Abstract 2460
Poster Board P526

A New E-cigarette Aerosol pH Technique with Improved Toxicological Relevance

John H. Lauterbach, Ph.D., DABT, and Sebastian J. Lauterbach, Lauterbach & Associates, LLC, Macon, GA 31210 USA

Abstract

In the past, the determination of pH-values of mainstream cigarette smoke was controversial both in terms of analytical methodology and interpretation of the results in terms of nicotine addiction and other toxicological effects. The controversy has continued with the introduction of e-cigarettes (e-cigs) and related devices and the e-liquids (e-liqs) used with them, but there is one main difference. The aerosol generated by e-cigs does not contain carbon dioxide and other acids generated from combustion of the tobacco. Since there are no products of combustion, e-liq pH should equal e-cig aerosol pH. This is not the case. E-liquids contain little or no water, and must be diluted with water to achieve the dilute aqueous solution needed for accurate pH measurements and possible use of the Henderson-Hasselbalch equation. However, dilution of mentholated and highly flavored e-liquids results in cloudy mixtures. Attempts to measure the pH on undiluted e-liq is technically incorrect as e-liquids pick up water from ambient drawn in by users puffing on the devices, sometimes increasing water concentration as high as 10%, and there may be precursors of volatile acids in e-liquids that are activated by the e-cig heater assembly. Thus, pH determinations should be conducted on the undiluted aerosol emitted by the e-cigs. Health Canada Test Method T-113 has been used for e-cig aerosols, but T-113 specifies a modified pH electrode and sampling chamber that is atypical of the human mouth. These disadvantages can be overcome by using nonstandard pH electrodes in a glassmouth such that the active portion of the electrode is in the smoke stream. One example system is based around a Hanna Instruments HI99171 Leather and Paper pH Meter and probe. Without an aerosol stream, resting pH ≈7, with the aerosol (4-sec 55-mL puff every 30 sec) from popular brand 1, pH ≈7.2 and from popular brand 2 (known to contain organic acids), pH ≈6.8; nicotine at 50 mg/mL in PG, pH ≈7.6. These values are lower than obtained with other techniques and indicate that likelihood of adverse health effects from high pH may have been overestimated.

Introduction

The observed pH-values of mainstream cigarette smoke (MSS) have been a topic of considerable debate for several decades. First, it is extremely important to note that pH is only defined for dilute aqueous solutions. However, there are many industrial applications of pH where the matrix is not a dilute aqueous solution. Examples include paper, leather, fabric, many types of food, and latex paints. These applications of pH tend to be noncontroversial even though they bend the definition of pH. Another very important use of pH that does not fit the classical definition of pH is the determination of the pH of whole blood. Whole blood is not a true solution. Moreover, it contains dissolved carbon dioxide and the equilibrium between the carbon dioxide and bicarbonate anion is physiologically important.

The MSS smoke aerosol also contains carbon dioxide and water vapor in the gas-vapor phase (GVP) of MSS and carbon dioxide [as carbonic acid dissolved in the aqueous phase of the particulate matter (PP)]. There is more than sufficient water in the GVP and PP phases of the MSS aerosol to hydrate all the carbon

Introduction (con't)

dioxide (around 45 mg/cig) delivered by typical US-blend lights cigarettes smoked under ISO smoking conditions (35-mL puff volume, 2-second puff duration, 60-second puff interval; Counts et al., 2005). The MSS delivery of hydrated carbon dioxide (carbonic acid) for a typical US-blend cigarette far exceeds the amounts of nicotine, ammonia, and other bases in the smoke. Thus, the MSS of most all cigarettes should be acidic. Indeed, this has been found to be the case, especially when cigarettes are smoked under intensive smoking conditions that regulators believe to be more typical of actual human smoking behavior [so-called Canadian Intensive (CINT): 55-mL puff volume, 2-second puff duration, 30-second puff interval, with complete blocking of filter ventilation; Counts et al., 2005). However, there have those who alleged that the use of ammonia and/or its compounds can increase MSS pH (Chen and Pankow, 2009; Pankow 2001). One of the concepts reported by Pankow was that nicotine had to be in the non-ionized form to have a physiological effect on the smoker and that the amount of non-ionized nicotine in the MSS aerosol was controlled by gas-particle partitioning theory (Pankow, 2001). A key parameter in the equations used to apply this theory to the MSS aerosol is the number-average molecular weight of the particulate phase. Another key parameter is the concentration of the particulate matter in the aerosol. Conditions favorable for formation of non-ionized nicotine in the GVP occur when very low delivery cigarettes (very high filter ventilation) are smoked under ISO conditions. Such conditions yield very dilute aerosols and relatively dry particulate matter. However, these effects are not observed under CINT conditions, where the particulate phase concentration is high and the particulate matter generally contains more than 30% water (Lauterbach et al., 2010).

