Report Title:
Development of Advanced Raman Spectroscopy Methods and Databases For The Evaluation of Trace Evidence and The Examination of Questioned Documents *(Phase I)*

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Abstract:

Researchers from the City University of New York, Fitzpatrick Institute of Photonics at Duke University, the New York Police Department Crime Lab, and The Metropolitan Museum of Art formed a multidisciplinary collaborative to further the application of Raman spectroscopy and non-destructive surface enhanced Raman scattering (SERS) analysis techniques as applied to the evaluation of trace evidence and the examination of questioned documents. Through the sharing of instrumentation (e.g. the only multiwavelength open architecture Raman/FT-Raman microscope currently available), reference materials (e.g. a collection of natural and synthetic pigments and inks dating to the first half of the century), and their respective research expertise the team developed a much needed reference database of spectra for forensic applications and cross-validated newly developed techniques for non-destructive SERS analysis.

Raman spectroscopy is an established and increasingly utilized technique for the rapid and non-destructive analysis of paints, inks, fibers, mineral residues, pharmaceuticals, and controlled substances. The technique is applicable to a variety of substances, but analytes of great forensic interest such as some natural and synthetic dyes found in textiles, inks, and paints display an excessive fluorescent background, which limits Raman efficiency in most situations, leading to poor analytical results.

The team's goal is to further explore the application of Raman spectroscopy to the evaluation of trace evidence and the examination of questioned documents, with the aim of solving the problems that have so far limited the applicability of this technique to the identification of organic colorants and other materials in trace amounts. This project is divided into two phases. Phase I methods and results are covered in this report. Based on the success of Phase, funding for Phase II is currently being requested.

The work in Phase I of the project successfully demonstrated that surface enhanced Raman scattering (SERS) can be applied to the identification of organic colorants present in inks, paints, and textile fibers. The techniques we developed

are especially suited for handling microscopic samples: textile dyes were successfully identified from samples as small as a one-millimeter section of a single silk fibril of fifty-micrometer diameter and even from textiles severely degraded by burial.

The research conducted in Phase I also demonstrated that SERS is a valuable technique for the identification of organic colorants used in inks and for the dyeing of textile fibers, as well as for trace analysis of pharmaceuticals and of drugs of abuse. Analytical procedures for SERS of a number of representative dyes were developed, the core of a high quality spectral database was assembled as a proof of concept experiment, and innovative non-destructive approaches were investigated. To date our colorants database includes approximately 50 natural and synthetic dyes, and it is, to our knowledge, the first SERS spectral database ever assembled (in the course of the work, a normal Raman database of approximately 100 spectra of organic colorants was also assembled). The database is both chemically inclusive (as dyes belonging to the principal classes are represented) and spectrally comprehensive, since both SERS spectra obtained at different wavelengths (488, 633, 785 nm) and normal dispersive Raman (488, 633, 785 nm), and FT-Raman (1064 nm) are included. Scientific articles describing this first phase of the work were accepted for publication in the Journal of Forensic Sciences.

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Executive Summary

Raman spectroscopy is a well-established technique in the forensic laboratory. It is an ideal tool for its ability to rapidly identify organic and inorganic compounds in small samples without any need for cumbersome preparations. Conventional Raman spectroscopy is however plagued by poor sensitivity and interference from background luminescence and it is not easily applied to the analysis of some pharmaceuticals and of most organic colorants.

Raman spectroscopy provides a technique for the rapid and non-destructive analysis of paints, inks, fibers, mineral residues, pharmaceuticals, and controlled substances. While advances in commercial instrumentation have rendered the technique routinely accessible and reliable to use, development of reference databases has not kept pace with the increased use of the technique. There is a special need for high quality, and comprehensive databases of pigments, dyes, and inks, which are not available commercially. Moreover, while the technique is applicable to a variety of substances, some analytes of forensic interest, such as some natural and synthetic dyes found in textiles, inks, and paints produce a large fluorescent background obscuring the Raman signal, often leading to poor analytical results. We have investigated the application of a new ultra-sensitive technique, surface enhanced Raman scattering (SERS), to the evaluation of trace evidence and questioned documents.

Raman spectroscopy is characterized by very high spectral resolution, leading to effective discrimination among various species, and it has the added advantage of allowing non-destructive, *in-situ* detection. It is also preferable to infrared (IR) spectroscopy in that Raman spectra may readily be obtained in aqueous solution, while the large IR absorption of water precludes use of IR for many practical applications. However, the low intensity of normal Raman scattering has prevented its wide application as a sensitive spectroscopic probe. Furthermore, interference from fluorescence often obscures the much weaker Raman signal. Surface enhanced Raman scattering (SERS) has been found useful to overcome these restrictions. The SERS effect is characterized by an enormous

increase in the Raman intensity ($>10^8$) for species adsorbed on rough metal surfaces compared to that obtained from the same number of molecules in solution or the gas phase. At the same time, proximity to the surface provides a non-radiative pathway for relaxation from the excited state, which successfully quenches fluorescence.

Organic molecules that perform most strongly under SERS conditions are generally characterized by extensive aromatic systems carrying nitrogen and oxygen substituents with lone electron pairs. This is often the case for several molecules of forensic interest such as alkaloids and basic dyes (textile dyes and inks). It is now generally accepted that there are two major contributions to SERS. One is due to enrichment of the electric field (EM enhancement) caused by surface plasmon resonances, while the second involves charge transfer.

Both SERS theories predict that the SERS enhancement requires nanoscale surface roughness. This may be achieved by several techniques, including mechanical roughening, chemical etching, vapour deposition of silver islands, electrochemical roughening and use of metallic colloids in aqueous solution. The first three techniques are especially useful for the construction of portable probes for utilization in fieldwork. Colloids, on the other hand, are quite easy to make, and provide a large surface area, for convenient Raman studies in the laboratory. Under certain circumstances they may also be useful for portable probes or *in-situ* studies. In addition the effects of pH and electrolyte can be precisely measured.

The potential of SERS in forensic science and in particular in the trace evidence, controlled substances, and questioned documents fields has been recently pointed out in a review of analytical tools for forensic science. Research so far conducted mostly in Europe has shown that SERS can be effectively applied to problems such as the discrimination of jet printer inks *in situ*, the fast identification of synthetic dyes on fiber samples, and the sensitive determination of narcotics. Recent work conducted at the Department of Scientific Research of the Metropolitan Museum of Art, in collaboration with CCNY on the somewhat

germane topic of dye and ink identification on ancient and modern works of art shows great promise for forensic applications.

We conducted an in depth experimental review of the applicability of an innovative Raman technique, surface enhanced Raman scattering (SERS) to the rapid and ultrasensitive detection of analytes of forensic interest in trace evidence. Focusing on organic colorants typically found in inks or dyed textile fibers and on a selected group of controlled pharmaceutical substances, we demonstrated that surface enhanced Raman scattering can be reliably used to identify these analytes in samples of microscopic dimensions.

As SERS is a relatively new technique, analytical protocols and reference databases are not easily available. In order to foster its application as an investigative tool we developed specific sample handling and treatment protocols, and we assembled a demonstrative SERS spectral database of organic colorants. The reference database includes approximately 50 natural and synthetic dyes, and it is, to our knowledge, the first SERS spectral database ever created (in the course of the work, a normal Raman database of approximately 100 spectra of organic colorants was also assembled). The SERS database is both chemically inclusive (as dyes belonging to the principal classes are represented) and spectrally comprehensive, since both SERS spectra obtained at different wavelengths (488, 633, 785 nm) and normal dispersive Raman (488, 633, 785 nm), and FT-Raman (1064 nm) are included.

To enhance the reproducibility of SERS analysis, in addition to using solution reduced silver colloids, we will continue to investigate methods developed in phase I to produce nanostructures to permit enhanced field detection of model compounds relevant to trace evidence analysis, questioned document examination and controlled substances identification. The methods investigated in this task will produce plasmonic materials that can provide suitable Raman enhancement for ultra-sensitive detection (part-per billion and sub-part-per billion), and also be readily produced on an array platform. Several different methods will be investigated and evaluated: (i) deposition of metal-island films on smooth surfaces, (ii) photoreduction of silver solutions to produce nanoparticles

with controlled geometries. The objective of both approaches is the production of metallic nanostructures with protrusions of specific nanoscale size and shape for optimal plasmon enhancement.

Using the methods developed in the course of this project we obtained three significant proof-of-concept results:

i) We were able to demonstrate that spectra of organic dyes can be obtained from microscopic samples, by identifying the natural dye alizarin in a single wool fiber measuring 50 micrometer by 2 millimeter;

ii) We were able to demonstrate that the technique is not affected by chemical contamination or colorant degradation due to burial, by identifying the colorant present in a textile that had been buried for the past 1,000 years;

iii) We were able to obtain high quality spectra of the main natural and synthetic opiates, notoriously poor Raman scatterers.

Furthermore, we demonstrated that SERS is a technique is of easy applicability and does not require specialized instrumentation over what a normal Raman laboratory may possess. We successfully reproduced the results obtained using three different experimental setups (a research grade optical bench and two compact commercial systems), and we feel that the great sensitivity of SERS would even allow the use of economic portable devices. Sample handling and preparation is considerably less complex than for chromatographic techniques, and the procedures developed can be quickly taught to laboratory personnel.

Finally, the project had a significant educational and dissemination component. Two Master Theses were completed as a result of our collaboration with faculty of John Jay College of Criminal Justice; two articles were accepted for publication in the Journal of Forensic Science; and several presentations at conferences were made. A workshop on SERS was also held at the 2008 conference of the International Association of Forensic Science in New Orleans. We are currently in the process of preparing a searchable digital version of our SERS spectral library for free distribution.

I. Introduction

1. Statement of the Problem

1.1 Surface Enhanced RAMAN Spectroscopy (SERS)

Raman spectroscopy provides a technique for the rapid and non-destructive analysis of paints, inks, fibers, mineral residues, pharmaceuticals, and controlled substances[1]. While the technique is applicable to a variety of substances, some analytes of forensic interest, such as some natural and synthetic dyes found in textiles, inks, and paints produce a large fluorescent background obscuring the Raman signal, often leading to poor analytical results. We propose here to investigate the application of a new ultra-sensitive technique, surface enhanced Raman scattering (SERS), to the evaluation of trace evidence and questioned documents, and to develop high quality, and comprehensive databases of pigments, dyes, and inks, which are not available commercially.

Raman spectroscopy is characterized by very high spectral resolution, leading to effective discrimination among various species, and it has the added advantage of allowing non-destructive, *in-situ* detection. It is also preferable to infrared (IR) spectroscopy in that Raman spectra may readily be obtained in aqueous solution, while the large IR absorption of water precludes use of IR for many practical applications. However, the low intensity of normal Raman scattering has prevented its wide application as a sensitive spectroscopic probe. Furthermore, interference from fluorescence often obscures the much weaker Raman signal. Surface enhanced Raman scattering (SERS) has been found useful to overcome these restrictions. The SERS effect is characterized by an enormous increase in the Raman intensity by many orders of magnitude for species adsorbed on rough metal (usually silver) surfaces compared to that obtained from the same number of molecules in solution or the gas phase[2,3,4,5]. At the same time, proximity to the surface provides a non-radiative pathway for relaxation from the excited state, which successfully quenches fluorescence.

Organic molecules that perform most strongly under SERS conditions are generally characterized by extensive aromatic systems carrying nitrogen and

oxygen substituents with lone electron pairs[6]. This is often the case for several molecules of forensic interest such as alkaloids (drugs of abuse) and dyes (textile dyes and inks). Although the enhancement process is not yet understood in full detail, it is now generally accepted that there are two major contributions to SERS. One is due to enrichment of the electric field (EM enhancement) caused by surface plasmon resonances induced by the laser light in nano-sized metal clusters on the surface[7]. The second factor is associated with chemisorption, and involves either molecule-metal or metal-molecule charge transfer[8].

Both electromagnetic and chemical SERS theories predict that the SERS enhancement requires nanoscale surface roughness. This may be achieved by several techniques, including mechanical roughening, chemical etching, vapor-deposition of silver islands, electrochemical roughening and use of metallic colloids in aqueous solution. The first three techniques are especially useful for the construction of portable probes for utilization in fieldwork. Colloids, on the other hand, are quite easy to make, and provide a large surface area, for convenient Raman studies in the laboratory. Under certain circumstances they may also be useful for portable probes or *in-situ* studies. Electrochemical studies in SERS are more difficult for practical applications due to the bulk and electronic equipment needed, but since the electrochemical potential can be controlled they are quite useful for basic scientific studies, such as determining the effect of oxidation state on the Raman spectrum. In addition the effects of pH and electrolyte can be precisely measured. Electrochemical studies are especially sensitive to the charge-transfer contributions to SERS, and since this is associated with chemisorbed molecules, they can elucidate the mode of adsorption on the surface as well as the molecular orientation with respect to the surface plane.

1.2 SERS and RAMAN Spectroscopy in Forensic Science

Raman spectroscopy has a long history of use in forensic science, originally as a complement to infrared (IR) spectra, which is used to identify, at least qualitatively, the specific chemical components in a sample of interest. Both techniques are highly specific for identification since the IR or Raman spectra are

of fairly high resolution and no two substances have the same vibrational signature. For example, the analyses and detection of explosives is of utmost importance in today's world. Even more important is a positive association of a suspect to the explosive. In 1995, Cheng, *et al.*[9] reported the *in-situ* detection of the high explosive SEMTEX-H. Using Raman instrumentation, they were able to identify both components of SEMTEX-H (RDX and PETN) from latent fingerprints produced by individuals who had handled SEMTEX-H. Remote analysis of the high explosive SEMTEX-H has been demonstrated. RDX and PETN, the two components of SEMTEX-H, were identified in fingerprints. The analysis was conducted using a fiber optic probe at a range of four meters from the instrument[10].

In 1999, Ryder[11] reported the identification and characterization of solid mixtures of the controlled substances cocaine, heroin, and MDMA using Near-IR and Raman spectroscopy. Quantitative results were obtained from these narcotics mixed with various amounts of diluents.

The main advantages of Raman spectroscopy over IR are that light sources (especially lasers) are more intense and detectors are more sensitive in the visible range of the spectrum than in the IR, but most importantly water, which has a weak Raman signal but strong IR signal, can be used as a solvent. However, Raman spectroscopy suffers in that it is inherently weak, and even worse, suffers from fluorescence interference for many compounds of interest to the forensic community. The application of surface enhanced Raman spectroscopy (SERS) overcomes these two problems. The proximity of a metal surface has been shown to increase the Raman signal by more than six orders of magnitude, while at the same time suppressing the fluorescence signal.

The potential of SERS in forensic science, and in particular in the trace evidence, controlled substances, and questioned documents fields has been recently highlighted in a review of analytical tools for forensic science[12]. Research so far conducted mostly in Europe has shown that SERS can be effectively applied to problems such as the discrimination of jet printer inks *in situ*[13], the fast identification of synthetic dyes on fiber samples[14,15], and the

sensitive determination of narcotics[16,17]. Further work has involved SERS as applied to inks[18,19], as well as in an in-situ analysis of lipstick stains[20]. SERS was needed in this latter study due to strong fluorescence of the sample. Recent work conducted at the Department of Scientific Research of the Metropolitan Museum of Art, in collaboration with CUNY-CCNY and ORNL on the somewhat germane topic of dye and ink identification on ancient and modern works of art shows great promise for forensic applications.

As for forensic scientists, preserving the integrity of the object, to analyze, is paramount when working in art authenticity testing. SERS has been successfully applied to the study of ancient textiles and documents at the Metropolitan Museum of Art, and analytical protocols and references databases have been developed for organic dyes and inks. Moreover, a relatively non-destructive technique for examining textile fibers and documents has been developed. An example of the potential of the work so far conducted is given by the successful identification of the dye contained in a silk textile unearthed in an archaeological expedition: the textile, a silk fragment measuring less than one cm^2 was severely degraded after ten centuries of burial. The color of the textile was dark brown and the silk was extremely brittle. UV-Vis Spectroscopy or ordinary Raman spectroscopy could not detect any colored compound. Analysis of a fragment of the textile measuring less than 2 mm in length following the SERS protocol developed at the museum proved that the textile had originally been dyed with the natural dye madder[21]. A further development of the SERS protocol in use at the museum allows preservation of the integrity of the object examined, by means of non-sampling analysis. Dyes and inks present in a textile sample or a questioned document can be extracted from the object using a specially designed hydroxygel polymer, which is then subsequently analyzed by SERS. The matrix transfer procedure does not require that a fragment be detached from the object, nor does it affect the appearance or integrity of the object. The amount of dye transferred to the gel is so small that no fading or adverse colorimetric effect can be perceived; yet due to the extreme sensitivity of SERS, dye identification is still possible[22].

Work conducted in collaboration with ORNL shows that the sensitivity of SERS for the analysis of dyes is in the femtogram range, and that excellent reproducibility can be obtained with the proper nanosupports[23]. The possibility of using SERS with a variety of textile dyes and organic colorants has been likewise demonstrated[24].

We are aware that if this technique is to be successfully applied in a forensic setting, it must be properly validated for use in actual crime laboratories, as opposed to the academic laboratories in which much of the basic research has been conducted. To this end, in Phase II we intend to put greater emphasis on "real world" applicability. In addition to the expert help of Phil Antoci of the NYPD Crime laboratory, we have recruited Patrick Buzzini of the University of West Virginia, who is an expert on Forensic applications of Raman spectroscopy, to assist in this effort.

2. Statement of Hypothesis or Rationale for the Research

2.1 Objectives

2.1.1 To show the validity of SERS in a forensic context, and to show the conditions under which Raman spectroscopy and especially SERS contribute to the value of forensic science.

2.1.2. To compare the results obtained from SERS with the results obtained with normal Raman spectroscopy, and to explore the conditions in which each may be of value.

2.1.3 To validate certain specialized SERS techniques (see below).

2.1.4 To provide useful protocols for use of field workers.

2.1.5 To provide a searchable database for rapid and reliable field use.

2.2 Development of Analytical Protocols and Creation of a SERS Database

Drawing from our experience in the analysis of natural dyes on textiles, we plan to test the efficiency of silver colloids, silver nanoisland films, silver-over-alumina supports with a variety of dyes found in inks. Initially, we will work on a limited number of dyes of the arylmethane, azo, azine, anthraquinone,

phthalocyanine, and flavone classes. We will expand our work to include pigment dyes and lake dyes. This part of our work will entail determining the correct hydrolysis and/or extraction conditions for these colorants, to ensure maximum efficiency in SERS detection. The database of SERS spectra will be established as we proceed with this phase of the work, and it will be enhanced by systematic inclusion of new dyes, and by testing of pen, printer ink, and dyed textile spectra. Experiments with artificially aged written and printed samples will also be conducted (an Atlas Fade-o-meter is available at The Metropolitan Museum of Art for accelerated aging experiments). The database will contain dye spectra together with information on the source, state (neat, extracted, type of support, etc), and on the SERS methodology and eventual extraction conditions. Experiments on controlled substances are conducted at the NYPD Crime Lab using the same materials and techniques investigated for dye analysis applications.

2.3 Non-Destructive Analysis with SERS Nanoprobes

A new tool for SERS analysis recently developed at ORNL, the SERS nanoprobe, will be tested and further developed for forensic analysis of inks and trace evidence. The probe is an optical fiber tapered to a tip measuring 100 nanometers with an extremely thin coating of nanoparticles of silver, which induces the SERS effect. The ORNL nanoprobe combines in one tool the functions of delivering and collecting laser light and providing a SERS support.

The significance of this work is that the nanoprobe could potentially be used to perform direct analysis of samples (even dry samples) with no preparation of the surface. The small scale of the nanoprobe demonstrates the potential for detection at the microscopic scale, thus facilitating the analysis of very small details and samples.

The applicability of the nanoprobe approach to the analysis of inks and of textile dyes will be verified in parallel with the work conducted to build the SERS database using silver colloids; we expect that the nanoprobes will be very useful for a variety of inks, and in particular for those using pigment dyes. In the case of lake pigments (which require hydrolysis prior to analysis), we will experiment

with gel-coated nanoprobes, combining the nanoprobe approach with the matrix transfer technique described below.

2.4 Non-Destructive Analysis with Matrix Tranfer SERS

We will expand our previous work on the use of solvent hydroxygels in the analysis of textiles to include inks on documents. The application of this method to works of art has demonstrated that it is a reliable technique for the non-destructive identification on dyes on irreplaceable objects.

2.5 Sensitivity Enhancement with Immobilized Colloids

Preliminary experiments in our laboratory have demonstrated that an increment in sensitivity of up to 3 orders of magnitude can be obtained by immobilizing, on the tip of a glass fiber, or on a glass microscope slide, a small amount of colloid (a dot about 0.5 mm in diameter). The active surface thus obtained is left in contact with a large amount of diluted dye solution (10^{-9} M concentration) for a relatively long time. The silver nanoparticles act as an extraction and concentration device, removing from the solution the dye molecules. The advantage of using a small amount of colloid concentrated in a small space is that colloid saturation can be achieved even at very low analyte concentrations, leading to a situation in which a large number of silver nanoparticles carrying adsorbed dye molecules are concentrated within the instrument's excitation beam. We plan to develop this procedure so that it can be used for the analysis of microsamples extracted from specimens on ink writing, or other evidence.

II. Methods

1. Preparation of Ag Colloids

Ag colloid is prepared following the method of Lee and Meisel [16] by reduction of silver nitrate (Aldrich 209139 Silver Nitrate 99.9%) with sodium citrate (Aldrich W302600 Sodium Citrate Dihydrate). The colloid thus prepared shows an absorption maximum at 406 nm and FWHM of 106 nm, as measured with a Cary 50 UV-Vis Spectrophotometer (after a 1:4 dilution with ultrapure water to keep maximum absorbance within the instrumental range). To further concentrate the colloid for use, a volume of 10 ml of the original colloid was centrifuged at 5000 rpm for 2 minutes. The supernatant was discarded and the settled portion was resuspended in 1 ml of ultrapure water. All glassware was cleaned with Pierce PC54 cleaning solution, rinsed with ultrapure water and finally in acetone and methanol. This method proved to be as effective as the use of aggressive cleaning agents such as aqua regia or piranha solution, and was preferred for health and safety reasons. Only ultrapure water was used for the preparation of the various solutions. SERS measurement were made simply by adding 1 μl of dye solution to a 2 μl drop of colloid deposited on a gold coated microscope slide, followed by addition of 2 μl of a 0.2 M KNO_3 solution. Raman measurements were taken directly from the drop using a 50 or 100x microscope objective and focusing on the microscope slide surface. SERS spectra could be obtained two or three minutes after addition of the KNO_3 and remained constant in quality until evaporation of the liquid.

2. Raman Spectra

The NR spectra of solids are obtained in the region of 100 to 4000 cm^{-1} directly from pure powder samples. Since the fluorescence of the dyes prevented the acquisition of a Raman spectrum, FT-Raman spectroscopy are carried out using a Bruker Ram II FT-Raman-Vertex 70 FTIR Micro spectrometer. The 1064 nm line of an Nd:YAG laser is used as the excitation line. The resolution was set to 4 cm^{-1} in back scattering mode. A liquid nitrogen cooled Ge detector was used

to collect 100 scans for a good Raman spectrum. The laser output was kept at 150 mW for the SERS spectra and 50 mW for the solid samples.

Additional SERS work on Ag colloids is carried out using a Bruker Senterra Raman microscope using 785 nm excitation, a 1200 rulings/mm holographic grating, a CCD detector and power at the sample ranging from 8 to 80 mW.

3. Matrix Transfer SERS

Using a gel as a medium for the solvent mixture confines its action only to the areas of the substrate covered by the gel bead. The extraction is carried out for 2-8 hours at room temperature. The gel bead is then removed, transferred to a microscope slide, covered with a drop of Ag colloid, and examined with the Raman microscope. Transfer of the dye from the substrate to the gel does not require removing a fragment from the object; the amount of dye removed is so small that no appreciable fading is detected by the eye, and; the size of the polymer bead can be reduced to a fraction of a millimeter in order to minimize any impact on the substrate, without detriment on the effectiveness of the method. The one-step procedure combining extraction and hydrolysis is extremely efficient and time saving. Additionally, problems previously encountered in obtaining SERS spectra from alizarin[25] and due to its difficult adsorption on silver nanoparticles do not appear to affect its analysis by MT-SERS. In an alternative procedure, instead of using a silver colloid to obtain the SERS effect, the gel fragment is coated with silver nanoislands by thermal evaporation of silver in a high vacuum evaporator (Edwards E306A). The method has been found to yield the same results as the use of a colloid.

Different hydrogels can be used for the MT procedure. We have so far worked with either a 1:1 random copolymer of 2,3-dihydroxypropyl methacrylate with 2-hydroxyethyl methacrylate (Benz 5X, also known as GMA) or 2-hydroxyethyl methacrylate (known as p(2-HEMA) or Benz 38 or HEMA). The gel is prepared for use by soaking for 10 minutes in a solution of dimethylformamide (DMF) and water with EDTA (1% w/w), with water and DMF in a 1:1 ratio. This solution is known as a solvent for extraction of dyes

from fibers. Different solvents however could be used: the possibility of using pure DMF for the extraction of vat dyes has been demonstrated in our tests.

The procedure described above is the first example of the use of a solvent gel to hydrolyze/extract insoluble dyes from an object for the purpose of non-invasive identification by SERS techniques. The current standard procedure for dye extraction when HPLC is used is a destructive procedure based on the removal of a sample and its treatment with an appropriate reagent capable of dissolving the dye-aluminum complex and removing the dye from the textile fiber or other support. This reagent can be an acidic solution, or the same H_2O/EDTA/DMF mixture used here, with the difference that the procedure of reference is carried out at boiling.

Gel supports for SERS analysis have been described before[26] and are commercially available[27]. The bulk of the published literature however deals with gels containing silver particles, and having the specific functions of stabilizing the silver particles to increase their efficiency for SERS analysis. The innovative aspect of the MT-SERS procedure is that it is the first method ever proven effective for the non-invasive analysis of insoluble dyes contained in textiles, paintings, documents, etc. We expect this technique to be especially useful in the analysis of writing and printing inks containing lake pigments and pigment dyes, because of the unique ability of the solvent gel system to hydrolyze and solubilize such materials.

4. Immobilized Colloids

In a typical application, a microscopic fragment, such as a paper fiber with ink traces, or a textile fiber is pre-treated with HF vapor to facilitate the solubilization of the dye, and then deposited on a microscope slide (or other appropriate surface) and covered with a drop of water (or other appropriate development solvent). The immobilized silver colloid (on the tip of a glass fiber, or on a microscope coverslip) is then placed in contact with the solvent and left there for a suitable time. We eventually plan to develop this technique into a microfluidic system able to handle microsamples pretreated with HF vapor.

III. Results

1. Statement of Results

1.1 RESULTS FROM THE METROPOLITAN MUSEUM OF NEW YORK SITE-

Normal Raman spectroscopy and surface-enhanced Raman scattering were used to collect spectra of ten synthetic dyes commonly found in ballpoint pen ink. The dyes included Acid blue 1, Acid Orange 10, Acid Red 52, Aniline Blue, Crystal Violet, Methyl Violet, Pararosaniline, Rhodamine B, Sudan Black B, and Victoria Blue. The normal Raman spectra were collected using 633 nm, 785 nm, and 1064 nm lasers. The SERS spectra were obtained with 633 nm and 785 nm lasers on a silver colloid substrate aggregated with 0.5 M potassium nitrate solution. The surface-enhanced Raman spectra and the majority of the normal Raman spectra were found to be of excellent quality with highly resolved and intense peaks.

Additionally, the above dye standards and ink extracted from a readily available ballpoint pen were developed on a thin-layer chromatography plate following the procedure described in the ASTM Guide E 1422-05 (standard guide for test methods for forensic writing ink comparison). The SERS spectra were collected directly from the plate with a 785 nm laser after the colloid and the aggregate were added to the dye spots. The ink dyes spectra were found to correspond to the spectra of the standards on the plate and the dyes spectra collected earlier on the colloid drop. The collected spectra were of high quality and the technique was found applicable for the *in situ* analysis of ink dyes separated on a thin-layer chromatography plate.

Other synthetic dyes that the NY site has studied using SERS (at 633 and 785 nm) are: Eosin Y Na salt, Eosin, Erythrosin, Flourescein, Fisihin, Rhodamine 6G, Rosaniline, Mauve, Nile blue A, and Safranin. The studies have been performed using citrate reduced Ag colloids, and different aggregating agents (KNO_3, HCI, NaOH and HNO_3). Several different conditions (wavelength and

aggregation agents) were used in order to determine the best conditions to detect the dyes by SERS.

SERS analysis was used to identify opiates, which are commonly submitted to forensic laboratories. The research has focused primarily on three opiates – Morphine & codeine, which are both natural opiates, and hydrocodone a semi-synthetic opiate. The plan is to continue the research using SERS to identify opiates by relating it to "real-world" situations through the use of these techniques on actual samples received by the NYPD Crime Laboratory.

In comparing the results obtained on organic colorants by normal Raman (both with the dispersive spectrometer and with the Fourier Transform spectrometer) and by SERS we have found that fluorescence and low Raman signal intensity are major drawbacks in dye analysis. The conclusion could be drawn from our initial results that FT-Raman is the preferred option for identification of dyes in inks, paint and fiber evidence, as it significantly reduces fluorescence. The FT-Raman data presented in our study however was obtained with a macro-sampling configuration, from pure dye or pigment samples mounted in 2mm diameter cells. When attempting to analyze microscopic samples, such as paint chips or single textile fibers using an FT-Raman microscope we encountered significant problems due to reduced throughput. FT-Raman spectrometers perform best as apertureless detectors, and suffer from extremely low throughput when a small aperture – such as a microscope- is used. In addition, the use of high magnification optics carries a higher risk of thermal degradation of the sample due to the concentration of the beam energy on a smaller spot (this is somewhat of a lesser problem in dispersive Raman spectroscopy as lower intensity lasers can be used). Finally, FT-Raman is generally less sensitive than dispersive Raman with visible light laser excitation, as the intensity of Raman scattered light increases with the 4^{th} power of the exciting laser frequency. This would be a significant factor when going from the pure reference dyes and pigments in the survey to real samples and trace evidence.

SERS presents the advantage of extremely high sensitivity, complete fluorescence rejection, and specificity towards organic colorants (inorganic

pigments and extenders are not enhanced, nor are paint and ink binding media). It does generally require an additional step, either an extraction or the pretreatment of the sample with acidic vapors, but the procedure is straightforward and the analysis can be carried out on sample as small as 50 micrometer as a matter of routine. The HF pretreatment procedure used in this study is a gas-solid reaction specifically developed to hydrolyze lake pigments. As no liquid phase is involved, there is no risk of losing analyte due to dilution, transfer losses, or adhesion to container walls. The procedure is carried out directly on a microsample in a small sample holder (obtained from the cap of a Beem size 3 embedding microvial). The sample holder is then inserted in a micro-chamber obtained from a Beem size 0 embedding microvial, previously loaded with a 10 μL drop of HF. We found this to be a convenient and safe experimental setup. The HF drop is retained in the pyramidal bottom of the micro-chamber and the sample in its holder is exposed to an HF saturated atmosphere without coming in contact with the liquid phase. Retrieving the sample holder is easily achieved and there is no risk of operator exposure to HF. In our experience we noticed that the micro-chamber needs to be refilled only every two weeks, further diminishing the dangers associated with HF use. We are currently experimenting with other acids, but HF is an ideal reagent for a number of reasons: it does not have any oxidizing action, as HNO_3 would have, and it forms insoluble or non-hygroscopic salts with the metal ions used as lake bases, unlike HCl, thus diminishing any interference due to those metals. In any case, the sample treatment is no more complex than what necessary for traditional analysis of inks by thin layer chromatography.

We have also found that although the production of colloid is not exactly reproducible from batch-to-batch, the spectra are reproducible. There are no variations in either measured Raman wavenumbers, or the relative intensities. Therefore there is no doubt as to the identification of the species. Furthermore, the limits of detection do not vary from colloid samples.

We are currently working on alternative methods for colloid preparation (including use of microwave ovens, both scientific grade and standard domestic

ones), and we have increasing evidence for additional sensitivity enhancements with properly tailored colloids.

1.2 RESULTS FROM THE DUKE UNIVERSITY SITE

Detection of Fluorescent Dye Molecules using SERS and SERRS

Our results indicate that ultra-high sensitivity is achievable when combining surface-enhanced Raman scattering (SERS) and resonance-enhanced Raman. In addition, our earlier work illustrated the technical limitations inherent to excitation external to the SERS nanoprobe. More specifically, we demonstrated that detection of thousands, hundreds or even tens of molecules is eminently practical (Table 1) if the plasmonic enhancement generated in SERS couples with molecularly-dependent signal enhancement in surface-enhanced resonance Raman spectroscopy (SERRS)—but only if the intense fluorescence associated with excitation of the bulk matrix (Figure 1) can be prevented. To pursue both these

Table 1: Estimated Limits of Detection (L.O.D.) for several sample dyes

Dye	Estimated L.O.D. (molecules)
Methylene Blue Hydrate	12000
Sudan III	4500
Crystal Violet	1500
Toluidine Blue 0	225
Brilliant Cresyl Blue	80
Cresyl Violet Perchlorate	12

goals, in the current reporting period we have focused our efforts on construction of a highly-sensitive Raman spectrometer which will allow efficient fiber-coupled plasmonic excitation.

Our initial attempts to use our laboratory's existing intensified charge-coupled detectors (ICCDs) were thwarted by the use of continuous wave (CW) excitation and the high noise levels inherent to the use of an ICCD in CW mode. To circumvent these fundamental limitations, we have purchased an electron-multiplying charge-coupled detector (EMCCD, ~$30k) and spectrograph (~$10k) which are able to provide the high signal enhancement typically associated with an ICCD—but with the near-single-photon noise limits typically associated with non-intensified CCDs, photomultiplier tubes (PMTs) or avalanche photodiodes (APDs). Figure 2 shows the current instrumental setup for our proof-of-concept EMCCD-based Raman spectrometer. Note that the lack of optics to focus the HeNe laser on the sample is intentional: The reduced laser fluence, the limited field of view of the fiber-based signal collection optics and a properly-chosen slit width can conveniently mimic the laser attenuation which occurs if laser light is coupled into a fiber-optic nanoprobe with a sub-wavelength tip diameter.

Figure 3 shows normalized SERRS spectra for several resonance Raman-active dyes physisorbed to SERS-active silver island films. After correction for the lack of laser focusing, the limited field of view associated with fiber-optic signal collection and the

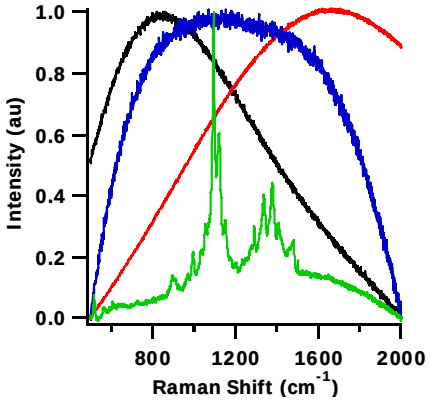


Figure 1. Normalized fluorescence spectra of Acid Blue 1 (black), Victoria Blue (blue) and Aniline Blue (red) deposited on filter paper and excited at 633 nm, along with the normal (non-SERS) Raman spectrum of filter paper.

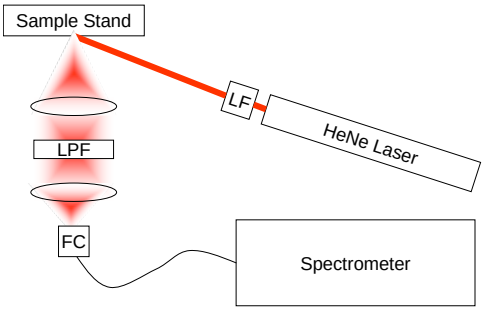


Figure 2: EMCCD-based SERS / SERRS spectrometer. LF: laser line filter. LPF: long-pass Raman filter. FC: Fiber-optic coupler.

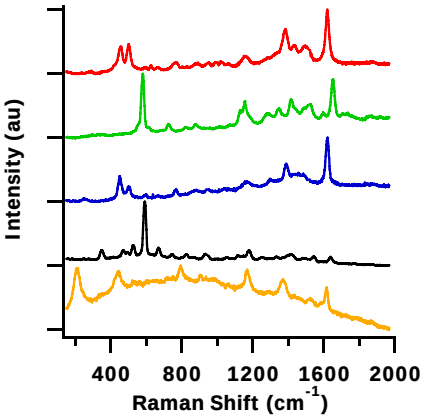
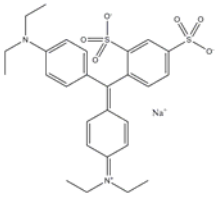


Figure 3: Normalized SERRS spectra of Toluidine Blue 0 (red), Brilliant Cresyl Blue (green), Methylene Blue Hydrate (blue), Cresyl Fast Violet (black) and Crystal Violet (orange). Raman shift is with respect to excitation at 633 nm.

narrow slit width of the spectrograph (10 μm, in this case), the spectra in Figure 3 are similar to those which can be generated with a few hundred nanowatts of 633-nm laser light and an acquisition time of less than 60 seconds. We expect that optimization of this Raman spectrometer and incorporation of through-fiber excitation will allow efficient probe-enhanced SERS and SERRS of real-world samples.

2. Tables and Figures



Acid Blue 1

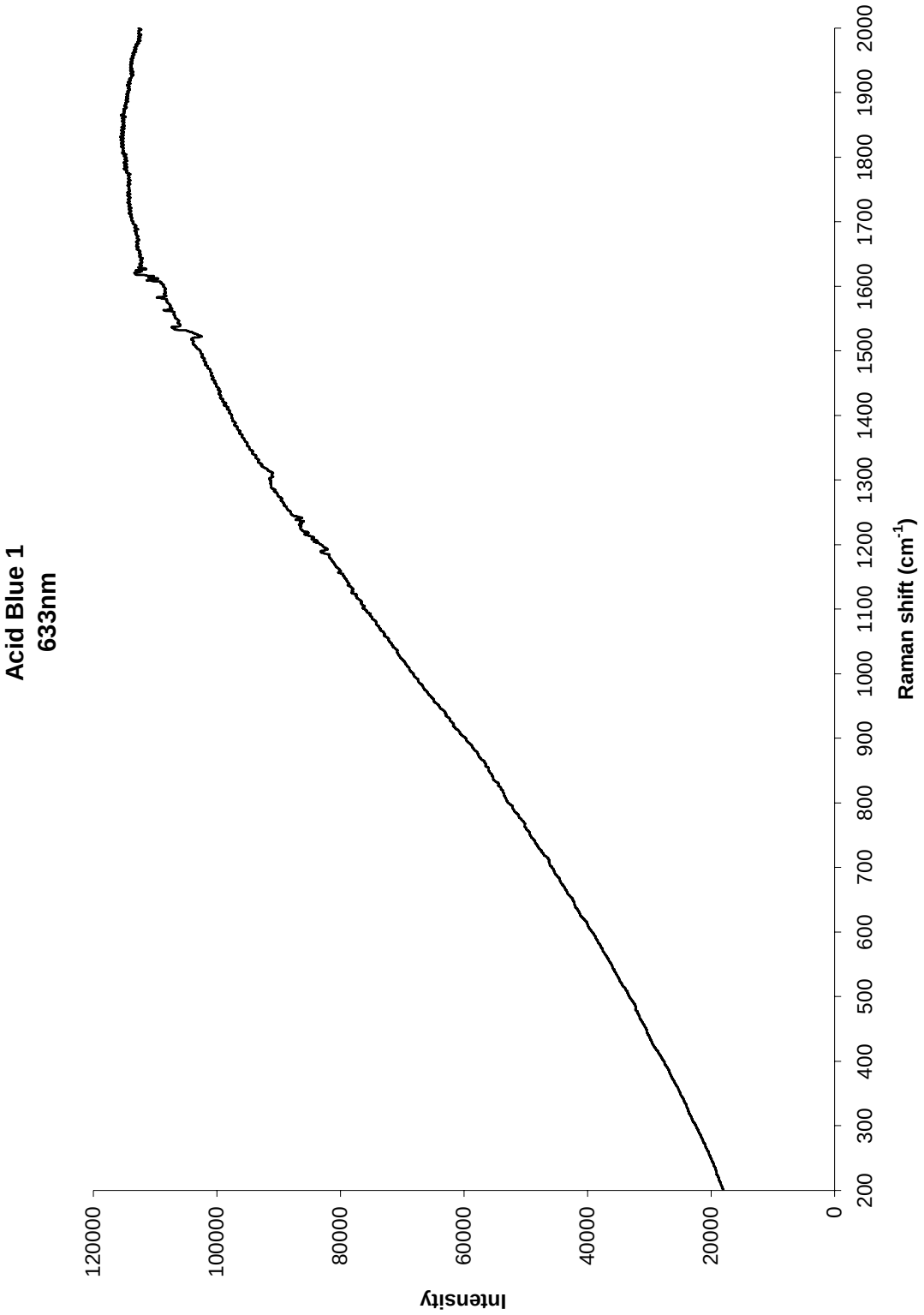
CAS 129-17-9; CI 42045

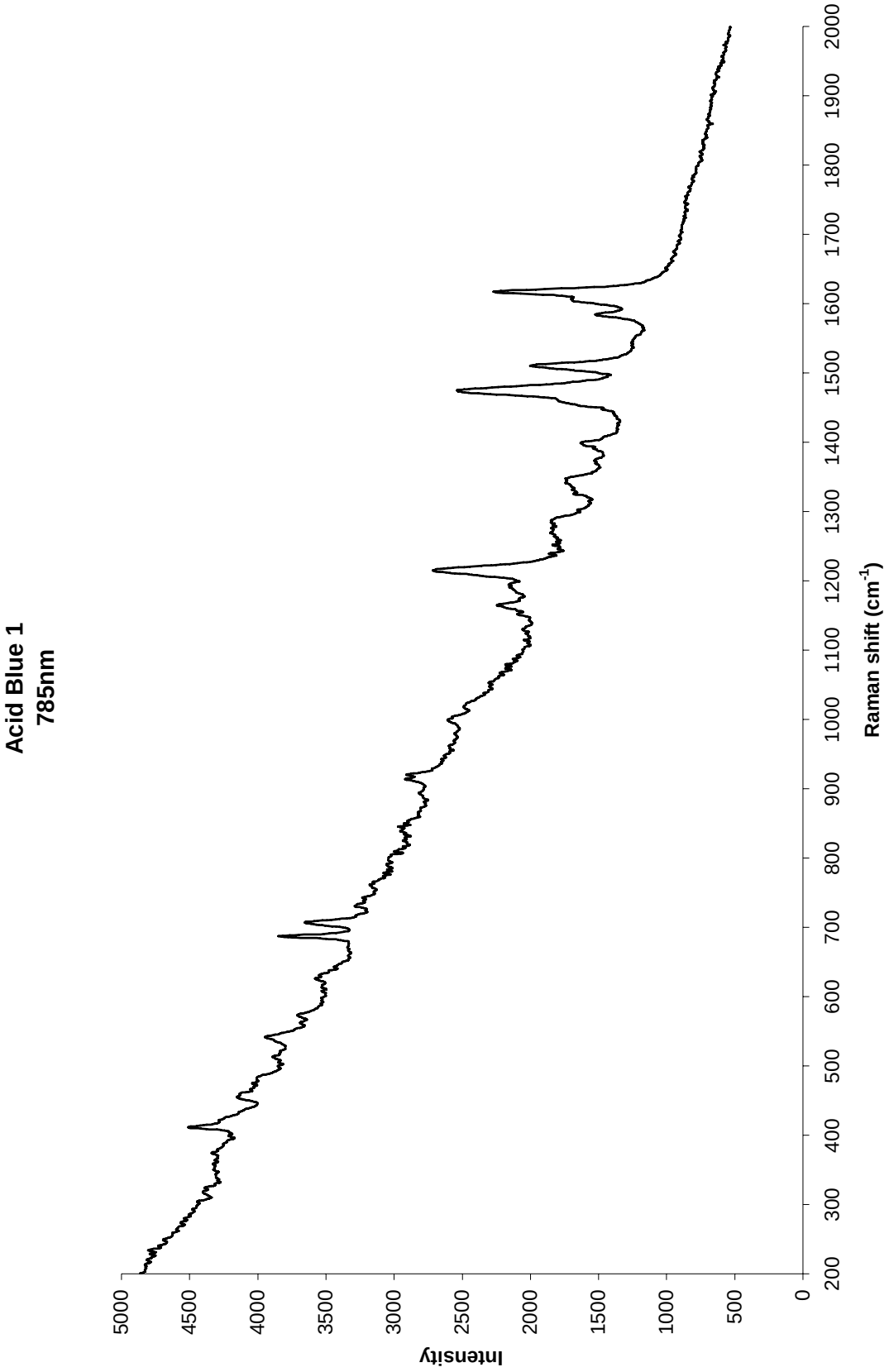
Alternate name: Patent Blue VF

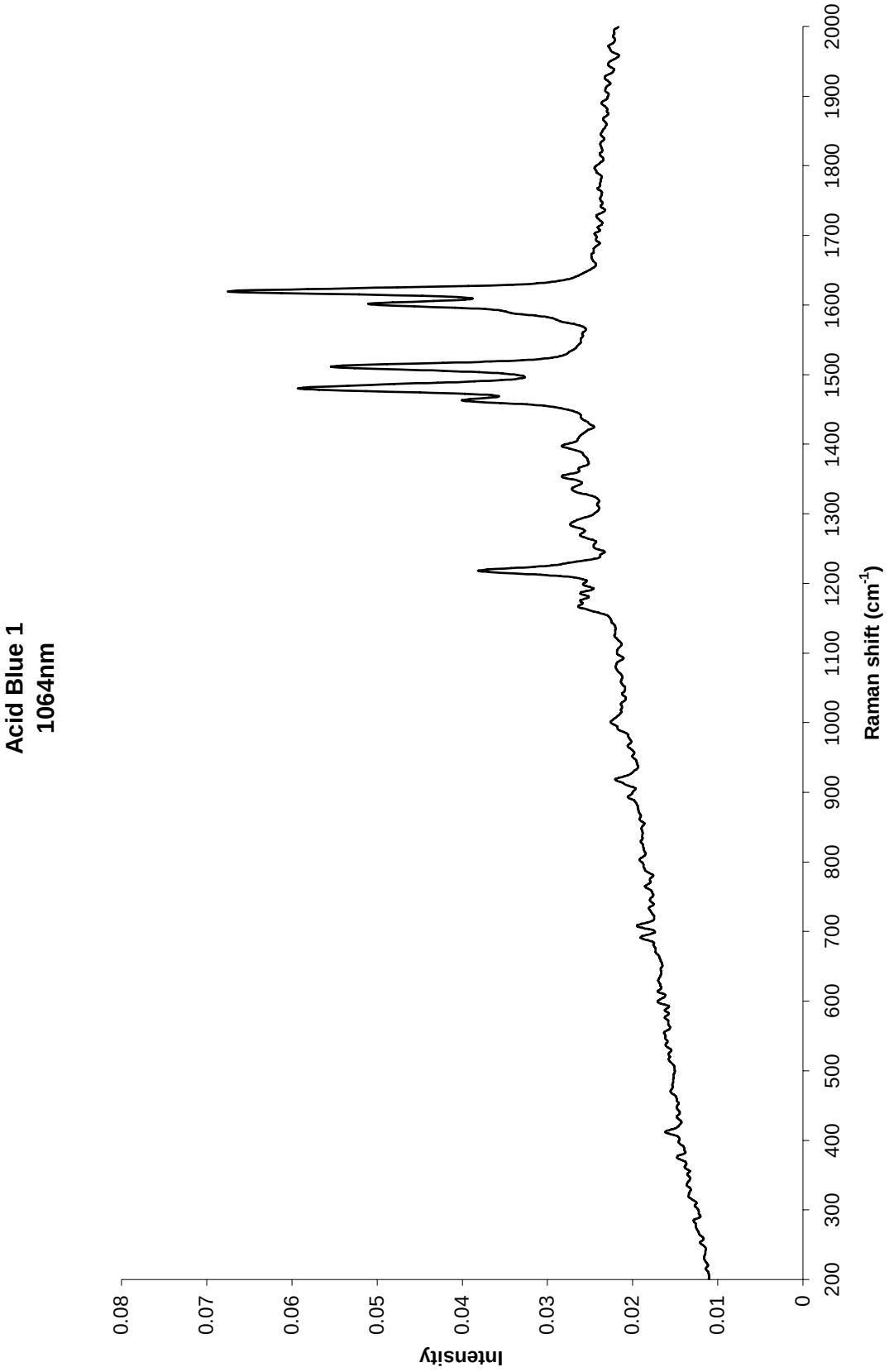
Table 1. Acid Blue 1 Raman peaks (cm^{-1})

