Human Hair in the Identification of Cocaine Abuse with Cantilever-Enhanced Photoacoustic Spectroscopy and Principal Component Analysis

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In this study, a novel approach combining different techniques, including Fourier transform infrared (FT-IR) spectroscopy, photoacoustic spectroscopy (PAS) with an interferometric cantilever microphone, and principal component analysis (PCA) along with a proper data preprocessing procedure, have been used in the investigation of hair samples for cocaine abuse. Hair fibers from cocaine-overdose patients have been measured using a simple procedure involving cantilever-enhanced photoacoustic Fourier transform infrared spectroscopy. In addition, a reference group of hair samples from subjects with no cocaine abuse has been measured. We present a first approach to discriminate the cocaine users from the reference group with the photoacoustic method and PCA. With proper data preprocessing methods, the two groups were successfully separated according to their spectra. The results were confirmed with two different classification methods independent of the principal component data analysis.

Index Headings: **Photoacoustic spectroscopy; PAS; Fourier transform infrared spectroscopy; FT-IR spectroscopy; Cantilever microphone; Hair; Cocaine; Principal component analysis; PCA.**

INTRODUCTION

Cocaine is difficult to detect by quick observation; blood or urine tests are always needed for certainty.[1] The tests can be expensive and difficult to perform.[2] Still, the accuracy is good only to a certain limit, and expertise has to be included in the interpretation of results. Hair analysis for drugs started in 1979, when opiates were successfully detected in hair.[3] There are some advantages of using hair samples as opposed to the more commonly used blood or urine samples. Perhaps the most important advantage is a larger detection window. With blood or urine samples, the detection window is typically 2–4 days for most drugs, compared to years with hair samples.[2,3] The record of substances used could be nearly permanent in hair,[4,5] but generally this is not realistic given normal hygiene practices.[2] Once the hair is cut and stored, its condition can be regarded as quite stable.[4,6,7] The larger detection window gives a possibility to examine a person's drug history for systematic, occasional, or coincidental abuse. Hair sampling is also considered a noninvasive technique by most.[4,8] Hairs are easily collected and do not suffer from the preparation and storage problems often encountered with blood and urine samples. There are also cases where hair is the only available sample material, for example, in a crime scene. Generally, this applies in cases where urine or blood samples are not available or are somehow inappropriate. The drawbacks of using hair samples relate to some uncertainties, even in the basics, when it comes to hair as a matrix for the drugs. These include drug incorporation into hair, the dose–concentration relationship, and external effects and contamination.[2,4,9]

This study focuses on the use of cantilever-enhanced photoacoustic Fourier transform infrared (FT-IR) spectroscopy. Cocaine has several infrared absorption bands, with two characteristic bands around 1750 and 720 cm^{-1}, which can be used for identification. Some cocaine metabolites, such as benzoylecgonine or methylecgonine, can be used for identification as well[1,8,10,11] but are not investigated in this study. Normally, the metabolites appear in significantly lower concentrations, 10% at most, compared to cocaine.[8,12] However, higher levels of metabolites can be found in some cases.[11] Hair has been a problematic sample material for measurement by common infrared spectroscopic techniques. Lack of sensitivity and difficult sample preparation procedures of the commonly used transmission-based infrared techniques, attenuated total reflectance (ATR) spectroscopy and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), lead to low reproducibility and long sample preparation times. Since hair is a highly inhomogeneous material, a microtome or similar mechanical cutting device operating at micrometer level is usually needed to perform the measurement at the right section of the sample. Photoacoustic spectroscopy (PAS) has some advantages over the more commonly used transmission- or reflection-based infrared techniques: No sample preparation is usually needed, depth profiling can be performed for layered samples, and the technique is nondestructive and suitable for opaque samples. In cantilever-enhanced PAS, an interferometric silicon cantilever microphone is used as a pressure sensor instead of the condenser microphone in most of the commercial photoacoustic detectors. Condenser microphones can be constructed to achieve a several orders of magnitude higher sensitivity than that of the condenser microphone.[13,14] High sensitivity and no need for additional sample preparation makes cantilever-enhanced PAS an ideal infrared technique for measuring hair fibers. There are, of course, drawbacks to using photoacoustic detection and to infrared spectroscopy in general. There are no established practices when it comes to measuring hair samples, not to mention measuring drugs, which complicates the study of hair samples and comparisons between different studies. In infrared spectroscopy, hair

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TABLE I. Amount of cocaine measured by GC/MS.

