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High dilution surface-enhanced Raman spectroscopy for rapid determination of nicotine in e-liquids for electronic cigarettes

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Abstract

The rise in popularity of electronic cigarettes and the associated new legislation concerning e-liquids has created a requirement for a rapid method for determining the nicotine content of e-liquids in the field, ideally at the point of sale. Here we have developed a rapid method based on surface-enhanced Raman spectroscopy (SERS) with Au colloid and an isotope-labeled nicotine (d₄-nicotine) internal standard for measurement/quantification of samples which contain 10's of mg mL⁻¹ nicotine in a complex viscous matrix. The method is novel within the area of SERS because it uses high dilution (ca. 4000×) in the sample preparation which dilutes out the effects of the viscous glycerin/glycerol medium and any flavouring or colouring agents present but still allows for very accurate calibration with high

reproducibility. This is possible because the nicotine concentration in the e-liquids (≤ 24 mg mL⁻¹) is several orders of magnitude above the working range of the SERS measurement. The method has been tested using a portable Raman spectrometer and a very large set of 42 commercial e-liquids to check there is no matrix interference associated with different manufacturers/flavourings/colouring agents etc. Finally, as an alternative to determining the nicotine concentration by measuring peak heights in the spectra, the concentration was also estimated by comparing the sample spectra with those of a set of standard sample which prepared at known concentrations and held in a spectral library file in the spectrometer. This simple approach allows concentration to be estimated without any complex data analysis and lends itself readily to handheld Raman system which are typically designed to carry out library searching using the internal software for materials identification. Library searching against standards correctly classified 41 of the 42 test liquids as belonging to the correct concentration group. This high dilution SERS approach is suitable for analysis of sample types that have reasonably high concentrations of analytes but suffer from matrix problems and it therefore has broad potential for applications across the food, pharmaceutical and nutraceutical areas.

Introduction

Electronic nicotine delivery systems, commonly called electronic cigarettes (ECs), are battery-powered devices that simulate tobacco cigarettes by converting nicotine-containing liquid into an aerosol. ECs have gained popularity in the past few years, primarily among smokers who want to reduce the risks of smoking because ECs do not produce the numerous chemicals found in conventional tobacco smoke.¹⁻³ ECs use e-liquids which contain nicotine, flavouring/colouring components and a base such as propylene glycol, glycerin, or a mixture of these two substances. The nicotine concentrations of available e-liquids typically range from 0 mg mL⁻¹ to 24 mg mL⁻¹ and

51 numerous different flavours are available, ranging from tobacco flavours (which are similar to
52 cigarettes) to menthol, fruits and coffee.⁴⁻⁷

53 Because the nicotine contained in e-liquids is both addictive and toxic,⁸ some countries
54 have banned/regulated the use of ECs, e-liquids containing nicotine.^{9, 10} This has created a
55 requirement for analytical methods which can be used to determine the nicotine concentrations in e-
56 liquids. The production and labelling of many of these products is not regulated at source so
57 independent methods are required by authorities who have a legal duty to enforce legislation for
58 public health or taxation reasons. This could be, for example, detecting nicotine in supposedly
59 nicotine-free e-liquids or checking that the e-liquids actually contain the concentrations of nicotine
60 stated on their containers by the manufacturers.^{5, 6, 11-13}

61 Nicotine concentrations in e-liquids have been widely quantified by gas chromatography
62 (GC) or high-performance liquid chromatography (HPLC). Sample solutions for these instruments
63 are commonly prepared by the pipetting of e-liquids followed by dilution/extraction and are mixed
64 with/without internal standards such as quinoline.^{4-7, 11-15} These methods are well-established and
65 accurate but they are time-consuming (usually more than 30 min for each sample) and not suitable
66 for rapid field testing at point of sale.