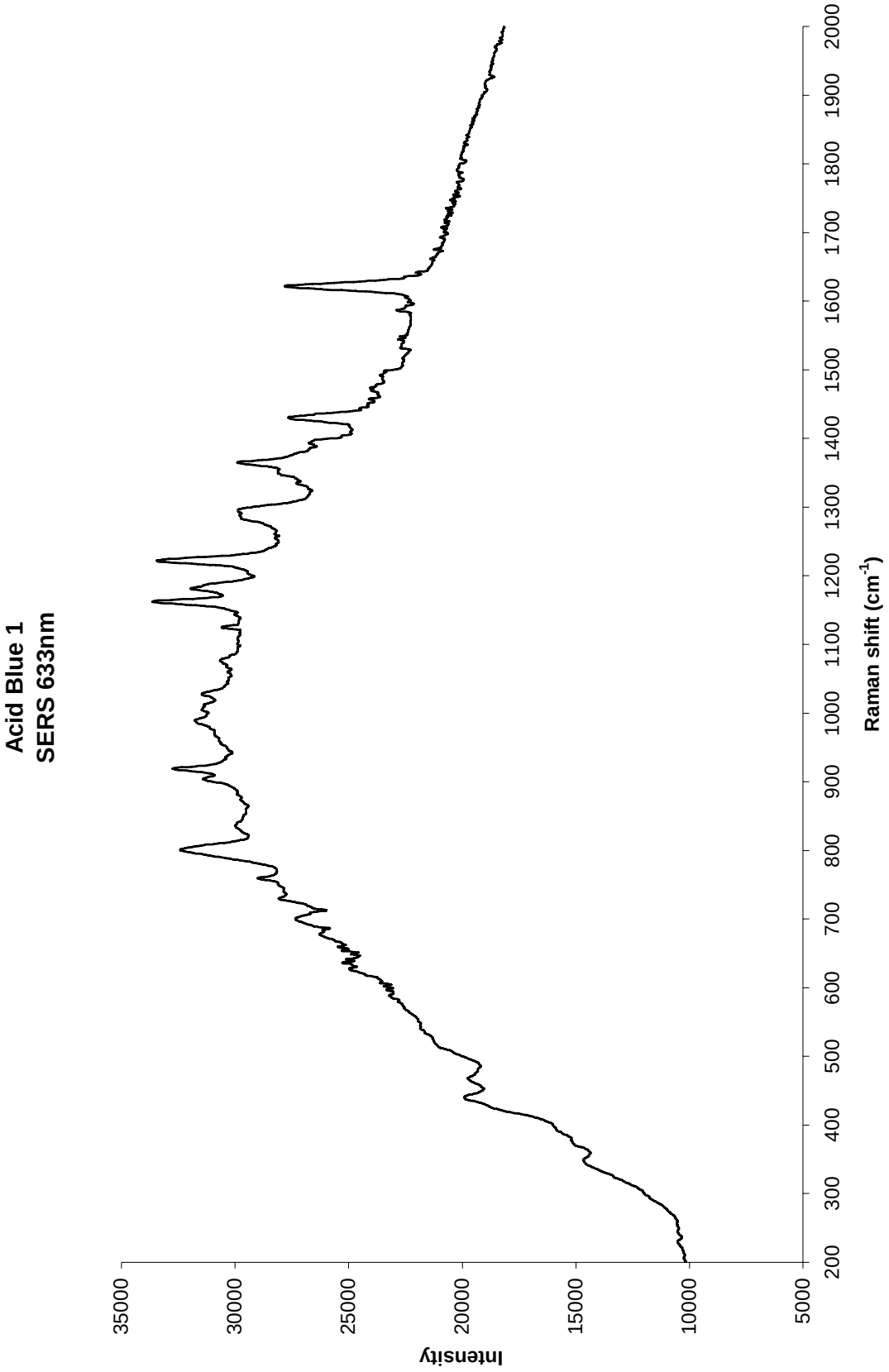
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
			231	230.5
			349.5	346.5
		375.3		
	411.5	411.9		
				423
			440.5	
	455			
	461		468	468
	513.5			
				535.5
	541.5			
				559.5
	573.5			
		599.0		599.5
	632		636.5	
	646		659.5	
			678	
	687	691.6		
			700.5	700
	707	708.9		
	730.5		729.5	728
		764.9	759.5	759
			801	798.5
			836.5	
	845.5			
	894	894.1		
			904	904.5
	914	919.1	919	920
				950

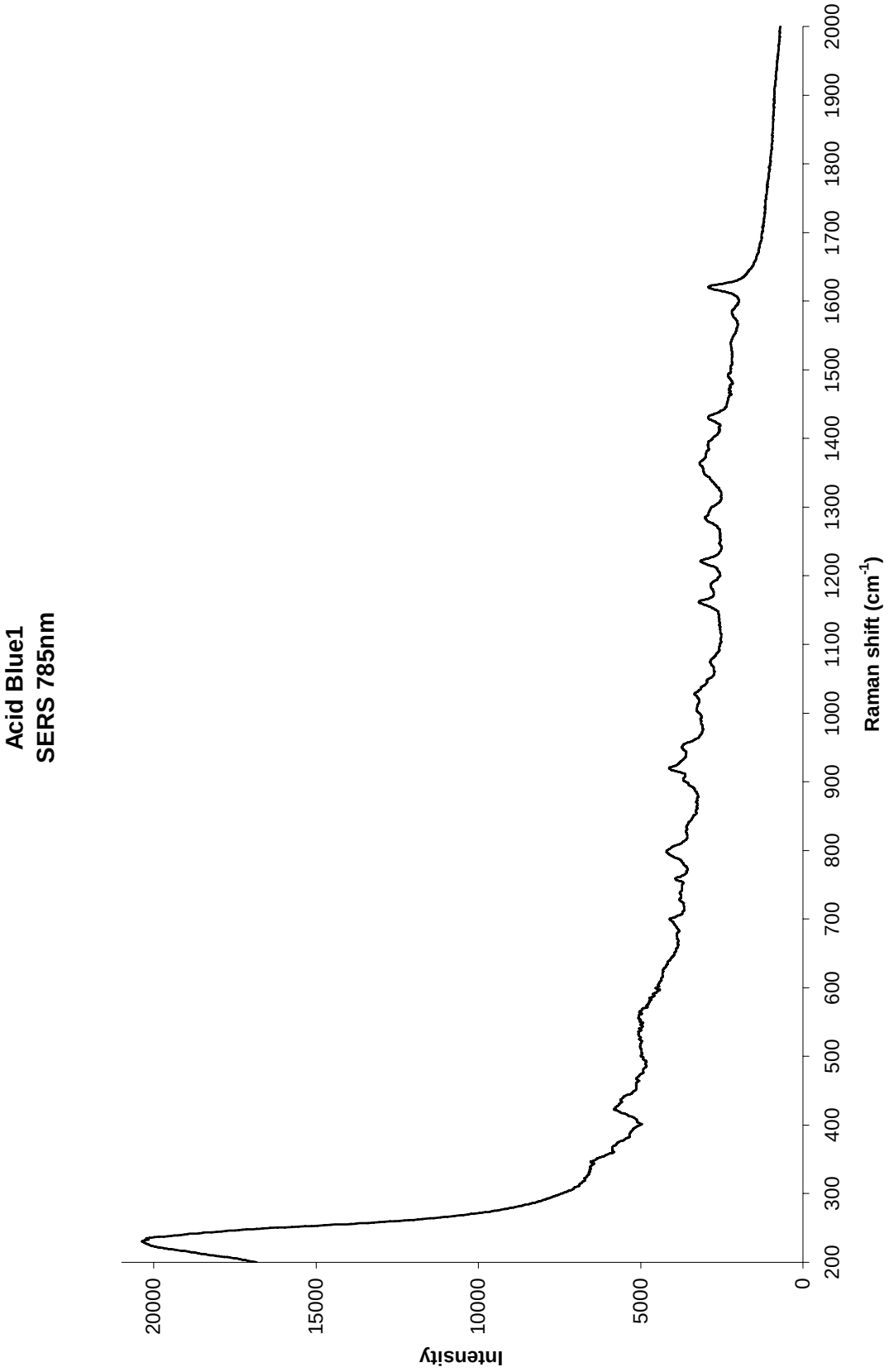
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
			989.5	
	999.5	1000.1	1005	
			1027.5	1028
			1076.5	1074.5
1126	1130		1124.5	
	1165	1174.0	1162.5	1161.5
1189.5	1194.5	1187.2	1181.5	1191
	1215	1218.1	1222	1221.5
1302	1293	1285.6	1294	1284.5
		1335.7		
	1347.5	1353.1		
			1365	1364
	1399	1397.4	1397	1397
			1430.5	1429.5
		1463.0		
	1474.5	1480.3	1473.5	1474
			1492	1491
1518	1510.5	1511.2		
1582.5	1584.5			
1609.5		1601.8		
1621	1617	1619.2	1622	1620



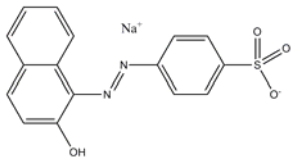








Acid Orange 10



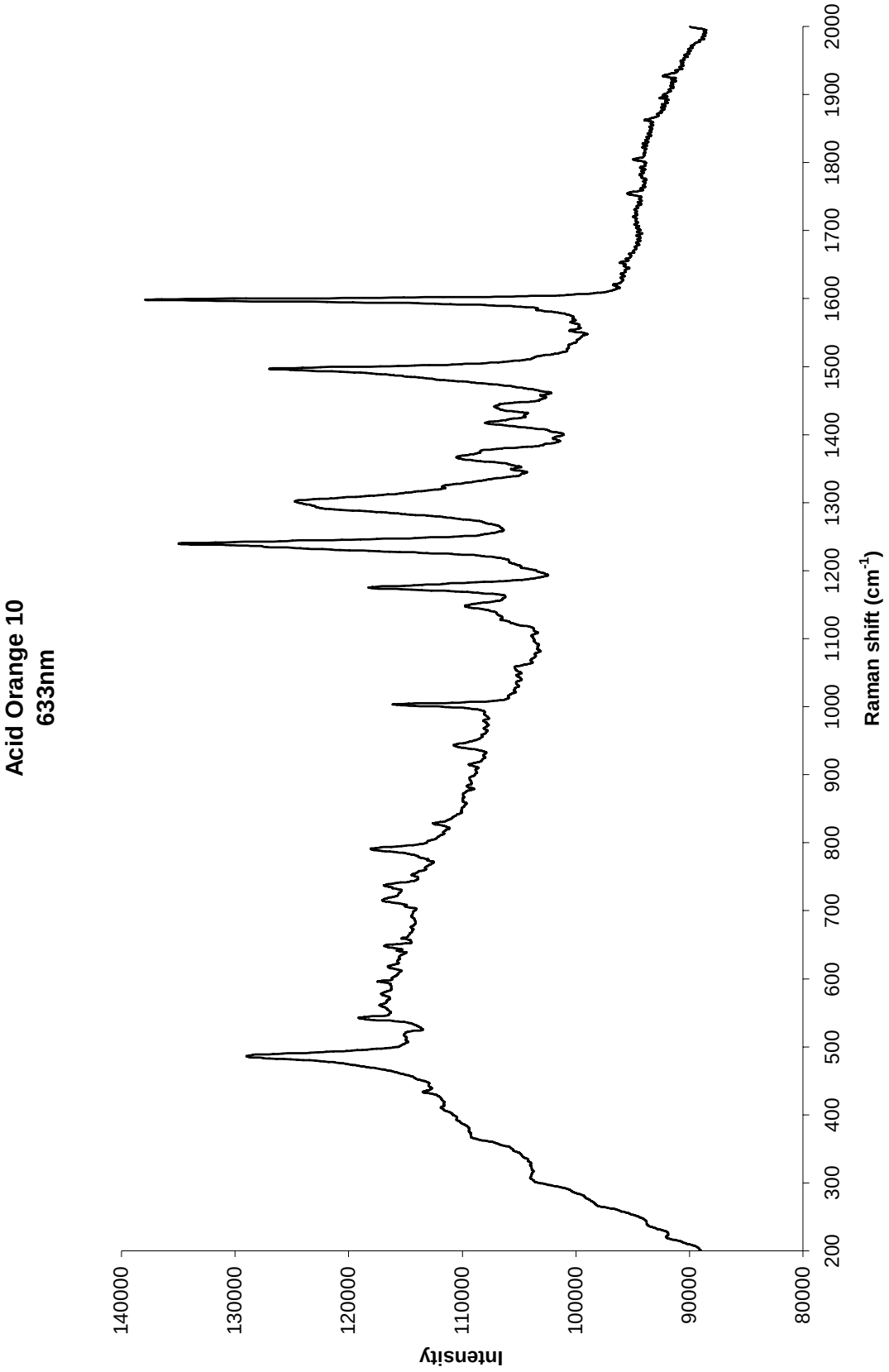
CAS 1936-15-8; CI 16230

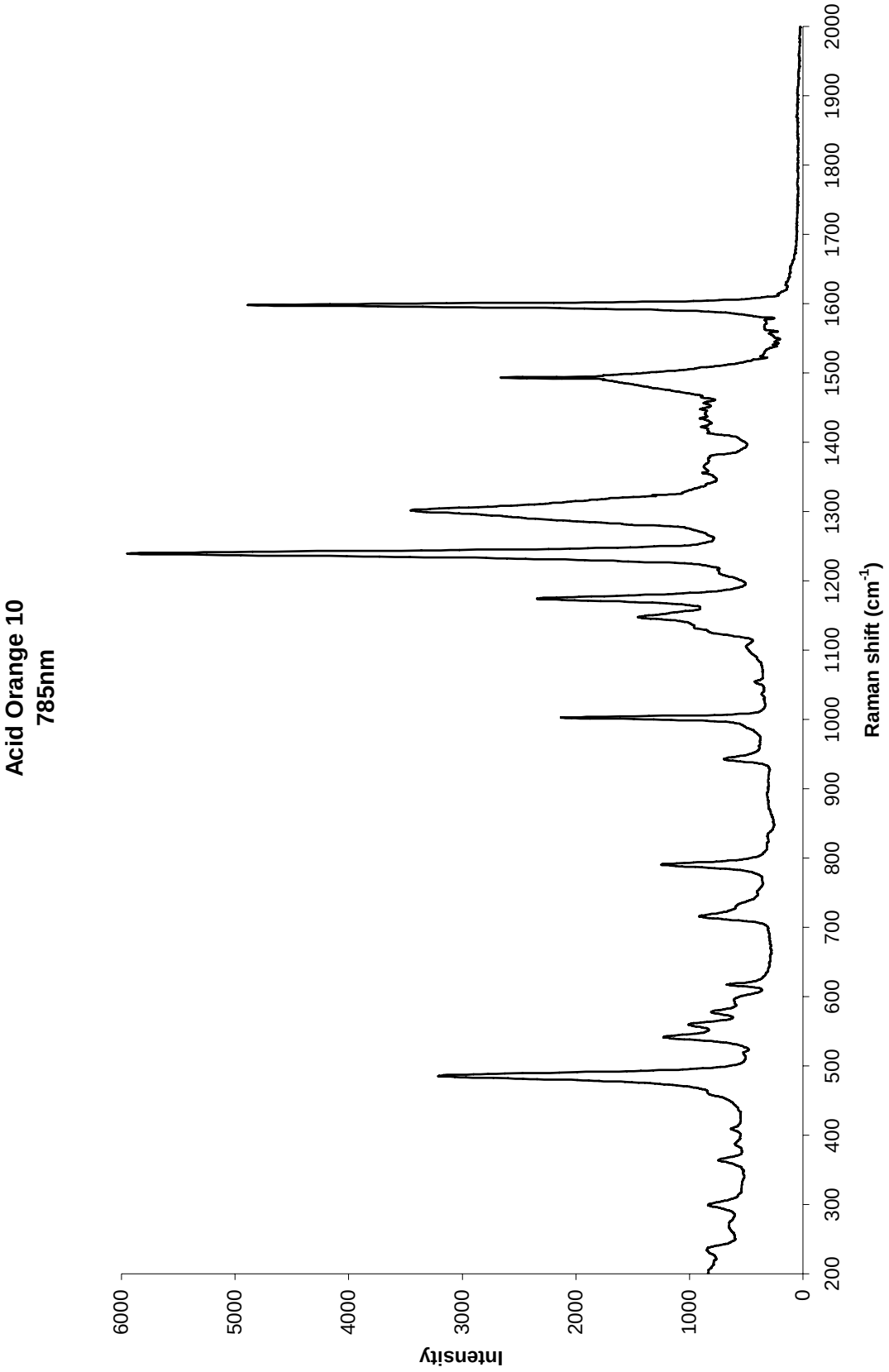
Alternate name: Orange G certified

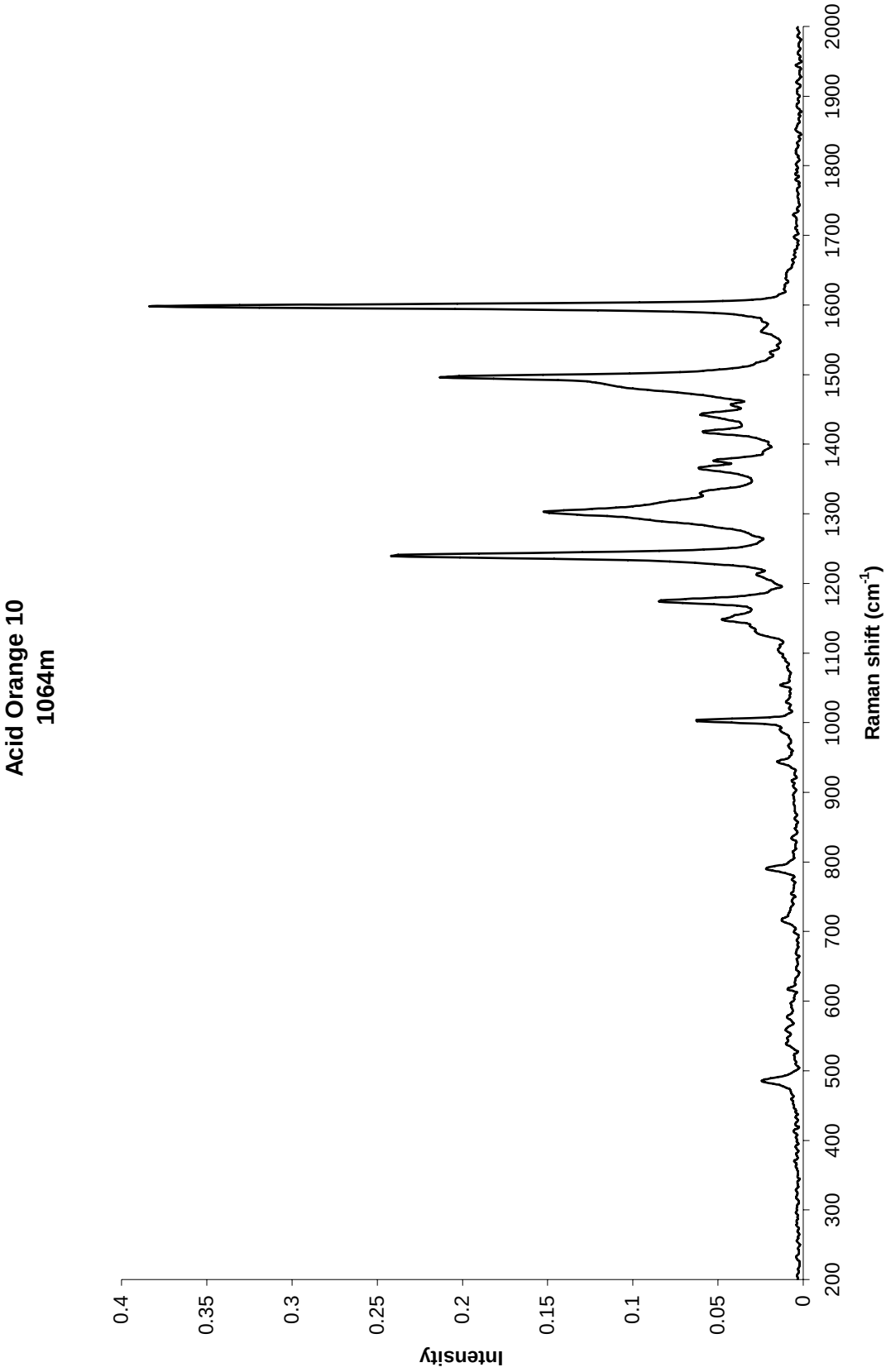
Table 2. Acid Orange 10 Raman peaks (cm^{-1})

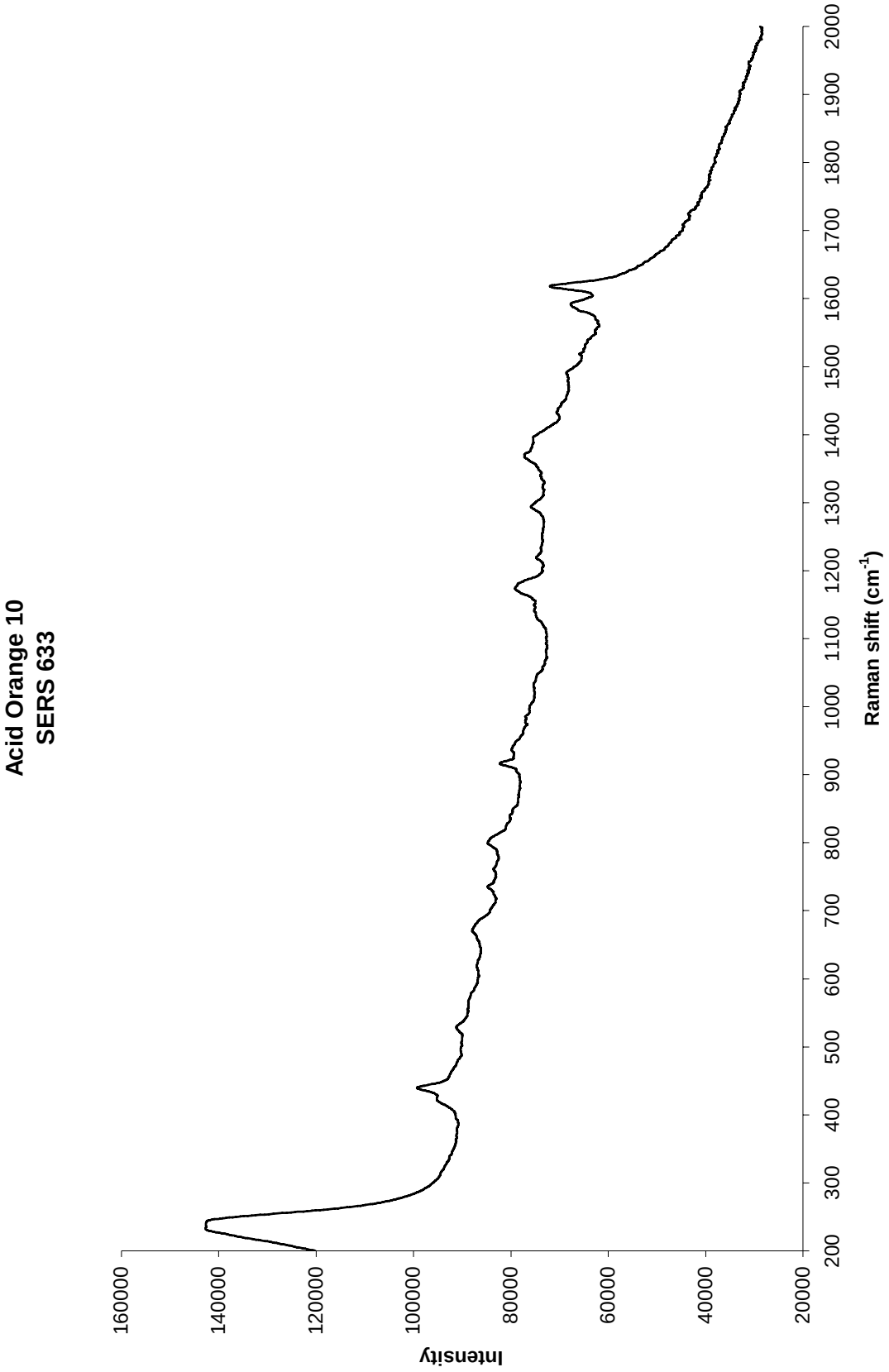
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
	234.5			240.5
	299.5			
	364			
				421
			439.5	440
486.5	485.5	485.2		
			529	527
542.5	541.5			
561	560			
577.5	578			
596				
618	617.5	616.4		
648.5				
			671.5	
715.5	716	716.6		
737			735	731
			761	760
791	790.5	789.9		
			799.5	800
828.5				
915			916.5	915.5
943.5	942.5	944.2		940
1003	1003	1004.0		1000
1058	1054.5	1054.1		
1148	1147.5	1148.6		
1175	1174.5	1173.7	1173.5	1172.5
			1219.5	1218
1239.5	1239.5	1239.3		
1302	1301.5	1302.9	1294	1297.5
1367		1366.6	1370.5	1365.5
				1394.5

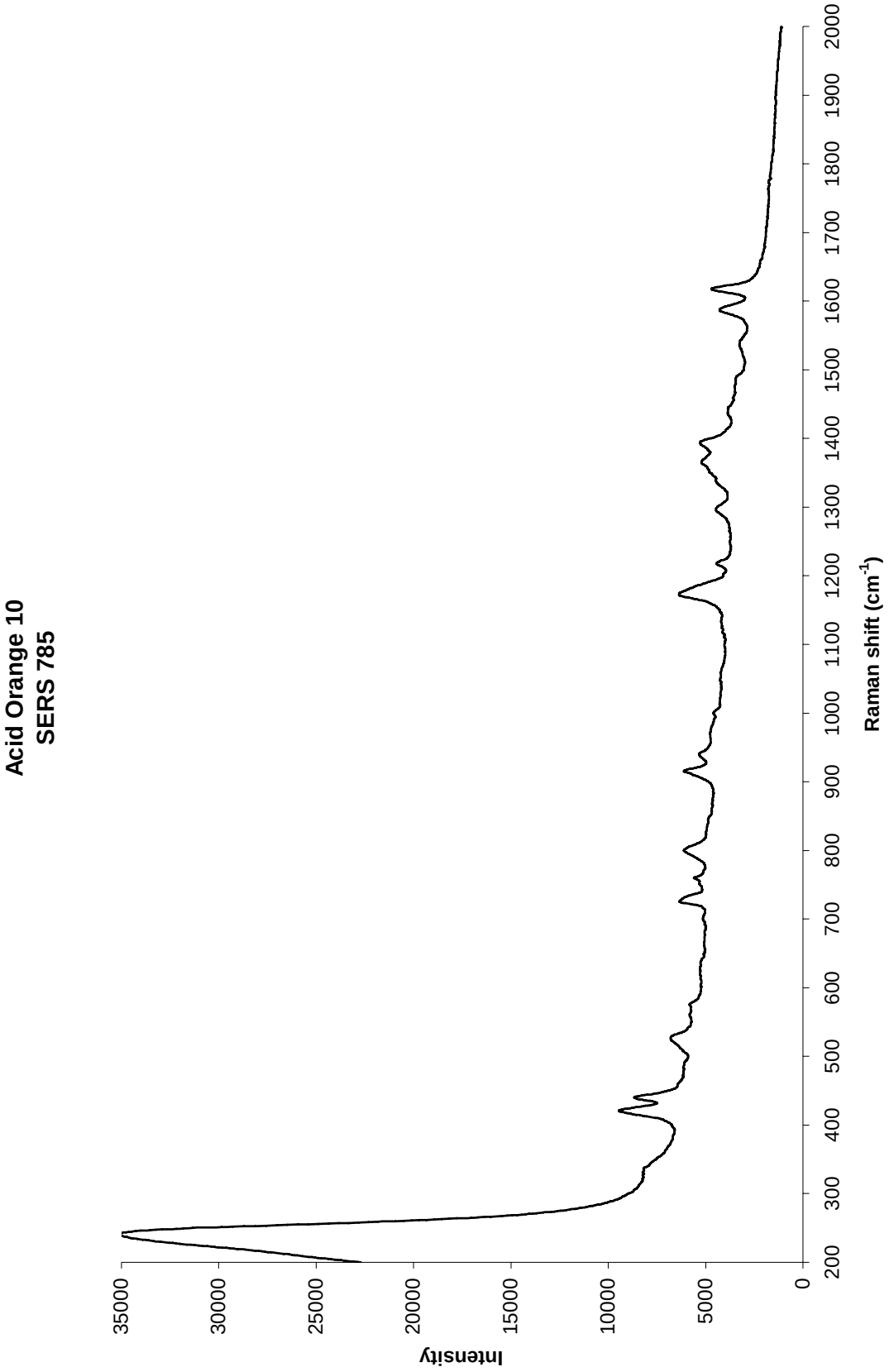
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
1417.5	1422.5	1418.6		
			1433	
1441.5		1441.8		
1496.5	1493.5	1495.8		
			1518.5	
1598.5	1598	1598.0	1592	1587
			1618	1617.5



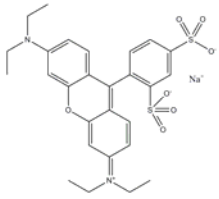








Acid Red 52



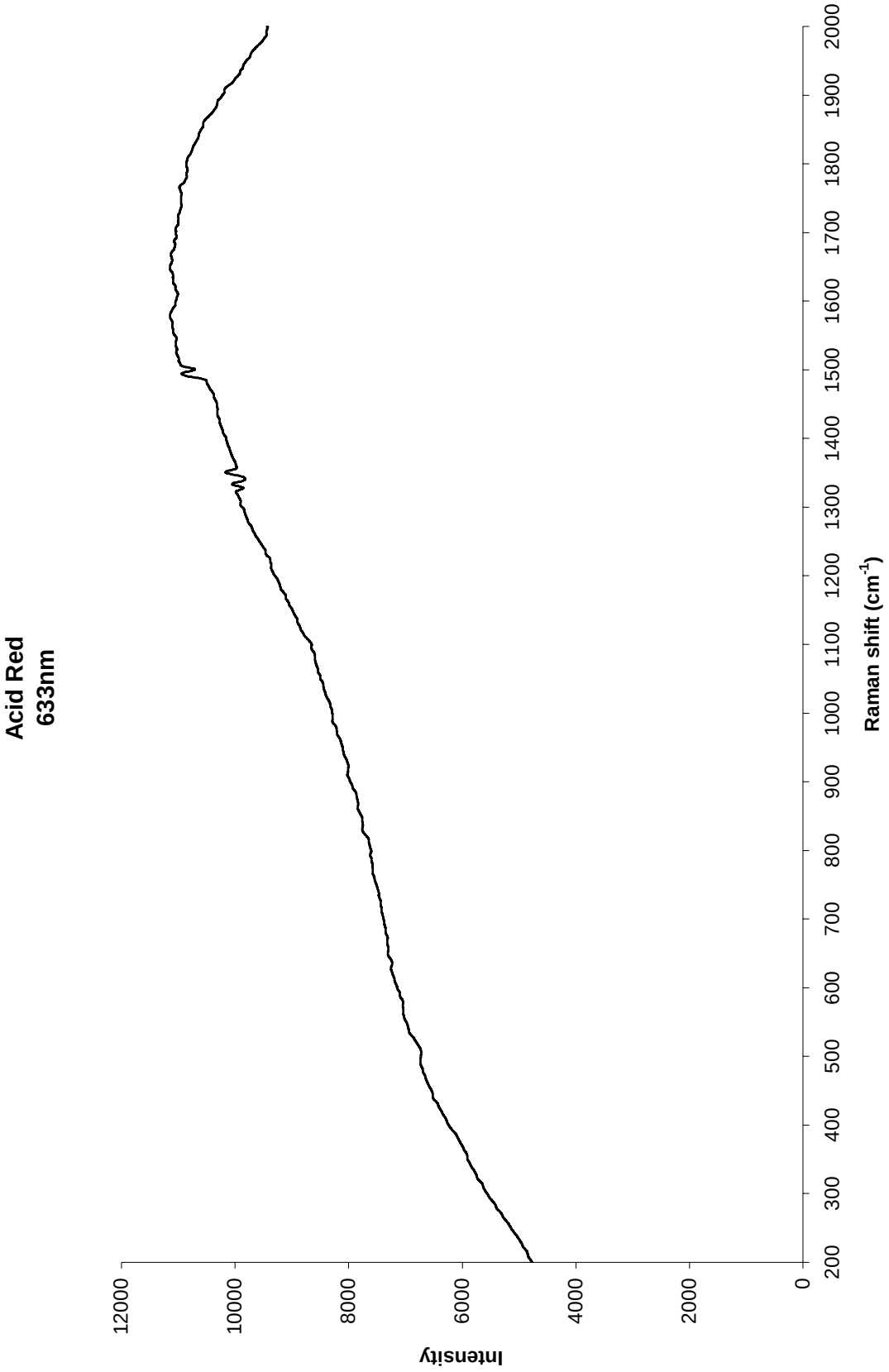
CAS 3520-42-1; CI 45100

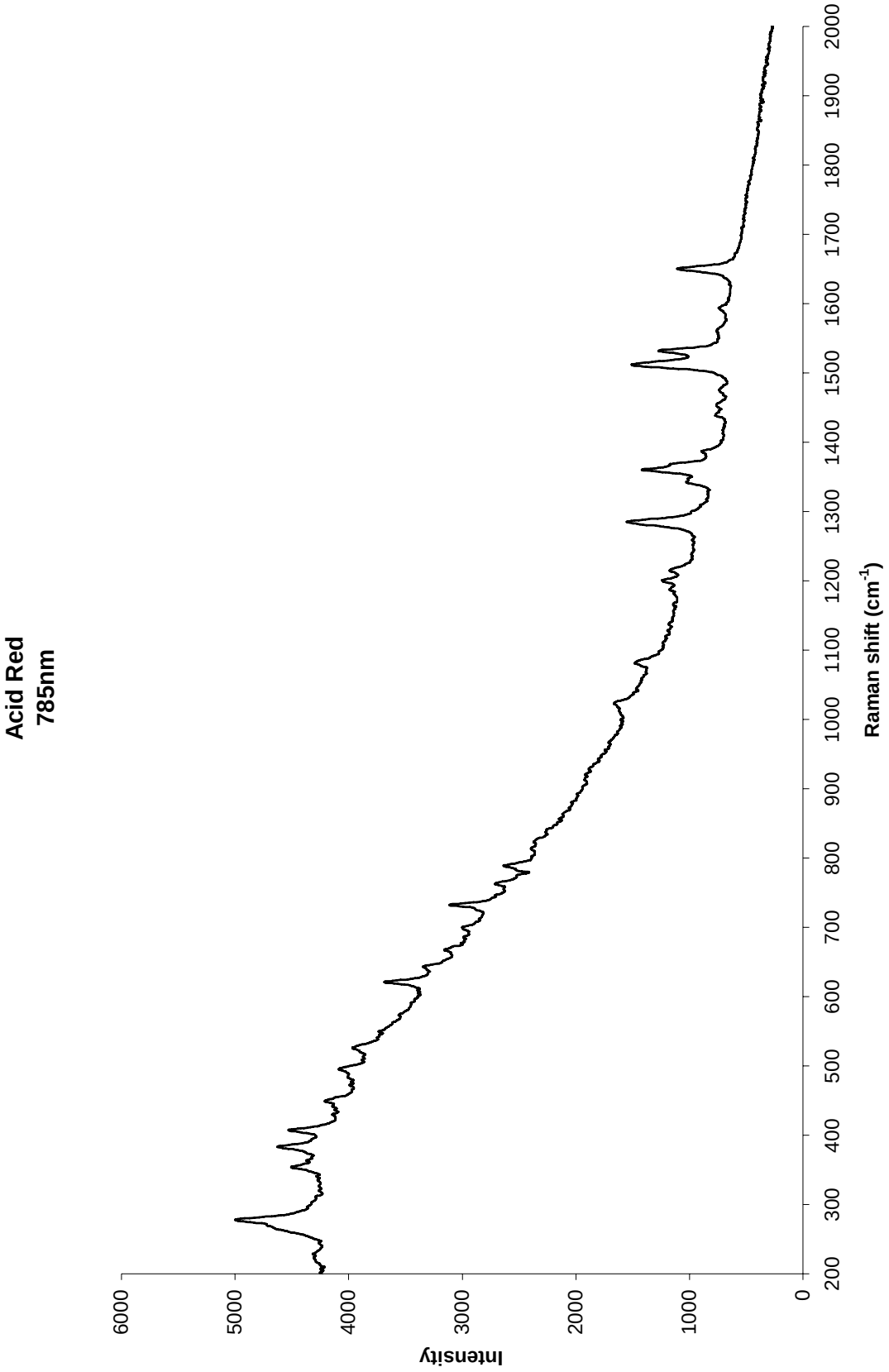
Alternate names: Food Red 106, Sulforhodamine B, Xanthylium

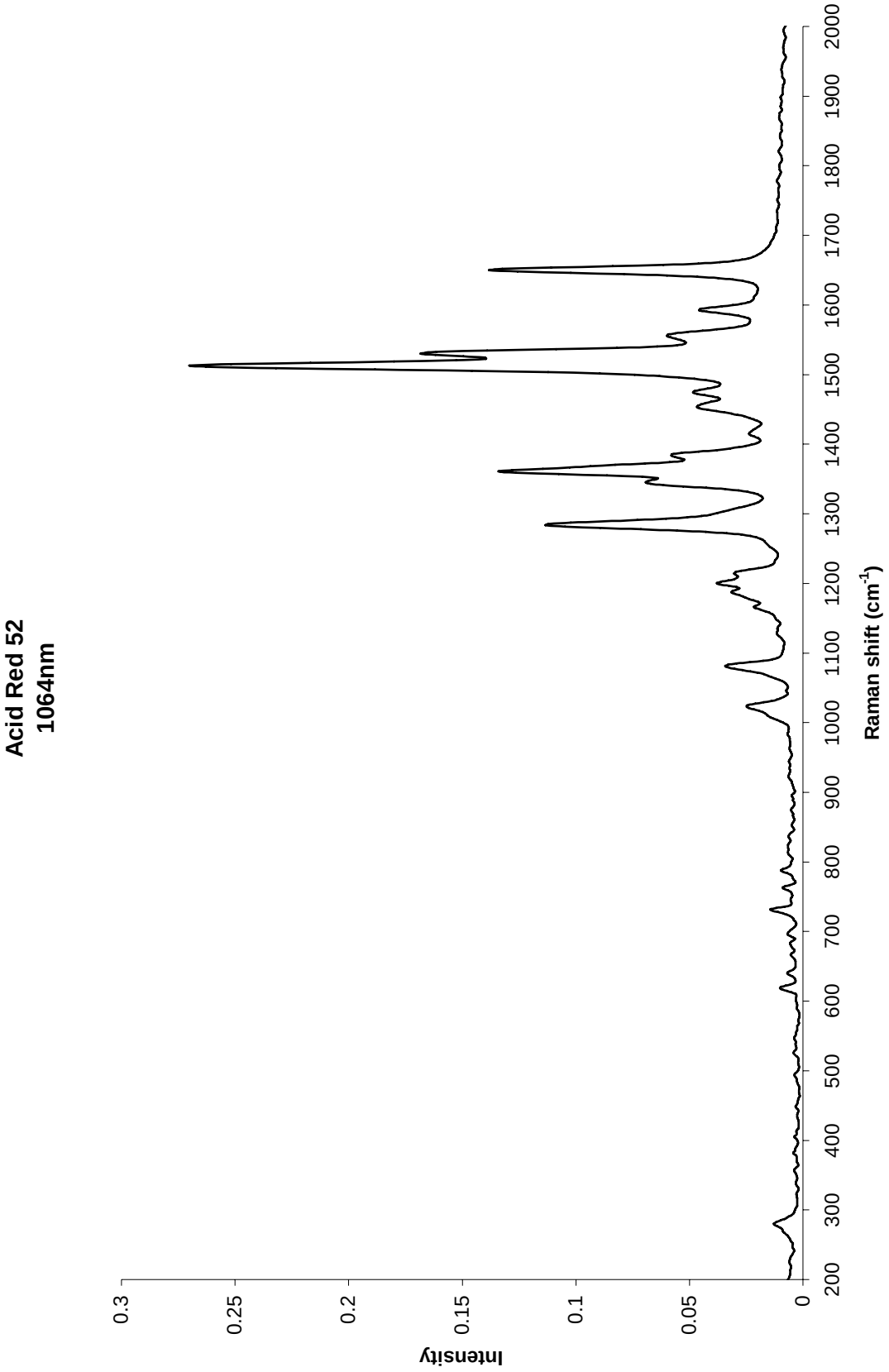
Table 3. Acid Red 52 Raman peaks (cm^{-1})