Sample no.	1	2	3	4	5	6	7	8	9	10	11	12
Cocaine content (ng/mg)	30.2	38	9.5	6.4	6	4.8	14	9.3	17	1.5	16.8	15

samples have been mostly studied for the effects of bleaching and other chemical treatments, with only a couple of studies made about drug detection.[4,15] Using PAS, there have been only few studies of hair in general.[16,17] The second drawback is a common problem of infrared spectroscopy and relates to the interpretation of spectra.[4] Mathematics is usually required to discriminate the target substance from the infrared spectrum. It is also difficult to determine the actual concentrations of the drug from the spectrum.

In this study, we present a first approach to discriminating between a group of cocaine users and a reference group using photoacoustic FT-IR spectroscopy and principal component analysis (PCA). The aim of this study is to discriminate between the two groups using a fast and simple photoacoustic measurement in which additional sample preparation methods and chemical extractions are not required.

EXPERIMENTAL

Materials. Hair fibers from cocaine-overdose patients were investigated in this study. The subjects were frequent users of cocaine, which implies that cocaine can be found virtually in the full length of hairs and also in high concentrations. Cocaine concentration in the hairs was measured previously with gas chromatography/mass spectrometry (GC/MS) by a research group headed by Kathryn S. Kalasinsky. These premeasured values can be found in Table I. High concentrations of cocaine in the samples eliminate the need for any cutoff levels for minimum detectable concentration as the measured concentrations are far above the usual concentrations among cocaine users[11] and the cutoff level of 0.5 ng/mg, suggested by the Substance Abuse and Mental Health Services Administration (SAMSA) and the Society of Hair Testing (SOHT).[9]

Measurements. Hair samples were measured using a PA301 (Gasera Ltd.) cantilever-enhanced photoacoustic detector coupled with a Tensor 37 FT-IR spectrometer (Bruker Optics Inc.). The PA301 was used as an external detector for the FT-IR instrument. Wavenumbers from 400 to 4400 cm^{-1} were recorded in each spectrum with 8 cm^{-1} resolution, 2.2 kHz mirror velocity, and 50 scans. With these parameters, performing a measurement took approximately 90 s. As solid matter is under investigation, the peaks are generally broad and further increasing the resolution from 8 cm^{-1} does not notably enhance the result. The lowest mirror velocity of the FT-IR instrument was used in the measurements to achieve the highest possible penetration depth into sample. Carbon black was used as a reference material in all the measurements, and the photoacoustic cell was purged with helium to increase the signal because helium has a higher heat conductivity than that of nitrogen. The samples measured were 12 hair samples from cocaine users and 12 reference samples from non-cocaine users. The hair sampling was performed in a similar manner as that of a previous study.[17] Hair fibers were cut into approximately 2 mm pieces and laid on the bottom of the cylindrical sample cup with a 10 mm diameter and a height of 2 mm. Samples of 20–30 mg of hair material were used in the measurements. The sample cups of PA301 were cleaned with an ultrasonic cleaner before use to remove possible contamination in the cups. For each subject, a sample composed of mixed hair fibers was measured to obtain a good average. This was done to reduce the effects of variation among individual hair fibers, which were a problem in some previous infrared measurements.[18] The hair samples came prewashed to remove, for example, hair-care products, sweat, and sebum from the hair fibers. No additional chemicals or sample-preparation methods were used in this study. The normal measurement procedure of hair samples includes decontamination, isolation, extraction, and analysis phases in most of the techniques. In each of these techniques, decontamination, isolation, and extraction require the use of different chemicals, which may have an effect in the spectrum. Photoacoustic detection does not require isolation and extraction steps, which effectively reduces the amount of chemicals and treatments needed.

