Radiolabeling an Electronic Cigarette Aerosol Using Technetium Carbon Ultrafine Particles

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Abstract

Background: Electronic cigarettes (ECIGs) are widely used, but their health effects are not well known. ECIG exposure is difficult to quantify, and a direct measurement of deposition would be beneficial to *in vivo* and *in vitro* toxicity studies. The aim of this study was to demonstrate effective radiolabeling of an ECIG.
Methods: A technetium-99m-labeled carbon ultrafine (TCU) aerosol was generated and introduced to a fourth-generation ECIG before nucleation and aerosol formation. The aerosolized e-liquid was a commercially available strawberry flavor containing 1.2% nicotine in a 55% propylene glycol and 45% vegetable glycerine base. An ECIG power setting of 100 W was selected. Mass and radioactivity were measured on each stage within a Sierra Cascade Impactor at 14 L/min to verify the labeling technique using the calculated aerodynamic diameters. A strong positive correlation ($R^2 > 0.95$) between the percent activity and percent mass deposition on each stage provides a reliable validation of colocation.
Results: Unlabeled ECIG aerosol from the chosen e-liquid produced a mass median aerodynamic diameter (MMAD) of 0.85 μm. An ECIG labeled with TCU produced an aerosol with an activity median aerodynamic diameter of 0.84 μm and an MMAD of 0.84 μm. The relative mass versus radioactivity on each plate was highly correlated (average $R^2 = 0.973$, $p < 0.001$).
Conclusion: A TCU radiolabel was generated and shown to associate with the mass of an aerosol produced by a typical commercially available ECIG. Thus, the radioactivity of the deposited aerosol may be used to determine ECIG aerosol deposition for the future *in vivo* and *in vitro* dosimetry studies of the third- and fourth-generation ECIGs.

Keywords: e-liquid, ECIG, electronic cigarettes, gamma scintigraphy, respiratory deposition imaging, technetium 99m, vaping

Introduction

The potential health effects of short- or long-term electronic cigarette (ECIG) use are not well known across the currently available four generations of ECIG devices.[1] Deposition and imaging studies could connect the *in vitro* properties of the aerosol to its toxicology and safety.[2] However, tools to accurately assess the deposition of ECIG aerosols *in vitro* or *in vivo* are lacking for ECIG users. On a cellular level, exposure to ECIGs has temporarily reduced ciliary beat frequency in the same way as traditional cigarettes, which suggests that repeated ECIG use may damage the lungs over time.[3] The total and regional respiratory deposition of inhaled aerosols depends on the mass median aerodynamic diameter (MMAD), as well as breathing frequency, inhaled volume, and inhaled flow rate.[4,5] *In vivo* experiments have defined the relationship between breathing patterns and respiratory deposition for stable and monodisperse particles with MMADs between 5 nm and 15 μm.[6] *In vitro* measurement techniques such as cascade impaction can be used to guide toxicological decisions, but these methods are not consistently predictive of *in vivo* lung deposition.[7]

An ECIG can be used to generate aerosols containing nicotine and/or flavoring without the combustion by-products associated with traditional cigarettes. ECIGs generate aerosols by wicking an e-liquid mixture into an electrically heated coil. Air flows through the center of the coil and the e-liquids are vaporized in the high temperature region. This vapor is drawn out of the heating coil by inhalation and droplet formation commences. A single ECIG inhalation contains a

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semivolatile aerosol with condensing/evaporating droplets and/or nucleating vapor for multiple e-liquid components simultaneously.[8] Droplet formation occurs through homogeneous nucleation.

As collisions within the vapor phase happen more frequently than disintegration, transient agglomerate seeds are created that are large enough to maintain mass transfer equilibrium and permit stable growth by condensation.[9] This aerosolization mechanism can be better understood through the functional similarity of ECIGs to condensation aerosol generators.[10]

The objective of this study was to demonstrate effective radiolabeling of an ECIG for the future *in vivo* and *in vitro* studies. This method was developed from one previously used to deliver ultrafine carbon technetium-99m particle aggregates to human subjects,[11,12] which was used to quantify *in vivo* deposition of aerosols in healthy adults and patients with chronic obstructive pulmonary disease.[12] Nebulizers, pressurized metered dose inhalers, and dry powder inhalers have all been successfully radiolabeled with technetium-99m (Tc99m) through physical rather than chemical binding to elucidate the effect of patient population and drug formulation on aerosol delivery.[13]

