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Investigation of the chemical origin and evidential value of differences in the SERS spectra of blue gel inks

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Highly swellable polymer films doped with Ag nanoparticle aggregates (Poly-SERS films) have been used to record very high signal:noise ratio, reproducible surface-enhanced resonance Raman (SERRS) spectra of *in situ* dried ink lines and their constituent dyes using both 633 and 785 nm excitation. These allowed the chemical origins of differences in the SERRS spectra of different inks to be determined. Initial investigation of pure samples of the 10 most common blue dyes showed that the dyes which had very similar chemical structures such as Patent Blue V and Patent Blue VF (which differ only by a single OH group) gave SERRS spectra in which the only indications that the dye structure had been changed were small differences in peak positions or relative intensities of the bands. SERRS studies of 13 gel pen inks were consistent with this observation. In some cases the inks from different types of pen could be distinguished even though they were dominated by a single dye such as Victoria Blue B (Zebra Surai) or Victoria Blue BO (Pilot Acroball) because their predominant dye did not appear in the other inks. Conversely, identical spectra were also recorded from different types of pen (Pilot G7, Zebra Z-grip) because they all had the same dominant Brilliant Blue G dye. Finally, some of the inks contained mixtures of dyes which could be separated by TLC and removed from the plate before being analysed with the same Poly-SERS films. For example, Pentel EnerGel ink pen was found to give TLC spots corresponding to Erioglaucine and Brilliant Blue G.

Overall, this study has shown that the spectral differences between different inks which are based on chemically similar, but nonetheless distinct dyes, are extremely small, so very close matches between SERRS spectra are required for confident identification. Poly-SERS substrates can routinely provide the very stringent reproducibility and sensitivity levels required. This, coupled with the awareness of the reasons underlying the observed differences between similarly coloured inks allows a more confident assessment of the evidential value of inks SERS and should underpin adoption of this approach as a routine method for the forensic examination of inks.

Introduction

Document examination is an important area in forensic science since it covers evidence including threatening letters, forged certificated and altered cheques. Within this area video spectral comparison (VSC)¹ is very commonly used because it is rapid and non-destructive but its discrimination is limited because it compares the optical properties of inks rather than their chemical composition. Conversely thin layer chromatography (TLC)²⁻³ gives much more detailed information on the composition of the ink but it is both more time consuming to carry out than VSC and also can only be used for dye-based inks as pigment based inks are, by definition, insoluble in common solvents and so are not suitable for TLC analysis. Other, more specialized methods which have been used for ink analysis include HPLC⁴⁻⁵ desorption electrospray ionization mass spectrometry⁶, matrix assisted laser-desorption/ionization reflector time of flight mass spectrometry⁷ and capillary electrophoresis⁸, however none of these have replaced conventional VSC and TLC. Raman spectroscopy and surface-enhanced Raman spectroscopy

(SERS) have real potential in forensic document examination since they can provide chemical information without the long analysis times associated with TLC. There are numerous reports⁹⁻¹¹ which use Raman and/or resonance Raman spectroscopy for the analysis of ink samples however these have been limited to pigment based inks since the dye-based inks are highly fluorescent even with red excitation. It is possible to use SERS to overcome the problem of fluorescence and SERS has already been used for the analysis of dye standards or dyes in ballpoint inks¹²⁻¹⁵ however, the analysis of gel inks has been much more limited.¹¹ For SERS sampling of inks the sample could be mixed with the colloid in the conventional way but for dried ink lines a better approach is to apply small droplets of concentrated colloid on top of the ink line before probing^{11-13,15} Typically the measurement time of this method is quite long, c.a. 3 h, because in most cases it required the droplet to completely dry before SERS analysis. Alternatively, Ag nanoparticle-doped agarose disks have been shown to be capable of detecting the predominant dyes in a red ink (Rhodamine 6G) and a blue ink (crystal violet).¹⁵ However, the complexity of the preparation and pretreatment processes required mean this is unlikely to be widely adopted as a routine method.

