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Evaluation of the Use of Raman Spectroscopic Techniques in Ink Analyses

Patricia T. Elswick

Thesis submitted to the
Eberly College of Arts and Sciences
at West Virginia University
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for the degree of

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ABSTRACT

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Patricia T. Elswick

The inelastic scattering effects seen in Normal Raman (NR) spectroscopy were first observed in 1928 by Raman and Smekal. One problem encountered with Raman spectroscopy was the Raman scattering being masked by the intense fluorescence produced when the sample was exposed to the laser. One discovery in 1974 by Fleischmann *et al.* helped to maximize the Raman signal and came to be known as surface-enhanced Raman spectroscopy (SERS). In this research, spectral libraries of dyes used in pens and Raman spectra produced from the analysis of inks were evaluated in order to determine the extent to which inks can be discriminated using Raman spectra. The acid dyes were analyzed at 532 nm using NR and SERS and at 785 nm using NR. The results of the acid dye analyses indicated that there was not a single method that could generate usable spectra for all the dyes. NR at 532 nm produced the most usable spectra. The inks were analyzed both *in situ* and by extraction with methanol. They were analyzed at 532 nm using NR and SERS. The *in situ* analyses only produced usable results for 7 of the inks analyzed. The results of the ink analyses showed the potential for SERS to produce enhanced spectra over NR. Extraction SERS yielded usable spectra for 28 of the 31 inks analyzed, compared to 7 inks and 2 inks for *in situ* NR and extraction NR, respectively. The extent of the SERS enhancement varied between samples, and some of the spectra generated could not be differentiated.

TABLE OF CONTENTS

ABSTRACT	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
INTRODUCTION.....	1
METHODS	6
RESULTS	8
DISCUSSION	16
CONCLUSION	18
BIBLIOGRAPHY	19
APPENDIX A	21
APPENDIX B	23
APPENDIX C	25
APPENDIX D	27

LIST OF TABLES

Table 1 Counts of usable spectra for each acid dye color group under the three analysis scenarios

Table 2 Results of each analysis scenario for each pen brand

LIST OF FIGURES

Figure 1	Energy states of molecules in Rayleigh scattering, Stokes shift and anti-Stokes shift
Figure 2	Raman spectra of reference dye C.I. Acid Blue 113 and C.I. Acid Black 24
Figure 3	Acid Black 63 spectra using SERS at 532 nm and NR at both 532 nm and 785 nm
Figure 4	Three Acid Blue dyes analyzed at 532 nm using NR and their corresponding structures
Figure 5	Acid Yellow 19 spectra at 532 nm using NR and SERS
Figure 6	Test 18 <i>in situ</i> NR
Figure 7	Example of increased fluorescence between <i>in situ</i> NR and SERS
Figure 8	Spectra showing the difference between NR and SERS extraction
Figure 9	Four inks showing similar spectra analyzed using SERS extraction
Figure 10	Spectra of Test 19 analyzed <i>in situ</i> NR and SERS and extraction SERS
Figure 11	Spectra for Test 18 using <i>in situ</i> NR and SERS and extraction SERS
Figure 12	Chemical structures of three acid dyes and their associated spectra

INTRODUCTION

Raman spectroscopy was first observed by Raman and Smekal in 1928 (1), and it is often considered the complement of Fourier Transform Infrared (FTIR) spectroscopy. Both techniques irradiate the sample and provide a spectrum indicative of the chemical bonds that are present based on the vibrational modes of the bonds. However, the two techniques differ greatly in how the spectra are obtained. FTIR spectroscopy depends on a permanent change in the dipole moment because of the absorption of light. Raman spectroscopy involves polarization to produce scattering of the light (2).

When photons interact with the molecule they can be scattered in two ways: elastically or inelastically. Elastic scattering, also known as Rayleigh scattering, is when the photon interacts with the sample and there is no energy exchange. With inelastic scattering, or Raman scattering, the photon either loses energy or gains energy depending on the vibrational state of the molecule. A molecule at the ground vibrational level will take energy from the photon producing a Stokes shift. However, if a molecule is already in an excited vibrational state, energy can be transferred to the photon; this is known as anti-Stokes shift (1, 2). Figure 1 diagrams the energy states of the molecule in each type of scattering. E_0 represents the ground vibrational state, and E_1 represents the excited vibrational state. The dashed lines denote the virtual state of the molecule. Raman spectra show the change in the amount of energy detected in the light after interacting with the sample.

