Surface-Enhanced Raman Scattering Detection of Amphetamine and Methamphetamine by Modification with 2-Mercaptonicotinic Acid

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We have demonstrated the use of surface-enhanced Raman scattering (SERS) spectroscopy for the detection of the phenylalkylamines amphetamine and methamphetamine. This work can be viewed as the first phase of development toward a one-step drug detection method with a selective reactive coating on a SERS substrate. This work involves a fairly complicated coupling reaction prior to surface derivatization. Future efforts will be directed at creating a reactive coating directly on the surface. The amines were derivatized by a coupling reaction with 2-mercaptonicotinic acid (2-MNA) with the use of dicyclohexylcarbodiimide (DCC) as the coupling reagent to form the amide compounds AMNA [N-(1-methyl-2-phenylethyl)-2-mercaptopyridine-3-carboxamide] and MMNA [N-methyl-N-(1-methyl-2-phenylethyl)-2-mercaptopyridine-3-carboxamide]. The amines were qualitatively identified from SERS spectra. Quantification of the amines was accomplished by adding the internal standard pentachlorophenol (PCTP) and measuring the intensity of Raman bands of the analyte relative to a Raman band of the internal standard. Calibration curves were plotted of the relative peak intensity ratios as a function of analyte concentration. Detection limits of 19 ppm and 17 ppm were found for amphetamine/2-MNA (AMNA) amide and methamphetamine/2-MNA (MMNA) amide, respectively.

Index Headings: Raman; SERS; Drugs; Methamphetamine; Amphetamine.

INTRODUCTION

There is considerable interest in the detection of illicit drugs, especially the alkaloids heroin, cocaine, and methamphetamine. The ability to detect the presence of a drug in bodily fluids is vital in the area of drug enforcement. Many government agencies and private industries test for drug use. In many instances, immunoassay techniques are used as screening tests that may indicate the presence of a drug in a sample; however, these tests are not intended for quantitative purposes.

The current National Institute on Drug Abuse (NIDA) limit range for drugs present in biological fluids is in the low parts-per-million (ppm) to parts-per-billion (ppb) levels. These trace amounts, coupled with the complex matrix of the fluid, can lead to difficulties in detection, depending on the method chosen for analysis. Additionally, both the parent drug and its metabolite may be present in the sample, and it may be necessary to distinguish between them.

A phenylalkylamine is a broad classification of stimulant drugs derived from, and including, the parent drug amphetamine. They are synthetic drugs more appropriately classified as sympathomimetics, referring to their mimicking action of the sympathetic nervous system that can increase the rate of body functions. Included in this category are amphetamine (Benzedrine), dextroamphetamine (Dexedrine), and methamphetamine (Methedrine or Desoxyn), as well as norephedrine, ephedrine p-chloroamphetamine, and fenfluramine.

The detection and quantification of amphetamine in biological fluids has been accomplished by utilizing spectrophotometric and fluorometric methodologies. Gas chromatography is used quite often with flame-ionization detection of amphetamine and by electron-capture detection of some amphetamine derivatives. Recent efforts to derivatize amphetamine and methamphetamine with ortho-phthalaldehyde (OPA) and with naphtoquinone sulfonic acid (NQS) for HPLC analysis have met with success.

The purpose of this work is to develop a rapid screening method for illicit drug detection. The work described herein is the first phase of the development of a drug-selective surface. The approach we are taking to solving this problem is to first carry out the reaction in solution and attach the drug-coating adduct to the surface. This approach demonstrates the feasibility of the reaction scheme and the selectivity of the SERS spectra for a given drug. Following this first stage of experimentation we will place the coating, in this case MNA, on the surface and react it with dicyclohexylcarbodiimide (DCC). This product will then be reacted with the sample containing the drug. Should the second stage of development prove feasible, we will have produced a simple one-step drug detection test.

We have developed a technique for the detection and quantification of amphetamine and methamphetamine utilizing surface-enhanced Raman scattering (SERS) spectroscopy. SERS is effective in transforming Raman spectroscopy into a highly sensitive technique capable of ultratrace analysis. The molecular specificity of the SERS technique has potential for resolving a mixture into its individual active components; thus, it could be a viable method for the detection of illicit drugs. Due to the large surface enhancement and the small volumes required, SERS is a very sensitive technique. The SERS substrate enhances the Raman signal produced by the component(s) of the sample so that nanogram (ng) amounts of material can routinely be detected.