Data Analysis. Principal component analysis was used for the separation of the spectra. PCA results were compared with results acquired from two other classification methods: *k*-nearest neighbors (KNN) and support vector machines (SVM).

RESULTS AND DISCUSSION

Hairs are biological samples with a broad chemical composition. Hair fibers consist mainly of keratin, which is a general name for fibrous proteins consisting of long amino acid chains, where individual amino acids are linked together by an amide bond. The remaining components are water, lipids, pigment (eu- and pheomelanins), and trace elements.[6] Main features in the infrared spectrum of hair have been explained in a previous publication.[17] Drugs can enter the hair via the bloodstream, sebaceous and eccrine secretions, and external contamination.[19] Definite information about the binding sites of drugs in hair remains uncertain at present. The binding sites are believed to be keratin, most probably where it attaches to the amide bonds, melanin, and lipids.[20,21] Eumelanin has been proved to provide the highest binding.[19] Basic drugs, such as cocaine, appear to attach easier to keratin, while cationic drugs attach to melanin.[20] There are also results that cocaine binds mainly to the medulla, which is the innermost region of hair.[4]

The mean photoacoustic spectra of measured hair samples for both cocaine users and reference subjects, as well as the absorbance spectrum for pure cocaine, can be found in Fig. 1. When we investigate the spectra in Fig. 1, no cocaine-related visual differences can be seen. The same situation occurs when we investigate the individual spectra. Therefore, data analysis methods are needed to determine whether cocaine is present in the hair sample. Prior to this analysis, data preprocessing is done.

Data Preprocessing. The preprocessing procedure had four steps. First, the suitable spectral range was selected. To work out the differences between cocaine- and non-cocaine-containing hair spectra, the spectral part not contributing to the discrimination was left out. The spectral range was selected to incorporate the wavenumber areas where the spectrum of

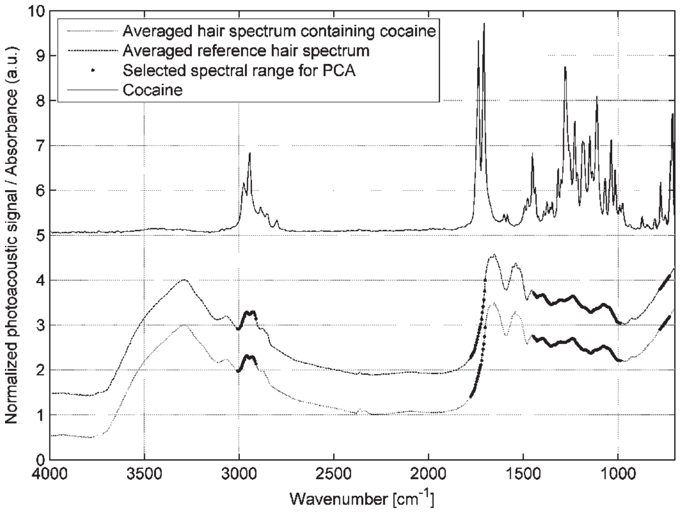

Fig. 1. Top: Pure cocaine spectrum (from Bruker drug library). Bottom: Average of measured hair spectra for both cocaine-containing and non-cocaine-containing hair samples with the spectral filter for data analysis. For better visibility, the cocaine spectrum is multiplied by 5 and plotted with an offset of 4.