67 While conventional vibrational spectroscopy has some of the aspects required for field
68 testing, such as portability and acceptable cost, the nature of the sample makes conventional
69 vibrational analysis of e-liquids difficult. For IR the aqueous/glycerol medium will interfere while
70 the nicotine concentration is too low for normal Raman analysis, moreover the samples can give
71 strong fluorescence backgrounds with common excitation wavelengths. In principle, surface-
72 enhanced Raman spectroscopy SERS should have appropriate sensitivity but there are potential
73 problems due to the oily and highly viscous nature of the e-liquids (propylene glycol: 40.4 mPa•s at
74 25 °C; vegetable glycerin: 934 mPa•s at 25 °C)¹⁶ which could hinder aggregation and also interfere
75 with adsorption of the analyte to the enhancing surface. In addition, the numerous different
76 colouring/flavouring compounds can also potentially give their own interfering SERS signals.¹⁷

77 Here we show that these problems in the SERS analysis can be overcome because the
78 sensitivity of SERS is vastly better than is required to detect the analyte in the unprocessed samples.
79 Literature data has shown SERS nicotine detection at the low ppm level¹⁸⁻²¹ while the e-liquids are 4
80 orders of magnitude higher. This means the samples can be diluted down dramatically, which removes
81 problems associated with the glycerin/glycerol medium and similarly reduces the flavouring
82 compounds to undetectably low concentrations. This has allowed us to develop a convenient
83 procedure for nicotine screening in e-liquids suitable for field use which combines high dilution in
84 the sample preparation with very straightforward data analysis that can be carried out on simple
85 portable Raman instruments where sample spectra are automatically compared to a library of standard
86 spectra of samples prepared at different concentrations.

87

88 **Experimental**

89 *Chemicals and samples*

90 Nicotine, deuterium-labeled nicotine (d₄-nicotine), and magnesium sulfate (MgSO₄) were
91 obtained from Sigma-Aldrich (St. Louis, MO, USA). The Au colloid (particle size: 50 nm, 4.50 ×
92 10¹⁰ particles mL⁻¹) solution was obtained from BBI solutions (Cardiff, UK). Monopropylene glycol
93 (PG, Pharma grade) and vegetable glycerin (VG, USP Kosher grade) were obtained from Classikool
94 (Essex, UK). Deteriorated nicotine was a sample of pure nicotine which had been stored for more
95 than 10 years at room temperature in the reagent cabinet of our laboratory.

96 E-liquid solutions were obtained from manufacturers in the United Kingdom (Table S1). A
97 set of samples comprising eight flavours, each at 4 different nicotine levels were purchased so the
98 calibration could be tested with a range of flavours. A further set of 10 assorted liquids with different
99 flavours were also used to allow the influence of colourings and flavours as well as different types of
100 bases to be examined over a broad range of liquid types. All of the e-liquids obtained were stored at
101 room temperature in the dark.

102

103 *Preparation of solutions*

104 Nicotine reagents were diluted with double distilled water (DDI). To avoid pipetting the
105 viscous liquid, a fixed amount, approximately 200 μL , of nicotine solution or e-liquid was measured
106 by pouring it into the upturned cap of a 2 mL shell vial until the cap was full. The nicotine solution
107 was transferred to a glass vial, which was previously filled with 25 mL of DDI (Solution A). Then,
108 20 μL of solution A was transferred to another 1 mL glass vial that contained 600 μL of 0.01 mM d_4 -
109 nicotine (Solution B). Finally, 20 μL of solution B was transferred to another 1 mL glass vial that
110 contained 180 μL of Au colloid solution. Nicotine solutions and e-liquids were diluted ca. 4000 times
111 throughout this preparation process. 50 μL of 0.1 M MgSO_4 was added to aggregate the colloids
112 before their SERS spectra were recorded with a portable Raman spectrometer. The overall procedure
113 is illustrated in Fig. S1.

114

115 *Measurement and classification*

116 The aggregated solutions were analyzed with a portable Raman spectrometer (ReporteR,
117 DeltaNu, WY, USA). The laser wavelength was 785 nm, and the spectral range 200 cm^{-1} to 2000
118 cm^{-1} . Spectrometer cm^{-1} was calibrated with a standard polystyrene accessory. The acquisition time
119 was 2 sec x 3 accumulations. The data were analyzed with NuSpec software and no subtraction of
120 background spectra was carried out.