Our group has developed a number of coatings for SERS substrates that can detect and quantify specific analytes. We have termed these coatings as either passive or active. The passive coatings simply provide an affinity for the analyte and can act as an internal standard for
normalizing the signal. Detection of chlorinated ethylenes on octadecylthiol-coated surfaces is an example of a passive coating. Active coatings exhibit a different Raman spectrum in the presence of the analyte. An example of an active coating is our work with the pH active coatings on SERS substrates. This work presents the first step to a new type of coating. We have termed this type of coating as reactive. The concept is to put a reactive molecule onto a SERS substrate and analyze the reaction product with SERS.

We propose a technique for the detection and quantification of amphetamine and methamphetamine utilizing SERS spectroscopy. This work describes a DCC coupling reaction whereby an amide is formed by coupling the amine with 2-mercaptonicotinic acid (2-MNA). By derivatization of the amine with 2-MNA, a new compound is formed possessing functionalities useful for SERS analysis. 2-MNA was chosen due to its ability to bind to noble metals through both the thiol sulfur and the pyridine nitrogen. We have recently shown that Raman spectroscopy can be used to examine the responsivity to alkali metal cations of diaminodibenzo-18-crown-6, which had been coupled to 2-MNA and attached to a SERS surface.

The product formed between amphetamine or methamphetamine with 2-MNA can be coated onto a SERS substrate and a Raman spectrum obtained from the coated silver surface. Our detection scheme is based on bands observed in the Raman spectrum of the amide compared to the spectra of the starting materials. Quantification of the drug can be accomplished by adding a known quantity of pentachlorothiophenol (PCTP) as an internal standard. The co-adsorption of the amide and PCTP is a competitive process; however, within the range of our experimental concentrations the PCTP appears to cover the surface with a constant fractional coverage. The intensity of the Raman bands associated with the drug/2-MNA complex is measured relative to the intensity of a Raman band associated with the internal standard. An important advantage of this technique is that the coupling reaction is not moisture sensitive so that it may be possible to apply this scheme to the detection of amphetamine and methamphetamine in biological fluids.

**SYNTHESIS OF 2-MNA DERIVATIZED AMINES**

**Materials and Solvents.** d-Amphetamine sulfate and (+)methamphetamine hydrochloride were purchased from Sigma. Absolute diethyl ether, 2-MNA, and DCC were purchased from Aldrich. Dichloromethane and ethyl acetate were purchased from Spectrum and EM Science, respectively. PCTP was purchased from TCI America. Absolute ethanol and methanol were purchased from Pharmco and Spectrum, respectively.

**Synthesis.** The basic synthetic scheme is illustrated in Fig. 1. Reaction conditions were varied to examine the synthesis of the derivatized methamphetamine in chlorinated organic and aqueous alcohol solvent systems. The procedure for each will be provided in this section.

**Chlorinated Organic Solvent Conditions.** Amphetamine sulfate (363 mg, 1 mmol) or methamphetamine hydrochloride (186 mg, 1 mmol) was dissolved in 10 mL of an aqueous saturated solution of K$_2$CO$_3$ to neutralize the sulfate or hydrochloride. The freebase drug was then extracted from the aqueous solution by using 3 mL of CH$_2$Cl$_2$. 2-MNA (155 mg, 1 mmol) and DCC (206 mg, 1 mmol) were added to the combined extracts (6 mL) of CH$_2$Cl$_2$ containing the drug. A condenser was attached to the reaction flask. The reaction mixture was gently refluxed overnight under a nitrogen atmosphere. The mixture was transferred to a separatory funnel with 20 mL of CH$_2$Cl$_2$ and washed with an aqueous saturated solution of NaHCO$_3$ (3 mL) to neutralize unreacted 2-MNA, followed by H$_2$O (2 mL) and a wash with a 10% HCl (1 mL) to neutralize unreacted freebase and DCC, and finally, dried over Na$_2$SO$_4$. The solvent was removed under reduced pressure.