In this work, we exploit the recently reported Poly-SERS films which are made of a highly swellable polymer, hydroxyethylcellulose (HEC), containing a high loading of SERS-active nanoparticle aggregates. On contact with aqueous analyte these films swell which

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allows the analyte to come into contact with the enhancing particles. These films have been well characterized in previous work¹⁶⁻¹⁷ and have been shown to work well with a range of analytes, not only those which are more commonly used as test analytes in SERS, such as thiophenol or rhodamine 6G¹⁶ but also with more challenging targets such as phenytoin¹⁸ and cathinone derivatives¹⁹. They also have practical advantages; most notably they can be stored (with a lifetime of more than 9 months) and used off-the-shelf when required, since the SERS active particles are protected in a dry and solid polymer matrix. In addition, since they function by drawing the analyte into the film, they can potentially be used *in situ* to extract the dye from ink lines towards the enhancing nanoparticles rather than being applied to the lines.

Here we show that these Poly-SERS films not only provide a highly convenient method for the analysis of inks in dried ink lines but that they combine ease of use with very high sensitivity and reproducibility. These factors have allowed us to carry out detailed studies on the SERRS of writing inks, using blue dye based gel inks as an example. This involved both identifying the dominant dyes by comparison with the spectra of commercially available dye standards and combining Poly-SERS analysis with TLC separation of constituents inks prepared with mixtures of dyes. This approach is more sophisticated than simply comparing spectra of a given set of different inks and looking for differences which can be quantified by the discriminating power because it aims to provide a more complete understanding of the chemical origin of these differences. This is important for correctly interpreting the significance of the spectroscopic data and deciding how much weight can be placed on the evidence such data can provide.

Experimental

Materials and methods

SERRS spectra were obtained using a PerkinElmer RamanMicro 200 microscope with 633 or 785 nm excitation (spectral resolution of 6 and 4 cm^{-1} , respectively). Some spectra were also recorded using a handheld 785 nm Delta Nu Reporter system which had a spectral range of 300–2000 cm^{-1} and spectral resolution of 12–15 cm^{-1} .

All samples were analysed using Poly-SERS films containing citrate reduced silver colloid (CRSC).¹⁶ Briefly, CRSC was prepared using the Lee and Meisel method (20) and aggregated with MgSO_4 before finally adding hydroxyethylcellulose (HEC) powder whilst stirring vigorously. The mixture was allowed to stir for a further 50 minutes or until a thick and smooth gel consistency was obtained. This was then poured into a 100 × 100 mm mould and left to dry at which point the film could be peeled away. In use, the Poly-SERS film was cut to size (ca. 1 × 1 mm) using scissors and placed on top of a dried ink line (drawn on normal white printing paper) or powdered dye standard (on a glass slide covered in aluminium foil). A 20 μL droplet of 30 % methanol in water was then applied directly on top of the film. After 1–2 minutes, the film was then laser probed using either 633 or 785 nm Raman excitation with 30 s (Raman microscope) or 3 s (handheld Delta Nu Reporter) accumulation times. All spectra shown in this report were processed using GRAMS/AI v7.02.

TLC separation was carried out using Merck TLC silica gel 670 F_{254} TLC plates and N-butanol:ethanol: water (2:1:1) as the eluent. Small concentrated circles were drawn onto aluminium foil with the pens to be tested and 200 μL methanol was added to make a dye solution. This dye-methanol mixture was then spotted onto the TLC plate for separation. For TLC-SERRS, the separated ink component was

removed from the TLC plate by scraping its surface with a sharp scalpel and the resulting solid was then placed onto a glass slide covered in aluminium foil before the Poly-SERS film and methanol solution were applied.

