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

Nobuyasu Itoh*[a] and Steven E.J. Bell[b]

[a] *National Metrology Institute of Japan (NMIJ), National Institute of Advanced Industrial Science and Technology (AIST), Central 3, 1-1-1 Umezono, Tsukuba, Ibaraki 305-8563, Japan*

[b] *Innovative Molecular Materials Group, School of Chemistry and Chemical Engineering, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast BT9 5AG, United Kingdom*

*Corresponding author. National Metrology Institute of Japan (NMIJ), National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Umezono, Tsukuba, Ibaraki 305-8563, Japan.

E-mail address: nobuyasu-itoh@aist.go.jp (N. Itoh).

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 (d_4-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

numerous different flavours are available, ranging from tobacco flavours (which are similar to cigarettes) to menthol, fruits and coffee.[4-7]

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

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

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

Here we show that these problems in the SERS analysis can be overcome because the sensitivity of SERS is vastly better than is required to detect the analyte in the unprocessed samples. Literature data has shown SERS nicotine detection at the low ppm level[18-21] while the e-liquids are 4 orders of magnitude higher. This means the samples can be diluted down dramatically, which removes problems associated with the glycerin/glycerol medium and similarly reduces the flavouring compounds to undetectably low concentrations. This has allowed us to develop a convenient procedure for nicotine screening in e-liquids suitable for field use which combines high dilution in the sample preparation with very straightforward data analysis that can be carried out on simple portable Raman instruments where sample spectra are automatically compared to a library of standard spectra of samples prepared at different concentrations.

Experimental

Chemicals and samples

Nicotine, deuterium-labeled nicotine (d_4-nicotine), and magnesium sulfate ($MgSO_4$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The Au colloid (particle size: 50 nm, 4.50×10^{10} particles mL^{-1}) solution was obtained from BBI solutions (Cardiff, UK). Monopropylene glycol (PG, Pharma grade) and vegetable glycerin (VG, USP Kosher grade) were obtained from Classikool (Essex, UK). Deteriorated nicotine was a sample of pure nicotine which had been stored for more than 10 years at room temperature in the reagent cabinet of our laboratory.

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

Preparation of solutions

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

Measurement and classification

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

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

is based on Pearson's correlation.

Results and discussion

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

Influence of the nicotine freshness on the Raman spectrum

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

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

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

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

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

nicotine (from 0 to 40 μM nicotine with 10 μM d_4-nicotine). The signal intensity of d_4-nicotine at 994 cm^{-1} is distinct from that of nicotine at 1027 cm^{-1} and grows as expected with increasing nicotine concentration. For quantitation, the ratio of the peak heights due to nicotine and d_4-nicotine at 1027 and 994 cm^{-1}, respectively, were measured. Over the range 0−40 μM nicotine the reproducibility was good (< 5% relative standard deviation at each concentration over the range examined), however this decreased noticeably at 50 μM, possibly due to the influence of nicotine's small signal intensity at 994 cm^{-1} and saturation effects, so the calibration range was limited to 0−40 μM. Over this range the calibration is excellent, the plot of relative signals versus relative concentration is liner with an intercept at 0.06 and r^2 = 0.9996, so that SERS is clearly suitable for quantification of nicotine in aqueous solution.

E-liquids are quite difficult to pipette and disperse in exact volumes because they are typically oily and highly viscous.[16] Furthermore, the concentration range of SERS that is applicable for the reliable quantification of nicotine is limited (from 0 to 40 μM). To overcome these problems, we developed an easy sample preparation process using the internal volume of vial caps (see Experimental and Fig. S1). This preparation process avoids accurate pipetting of e-liquids and involves just mixing with DDI and other aqueous solutions, resulting in aqueous solutions containing d_4-nicotine and Au colloid. This sample preparation process takes only a few minutes.

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

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

colourings and bases (Table S1). Fig. 4 shows the results of nicotine quantification in real e-liquids obtained from measurements of the relative peak heights of nicotine and d_4-nicotine in their spectra. Because the repeatability of this method was good over this range, as discussed above, we applied only duplicate analyses for each sample. Analytical results of the nicotine concentrations in all of the e-liquids were comparable to those shown on their containers in samples with different nicotine 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 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, different colors and different types of bases (Fig. S6 and Table S1). This fact suggests that the analytical results obtained by this method are free from interference due to flavours, colourings and types of base.