Either the amide of AMNA or the amide of MMNA was redissolved in 10 mL of CH$_2$Cl$_2$ and then further diluted with additional CH$_2$Cl$_2$ to make standard amide solutions. The internal standard of PCTP in CH$_2$Cl$_2$ was added to each solution for a final concentration standard of 3 ppm per each amide solution.

**Aqueous/Ethanol Solvent Conditions.** Methamphetamine hydrochloride (186 mg, 1 mmol) was dissolved in 15 mL of saturated aqueous K$_2$CO$_3$ to neutralize the hydrochloride. The freebase of methamphetamine was then extracted from the aqueous solution by using 3 mL of diethyl ether. The solvent was removed under reduced pressure and the methamphetamine redissolved in 8 mL of ethanol. 2-MNA (169 mg, 1.1 mmol) and DCC (228 mg, 1.1 mmol) in 2 mL of H$_2$O were added to the methamphetamine/ethanol mixture. A condenser was attached to the flask, and the mixture was gently refluxed for 2 h under a nitrogen atmosphere. The reaction was followed by thin-layer chromatography (TLC) using a 50% ethanol/ethyl acetate eluent to monitor the disappearance of starting materials. The ethanol was removed under reduced pressure. The reaction mixture was transferred to a separatory funnel with 10 mL of diethyl ether and extracted with additional ether (3 mL). The combined ether extracts were washed with saturated aqueous

![Fig. 1. Synthetic scheme for the derivatization of amphetamine (R=H) or methamphetamine (R=CH$_3$) with 2-mercaptonicotinic acid.](image-url)
NaHCO$_3$ (3 x 20 mL), followed by a wash with H$_2$O (2 x 10 mL), and then a wash with 10% HCl (1 x 10 mL). The organic layer was dried over Na$_2$SO$_4$. The solvent was removed under reduced pressure.

Ten milligrams (10 mg) of MMNA was dissolved in 5 mL methanol, then further diluted with additional methanol to make standard amide solutions. The internal standard of PCTP in CH$_2$Cl$_2$ was added to each solution for a final concentration standard of 2 ppm per amide solution.

**Substrate Preparation.** The surfaces were prepared by first roughening 0.1 mm silver foil (99.9%, Aldrich) with 12 µm optical polishing paper. The largest enhancement was found when the silver foil was polished prior to etching; this procedure leads to a surface that appears homogeneous. The silver foil was then etched in a rapidly stirred 40% nitric acid solution for 10 to 20 s. The etched foil was rinsed first in deionized water (Millipore, 18 Ω) followed by an ethanol rinse to remove traces of polishing material and any residual acid solution from the surface. The ethanol may also serve to reduce the silver sites (Ag+) in preparation for the coating process. The SERS surface appears light gray in color after proper roughening and etching. A coated substrate was prepared by soaking the roughened/etched silver foil in the amide/PCTP solutions for 20 min. The coated substrates were removed from the amide solutions, rinsed with ethanol to wash off excess unbound amide and PCTP, then air dried. Raman spectra of the amide coated substrates were obtained by cutting the silver foil substrates to fit diagonally in a 10 mm glass cuvette.

**Instrumentation.** A Spectra-Physics (Mountain View, CA) 2025 Kr$^+$ ion laser provided 647 nm light and was used to excite Raman scattering. The design of this system consists of the power supply, Kr$^+$ ion laser, spectrograph, charge-coupled device (CCD), and PC. The optics-based Raman system consists of the following, all optimized for 647 nm excitation: a Kr$^+$ ion high-power excitation source (5–180 mW), beam steering and focusing optics with sample illumination provided by a 50 mm cylindrical lens (Melles Griot, Irvine, CA), a glass cuvette/sample holder, and optics for Raman scattering collection. Laser power was maintained between 5 and 30 mW. Laser powers were measured at the sample, after the beam passed through an appropriate interference filter to remove plasma lines. The collection optics consisted of an F1.8 Minolta camera lens and approximately 3:1 magnification for f-number matching with the f/5.4 mirrors of the spectrograph. The excitation source and Rayleigh line are rejected with a holographic notch filter (Kaiser) designed for the specific excitation source. Spectra were collected with an HR320 (ISA) spectrophotograph with a 1200 grooves/mm grating and a Photometrics CCD 9000 spectroscopic system for a TK512 CCD detector cooled to −102 °C. The spectrophotograph entrance slit was kept at 30 µm and the grating was set from 695 to 717 nm for a spectral range of approximately 800 to 1650 cm$^{-1}$. Integration times varied between 5 and 30 s. The spectra were collected and stored with a PC workstation and analyzed by LABCALC (Galactic) and GRAMS/32 (Galactic) programs. A multipoint baseline correction and eleven-point Savitzky–Golay smoothing routine were employed for all spectra. Calibration curves were constructed with the use of an EXCEL (Microsoft) spreadsheet and GRAMS/32 PLS-IQ.

