotine. “Winter’s Bite” and “Aries” (containing vanillin and ethyl vanillin without nicotine) had a greater % flavorant converted into total PG- and GL-flavorant acetals compared to “Taurus” and “Snow White’s Demise” (containing vanillin and/or ethyl vanillin with nicotine; Table 5).

“Dragons Breath” contained ~3.3 times more *trans*-cinnamaldehyde than nicotine yet had the lowest % of flavorant-PG and -GL acetals (Table 5). Nicotine appeared to inhibit acetal formation in “Taurus,” “Snow White’s Demise,” and “Dragons Breath.” Commercial e-liquids may be complex and contain other additives that might inhibit acetal formation, but we compared simulated e-liquids with and without nicotine and found that nicotine inhibited acetal formation (Tables 2 and 3). However, “Snake eyes” and “Snake oil” contained nicotine and had the highest total acetal yield among e-liquids. “Snake eyes” and “Snake oil” had a higher ratio of GL than PG (1.6:1.0 GL:PG mol ratio) and contained organic acids (an unknown amount) to protonate nicotine,[30] both of which could increase the total % of acetals formed over time.

Commercial e-liquids with and without nicotine frequently contain a wide range of flavorants at varying concentrations to create the desired flavor.[33] The effects of flavorant mixtures on aldehyde flavorant-PG and -GL acetal formation in e-liquids without and with nicotine and organic acids require further study. We showed that nicotine generally delays individual *trans*-cinnamaldehyde-, vanillin-, and benzaldehyde-acetal formation in simulated PG e-liquids, even if benzoic acid is present at 10-fold the nicotine concentration. We also found that the rate of total flavorant-PG and -GL acetal formation increased as the ratio of GL increased (relative to PG) in e-liquids. Commercial e-liquids containing aldehyde flavorants with little water, without nicotine, with organic acid(s), or a higher ratio of GL than PG would generally form acetals at a faster rate compared to the opposite of these e-liquid conditions.

4. DISCUSSION

Erythropel et al.[22] determined the % acetal formed at equilibrium and half-life of 21 mg/mL *trans*-cinnamaldehyde, vanillin, and benzaldehyde in PG over a 2-week period via gas chromatography-flame ionization detection (GC-FID). They found that 92, 40, and 95% of *trans*-cinnamaldehyde, vanillin, and benzaldehyde were converted into acetals in PG in <1, ~7, and ~5 days, respectively. We found that acetal formation was the fastest in PG with *trans*-cinnamaldehyde, followed by benzaldehyde and vanillin by comparing the half-lives for PG e-liquids with 2.5 mg/mL flavorant (Tables 2-4). *trans*-Cinnamaldehyde (an α,β-unsaturated aldehyde) was the most reactive flavorant used in this study with two electrophilic sites at the β- and carbonyl-carbon which are available for nucleophilic attack. Benzaldehyde and vanillin are simpler aldehydes having one electrophilic site at the carbonyl-carbon. The acetal yield was highest for benzaldehyde, followed by *trans*-cinnamaldehyde and vanillin among PG e-liquids with 2.5 mg/mL flavorant. The order of the half-lives from fastest to slowest and acetal yield from most to least for flavorants in this study were thus consistent with Erythropel et al.’s results.

Agirre et al.[34] and Nanda et al.[35] showed that the rate and yield of GL-ketal and -acetal formation increased as the GL:ketone and aldehyde mol ratio increased (for either reactant), respectively. There were excess PG and GL relative to aldehyde flavorants in e-liquids, yet we still observed an increased reaction rate with a slight change in acetal yield for PG e-liquids with 31 versus 2.5 mg/mL vanillin and 10 versus 2.5 mg/mL *trans*-cinnamaldehyde (Tables 2 and 3). Yu et al.[36] found that *trans*-cinnamaldehyde can be oxidized to form 3.6% (by wt) *trans*-cinnamic acid at temperatures as low as 35 °C. Our samples were stored at room temperature, but we observed ~1.5-fold more *trans*-cinnamic acid in the PG e-liquid with 10 versus 2.5 mg/mL *trans*-cinnamaldehyde.