Dye standards: Brilliant Blue G, Erioglaucine, Ethyl Violet, Crystal Violet, Methyl Violet B base, Victoria Pure Blue BO, Victoria Blue R, Victoria Blue B, Patent Blue V, Patent Blue VF were all purchased from Sigma Aldrich. The gel pens were purchased from retail stores in the U.K., Taiwan (TWN) and Singapore (SIN) and are labelled according to the information printed on their bodies along with the designation TWN or SIN if they were purchased outside the UK.

Results and discussion

The method for obtaining spectra from ink lines was extremely simple, a small piece of the Poly-SERS film was placed on top of the dried ink and then a 20 μL droplet of 30% methanol in water was placed directly on top of the film, which was then allowed to dry for ca. 1–2 minutes before a SERRS spectrum was collected. The process caused simultaneous dissolution of the ink and swelling of the film, which allowed the dye analyte to interact with the nanoparticle aggregates.

Although this method did mean that there was some ink spread within the dried sample, the ink was still legible after the analysis and the Poly-SERS film could be easily removed. Figure 1 shows photographs of the procedure. A similar method was used for the dye standards where the film was placed on top of the sample and solvent applied. In either case this approach reliably produced very high quality spectra in minutes and without the need to optimize the experimental conditions for any given sample. Figure 2 shows exemplar data for a single pen at several positions along an ink line at two different excitation wavelengths. The high level of reproducibility in both the major bands and much smaller features shown in Figure 2 was observed throughout this work. The change in relative intensities of the bands of the same ink at the two excitation wavelengths shows that there is a resonance contribution to the enhanced spectra so these are SERRS, rather than SERS, spectra.

Since the primary objective of this study was to understand the chemical origin of the differences in the spectra of inks from different pens, the first step was to record the spectra of the dyes which were most likely to be present in blue inks, determined from the literature on ink preparation. The structures of many of these dyes are

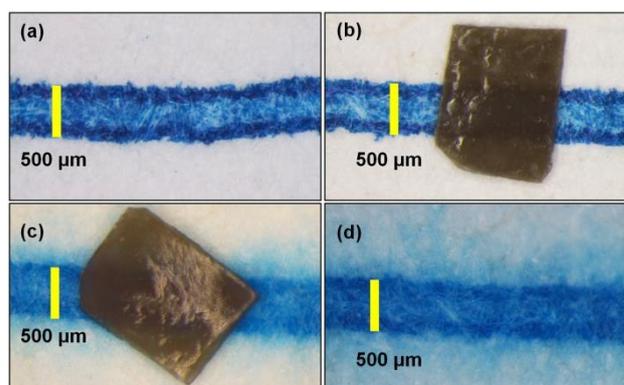


Figure 1: Photographs illustrating the SERRS method in which (a) the dried ink line has (b) a small section of Poly-SERS film placed on top before (c) a droplet of solvent is deposited directly on top. Image (d) shows the ink line after removal of the film.

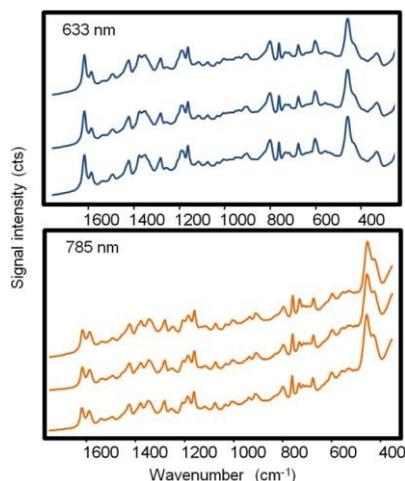


Figure 2: SERRS spectra of an ink line from a Staedtler Silver Ball pen, obtained at various points along line using (a) 633 and (b) 785 nm excitation.

very similar to each other so it was useful to determine the extent to which small changes in structure affected their SERRS spectra.