Despite its many advantages, Raman spectroscopy had one major limitation. Raman spectra could not be obtained from a number of materials because of the intense fluorescence observed in the visible region. Fluorescence occurs as a result of an electronic transition when a photon is absorbed by a molecule. The absorbed energy is then emitted as light of a longer

wavelength. The Raman spectra are usually obtained at the lower-energy side to show Stokes shift, but this is where fluorescence has the greatest interference. The fluorescent signal will be more intense than the Raman signal, in effect masking any Raman signal detected. One technique that is utilized to overcome the fluorescence is to record in the anti-Stokes region by selecting the laser wavelength. Using near infrared lasers decreases the amount of energy the molecule is exposed to and does not provide enough energy to excite the molecule to fluorescence. Fleischmann *et al.* discovered another solution to the fluorescence problem in 1974 when they observed strong Raman scattering from pyridine adsorbed onto a roughened silver electrode surface from aqueous solution (3). This discovery came to be known as surface-enhanced Raman spectroscopy (SERS).

Figure 1: Energy states of molecules in Rayleigh scattering, Stokes shift and anti-Stokes shift

SERS was first encountered in 1974 by Fleischmann *et al.* in their work to attempt to develop a probe that could be used to study electrochemical processes *in situ* (3). The mechanism

by which SERS works is still not completely understood, but electromagnetic and chemical enhancements are two proposed methods for how SERS increases the Raman scattering (4). Research done by Lombardi and Birke (5) presents a unified expression showing the two methods working together to produce the total SERS effects.

In the electromagnetic theory it is believed that the interaction of surface plasmons with the electromagnetic field of the light used during analysis causes amplification of the incident laser and the scattered Raman field. Surface plasmons are only excited by light if the surface is rough or curved. The application of the silver colloid in SERS analysis produces the necessary surface roughness for surface plasmon excitation and, in turn, enhancement of the Raman signal.

The chemical enhancement theory was suggested when molecules having nearly identical polarizabilities exhibited extreme differences in their SERS intensities. This difference was explained by charge-transfer (6) from molecule to metal or vice versa. The new electronic state that is formed acts as a resonant intermediate state in Raman scattering, increasing the sensitivity by two to four orders of magnitude (7).

The discovery of SERS introduced the possibility of the analysis of samples that once had a fluorescence that masked the Raman spectra. In forensic science, this includes ink samples encountered in the field of questioned documents.

According to the American Society for Testing and Materials, forensic document examiners make scientific examinations, comparisons and analyses of documents in order to (8):

- 1) Establish genuineness or non-genuineness, or to expose forgery, or to reveal alterations, additions or deletions,
- 2) Identify or eliminate persons as the source of handwriting,
- 3) Identify or eliminate the source of typewriting or other impressions, marks or relevant evidence and
- 4) Write reports or give testimony, when needed to aid the users of the examiner's services in understanding the examiner's findings.

As part of the duties involved in performing questioned document analyses an examiner may be required to analyze inks. The analysis of inks can indicate the presence of alterations, additions or deletions in a document and can be used to include or exclude a writing instrument as a possible ink source.

Inks are generally composed of vehicles, colorants and additives. The vehicle is the solvent in which the other components are suspended. The colorants consist of pigments, dyes or both. Additives provide the qualities desired by the manufacturer for the specific use of the ink. They can help to control the flow of ink, increase the thickness of the ink or alter the appearance and ink characteristics in other ways. The inks encountered in questioned documents usually originate from printers and pens. The focus of this study will be the ink found in pens.

There are four main classes of pens: ballpoint, felt tip, roller ball and gel. The ballpoint pen is constructed with a small steel ball encased in a housing which allows the ball to roll freely. Ink inside the reservoir of the pen adheres to the ball, and the ink is transferred from the ball to the writing surface as it rolls. The felt tip pen introduced water-soluble inks. The ink is stored in the pen reservoir and flows into the porous tip and on to the writing surface due to capillary action. The roller ball pen combines the structure of the ballpoint pen and the water-soluble ink of the felt tip pen. Gel pens are the newest class of pens developed in Japan. The use of pigments in gel pens differentiates them from most water-based inks that use dyes.