Finally, the portable Raman system used in this study can automatically compare newly acquired spectra with library data in real time. This function becomes possible with d_4-nicotine addition and is very convenient for rapidly estimating the approximate nicotine content, a task which is made easier by the fact that most of the available e-liquids contain nicotine levels which vary in multiples of 6, such as 0, 6, 12, and 18 mg mL^{-1}.[4-7] Here the SERS spectral data from the calibration curve was used to build a spectral library that the spectra for each e-liquid could be compared against. This allowed the nicotine level in the e-liquids to be classified by finding which spectrum in the library they matched most closely. Fig. 4 shows the classification of the nicotine levels in all 42 samples obtained by library matching, along with the results from the quantitative analysis. In the plot, the shape of each of the points is used to indicate which of the 5 concentration values the library matching gave. The approach was remarkably successful, only 1 of the 42 samples was incorrectly classified and in that case the sample was classified as belonging the nearest neighbor (actual 11 mg mL^{-1}, estimated value 6 mg mL^{-1}). This level of accuracy also meant that the method allowed samples which contained nicotine to be distinguished from those that did not with confidence (Tables S2 and S3).

Conclusions

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

Notes and references

1. P. Hajek, J.-F. Etter, N. Benowitz, T. Eissenberg and H. McRobbie, *Addiction*, 2014, **109**, 1801-1810.
2. J.-F. Etter and C. Bullen, *Addiction*, 2011, **106**, 2017-2028.
3. J.-F. Etter, *BMC Public Health*, 2010, **10**, 231.
4. M. L. Goniewicz, R. Gupta, Y. H. Lee, S. Reinhardt, S. Kim, B. Kim, L. Kosmider and A. Sobczak, *Int. J. Drug Policy*, 2015, **26**, 583-588.
5. B. Davis, M. Dong, J. Kim and P. Talbot, *Nicotine Tob. Res.*, 2015, **17**, 134-141.
6. M. L. Goniewicz, T. Kuma, M. Gawron, J. Knysak and L. Kosmider, *Nicotine Tob. Res.*, 2013, **15**, 158-166.
7. J.-F. Etter, E. Zäther and S. Svensson, *Addiction*, 2013, **108**, 1671-1679.
8. B. Mayer, *Arch. Toxicol.*, 2014, **88**, 5-7.
9. J. E. Henningfield and G. S. Zaatari, *Tob. Control*, 2010, **19**, 89-90.
10. W. H. Organization, *Conference of the Parties to the WHO framework convention on tobacco control*, 2014, **FCTC/COP/6/10 Rev.1**, 13 pp. (last accessed: August 9, 2016)
11. J. G. Lisko, H. Tran, S. B. Stanfill, B. C. Blount and C. H. Watson, *Nicotine Tob. Res.*, 2015, **17**, 1270-1278.
12. J. M. Cameron, D. N. Howell, J. R. White, D. M. Andnyak, M. E. Layton and J. M. Roll, *Tob. Control*, 2014, **23**, 77-78.
13. T. Cheng, *Tob. Control*, 2014, **23**, ii11-ii17.
14. M. Famele, C. Ferranti, C. Abenavoli, L. Palleschi, R. Mancinelli and R. Draisci, *Nicotine Tob. Res.*, 2015, **17**, 271-279.
15. M. L. Trehy, W. Ye, M. E. Hadwiger, T. W. Moore, J. F. Allgire, J. T. Woodruff, S. S. Ahadi, J. C. Black and B. J. Westenberger, *J. Liq. Chromatogr. Related Technol.*, 2011, **34**, 1442-1458.
16. *CRC Handbook of Chemistry and Physics*, CRC Press, 89th edn., 2009.

17. E. C. L. Ru and P. G. Etchegoin, *Principles of Surface-Enhanced Raman Spectroscopy*, Elsevier, Amsterdam, 2009.
18. S. E. J. Bell and N. M. S. Sirimuthu, *Analyst*, 2004, **129**, 1032-1036.
19. J. Jung, J. Choo, D. J. Kim and S. Lee, *Bull. Korean Chem. Soc.*, 2006, **27**, 277-280.
20. M. B. Mamián-López and R. J. Poppi, *Anal. Chim. Acta*, 2013, **760**, 53-59.
21. O. Alharbi, Y. Xu and R. Goodacre, *Analyst*, 2014, **139**, 4820-4827.
22. H. Schievelbein, *Beitr. Tabakforsch.*, 1962, 199-274.
23. Centers for Disease Control and Prevention, Nicotine: Systemic agent. Available at: http://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750028.html (last accessed: August 9, 2016).
24. A. Mechlińska, L. Wolska and J. Namieśnik, *Trend Anal. Chem.*, 2010, **29**, 820-831.