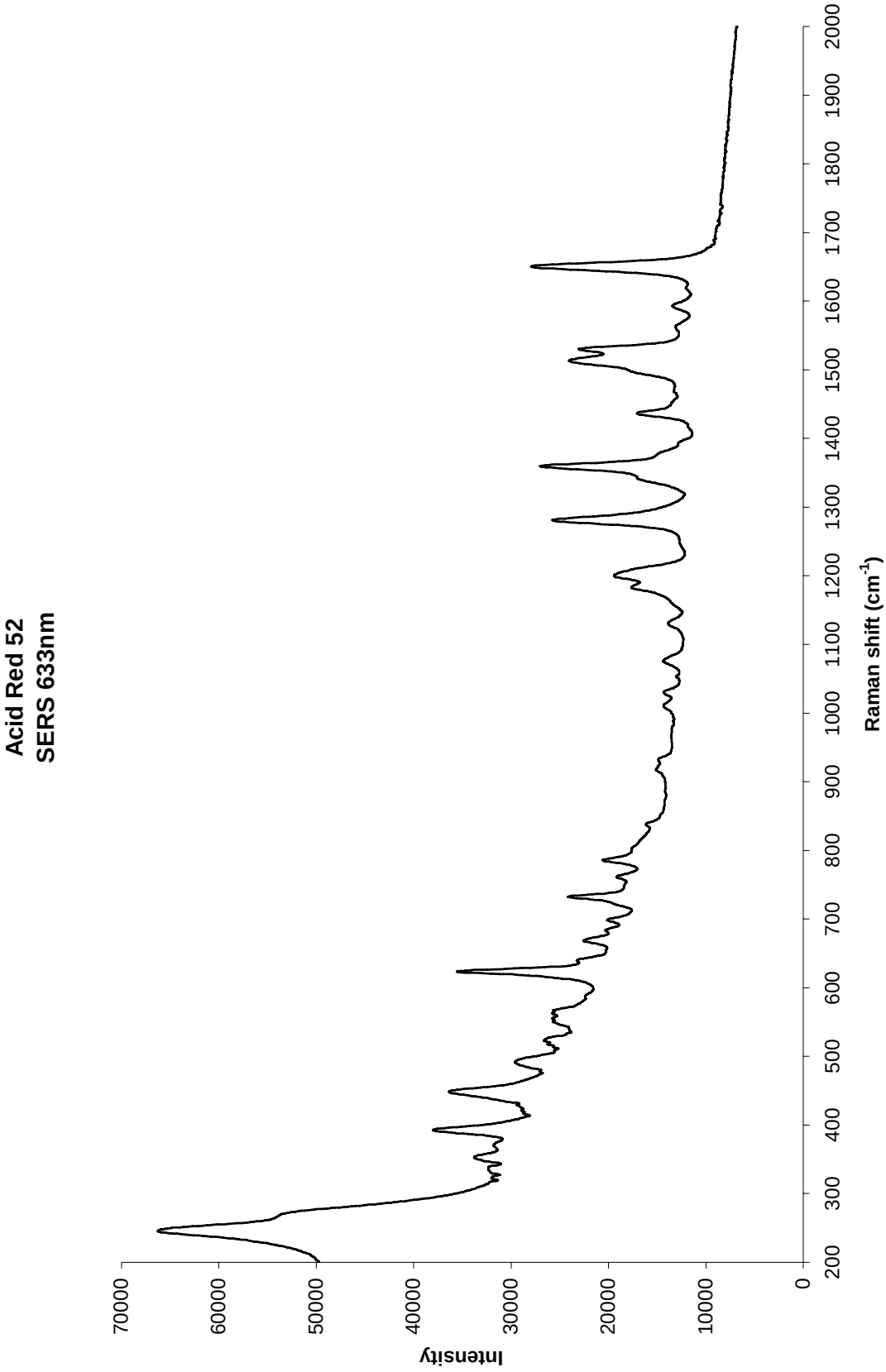
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
	228.5			
			245.5	241
	278	280.8		
	354		352.5	355
	383.5	381.1	393	389.5
	407.5			
	449		448.5	447
	495	494.9	492	
	526		523	
			562.5	566
628.5	621	618.3	623.5	
	643	641.4		
	667.5		668.5	
	700	697.4		
	732.5	732.1	732.5	730
	763	762.9	761.5	
	789	788.0	785.5	782.5
			842	
			923	926.5
			936	
			1012	1017
	1023	1023.3	1033	1028.5
				1048
	1081	1081.1	1076	1079
			1131	1128
	1200.5	1200.7	1200.5	1202.5
	1285.5	1283.6	1281	1281
1322				
1333.5				

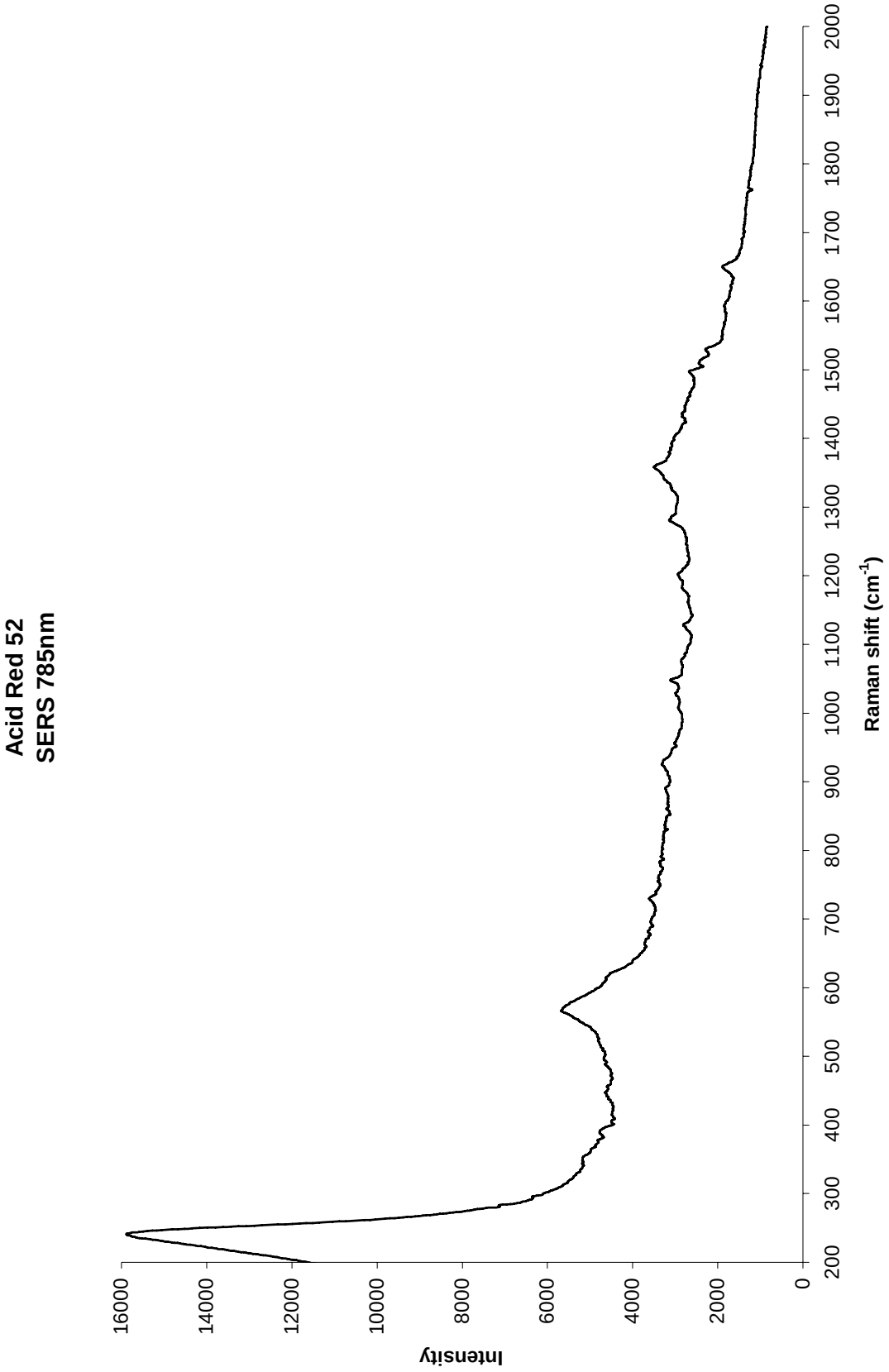
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
1354	1360.5	1360.8	1359.5	1358
	1446		1436.5	1436
	1457	1453.3		
	1475.5	1474.6		
1494				1498
	1512	1513.1	1513.5	1509.5
	1532		1531	1530
1584	1593.5	1592.2	1593	1599
1649	1650.5	1650.1	1650.5	1650



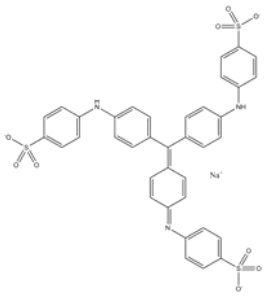








Aniline Blue



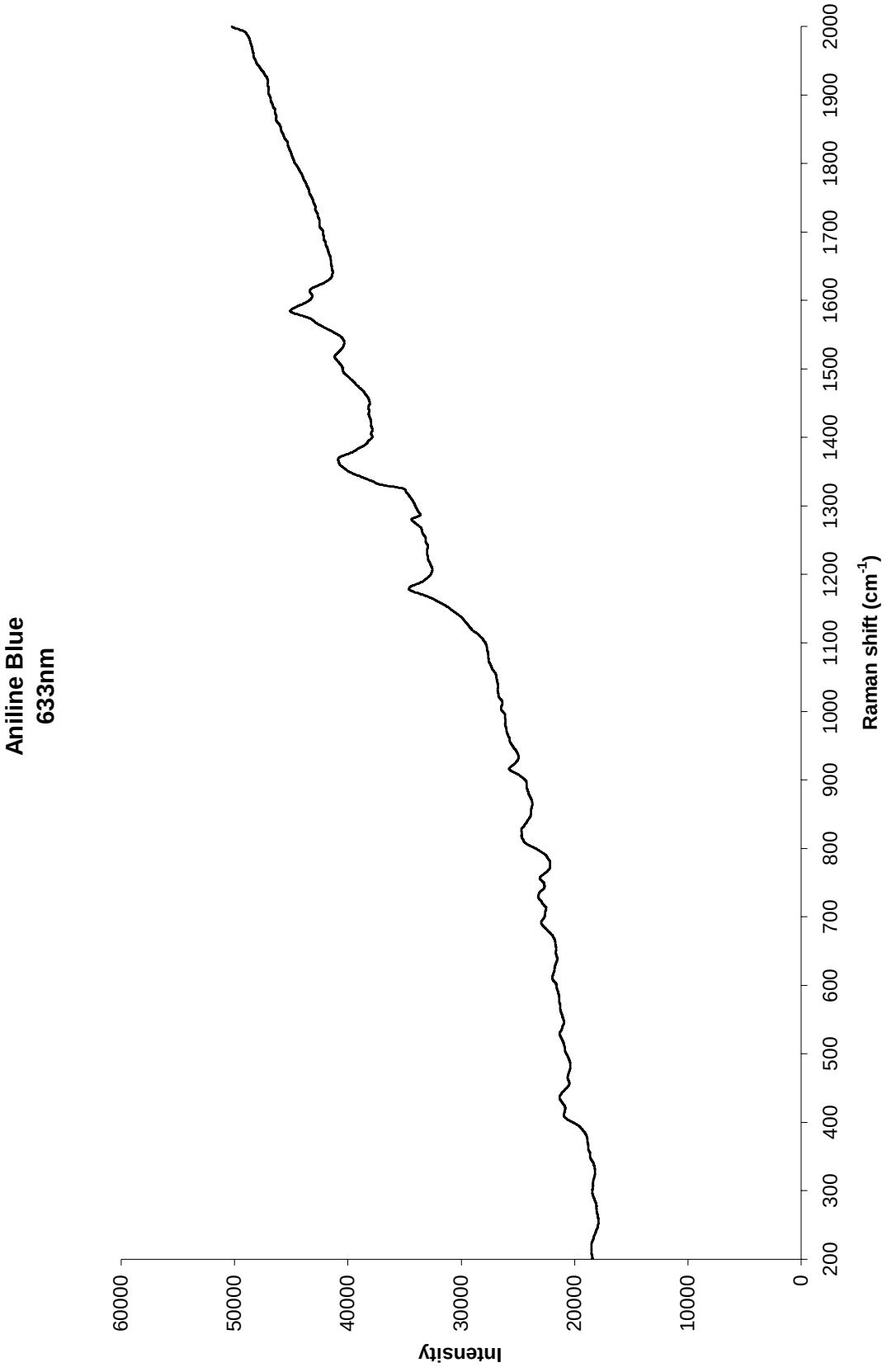
CAS 28631-66-5; CI 42755

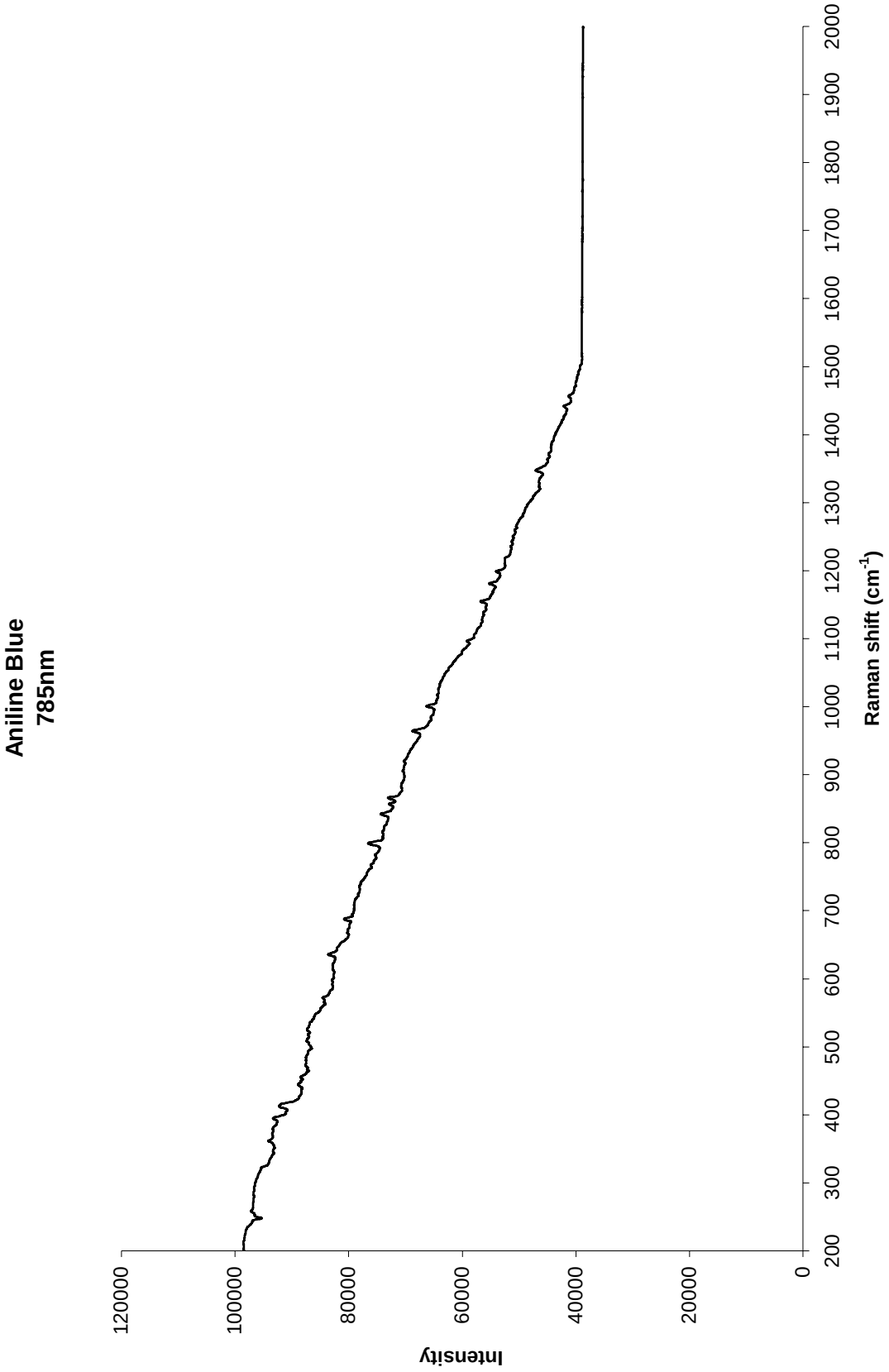
Alternate names: Acid Blue 22, Methyl Blue

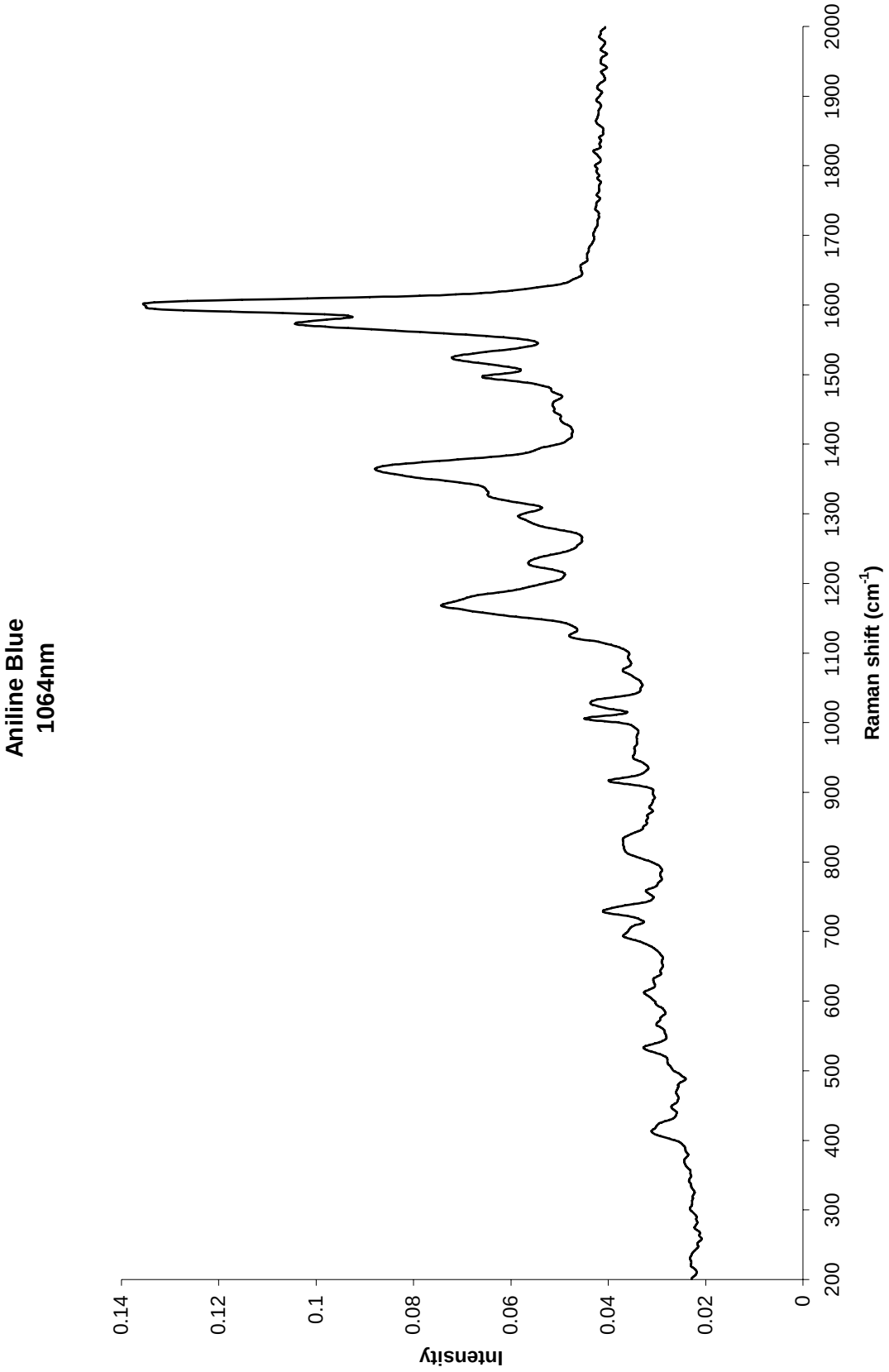
Table 4. Aniline Blue Raman peaks (cm^{-1})

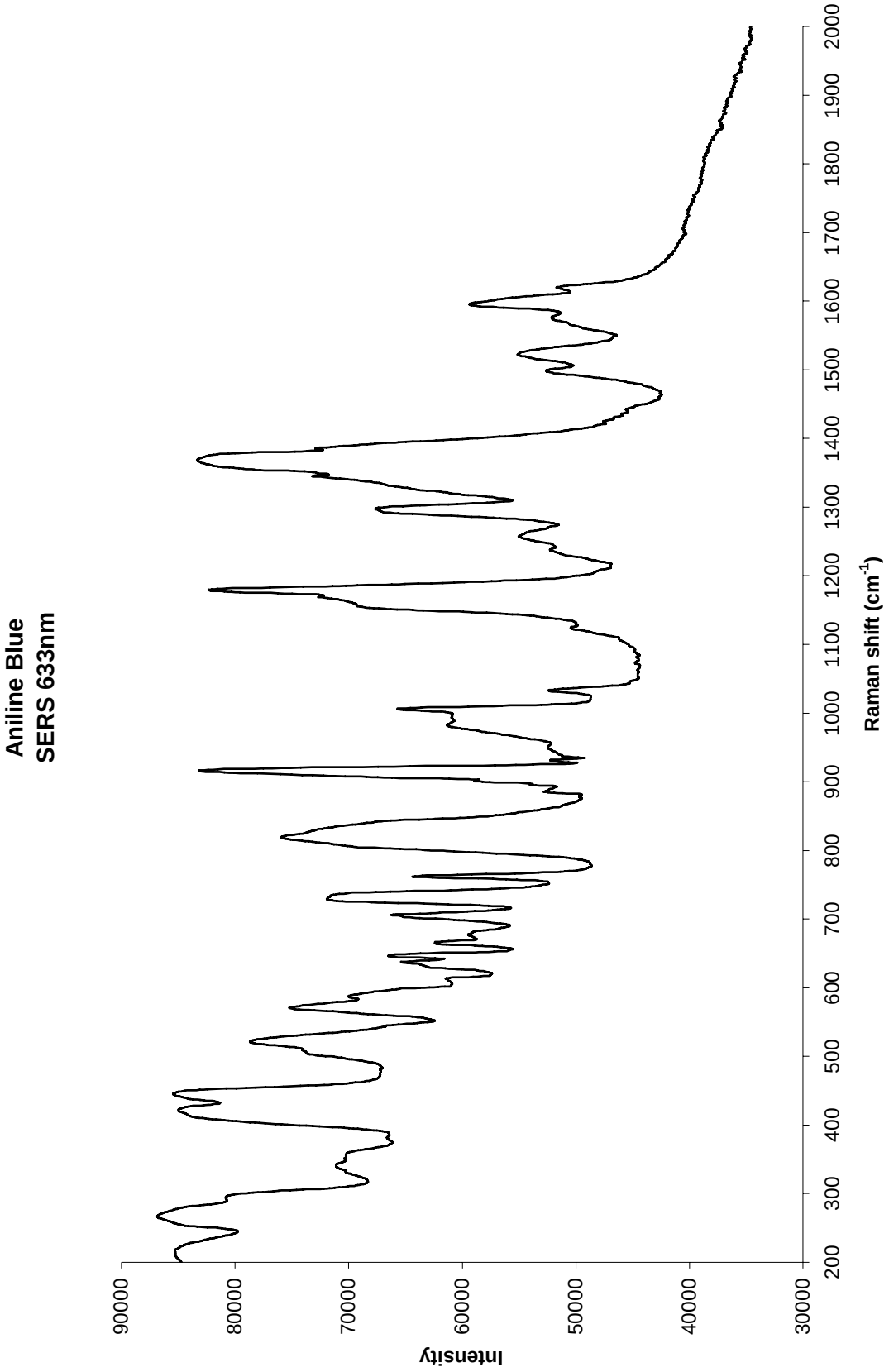
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
			222	219
			267	
			342	336.5
416	412.5	413.9	421.5	411.5
436.5	445	448.6	445	445.5
			521.5	522.5
529.5	526	533.4		
	572	566.2	571	568.5
			587.5	
619		612.5	613.5	
	636		637.5	625
			646	645
			664.5	
			677.5	672.5
691.5	687.5	693.5		
			706	702
730.5		730.1	729	730
756.5		759.1	762	760.5
	799			805.5
826		832.0	822	832.5
	842.5			
	856.5			
	866			
		878.6	885.5	
916		917.2	916	915
	964.5	959.0	951	951.5
			982.5	
1005	1001	1005.9	1006	1006
		1027.1	1033	1023
		1075.4	1073	
		1125.5	1124	1129

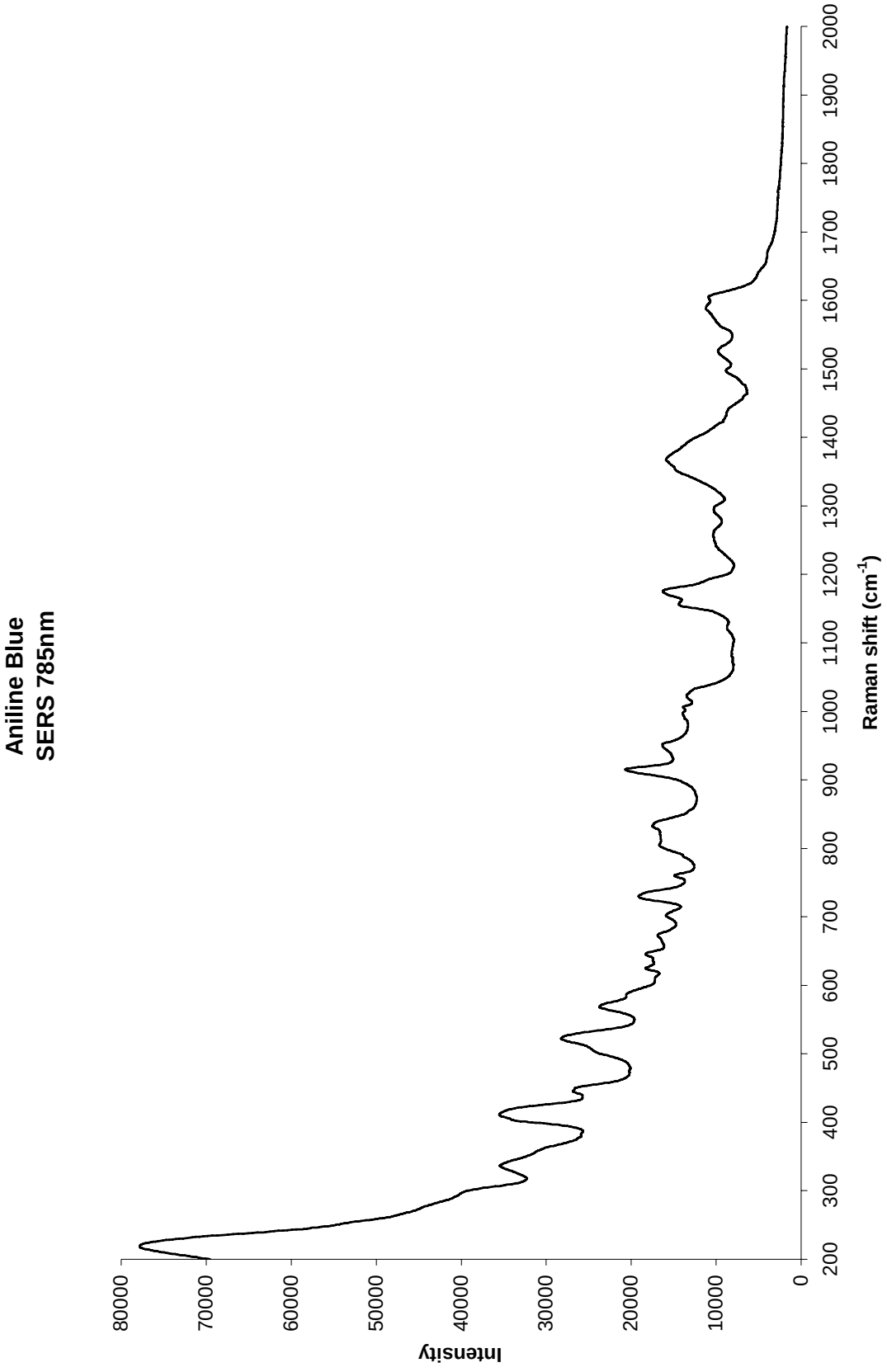
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
	1155			1156
		1167.9		
1179	1181		1179.5	1175.5
	1199			
		1229.6	1237.5	
			1257.5	1262.3
1280				
		1297.1	1297.5	1300
	1347.5		1345	
1368		1364.6	1368.5	1367
	1441.5			
	1456.5	1455.0		
1501		1495.8	1498.5	1497.5
1518		1524.7	1522	1526.5
		1572.9	1576.5	
1585				1589
		1601.8	1595.5	1605
1614			1620	

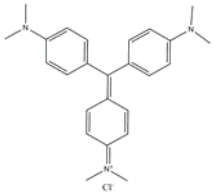








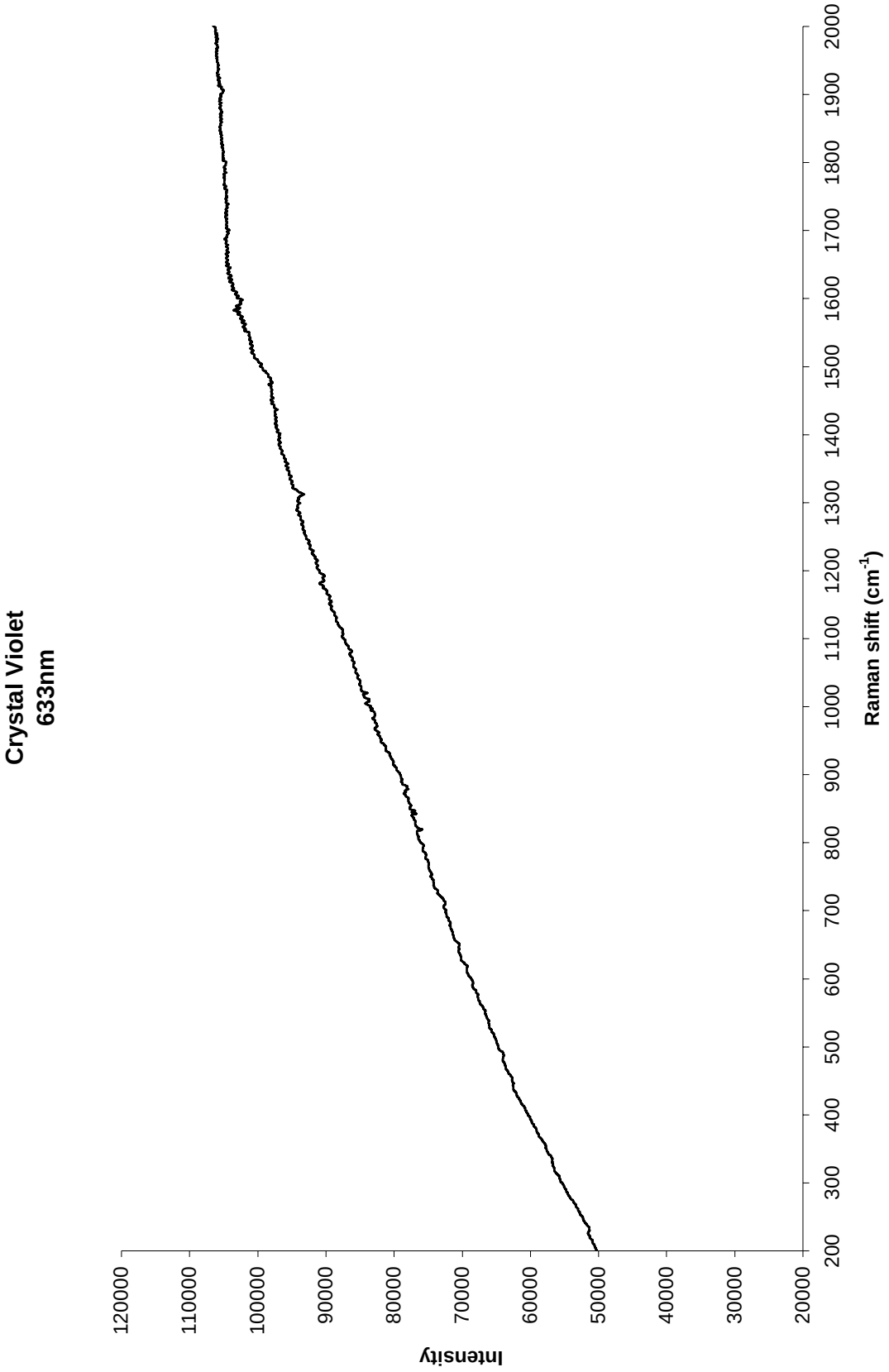


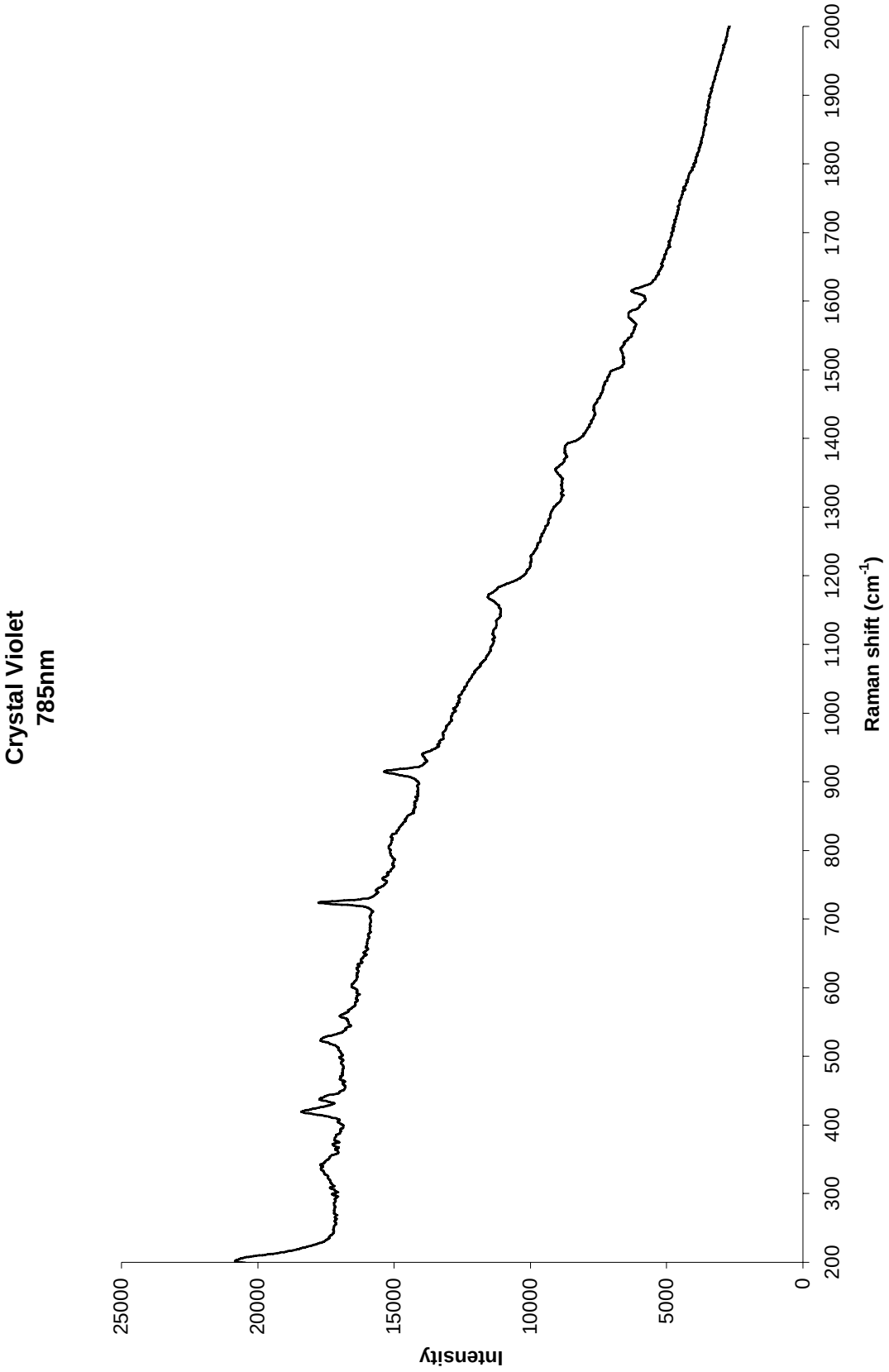


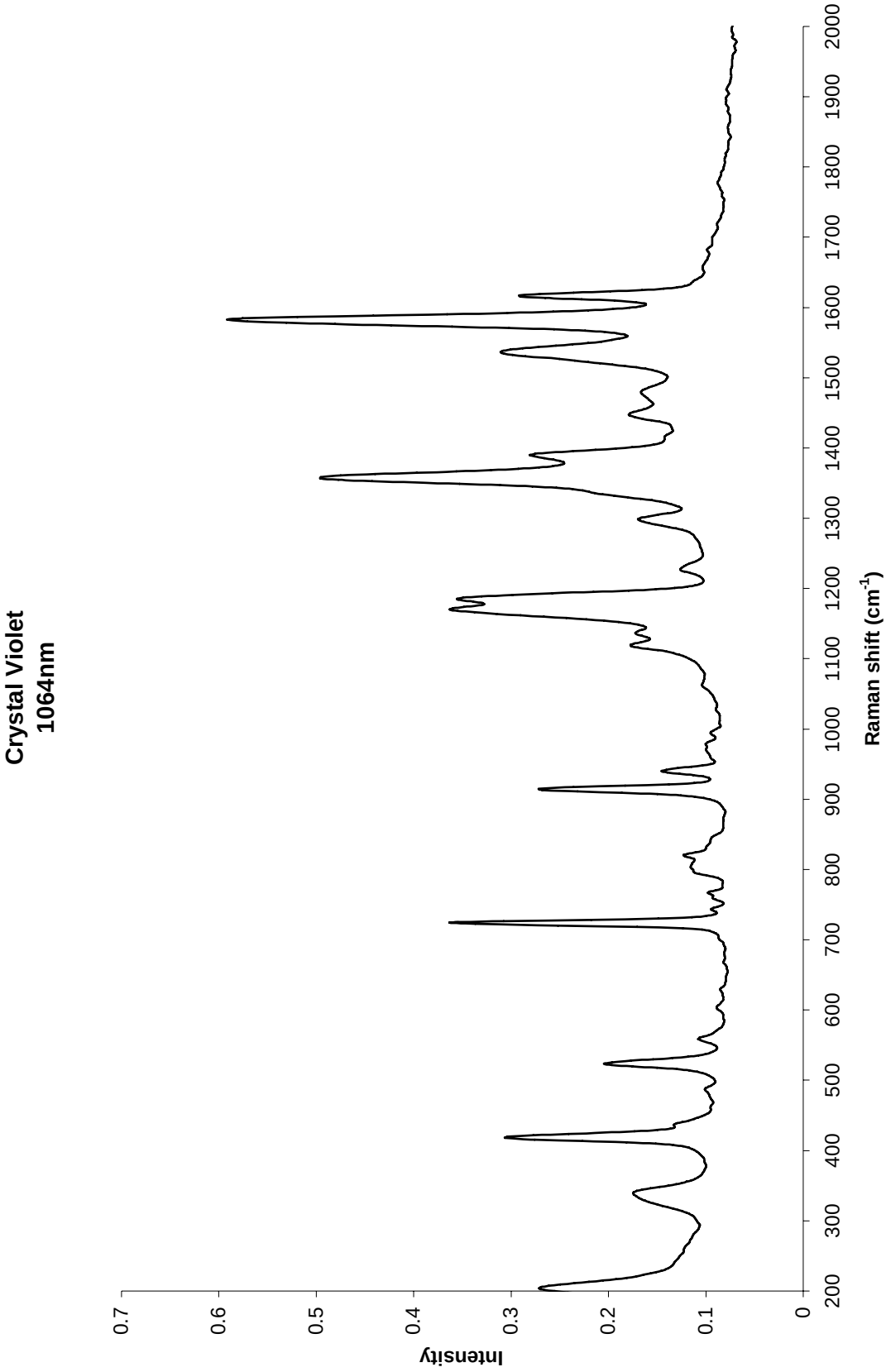
Crystal Violet
CAS 548-62-9; CI 42555
Alternate names: Basic Violet 3; Gentian Violet

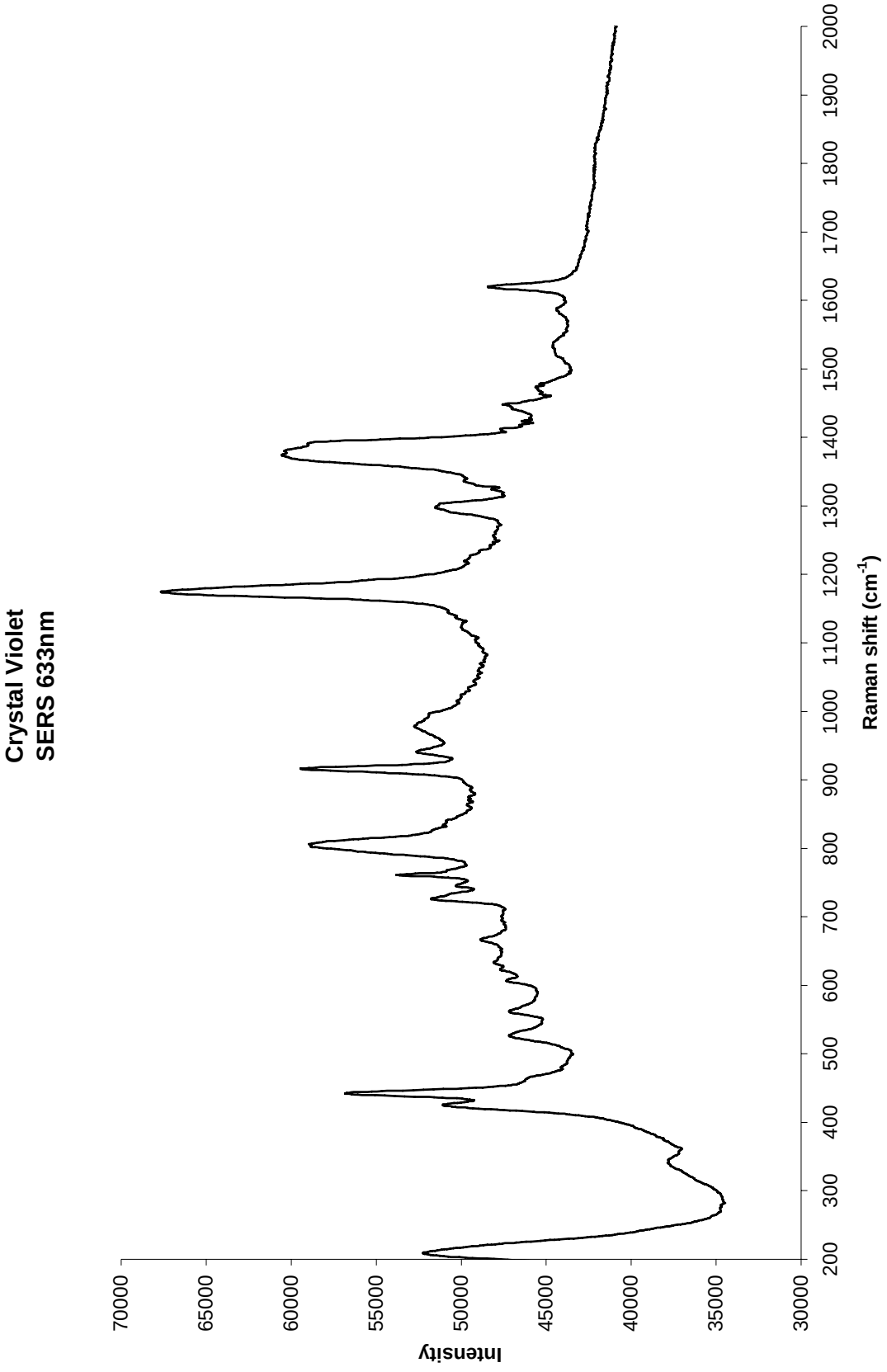
Table 5. Crystal Violet Raman peaks (cm^{-1})