Data were recorded using both 633 nm and 785 nm excitation to check if the SERRS spectra of the dyes would change significantly with excitation wavelength in the same way as was often observed for the inks. There are hundreds of commercially available dyes which can be used for preparing pen inks but here the study was confined to the ten most common ones, in fact we found (see below) that

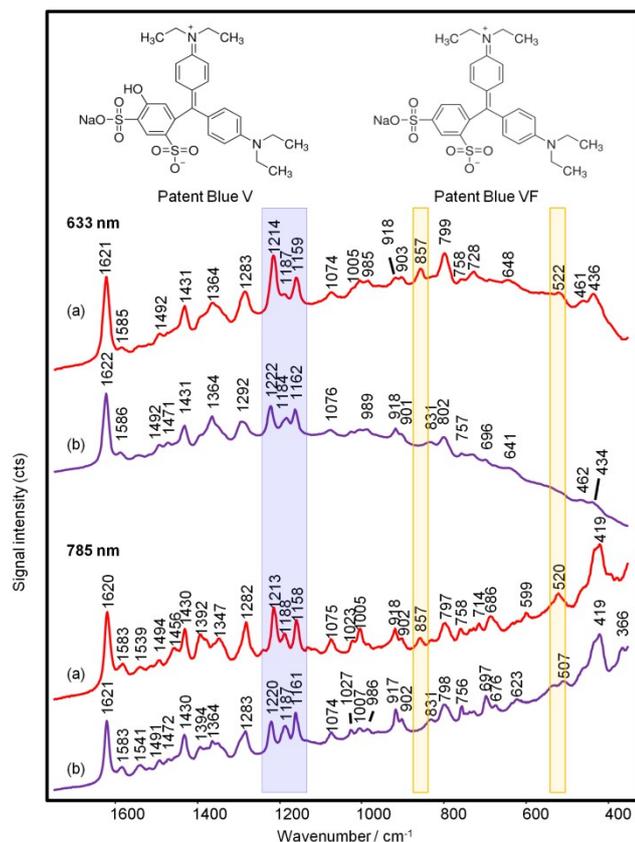


Figure 3: SERRS spectra of (a) Patent blue V and (b) Patent blue VF with 633 and 785 nm excitation. The coloured bars highlight the regions where the band position (yellow) and relative intensities (blue) are different between the dyes.

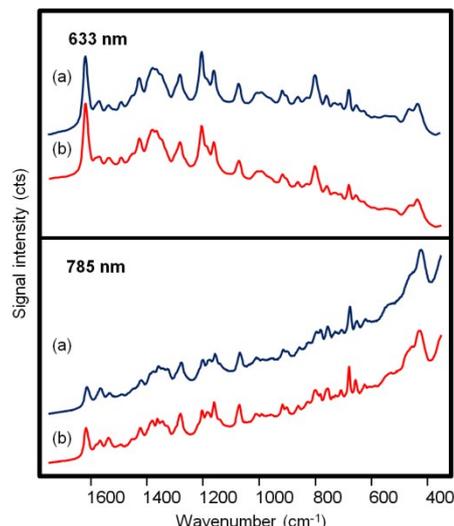


Figure 4: SERRS spectra of a (a) Pilot Acroball pen ink line and (b) Victoria Blue BO standard using 633 nm or 785 nm excitation.

almost all the dyes in the inks investigated came from this set. All the standard dyes have strong absorption bands at ca. 600 nm with λ_{max} ranging from 580 nm (Methyl Violet B) to 638 nm (Patent Blue V) so are strongly in resonance at 633 nm and pre-resonance with 785 nm excitation. A list of the dyes with their λ_{max} values is given in the Supplementary Information. Figure 3 shows examples of the spectra of two very similar dyes, Patent Blue V and Patent Blue VF, which differ only by a single OH group. Despite this small difference there are some distinct differences in band positions and relative intensities between the spectra, for example the peak at 857 cm^{-1} in the 633 nm spectrum of Patent Blue V shifts to 831 cm^{-1} in Patent Blue VF. This means that for ink analysis it is important to be able to detect even subtle spectral changes because these may indicate a difference in the structure of one of the main chemical constituents, rather than variation in some minor component. The SERRS spectra of the remaining 8 dyes which were tested are shown in the Supporting Information and these follow the same pattern; closely related dyes give similar but still distinctive spectra. The SERRS spectra (with 633 or 785 nm excitation) of thirteen dye-based pen ink samples (see Supporting Information for a list of the manufacturers and country purchased) were then recorded and