The goal of our study was to develop a technique for radiolabeling the fourth-generation ECIG without altering the aerodynamic properties of the aerosol when compared with the nonlabeled product. This radiolabeling technique may allow for the future *in vivo* measurements of ECIG aerosol deposition in the respiratory tract using gamma scintigraphy,[2] a method that has been used to quantify *in vivo* total and regional deposition of orally inhaled products in the respiratory tract,[14] but has never been used to measure *in vivo* deposition of ECIG aerosols.

Materials and Methods

Radiolabeling of aerosols can often be accomplished by simply mixing the radioactive material with the liquid to be aerosolized. ECIGs generate an aerosol by heating a coil to vaporize a liquid, which rapidly nucleates homogenously and grows through condensation to form an aerosol. Preliminary experiments that simply mixed the radioactive material with the e-liquid failed to radiolabel the ECIG aerosol. Admixing the label with the e-liquid produced an aerosol whose radioactive counts on a cascade impactor were not distinguishable from background radiation, that is, the radioactivity did not leave the device. The dissolved sodium pertechnetate salt could not reliably colocate with the aerosol when the ECIG was actuated in these preliminary experiments (data not shown).

Therefore, the ECIG could not generate the radiolabel by simply mixing the label with the e-liquid before vaporization, and alternative approaches needed to be developed. Instead, an approach was developed in which aerosols generated with a fourth-generation ECIG device were mixed with Tc99m-labeled carbon ultrafine (TCU) particles at the ECIG air inlet as described in more detail below.

Experimental system selection: ECIG device, parameters, and liquid

An ECIG has an electrical control system and user interface typically referred to as a box mod. This box mod transfers energy from a battery source to a heating element that is housed in the e-liquid reservoir or ''tank.'' The device that was used in these experiments was a fourth-generation ECIG composed of a temperature-controlled box mod (Fuchai 213W; Sigelei, Dongguan, China) paired with an appropriate tank (Crown 2; Uwell Technologies, City of Industry, CA) that utilized a 0.25Ω heating coil. This ECIG used was a popular model in a local ECIG shop at the time but was purchased online and is still available from https://www.elementvape.com. A commercially available strawberry-flavored liquid was purchased from The Vapor Girl Inc. (lot #1523987; Chapel Hill, NC). It contained 1.2% nicotine in a base fluid composed of 55% propylene glycol (PG) and 45% vegetable glycerine (VG).

The box mod allowed for adjustment of power supplied to the coil with an adjustment range of 10W–213W. A power of 100W was selected after preliminary testing at various power levels recommended by the packaging instructions. This power level was selected because it was the maximum suggested power, which also produced the maximum amount of ECIG aerosol. The duration of actuation was chosen to be 10 seconds because of system complexity and the need for timed coordination. Ten seconds were needed to generate the TCU, manually switch the TCU flow path, and actuate the ECIG. The ECIG was actuated twice for 10 seconds each at 100W.

Experimental system selection: generation of TCU radiolabel

A commercially available generator (Model GFG-1000; PALAS, Karlsruhe, Germany)[15] was used to generate the carbon nanoparticle aggregates (20–100 nm) that could be labeled with Tc99m as was previously described by Brown et al.[11] This generator has been used previously to study the deposition and retention of inhaled carbon nanoparticles in both human and animal studies.[12,16–19] An alternating high voltage was applied across graphite electrodes in an argon-filled environment to produce carbon nanoparticles. The arcing frequency was set to 50 Hz and argon flow at 3 L/min into an impactor at 14 L/min, which determined both the concentration[15] of the Tc99m-labeled carbon ultrafine (TCU) particles (1.2–5.5 mg/m^3) and the final activity median diameter of the ultrafine aerosol (62–87 nm).[11]

The arcing frequency also affected the amount of carbon vapor output from the PALAS generator. Upon release from the electrodes into the inert argon gas flowing through the generator, the carbon vapor condensed into small primary nanoparticles. From this point on, the primary nanoparticles steadily coagulated and produced larger particles until stabilized. Two parameters affected the coagulation size: time until stabilization and the concentration of the vapor. Since time was essentially held constant by the geometry of the transport tubing and the flow of the argon gas through it, only the carbon particle concentration was considered adjustable. The concentration could be adjusted by the arcing frequency: the higher the frequency, the higher the particle concentration and hence the final radiolabel particle size.