Ink can be analyzed using a number of methods including thin layer chromatography, UV/vis microspectrophotometry, mass spectrometry and Raman spectroscopy. Thin layer chromatography crudely separates the components of the ink allowing for preliminary comparisons. UV/vis microspectrophotometry utilizes the fact that inks of similar color can sometimes exhibit different luminescent properties at different wavelengths due to their chemical

components. This allows an examiner to visualize alterations on questioned documents. Mass spectrometry coupled with various techniques (9-12) has been used to characterize inks as well as study the degradation of the chemical components for age determination. Raman spectroscopy is also used to determine the chemical composition of the inks.

Studies using Raman spectroscopy on inks have yielded promising results. And the introduction of SERS improved the capabilities of the technique. A 2002 study on discriminating between inks (13) looked at 16 blue ballpoint pen inks which were arranged in 120 ballpoint pen pairs. Using normal Raman spectroscopy, 106 pairs were able to be differentiated. A similar study determined the discriminating power of the Raman technique to be 0.68 for thirty-six blue gel pen inks where the discriminating power was calculated by dividing the number of discriminated pairs by the total number of possible pairs (14). Geiman et al. conducted research (15) indicating that SERS analysis consistently provided increased signal strength and quenched fluorescence when compared to normal Raman spectroscopy in the analysis of pen ink.

To date, the research conducted on Raman spectroscopy has involved limited sample sizes (4, 12-15) and has typically used extraction methods for analysis. In forensic science, a non-destructive method would be preferred in order to preserve the integrity of the evidence. While SERS has been shown to have significant advantages over normal Raman spectroscopy in the analysis of inks, a study comparing *in situ* and extraction SERS analysis had not been carried out.

The first goal of this study was to build a spectral library of seventy-five acid dyes. The normal Raman spectra obtained for the acid dyes were evaluated for inter- and intra- spectral variations. There have been instances (Figure 2) in which normal Raman spectroscopy was unable to differentiate between two dyes.

The second aim of the study was to evaluate normal Raman spectroscopy and SERS, both in situ and by extraction. The efficiency of each technique was assessed in regards to the analysis of inks for use in the field of questioned documents. Efficiency was based on four factors: 1) ability to provide informative spectra 2) discriminating power 3) difficulty and 4) rapidity.

Figure 2: Raman spectra of the reference dye C.I. Acid Blue 113 (C.I. 26360) and C.I. Acid Black 24 (C.I. 42655) ($\lambda_{exc}=785$ nm). Courtesy of P. Buzzini

METHODS

Seventy-five powdered acid dyes from the ORCO Organic Dyestuffs Corporation (Concord, NC) were analyzed using normal Raman spectroscopy and SERS for the development of an acid dyes database (Appendix A). Preparation of the dyes and normal Raman spectroscopy were done as proposed by Buzzini (16). In order to avoid the problem of flooding the detector with signal, preparation of the acid dyes required the dilution of the powders with distilled water.

The diluted acid dye samples were pipetted onto welled, glass slides covered with a small piece of aluminum foil to reduce interference from the glass. The excess distilled water was evaporated off using a hot plate. The samples were analyzed using normal Raman spectroscopy at 532nm and 785nm. The silver colloids used for the SERS techniques were prepared according to the method described by Lee and Meisel (17) with modifications by Leona (18). One hundred milligrams of silver nitrate were dissolved in 5 mL of cold distilled water in a 50 mL conical vial. 10% sulfuric acid was added dropwise to precipitate the silver sulfate. The precipitate was washed twice with distilled water and then dissolved in 580 mL of distilled water. Twenty-five milliliters of the silver sulfate solution were placed in a 50 mL conical vial with 2 mL of a 1% w solution of glucose and 1 mL of a 1% w solution of sodium citrate. The solution was vortexed and then heated for 30 seconds in a conventional microwave. The solution was heated additionally in 15 second increments as needed to achieve a UV-Vis spectrum with maximum absorption around 400 nm. The solution was kept refrigerated and dark until use. For use, 1 mL of stock solution was centrifuged for 5 minutes at 16,060 x g relative centrifugal force. Nine hundred fifty microliters of the supernatant were removed and replaced with distilled water. The silver colloids were added directly to the samples after normal Raman analysis was complete. SERS analysis was performed only at 532nm.