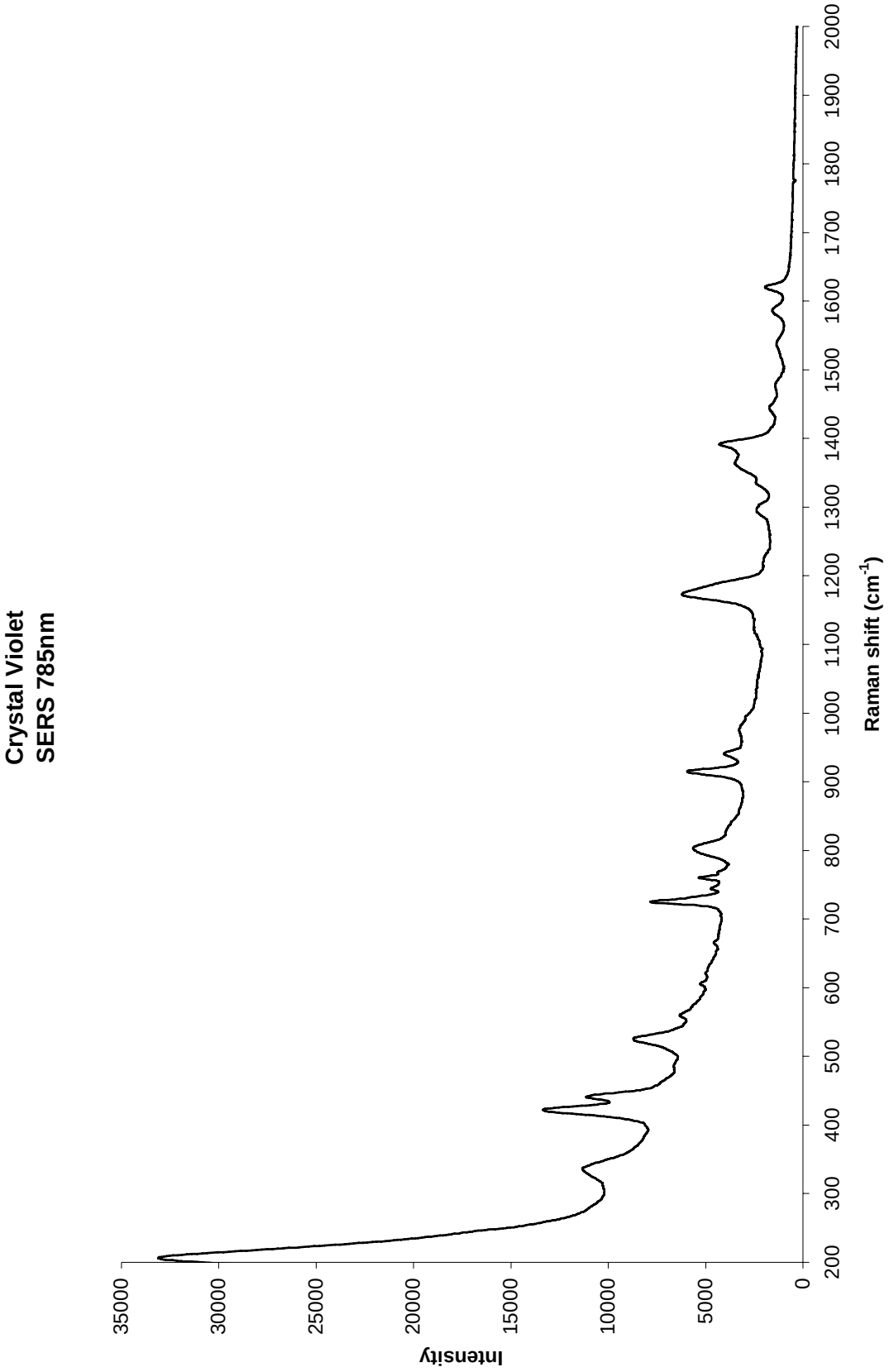
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
	205	209.0	206.5	208.5
	342	338.5	341.5	336.5
	419.5	417.7	425.5	422
	440		442	441
		487.2		491
	523.5	523.8	526.5	526
	559	558.5	562	560
598	601.5	604.8	607	605.5
				621
			633.5	
			666.5	666
	724	724.4	726	725
			745.5	744.5
	761.5	766.8	761	760.5
	803.5	809.0	806	803.5
		820.8		
	914.5	915.3	916	915
	940.5	940.4	940.5	941
982.5		978.9	978.5	975.5
1110		1119.7		
		1144.0		
1181	1169	1169.9	1174.5	1173
		1187.0		
		1227.7		
		1299.1	1298	1296.5
	1355	1356.9	1349	1364
	1393	1389.7	1387	1391.5
1457		1447.6	1448	1446
1473.5		1480.3	1473.5	1482
1538	1531	1536.3	1541	1545
1583	1582	1582.6	1588.5	1586
	1615	1617.3	1620	1620.5



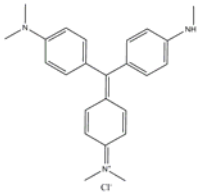








Methyl Violet



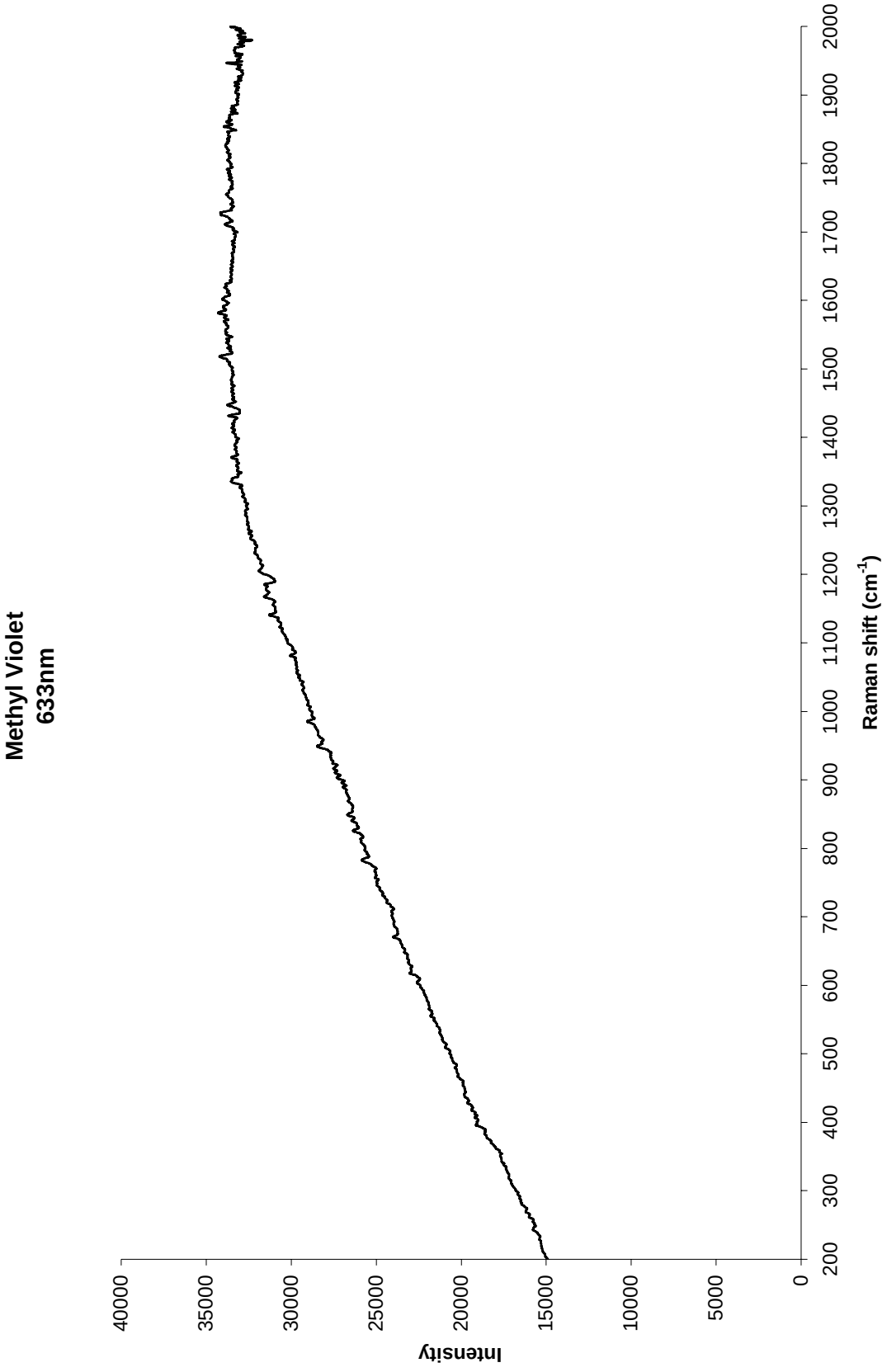
CAS 8004-87-3; CI 42535

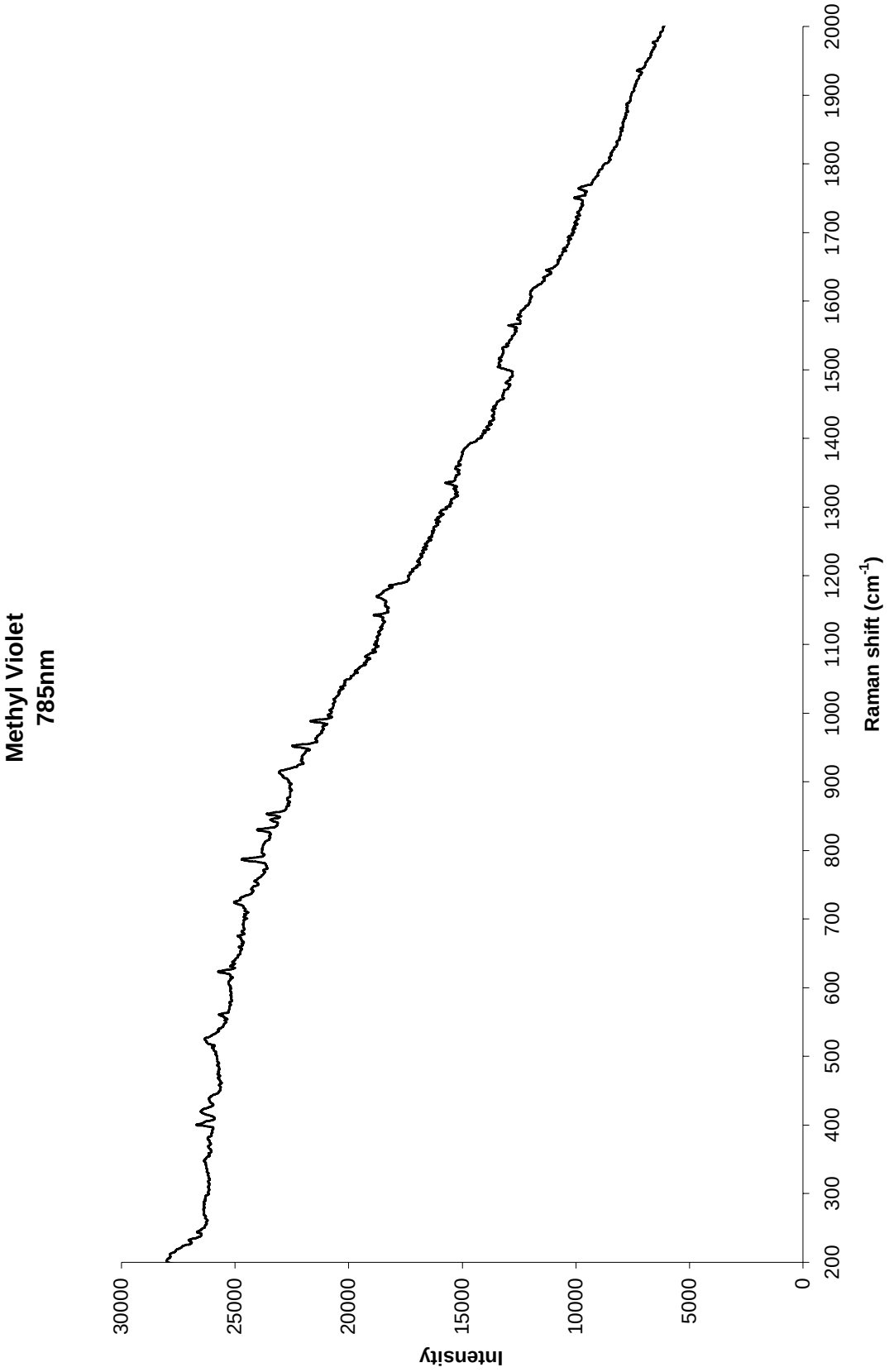
Alternate name: Methyl Violet 2B

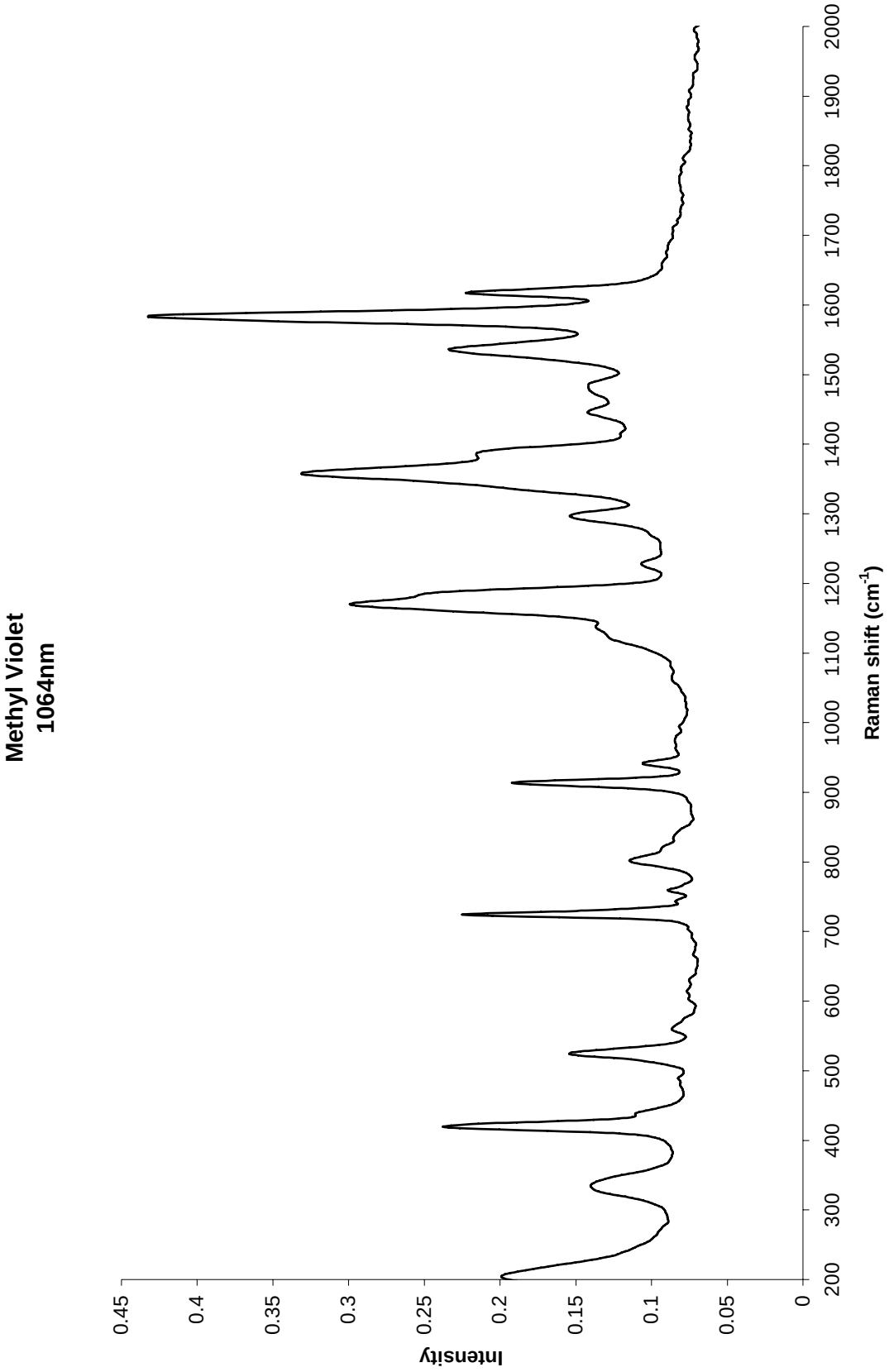
Table 6. Methyl Violet Raman peaks (cm^{-1})

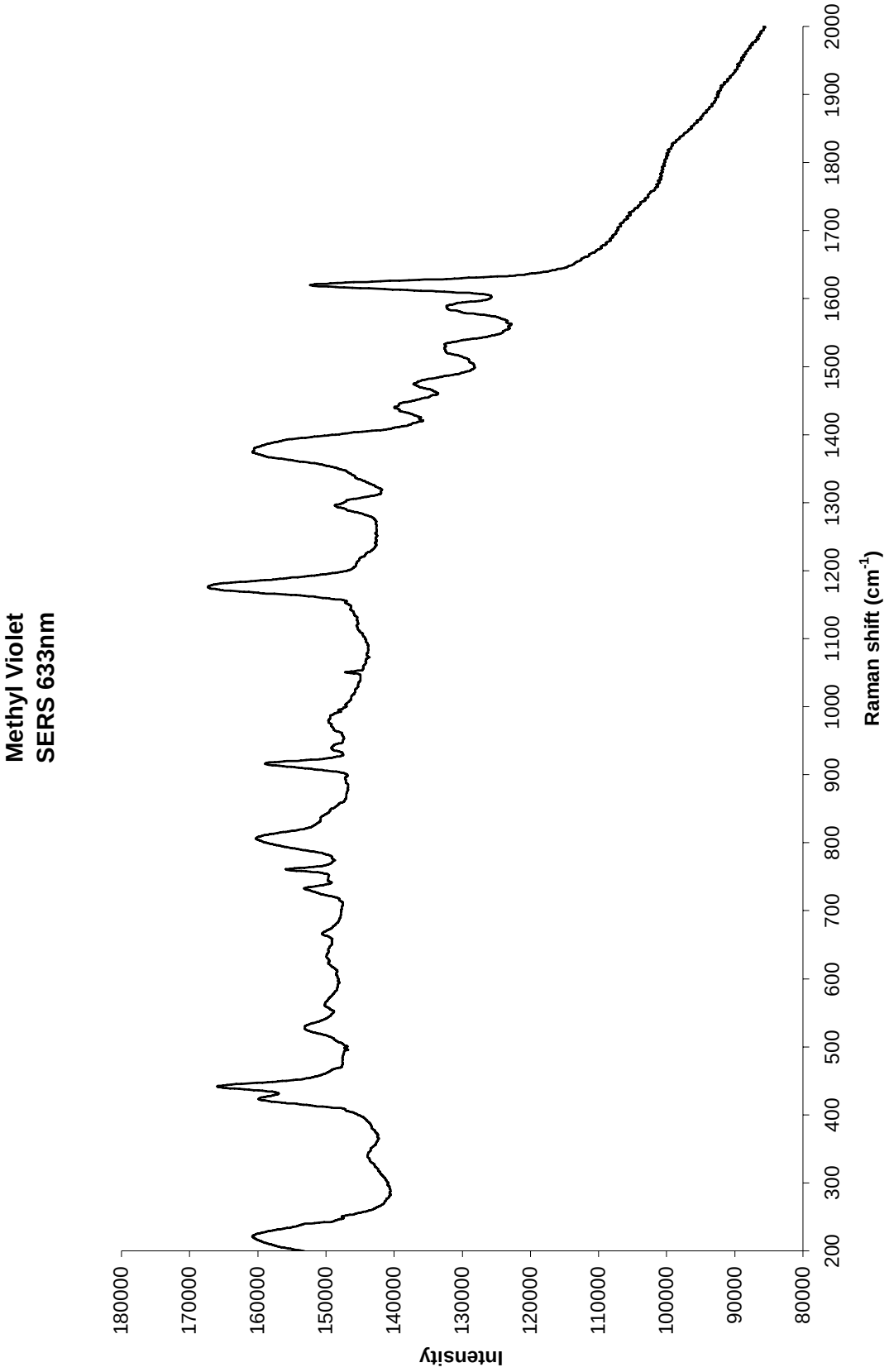
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
		207.0		
			221.5	222
		334.8	339.5	335.5
402	401			
	420	419.7	423.5	416
	443		441.5	438.5
	526	523.8	527.5	525.5
	561	560.4	561.5	
624	624			
			666.5	666.5
678	676			
	725	724.4		
			733	731
		759.1	761	760
786	787.5			
		801.5	806.5	800.5
833	830.5			
856	853.5			
	914	913.4	916.5	914
		942.3	939.5	938.5
954	952.5			
				965.5
985	989		983	996
1141	1143	1144.0		
1174	1170.5	1169.9	1176	1172.5
1185.5				
		1227.7		
		1297.1	1296	1295.5
1336	1335.5			
		1356.9		
1378			1384	

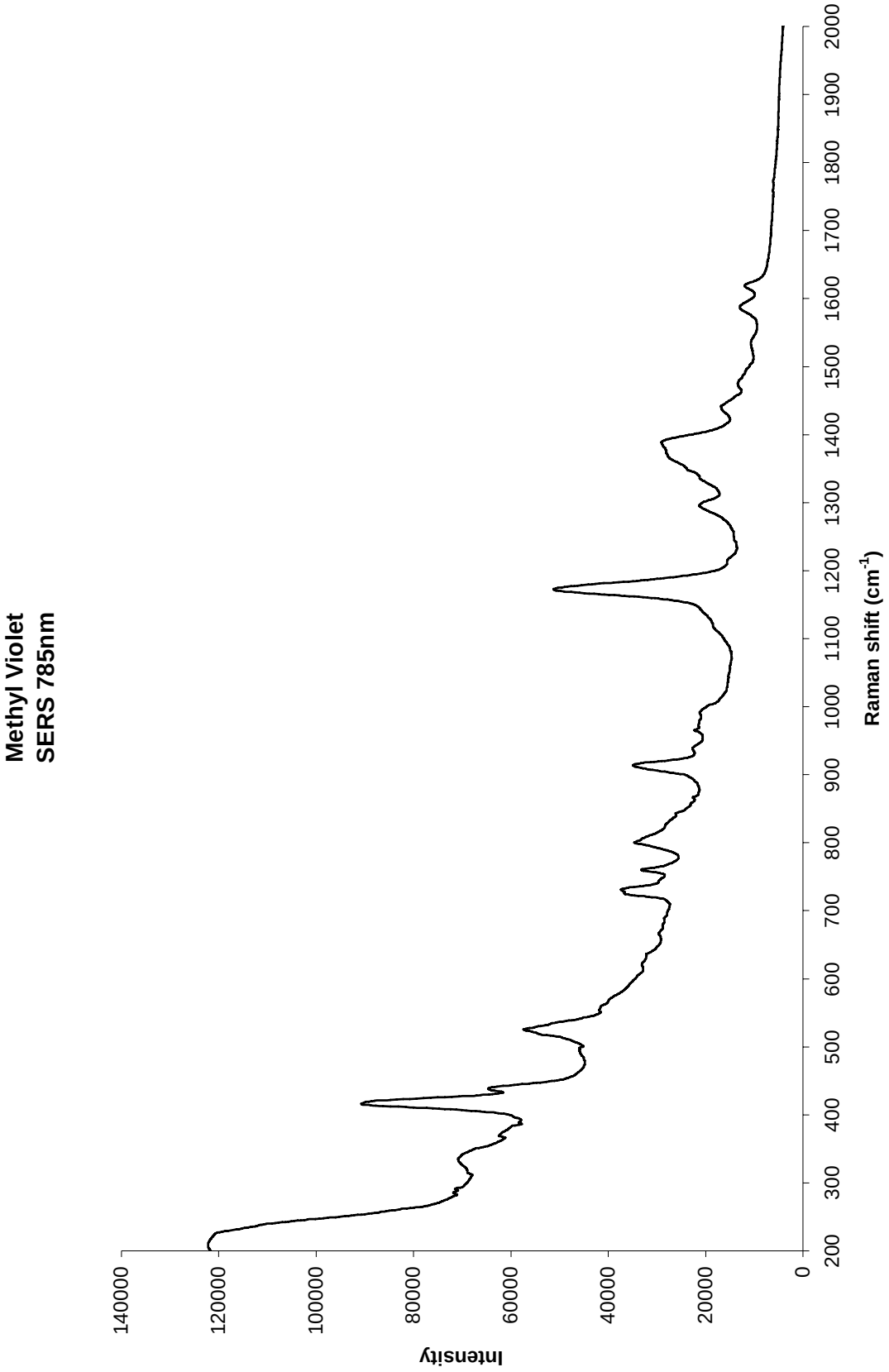
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
		1389.0		1389
1434				
1447.5		1445.6	1440	1441.5
		1484.2	1480	1488
	1509.5			
1520		1536.3	1535	1542
	1565			
1582		1582.6	1588.5	1587.5
1619		1617.3	1620	1618.5
1728				
1755	1751			
	1767			



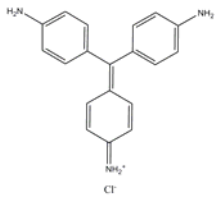








Pararosaniline

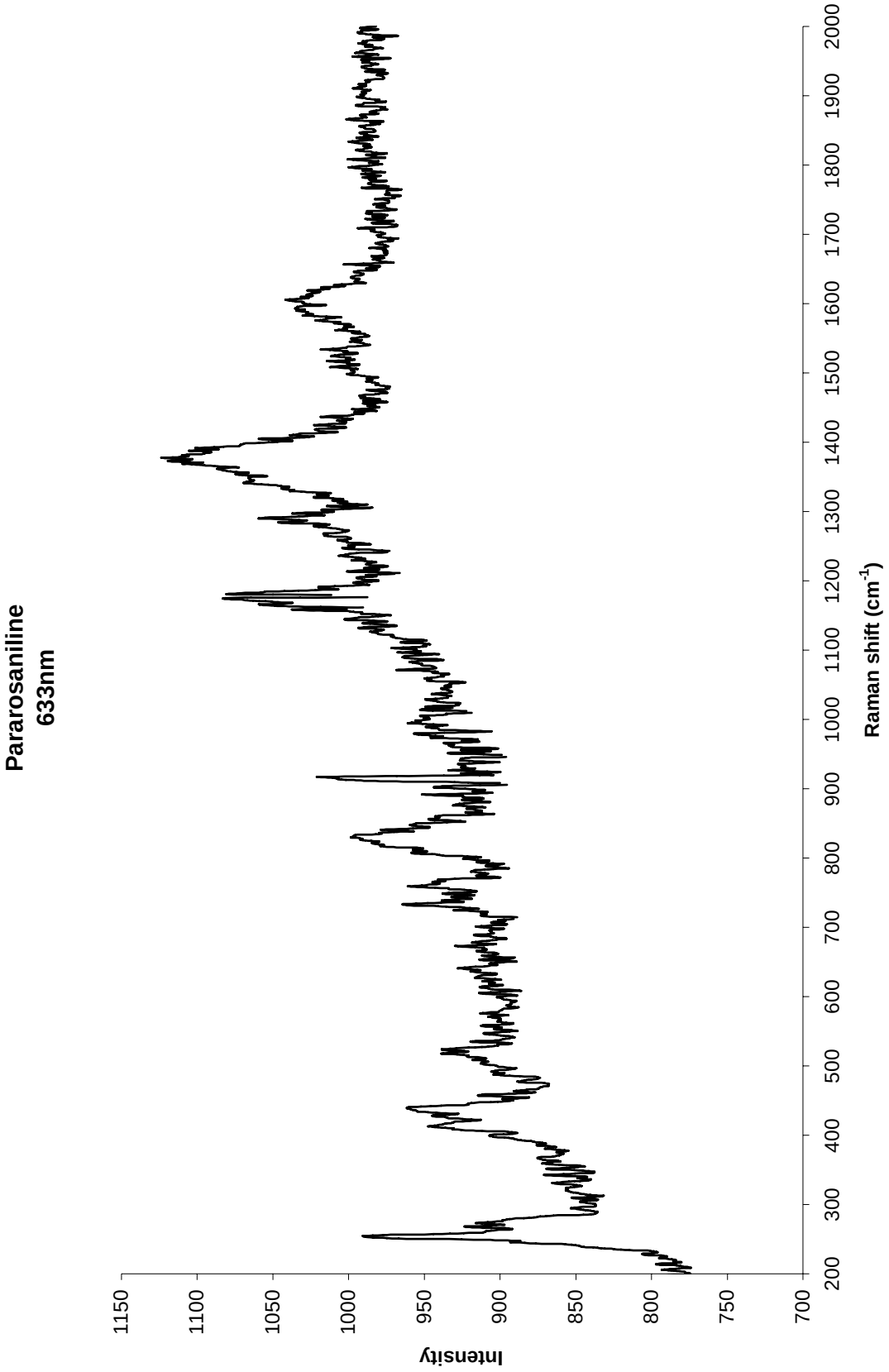


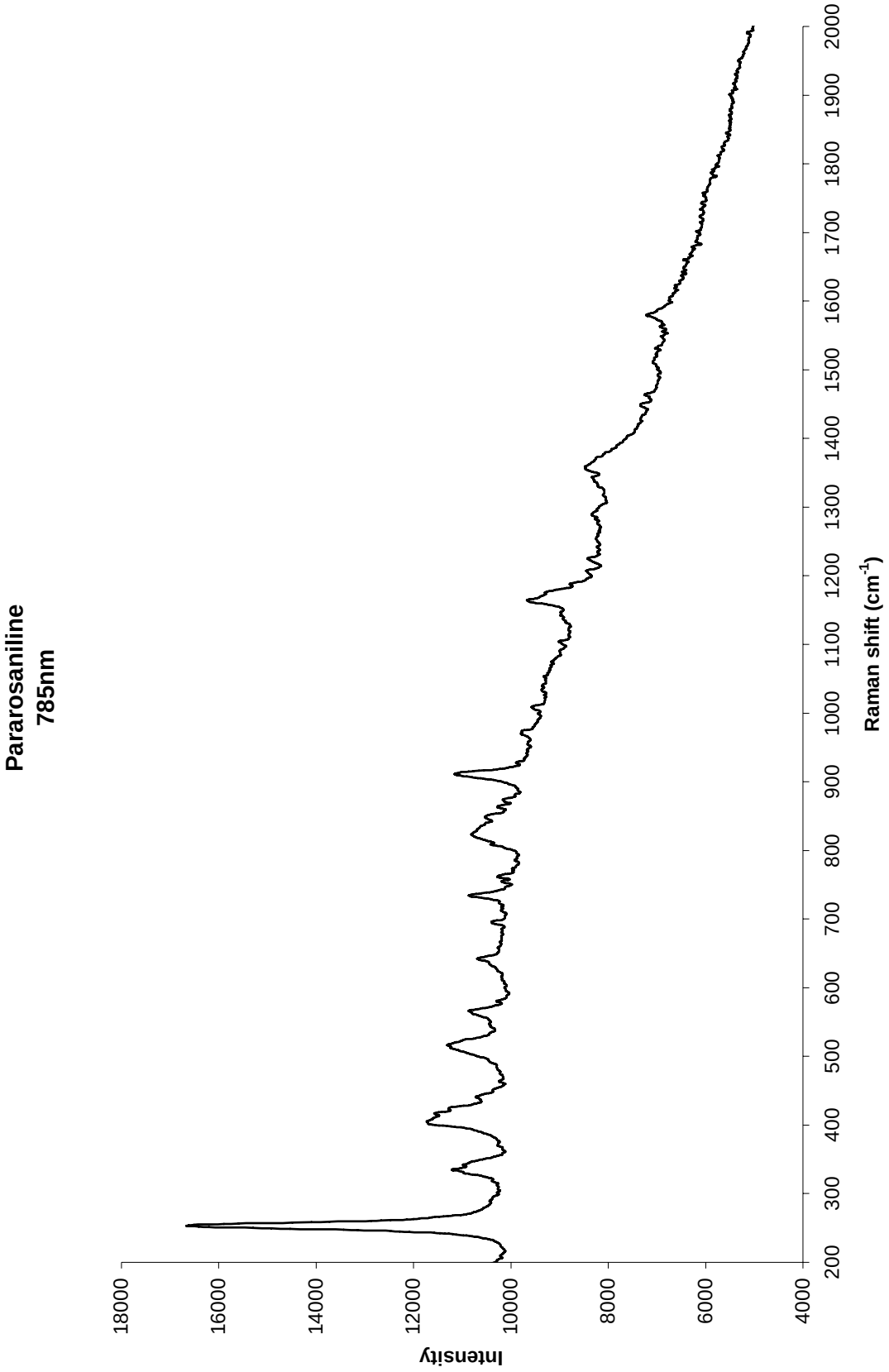
CAS 569-61-9; CI 42500
Alternate name: Basic Red 9

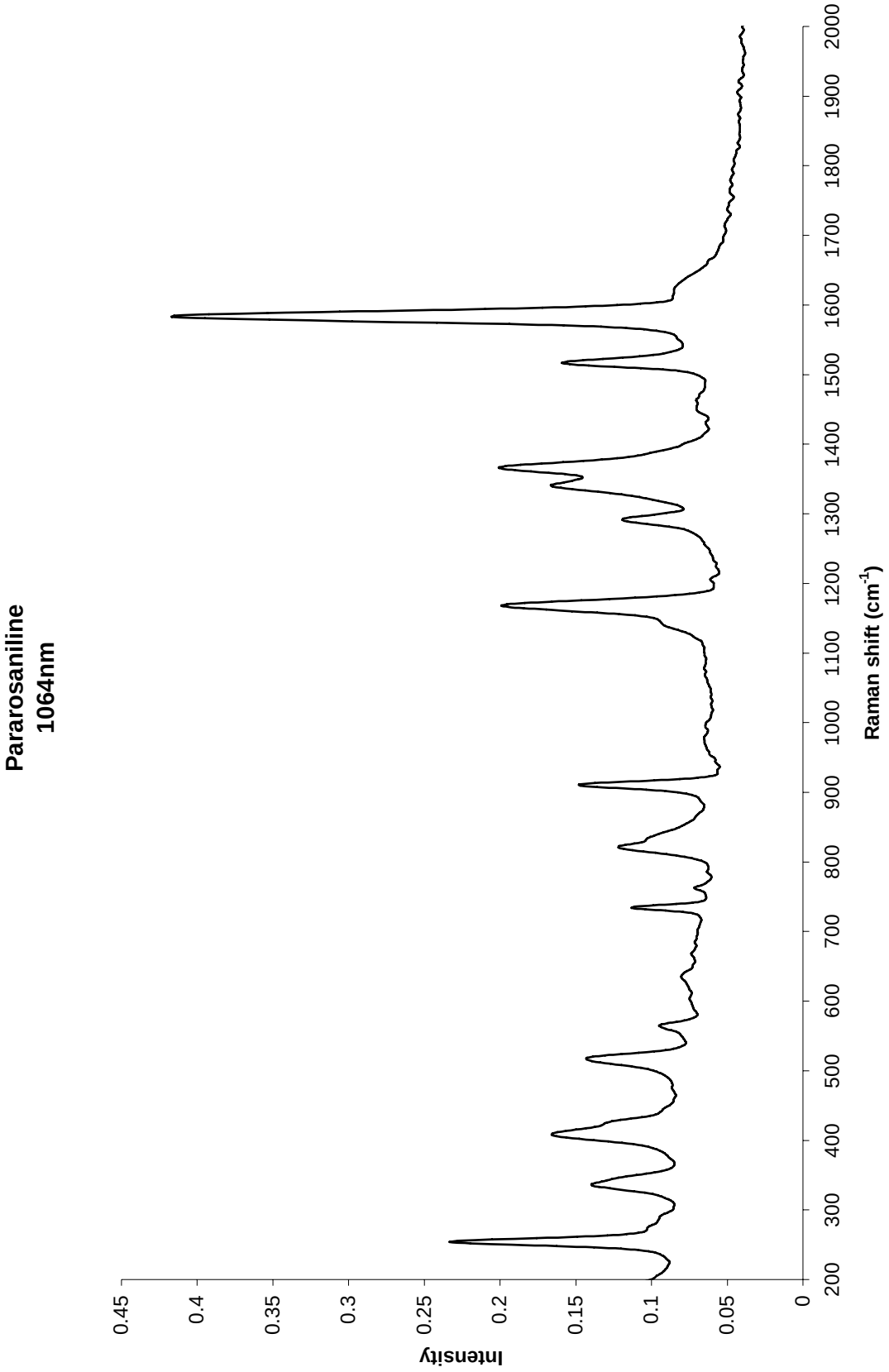
Table 7. Pararosaniline Raman peaks (cm^{-1})

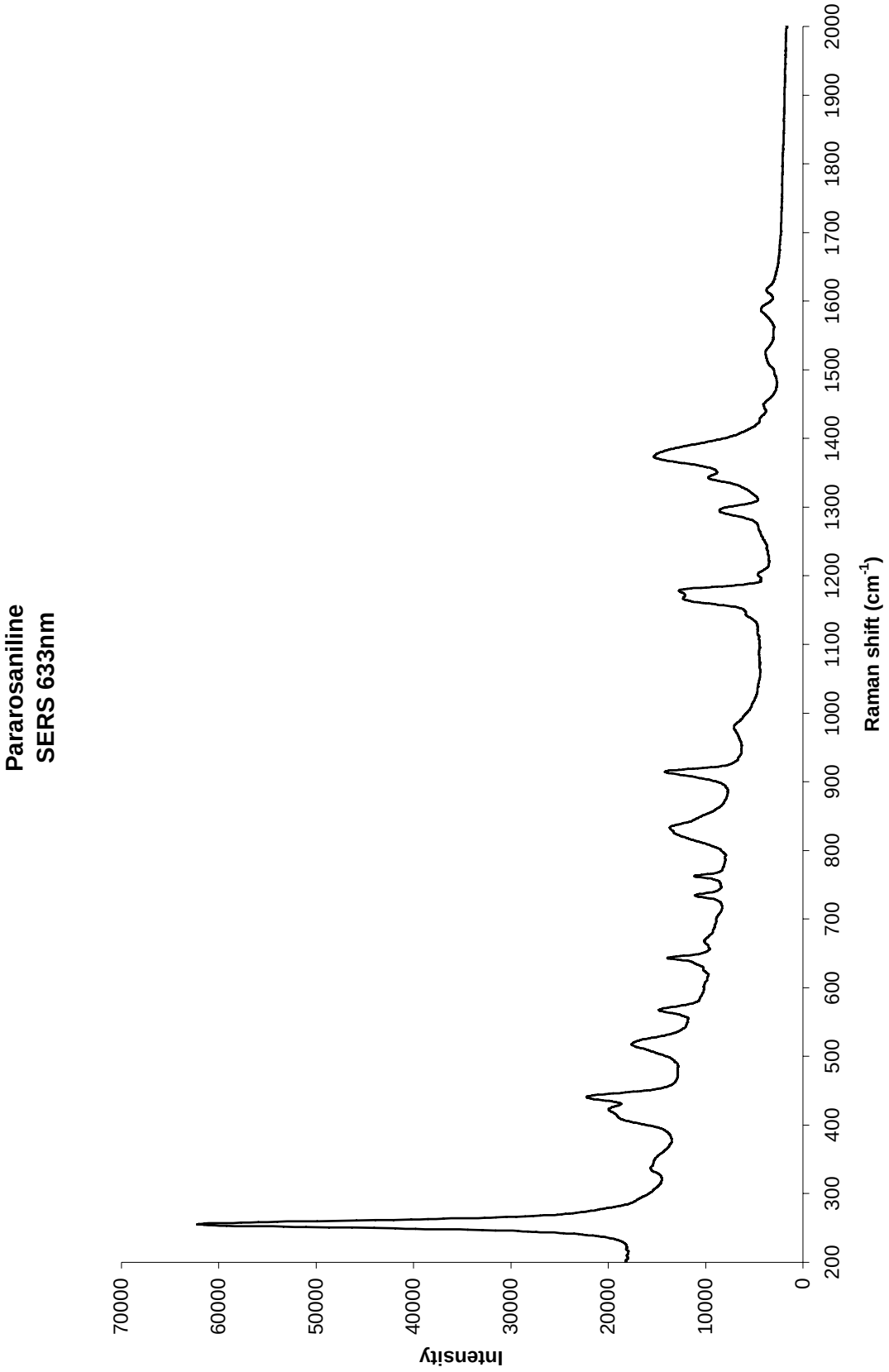
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
254.5	253.5	253.8	255.5	255.5
268				
	335	336.7	337.5	338.5
	405	408.1		
414				413
			422.5	
439.5	441		440.5	
	463.5			
518	516.5	518.0	517.5	520
	566	564.3	567.5	567.5
641.5	642	641.2	643.5	642.5
673			668	
	696			
733	734.5	734.0	734.5	734.5
760	761.5	762.9	762.5	762
	823	820.8		
830			833.5	833.5
	848.5			
917	911	911.4	914.5	906
	970			
979.5		977.0	980.5	977
	1009			
	1164.5	1167.9	1167	
1174.5			1178	1171.5
	1225			
1290	1290	1291.3	1295	1295
		1341.5	1343	1349
	1364	1366.6		
1377.5			1373.5	1379.5
	1448		1449.5	1451
	1463.5	1463.0		
		1517.0	1525	1521

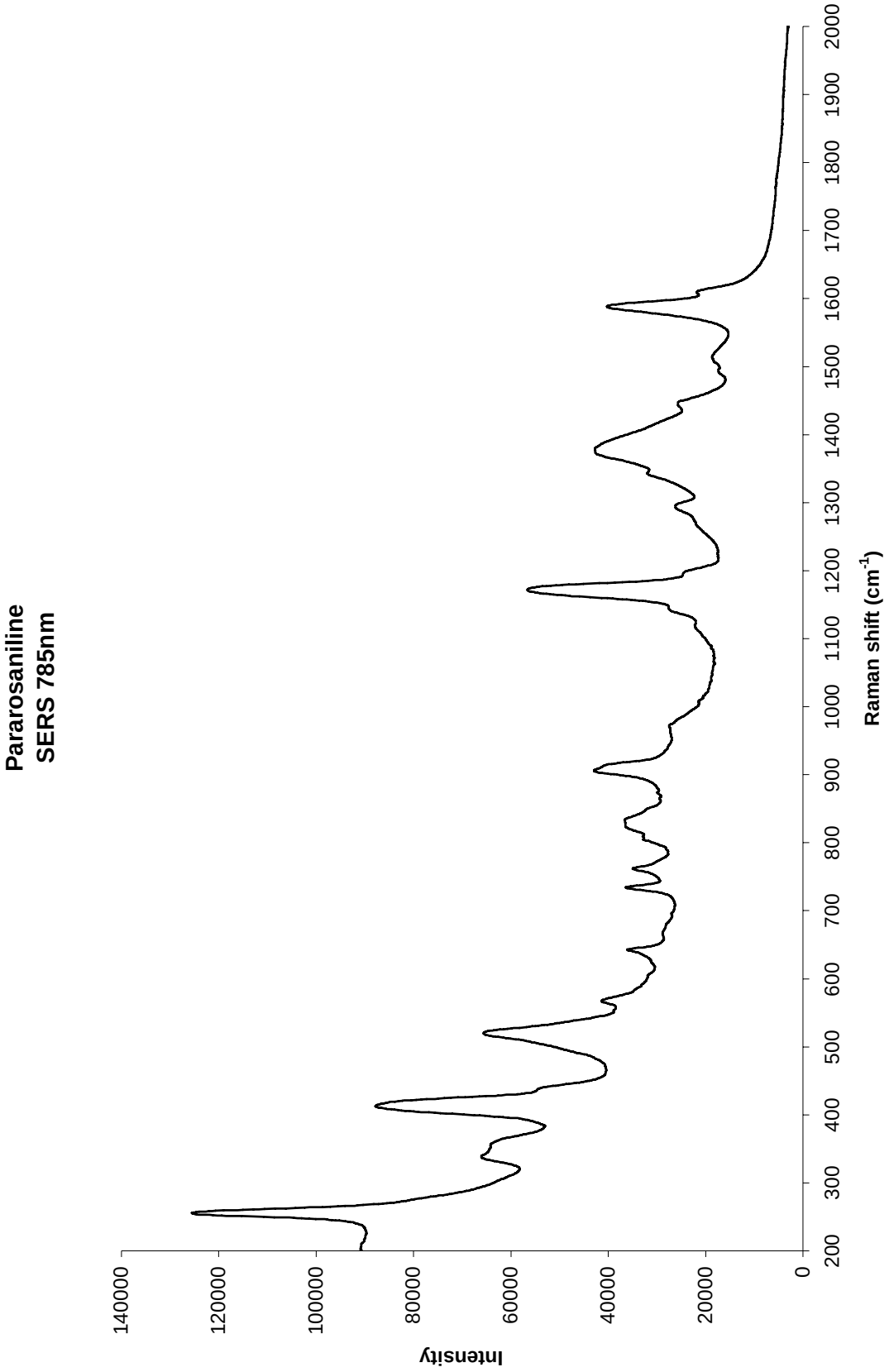
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
	1579.5	1582.6	1588	1588
1605.5				
			1616	1613