The size of the radio-nanoparticle mixed with the e-liquid vapor will affect its cotransport into the condensing e-liquid vapor after actuation. Radio-particles that are too large may not colocate well with the condensing e-liquid but may deliver high concentrations of radiolabel. However, radio-particles that are too small may colocate well within the condensed e-liquid after actuation but may deliver a very small concentration of the

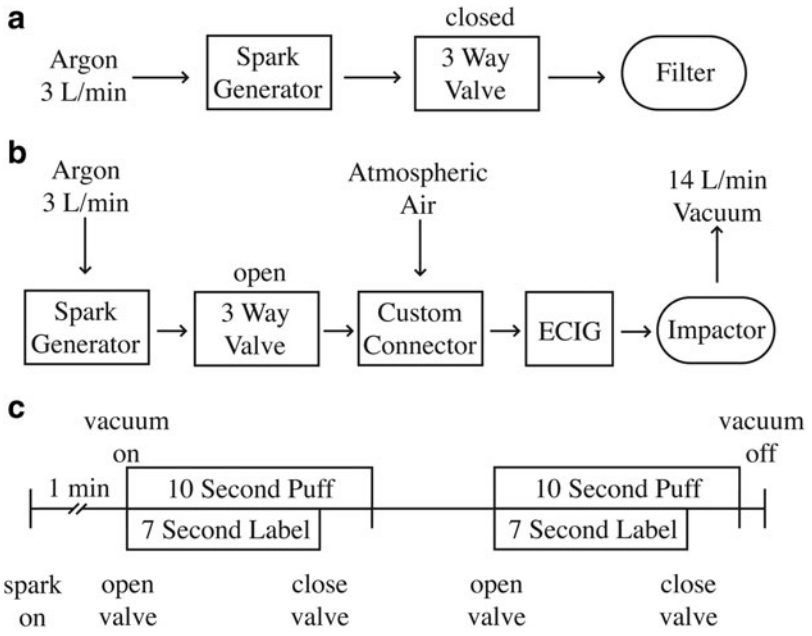

FIG. 1. System schematic and timing diagram for radiolabeled ECIG aerosol delivery. The three-way valve was (a) closed to direct the technetium carbon ultrafine aggregates directly to a filter or (b) open to direct the technetium carbon ultrafine aggregates to the ECIG for labeling. The timeline (c) outlines the actuation parameters used to minimize collection of label not associated with ECIG aerosol. ECIG, electronic cigarette.

label. An arcing frequency that resulted in sufficient radiolabel to be used as an inhalation deposition marker in the future *in vivo* studies along with satisfactory colocalization in the e-liquid output was selected from previous studies[11,12] and tested in a commercially available e-liquid.

A small aliquot (~0.5 mL normal saline solution) containing 10 mCi of sodium pertechnetate was desalted through a 3 mL chromatography column (Poly-Prep Prefilled, AG 50W-X8 100–200 mesh, hydrogen form; BioRad) and eluted with distilled water. Sequential aliquots of 0.2 mL were collected from the column eluent. The four aliquots with the highest activity were combined, representing about 6 mCi. The eluent was placed a few drops at a time on the tip of two upright carbon electrodes from the PALAS carbon nanoparticle generator.

A dry heat blower was directed carefully after each application of the pertechnetate solution to the electrode until the tip was dry. This was repeated until all the pertechnetate solution had been applied, requiring a total of about 1 hour. The carbon electrodes were then placed back into position in the PALAS generator. More complete details regarding the production of labeled carbon particles can be found in our previous publication.[11]

Experimental system selection: mixing TCU radiolabel with ECIG aerosols

The experimental setup incorporated an electronically controlled three-way valve that changed the path of airflow during labeling. The first minute of TCU generation was captured by a filter (Fig. 1a). Then, the three-way valve was opened, and the TCU was directed into the ECIG where nucleation could occur (Fig. 1b). The duration and timing of flow switching, ECIG actuation, and TCU production are shown in Figure 1c. As reported in previous studies,[11,12] the commercial e-liquid was run through a Krypton-85 charge neutralizer (Model 3012; TSI Incorporated, St. Paul, MN) to eliminate any TCU particle charge that may have been induced by sparking the carbon electrodes. To determine the effect of charge