Figures

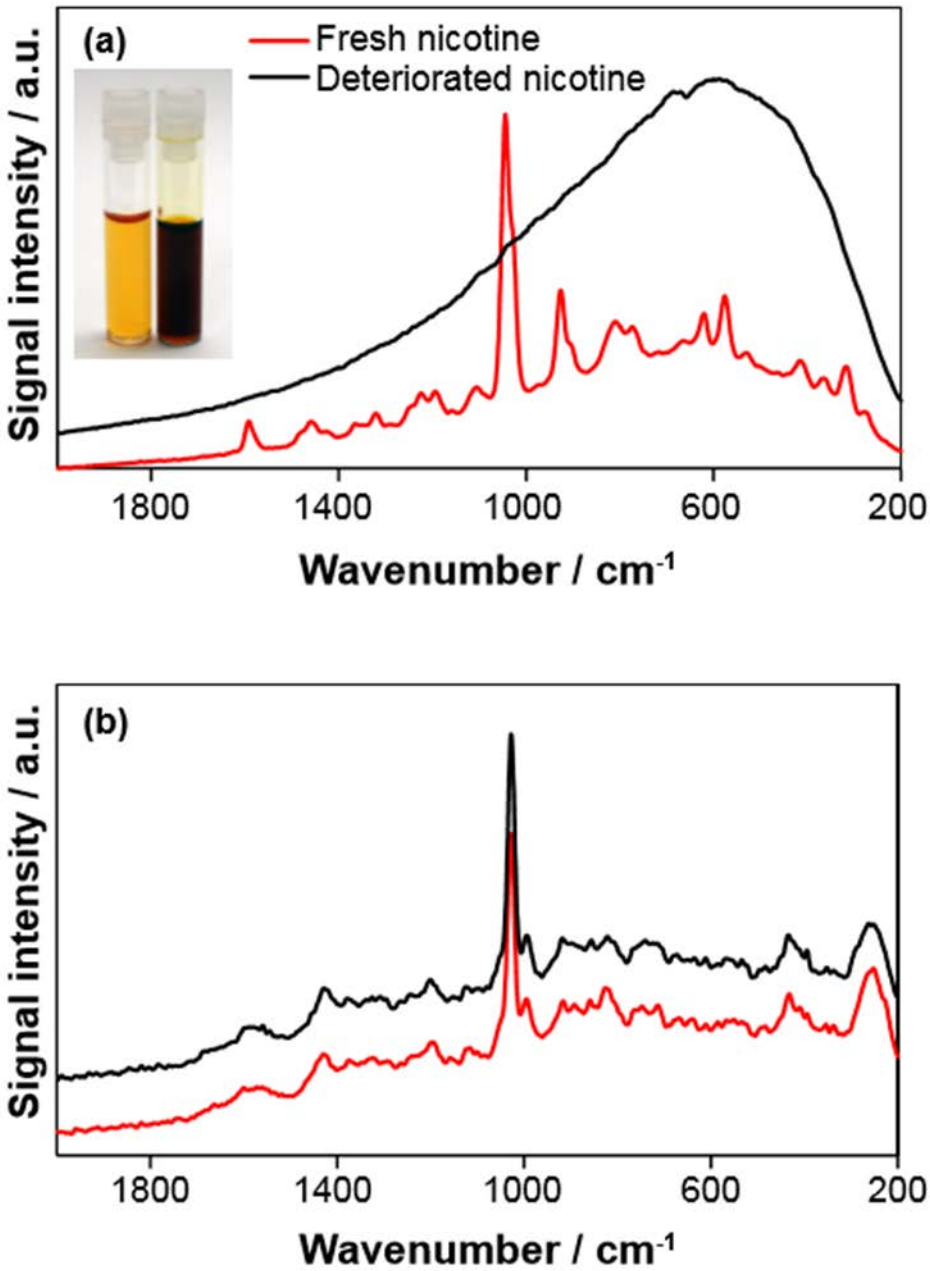

Fig. 1

(a) Raman spectra of fresh and deteriorated nicotine. The inset photograph shows fresh (left) and deteriorated (right) nicotine in glass vials. (b) SERS spectra of fresh and deteriorated nicotine.