Most e-liquids are aerosolized by metal coils (e.g., Kanthal = FeCrAl alloy, nichrome = NiCr, nickel = Ni, stainless steel = FeNiCr) in the tank or cartridge of an e-cigarette and remain in the reservoir until most of the e-liquid is consumed. Olmedo et al.[37] showed that metals from the coils can be transferred into the aerosol and then leach into the remaining e-liquid in the reservoir after aerosolization, but they did not specify that the metals could be oxides or salts. Saliba et al.[38] showed that different metal coil materials can affect the thermal degradation of PG via the surface chemistry during aerosolization. Subaramanian et al.,[39] da Silva and Teixeira,[40] and Dhakshinamoorthy et al.[41] have catalyzed the acetalization of various aldehydes and alcohols with a Ni^{II}-complex, transitionmetal salts (i.e., $FeCl_3$, $NiCl_2$, and $CuCl_2$), and metal-organic frameworks (containing Fe, Cu, and Al), respectively. E-liquids in contact with the metal coil could contain metals that catalyze the formation of aldehyde flavorant-PG and -GL acetals before, during, and after aerosolization.[24] The rates of acetal formation in e-liquids with aldehyde flavorants in the original container versus in the e-cigarette reservoir (before and after aerosolization) require further study.

PG and GL can thermally degrade into formaldehyde, acetaldehyde, and other toxicants upon aerosolization.[42] Formaldehyde can react with PG and GL in the aerosol to form formaldehyde-PG and -GL hemiacetals that can release formaldehyde before or after particle deposition in the respiratory tract.[43] Formaldehyde-PG and -GL hemiacetals can be converted into chemically stable cyclic acetals under acidic conditions.[44] Duell et al. [14] observed acetaldehyde and formaldehyde cyclic acetals in acidic aerosols produced from e-liquids with sucralose (i.e., sucralose can thermally degrade upon aerosolization to form HCl) by GC-MS. Acetalization can occur before and after e-liquid aerosolization.

5. CONCLUSIONS

In this study, we used ^{1}H NMR spectroscopy to determine the rate and yield of aldehyde flavorant-acetal formation in PG, GL, and PG + GL e-liquids and then PG e-liquids with water, nicotine, benzoic acid, and mixtures of nicotine+benzoic acid. Acetalization occurred at a faster rate and produced a higher yield in e-liquids with GL compared to PG. Acetal formation in PG e-liquids with flavorants was inhibited by nicotine and water (i.e., a base and an acetalization product, respectively) and catalyzed by benzoic acid. PG e-liquids containing nicotine and flavorants with 2, 4, and 10 times more benzoic acid than nicotine (by mol) generally formed acetals at a slower rate compared to e-liquids without nicotine and benzoic acid. Many of the flavorant-PG and -GL acetal peaks were assigned in their ^{4}H NMR spectra to identify the acetals in e-liquids. Flavorant acetals have unique toxicity profiles and can be more harmful than the parent flavorant.[22,24] The rate and yield of additional flavorant-acetal and -ketal formation in e-liquids with and without common additives and the impact on other reactions should also be explored to inform consumers and regulators about the HPHCs in e-liquids before and after aerosolization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

e-cigarette	electronic cigarette
e-liquid	electronic cigarette liquid
PG	propylene glycol
GL	glycerol
NMR	nuclear magnetic resonance
TMB	1,3,5-trimethoxy benzene
HPHC	harmful and potentially harmful constituents
GC-MS	gas chromatography–mass spectrometry
GC-FID	gas chromatography-flame ionization detector

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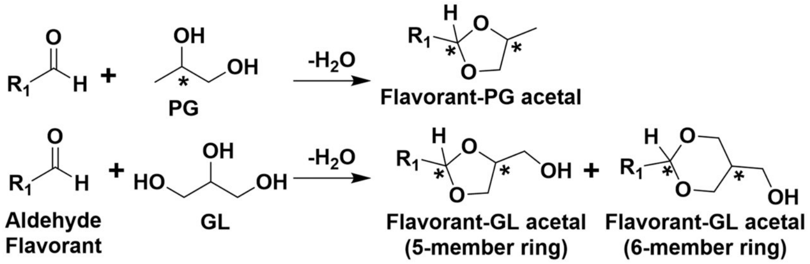

Figure 1.

General reactions for the formation of the flavorant-propylene glycol (PG) and -glycerol (GL) acetals. First, (a) the carbonyl oxygen is protonated (typically by an acid catalyst), then (b) the alcohol (e.g., PG or GL) attacks the carbonyl carbon to form a hemiacetal, and finally (c steps (a) and (b) are repeated once more to form the cyclic acetal. Each "*" on the structures indicates a stereocenter.

Table 1.