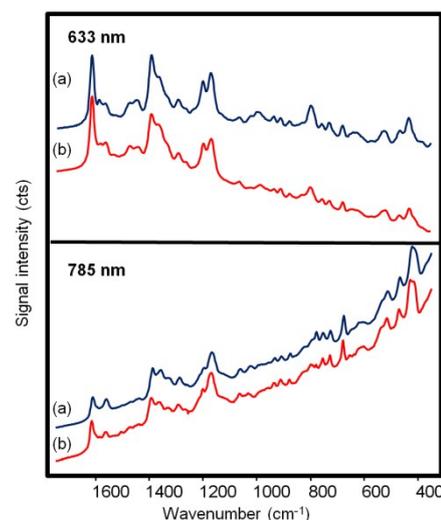


Figure 5: SERRS spectra of a (a) Zebra Surai pen ink line and (b) Victoria Blue B standard using 633 nm or 785 nm excitation.

compared to the spectra of the dye standards. Some of the ink spectra could be directly matched to a single standard dye, for example, the ink from a Pilot Acoball pen is dominated by bands due to Victoria Pure BO and the Zebra Surai SERRS spectrum had only Victoria blue B bands, as shown in Figures 4 and 5. The closeness of the matches between the spectra of the inks and the corresponding dye standards is impressive, particularly since they were observed at two different excitation wavelengths, where differences in the resonance contributions mean that these SERRS spectra are really quite different at each wavelength.

In most cases the spectra of the pens were found to consist of one main dye component which allowed them to be classified into different groups. For example, Figure 6 shows the SERRS spectra of a group of pens which were all found to have Brilliant Blue G. Again the match between ink and standard dye is striking. However, very close inspection of the spectra does reveal that in some of the pens in this group there is a very small additional peak at ca. 780 cm^{-1} which is not present in the standard dye or in the other inks. We are confident that this is not an artifact because it was reproducible (being found for Pentel G2 pens purchased in 3 different countries) and also because it was confined to Pilot G type pens only. Presumably it is due to a second component which is added only to the Pilot G pens during manufacture. However, the fact that only one very small peak is observed makes it very difficult to identify its structure on the basis of SERRS alone.

The data in Figures 4-6 showed examples where the differences between the inks were due to them being manufactured with different dyes (with the exception of the 780 cm^{-1} band discussed above). In these cases the differences between some of the spectra are subtle because the changes in the structure of the predominant

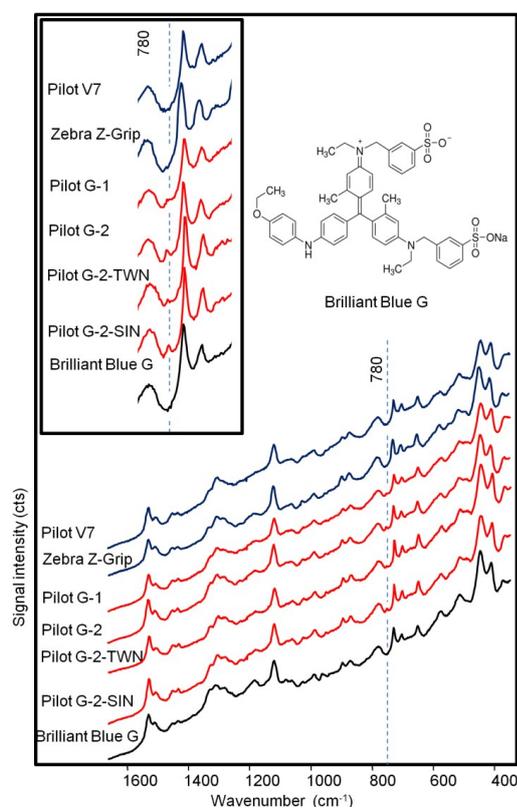


Figure 6: SERRS spectra of the ink lines from group of pens containing Brilliant Blue G standard using 785 nm excitation. The insets show an expanded view of the region around the 780 cm^{-1} peak.