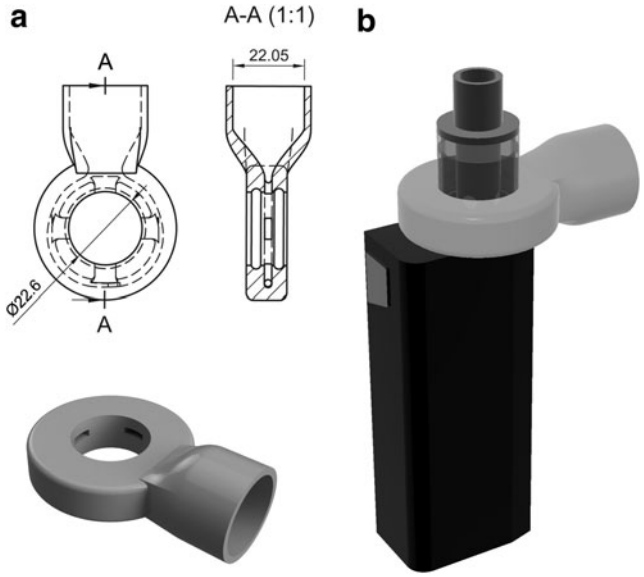

FIG. 2. The custom connector (a) is shown with dimensions in millimeters. The dashed hidden lines represent channels that allow airflow into the heating region by four vent inlets. The section view along the plane A-A shows the wall thickness of the connector by diagonal lines. The custom connector and the fourth-generation ECIG assembly (b) are shown with the connector sealed over the air inlets.

Table 1. Stage Cutoff Diameters Associated with the Sierra 210 Cascade Impactor and the Corresponding Grouping Assignments to Bins for Analysis

Stage No.	*Cutoff diameter (μm)*	*Grouping*
Filter	N/A	Bin 1
8	0.15	Bin 2
7	0.34	Bin 3
6	0.63	Bin 4
5	1.2	Bin 4
4	1.8	Bin 4
3	3.1	Bin 5
2	7.8	Bin 6
1	13	Bin 7

N/A, not applicable.

neutralization on the radiolabeling process, the results from those experiments were compared with the results from experiments that did not use the charge neutralizer.

An aerosol containing ultrafine carbon particle aggregates was radiolabeled with Tc99m-labeled carbon ultrafine (TCU) and introduced via a novel, but simple connector at the ECIG air inlet. The ultrafine aerosol was presented to the ECIG immediately upon actuation and was routed away before the end of actuation to clear the ECIG and connectors of TCU. The investigators designed and three-dimensional printed the custom connector illustrated with the dimensions specified in Figure 2a. The tight fit ensured a seal over the air inlet vents of the ECIG. The custom connector allowed for ultrafine particles to be precisely introduced before the site of nucleation within the ECIG (Fig. 2b). As ECIG design evolves, the connector may need to be adapted to the dimensions of a new ECIG mouthpiece.

Analysis of radiolabeled ECIG aerosol generation

An eight-stage Sierra Series 210 Cascade Impactor (Sierra Instruments, Carmel Valley, CA) was operated at 14 L/min to determine the MMAD gravimetrically and activity median aerodynamic diameter (AMAD) using a single crystal (NaI) scintillation detector (Model LMS-44; Nuclear Data, Inc., Smyrna, GA). This flow rate is one of the recommended flow rates for operation and one for which stage cutoff sizes are clearly provided. The 50% collection efficiency for the impactor stages span aerodynamic diameters from 0.15 to 13 μm at 14 L/min and are provided in Table 1.

The single crystal detector was placed a few inches from each impactor plate and counted for radioactivity in their sequential order, with distance from the plates kept constant. Radioactive counts deposited on each plate were used to calculate the AMAD of the particles found in the impactor according to the manufacturer's manual.