Thirty-one roller ball and ballpoint pens were collected for analysis (Appendix B). A pen mark from each pen was drawn onto a piece of printing paper. Each pen mark was analyzed at 532nm both *in situ* and through extraction using normal Raman and SERS. Extraction was carried out by the method used by Munro et al. (19). A small area of ink was scratched from the paper and placed into a vial containing methanol. The solution was vortexed and pipetted onto a welled, glass slide covered with a small piece of aluminum foil. The methanol was allowed to

evaporate prior to analysis. The silver colloids were added directly to the samples after normal Raman analysis was complete.

Each analysis was performed three times, and an average spectrum was generated from these analyses. Acetaminophen was used as a positive control prior to each batch of analyses. Blanks of the paper, the methanol and the silver colloids were also analyzed as negative controls.

The Raman spectrometer used was a Bruker (Bruker Optik GmbH, Ettlingen, Germany), model Senterra, coupled to a Bruker BX51M microscope equipped with two lasers at 532 nm (~20 mW power) and 785 nm (~100 mW power) as excitation sources. A CCD camera was used as a detector.

Three objective lenses were available for the visualization of samples: 20x (about 5 microns spot size), 50x (about 2 microns spot size) and 100x (about 1-3 micron spot size). The display and management of data was carried out with OPUS Spectroscopy Software, v. 6.0, (Bruker Optik GmbH, Ettlingen, Germany).

RESULTS

Table 1 shows the overall results of the acid dye analyses. The table indicates the total number of acid dyes in each dye color group and the number of usable spectra generated in each

Nom C.I.	# of Acid Dyes	NR@532nm	SERS@532nm	NR@785 nm
Acid Black	7	3	3	6
Acid Blue	16	15	7	2
Acid Brown	3	3	3	0
Acid Green	4	3	2	0
Acid Orange	5	4	4	0
Acid Red	22	8	5	0
Acid Violet	7	6	4	0
Acid Yellow	11	2	4	1
Total	75	44	32	9

Table 1: Counts of usable spectra for each acid dye color group under the three analysis scenarios

of the three analysis scenarios. Analysis at 785 nm with NR showed the best results for the acid black dyes. Figure 3 shows the spectra of Acid Black 63. Considerable fluorescence was observed at 785 nm in the acid blue dyes, and little to no signal was generated in the acid reds and yellows. Normal Raman at 532 nm generated good spectra in the acid blues. Figure 4

Figure 3: Acid Black 63 spectra using SERS at 532 nm and NR at both 532 nm and 785 nm

shows three Acid Blue dyes analyzed at 532 nm using NR. The chemical structures (20, 21) for the each acid dye are also seen in Figure 4 along with peak assignment for some of the chemical bonds. The addition of the silver colloids at 532 nm showed signal enhancement and decreased fluorescence in some acid dyes compared to NR at 532 nm. Figure 5 shows the fluorescence of Acid Yellow 19 using NR at 532 nm and the distinct spectra generated using SERS at 532 nm.

However, there were some instances, especially in the Acid Reds and Yellows, where SERS analysis increased the baseline or the fluorescence of the spectra.

Figure 4: Three Acid Blue dyes analyzed at 532 nm using NR and their corresponding structures

Figure 5: Acid Yellow 19 spectra at 532 nm using NR and SERS

Appendix C lists the seventy-five acid dyes and whether usable spectra were generated for each acid dye in each analysis scenario. Twenty-one of the acid dyes did not generate usable spectra using SERS or NR at both wavelengths. Thirteen of these were Acid Red dyes.

The count of usable spectra for each analysis method for the inks is shown in Table 2.

Appendix D shows which scenarios gave usable spectra for each ink. Some peaks were visible

Pen Brand	# of pens	<i>in situ</i> NR	<i>in situ</i> SERS	Extraction NR	Extraction SERS			
Papermate	7				7			
Uni-ball	8				2	2	6	
Pilot	4							3
Avery	1							
Zebra	5	4	4	1	5			
Parker	2				2			
BIC	4				1		1	4
Total	31	7	6	2	28			

Table 2: Results of each analysis scenario for each pen brand

through the fluorescence seen in the *in situ* NR analyses. Figure 6 shows an example of a spectrum generated *in situ* using NR. The results of the *in situ* SERS analyses did not show significant enhancement over the *in situ* NR analyses. The *in situ* SERS generated one less usable spectrum than the *in situ* NR. The fluorescence also increased with *in situ* SERS in some cases (Figure 7). Extraction with NR only produced two usable spectra. The most usable spectra were generated using extraction SERS. Thirteen inks that did not generate usable spectra using the other three methods produced usable spectra using the extraction SERS method. The three inks that did not generate usable spectra using extraction SERS were all from roller ball pens, and these inks did not produce usable spectra using any of the analysis methods.