121 For the classification of nicotine levels by library matching, the SERS spectra of mixtures
122 of nicotine/internal standard at the appropriate concentrations (0 mg mL^{-1} , 6 mg mL^{-1} , 12 mg mL^{-1} ,
123 18 mg mL^{-1} , 24 mg mL^{-1} and 30 mg mL^{-1}) were recorded and then used to create a small spectral
124 library using the instrument's internal NuSpec software. To test the nicotine levels of e-liquids the
125 spectra of the liquids prepared in the standard way with internal standard were recorded and searched
126 against the library of standard mixtures. The nicotine concentration of the e-liquid was then estimated
127 as being the same as that of the standard library spectrum which gave the closest match. The search
128 used the instrument's full range spectra (200 cm^{-1} to 2000 cm^{-1}) and the proprietary software which

129 is based on Pearson's correlation.

130

131 **Results and discussion**

132 Quantifying the nicotine content of e-liquids using normal Raman scattering measurements
133 is extremely difficult since the spectra are dominated by signals from the propylene glycol and
134 vegetable glycerin solvent, rather than the much lower concentration (mg mL^{-1}) nicotine component
135 (Figs. S2, S3, S4). Similarly, it is not possible to increase the intensity of the nicotine bands in the
136 spectra of undiluted e-liquids using SERS since the addition of the e-liquids prevents colloid
137 aggregation, as shown by the observation that even the background signals from the colloid's organic
138 stabilizing layer (which are readily detectable with simple aggregated colloid) disappear in samples
139 prepared with undiluted e-liquids (see Fig. S5). In contrast, SERS of highly diluted e-liquids and
140 nicotine samples was much more successful since it removed problems with aggregation, allowing
141 the nicotine to be preferentially enhanced and therefore detected, even in the presence of the other
142 components in the e-liquid.

143

144 *Influence of the nicotine freshness on the Raman spectrum*

145 One potential problem for nicotine analysis either by Raman or SERS methods is that
146 nicotine decomposes in air, turning from a very pale brown to a much darker brown liquid.^{22, 23} In
147 this study nicotine that had been stored for more than 10 years and was very dark brown (see Fig. 1a)
148 was tested alongside fresh nicotine to determine the effects of deterioration on the Raman and SERS
149 spectra. As shown in Fig. 1, the Raman spectrum of the fresh nicotine showed its characteristic peaks
150 originating from the stretching vibrations of the pyridine ring at 1027 cm^{-1} , whereas deteriorated
151 nicotine showed only broad emission due to fluorescence. In contrast, when the fresh and deteriorated
152 nicotine were diluted to $10\text{ }\mu\text{M}$, their SERS spectra were indistinguishable (Fig. 1b).

153 Even though the SERS spectra of the fresh and deteriorated samples were the same, it was
154 useful to check that deterioration did not create products that interfered with the magnitude of the

155 signals e.g. by blocking surface sites. Fig. 2 shows the changes in the signal intensity of nicotine at
156 1027 cm^{-1} between 0 and 2 mM. The signal intensity of fresh nicotine increased dramatically up to
157 $50\text{ }\mu\text{M}$ and then plateaued. The slight decrease in the signal intensity at 2 mM might be due to the
158 reduction of colloid aggregation caused by the presence of excess nicotine in the solution.
159 Deteriorated nicotine also showed an increase in the signal intensity with concentration up to $50\text{ }\mu\text{M}$.
160 However, the signal intensity dramatically decreased with a further increase in the concentration, and
161 it became less than half of the maximum intensity at $200\text{ }\mu\text{M}$. This was presumably due to self-
162 absorption of the excitation laser and Raman scattering by the dark-coloured deteriorated solutions,
163 although it is also possible that the affinity of deteriorated nicotine may be different from those of
164 fresh nicotine at higher concentrations ($> 50\text{ }\mu\text{M}$). Nonetheless, up to $50\text{ }\mu\text{M}$, as shown in Fig. 2b, not
165 only were the signal intensities of both nicotine samples comparable but both also showed almost a
166 linear relationship with concentration. Thus, we considered that both fresh and deteriorated nicotine
167 can be quantified comparably in the range from 0 to $50\text{ }\mu\text{M}$. These results are important because the
168 extent of deterioration of the aged sample is much larger than would be expected in the samples which
169 will be tested in actual field analysis, so interference from nicotine deterioration products should not
170 be a significant problem with the SERS analysis.