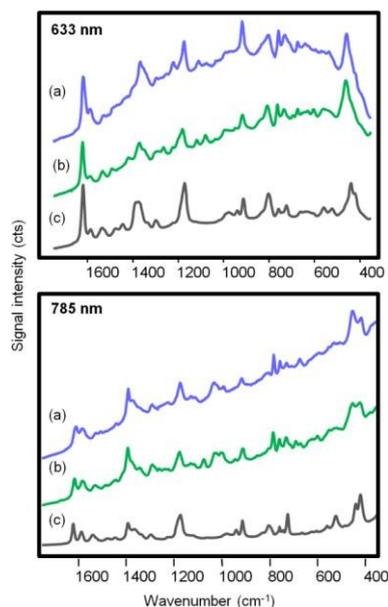


Figure 7: SERRS spectra of an ink line from (a) Pentel Energel, (b) Pentel Ball and (c) crystal violet standard using 633 and 785 nm excitation.

dye are small. However, another potential source of variation between inks is the possibility that they could be prepared from mixtures of dyes and that the relative proportions could be different in different inks. One indication that this is the case would be where the spectra of some samples match each other at one wavelength but not at another. We have found this in the spectra of the Pentel Ball and Pentel Energel pen inks, which were similar to each other with 785 nm excitation but more different to each other with 633 nm , as shown in Figure 7. The ink spectra at 785 nm were both sufficiently similar to those of crystal violet (also shown in Figure 7) that they might be mistaken for crystal violet based inks, however, the differences in the spectra recorded with 633 nm excitation (see the lowest frequency bands for example) clearly indicated that this is not the case, so the inks were then investigated by TLC.

Figure 8 shows the TLC plates for the two inks which indicated that the ink from a Pentel Ball pen was made up of two components (both blue dyes), whereas the Pentel Energel was made up of three (two blue and one red dye). Although TLC was useful in identifying that these inks contained a mixture of dyes, it did not allow for unambiguous identification of the dyes themselves. However, the same approach used for extracting the dyes from the inks using Poly-SERS films could also be used to obtain the SERRS spectra of the individual bands on the TLC plates. The bands corresponding to each of the separated dyes were scraped from the TLC plate onto a clean aluminum covered glass slide where the silica gel was then tested by pressing on a section of the Poly-SERS films and adding a drop of solvent. The spectra shown in Figure 8 demonstrate that this approach enabled the dyes to be extracted from the silica when brought into contact with the enhancing nanoparticles in much the same way as was observed with the ink lines. The results are excellent, the dye spectra were recorded with high signal:noise and cross-contamination between the dyes was minimal. For example, the peak at 919 cm^{-1} , which appears in the spectrum of Pentel Ball and is characteristic of the Erioglaurine component, is observed in the spectrum obtained from the light blue band on the TLC plate but not the spectrum of the dark blue band. This contrasts with previous