Composition of the Simulated E-Liquids Used to Study the Kinetics of Flavorant-Acetal Formation

	e-cigarette solvent	internal standard, TMB[a] (mg/mL)	flavorant (mg/mL)	nicotine (mg/mL)	benzoic acid (mg/mL)	water (% by wt)
trans-cinnamaldehyde	PG	0	10.0	0	0	0
	PG + GL	1.0	10.0	0	0	0
	GL	1.0	10.0	0	0	0
	PG	1.0	10.0	0	0	0
	PG	1.0	2.5	0	0	20.0
	PG	1.0	2.5	1.6	0	0
	PG	1.0	2.5	6.3	0	0
	PG	1.0	2.5	0	4.8	0
	PG	1.0	2.5	6.3	4.8	0
	PG	1.0	2.5	3.1	4.8	0
	PG	1.0	2.5	3.1	9.3	0
	PG	1.0	2.5	3.1	23.0	0
vanillin	PG	0	10.0	0	0	0
	PG + GL	3.1	31.0	0	0	0
	GL	3.1	31.0	0	0	0
	PG	3.1	31.0	0	0	0
	PG	1.0	2.5	0	0	0
	PG	1.0	2.5	5.4	0	0
	PG	1.0	2.5	0	4.0	0
	PG	1.0	2.5	5.4	4.0	0
	PG	1.0	2.5	2.7	4.0	0
	PG	1.0	2.5	2.7	8.1	0
	PG	1.0	2.5	2.7	20.0	0
benzaldehyde	PG	0	10.0	0	0	0
	PG + GL	1.0	2.5	0	0	0
	GL	1.0	2.5	0	0	0
	PG	1.0	2.5	0	0	0
	PG	1.0	2.5	0	11.0	0

	e-cigarette solvent	**internal standard, TMB[a] (mg/mL)**	**flavorant (mg/mL)**	**nicotine (mg/mL)**	**benzoic acid (mg/mL)**	**water (% by wt)**
	PG	1.0	2.5	7.0	11.0	0
	PG	1.0	2.5	7.0	21.0	0
	PG	1.0	2.5	7.0	51.0	0

[a] TMB = 1,3,5-trimethoxy benzene.

Table 2.

Experimental Details for E-Liquids Containing *trans*-Cinnamaldehyde Including the % Acetal Formed at Equilibrium, Initial Rate Constant, and Half-Life Determined under Various Conditions

e-cigarette solvent	*trans*-cinnamaldehyde (mg/mL)	nicotine:*trans*-cinnamaldehyde mol ratio	benzoic acid:*trans*-cinnamaldehyde mol ratio	water (% by wt)	% acetal formed at equilibrium	initial rate constant (h^{-1})	half-life (h)
PG + GL[a]	*10*	0	0	0	94	23.0×10^{-2}	3.0
PG[a]	*10*	0	0	0	91	11.0×10^{-2}	6.6
GL[a]	*10*	0	0	0	96	20.0×10^{-2}	3.4
PG[a]	*2.5*	0	0	0	90	5.2×10^{-2}	14.0
PG[a]	*2.5*	0	0	*20*	NR[h]	NR[h]	NR[h]
PG[a]	*2.5*	*0.5*[b]	0	0	NR[h]	NR[h]	NR[h]
PG[a]	*2.5*	*2*[c]	0	0	NR[h]	NR[h]	NR[h]
PG[a]	*2.5*	0	*2*[d]	0	54	22.0×10^{-2}	3.2
PG[a]	*2.5*	*2*[c]	*2*[d]	0	87[i]	0.8×10^{-3}	880.0
PG[a]	*2.5*	*1*[e]	*2*[d]	0	88	1.0×10^{-3}	690.0
PG[a]	*2.5*	*1*[e]	*4*[f]	0	91	1.8×10^{-3}	370.0
PG[a]	*2.5*	*1*[e]	*10*[g]	0	90	8.4×10^{-3}	82.0

[a] + 1.0 mg/mL 1,3,5-trimethoxy benzene (TMB).

[b] 1.6 mg/mL nicotine.

[c] 6.3 mg/mL nicotine.

[d] 4.8 mg/mL benzoic acid.

[e] 3.1 mg/mL nicotine.

[f] 9.3 mg/mL benzoic acid.

[g] 23.0 mg/mL benzoic acid.

[h] NR = no reaction.

[i] Forced to equilibrium by heating at 100 °C for 24 h and then verified by reheating for another 24 h.

Table 3.