Figure 6: Test 18 *in situ* NR

Figure 7: Example of increased fluorescence between *in situ* NR and SERS

Figure 8 shows an example of the difference in the spectra generated using NR and SERS after extraction. A number of the inks exhibited similar spectra when analyzed by extraction SERS (Figure 9). This same spectrum was also generated in some of the inks when analyzed *in situ*. Figure 10 shows the spectra for Test 19 analyzed *in situ* NR and SERS and extraction SERS. Similar peaks can be seen in all three spectra. Tests 15, 16 and 18 generated this common spectrum under extraction SERS, however, this spectrum was different from the spectra generated using *in situ* NR and SERS for these three inks. The spectra for Test 18 using *in situ* NR and SERS and extraction SERS are seen in Figure 11.

Figure 8: Spectra showing the difference between NR and SERS extraction

Figure 9: Four inks showing similar spectra analyzed using SERS extraction

Figure 10: Spectra of Test 19 analyzed *in situ* NR and SERS and extraction SERS

Figure 11: Spectra for Test 18 using *in situ* NR and SERS and extraction SERS

DISCUSSION

The initial goal of this study, to build a spectral library, produced some challenges. The complexity of the chemical structures of the acid dyes is demonstrated in Figure 12 (20, 21). The inability of a single method to produce usable spectra for all the acid dyes may be explained by the variability in the chemical structures. The varying chemical structures make the availability of lasers with different wavelengths very important. Analysis using all three methods may be useful in order to determine which method works best for each acid dye. A specific dilution for the dyes would control the amount of dye in the samples and potentially decrease the amount of fluorescence with and without the SERS chemicals. The SERS effects might also be enhanced by concentrating the colloids and using an aggregating agent to facilitate the binding of the colloids to the dye compounds.

Acid Black 52

Acid Orange 156

Acid Blue 1

Acid Red 111

Acid Red 50

Figure 12: Chemical structure of five acid dyes

Ink analysis using NR and SERS *in situ* and by extraction was the second focus of this study. The inks that did not generate usable spectra or that gave varying spectra depending on the analysis method were all from roller ball pens. The components of roller ball pen ink may require a different method for analysis to obtain more consistent results. Ink analysis may also benefit from the concentration of the colloids and the use of an aggregating agent. Adding these two steps may increase the effectiveness of the method *in situ*. This would help to avoid destruction of the sample. Analysis of the inks first by TLC and then analysis of each spot using SERS may help to determine which dye spot is being seen in the spectra using the extraction SERS method.

The common spectrum seen in the extraction SERS analysis of the inks can be explained for some of the inks due to the pens being the same brand. However, another situation arose where three inks, all from roller ball pens, generated different spectra depending on if the ink

was analyzed *in situ* or by extraction. When the three inks were analyzed using extraction, the spectra produced were the common spectrum. This indicates that a particular component of the inks is being extracted and enhanced by the SERS chemicals.

The inter- and intra-variability of the dye and ink samples become key in determining whether analysis using NR or SERS is useful. The intra-variability needs to be small enough to have reproducible spectra, while the inter-variability needs to be large enough to allow for differentiation between spectra from different samples. Further research into the statistical analysis of both inter- and intra-variability of the spectra will be useful in determining the discriminating power of the method (22).

CONCLUSION

The similarity of some of the spectra produced using extraction SERS makes differentiation extremely subjective. The dilemma comes in determining how different the spectra have to be to be considered different inks, and vice versa, how similar the spectra have to be to be considered the same ink. The results showed the definite potential for SERS to produce enhanced spectra over NR, but the inability to differentiate between the spectra produced makes the enhancement of little value. Further research needs to be done into the chemical structures of the acid dyes and the components that make up the ballpoint and roller ball inks. The chemical structures should also be looked at in conjunction with the peaks of the spectra to determine which bonds are represented. Concentration of the silver colloids and the use of an aggregating agent should also be investigated as potential ways to increase the effects of the SERS methods. With continued research, SERS may become a useful method of analysis for inks within the field of questioned documents.