It has been proposed that the acceptability of a radiolabeling method for regional deposition requires that the mean ratio of the radiolabeled drug to the reference drug should be within 0.85–1.18 per group of impactor stages with at least four groups or within ±2% if the deposited fraction of unlabeled aerosol is <10%.[20] The reference drug for this study was the unlabeled commercial e-liquid. In addition to testing the unlabeled ECIG, the distribution of mass and activity fractions of the labeled ECIG aerosol across the stages of the cascade impactor were partitioned into seven bins with bin 1 containing the filter; bin 2 containing stage 8; bin 3 containing stage 7; bin 4 containing stages 6, 5, and 4; bin 5 containing stage 3; bin 6 containing stage 2; and bin 7 containing stage 1. This is summarized in Table 1.

Grouping the deposition in seven bins instead of the minimum suggested four bins highlights the quality of the labeling method and demonstrates the ability to produce a radiolabeled ECIG aerosol in which the activity follows the mass for the majority of the mass.

To validate colocalization of radio-label versus the mass of the deposited aerosol, an R^2 value >0.95 was deemed acceptable. The R^2 value was determined from a simple linear regression of the mass of the deposited aerosol versus the activity of the label found on the stages of the Sierra impactor.

Results

Baseline unlabeled ECIG aerosol size distributions for the commercial e-liquid were measured in four replicate experiments. The average (standard deviation [SD]) MMAD of the commercial e-liquid emitted by the ECIG was 0.85 (0.00) μm. Mass-based sizing of the commercial e-liquid radiolabeled at 50 Hz resulted in an average (SD) MMAD of 0.84 (0.02) μm. These values and the corresponding geometric standard deviations are summarized in Table 2. Activity-based measurements were made using the same impactor techniques as mass-based measurements. When the TCU was introduced into an actuated ECIG, the average (SD) AMAD of the commercial e-liquid was 0.84 (0.01) μm.

The potential effect of TCU charge on ECIG particle size showed that the average (SD) MMAD of the ECIG aerosol with and without the charge neutralizer was 0.83 (0.02) and 0.85 (0.02) μm, respectively (Fig. 3). The mean (SD) AMAD of the ECIG aerosol with and without the charge neutralizer were 0.84 (0.02) and 0.84 (0.01) μm, respectively. The charge neutralizer was observed to have no effect on the output. Additional aerosol characteristics for the charge neutralizer experiments are listed in Table 3.

Table 2. Aerosol Size Characteristics of a Commercially Available Strawberry-Flavored e-Liquid Containing 1.2% Nicotine, 55% Propylene Glycol, and 45% Vegetable Glycerine With and Without Labeling Using Technetium Carbon Ultrafine Aerosol Generator

	MMAD (μm)	*Mass, GSD*	*AMAD (μm)*	*Activity, GSD*	R^2	*Replicates*
Unlabeled	0.85 ± 0.00	1.46 ± 0.01	N/A	N/A	N/A	4
Labeled	0.84 ± 0.02	1.48 ± 0.01	0.84 ± 0.01	1.69 ± 0.04	0.973 ± 0.01	7

AMAD, activity median aerodynamic diameter; GSD, geometric standard deviation; MMAD, mass median aerodynamic diameter.

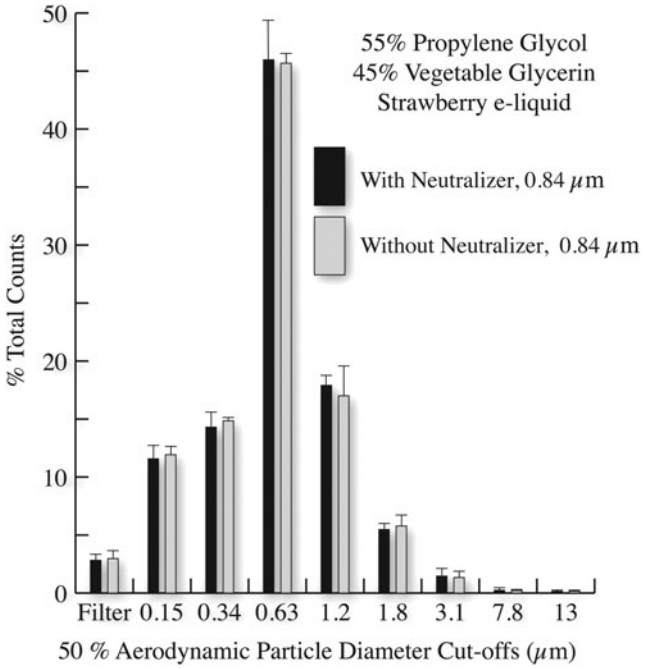

FIG. 3. Cascade impactor measurements of radiolabeled ECIG with and without a charge neutralizer. The counts on each stage were divided by the total number of counts to determine the % counts. Error bars represent 1 standard deviation in $n \geq 3$ replicates.