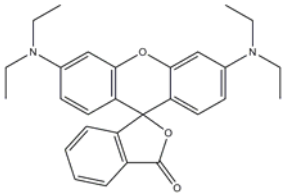








Rhodamine B base



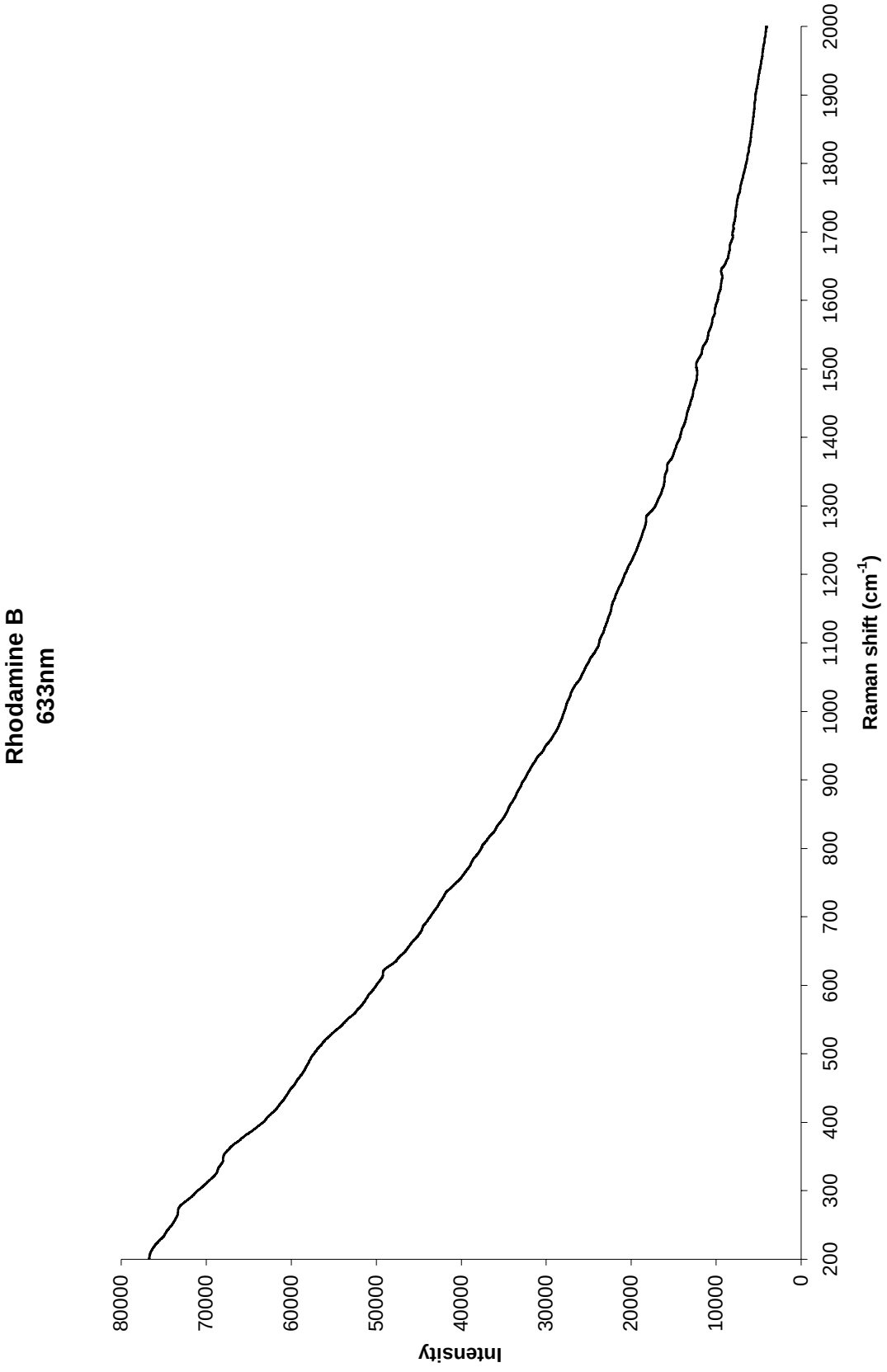
CAS 81-88-9; CI 45170

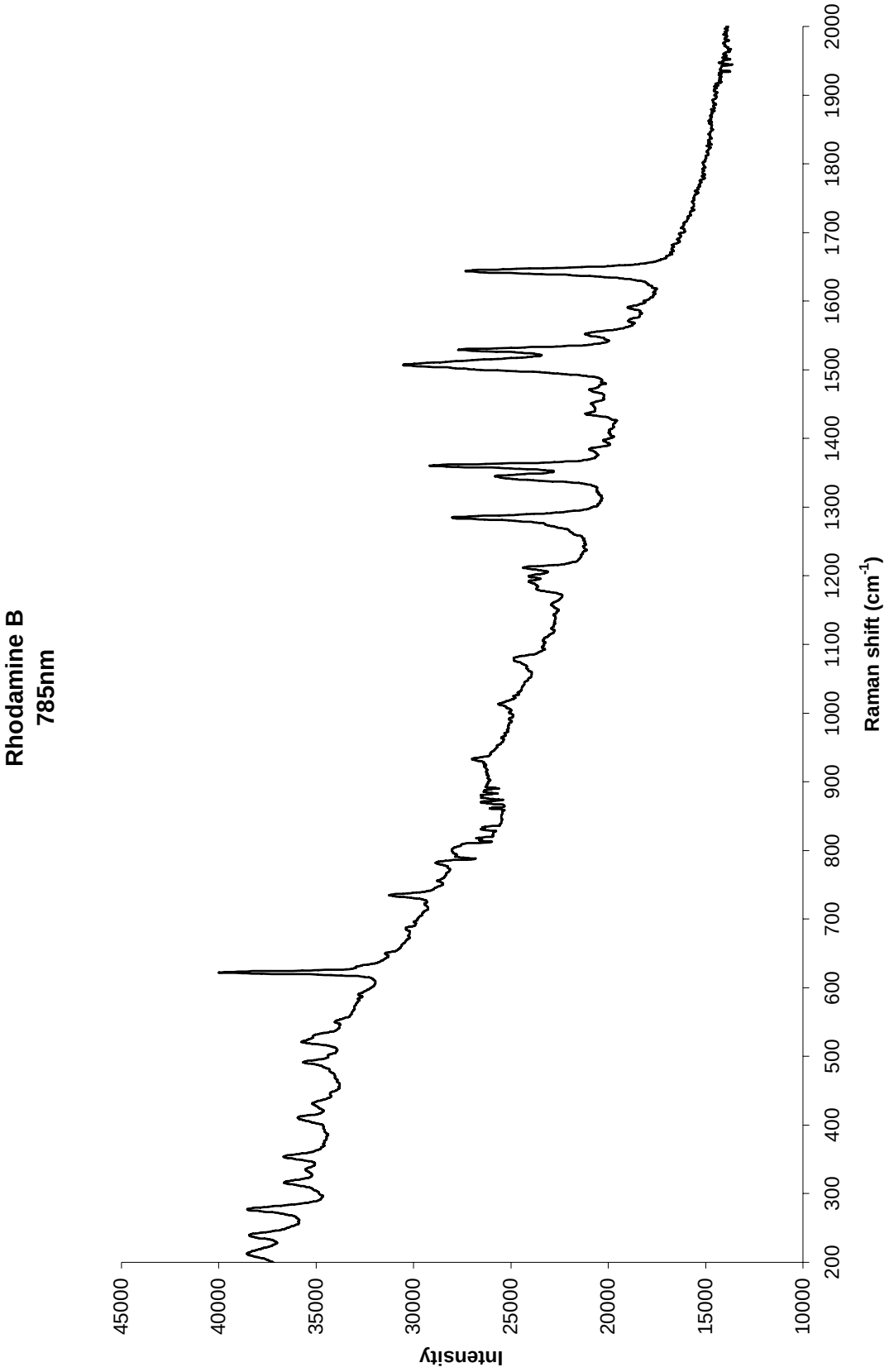
Alternate names: Basic Violet 10, Solvent Red

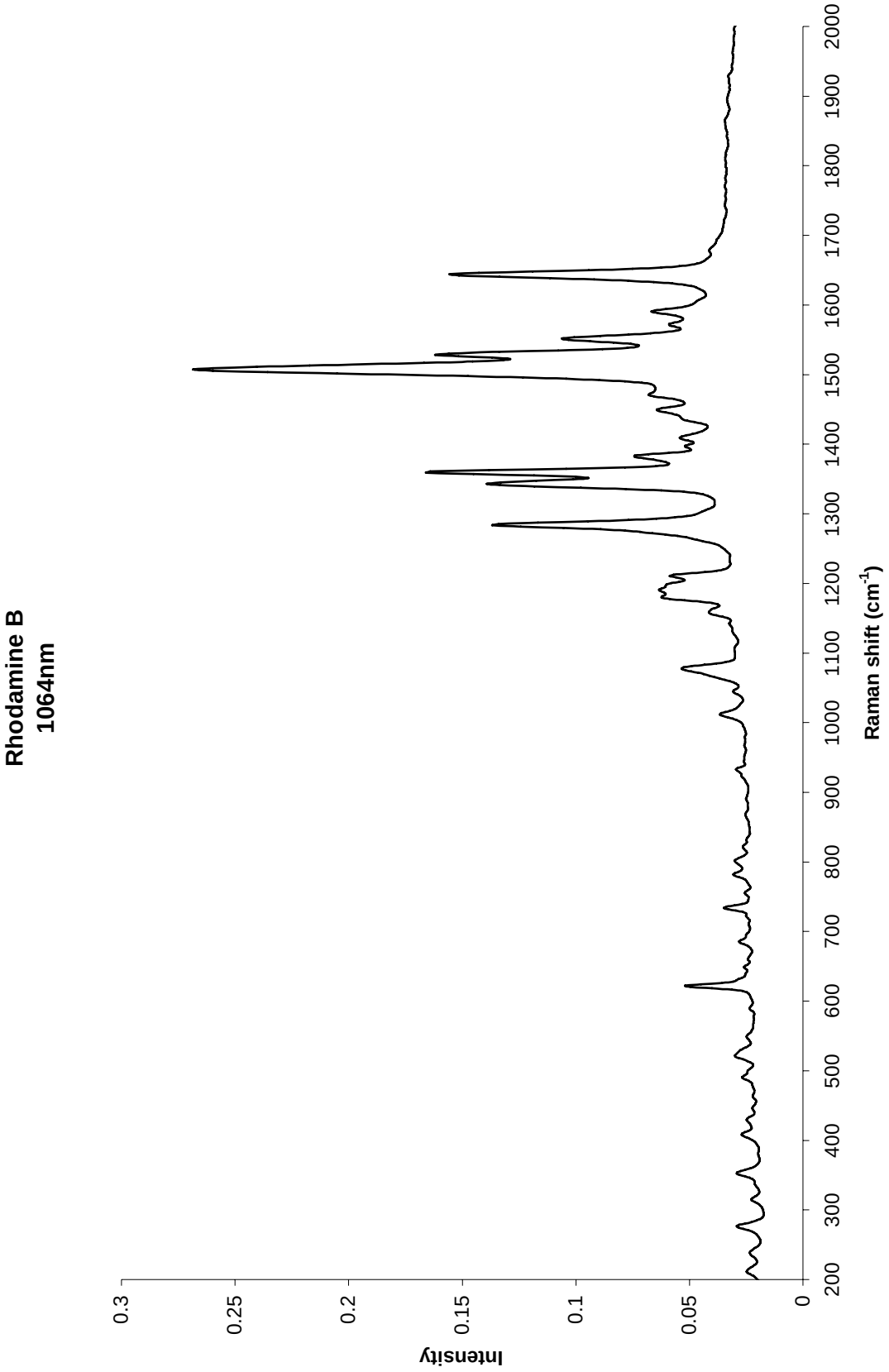
Table 8. Rhodamine B base Raman peaks (cm^{-1})

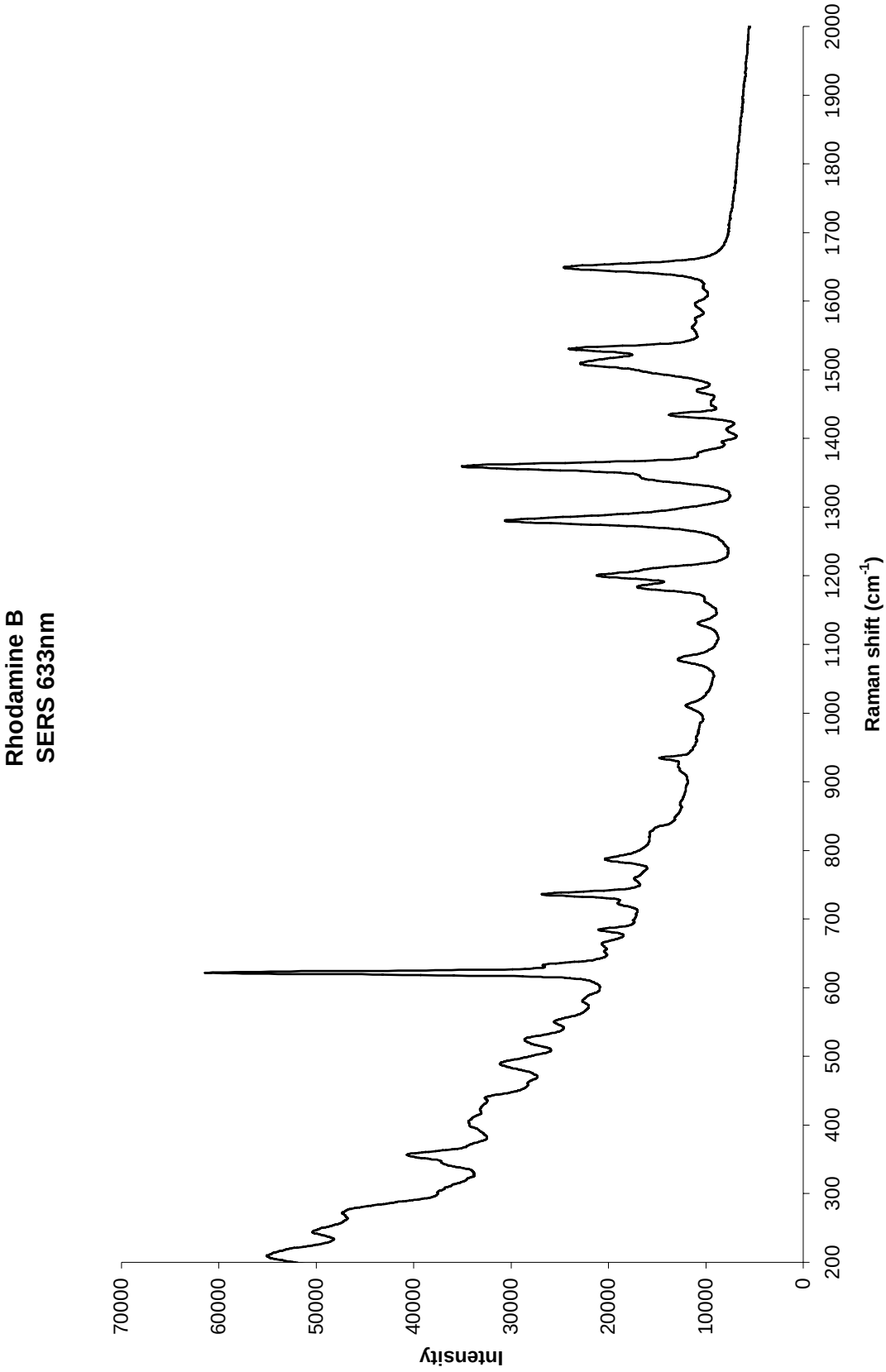
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
217.5	212.5	211.4	209.5	
	239.5	238.4	244.5	241
275.5	277	276.9	277	
	316	315.5		
354	354	352.2	356.5	356.5
	410.5	408.1	405	401.5
	431	435.0	433	
495	491.5	491.0	489	487
	521	521.9	524.5	521
			550.5	550
623	622	622.2	622	621.5
688.5		685.8	684.5	684.5
741	735	734.0	736	737
788	782	786.0	787	789
		806.0		
				823.5
925.5	933	932.6	934.5	924.5
1017.5	1013	1011.7	1011	1013.5
				1043
	1080	1077.3	1078.5	1076
1105				
			1131	1130
	1163	1164.0		1162.5
1177			1184	1183
	1192	1191.1		
	1214	1212.0	1200.5	1199
1286.5	1284.5	1283.6	1280	1280
1342.5	1345	1343.5		
	1360.5	1358.8	1359.5	1358.5
1367.5				
	1386	1385.8		1378

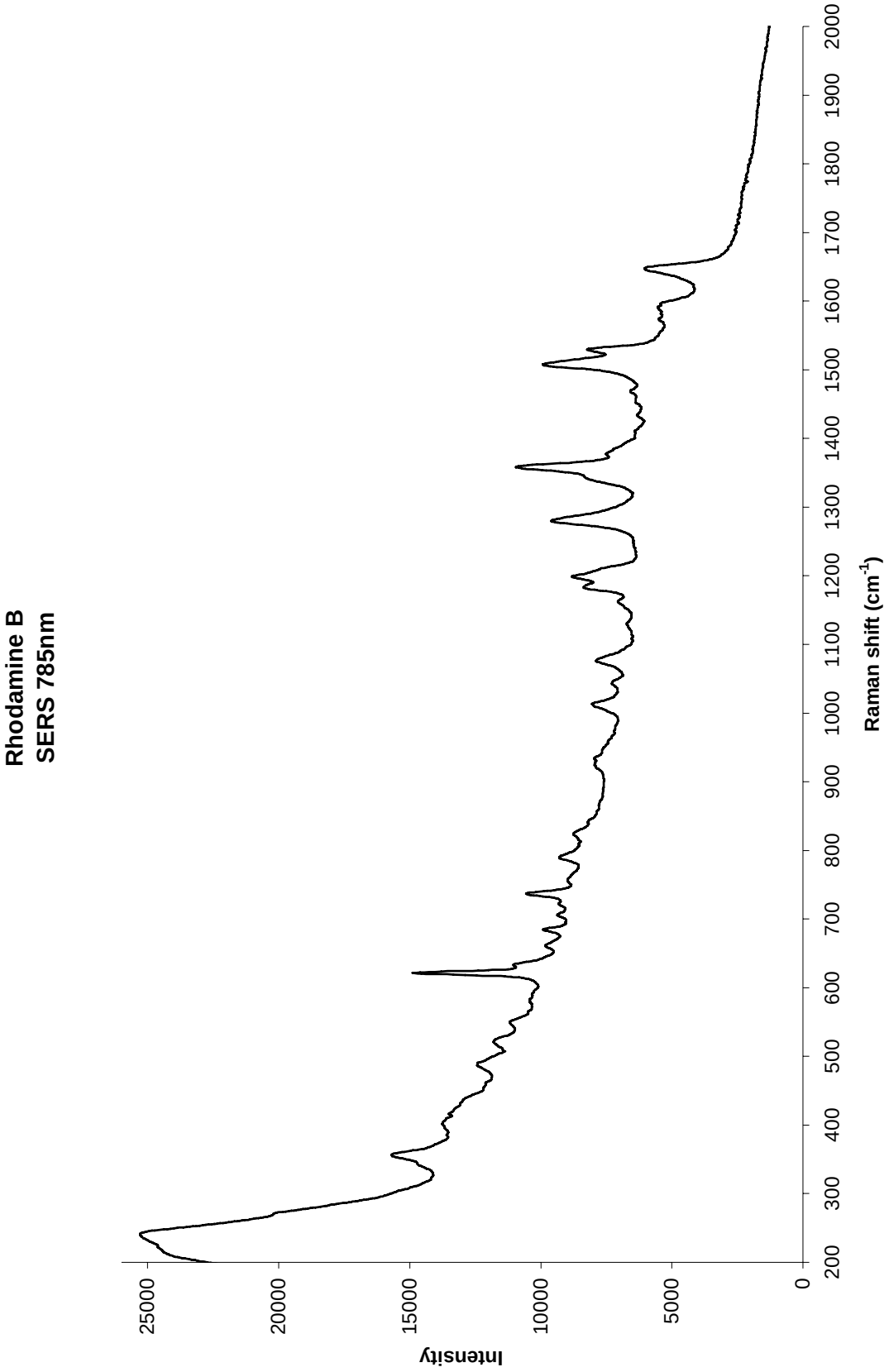
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
	1436		1434.5	1434
		1449.5		
	1471	1478.0	1470	1468
1509.1	1507	1507.3	1509.5	1507.5
1527	1529	1528.0	1530.5	1529.5
1549	1552.5	1553.6		
	1591	1590.3	1596.5	1591
1643	1644	1644.3	1649	1647.5



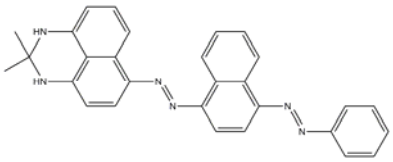








Sudan Black B

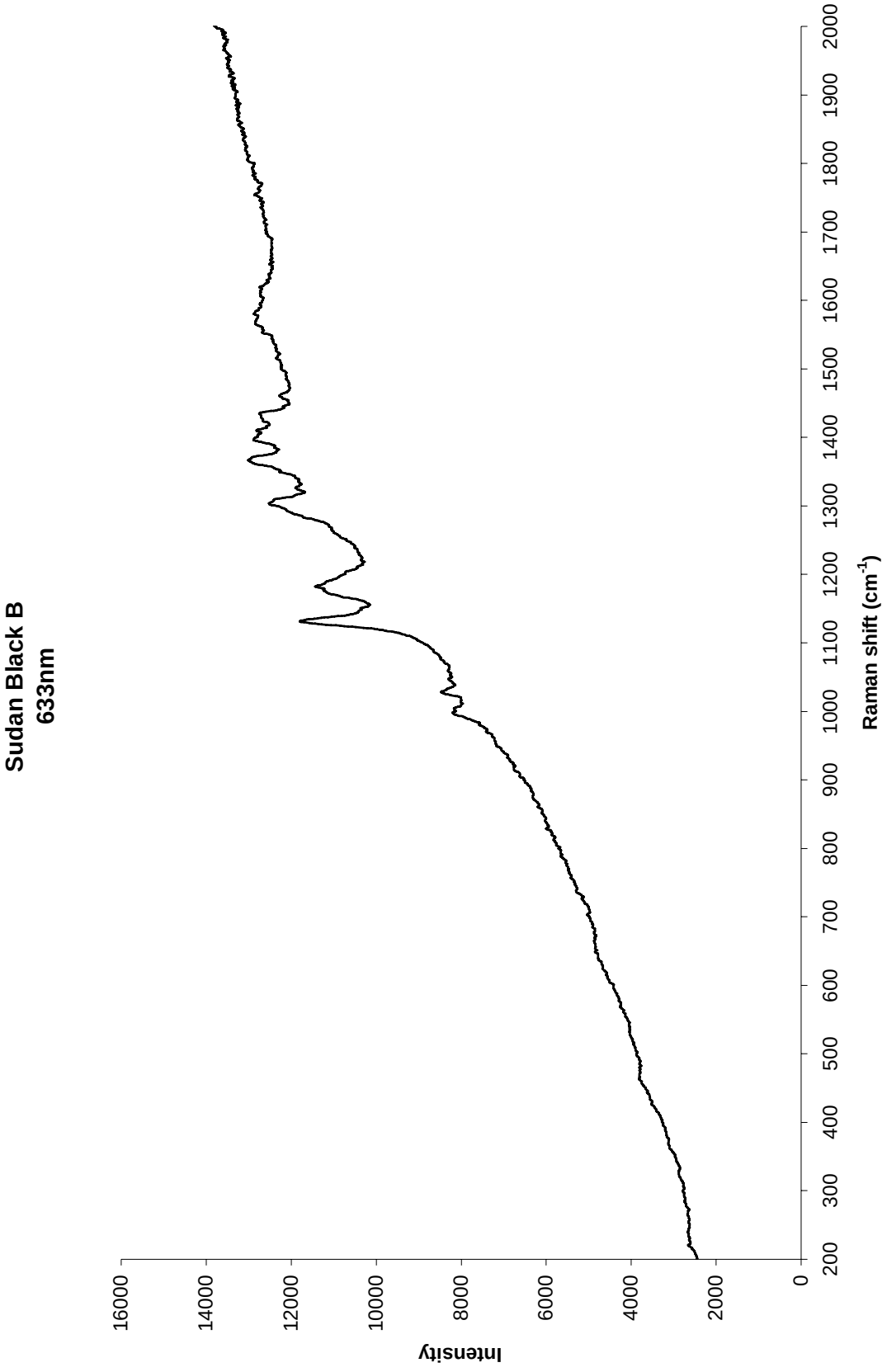


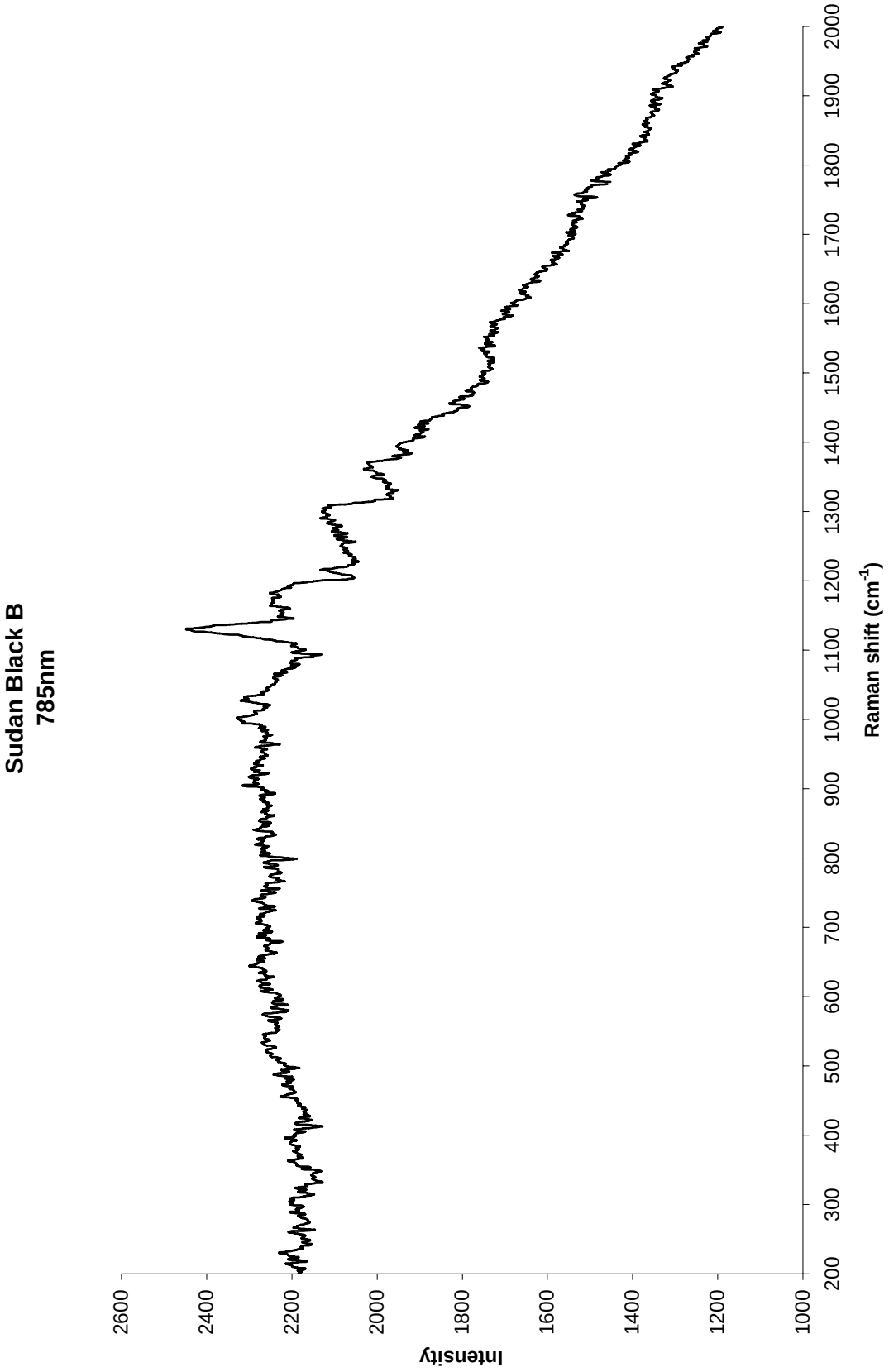
CAS 4197-25-5; CI 26150

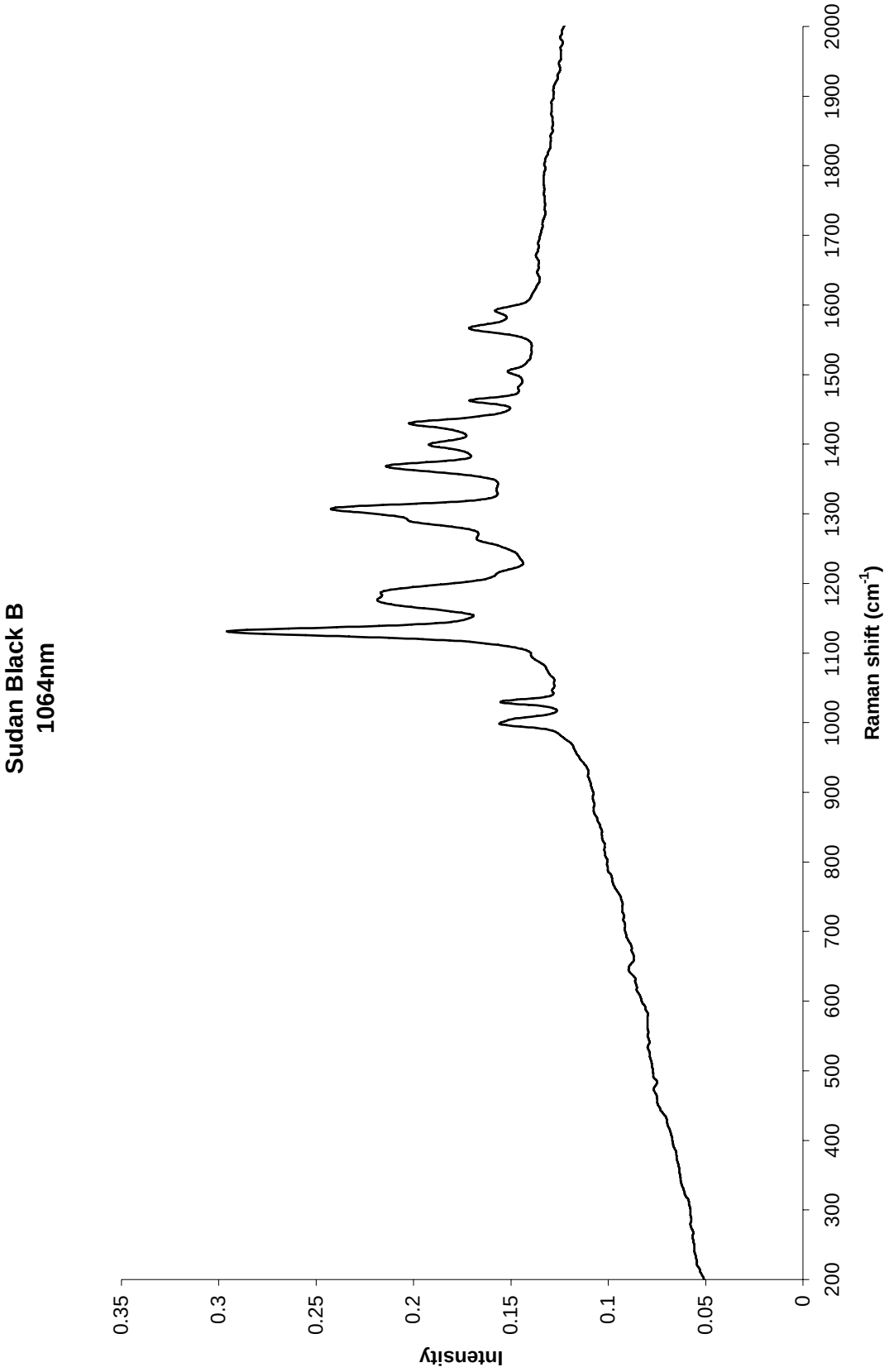
Alternate name: Solvent Black 3

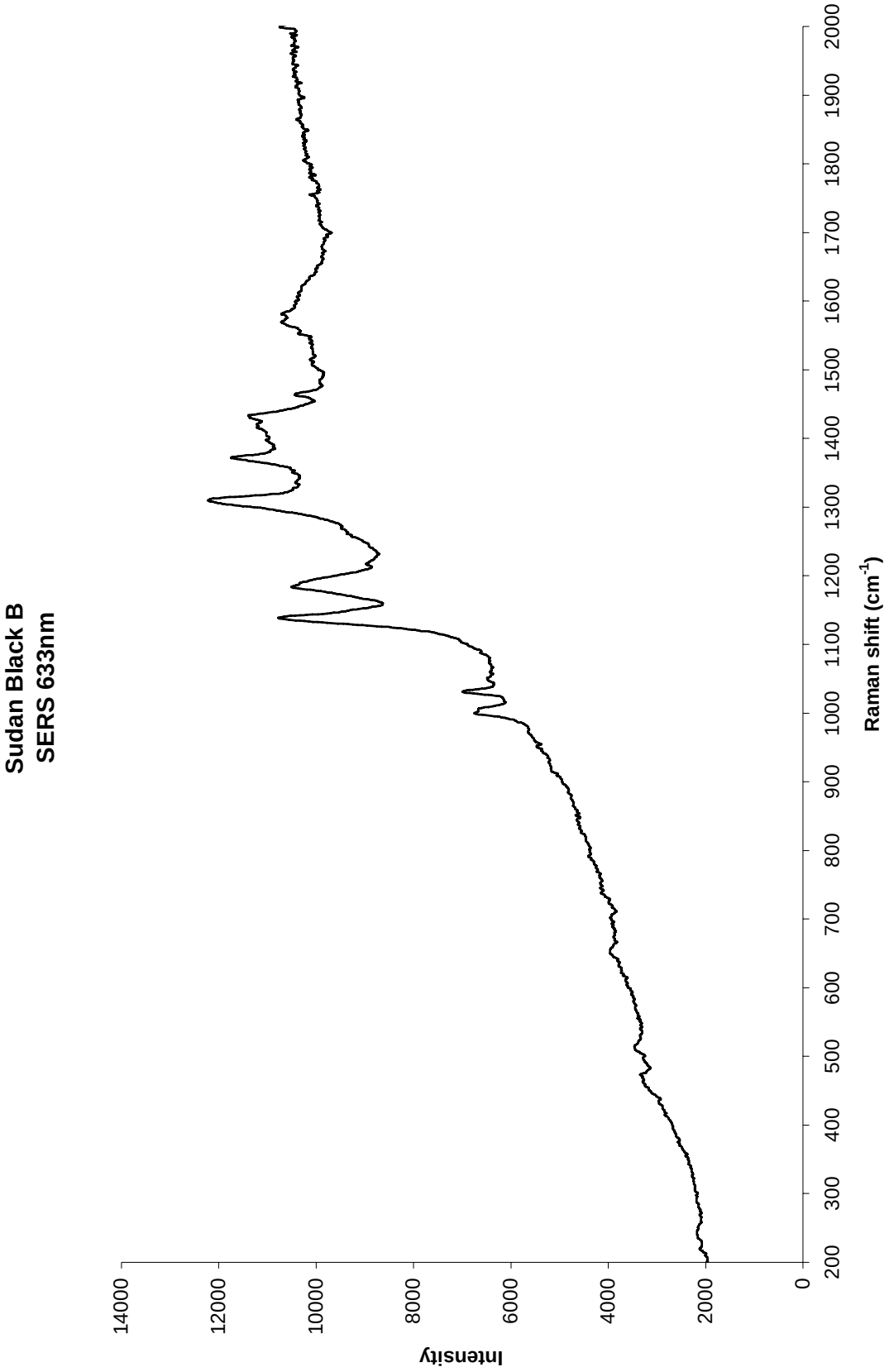
Table 9. Sudan Black B Raman peaks (cm^{-1})

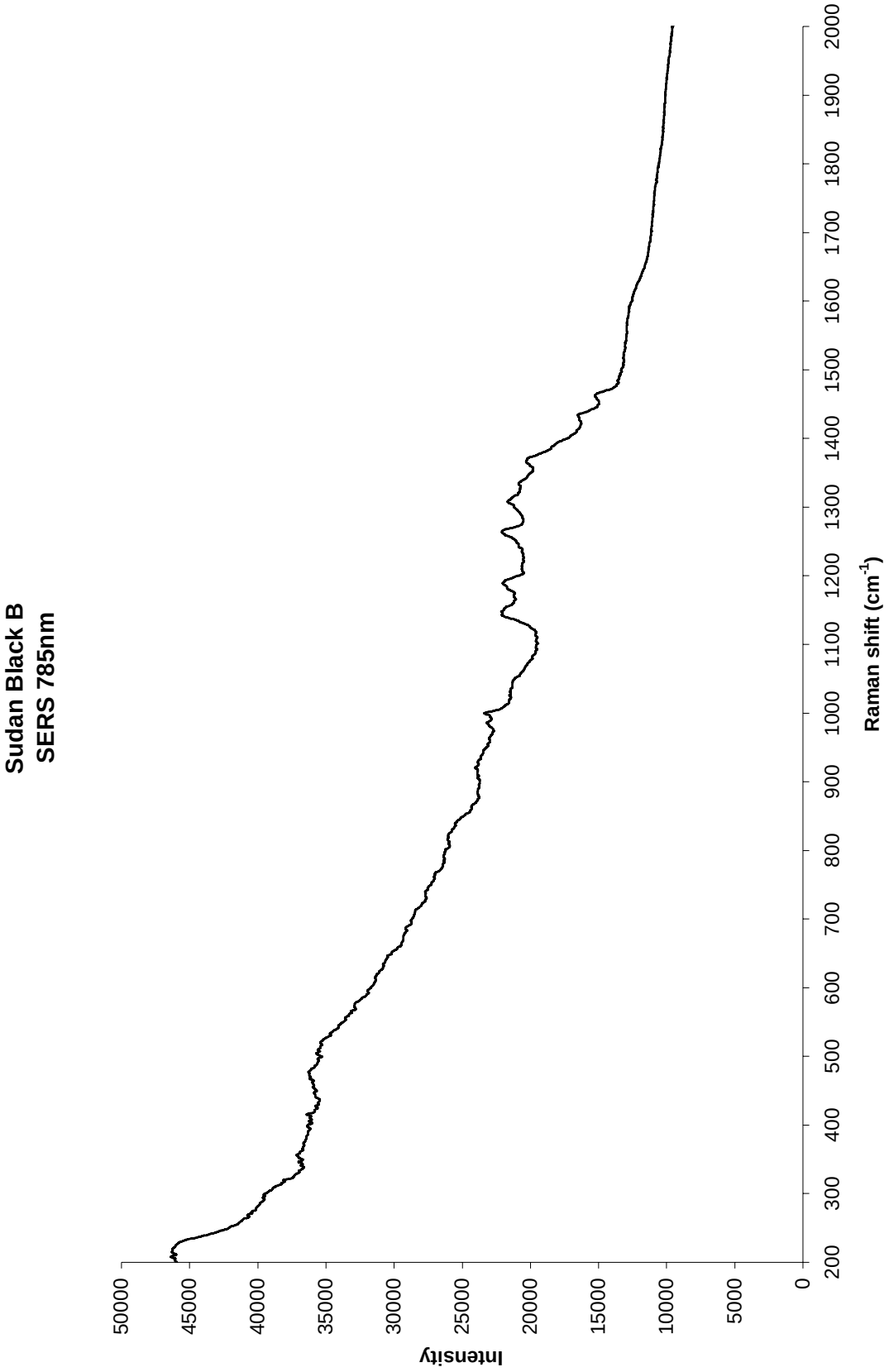
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
				208
			245	
				298.5
468		473.7	473.5	477.5
			514.5	516
655		653.0	654	653
			741.5	740
			798	793
				985.5
998	1005	998.2	1000	999.5
1028.5	1034	1029.1	1031.5	
1131.5	1133	1131.3	1138.5	
1182.5	1182	1185.0	1184	1189
		1264.4		1264
1303.5	1305	1306.8	1309.5	1308
1366.5	1371	1368.5	1372	1367
1396.5	1399	1399.3		
1434.5	1430	1430.2	1433	1434
1461	1461.5	1463.0	1464	1462
		1505.4		
1567.5	1568	1567.1		
1580.5			1581	
		1592.2		
1624				
		1646.2		
		1671.3		



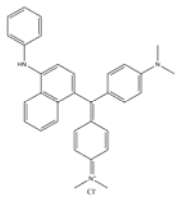








Victoria Blue



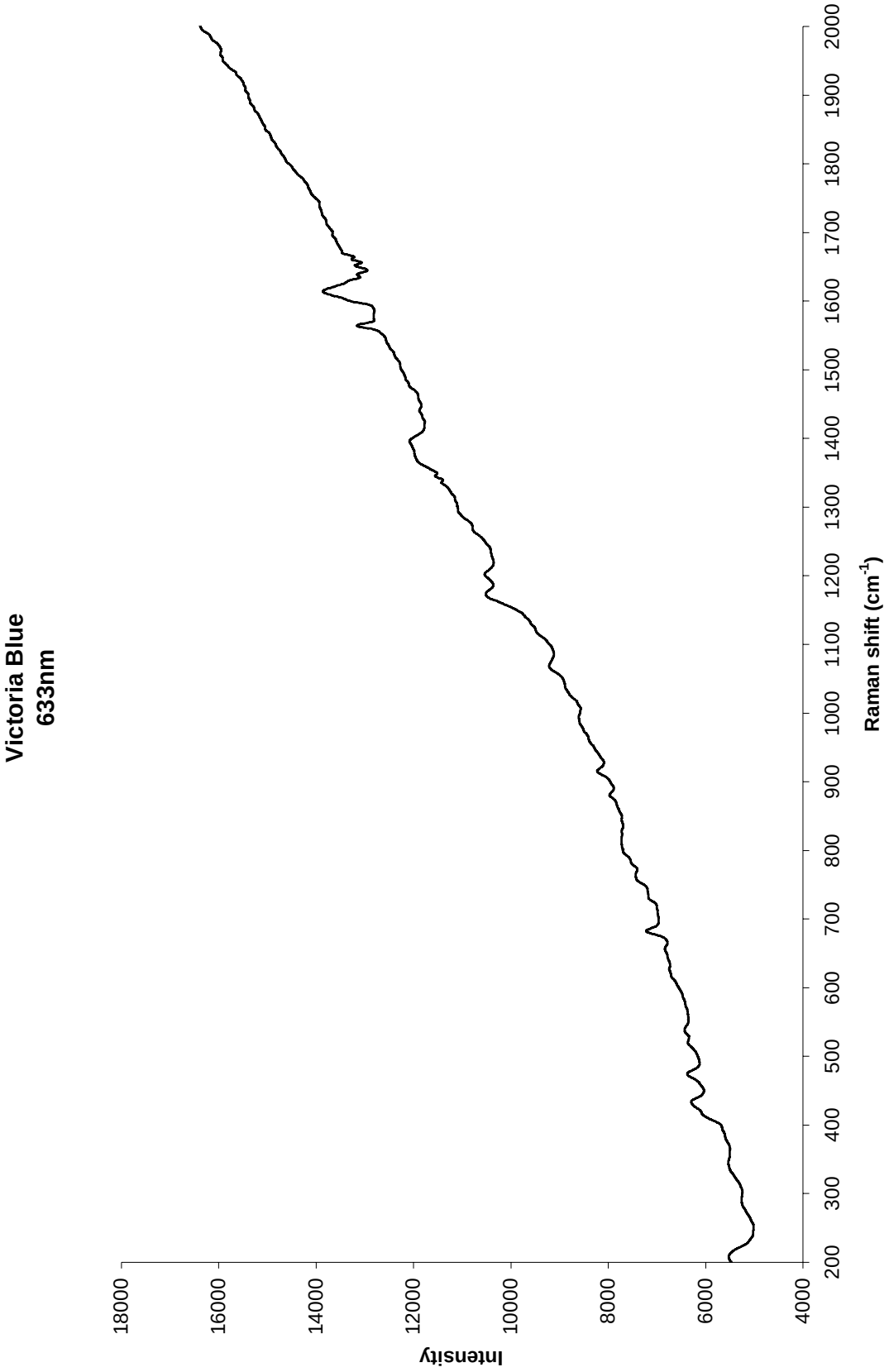
CAS 2580-56-5; CI 44045

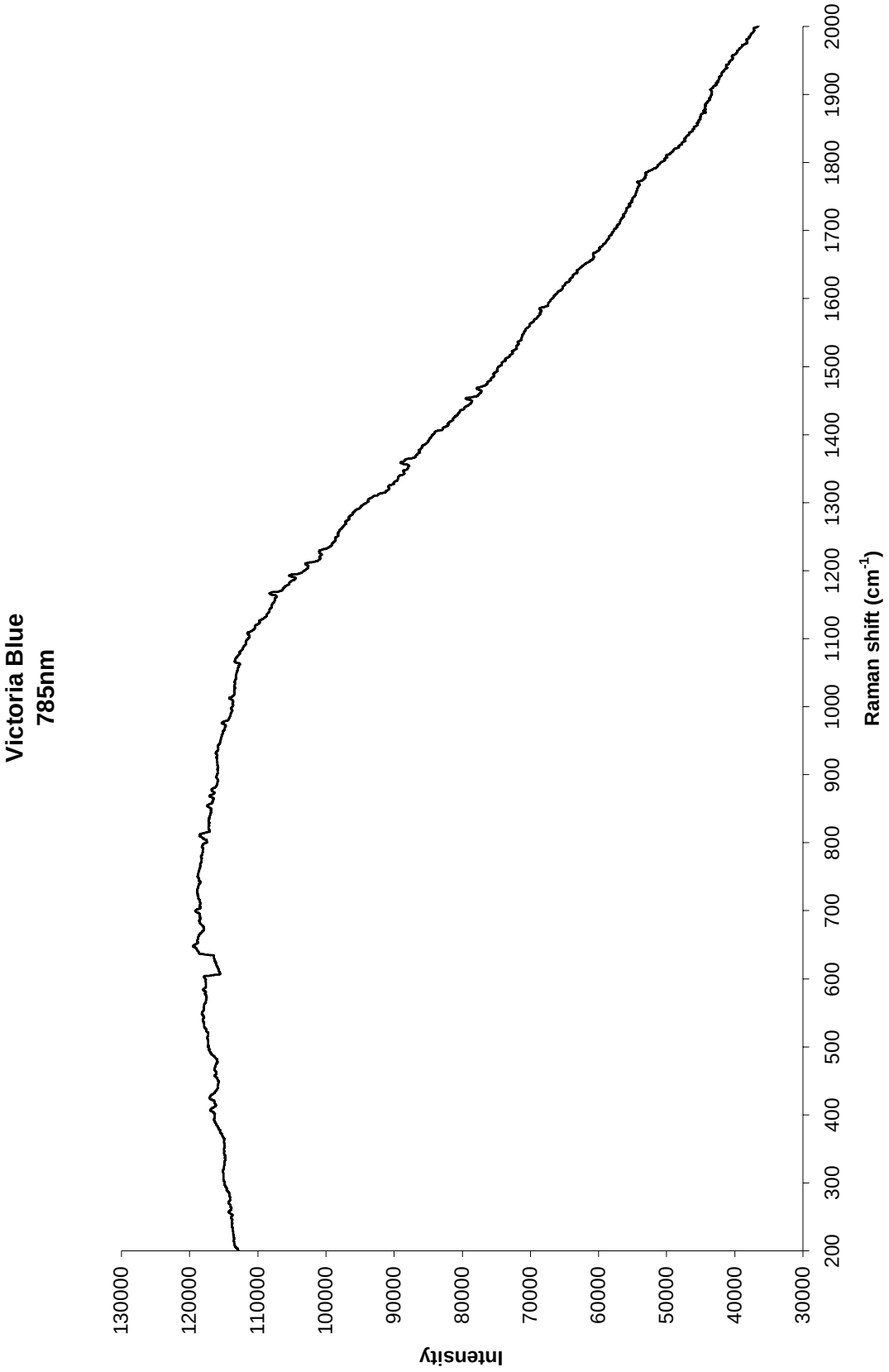
Alternate name: Victoria Blue B

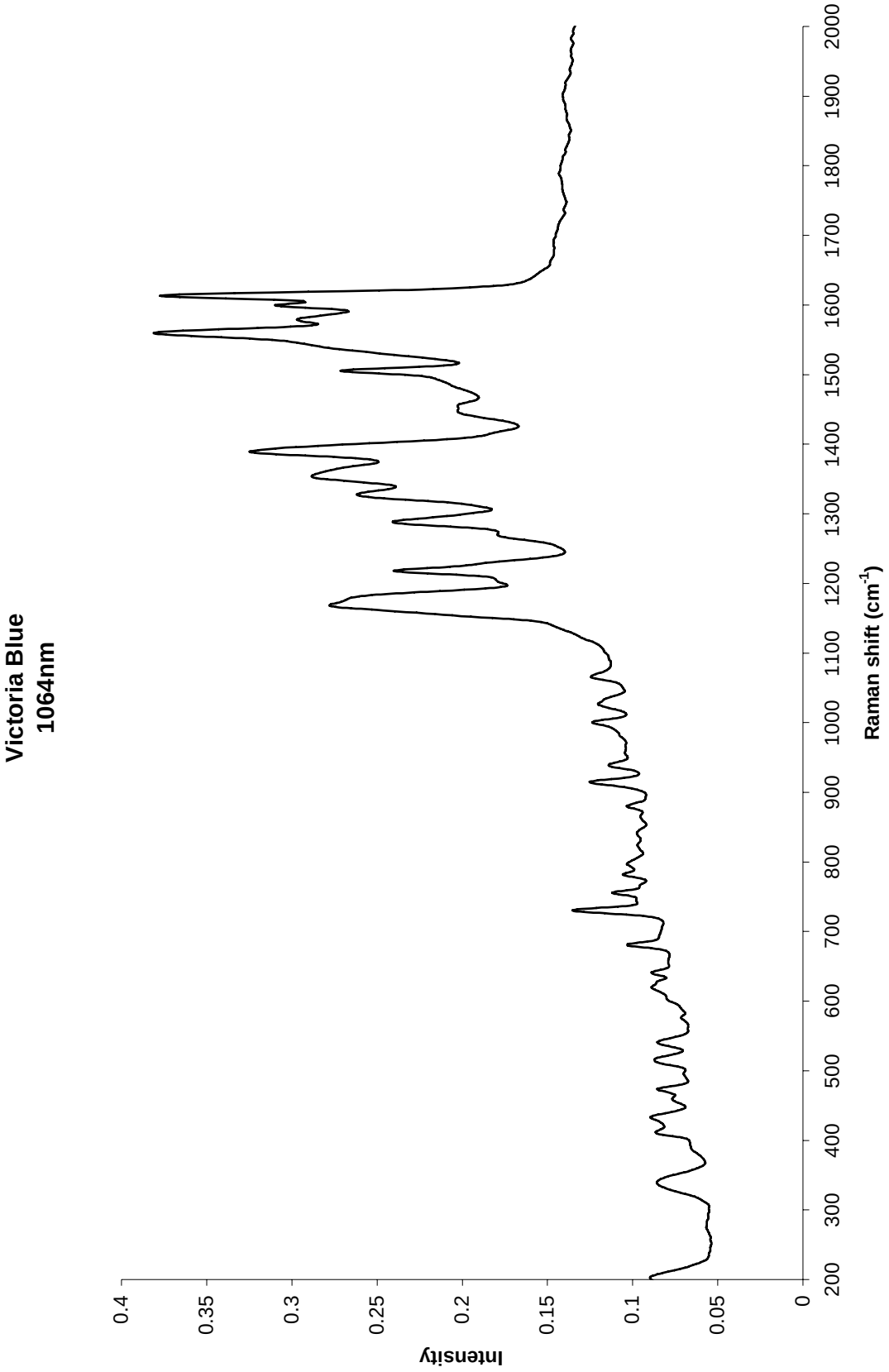
Table 10. Victoria Blue Raman peaks (cm^{-1})