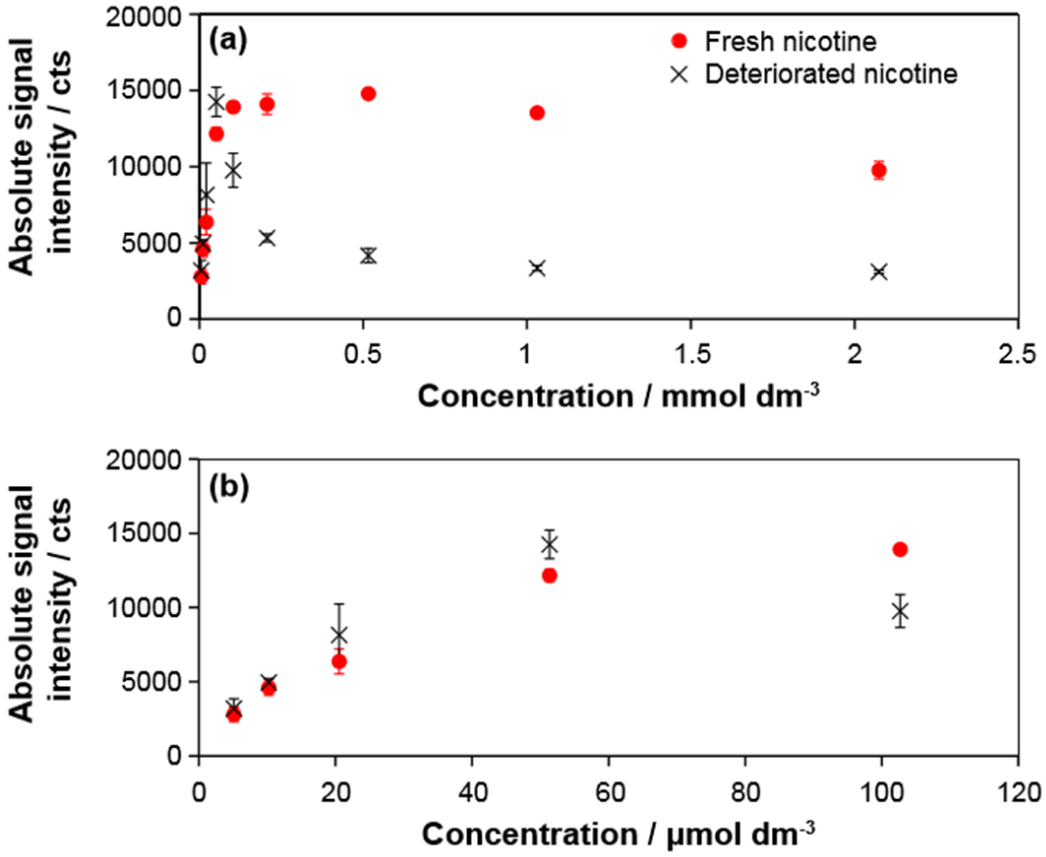

Fig. 2

Plot of the intensity of the SERS band at 1027 cm^{-1} and nicotine concentration (a) from 0 to 2 mmol L^{-1} and (b) magnified between 0 to 100 µmol L^{-1}. Data for fresh and deteriorated nicotine are shown. The error bars indicate the standard deviation in triplicate analyses.