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APPENDIX A

Nom C.I.	Commercial Name
Acid Black 107	ORCOLAN Neutral Black BGL 200%
Acid Black 164	ORCOLAN Neutral Black 3SBR
Acid Black 172	ORCOLAN Neutral Black DW
Acid Black 2	ORCOACID Nigrosine Jet L
Acid Black 52	ORCOLAN Fast Black WAN EX
Acid Black 60	ORCOLAN Neutral Grey B
Acid Black 63	ORCOLAN Fast Black BN Ex Conc
Acid Blue 1	ORCOACID Blue V Ex Conc
Acid Blue 127	ORCOLAN Neutral Blue GL
Acid Blue 158	ORCOLAN Fast Blue GGN 200%
Acid Blue 182	ORCOACID Alizarine Light Blue HRL
Acid Blue 193	ORCOLAN Neutral Dark Blue BR
Acid Blue 25	ORCOACID Alizarine Fast Blue SWA
Acid Blue 260	ORCO NYLOSOL Brilliant Blue RL 200%
Acid Blue 281	ORCO NYLOSOL Blue GLF
Acid Blue 324	ORCO NYLOSOL Blue BRL 200%
Acid Blue 40	ORCOACID Sapphire 2GL 200%
Acid Blue 41	ORCOACID Blue ARA
Acid Blue 45	ORCOACID Blue SAP
Acid Blue 62	ORCOACID Brilliant Alizarine Blue R 150%
Acid Blue 7	ORCOACID Blue A 150%
Acid Blue 83	ORCO MILLING Brilliant Cyanine 6B
Acid Blue 90	ORCO MILLING Brilliant Cyanine G
Acid Brown 19	ORCO NYLOSOL Brown
Acid Brown 282	ORCOLAN Neutral Brown GRS
Acid Brown 289	ORCOLAN Neutral Brown BRL
Acid Green 16	ORCOACID Napthalamine Green 2G 300%
Acid Green 20	ORCOACID Dark Green A Conc
Acid Green 28	ORCO NYLOSOL Green C3G
Acid Green 9	ORCOACID Fast Green B
Acid Orange 116	ORCO NYLOSOL Orange SLF 200%
Acid Orange 156	ORCO NYLOSOL Orange 3G Conc
Acid Orange 60	ORCOLAN Neutral Orange NR
Acid Orange 74	ORCOLAN Fast Orange GEN
Acid Orange 86	ORCOLAN Neutral Orange RL 250%
Acid Red 1	ORCOACID Phloxine GR 175%
Acid Red 111	ORCO NYLOSOL Scarlet GN
Acid Red 114	ORCO MILLING Red RS 125%
Acid Red 119	ORCO MILLING Bordeaux LFS
Acid Red 131	ORCO MILLING Brilliant Red 3NR
Acid Red 138	ORCOACID Red C-2B
Acid Red 14	ORCOACID Azo Rubine CWH
Acid Red 151	ORCO NYLOSOL Scarlet CL

Acid Red 18	ORCOACID Brilliant Scarlet 3R-L
Acid Red 182	ORCOLAN Neutral Red B
Acid Red 186	ORCOLAN Fast Pink BN Conc
Acid Red 194	ORCOLAN Fast Bordeaux RL
Acid Red 249	ORCO MILLING Brilliant Red B
Acid Red 266	ORCO NYLOSOL Red 2GP (N)
Acid Red 299	ORCO NYLOSOL Rubine 5BLF (N)
Acid Red 337	ORCO NYLOSOL Red GN 200%
Acid Red 4	ORCOACID Eosine G
Acid Red 414	ORCOLAN Neutral Red RLS
Acid Red 50	ORCOACID Sulpho Rhodamine G
Acid Red 57	ORCOACID Rubine 3GP
Acid Red 73	ORCOACID Scarlet MR
Acid Red 88	ORCOACID Fast Red A Conc
Acid Violet 1	ORCOACID Violet 2R Conc
Acid Violet 12	ORCOACID Carmine 4B Conc
Acid Violet 17	ORCOACID Violet 4B
Acid Violet 43	ORCOACID Violet WN
Acid Violet 48	ORCO MILLING Brilliant Violet BL 200%
Acid Violet 7	ORCOACID Fuchsine 6B Ex Conc
Acid Violet 90	ORCOLAN Neutral Bordeaux BSB
Acid Yellow 127	ORCO NYLOSOL Brilliant Yellow 5G
Acid Yellow 129	ORCOLAN Neutral Yellow 2GL Ex
Acid Yellow 151	ORCOLAN Neutral Yellow WN 250%
Acid Yellow 17	ORCOACID Fast Light Yellow 2G
Acid Yellow 184	ORCOACID Brilliant Flavine 8GN
Acid Yellow 19	ORCOACID Fast Light Yellow R
Acid Yellow 219	ORCO NYLOSOL Yellow 4GR 250% (N)
Acid Yellow 250	ORCO NYLOSOL Yellow Brillant FSC
Acid Yellow 38	ORCO MILLING Yellow O
Acid Yellow 49	ORCO NYLOSOL Yellow 9GL 200%
Acid Yellow 99	ORCOACID Fast Yellow GRN