Attempts have been made to extend the concept of pH to e-liquids and aerosols generated from e-liquids. Stepanov and Fujioka (2014) reported that many of the e-liquids they sampled had pH-values greater than 9 (with menthol varieties generally high than nonmenthol counterpart). Those authors reported that they adapted the method used for the determination of smokeless tobacco pH for their measurements. However, when other authors repeated the analyses reported by Stepanov and Fujioka, they found that the preparation of the menthol samples resulted in cloudy solutions and that the pH-values determined drift downwards over the time it took to make three replicated determinations. This drift was observed over several combinations of pH electrodes and meters (Lauterbach and Lauterbach, 2014). Lisko *et al*. (2015) also reported e-liquid pH determinations using a technique similar to that of Stepanov and Fujioka and also reported free nicotine percentages based on an incorrect use of the Henderson-Hasselbalch equation.

A recent article by El-Hallani *et al*. (2015) reported the use of toluene extraction of the quartz-fiber filter collected aerosol from commercial e-liquids to claim that most of the nicotine in e-liquids was in the unprotonated form. Moreover, it was suggested that unprotonated nicotine is formed from thermal decomposition of the protonated nicotine on the heated coil of the e-cigarette that is used to vaporize the e-liquid.

Experimental

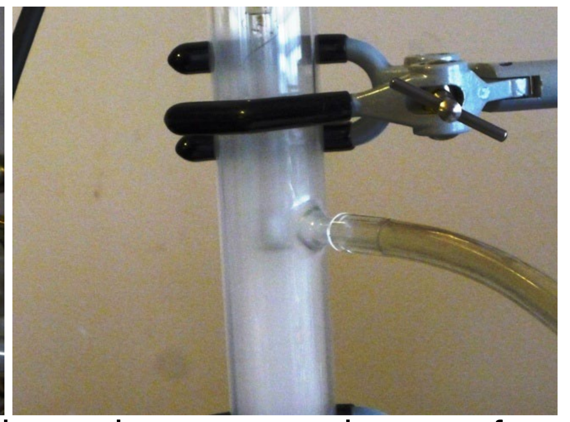
Our experimental plan was designed to eliminate the deficiencies in the cited prior work Two different systems were used for the determination of the pH-values of aerosols generated by e-cigarettes. The first system was adapted from Health Canada Method T-113, Determination of Mainstream Tobacco Smoke pH (Health Canada, 1999). This is a puff-by-puff method, and it was patterned after the technique developed by Sensabaugh and Cundiff (1967). In some ways, it was similar to the technique reported by Lauterbach in 2013 (Lauterbach, 2013). However, there were several important differences. The first difference was that the 55-mL, 3-second square-wave puff used (generated by one port of a Lauterbach & Associates four-port constant vacuum smoking machine) instead of the normal bell-shaped puff as specified in T-113. The smoking machine was operated in a manner compliant with CORESTA Recommended Method No. 81, Routine analytical machine for e-cigarette aerosol generation and collection – definitions and standard conditions (2015). The second difference was that the smoke trap was a modification of one specified T-113 that was designed and constructed by Prism Research Glass (Raleigh, NC). This modification focuses the aerosol stream emitted by the e-cigarette on the pH electrode, itself, which is the third important difference with T-113. The modified silver/silver chloride combination pH electrode specified in Method T-113 was replaced by a Hanna Instruments (Woonsocket, RI) HI1414D flat bottom combination electrode with internal temperature sensor. This electrode was designed determining the pH of paper, leather and fabric. This electrode was designed for use specifically with the Hanna Instruments HI99121 digital pH meter. This meter provides digital readouts of temperature-corrected pH and temperature, but such data cannot be uploaded to another device and the operator must manually record the readings of the pH meter. In most cases, 50 puffs per replicate sample were needed for full equilibration of the pH values of the aerosol incoming aerosol with that already in the smoke trap. The second system used a glassmouth with pH adapter that was constructed by Prism Research Glass based on the glassmouth reported by Honeycutt (1985). The pH electrode was a HI1053B conical tip probe, and was used with an IQ Scientific Instruments Model 150 pH meter. Only 25 puffs were taken for experiments with the glassmouth. Fresh human stimulated saliva (5 mL) was used in the glassmouth. V2 CIGS© with blank V2-brand cartomizers or V2-brand prefilled cartomizers were used. The blank cartomizers are dry and ready to be filled with solution used to generate vapor. V2 CIGS extended length automatic electronic cigarette batteries were used and a freshly charged battery was used for each run. When V2-brand e-liquids were used with blank cartridges or custom-formulated e-liquids were used, cartomizer loadings were either ~750 mg or ~ 900 mg.