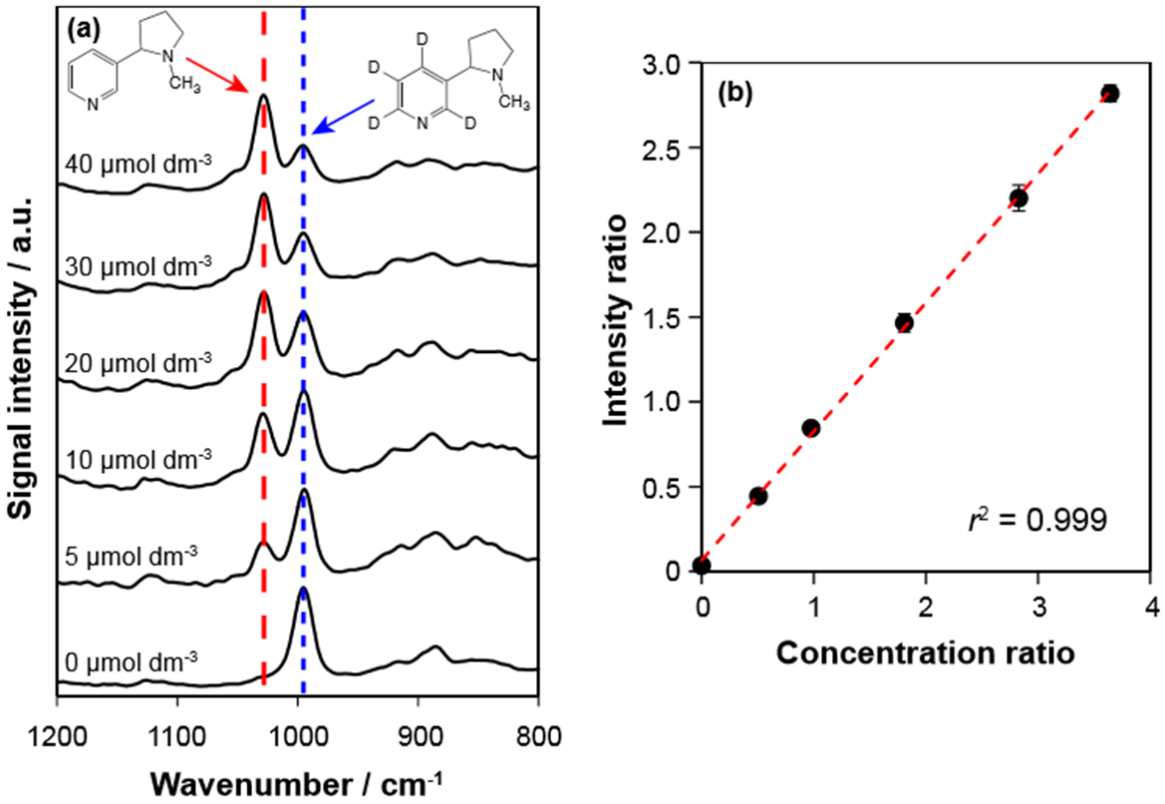

Fig. 3

(a) Changes in the SERS spectra of a nicotine (1027 cm^{-1}) and d_4-nicotine (994 cm^{-1}) mixture in 0 to 40 μM nicotine solutions with d_4-nicotine internal standard fixed at 10 μM. Inset shows the structures of nicotine and d_4-nicotine. (b) Plot of the concentration ratio of nicotine and d_4-nicotine against the ratio of their characteristic (1027: 994 cm^{-1}) bands. Error bars indicate the standard deviation in quintuplicate analyses.

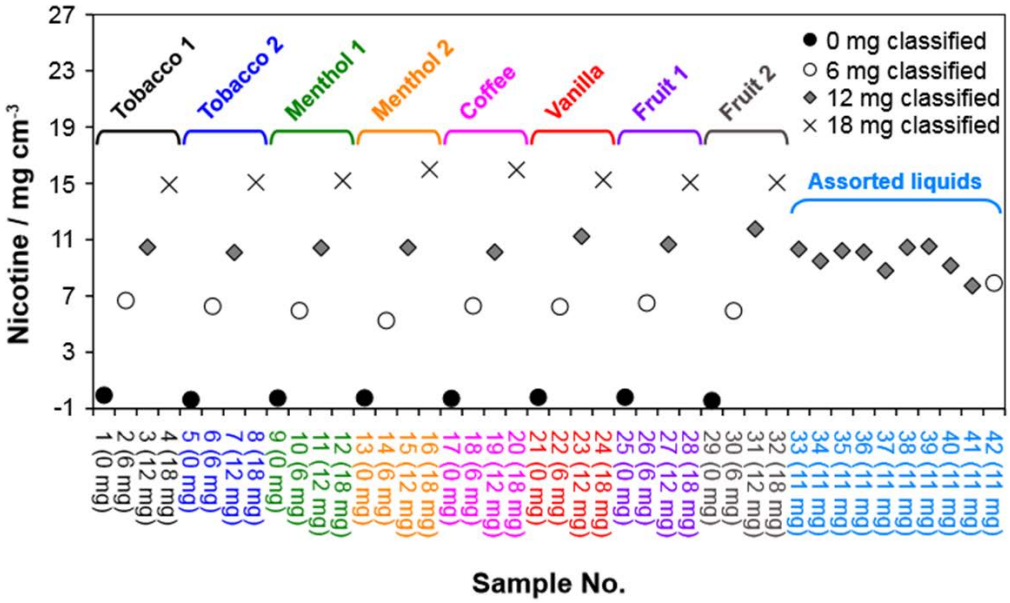

Fig. 4

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 d_4-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.