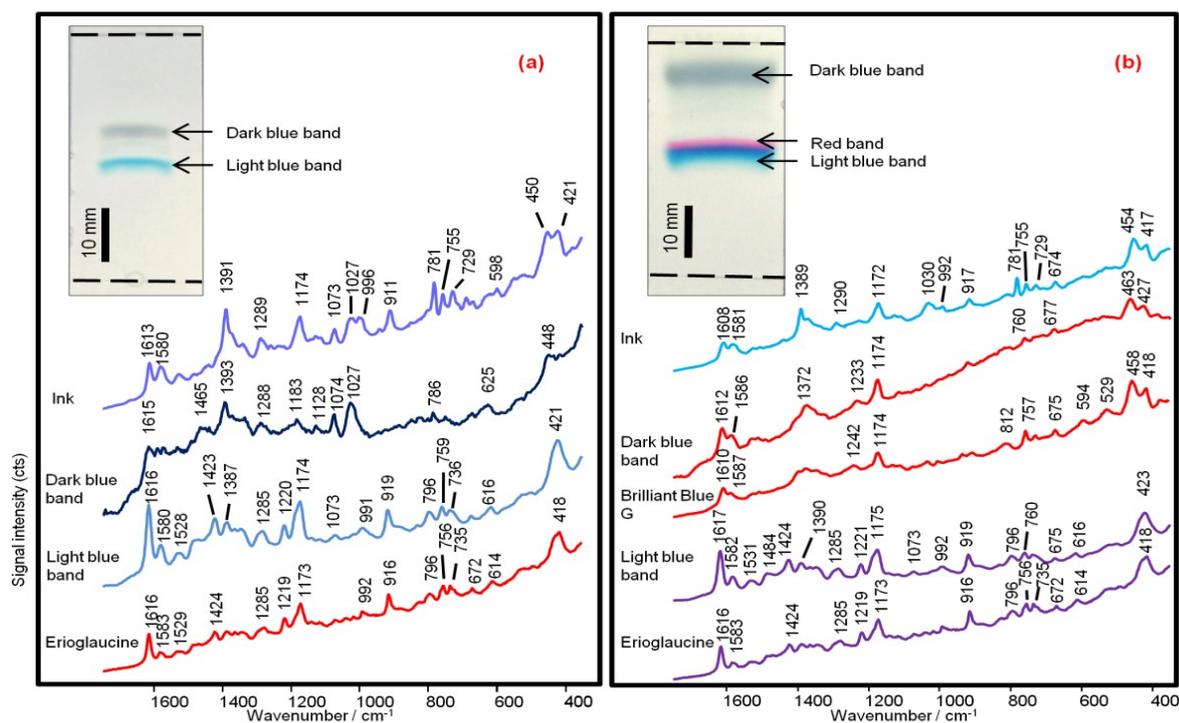


Figure 8: (a) The SERRS spectra of an ink line from a Pentel Ball pen and its separated components along with Erioglaucine standard. (b) The SERRS spectra of an ink line from a Pentel EnerGel pen and its separated components along with Erioglaucine and Brilliant Blue G standards. Both sets of data were recorded using 785 nm excitation. The insets show the inks separated on the TLC plates.

work¹⁴ where different TLC spots from an ink gave identical spectra, presumably due to cross-contamination. The advantage of having the spectra of the individual components is that they can be matched against the standards and then identified. With the Pentel EnerGel, TLC-SERRS was able to identify Erioglaucine and Brilliant Blue G as its two blue dye components. It was immediately clear that one of the main dye components of the Pentel Ball pen was also Erioglaucine but the other blue ink line did not match any of the 10 dye standards in our spectral library. Notably, despite a superficial resemblance, this analysis showed that neither of the two pens contained crystal violet, as originally believed. Moreover, in a previous SERRS study which used conventional aqueous colloid¹¹ the Pilot G pens shown in Figure 6 were not discriminated, since the spectral differences were barely larger than the noise level and in addition there was no background information that would support attributing small differences in the spectra of inks to the presence of chemically distinct dyes. In the current study the increased signal:noise ratio provided by Poly-SERS and awareness of the potential significance of even small spectral differences within the inks has allowed us to discriminate between these inks, which we previously could not be certain were different from each other. This clearly has implications for the discriminating power which SERRS (specifically Poly-SERS) can provide for analysis of these types of samples, since even subtle spectral changes can now be attributed to chemical differences in the samples with considerable confidence. The Supporting Information shows the complete set of ink SERRS spectra at both excitation wavelengths which have been grouped so that similar spectra are displayed alongside each other to allow easy comparison.