A Detection Limit, Inc. SOLUTION 633 Raman laser system provided 633 nm light and was also used to collect Raman data. This system is a portable Raman workstation that uses a fiber-optic probe to excite and collect the Raman scattered light. The SOLUTION 633 system provided 25 mW of laser power at the sample and used a thermoelectric (TE) cooled CCD (−10 °C) for detection. The fiber-optic designed system consists of the probe, HeNe laser, control unit, and compact PC. The epillumination probe provided 180° back-scattering. Excitation light enters the probe through a 50 µm silica fiber. Folding mirrors are used for beam steering. A bandpass filter removes plasma lines before the light reaches a dichroic beamsplitter that reflects 633 nm light with 85% efficiency. The light is focused onto the sample by using a 6 mm focusing lens. Back-scattered light (λ = 643 nm) is transmitted by the beamsplitter, through a long-pass filter, and focused onto a collection fiber. The control unit houses the power supply, a spectrograph with a 1200 grooves/mm blazed ruled grating, and a Kodak 0400 CCD detector. The spectral range observed was 500 to 1800 cm$^{-1}$, and integration times of 1 to 30 s were used to collect spectra. The Raman data were automatically transferred to GRAMS/32 for storage and analysis. A multipoint baseline correction was employed for data analysis.

**RESULTS AND DISCUSSION**

We have demonstrated the quantification of the phenylalkylamines amphetamine and methamphetamine, by derivatizing the amines with a reactive coating of 2-MNA by a DCC coupling reaction and by using SERS spectroscopy as a method of detection. The derivatized amines were isolated by solvent extraction with the use of either CH$_2$Cl$_2$ or diethyl ether. Reaction conditions were varied by using either an organic solvent or an aqueous alcohol solvent. The purpose of this was not only to eliminate the use of chlorinated solvents and to reduce amounts of waste solvent but also to reduce time-consuming steps by developing a reaction condition better matched to the sample. The advantages of using aqueous solvent conditions for the DCC coupling of amphetamine or methamphetamine with 2-MNA include a shorter reaction time, the solubility of the reactants, the elimination of chlorinated organic solvent, and the feasibility of carrying out the reaction in an aqueous biological matrix. It is necessary to use a separate SERS substrate for each concentration solution of either the AMNA or MMNA. Since the binding of the 2-MNA portion of the complex to the silver through both the thiol sulfur and the pyridine nitrogen is irreversible, it is not possible to wash the complex off the substrate before sampling the next solution. Additionally, the use of an internal standard that is added to the amide solutions prevents the use of a single substrate for all measurements.

The amide compounds were coated onto separate roughened/etched silver foil substrates and Raman spectra obtained. The compounds were qualitatively identified by Raman bands corresponding to the amide molecule adduct. Quantification of the compounds was accom-
plished by measuring the peak intensity of a Raman band of the amide molecule relative to the peak intensity of a Raman band of an internal standard and demonstrated with chemometrics. PCTP was added to solutions of varying amide concentration as the internal standard for quantification purposes. A Raman band associated with PCTP was used as the reference for the calibration curve of the peak intensity ratio as a function of concentration formed for each amide.