Results (modified T-113 smoke trap)

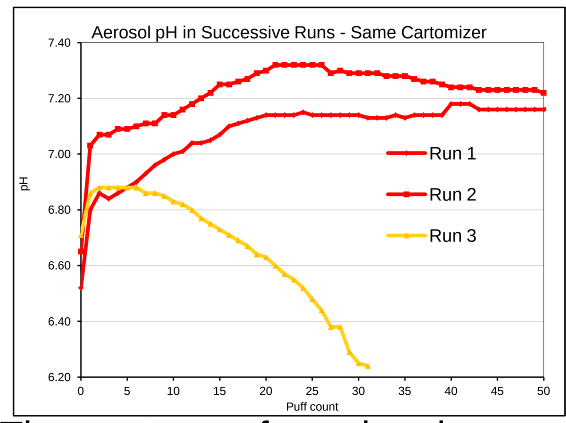
One important finding from this research is that the puff-by-puff deliveries from the V2-brand cartomizers is not constant, and that the apparent pH of the aerosol is a combination of the nicotine content of the aerosol, the amount of aerosol in the trap and the concentration of the aerosol of in the trap (determined visually).

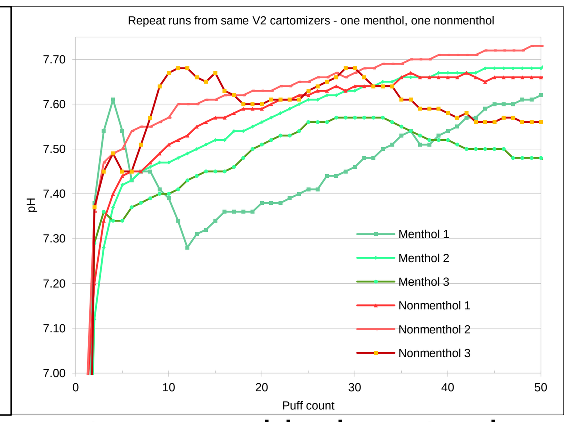
Results (modified T-113 smoke trap) (con't)

The following two pictures show the differences between a cartomizer giving a high amount of aerosol per puff (left) and one giving a low amount of aerosol per puff (right). The reasons for this are not clear except in cases where the amount of e-liquid in the cartomizer is low. An example of low e-liquid is shown in the