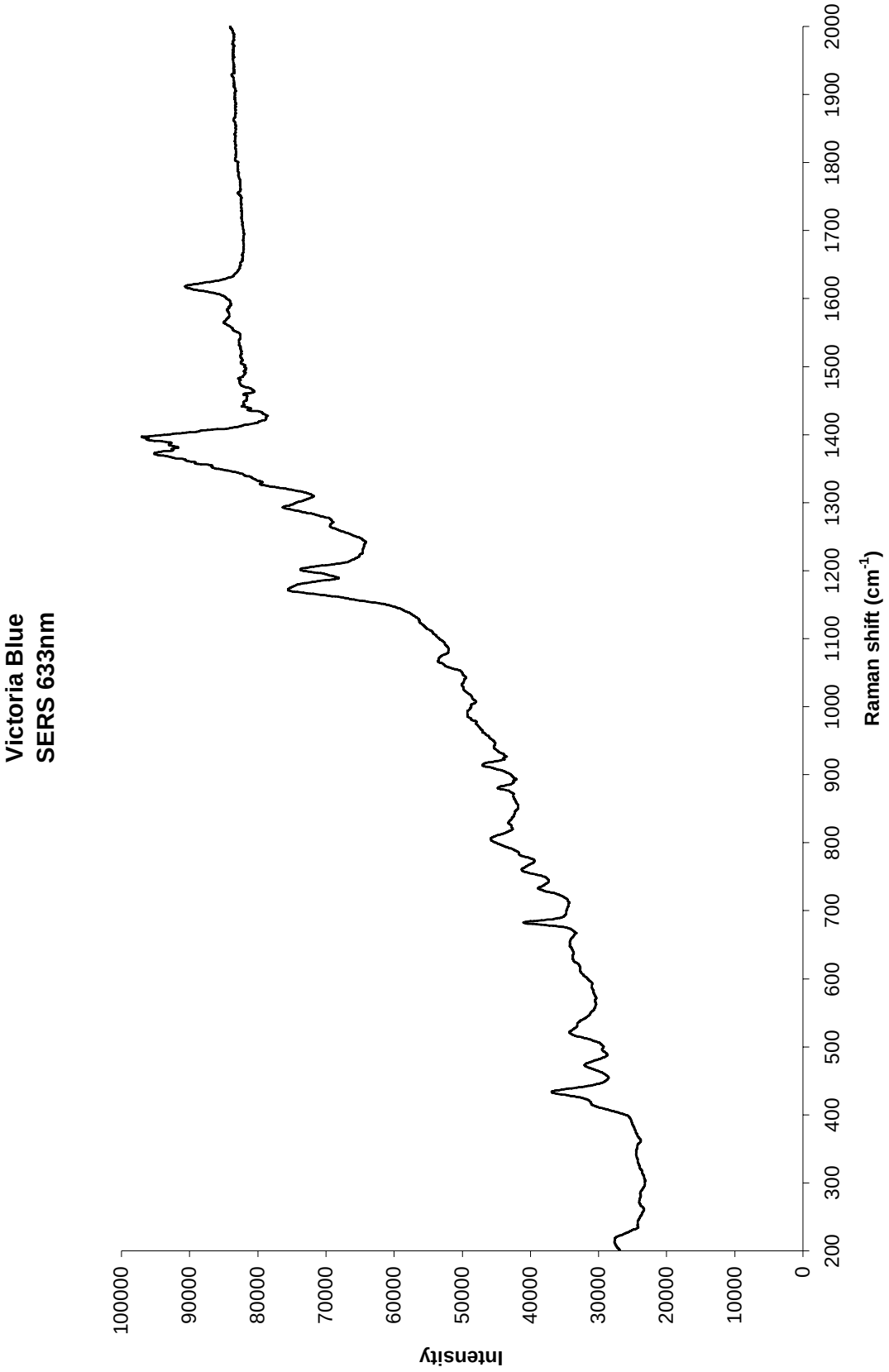
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
		205.0	213	206
342		338.7	343	336.5
	407	415.0		415
433.5	435	433.2	433.5	435
474		473.7	473	475
524		516.1	521.5	517
545	549	541.2		
		620.2	619	616.5
	648	641.4	648.5	
682.5		681.9	682.5	682
737		730.1	733	730.5
		755.2	760.5	757.5
786		782.2		781
807	811.5		806	796.5
			829	
880.5	878.5	880.6	880.5	880
915.5		915.3	914	913.5
		938.4	941	935.5
980.5	976.5			
996		1000.1	992	1005
	1012.5			
1032.5		1027.1	1032.5	1034
1071	1067.5	1065.7	1067	1065.5
1173.5	1166.5	1167.9	1172	1175.5
	1193			
1202			1202	
	1210	1218.1		1214.5
	1228.5			
1270			1265.5	
1301		1289.4	1293	1290.5
		1328.0		1338

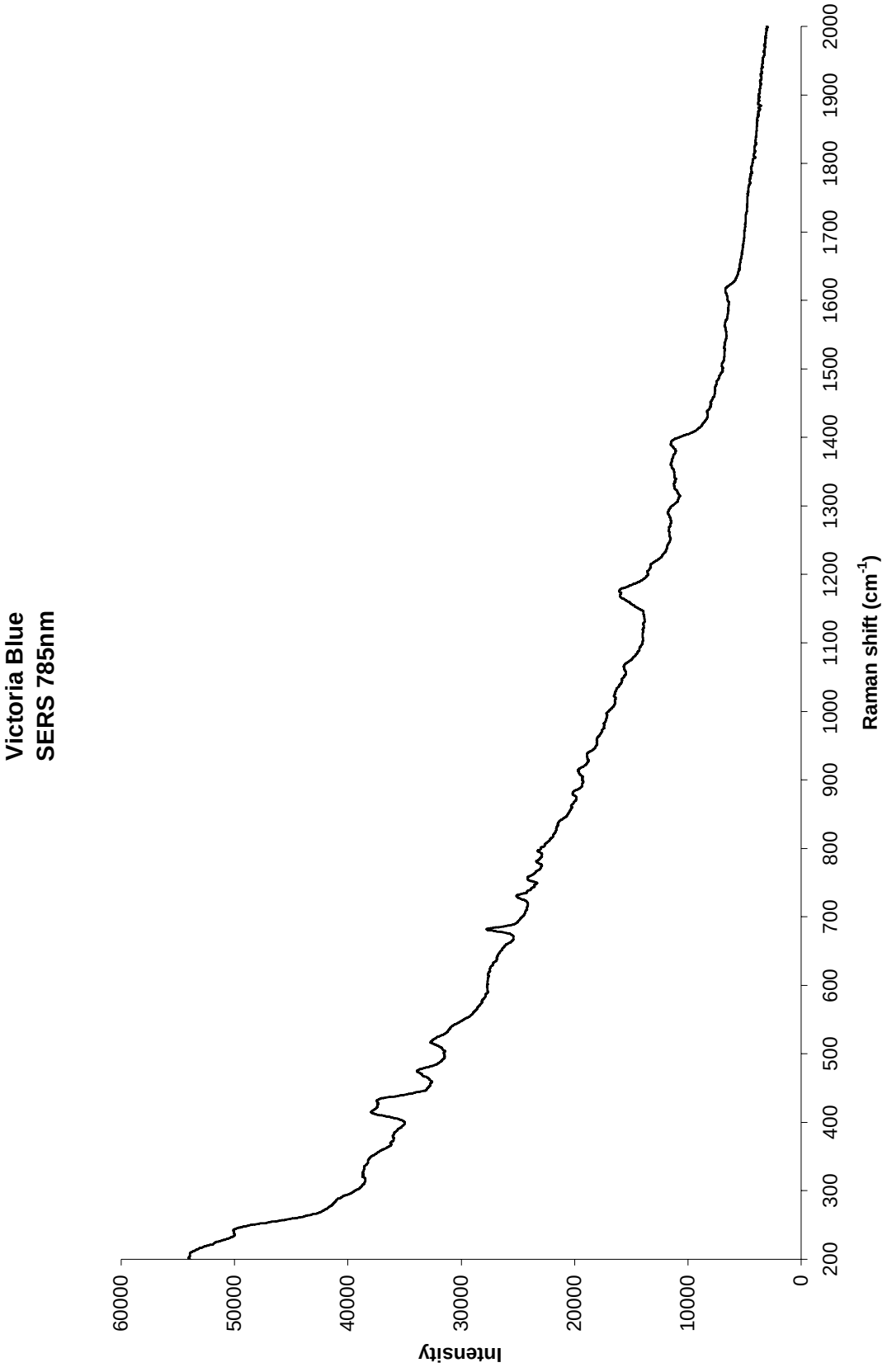
NR 633nm	NR 785nm	NR 1064nm	SERS 633nm	SERS 785nm
1364	1359	1353.1		
			1372	1370
1390		1389.7	1396.5	1390
	1453	1455.3	1459.5	
	1468			
		1505.4		
1564		1559.4	1565	
1614.5		1613.4	1617.5	1613.5







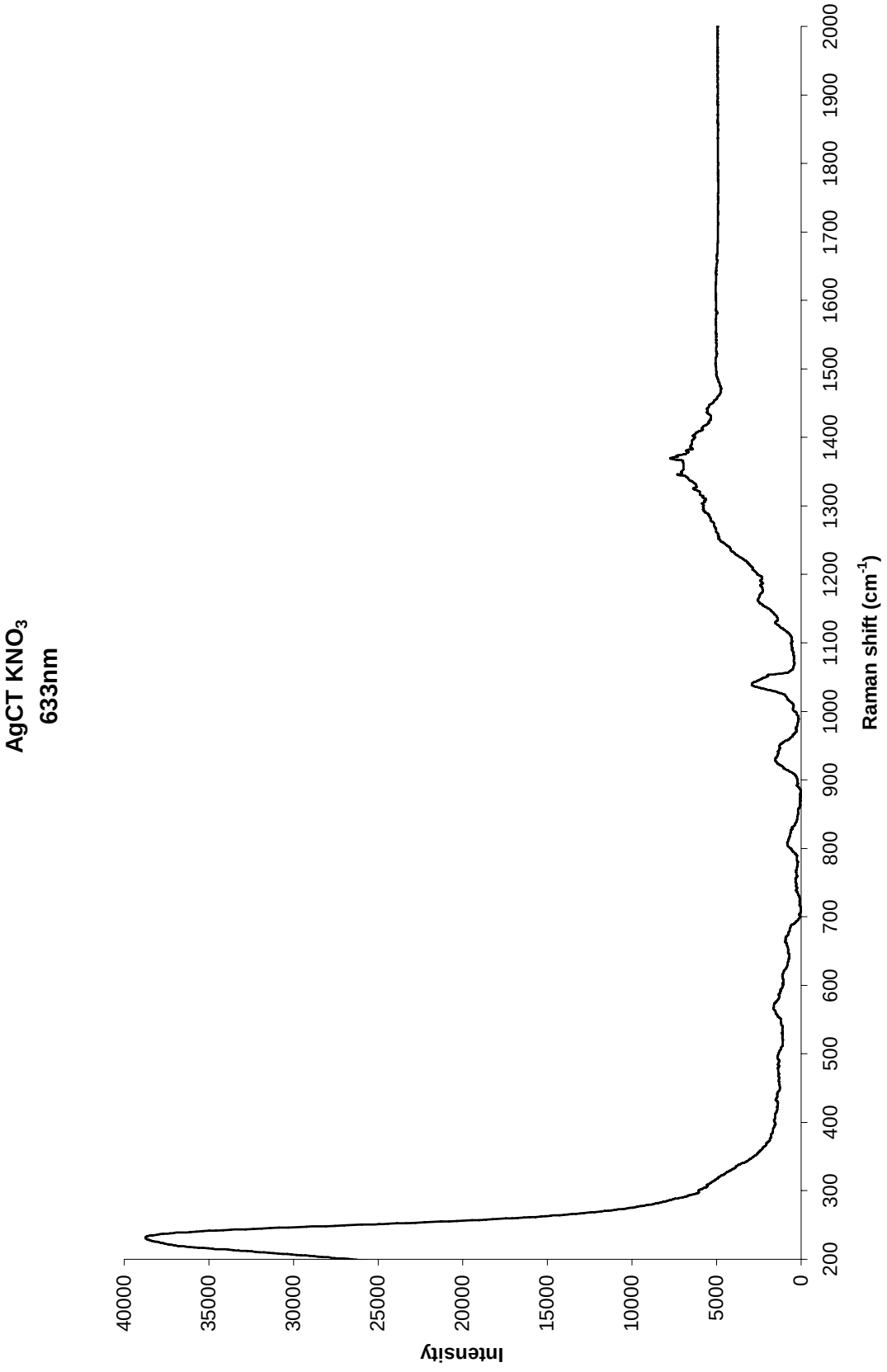


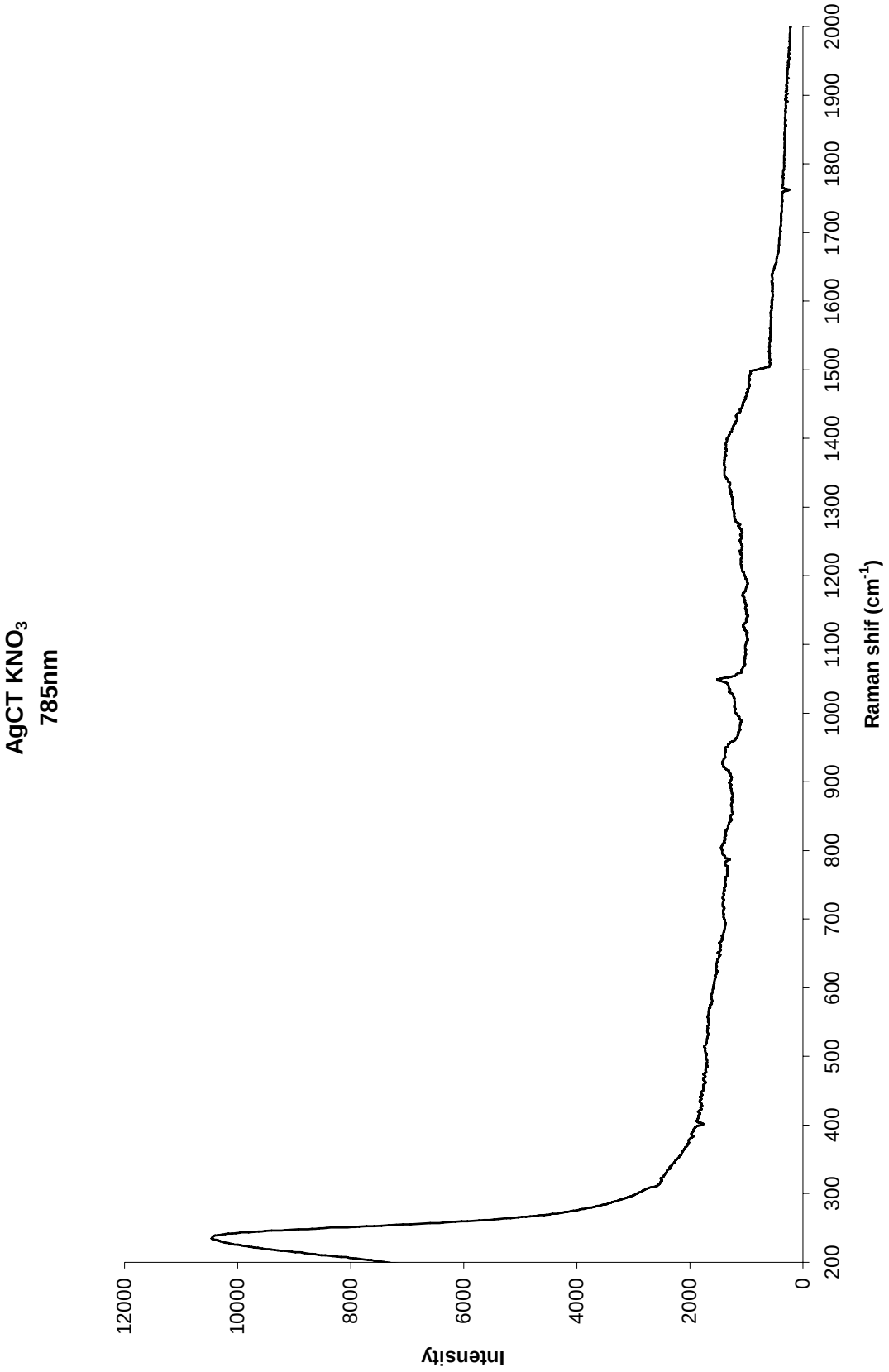


Ag colloid/KNO_3 mixture

Table 11. Ag colloid/KNO_3 Raman peaks (cm^{-1})

633nm	785nm
231.5	235
586.5	
622	617.5
672	665
808	801
936	930
956	954
1039.5	1031.5
	1048.5
1130	1127
1172	1170
1346	
1369.5	1372.5
1437	1442.5



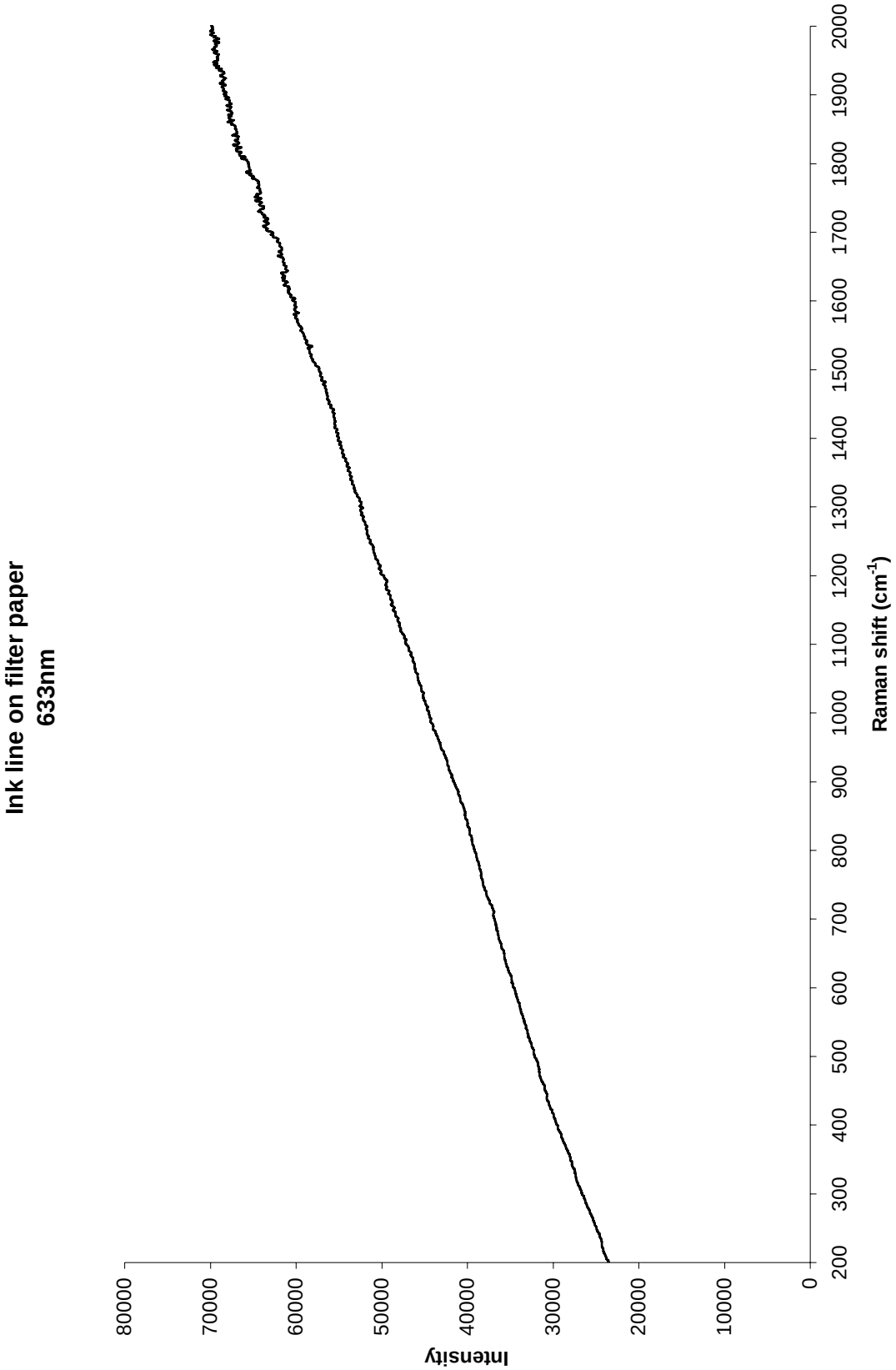


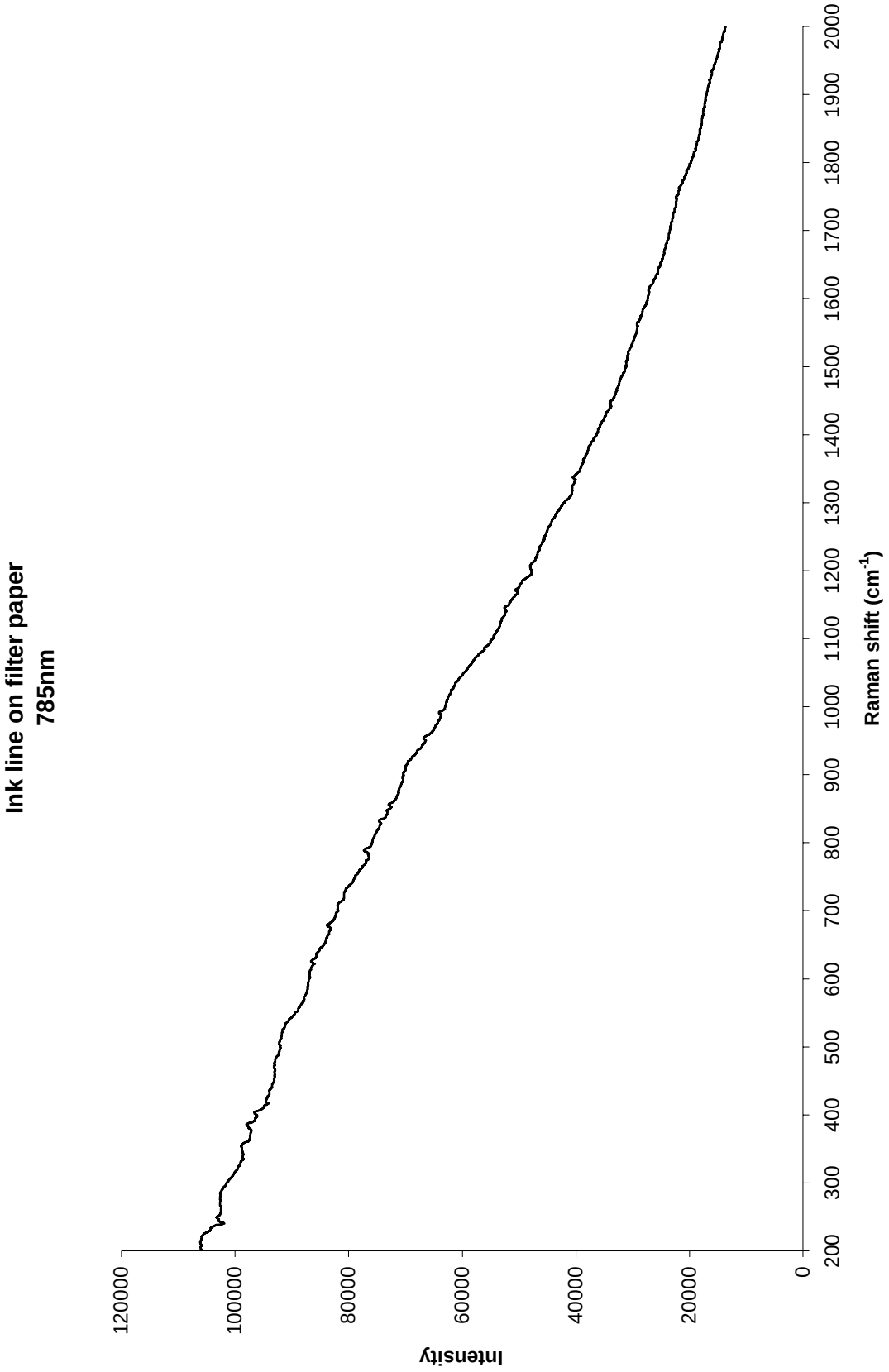
Ink line on filter paper

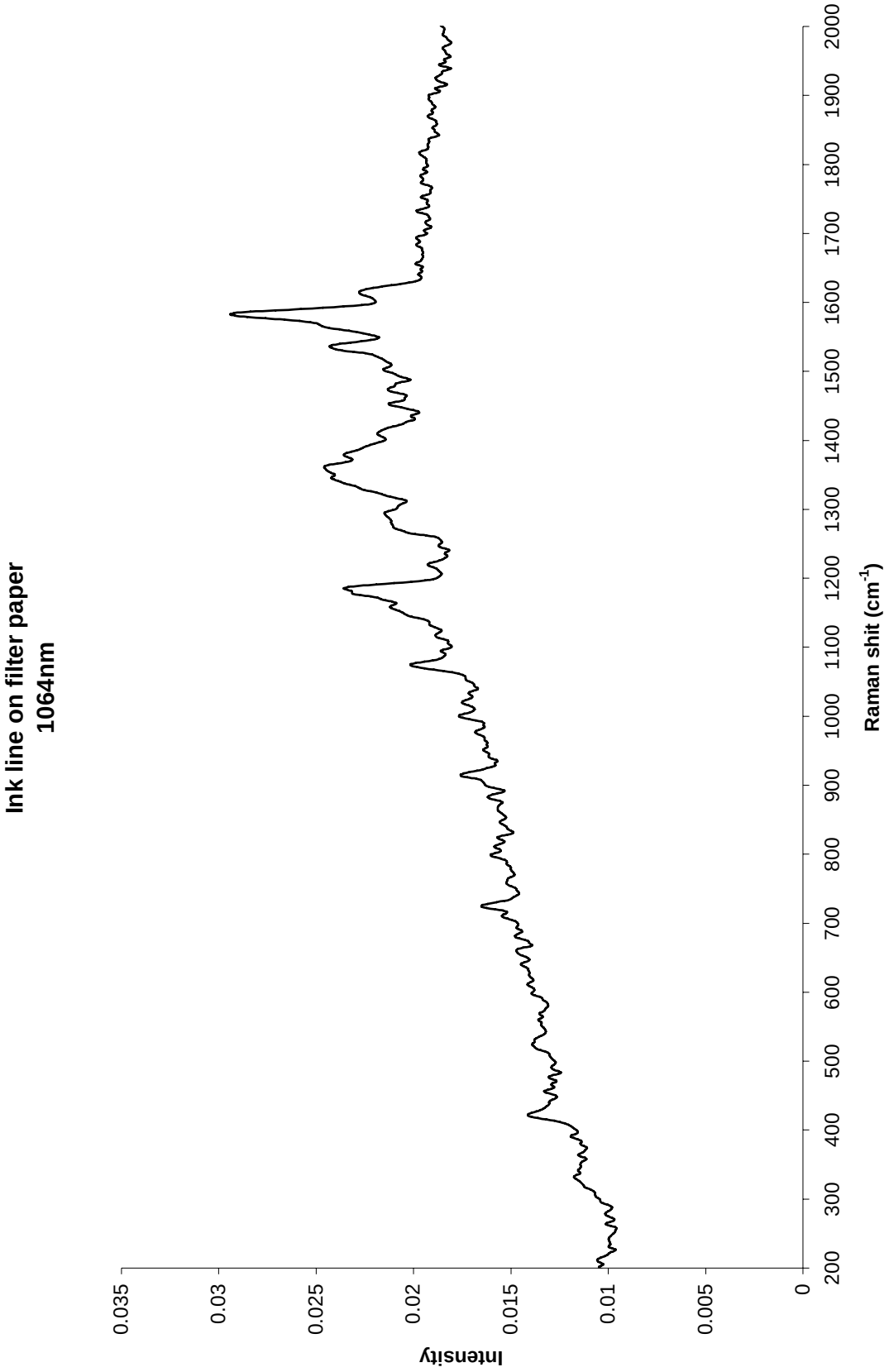
Table 12. Ink line Raman peaks (cm^{-1})

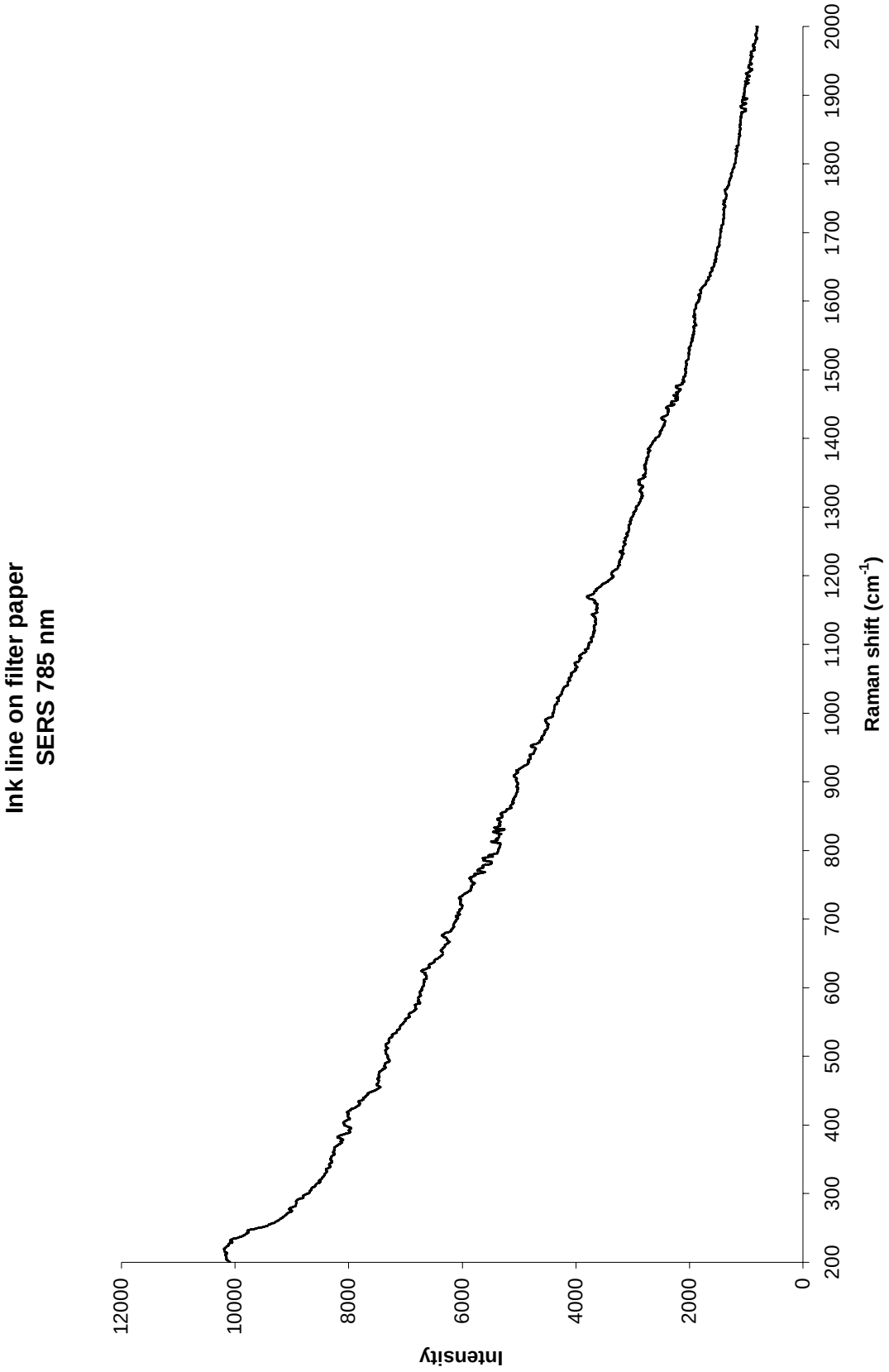
NR 633nm	NR 785nm	NR 1064nm	SERS 785nm
	205		
		211.4	219
	338.5	332.9	
	354		
	386	390.7	382.5
	403		403.5
		421.6	418.5
	470	475.6	480
	529	525.7	524
	625.5	620.0	624
	636.5	639.5	640
		660.7	654
	678.5	681.9	676
	714	710.9	
	737	724.4	731
		759.1	759.5
	789	799.6	788.5
		811.1	812.5
	832.5	824.6	827
		847.8	845.5
	856	865.1	
	876	882.5	
	902	907.0	908.5
	924	915.3	919
	954	951.9	952
	991	1000.1	993
		1019.4	
		1075.4	1072.5
		1094.6	1088
	1145.5	1158.3	1143.5
	1171.5	1185.3	1169.5
	1214	1220.0	1208
1296		1293.3	
	1337		1339

NR 633nm	NR 785nm	NR 1064nm	SERS 785nm
		1360.8	
		1380.1	1385
		1410.9	
	1431.5		1429.5
	1445		1445
		1453.3	1462.5
		1474.6	1475
		1503.5	
1531.5		1536.3	
	1563		
1580.5		1582.6	
			1596
	1612	1615.3	1624