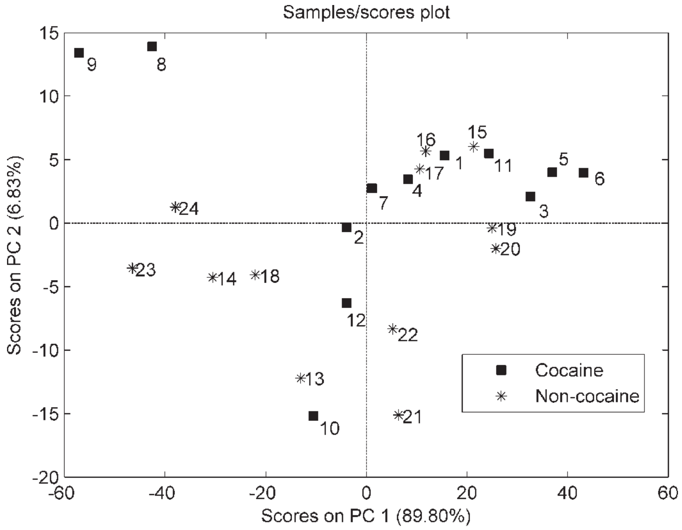

Fig. 2. Scores plot of PC1 and PC2 without the preprocessing. The percentages on the axis titles relate to the amount of explained variance in the PCA. The samples in the plot do not divide into groups, and the discrimination of cocaine users cannot be done.

cocaine shows absorption bands. The selection of the optimal spectral range is important because the discrimination of spectra is done according to the cocaine content, and this requires that the effects of natural variance among different hair samples be as low as possible. Some minor absorption bands of cocaine were omitted, since they did not contribute to the discrimination and overlapped with more intense absorption features. One example is the small peaks at amide I and II band area between 1450 and 1700 cm^{-1}. Choosing the best spectral points for the C=O peak of cocaine, around 1750 cm^{-1}, was particularly important for two reasons. First, the band interferes strongly with the amide I band at lower wavenumbers, and second, this band is the strongest and most important single feature in the spectrum of cocaine. Therefore, finding a balance between having the most of information in the C=O peak and having minimal interference from the amide I band is a key point in the selection of the range for successful analysis results. Several PCAs were calculated with different spectral ranges. The best discrimination was achieved with the wavelength filter containing 2912–3009, 1701–1782, 984–1497, and 729–783 cm^{-1}. Figure 1 shows the selected range in the averaged spectra.

The second step is the normalization of the spectra due to offset in the ordinate. Hair samples are not easy to handle because of their fiberlike structure. The offset in the spectra comes mainly from the different amounts of sample material in the cup between different measurements but also from the different filling heights of the samples in the cup and the different arrangements of the single hairs in the cup.[17,22,23] For a proper data analysis, the offset needs to be corrected. To normalize, every variable (spectral point) was divided by the sum of the value of all variables for the given sample. Normalization was done on the basis of all remaining spectral points after wavelength filtering.

The third step is the derivation of the spectra. To make the differences in shape more pronounced, a second derivative (polynomial order 2, filter width 15 spectral points) was calculated. The spectrum of hair is very rich in the mid-infrared region, which means there is a lot of information and broad peaks in the spectra. Broad peaks and a continuous background imply that the target substance (cocaine) and the sample matrix (hair) overlap and that the desired information lies in the band shape rather than in the absolute values of the peak maxima.

The fourth step is mean centering. To balance the scales of the different variables, the data set was mean centered. To do this, every sample value at a variable (spectral point) was divided by the mean of all the sample values at that variable.

Discrimination Based on Principal Component Analysis. PCA was performed on the data with and without the preprocessing explained above. The plot of the scores of the principal component (PC) 1 and PC2 of the calculated PCA model without preprocessing is shown in Fig. 2. The loadings plots are not shown here as they are difficult to interpret after the derivative preprocessing. As we can see from the plot, the data points seem rather randomly organized and the discrimination between cocaine and non-cocaine samples cannot be done without using the preprocessing. The discrimination does not improve even if different combinations of higher PCs are used. In Fig. 3, PC1 and PC2 of the calculated PCA model with preprocessing is shown. This time, the points in the plot separate into two groups, and a clear discrimination between cocaine and non-cocaine samples can be seen. Even though ten PCs were calculated, only the first two are used for the discrimination of the two classes of hair samples. Using higher PCs does not give any better discrimination. On the basis of PC1 and PC2, the hair samples can be discriminated according to their cocaine content, although the distance between the two classes in the PC1–PC2 space is narrow. PC1 has an explained variance of 46.96% and PC2 14.67%. It is obvious that not all the PC1 explained variance is due to cocaine because PC2 has approximately one-third the explained variance but still contributes at least as much as PC1 to the classification. Consequently, part of the explained variance must be due to other factors. These can include, at least, natural variation between different individuals, and chemical exposure and weathering of the hair fibers.