Experimental Details for E-Liquids Containing Vanillin Including the % Acetal Formed at Equilibrium, Initial Rate Constant, and Half-Life Determined under Various Conditions

e-cigarette solvent	vanillin (mg/mL)	nicotine:vanillin mol ratio	benzoic acid:vanillin mol ratio	% acetal formed at equilibrium	initial rate constant (h^{-1})	half-life (h)
PG + GL[a]	*31*	0	0	81	1.3×10^{-2}	52.0
PG[a]	*31*	0	0	63	0.3×10^{-2}	240.0
GL[a]	*31*	0	0	87	3.7×10^{-2}	19.0
PG[b]	*2.5*	0	0	61[i]	0.4×10^{-3}	1700.0
PG[b]	*2.5*	*2*[c]	0	NR[h]	NR[h]	NR[h]
PG[b]	*2.5*	0	*2*[d]	73	18.0×10^{-2}	3.8
PG[b]	*2.5*	*2*[c]	*2*[d]	71[i]	0.3×10^{-3}	2100.0
PG[b]	*2.5*	*1*[e]	*2*[d]	79	1.2×10^{-3}	590.0
PG[b]	*2.5*	*1*[e]	*4*[f]	80	1.8×10^{-3}	390.0
PG[b]	*2.5*	*1*[e]	*10*[g]	77	4.1×10^{-3}	170.0

[a] + 3.1 mg/mL 1,3,5-trimethoxy benzene (TMB).

[b] + 1.0 mg/mL TMB.

[c] 5.4 mg/mL nicotine.

[d] 4.0 mg/mL benzoic acid.

[e] 2.7 mg/mL nicotine.

[f] 8.1 mg/mL benzoic acid.

[g] 20.0 mg/mL benzoic acid.

[h] NR = no reaction.

[i] Forced to equilibrium by heating at 100 °C for 24 h and then verified by reheating for another 24 h.

Table 4.

Experimental Details for E-Liquids Containing Benzaldehyde Including the % Acetal Formed at Equilibrium, Initial Rate Constant, and Half-Life Determined under Various Conditions

e-cigarette solvent	benzaldehyde (mg/mL)	nicotine:benzaldehyde mol ratio	benzoic acid:benzaldehyde mol ratio	% acetal formed at equilibrium	initial rate constant (h^{-1})	half-life (h)
PG + GL[a]	*2.5*	0	0	>99	0.3×10^{-2}	240.0
PG[a]	*2.5*	0	0	>99	0.1×10^{-2}	580.0
GL[a]	*2.5*	0	0	>99	4.2×10^{-2}	16.0
PG[a]	*2.5*	0	*2* [b]	>99	5.4×10^{-2}	13.0
PG[a]	*2.5*	*1* [c]	*2* [b]	>99[f]	2.7×10^{-4}	2600.0
PG[a]	*2.5*	*1* [c]	*4* [d]	>99[f]	3.6×10^{-4}	1900.0
PG[a]	*2.5*	*1* [c]	*10* [e]	>99[f]	7.9×10^{-4}	880.0

[a] + 1.0 mg/mL 1,3,5-trimethoxy benzene (TMB).

[b] 11.0 mg/mL benzoic acid.

[c] 7.0 mg/mL nicotine.

[d] 21.0 mg/mL benzoic acid.

[e] 51.0 mg/mL benzoic acid.

[f] Forced to equilibrium by heating at 100 °C for 24 h and then verified by reheating for another 24 h.

Table 5.

% Aldehyde Flavorant Converted into PG- and GL-Flavorant Acetals in Commercial E-Liquids with and without Nicotine

commercial e-liquid		**initial nicotine and flavorant concentrations**				
brand	**flavor**	**nicotine (mg/mL)**	**vanillin (mg/mL)**	**ethyl vanillin (mg/mL)**	***trans*-cinnamaldehyde (mg/mL)**	**% flavorant converted into total PG- and GL-flavorant acetals**
Twelve	Taurus	3	3	6	ND[a]	6
Vapor	Aries	ND[a]	13	5	ND[a]	14
Seduce	Snake Eyes	6	11	9	ND[a]	22
Juice	Snake Oil	12	7	11	ND[a]	20
The Mad Alchemist	Snow White's Demise	11	7	ND[a]	ND[a]	11
	Winter's Bite	ND[a]	9	20	ND[a]	17
	Dragons Breath	12	ND	ND[a]	39	4

[a] ND = not detected.