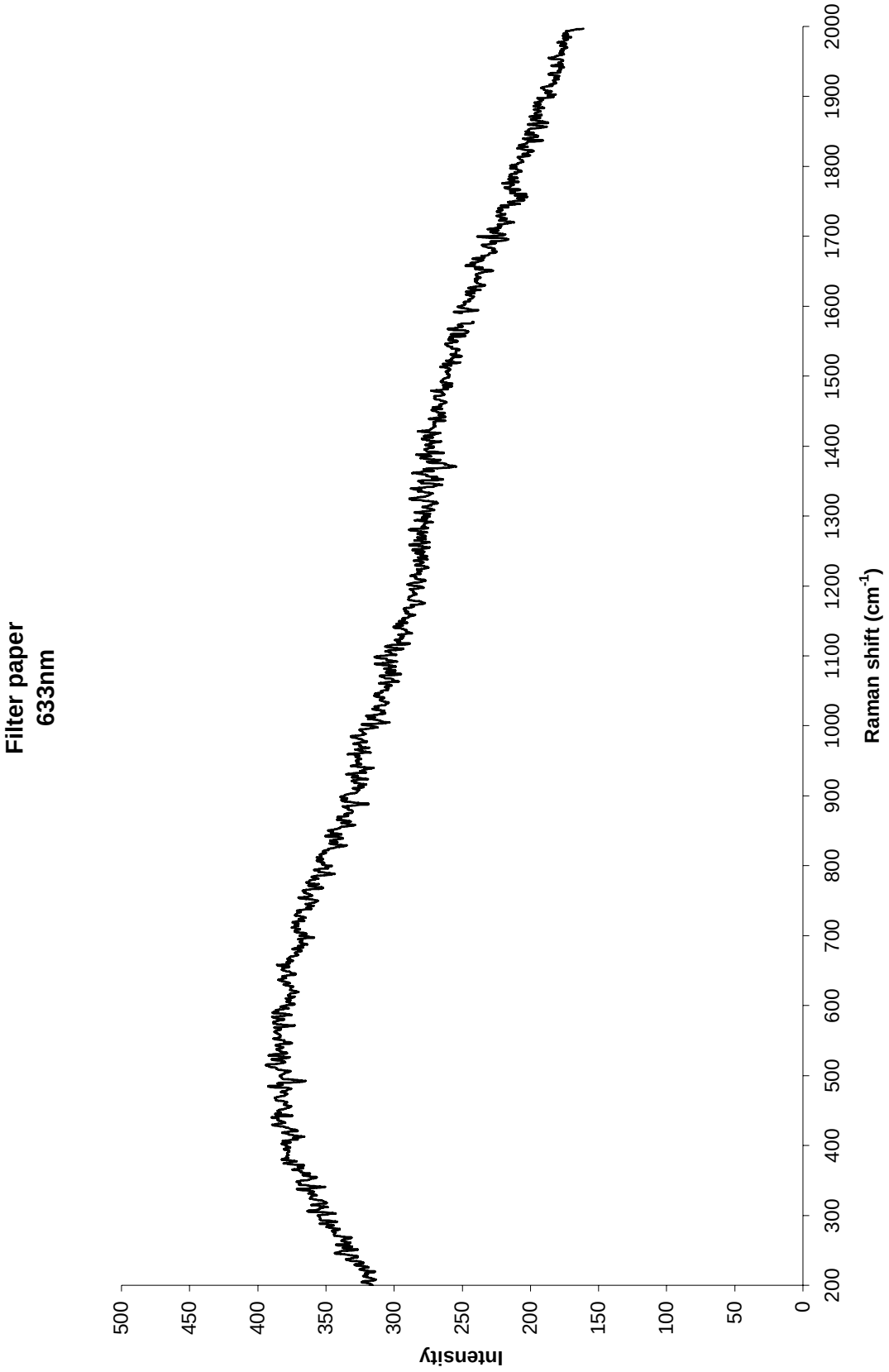


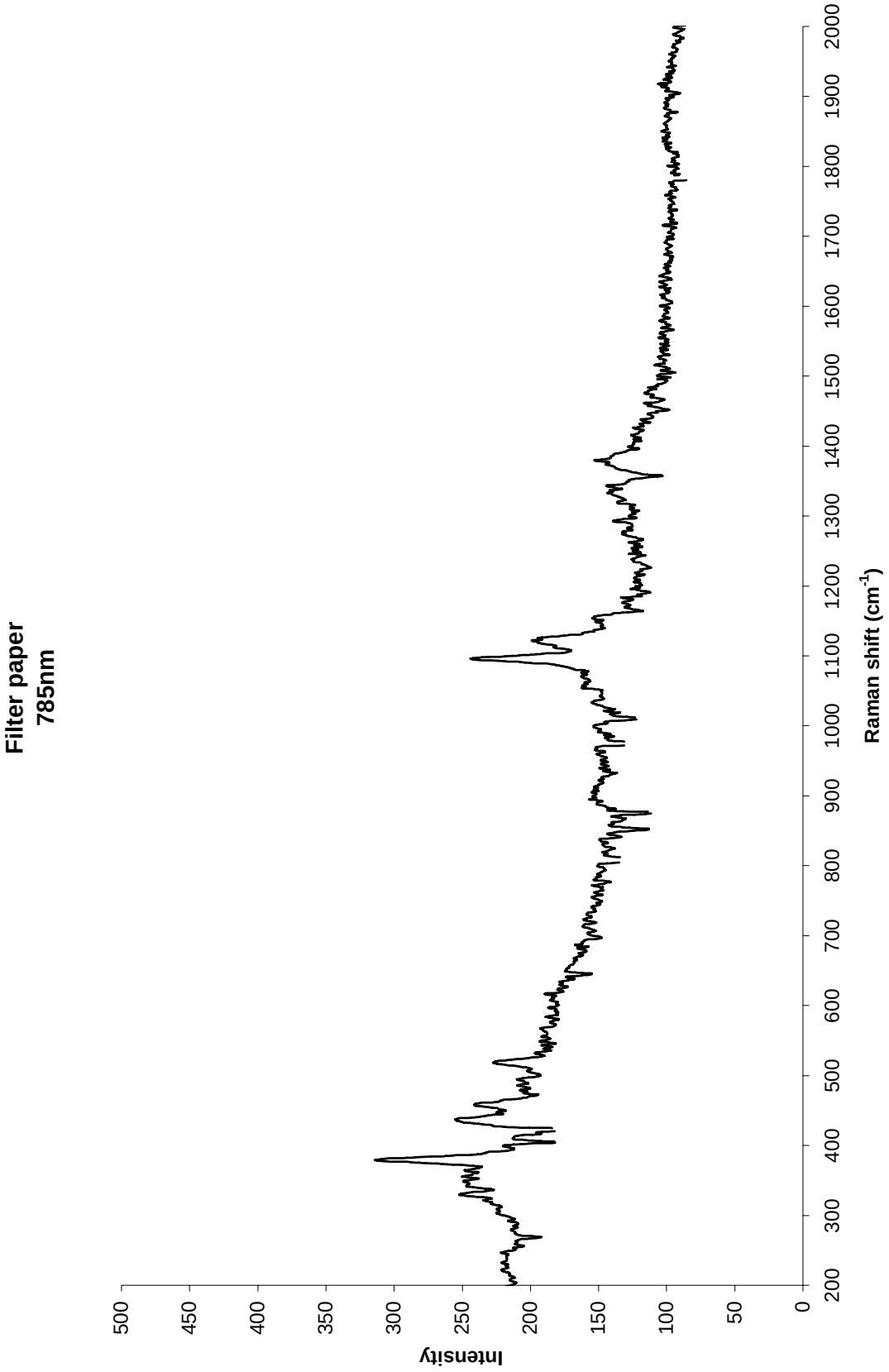


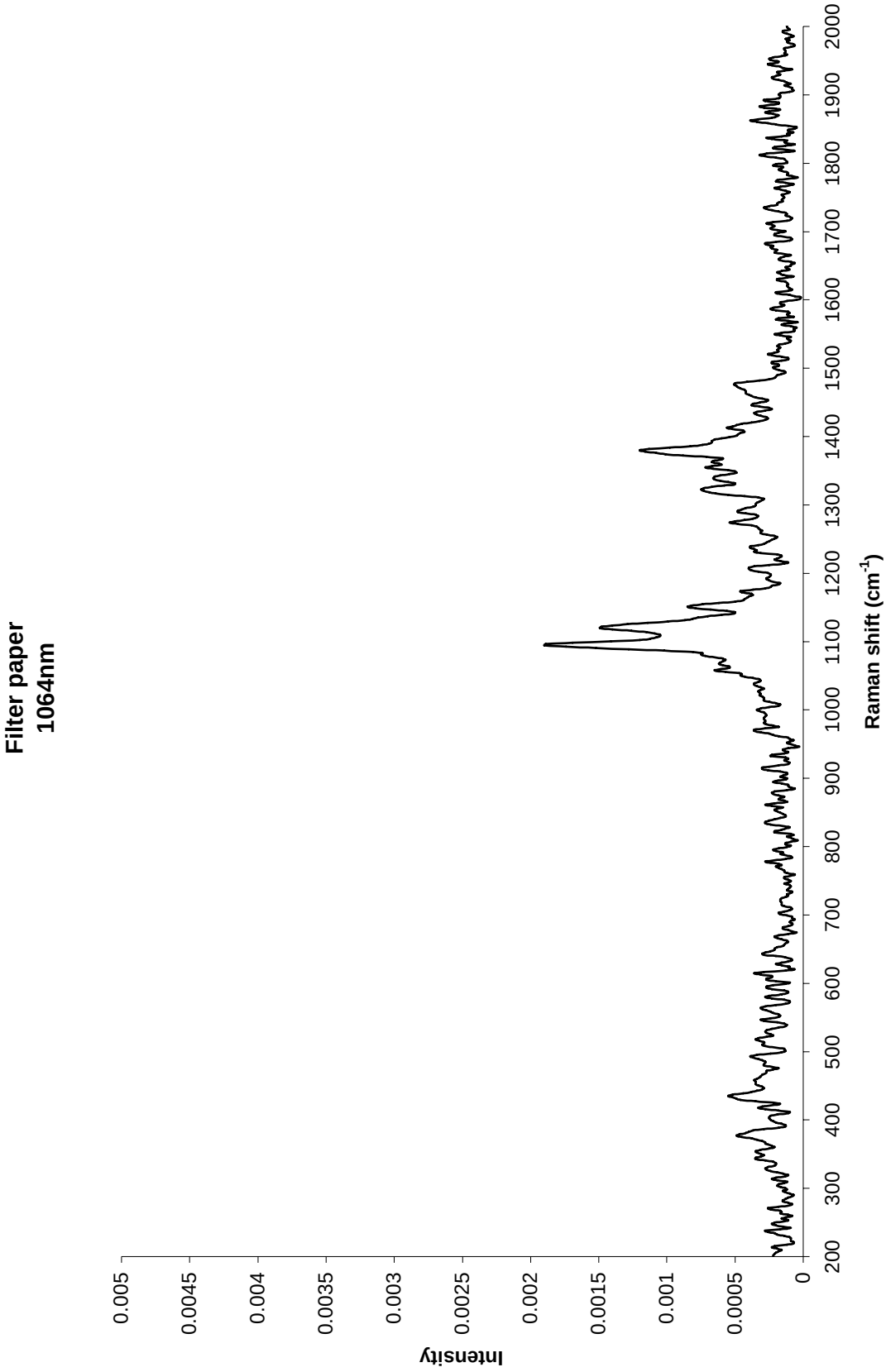
Filter paper

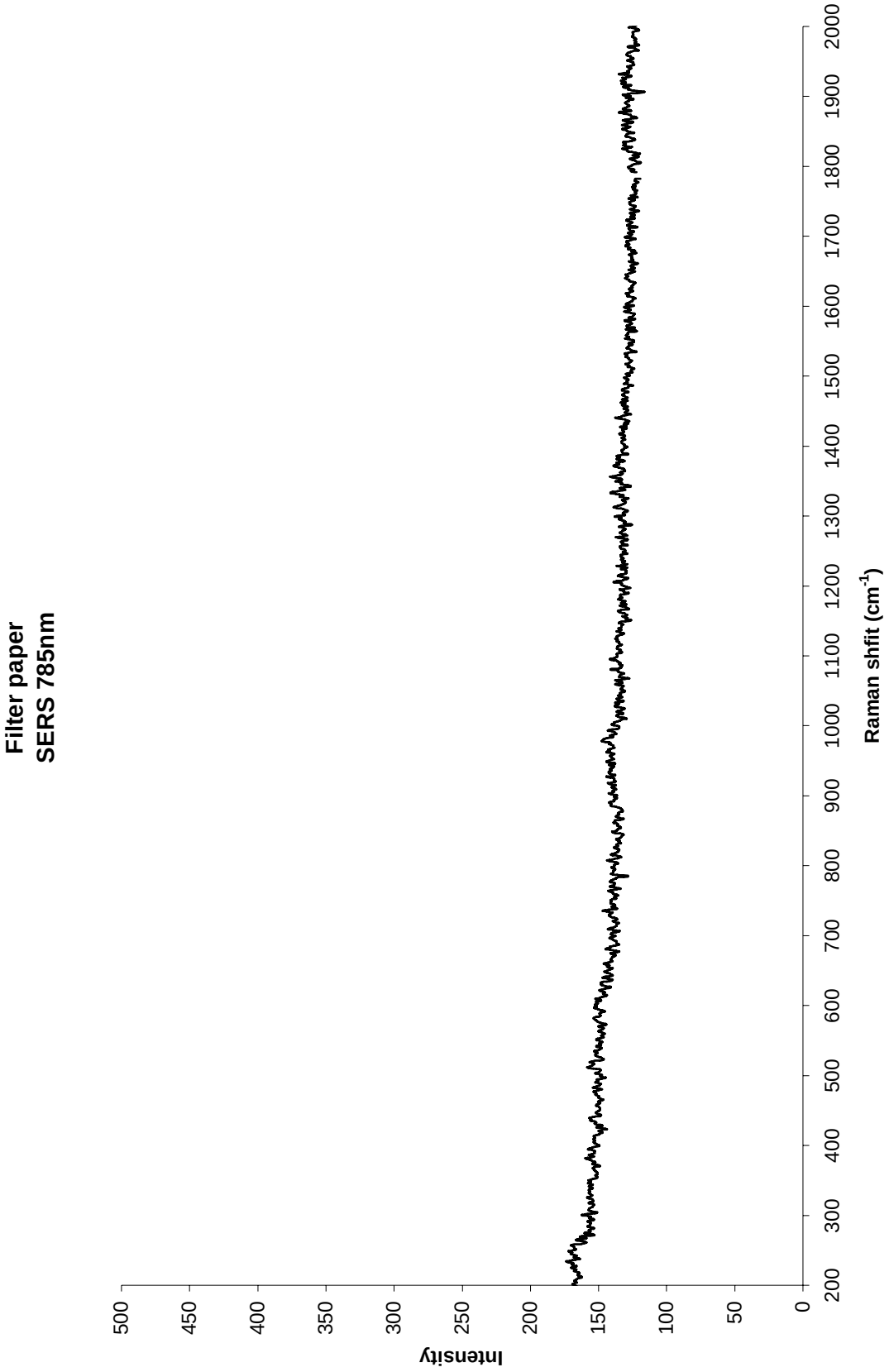
Table 13. Filter paper Raman peaks (cm^{-1})

NR 633nm	NR 785nm	NR 1064nm	SERS 785nm
	380.5	379	
	439	437	
	460		
	521		
	1097.5	1096.5	
	1122.5	1121.6	
	1157.5	1152.5	
	1343.5	1324.1	
	1381	1380	
		1478	









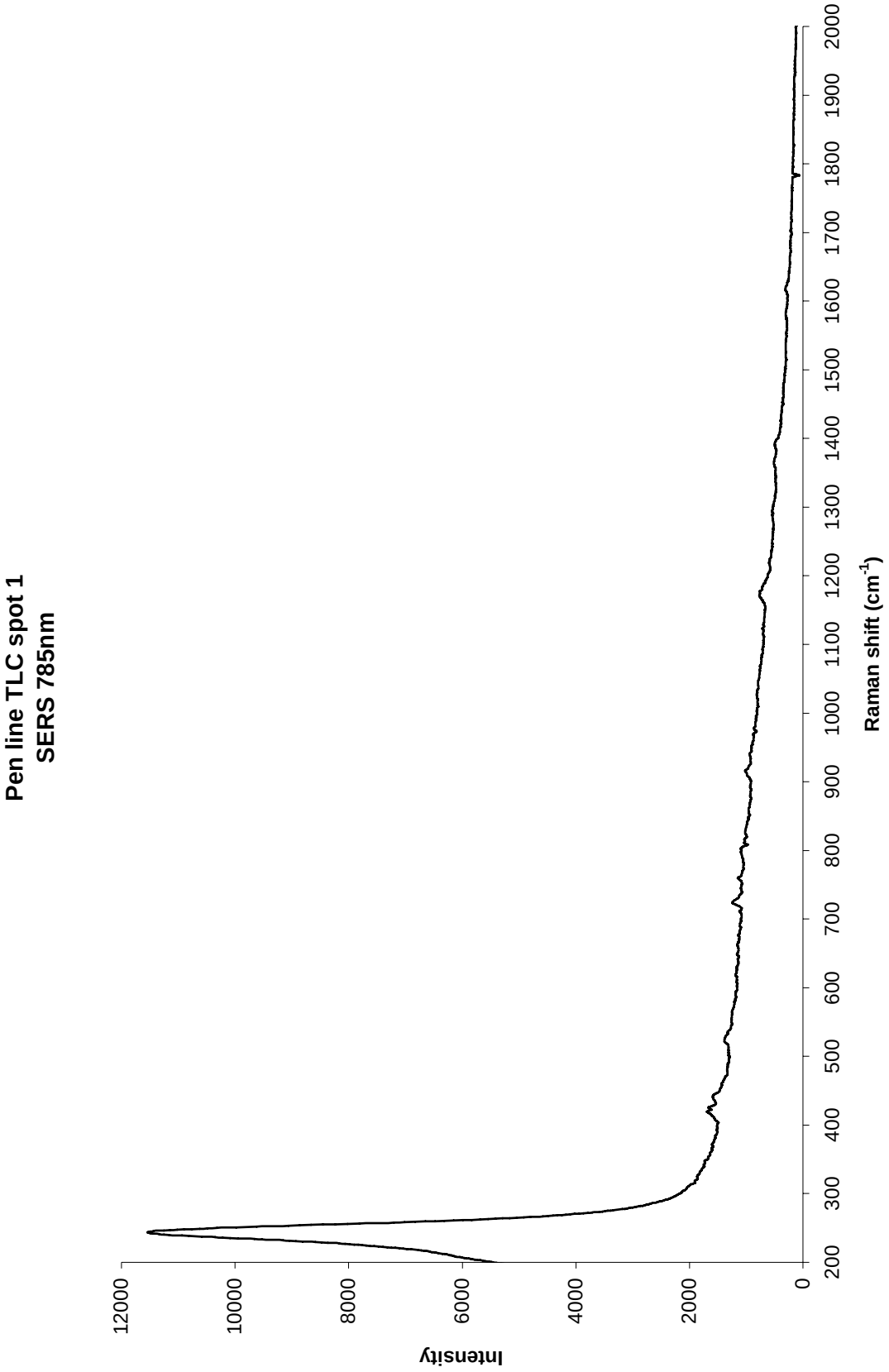
Ink TLC separation

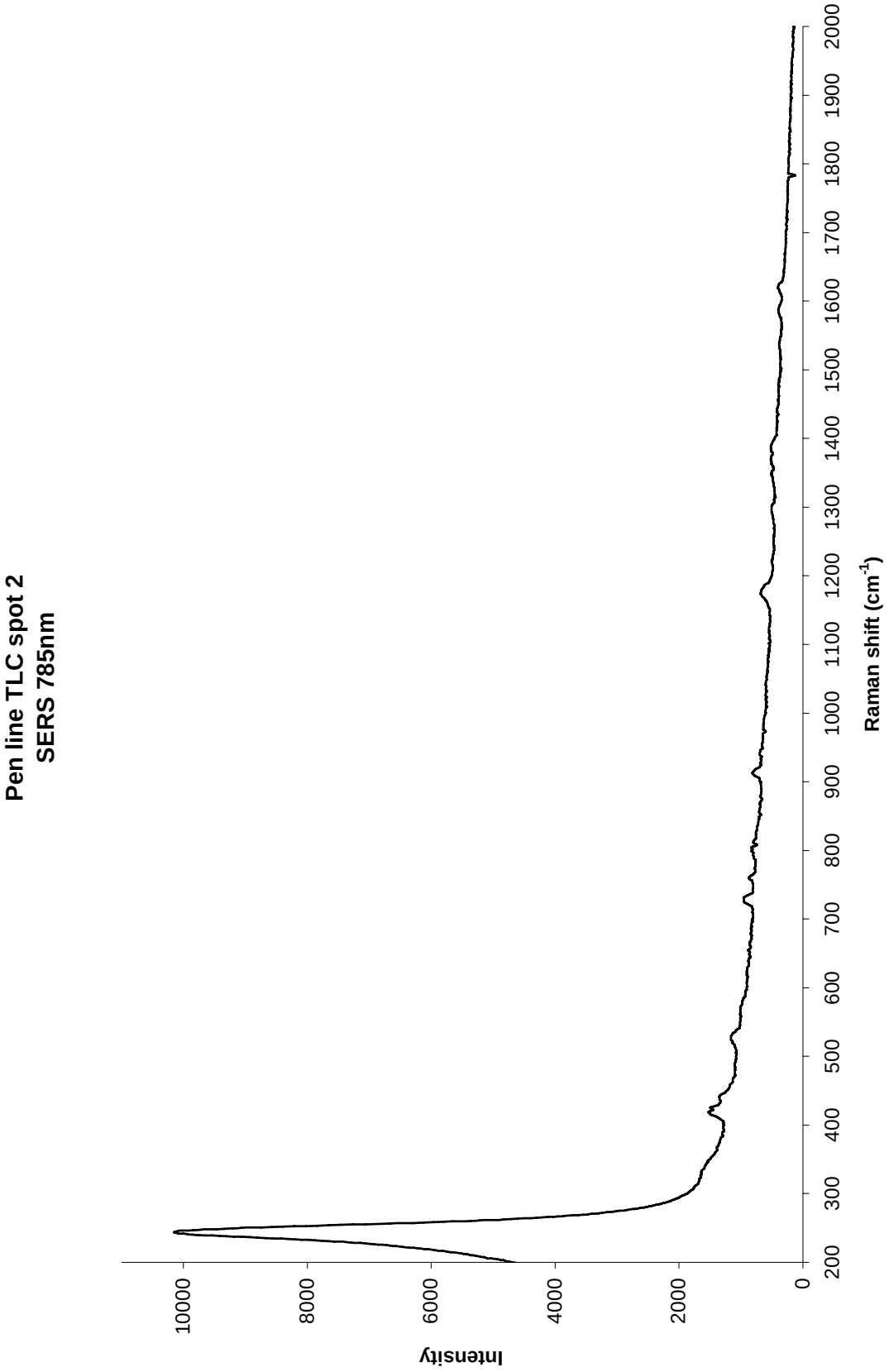
Table 14. Ink TLC Rf values
Spot 1 (light purple) – 0.816
Spot 2 (light purple) – 0.859
Spot 3 (light blue) – 0.912

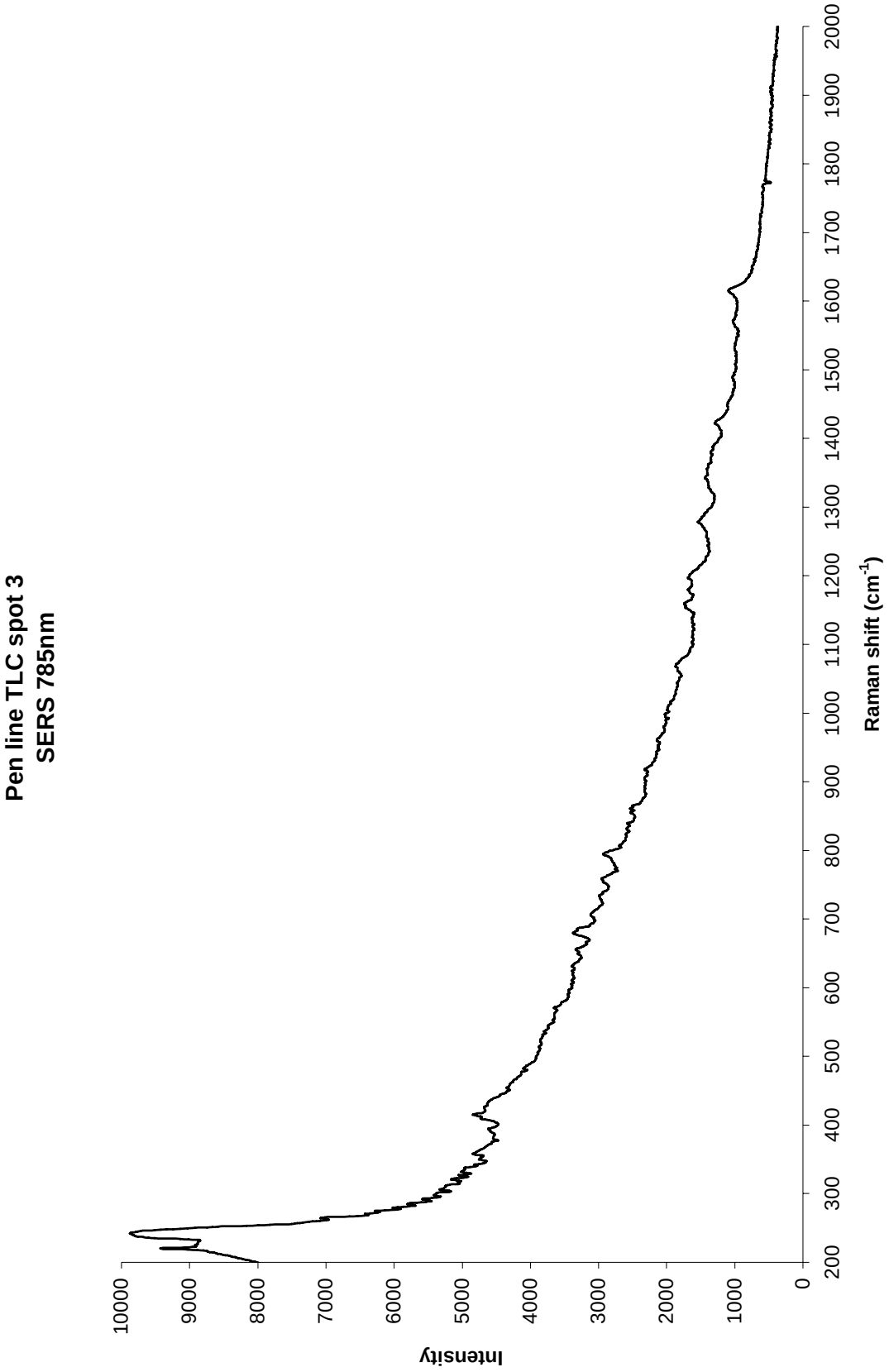
Table 15. Ink TLC Raman peaks (cm^{-1})

SERS Spot 1	SERS Spot 2	SERS Spot 3
244	244	242
		358
		394
419.5	419	415
441.5	441.5	
		571
		632
		656
		680
		705.5
724	726.5	734
759.5	759.5	758.5
802.5	799.5	794.5
816	812	
826.5	821	
		865
915.5	913	918
941	955.5	
		960.5
978	980.5	
		998.5
1043	1052	
		1068
1123	1132	
		1159.5
1172	1174.5	1180
		1197
1219.5	1221.5	
		1278.5
1289	1298	
1350.5	1348	1342.5
1364.5	1371.5	
1392.5	1388	

SERS Spot 1	SERS Spot 2	SERS Spot 3
		1422
		1489
	1537.5	1539
1583	1588	1580
1617.5	1619	1614.5







Crystal Violet, Methyl Violet TLC separation

Table 16. Crystal Violet TLC Rf values

Spot 1 (light purple) – 0.818

Table 17. Methyl Violet TLC Rf values

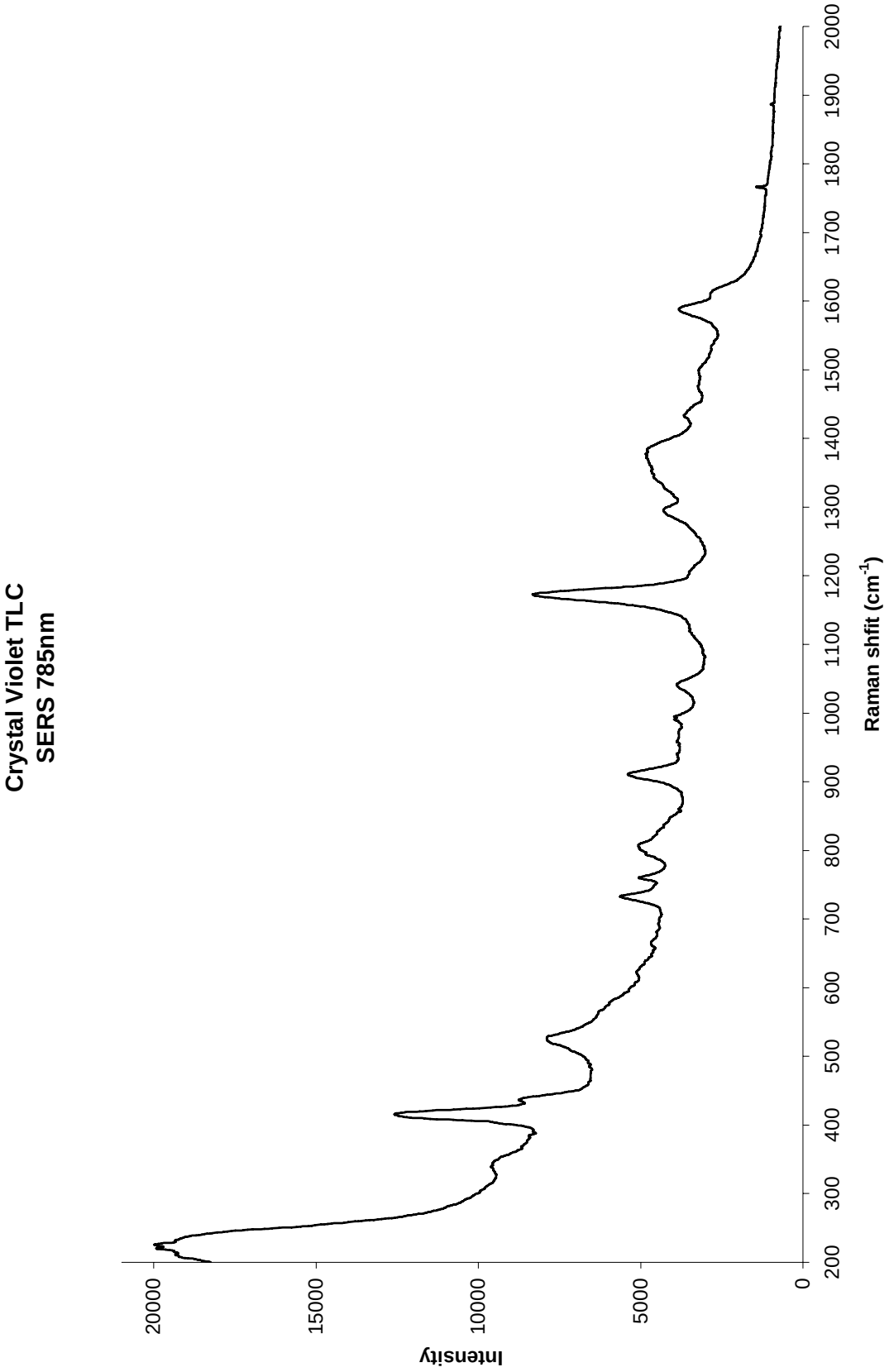
Spot 1 (light purple) – 0.815

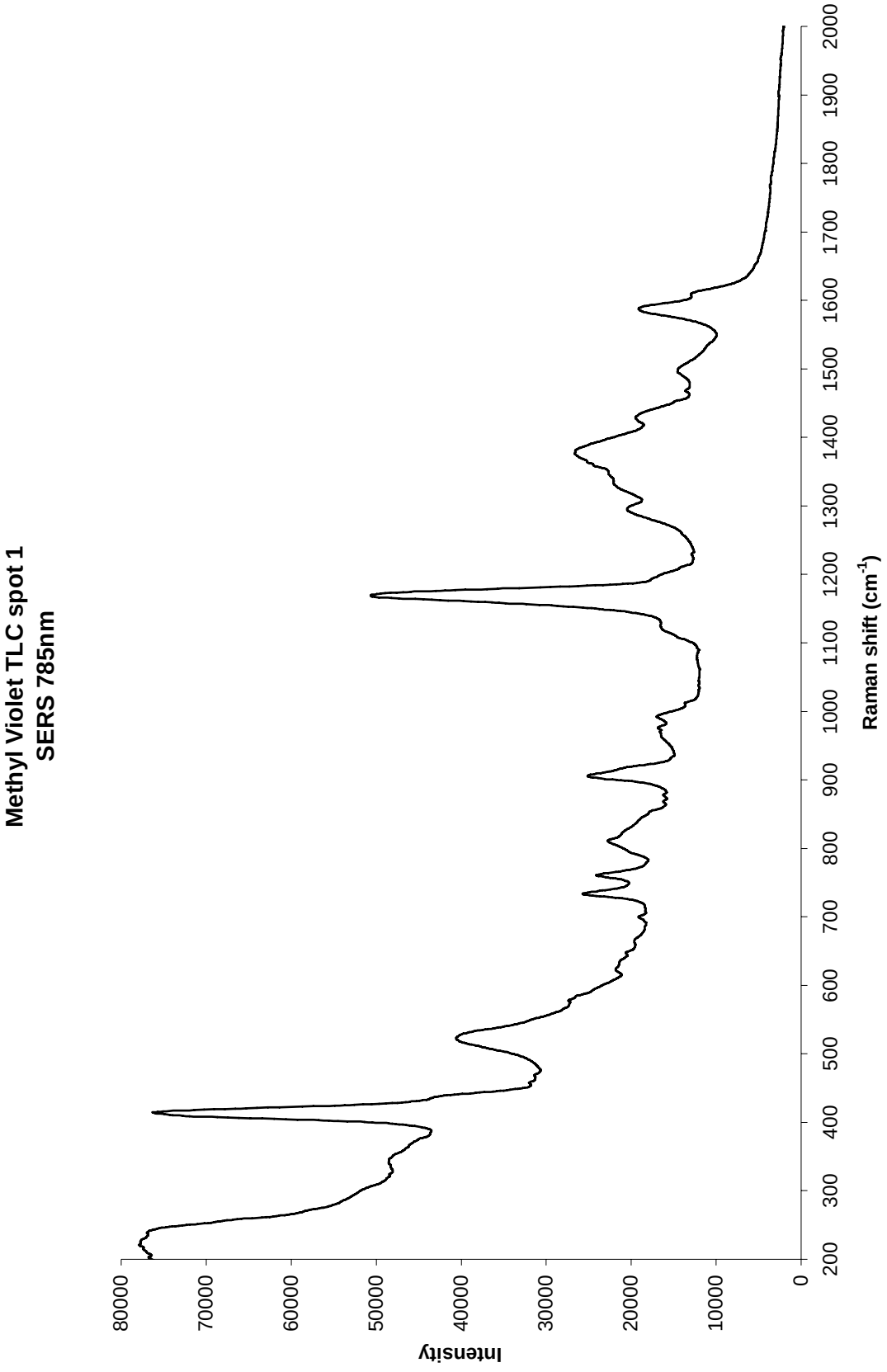
Spot 2 (light purple) – 0.862

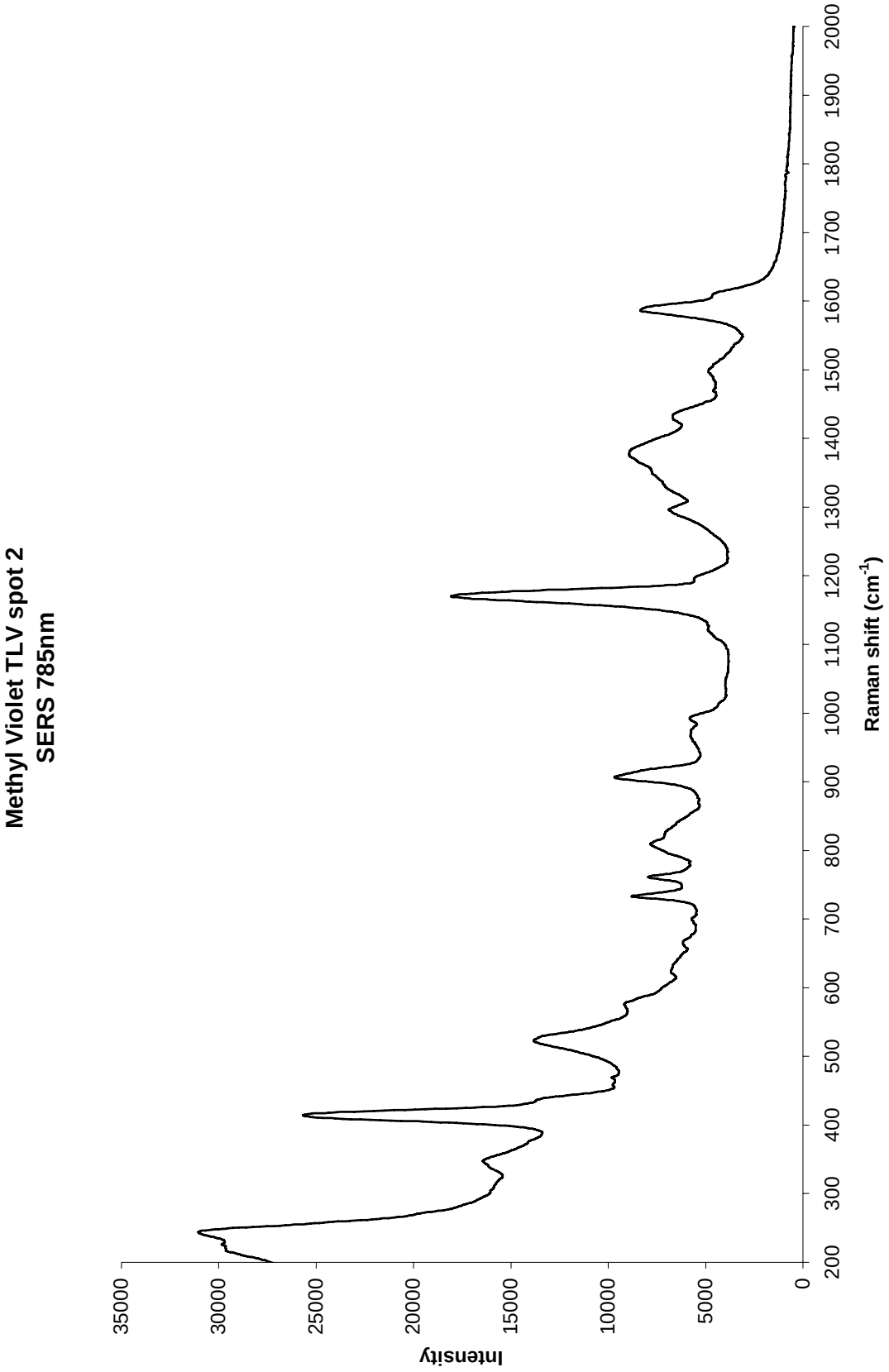
Spot 3 (light blue) – 0.892

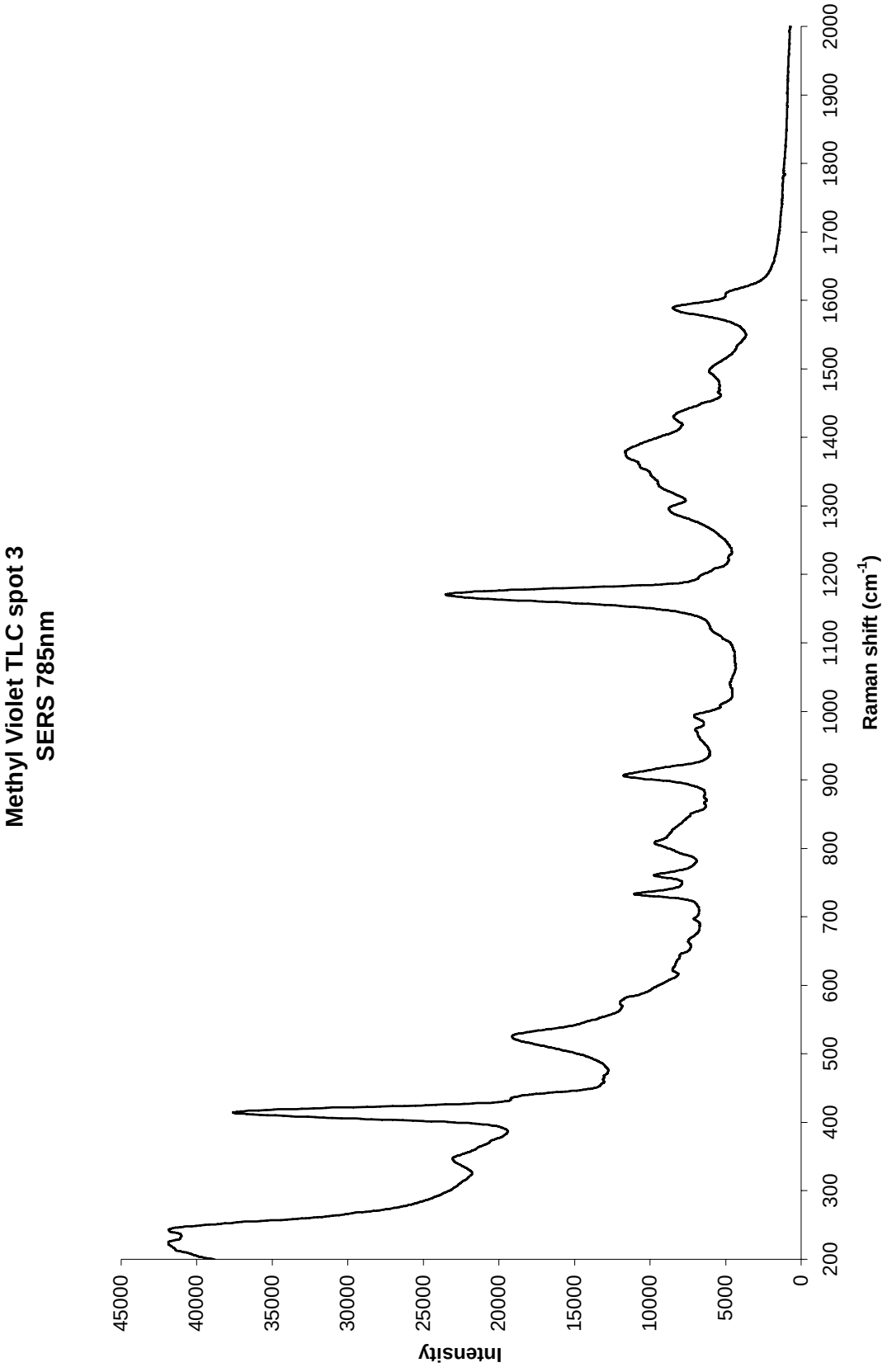
Table 18. Crystal Violet, Methyl Violet TLC Raman peaks (cm^{-1})

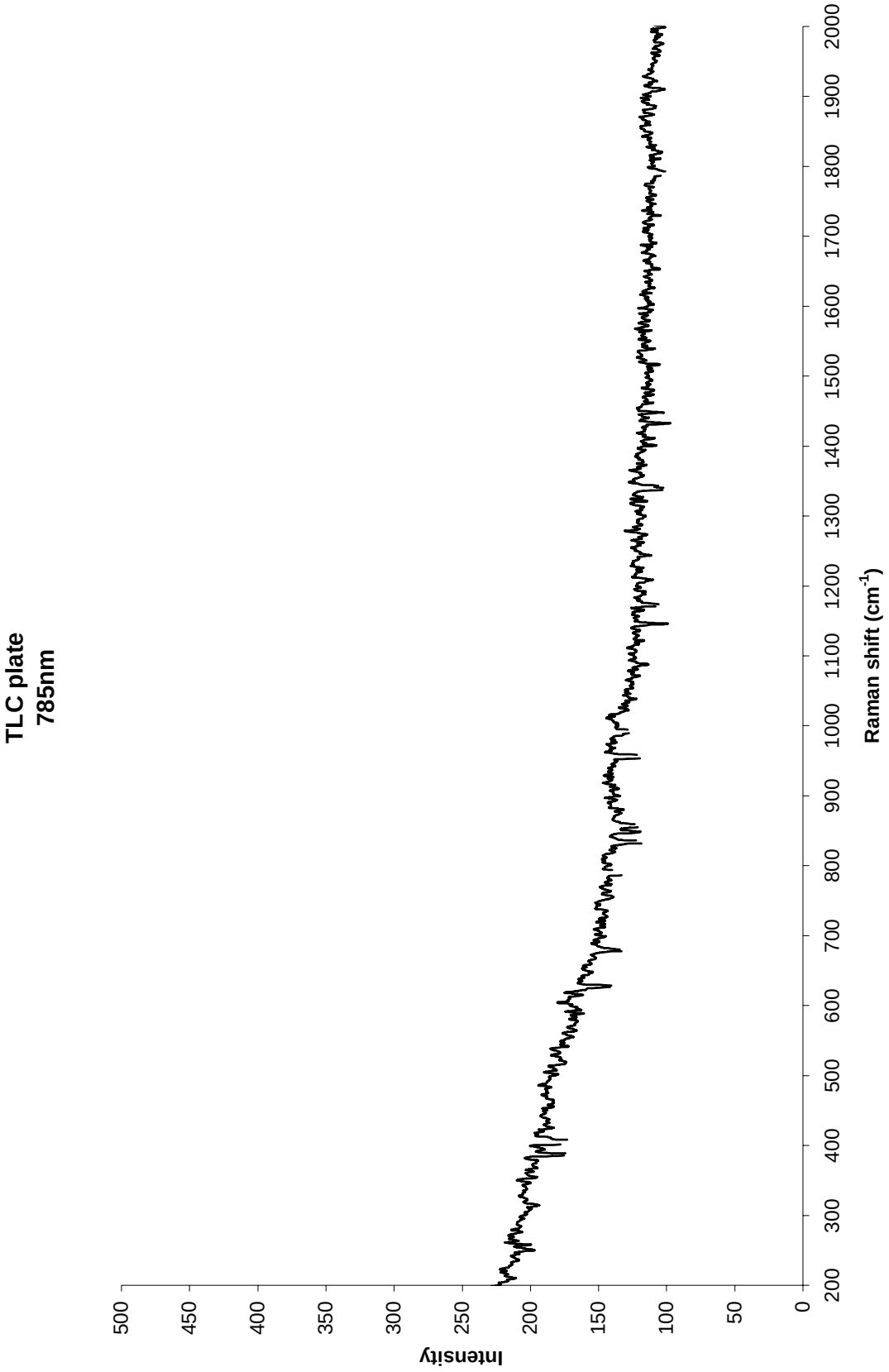
SERS CV	SERS MV spot 1	SERS MV spot 2	SERS MV spot 3
225.5	220.5	224	225
	244	244	243
339.5	352.5	347.5	347
416	414.5	414.5	414.5
439.5			
526	522.5	523	525
		580	582
733	733.5	733	733.5
760	760.5	761	760.5
806.5	811	809.5	808
911	905.5	906.5	906.5
	976	968	973
995	992.5	992	992.5
1041.5			
1172.5	1169	1170	1170.5
1295	1295	1296.5	1296
1380	1376.5	1377.5	1379
1433.5	1429	1431.5	1430.5
1507	1496	1497	1497
1588	1588	1586.5	1589
1619	1614	1613.5	1614











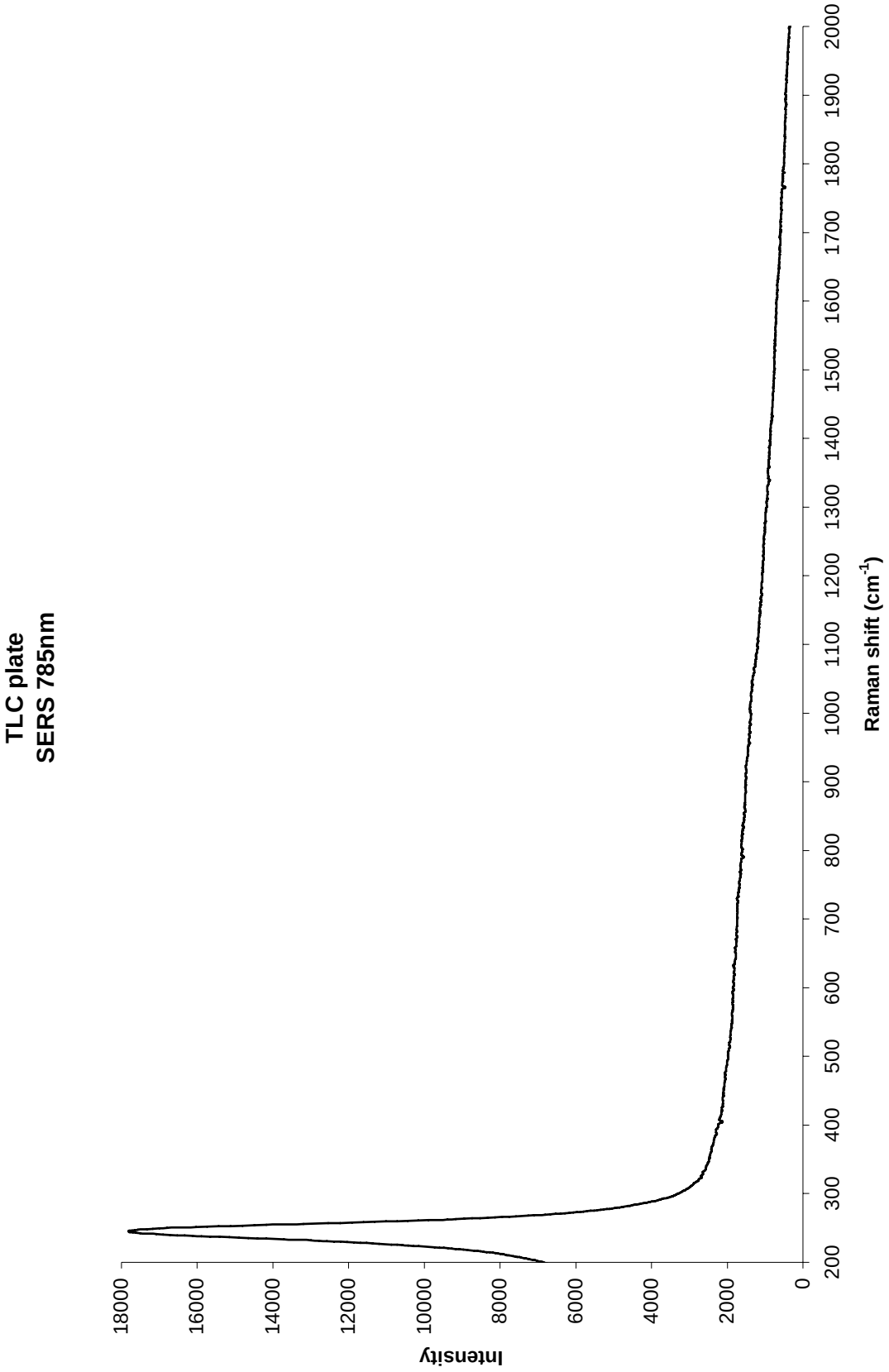


Table 1 – Frequencies and assignments of the Raman and SERS spectrum of Morphine

		Morphine		
Intensity	**Bond Stretch**	**DFT**	**Regular**	**SERS**
m	C-O-H bend on ring A; C-N-C bend	309	330	
w	C-O-H bend on ring C; C-H rock in ring B	343	353	
w	CH_3-N rock; O-H bend on ring A	405	401	
w	CH_3-N rock and wag	462	441	443
w	C-H rock throughout molecule	542	528	531
s	C-C-O bend in ring C; C-H rock on ring C	609	607	607
vs	ring bend (cage); no N movement	625	630	628
m	C=C rock in ring A	667	676	672
w	C-H rock on ring C & ring B	777	788	781
vw	C-H rock on ring B	882		
m	all C-Hs rock	1031	1048	1035
s	C-H around N rock and wag	1068	1089	1089
s	O-H bend on ring A; H-C=C-H rock	1113		
s	C-H rock on ring A	1121	1121	1111
m	O-H rock on ring C; C-H on ring B; C=C-H	1177	1178	1155
s	C-C bend; C-H & O-H rock on ring C	1230	1246	1227
m	C-H rock on ring B	1263	1279	1296
w	C-H rock on ring A	1293		
vs	C-H stretch on ring C; C-H stretch on ring B	1350	1327	1321
vs	More C-O-C and C-H stretch on ring C	1378		
s	O-H & C-H rock on ring A	1416		
s	C-H scissor on ring B	1469	1476	1444
s	ring C deformation; O-H bend on ring C	1654	1634	1627
vs	ring C quinoidal stretch	1684		
vs	C_a=C_b symmetric; H-C_a=C_b-H rock on ring A	1699		

vs: very strong
s: strong
m: medium
w: weak
vw: very weak

Table 2 – Frequencies and assignments of the Raman and SERS spectrum of Codeine

	Codeine			
Intensity	**Bond Stretch**	**DFT**	**Regular**	**SERS**
vw	C-H rock on ring B; O-CH_3 rock on ring C	392	383	375
w	O-H rock on ring A; C-H stretch by N	407		424
vw	ring A twist	469	433	443
w	C=C swing on ring A; C-H bend on ring C	542	532	531
s	Breathe bend of ring B & ring C; no N movement	619	628	629
s	Breathe bend of ring B & ring A; no N movement	634	649	650
w	H-C_a=C_b-H rock on ring A	668	677	675
w	ring C Carbon rock; H-C=C-H bend on ring A	710	720	734
vw	C-H twist on ring B	750	769	761
w	C-H bend on ring B	783	788	
vw	C-H in ring C rock in plane	816		816
vw	C-H structure rock; C=C, C-H wag	833	831	
vw	C-H rock on bridge, ring A, & ring C	855	854	
vw	C-H on ring C rock out plane	937		915
vw	C-H twist on ring C; C-H rock on ring B	951	953	
w	C-H twist on bridge & rock on ring A	996	989	
w	C-H stretch on ring A & bridge	1123	1124	
m	C=C scissor; C-O-H bend	1199	1188	1182
m	C-H wag on ring B & ring C	1238		1229
m	O-H bend on ring A	1255	1253	
s	C-H twist on bridge	1281	1279	1289
vs	C-O-Me bend on ring C	1294		
m	C-H rock on ring B	1348	1336	1352
s	C-H wag on ring A & ring B	1379		1369
s	N-Me rock	1466		
s	O-Me rock; C-H symmetric stretch on ring B	1476		
vs	O-Me asymmetric stretch; N-Me wag	1494		
w	ring C deformation	1634	1635	1619
vs	ring C quinoidal stretch	1671		
vs	C_a=C_b symmetric stretch; H-C_a=C_b-H rock	1699		

vs: very strong
s: strong
m: medium
w: weak
vw: very weak

Table 3 – Frequencies of the Raman and SERS spectrum of Hydrocodone

Hydrocodone	
Regular Raman	**SERS**
312	308
348	
377	378
416	417
440	440
513	514
567	568
594	595
639	640
693	695
766	762
901	904
961	964
1037	
1108	1110
1227	1228
1269	1278
1361	1350
1437	1449
1567	1539
1613	
1638	