Natural Variances in the Sample Matrix. When we investigate the selected spectral region of the mean spectra in

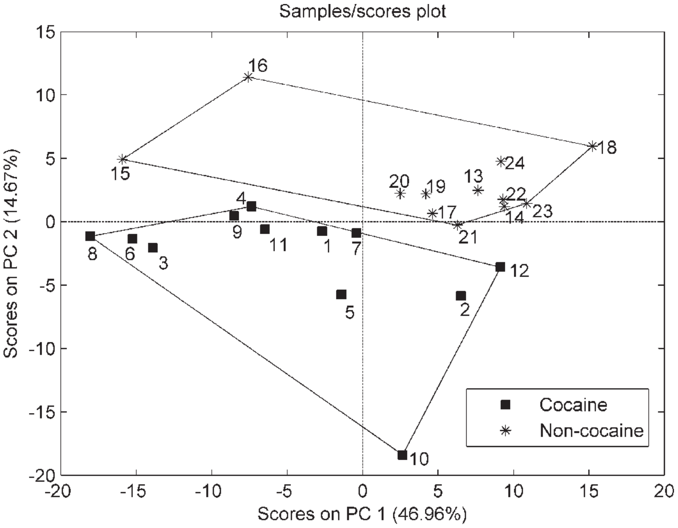

FIG. 3. Scores plot of PC1 and PC2 with the preprocessing methods developed in this study. In this plot, the samples divide into two groups according to cocaine content and the discrimination of cocaine users can be done.

Fig. 1 starting from higher wavenumbers, a small area around 2900–3000 cm^{-1} is seen first. The two major peaks in this area arise from methylene group (CH_2) and methyl group (CH_3) stretchings. In the infrared spectrum of hair, these vibrations are mainly assigned to fatty acids and lipids, and the levels and compositions change from individual to individual.[6] When we move to lower wavenumbers, a $C{=}O$ vibration around 1750 cm^{-1}, discussed earlier in this section, can be seen and then the fingerprint area of cocaine between 984 and 1497 cm^{-1}. Starting from lower wavenumbers, there are CH_2 and CH_3 bendings around 1400–1450 cm^{-1}, which are also assigned to lipids and fatty acids; the amide III peak around 1240 cm^{-1}; and various absorptions of cystine and cysteine derivatives around 1000–1220 cm^{-1}, from which we can, in particular, point out $S{=}O$ stretching around 1040 cm^{-1}, which is usually used to monitor the effects of chemical treatments and weathering in hair.[24,25] Below 700 cm^{-1}, the instrumental limitations start to occur, and carbon black, which is used as a reference material, starts to lose its absorptivity and become slightly transparent. This can be seen as the rising slope in each spectrum. The findings are in compliance with what the authors expected; that is, only a tiny fraction of the whole variance accounts for the cocaine content.

In this first approach to FT-IR photoacoustic cocaine detection, the discrimination of the cocaine-containing samples worked acceptably. On the basis of the PC1–PC2 space, the cocaine-containing samples can be separated from the non-cocaine-containing samples. The key to this separation was the right data preprocessing to extract the cocaine-related information from the spectra. Even though using this method the authors show a discrimination of the data according to cocaine content, the method is not yet robust enough to be used for confirming the use of cocaine by testing hair. First, the classes need to be separated further from each other in the model space, and second, the spread of the few samples lying far away from their groups need to be reduced. To make a more general model for measuring cocaine in hair, more samples are needed.