171 Although, as shown in Fig. 2, the absolute signal intensity varied linearly with
172 concentration, an appropriate internal standard was added because this makes the calibration more
173 robust by eliminating errors due to changes in the enhancing medium or the performance of the
174 instrument used to read the signals. In this study, we used deuterium-labeled nicotine (d_4 -nicotine),
175 since using an isotopomer of the target compound is known to be the best way to obtain accurate
176 quantification in SERS because the signals for the target and standard are both affected equally by
177 changes in measurement conditions.²⁴ Furthermore, the presence of the d_4 -nicotine peak in the spectra
178 of sample solutions makes it possible to quantify/classify the nicotine concentration by comparing
179 with library data (see below).

180 Fig. 3a shows the changes in the SERS spectra for a mixture of fresh nicotine and d_4 -

181 nicotine (from 0 to 40 μM nicotine with 10 μM d₄-nicotine). The signal intensity of d₄-nicotine at 994
182 cm^{-1} is distinct from that of nicotine at 1027 cm^{-1} and grows as expected with increasing nicotine
183 concentration. For quantitation, the ratio of the peak heights due to nicotine and d₄-nicotine at 1027
184 and 994 cm^{-1} , respectively, were measured. Over the range 0–40 μM nicotine the reproducibility was
185 good (< 5% relative standard deviation at each concentration over the range examined), however this
186 decreased noticeably at 50 μM , possibly due to the influence of nicotine's small signal intensity at
187 994 cm^{-1} and saturation effects, so the calibration range was limited to 0–40 μM . Over this range the
188 calibration is excellent, the plot of relative signals versus relative concentration is liner with an
189 intercept at 0.06 and $r^2 = 0.9996$, so that SERS is clearly suitable for quantification of nicotine in
190 aqueous solution.

191 E-liquids are quite difficult to pipette and disperse in exact volumes because they are
192 typically oily and highly viscous.¹⁶ Furthermore, the concentration range of SERS that is applicable
193 for the reliable quantification of nicotine is limited (from 0 to 40 μM). To overcome these problems,
194 we developed an easy sample preparation process using the internal volume of vial caps (see
195 Experimental and Fig. S1). This preparation process avoids accurate pipetting of e-liquids and
196 involves just mixing with DDI and other aqueous solutions, resulting in aqueous solutions containing
197 d₄-nicotine and Au colloid. This sample preparation process takes only a few minutes.

198 To test the efficacy of this method for e-liquids rather than aqueous nicotine solutions, the
199 nicotine concentration in tobacco flavoured e-liquids was measured. Among the examined e-liquids
200 at 0, 6, 12, and 18 mg mL^{-1} , the relative standard deviations in quintuplicated analyses through the
201 whole process were 2.2% for 6 mg mL^{-1} , 5.0% for 12 mg mL^{-1} , and 4.3% for 18 mg mL^{-1} , this
202 repeatability is comparable to that for pure aqueous solutions, so there were no problems in extending
203 the measurements using this technique to real e-liquids.

204 The method was tested using e-liquids with 8 flavours at 0, 6, 12, and 18 mg mL^{-1} (32
205 samples) and also for another 10 flavours at 11 mg mL^{-1} to examine its ability to obtain nicotine
206 concentrations both at different nicotine concentrations and with different interfering flavours,

207 colourings and bases (Table S1). Fig. 4 shows the results of nicotine quantification in real e-liquids
208 obtained from measurements of the relative peak heights of nicotine and d₄-nicotine in their spectra.
209 Because the repeatability of this method was good over this range, as discussed above, we applied
210 only duplicate analyses for each sample. Analytical results of the nicotine concentrations in all of the
211 e-liquids were comparable to those shown on their containers in samples with different nicotine
212 concentrations (0 mg mL⁻¹: -0.4–0.0 mg mL⁻¹, 6 mg mL⁻¹: 5.7–7.2 mg mL⁻¹, 12 mg mL⁻¹: 11.2–13.3
213 mg mL⁻¹, 18 mg mL⁻¹: 17.2–18.6 mg mL⁻¹, and 11 mg mL⁻¹: 8.5–11.7 mg mL⁻¹), various flavours,
214 different colors and different types of bases (Fig. S6 and Table S1). This fact suggests that the
215 analytical results obtained by this method are free from interference due to flavours, colourings and
216 types of base.