APPENDIX B

Pen Number	Pen Brand	Pen Type	Ink Color
1	Papermate Comfortmate	Ballpoint	Black
2	Papermate Ball Point	Ballpoint	Black
3	Papermate PhD	Ballpoint	Black
4	Papermate PhD Multi	Ballpoint	Black
5	Uni-ball Vision Micro	Roller ball	Black
6	Uni-ball Vision Fine	Roller ball	Black
7	Uni Power Take 1.0	Ballpoint	Black
8	Pilot Precise Rolling Ball V5 Extra-Fine	Roller ball	Black
9	Pilot Dr. Grip	Ballpoint	Black
10	Pilot Dr. Grip	Ballpoint	Black
11	AVERY Triple Click	Ballpoint	Black
12	Zebra F-301	Ballpoint	Black
13	Parker	Ballpoint	Black
14	Papermate Comfortmate	Ballpoint	Blue
15	Uni-ball Vision Micro	Roller ball	Blue
16	Uni-ball Vision Fine	Roller ball	Blue
17	Uni Power Tank 1.0	Ballpoint	Blue
18	Zebra GR8 Roller 0.7	Roller ball	Blue
19	Zebra F-301	Ballpoint	Blue
20	Parker	Ballpoint	Blue
21	Papermate Comfortmate	Ballpoint	Red
22	Papermate Comfortmate	Ballpoint	Red
23	Uni-ball Vision Fine	Roller ball	Red
24	Pilot Precise Rolling Ball V5 Extra Fine	Roller ball	Red
25	Uni-ball Vision Fine	Roller ball	Purple
26	BIC Multi-Color	Ballpoint	Black
27	BIC Multi-Color	Ballpoint	Green
28	BIC Multi-Color	Ballpoint	Blue
29	BIC Multi-Color	Ballpoint	Red

30	Zebra Clip-On 2-Color	Ballpoint	Black
31	Zebra Clip-On 2-Color	Ballpoint	Red

APPENDIX C

Nom C.I.	NR @ 532nm	SERS @ 532nm	NR @785 nm
Acid Black 107	N	N	Y
Acid Black 164	N	N	Y
Acid Black 172	Y	Y	Y
Acid Black 2	Y	Y	N
Acid Black 52	Y	Y	Y
Acid Black 60	N	N	Y
Acid Black 63	N	N	Y
Acid Blue 1	Y	N	N
Acid Blue 127	Y	N	N
Acid Blue 158	Y	Y	Y
Acid Blue 182	Y	N	N
Acid Blue 193	Y	Y	Y
Acid Blue 25	Y	N	N
Acid Blue 260	Y	Y	N
Acid Blue 281	Y	N	N
Acid Blue 324	N	N	N
Acid Blue 40	Y	Y	N
Acid Blue 41	Y	Y	N
Acid Blue 45	Y	N	N
Acid Blue 62	Y	N	N
Acid Blue 7	Y	N	N
Acid Blue 83	Y	Y	N
Acid Blue 90	Y	Y	N
Acid Brown 19	Y	Y	N
Acid Brown 282	Y	Y	N
Acid Brown 289	y	Y	N
Acid Green 16	Y	Y	N
Acid Green 20	Y	N	N
Acid Green 28	Y	N	N
Acid Green 9	N	Y	N
Acid Orange 116	Y	Y	N
Acid Orange 156	N	N	N
Acid Orange 60	Y	Y	N
Acid Orange 74	Y	Y	N
Acid Orange 86	Y	Y	N
Acid Red 1	Y	N	N
Acid Red 111	N	N	N
Acid Red 114	N	N	N