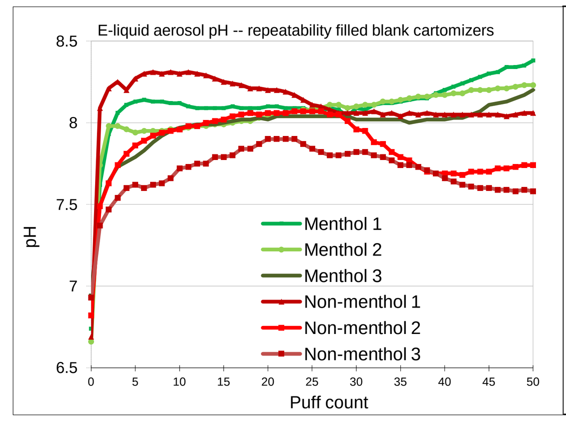


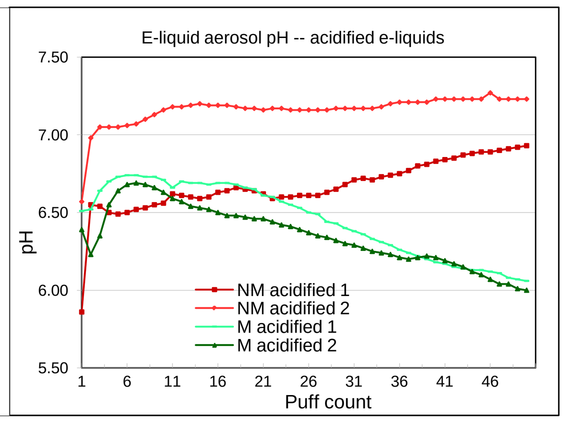
first graph below on the left. It shows three successive runs from one cartomizer. The graph on right shows repeat runs.





The next set of graphs shows repeat runs on blank cartomizers filled with V2 2.4% menthol and nonmenthol (Red) e-liquids (left) and the effects of added malic acid (equimolar with nicotine) to an e-liquid (right). The difference in the effect of malic acid between nonmenthol and menthol e-liquids is not known.





Results (glassmouth)

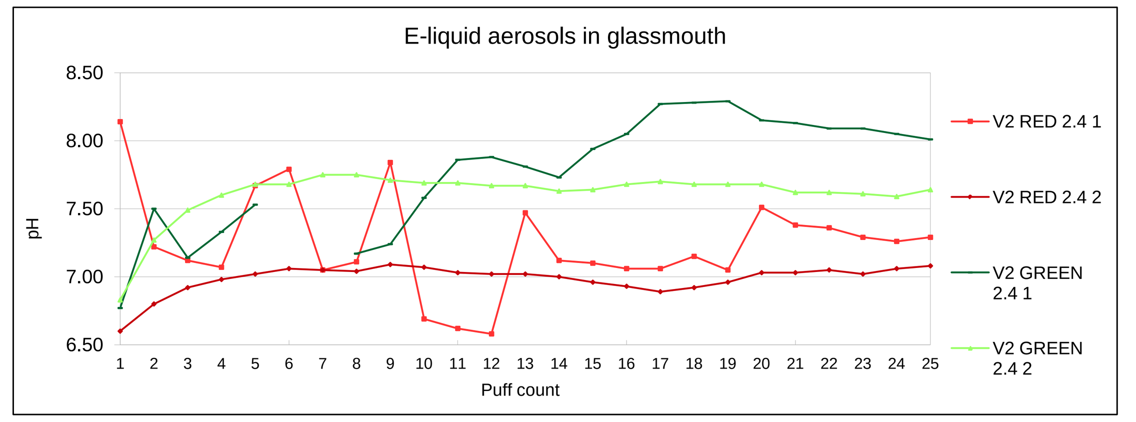
There were two objectives for the glassmouth: 1) determination of aerosol pH under more realistic conditions than could be obtained with the modified T-113 smoke trap; and 2) exposure of saliva to the e-liquid aerosol to determine change, if any, in the pH of the saliva. Pictures of the apparatus are shown below.