Classification Methods. To verify the results of the PCA, classification methods such as KNN and SVM are used. For both methods, the same data set as that used in the PCA method with the same preprocessing was used. Since the data set of 24 hair samples is rather small, no test set was used and the predictions shown here are based on the samples used for building the model. The prediction plot of the KNN (three neighbors) model is shown in Fig. 4 and that of SVM (C-support vector classification, PCA compression with two PC factors) model in Fig. 5. Samples 1–12 are those from the cocaine users and 13–24 are those from the reference group (without cocaine). The SVM model predicted all the samples correctly, which means that the samples divided into two groups according to their cocaine content. The KNN model shows three misclassified samples: 12, 15, and 16. Sample 12 contains cocaine but was classified as non-cocaine, and vice versa for samples 15 and 16. Samples 15 and 16 are the non-cocaine samples in the upper left quadrant of the PCA scores plot in Fig. 3; the cocaine sample 12 has the highest value ($\sim$10) on the PC1 axis. The KNN and the SMV results are in compliance with the PCA results. Just as in the PCA, the classification results suffer from this being a small data set, which should be enlarged in further investigations when a more robust model is constructed.

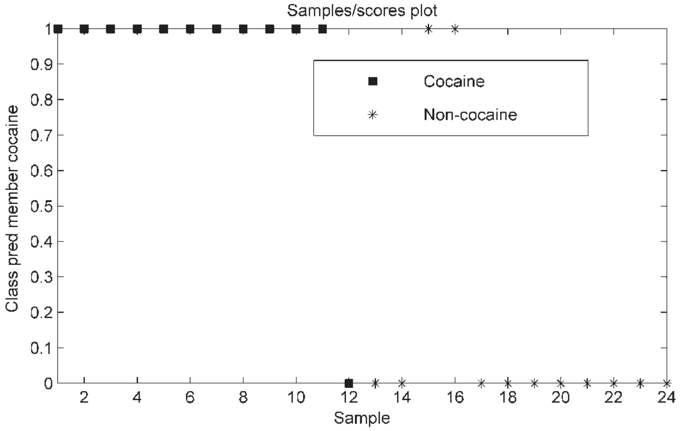

FIG. 4. Prediction of the KNN model. Samples 1–12 contain cocaine and 13–24 do not contain cocaine. All samples, with exception of samples 12, 15, and 16, were predicted correctly.

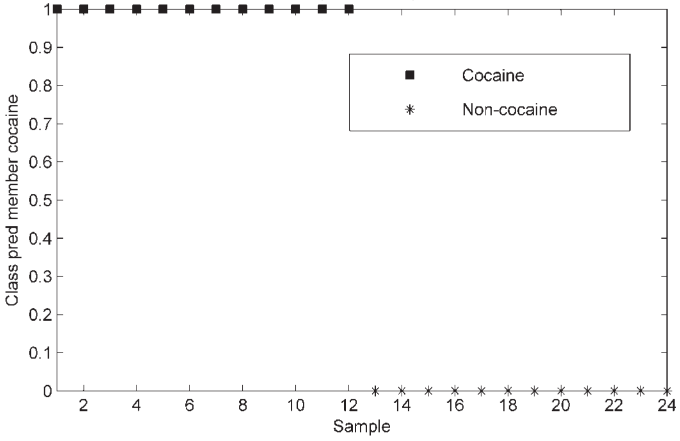

FIG. 5. Prediction of the SVM model. Samples 1–12 contain cocaine and 13–24 do not contain cocaine. All samples were predicted correctly.

CONCLUSION

In this article, it was shown that hair samples from cocaine-overdose patients can be discriminated from the hair samples from the reference group of non-cocaine users using a simple photoacoustic measurement combined with PCA. The measurement technique can be further developed using microsampling techniques in photoacoustic spectroscopy to measure single hair fibers with a length of only a few millimeters. The precision can also be improved by using a step-scan FT-IR interferometer or a tuneable laser source for a greater and more controlled sampling depth.

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