Acid Red 119	N	N	N
Acid Red 131	N	N	N
Acid Red 138	N	N	N
Acid Red 14	N	N	N
Acid Red 151	N	Y	N
Acid Red 18	N	N	N
Acid Red 182	Y	Y	N
Acid Red 186	Y	Y	N
Acid Red 194	Y	Y	N
Acid Red 249	N	N	N
Acid Red 266	Y	N	N
Acid Red 299	N	N	N
Acid Red 337	Y	N	N
Acid Red 4	N	N	N
Acid Red 414	Y	Y	N
Acid Red 50	N	N	N
Acid Red 57	Y	N	N
Acid Red 73	N	N	N
Acid Red 88	N	N	N
Acid Violet 1	Y	Y	N
Acid Violet 12	Y	N	N
Acid Violet 17	Y	Y	N
Acid Violet 43	Y	Y	N
Acid Violet 48	N	N	N
Acid Violet 7	Y	N	N
Acid Violet 90	Y	Y	N
Acid Yellow 127	N	N	N
Acid Yellow 129	N	N	N
Acid Yellow 151	Y	N	N
Acid Yellow 17	N	N	N
Acid Yellow 184	N	N	N
Acid Yellow 19	N	Y	N
Acid Yellow 219	N	Y	N
Acid Yellow 250	N	Y	N
Acid Yellow 38	N	Y	Y
Acid Yellow 49	N	N	N
Acid Yellow 99	Y	N	N

APPENDIX D

Pen Number	Pen Brand	Pen Type	Ink Color	<i>in situ</i> NR	<i>in situ</i> SERS	Extraction NR	Extraction SERS
1	Papermate Comfortmate	Ballpoint	Black	N	N	N	Y
2	Papermate Ball Point	Ballpoint	Black	N	N	N	Y
3	Papermate PhD	Ballpoint	Black	N	N	N	Y
4	Papermate PhD Multi	Ballpoint	Black	N	N	N	Y
5	Uni-ball Vision Micro	Roller ball	Black	N	N	N	Y
6	Uni-ball Vision Fine	Roller ball	Black	N	N	N	N
7	Uni Power Take 1.0	Ballpoint	Black	N	N	N	Y
8	Pilot Precise Rolling Ball V5 Extra-Fine	Roller ball	Black	N	N	N	N
9	Pilot Dr. Grip	Ballpoint	Black	N	N	N	Y
10	Pilot Dr. Grip	Ballpoint	Black	N	N	N	Y
11	AVERY Triple Click	Ballpoint	Black	N	N	N	Y
12	Zebra F-301	Ballpoint	Black	Y	Y	N	Y
13	Parker	Ballpoint	Black	N	N	N	Y
14	Papermate Comfortmate	Ballpoint	Blue	N	N	N	Y
15	Uni-ball Vision Micro	Roller ball	Blue	Y	Y	N	Y
16	Uni-ball Vision Fine	Roller ball	Blue	Y	Y	N	Y
17	Uni Power Tank 1.0	Ballpoint	Blue	N	N	N	Y
18	Zebra GR8 Roller 0.7	Roller ball	Blue	Y	Y	N	Y
19	Zebra F-301	Ballpoint	Blue	Y	Y	N	Y
20	Parker	Ballpoint	Blue	N	N	N	Y
21	Papermate Comfortmate	Ballpoint	Red	N	N	N	Y

22	Papermate Comfortmate	Ballpoint	Red	N	N	N	Y
23	Uni-ball Vision Fine	Roller ball	Red	N	N	N	N
24	Pilot Precise Rolling Ball V5 Extra Fine	Roller ball	Red	N	N	N	Y
25	Uni-ball Vision Fine	Roller ball	Purple	N	N	N	Y
26	BIC Multi-Color	Ballpoint	Black	N	N	N	Y
27	BIC Multi-Color	Ballpoint	Green	N	N	N	Y
28	BIC Multi-Color	Ballpoint	Blue	Y	N	Y	Y
29	BIC Multi-Color	Ballpoint	Red	N	N	N	Y
30	Zebra Clip-On 2-Color	Ballpoint	Black	Y	Y	Y	Y
31	Zebra Clip-On 2-Color	Ballpoint	Red	N	N	N	Y
Total usable spectra				7	6	2	29