Results (glassmouth) (con't)

Only two V2 cartomizers were evaluated with the glassmouth: 1) menthol (green) and 2) nonmenthol (red). Both were listed as 2.4% nicotine. Each was evaluated twice with 25 puffs, and then a second 25 puffs from the same cartomizers. In the first evaluation, fresh human stimulated saliva (~5 mL) with a pH of ~7.7 was used. This was placed in a depression in the bottom of the mouth area of the glassmouth. In the second evaluation, fresh human stimulated saliva with a pH of ~6.8 was used. Results are shown in the table below.

	V2 2.4 Red 1	V2 2.4 Red 2	V2 2.4 Green 1	V2 2.4 Green 2
Aerosol weight (mg)	121	76	112	165
Initial saliva pH	7.72	6.82	7.70	6.82
Final saliva pH	8.18	7.01	8.22	7.41

The data above should be considered very preliminary. Additional replication is needed both with the V2 systems and other products. The graph below shows the maximum aerosol pH at each puff. Additional replication is needed.



However, the data appear to show that one can determine the pH of an e-cigarette aerosol without need for expensive instrumentation, and still have experimental conditions for the aerosol collection that are within internationally accepted standards and do not require modification of the e-cigarettes.

Conclusions

First, we have shown that it is possible to determine the pH of e-cigarette aerosols using simple equipment, but at the same time keeping the generation of the aerosol within the bounds of CORESTA Recommended Method No. 81. Second, our data appear to indicate that the high e-liquid aerosol pH values reported by others using indirect measures are not correct.

References

Chen C, Pankow JF. Gas/particle partitioning of two acid-base active compounds in mainstream tobacco smoke: nicotine and ammonia. J Agric Food Chem. 2009 Apr 8;57(7):2678-90. Erratum in: J Agric Food Chem. 2010 Aug 25;58(16):9289.

Counts ME, Morton MJ, Laffoon SW, Cox RH, Lipowicz PJ. Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine-smoking conditions. Regul Toxicol Pharmacol. 2005 Apr;41(3):185-227.

El-Hellani A, El-Hage R, Baalbaki R, Salman R, Talih S, Shihadeh A, Saliba NA. Free-Base and Protonated Nicotine in Electronic Cigarette Liquids and Aerosols. Chem Res Toxicol. 2015 Aug 17;28(8):1532-7.

Honeycutt RH. Sampling of cigarette smoke from a glass model of the human mouth: effect of sampling location on relative "tar" and nicotine levels. Brown & Williamson Tobacco Corp. report. 1985; available at http://industrydocuments.library.ucsf.edu/tobacco/docs/jfbp0135.

Lauterbach JH, Lauterbach SJ. E-liquid pH as an important element for research and regulation. Are mentholated e-liquids really different? CORESTA Congress, Quebec City, Canada, October 12-16, 2014. Presentation ST 48.

Lauterbach J. Comparison of mainstream cigarette smoke pH with mainstream e-cigarette aerosol pH. 67th Tobacco Science Research Conference, Williamsburg, VA, September 15-18, 2013. Program Booklet and Abstracts, Vol. 67, No. 78, pp. 61-62.

Lauterbach JH, Bao M, Joza PJ, Rickert WS. Free-base nicotine in tobacco products. Part I. Determination of free-base nicotine in the particulate phase of mainstream cigarette smoke and the relevance of these findings to product design parameters. Regul Toxicol Pharmacol. 2010 Oct;58(1):45-63.

Lisko JG, Tran H, Stanfill SB, Blount BC, Watson CH. Chemical composition and evaluation of nicotine, tobacco alkaloids, ph, and selected flavors in e-cigarette cartridges and refill solutions. Nicotine Tob Res. 2015 Oct;17(10):1270-8.

Pankow JF. A consideration of the role of gas/particle partitioning in the deposition of nicotine and other tobacco smoke compounds in the respiratory tract. Chem Res Toxicol. 2001 Nov;14(11):1465-81.

Sensabaugh AJ, Jr., Cardiff, RH. A New Technique for Determining the pH of Whole Tobacco Smoke, Tob. Sci. 1967 11:25-30.

Stepanov I, Fujioka N. Bringing attention to e-cigarette pH as an important element for research and regulation. Tob Control. 2015 Jul;24(4):413-414.