Overall, the results from even this relatively small sample set show that the inks can be divided into three different categories. The

first category are inks whose spectra are characteristic of a single predominant dye which is unique to each sample, examples here included Pilot Acroball and Zebra Surai. The second category of inks are those prepared with mixed dyes and which are distinct from the other samples in the set because they either contain different dyes in the mixture (e.g. Pentel Ball, Pentel EnerGel) or, potentially, the same dyes but in different proportions, although we have found no examples of the latter. The final category is samples which match each other because they are based on the same predominant dye. In this study several inks were found to give spectra which were dominated by bands which matched that of Brilliant Blue G (as shown in Figure 6). In this case, there were very small spectral changes which allowed some of the inks to be discriminated by SERRS but in general TLC would be required to discriminate between samples which are dominated by the same single component. Even in this case, SERRS can add extra evidential value because SERRS of the separated TLC bands can be used to identify the individual dyes in the mixtures. This removes any possibility that samples may contain dyes which are chemically distinct but have the same R_f values because they have similar chemical structures.

Finally, although the studies shown above were carried out using a research grade Raman microscope, the high signal levels and ease of use of the Poly-SERS films means that they are equally suited to applications using lower cost, portable Raman instruments. Figure 9 compares the SERRS spectra of two inks and two dyes obtained using the Poly-SERS films and a portable Raman instrument. As was the case with the spectra obtained with the Raman microscope it is clear that the spectra of the inks match very closely to those of the individual dyes, and that the signal:noise ratio is comparable to that obtained using the Raman microscope (see Figures 4 and 5), although

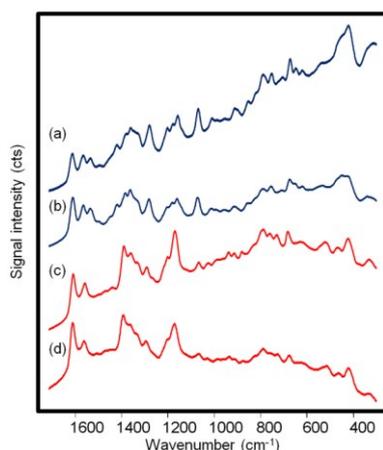


Figure 9: SERRS spectra of a (a) Pilot Acroball pen ink line (b) Victoria Pure Blue BO standard (c) Zebra Surari pen ink line and (d) Victoria Blue B base standard using 785 nm excitation and the ReporterR instrumentation.

there is a broad background signal which was not observed in the higher specification instrument.

Conclusion

This study has shown that because the chemical differences between the constituent dyes in blue gel inks are often small the differences between their SERRS spectra which need to be detected are also small, so very close matches between SERRS spectra are required for confident identification. Changes in the relative intensities of a just a few bands or shifts in one or two bands of a few cm^{-1} may be all that indicates a differently substituted dye. Fortunately, Poly-SERS substrates routinely give the levels of reproducibility and very high signal:noise ratios which are required for detecting such changes and they therefore have the potential to significantly increase the discriminating power of SERRS over previous studies. The same general approach can also be used to identify the individual dyes separated on TLC plates. This, coupled with their long shelf life and ease of use means that Poly-SERS films have the potential to make SERRS a routine technique for the forensic examination of inks.

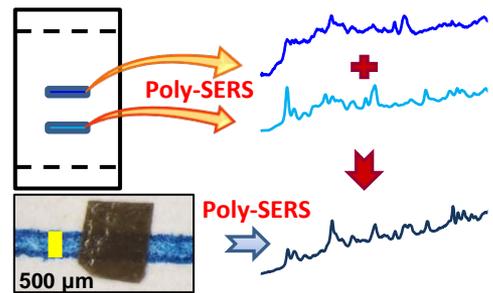
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