Table 4 – Frequencies of the SERS spectrum of Codeine, Morphine and Hydrocodone

Codeine **SERS**	***Morphine*** **SERS**	***Hydrocodone*** **SERS**
		308
375		378
424		417
443	443	440
531	531	514
		568
		595
	607	
629	628	640
675	672	695
734		
761	781	762
816		
		904
915		964
	1035	
	1089	
	1111	1110
	1155	
1182		
1229	1227	1228
1289	1296	1278
1369	1321	1350
	1444	1449
		1539
1619	1627	

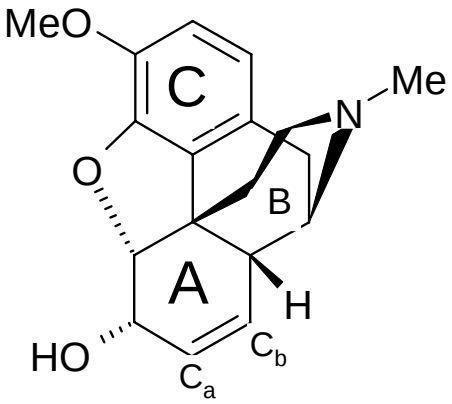


Figure 1. Structure of Codeine molecule

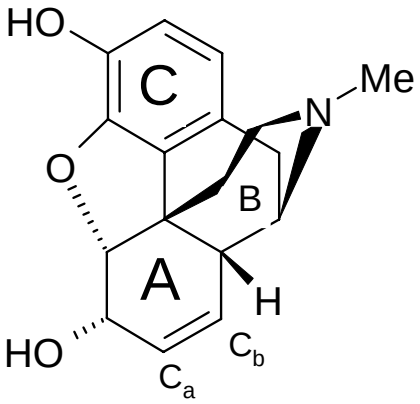


Figure 2. Structure of Morphine molecule

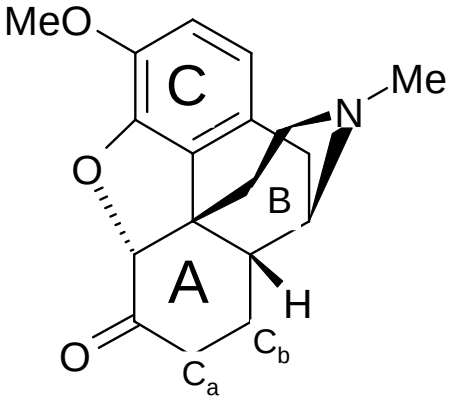


Figure 3. Structure of Hydrocodone molecule

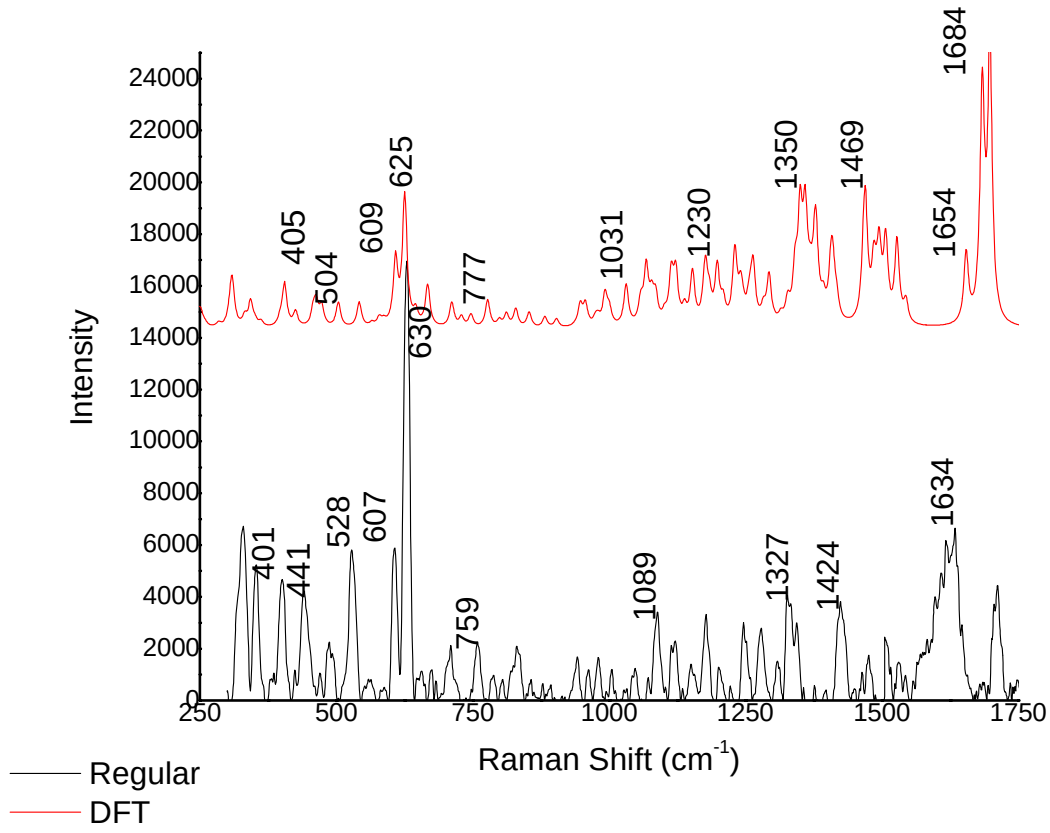


Figure 4. Comparison of the fluorescence rejection regular Raman spectrum of morphine to its calculated DFT spectrum in the region of 400-1750 cm^{-1}

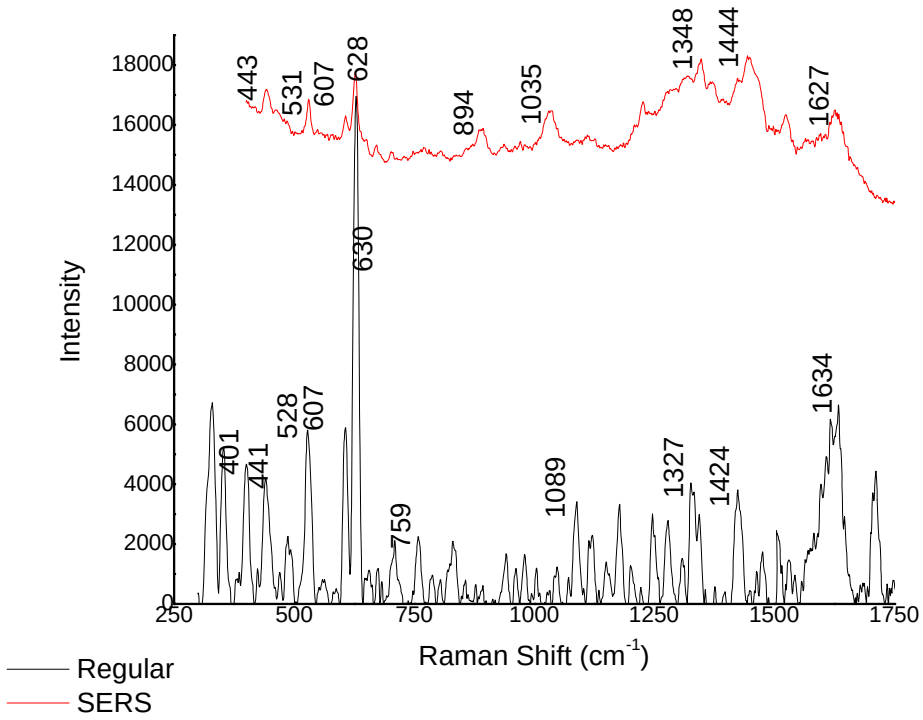


Figure 5. Comparison of the SERS spectrum of morphine to the fluorescence rejection regular Raman spectrum of morphine in the region of 400-1750 cm^{-1}

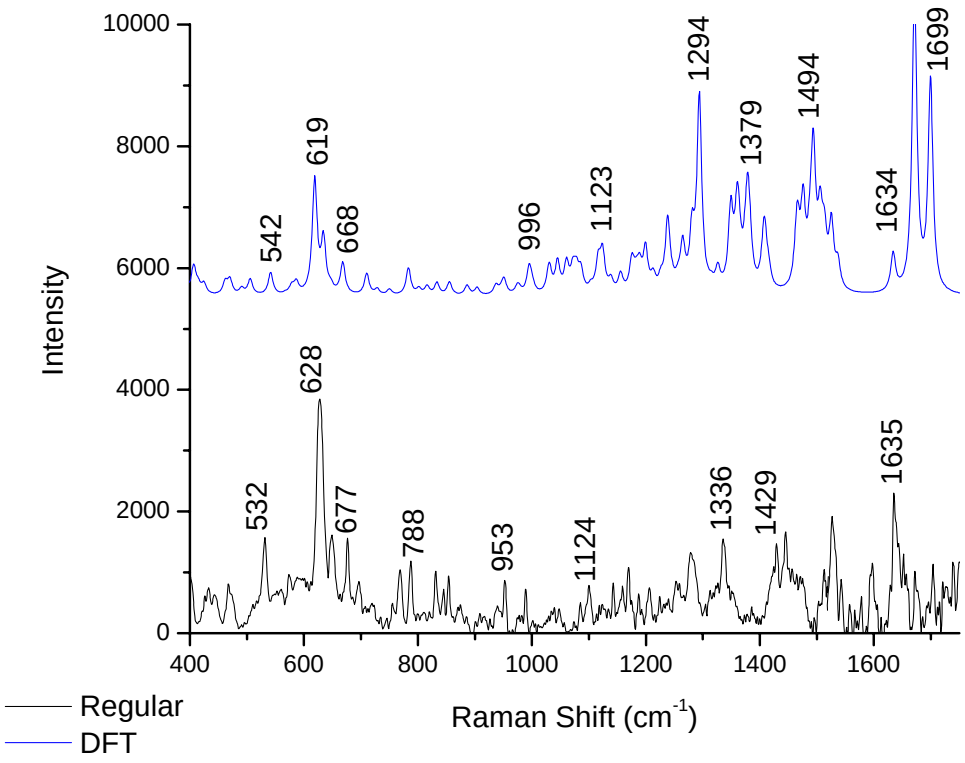


Figure 6. Comparison of the fluorescence rejection regular Raman spectrum of codeine to its calculated DFT spectrum in the region of 400-1750 cm^{-1}

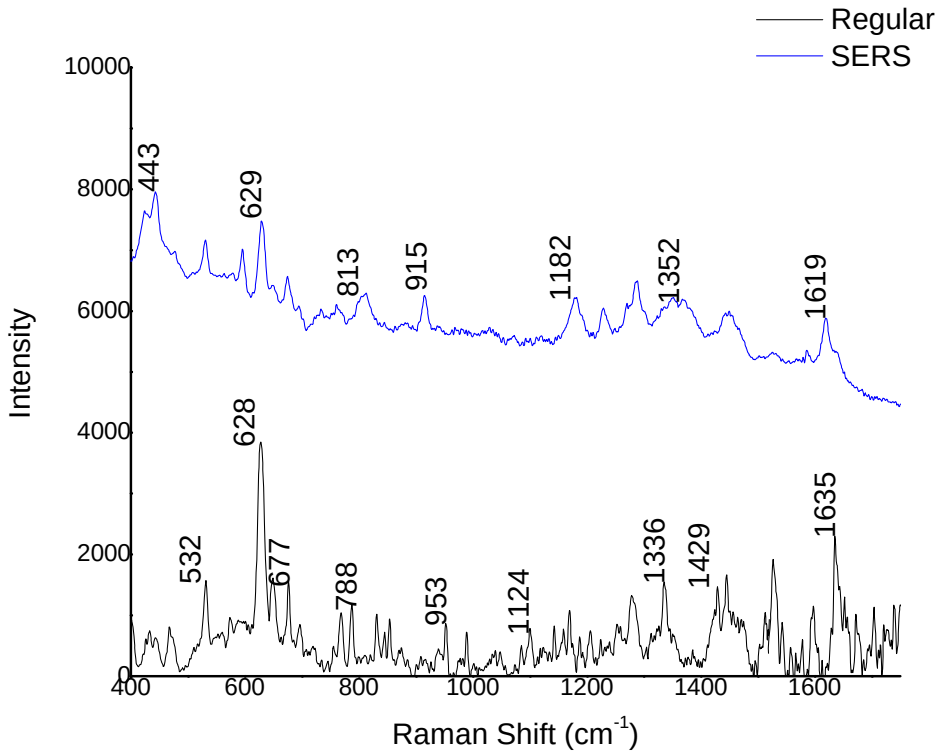


Figure 7. Comparison of the SERS spectrum of codeine to the fluorescence rejection regular Raman spectrum of codeine in the region of 400-1750 cm^{-1}

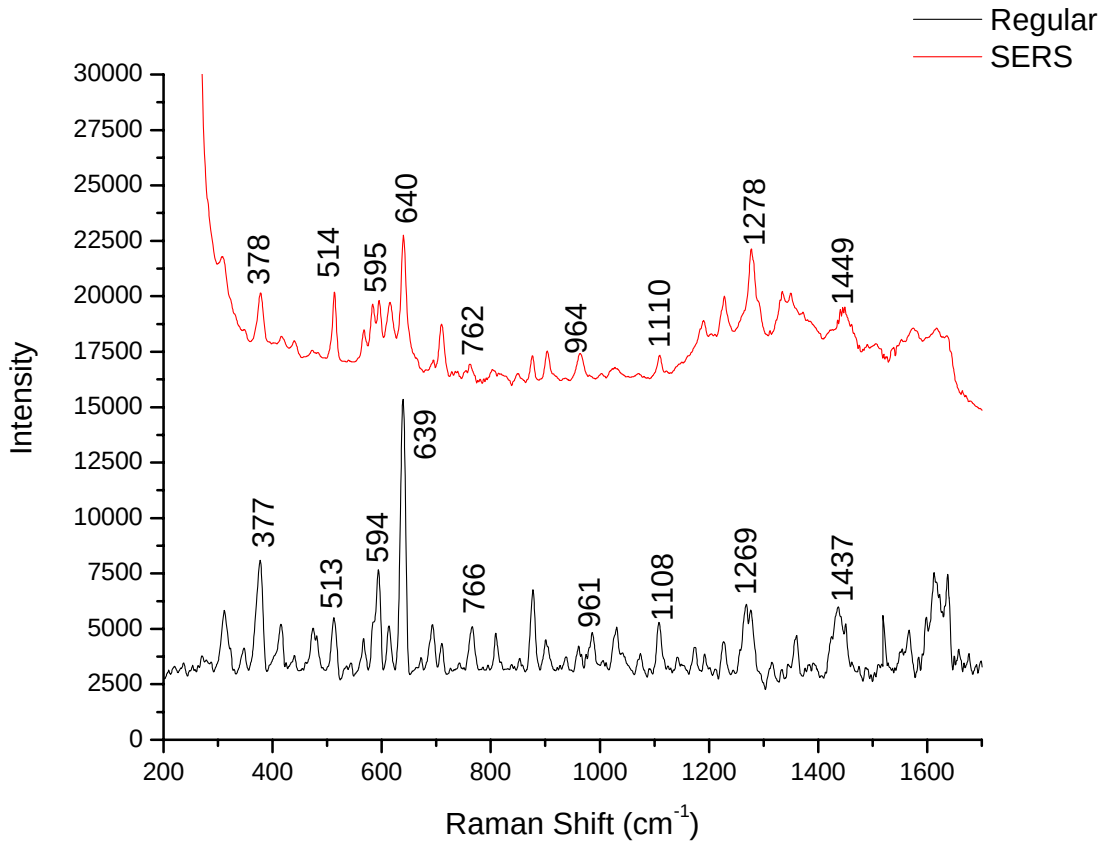


Figure 8. Comparison of the SERS spectrum of hydrocodone to the regular Raman spectrum of hydrocodone in the region of 400-1750 cm^{-1}

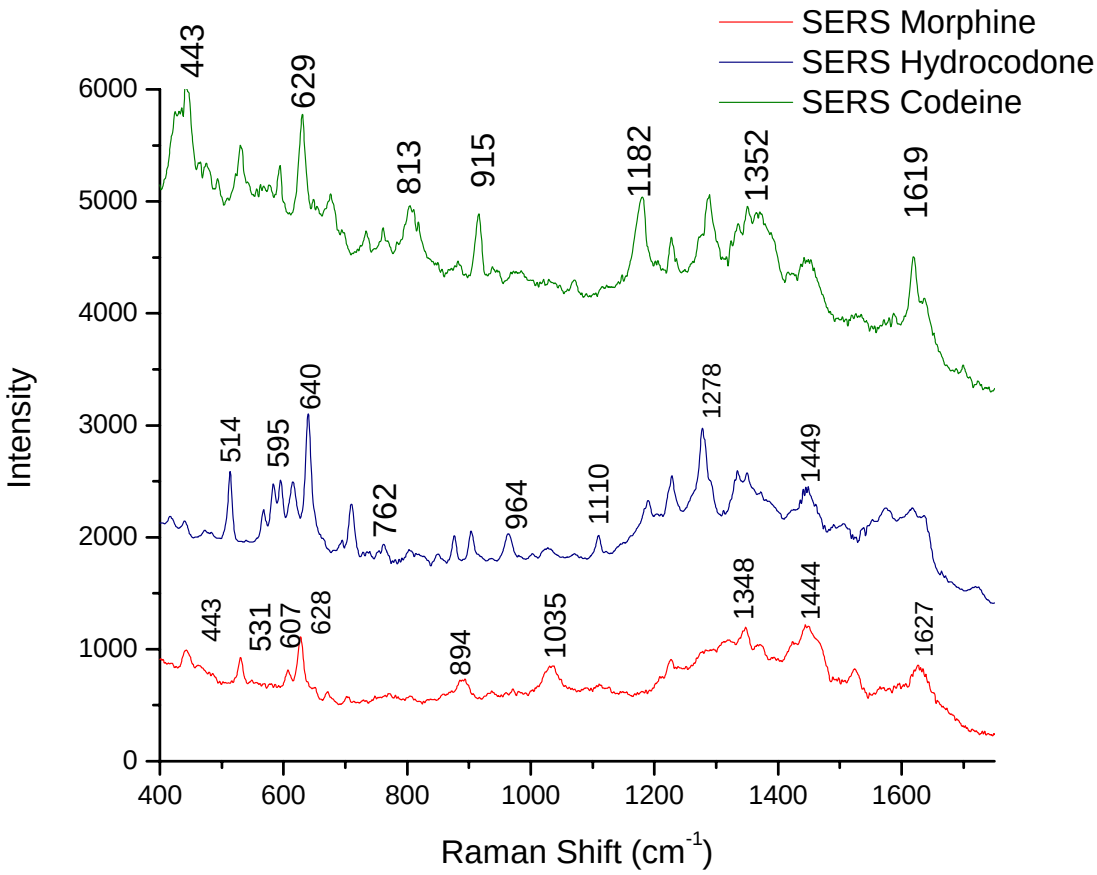


Figure 9. Comparison SERS spectra of codeine, morphine and hydrocodone in the region of 400-1750 cm^{-1}

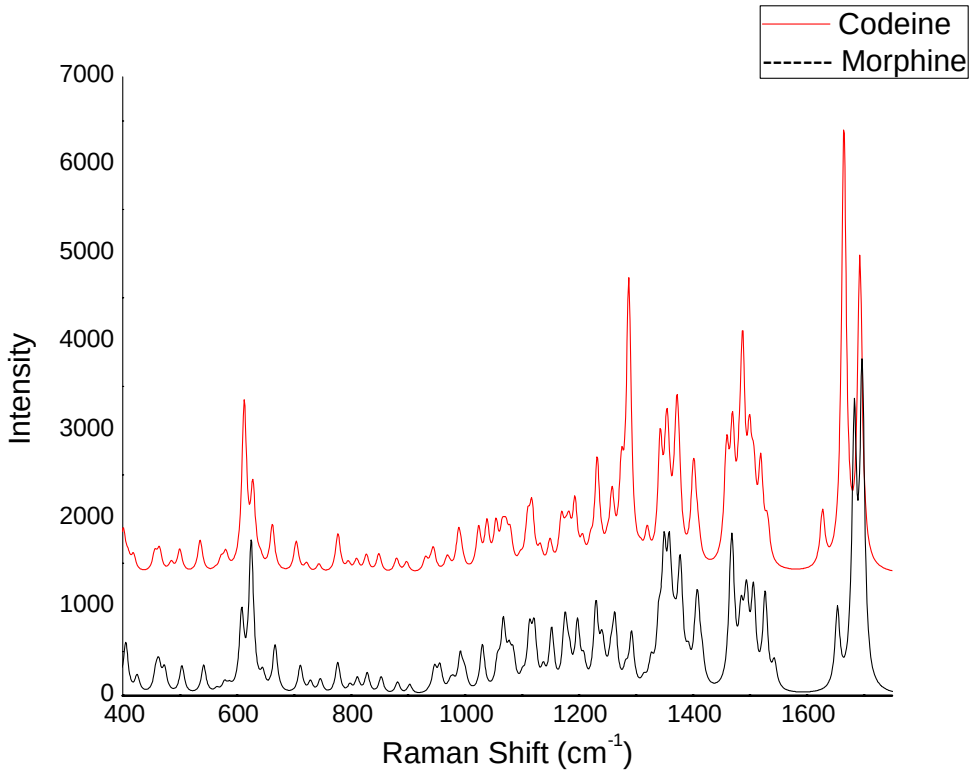


Figure 10. Comparison DFT calculated spectrum of codeine and morphine in the region of 400-1750 cm^{-1}

IV. Conclusion

1. Discussions of Findings

Although normal Raman spectra were obtained for all the dyes, the study showed that only the FT system with the 1064 nm Nd/YAG laser performed consistently. All the 1064 nm spectra had excellent signal intensity and signal to noise ratios. At the same time, the spectra determined with the 633 nm and 785 nm lasers differed in their signal clarity for each of the dyes. Only acid orange 10 responded well to both laser wavelengths. The 633 nm laser exceeded in its performance over the 785 nm wavelength for aniline blue, victoria blue, and sudan black b. These results can be explained by resonance Raman as the three dyes are similar in their deep blue color. Additionally, high levels of fluorescence were observed in all the 633 nm spectra. The 785 nm laser provided clear spectra for the rest of analytes including both red and blue colored dyes. Overall, the results contained enough individualizing peaks to easily differentiate the dyes. The exceptions were the spectra of crystal violet and methyl violet which are extremely similar, but so are the molecular structures of the compounds which differ only in the number of methyl groups. It should be noted that the analyte spectra obtained at different laser wavelengths did not correspond exactly. Such results may be due to low quality of many of the normal Raman spectra, differences in spectral resolutions used, and variations in peak identification by Origin® data analysis software. Noise may also account for some of the peak outliers observed in the spectra.

Excellent surface-enhanced Raman spectra were obtained for all the dyes with both 633 nm and 785 nm lasers. Approximately 4x increase in signal intensity was observed with the use of the 633 nm wavelength, although pararosaniline and sudan black b presented higher intensity spectra with the 785 nm laser. Differences between NR and SERS spectra were noted in the change of intensity of individual peaks, and the appearance of certain peaks in one type of the spectrum, but not the other. Occasional peak shifts (within 10 cm^{-1}) were also observed. These peak variations are explained by selection rules that dictate

which molecular bonds are Raman and SERS active. Adsorption of the analyte to the surface of the colloid and its orientation there changes the molecular symmetry altering the activity of the bonds. Peak shifts are also not uncommon and a bathochromic shift is particularly apparent with the adsorbed aromatic compounds such as dyes. (Smith & Dent, 2005).

The normal Raman and surface-enhanced Raman spectra obtained directly from the ink line were of poor quality; although the 1064 nm spectrum presented a recognizable peak pattern. As only the 785 nm wavelength was utilized for SERS in this part of the study, perhaps cleaner, higher signal/noise spectra could have been determined with other lasers. The TLC analysis showed that the methyl violet standard separated into three spots on the plate while the ink extraction presented only two spots corresponding to methyl violet in color, Rf values, and SERS spectra. Such results were probably due to different combinations of the demethylated pentamethyl pararosaniline found in both the standard and the ink. The surface-enhanced Raman spectra of the ink dyes obtained from the thin-layer chromatography plate contained high intensity peaks and were consistent with the SERS spectra of crystal and methyl violet standards determined from both drop on a microscope slide and TLC substrates. The spectrum of the third ink dye spot did not correspond to any of the dyes in this study.

As only one surface-enhanced Raman scattering condition was explored, other colloids and/or aggregants may provide higher quality spectra and should be considered for future studies. FT-Raman SERS research may also be beneficial in evaluating applicability of the 1064 nm laser. Additionally, surface-enhanced Raman evaluation of TLC ink separations with various laser wavelengths may provide higher quality spectra. A quantitative study with both NR and SERS of the dyes would be valuable in establishing limits of detection. Density functional theory calculations should also be conducted to properly assign peaks in both normal Raman and surface-enhanced Raman spectra.

DFT Comparison of Codeine and Morphine

A comparison DFT spectrum was constructed to show how the two drugs shared some peaks and differentiated in others. (Figure 10) The peaks in morphine and codeine found in the 600 cm^{-1} and 1600 cm^{-1} range are similar because the same vibrations are present. The 600 cm^{-1} vibration were associated with breathing of the whole structure, hence a strong intense peak was seen. On the other hand, the early vibrations in the 1600 - 1700 cm^{-1} range correspond to a deformation of ring C in both morphine and codeine, while the higher frequency vibrations in this region corresponded to a vibration of the double bond found in ring A. The bands that differentiate these two opiates correlated to the C-H rock and the C-H twist throughout the structure. In the morphine, these vibrations were observed around 1000 cm^{-1}, while in codeine, they are around 900 cm^{-1}. Also, the intense peak at 1294 cm^{-1} in codeine was a distinguishing characteristic to that drug because the morphine spectrum did not have an intense peak there.

SERS Comparison

A comparison of SERS spectra showed that these three compounds can be readily distinguished from each other see Figure 9. Codeine had intense bands at 629 cm^{-1}, 915 cm^{-1}, 1182 cm^{-1}, and 1619 cm^{-1}, while morphine had intense bands at 628 cm^{-1}, 1035 cm^{-1}, 1348 cm^{-1}, and 1627 cm^{-1}. For hydrocodone, the intense bands are 514 cm^{-1}, 640 cm^{-1}, 964 cm^{-1}, and 1278 cm^{-1}. These intense bands alone distinguish between these three drugs, and can be employed to indicate the presence of them. The region that helped in discrimination was around 600 cm^{-1}, where the most intense band was found in all three opiates. In hydrocodone, an intense peak was seen at 640 cm^{-1} but it was complemented with additional peaks in the high 500 cm^{-1} range, which was not seen in either of the other two drugs. In this same region, codeine was indicated because its 629 cm^{-1} peak had a small shoulder peak at 650 cm^{-1}. While in morphine, its 628 cm^{-1} peak had a smaller shoulder peak at 607 cm^{-1}, which was different from both codeine and hydrocodone. Therefore, using this region first and then looking at the rest of the spectrum, one should be able to distinguish between these three drugs.

2. Implications for Policy and Practice

With over 2,600 articles published on SERS since its discovery in 1974, it is clear that SERS is a generally accepted scientific technique. Thus, we believe that the basic criteria of Court Admissibility as put forth in *Frye*[28] are met by this technique. However, as indicated by the number of cases in which attempts at questioning techniques with a much longer track record than SERS were made[29], careful validation will be needed for the technique to be of use in forensic science.

The validation of SERS should be based upon the data obtained by CCNY, ORNL, NYPD, and MMA. Validation should include documentation to demonstrate that the application of SERS to forensic samples is a reliable, reproducible, and powerful technique. Also useful is the methodology delineated in the American Society of Crime Laboratory Directors Manual[30] and the ISO (the International Organization for Standardization) and IEC (the International Electrotechnical Commission) Manual[31]. A series of round robin samples should further support the use of SERS in the analysis of forensic samples. The initial data obtained must show that the methodology provides quality analytical data. Validation of the database will also be required. This research will result in providing greater discrimination between questioned and known forensic samples. The ability to provide greater discrimination between similar materials will result in a reduction of false inclusions, and an analysis that shows consistency between samples will result in a stronger association. The result of this research will provide the qualification and validation necessary for Raman spectroscopy to meet the standards delineated in *Frye* and *Daubert*. Calibration of the instruments should be performed prior to use by following individual instrument manufacturers recommendations and by following the American Society of Testing and Materials (ASTM) standard guide for calibrating Raman spectrometers, ASTM E1840 (1996)[32]. Quality Raman spectra depend on optimum instrumental parameters. Two Raman spectrometers have been used in this study, a Renishaw Dispesive Micro-Raman spectrometer (750nm laser, CCD detector) and a Nicolet FT-Raman system (1064nm laser, InGaS and Ge detectors). Both spectrometers are be adjusted to obtain high quality spectra. The

parameters for the Renishaw Raman instrument that can be adjusted include: the use of neutral density filters, the number of scans, exposure times, changing the laser spot size, and using different microscope objectives. The parameters for the Nicolet Raman instrument that can be adjusted include: the number of scans, the use of a neutral density filter, and the laser power. A reliable database will facilitate the identification of individual components present in complex matrices. The qualification and validation SERS and the data base(s) developed will be modeled to meet the standards delineated in *Frye* and *Daubert*[33].

This study provides analytical data on forensic samples that does not appear in the literature: in fact, no systematic library of SERS spectra is currently available. This research will result in providing greater discrimination between questioned and known samples. The ability to provide greater discrimination between similar samples will result in a reduction of false inclusions, and an analysis that shows consistency between samples will result in a stronger association.

3. Implications for Further Research

The work carried out in Phase I of the research project demonstrated that SERS is a valuable technique for the identification of organic colorants used in inks and for the dyeing of textile fibers, as well as for trace analysis of pharmaceuticals and of drugs of abuse. Analytical procedures for SERS of a number of representative dyes were developed, the core of a high quality spectral database was assembled as a proof of concept experiment, and innovative non-destructive approaches were investigated. To date our colorants database includes approximately 50 natural and synthetic dyes, and it is, to our knowledge, the first SERS spectral database ever assembled (in the course of the work, a normal Raman database of approximately 100 spectra of organic colorants was also assembled). The database is both chemically inclusive (as dyes belonging to the principal classes are represented) and spectrally comprehensive, since both SERS spectra obtained at different wavelengths (488, 633, 785 nm) and normal dispersive Raman (488, 633, 785 nm), and FT-Raman (1064 nm) are included.

For SERS to become a routine technique, the database must be expanded significantly, preferably by including well characterized ink compositions.

Sample handling techniques must also be improved, with the goal of matching the non-destructive approach of conventional Raman spectroscopy. Two promising methods have been developed, hydroxygel transfer SERS (HT-SERS) and inkjet nanoparticle delivery SERS (IND-SERS), and their use in the analysis of written and printed documents should be carefully explored.

Finally, further attention should be given to the analysis of problematic ink systems, such as gel pen inks and black inks. We have received a set of 45 black gel pen ink samples, and we plan to develop extraction techniques for gel inks suitable for SERS analysis, and in particular we will refine the hydroxygel transfer method to improve its applicability to non-destructive sampling of gel inks.

The rapid identification of drugs of abuse is another area in which SERS can be of significant interest. We see here two directions for development: detection of drugs of abuse in presence of extenders and cutting agents (which can be fluorescent and therefore obscure the conventional Raman spectrum of the illicit components), and, in a complementary fashion to gas-chromatographic analysis, identification of closely related precursors of designer drugs. Because of its high sensitivity SERS can be used with low cost portable devices; these devices generally suffer from poor fluorescence rejection and inadequate sensitivity. Using SERS and normal Raman on samples of unknown substances, it is feasible to detect both SERS-inactive inorganic and organic extenders, as well as SERS active ingredients. An example is the analysis of Vicodin. Besides a variety of inactive ingredients, the drug has two main components, acetaminophen and hydrocodone. The concentration of acetaminophen, a strong Raman scatterer is 100 times higher than that of hydrocodone, a weak scatterer. Acetaminophen does not adsorb on silver nanoparticles efficiently, leading to poor SERs activity. Therefore, analyzing a suspect tablet in normal Raman and in SERS conditions (for instance by simply dropping some silver colloid on the tablet) would reveal the presence of the opiate. The situation is probably a good model for that of any

suspect composition, where the illicit ingredient could be masked by several extenders.

The second area of development for SERS in the analysis of drugs of abuse is as a specific probe for molecular structure. We demonstrated in our work on berberine alkaloids (insert reference) that small variations in molecular structure can result in significant changes in the SERS spectrum. The example of the berberine alkaloids is actually quite interesting in a forensic context. These compounds can be seen as model compounds for metamphetamines, as they have methylenedioxy groups in their structure. Thus SERS could be used to distinguish precursor for methamphetamine drugs if a suitable spectral library was developed.

During phase II we will work to place SERS in an analytic flow of evidentiary chain, and to complete the Raman library. We also plan to test the validation techniques and the library data base. In order to carry out phase II more effectively Patrick Buzzini at West Virginia University has agreed to join our effort. His focus is on research and development of methods for the exploitation of the evidence, with primary interest in the comprehension of the contribution of the evidence in a legal context. We believe this additional expertise is needed for the satisfactory continuation of this project. Dr. Buzzini has experience in Raman spectroscopy on the analysis of fiber dyes and has also applied the Raman technique to his caseworks in paint evidence involving the detection of pigments. In phase II, he will participate in acquiring ordinary Raman spectra of reference dyes. Students and a post-doctoral fellow will be recruited to work on this project from CCNY, John Jay and VWU. With the aid of funding from the City University of New York, we have already begun work on expansion of the data base to produce a searchable library of SERS spectra needed for positive identification of trace samples and test limits of detection to ensure clear positive or negative determinations. The work on controlled substances will be expanded to include controlled substances commonly encountered in a crime laboratory, pharmaceutical preparations, counterfeit or clandestine substances, precursors, and degradation products. We will also examine real samples obtained from crime scenes provided by Philip Antoci at the NYPD, these methods and techniques will

be applied, when feasible, to the examination of related casework samples submitted to the NYPD crime laboratory. We will carry out further validation studies using 45 black gel pen ink samples kindly supplied to us by the Secret Service.

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VI. Dissemination of Research Findings

1. Presentations and Information Exchange

- On November 30, 2007, the project's Principal Investigators Dr. John R. Lombardi (CCNY) and Dr. Marco Leona (The Metropolitan Museum of Art) hosted a meeting of the Society of Applied Spectroscopy at the museum. The program included a tour of the facilities; a tutorial on Surface Enhanced Raman Spectroscopy (SERS) and Dr. Leona gave a talk regarding the project results and detection methods.
- Principal Investigator Dr. Tuan Vo-Dinh gave a presentation on "Nanobiosensing Using Plasmonics and SERS Nanoprobes" at the LEOS 2007 conference in Orlando, FL, October 23, 2007.
- Dr. Marco Leona gave a presentation on Oct. 17, 2007 at the General Forensic TWG: Fall Meeting.
- "Plasmonics Nanostructures and Nanoprobes for Chemical, Biological, and Medical Sensing," (Dr. Tuan Vo-Dinh), Fitzpatrick Institute for Photonics, 7th Annual Meeting in Durham, NC, October 11, 2007.
- The CCNY based post-doc. Dr. Maria Vega Canamares attended the LACONA VII Conference (Laser for the Conservation of Artworks) in September 2007. At the conference Dr. Canamares not only learned about other applications of SERS to the analysis of dyes, but she was able to discuss the work of this project and how evolving techniques in art conservation might also apply to direct analysis of dyes on substrates such as paper.
- "From Art and Archaeology to Forensic Science: Surface-Enhanced Raman Scattering as an Analytical Tool" (Dr. Marco Leona and Dr. John R. Lombardi) was presented at the Boston National Meeting of the American Chemical Society, August 23, 2007.
- On August 21, 2007, the Principal Investigator and NYPD Forensic consultant, Philip Antoci, hosted a tour of the NYPD Crime Laboratory in Jamaica, Queens. Several of the projects graduate and undergraduate students were in attendance as well as post-doc Maria Vega Canamares.
- Attend IRUG-8 Conference Vienna, March 2008. John Lombardi and MariaVega Canamares. Talk: Raman and Surface Enhanced Raman Spectra of 7 and 3',4' Hydroxyflavone"

2. Publications Resulting from this Award

Published or Accepted for Publication:

1. "Raman and Surface Enhanced Raman Scattering of 3-Hydroxyflavone", Minfa Wang, Tatyana Teslova, Fen Xu, John R. Lombardi, Ronald L. Birke, and Marco Leona, J. Phys. Chem.C, 111, 3044-3052 (2007).
2. "Raman and Surface Enhanced Raman Spectra of Flavone and Several Hydroxy- Derivatives", Tatyana Teslova, Charlie Corredor, Richard Livingstone, Tudor Spataru, Ronald Birke, John R. Lombardi, Maria Vega Canamares, and Marco Leona, J. Raman Spectroscopy, 38, 802-818 (2007). DOI:10.1002/jrs.1695.
3. "Time Resolved Surface Enhanced Raman Scattering Studies of 3-hydroxyflavone on a Ag Electrode", Minfa Wang, Tudor Spataru, John R. Lombardi, Ronald L. Birke, J. Phys. Chem. C, 111, 3038-3043 (2007).
4. "Time-Dependent Picture of the Charge-Transfer Contributions to Surface Enhanced Raman Spectroscopy", John R. Lombardi and Ronald L. Birke, J. Chem. Phys. 126, 244709 (2007).
5. "Identification of Berberine in Archaeological Textiles by Surface Enhanced Raman Spectroscopy", Marco Leona, John R. Lombardi, J. Raman Spec. 38, 853-858 (2007). DOI:10.1002/jrs.1726.
6. "ZnO Nanoparticle Size-dependent Excitation of Surface Raman Signal from Adsorbed Molecules: Observation of a Charge-Transfer Resonance", Zhihua Sun, Bing Zhao, John R. Lombardi, *Appl. Phys. Lett*., 91,221106 (2007).
7. "Effect of Fluence on the Discoloration of Marble Cleaned with UV Lasers"; Jie Zhang, Andrew J. Birnbaum, Y. Lawrence Yao, Fen Xu, John R. Lombardi, Applied Surface Science, 253, 3083-3092 (2007). DOI: 10.1016/j.apsusc.2006.06.056
8. "Raman Scattering Study of Molecules Adsorbed on ZnS Nanocrystals", Yanfei Wang, Zhihua Sun, Hailong Hu, Shengyu Jing, Bing Zhao, Weiqing Xu, Chun Zhao, John R. Lombardi, J. Raman Spectroscopy, 38, 34-38 (2007).
9. "Mechanism and Prediction of Laser Wet Cleaning of Marble Encrustation", John R. Lombardi, Fen Xu, Y. Lawrence Yao, Jie Zhang, Proceedings of ICALEO Conference, November, 2006.
10. "Surface Enhanced Raman Scattering on Mercaptopyridine-Capped CdS Microclusters", Y. Wang, Z. Sun., Y. Wang, H. Hu, B. Zhao, W. Xu, J.R. Lombardi, *Spectrochimica Acta A*., 66A, 1199-1203 (2007).

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12. "Photoinduced Shape Evolution: From Triangular to Hexagonal Silver Nanoplates" Jing An, Bin Tang, Xiaohua Ning, Ji Zhou, Shupng Xu, Bing Zhao, Weiqing Xu, Charlie Corredor, John R. Lombardi, J. Phys. Chem. C., 111, 18055-18059, (2007).
13. " Laser-Induced Growth of Monodisperse Silver Nanoparticles with Tunable SPR Properties and Wavelength Self-Limiting Effect", Xianliang Zheng, Weiqing Xu, Charlie Corredor, Shuping Xu, Jing An, Bing Zhao, John R. Lombardi, J. Phys. Chem. C, 111, 14962-14967, (2007).
14. "Mercaptopyridine Surface-Functionalized CdTe Quantum Dots: SERS Activity", Yanfei Wang, Junhu Zhang, Huiying Jia, Minjie Li, Jianbo Zeng, Bai Yang, Bing Zhao, Weiqing Xu, John R. Lombardi, J. Phys. Chem.C, 112, 996-1000 (2008).
15. "Surface-Enhanced Raman Scattering Study of the Red Dye Laccaic Acid" M.V. Cañamares and M. Leona. J. Raman Spectroscopy, 38, 1259-1266 (2007).
16. "Study of Laccaic Acid and Other Natural Anthraquinone Dyes by Surface-Enhanced Raman Spectroscopy" M.V. Cañamares and M. Leona. Accepted for publication in Proceedings of Lacona VII.
17. "Raman and Surface Enhanced Raman Spectra of Chrysin, Apigenin and Luteolin" Charlie Corredor, Tatyana Teslova, Zhanguo Chen, Jie Zhang, Maria Vega Cañamares, John R. Lombardi and Marco Leona. (in press Journal of Raman spectroscopy)
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In Preparation:

1. Surface-Enhanced Raman Spectroscopy for Trace Identification of Controlled Substances: Morphine, Codeine, and Hydrocodone, Vinesh Rana, Maria Vega Cañamares, Thomas Kubic, Marco Leona, John R. Lombardi, Submitted for publication, *Journal of Forensic Sciences.*
2. "Application of Surface-Enhanced Raman Scattering to the Detection of Representative Ink Dyes" Irina Geiman, Maria Vega Cañamares, Marco Leona and John R. Lombardi. (in press Journal of Forensic Sciences, June 2009)
3. "A Non-Destructive Approach for the Identification of Organic Colorants by Surface Enhanced Raman Scattering: Hydrogel Matrix Transfer" Marco Leona (to be submitted to Analytical Chemistry)
4. "Inkjet Colloid Deposition as an Aid in the Microanalytical Application of SERS" Marco Leona, Dario Benedetti and Jie Zhang. (to be submitted to Analytical Chemistry)
5. "Detection of Forgeries by Surface-Enhanced-Raman Scattering" Maria Vega Cañamares, Alessa Gambardella and Marco Leona. (to be submitted to Journal of Raman Spectroscopy).
6. "TLC-SERS of Mauve, the First Synthetic Dye" Maria Vega Cañamares, David Reagan and Marco Leona. (to be submitted to Journal of Raman Spectroscopy)