217 Finally, the portable Raman system used in this study can automatically compare newly
218 acquired spectra with library data in real time. This function becomes possible with d₄-nicotine
219 addition and is very convenient for rapidly estimating the approximate nicotine content, a task which
220 is made easier by the fact that most of the available e-liquids contain nicotine levels which vary in
221 multiples of 6, such as 0, 6, 12, and 18 mg mL⁻¹.⁴⁻⁷ Here the SERS spectral data from the calibration
222 curve was used to build a spectral library that the spectra for each e-liquid could be compared against.
223 This allowed the nicotine level in the e-liquids to be classified by finding which spectrum in the
224 library they matched most closely. Fig. 4 shows the classification of the nicotine levels in all 42
225 samples obtained by library matching, along with the results from the quantitative analysis. In the
226 plot, the shape of each of the points is used to indicate which of the 5 concentration values the library
227 matching gave. The approach was remarkably successful, only 1 of the 42 samples was incorrectly
228 classified and in that case the sample was classified as belonging the nearest neighbor (actual 11 mg
229 mL⁻¹, estimated value 6 mg mL⁻¹). This level of accuracy also meant that the method allowed samples
230 which contained nicotine to be distinguished from those that did not with confidence (Tables S2 and
231 S3).

232

233 **Conclusions**

234 We have developed a new method for the screening of nicotine in e-liquids which combines
235 an easy sample preparation process with SERS and a portable Raman spectrometer. The method can
236 be used either for full quantitation of nicotine concentration or for rapid estimation of the nicotine
237 level by library matching. Importantly, the results are not affected by flavours, colourings, type of
238 base or the freshness of the nicotine. This was possible because the high sensitivity of SERS meant
239 that the sample could be significantly diluted (ca. 4000×) in the sample preparation which diluted out
240 matrix effects from the glycerol present and also reduced interference from flavouring and colouring
241 compounds below detectable levels. This approach of combining high sample dilution with SERS
242 clearly has the potential to be applied to other sample types where matrix effects may be significant,
243 such as foodstuffs or topical pharmaceuticals.

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247 **Notes and references**

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298 **Fig. 2**299 Plot of the intensity of the SERS band at 1027 cm⁻¹ and nicotine concentration (a) from 0 to 2 mmol300 L⁻¹ and (b) magnified between 0 to 100 μmol L⁻¹. Data for fresh and deteriorated nicotine are shown.

301 The error bars indicate the standard deviation in triplicate analyses.

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312 **Fig. 3**

313 (a) Changes in the SERS spectra of a nicotine (1027 cm⁻¹) and d₄-nicotine (994 cm⁻¹) mixture in 0 to
 314 40 μM nicotine solutions with d₄-nicotine internal standard fixed at 10 μM. Inset shows the structures
 315 of nicotine and d₄-nicotine. (b) Plot of the concentration ratio of nicotine and d₄-nicotine against the
 316 ratio of their characteristic (1027: 994 cm⁻¹) bands. Error bars indicate the standard deviation in
 317 quintuplicate analyses.

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Fig. 4

Plot illustrating the results of nicotine SERS analysis in commercial e-liquids. Values in parentheses on the x-axis are the nicotine concentrations shown on each container. The positions of the points show the analytical values obtained by measuring relative peak heights of nicotine and d₄-nicotine for each of the samples. The symbols used to mark the points indicate which standard spectrum the unprocessed sample spectra matched in the spectral library. These latter values can be used to estimate the nicotine concentration without explicitly measuring peak heights in the spectra.