Figure 2a shows the overlaid spectra for the compound AMNA co-coated with PCTP onto SERS substrates for the concentration range of 47.2 ppm to 472 ppm. The Raman bands indicated are attributed to the phenyl ring breathing mode (998 cm$^{-1}$),$^{19-21}$ the pyridinyl ring breathing mode (1055 cm$^{-1}$),$^{22}$ the asymmetric C–N–C stretch (1125 cm$^{-1}$),$^{23}$ the phenyl–C stretch (1205 cm$^{-1}$),$^{19-21}$ the pyridinyl–C stretch (1223 cm$^{-1}$)$^{19,20}$ the amide C–N stretch (1389 cm$^{-1}$),$^{24}$ and the ring stretch (1569 cm$^{-1}$).$^{19-21}$ Also shown (Fig. 2b) are the mean-centered subtraction spectra for AMNA. The spectra were averaged and the mean spectrum subtracted from the original spectrum for the concentration range of 47.2 to 472 ppm. This figure highlights the analytical response of the SERS substrate from high to low concentration.

An overlay of the SERS spectra for the compound MMNA co-coated with PCTP onto SERS substrates is shown in Fig. 3a. The concentration range of the amide is 60.9 to 2435 ppm. The Raman bands of interest include the phenyl ring breathing mode (1000 cm$^{-1}$),$^{19-21}$ the pyridinyl ring breathing mode (1058 cm$^{-1}$)$^{22}$ the asymmetric C–N–C stretch (1126 cm$^{-1}$),$^{23}$ the pyridinyl–C stretch (1219 cm$^{-1}$),$^{19,20}$ the amide C–N stretch (1389 cm$^{-1}$),$^{24}$ and the ring stretch (1572 cm$^{-1}$).$^{19-21}$ Figure 3b is an overlay of the mean-centered subtraction spectra for MMNA. A relatively strong band at 1514 cm$^{-1}$ may be noted in the spectra of both amide compounds and corresponds to the ring stretch of PCTP.$^{25}$ Also noted, in the spectra for MMNA, is a strong Raman band at 1047 cm$^{-1}$ that is
attributed to residual nitrate on the silver foil surface as a consequence of the etching process. This band was eliminated in subsequent experiments by a more thorough rinsing of the etched silver foil prior to surface coating.

Peak intensities of the 998 and 1000 cm$^{-1}$ Raman bands of the AMNA and MMNA amide compounds, respectively, were measured relative to the peak intensity of the 1514 cm$^{-1}$ band of PCTP. The peak intensity ratios $I_{998}/I_{1514}$ and $I_{1000}/I_{1514}$ were plotted as a function of concentration of the respective amide (Figs. 4 and 5). Average detection limits (DLs) of 19 ppm for AMNA and 17 ppm for MMNA were determined. Detection limits were calculated by using the point at which the analyte peak is three times the noise level of the background. The error bars in Figs. 4 and 5 represent the spectroscopic noise expected from the measurement. The variation around the best-fit line indicates that the precision of this method is not limited by the instrument, but rather by the reproducibility of the SERS substrates.

We have also demonstrated the use of chemometrics for quantitation. Figure 6 illustrates development of a partial least-squares (PLS) model for AMNA. Prior to developing the model, we normalized the spectra by using the PCTP peak. Figure 6a shows the standard error of calibration as a function of the numbers of factors used. In this case, one factor produced the lowest error. Figure 6b shows the results of a cross-validation plotted as predicted vs. actual concentration. The $R^2$ of 0.987 shows excellent correlation. Figure 6c shows the matrix representing the first factor; comparison with Fig. 2a shows that this factor contains most of the spectral information. Using this method we found a DL of 18.4 ppm, which corresponds well with the 19 ppm value we obtained by using a simple ratio. The utility of using PLS is that it is easily automated and can solve for more than one component.

CONCLUSION

This work represents an extension of the use of specific surface coatings in SERS detection of illicit drugs. We developed a procedure for the derivatization of amphetamine and methamphetamine with 2-mercaptonicotinic acid to form the amide compounds $N$-(1-methyl-2-phenylethyl)-2-mercaptopurindine-3-carboxamide (AMNA) and $N$-methyl-$N$-(1-methyl-2-phenylethyl)-2-mercapto-purindine-3-carboxamide (MMNA). The derivatized amines can be identified and quantified from SERS spectra. The appearance of the C–N stretch of the amide is evidence of the new compounds.

The DCC coupling reaction employed in this procedure is selective for primary and secondary amines; thus, alkaloids such as morphine, cocaine, and their respective derivatives would not form an amide bond with 2-MNA.

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