The linearity of mass fraction versus activity fraction describes the association between the label and the bulk aerosol. The mean (SD) of R^2 values was 0.973 (0.01) for the commercial strawberry-flavored e-liquid with 1.2% nicotine in a 55% PG/45% VG base. These values demonstrate the robustness of the labeling method.

The mass distributions in the unlabeled ECIG were compared with the mass and activity distributions in the radiolabeled ECIG (Fig. 4) and were within the suggested acceptance criteria for all bins except for bin 2. The ratio of labeled mass to unlabeled mass in bin 4 was 0.968. The unlabeled aerosol had 7.3% mass deposited in bin 2, which produce the acceptance criteria[20] of 5.3%–9.3%. The labeled mass fraction was within this acceptance criteria at 8.3%. The labeled activity deposited in bin 2 was 11.8%, meaning that the labeled activity was 2.5% above the guidelines provided for bin 2.

Discussion

Dosimetry and toxicology of ECIG aerosols could be better understood by *in vivo* studies, but there are few tools available to quantitatively assess ECIG aerosol deposition. The methods implemented in this study provide a radiolabeling technique that can be used to measure the deposited dose of ECIGs for toxicology studies and supply benchmark data for the future *in silico* work. Unlabeled ECIG aerosol measurements of MMAD were similar to radiolabeled ECIG aerosols for the commercial e-liquid. Labeling the commercial e-liquid produced an aerosol with a similar mass distribution to that of the unlabeled reference (Table 2). The introduction of the radiolabel to the ECIG aerosol did not alter the aerosol distribution for the conditions considered.

Repeated measurements of the ECIG formulation demonstrated a low SD and consistent methodology. Future studies could use this approach to measure the size distribution for aerosols produced by other e-liquids and devices. Previous impactor measurements of ECIG aerosols have shown MMADs ranging from 0.53 to 0.96 μm.[21] Results from the present study are within that range. Other methods of measurements that did not collect the entire generated semivolatile aerosol resulted in smaller MMADs (0.12–0.18 μm).[22,23] This is expected due to the large air dilution volumes and sampling required for the measurement techniques employed in these previous studies.

When the TCU label passed through the aerosol generation region (heating coil) of the ECIG, the diameter shifted from the expected 0.06–0.09 μm to 0.84 μm. The TCU successfully provided nucleation sites for the condensing e-liquid and produced AMADs that were much larger than the initial TCU aerosol size. The TCU was shown to associate with the ECIG aerosol in (1) the measured AMAD of 0.84 μm, (2) the agreement between the AMADs and MMADs for the commercial e-liquid, and (3) the linear relationship between mass and activity fractions having an R^2 of >0.95.

Greater than 99% of the radioactivity, as well as the labeled and unlabeled mass of the aerosol, was in the fine particle fraction, as shown in Figure 4. A single bin in Figure 4 was slightly greater than the suggested ±2% acceptance criterion defined by Devadason et al.[20] for deposition fractions of unlabeled mass <10%. The suggested acceptance criterion[20] of a mass and activity ratio of 0.85–1.18 was met by all bins with deposition fractions >10%. The labeled activity measurement in bin 2 in Figure 4 was likely higher than the reference mass measurement due to evaporation of the deposited mass.

Previous studies have charge neutralized the TCU agglomerate before subject inhalation.[11,12] The use of the charge neutralizer in our study had no effect on the aerodynamic diameter as measured by mass or activity. Removing the charge neutralizer reduces complexity and makes it easier for this inhalation technique to be replicated by additional researchers without affecting the validity of the method.

TABLE 3. CASCADE IMPACTOR MEASUREMENTS USED TO DETERMINE THE NECESSITY OF ELECTRICALLY NEUTRALIZING THE RADIOACTIVE AEROSOL BEFORE LABELING THE ELECTRONIC CIGARETTE CONTAINING A COMMERCIALLY AVAILABLE STRAWBERRY FLAVORED E-LIQUID COMPOSED OF 1.2% NICOTINE, 55% PROPYLENE GLYCOL, AND 45% VEGETABLE GLYCERIN

	MMAD (μm)	*AMAD (μm)*	*Mass, GSD*	*Activity, GSD*	*Mass, collected (mg)*	R^2
Without charge neutralizer	0.85 ± 0.02	0.84 ± 0.01	1.49 ± 0.03	1.69 ± 0.08	467.2 ± 24.8	0.973 ± 0.02
With charge neutralizer	0.83 ± 0.02	0.84 ± 0.02	1.48 ± 0.01	1.69 ± 0.02	469.5 ± 29.6	0.973 ± 0.01

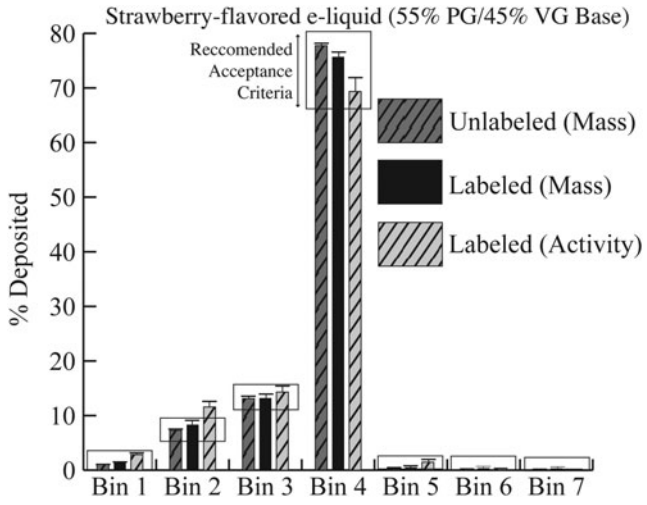

FIG. 4. Mass and radioactivity cascade impactor measurements of the unlabeled and radiolabeled ECIG grouped into seven bins. Bin 1 corresponds to the filter below the 0.15 μm stage, bin 7 corresponds to stage 1 with a cutoff size of 13 μm, and the remaining details are specified in Table 1. The recommended acceptance criteria are defined as a mean ratio of the labeled drug to unlabeled drug between 0.85 and 1.18 for deposition fractions >10% and within ±2% for deposition fractions <10%. PG, propylene glycol; VG, vegetable glycerine.

The ECIG radiolabeling method developed here has the potential to investigate *in vivo* aerosol deposition within the respiratory tract, validate *in silico* and *in vitro* deposition models, and quantify ECIG aerosol deposition in *in vitro* cellular work. The mechanism of deposition for stable monodisperse aerosols with MMADs similar to the present study are expected to be equivalent,[6] but the size of the semivolatile aerosol produced by an ECIG could change over time due to evaporation and condensation in the lungs.

To test the effect of condensational growth, radiolabeled ECIG aerosol deposition could be measured in a study designed to compare regional deposition of e-liquids with different ratios of PG/VG, which could differ due to condensational growth of the aerosol in the respiratory tract. *In vivo* regional deposition data could also be used to validate and improve numerical predictions of ECIG aerosol dosimetry.[8] Finally, quantifying the radiolabeled ECIG delivery to *in vitro* cell cultures could allow for better characterization of the dose–response associated with the ECIG toxicity in these *in vitro* models.[24]

In conclusion, a method for radiolabeling an ECIG aerosol was developed and tested in a commercially available strawberry-flavored e-liquid containing 1.2% nicotine and 55% PG and 45% VG. One limitation of the current system is the 10-second puff duration. This will be reduced to realistic puff durations in the future studies by streamlining and automating the three-way valve switching system. This automation will also allow consistent timing of label delivery when operated by ECIG users *in vivo*. For shorter puff durations, an arcing frequency of 100 Hz, with its higher activity output, is suggested. Implementation of the new TCU-ECIG labeling system is recommended as a useful tool for characterizing the deposition of aerosols produced by ECIGs in the future *in vivo* or *in vitro* studies.

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Author Disclosure Statement

The authors declare that no competing financial interests exist.

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