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RESEARCH ARTICLE

Measurement of methamphetamine on surfaces using surface plasmon resonance

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Abstract

Field methods are needed to assess the contamination of surfaces by methamphetamine from illicit drug manufacturing. This study performed a feasibility study on the use of a surface plasmon resonance (SPR) based instrument (SensiQ Discovery) in the evaluation of surface contamination by methamphetamine. The main goal was to see if the method could be sensitive enough for field measurements. A competitive immunochemical assay was developed for the instrument which was able to measure methamphetamine at 9 ng/ml with a range of 9–250 ng/ml. Methamphetamine was spiked onto ceramic tiles and the assay was able to detect methamphetamine contamination at 25 ng/100 cm^2, which is below the 50 ng/100 cm^2 standard used for surface cleanup assessment. The instrument is compact and mobile and is sensitive enough for use for measurement of methamphetamine on surfaces, so it is a candidate for a field method for methamphetamine surface contamination. Its use for this application will require further development of the instrument to make it more convenient to use. Also further evaluation of ruggedness and use of the instrument under various environmental conditions such as temperature and humidity are needed to define conditions under which the instrument can be employed in field measurements.

Keywords: *Competitive immunoassay; methamphetamine surface measurement; surface plasmon resonance (SPR)*

Introduction

There is currently interest in the field measurement of methamphetamine on surfaces which can become contaminated during illicit manufacture in facilities such as clandestine methamphetamine labs. Law enforcement and clean-up personnel can become exposed to methamphetamine from these surfaces (Martiny et. al. 2007). Three levels are currently used in different states in the US as methamphetamine clean-up standards: 500 ng/100 cm^2, 100 ng/100 cm^2, and 50 ng/100 cm^2 (Colorado Department of Public Health and Environment 2005). We evaluated the 50 ng/100 cm^2 level as a target in the present work. There are a number of analytical methods available for methamphetamine surface measurement such as LC-MS (Data Chem Laboratories, 2004). These are sensitive and specific but require the samples to be sent to the laboratory which can delay clean-up activities. In response to the need for the rapid evaluation of surface contamination, our group has developed a lateral flow device that can be used with methamphetamine wipe samples in the field (MethChek™ 50, SKC Inc., Eighty Four, PA). This device allows rapid assessment of surface contamination in the field to prevent exposure of personnel. A limitation of the lateral flow device is that it is qualitative and results require interpretation by the operator. In the past, a competitive immunoassay for methamphetamine on a piezoelectric crystal surface has been developed (Miura et. al. 1993) and a competitive immunoassay has been developed using surface plasmon resonance (SPR) (Miura et. al. 2002). In the present investigation, the use of SPR as a field method for methamphetamine surface contamination was evaluated.

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SPR is very sensitive method for measuring refractive index (Hutter and Fendler 2004). It has been useful for assessing molecular interactions at the sensor surface, since a very small amount of mass binding to the surface will result in a measurable change in refractive index by SPR. It has been used to study biomolecular interactions such as antibody-antigen and protein drug interactions. One advantage of SPR is that it can use a one step procedure to detect binding of an antibody to an antigen and therefore limits the number of reagents and steps needed in an assay. An enzyme-linked immunosorbent assay (ELISA) based procedure usually requires one or two addition steps such as binding of a secondary antibody and conversion of a substrate to a product to detect binding of an antibody to an antigen. In the methamphetamine assay, we explore the use of SPR in a competitive format for measurement of a small molecule (methamphetamine) in solution. The assay can than be used to assess surface contamination by sampling the surface with a technique that results in methamphetamine in solution. The principle of the competitive format for the SPR sensor is shown in Figure 1. The assay uses the competition of methamphetamine in solution for anti-methamphetamine antibodies that bind to the methamphetamine-BSA on the sensor surface. This results in a lower effective concentration of methamphetamine antibody at higher methamphetamine concentrations which results in a decrease in signal.

Methods

Reagents

The SPR running buffer was 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4), 150 mM NaCl, 3.4 mM ethylenediamine tetraacetic acid (EDTA), and 0.005% Tween 20. The anti-methamphetamine antibody (part number ABMET-0401, 5.1 mg/ml) and the methamphetamine-BSA conjugate (part number AGMET-0300) were obtained from Arista Biologicals, Inc. (Allentown, PA). Methamphetamine was obtained from Sigma-Aldrich (St. Louis, MO). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl(EDC) and N-hydroxysulfosuccinimide (NHS) were obtained from Pierce Chemical Company (Rockford, IL) All chemicals were reagent grade and doubly deionized water was used in preparing solutions.

SPR instrument

The measurements were made using a SensiQ Discovery SPR instrument (ICX Nomadics, Oklahoma City, OK). This is a compact instrument employing a dual channel SPR sensor (catalog number SS00 COOH1) which has free carboxylate groups (COOH) on its surface. The device uses a syringe pump to provide flow to the sensors. One channel is used for the measurement and the other channel is used as a reference to compensate for other effects not related to surface binding such as temperature. The instrument employs a manual 12 port sampling valve with a separate sampling loop for each of the channels which allows for the same or different samples to be injected. Figure 2 is a schematic diagram showing the valve in the load and inject positions. The sampling loops for the channels are larger than the volume to be injected. The sample volume to be injected is loaded into each sampling loop and then the sampling valve is switched from the load to the inject position. In the inject mode, the flow from the syringe pump pushes the loaded sample into the flow cell containing the sensor. The software for the instrument accurately measures the injection time so that an accurate volume is

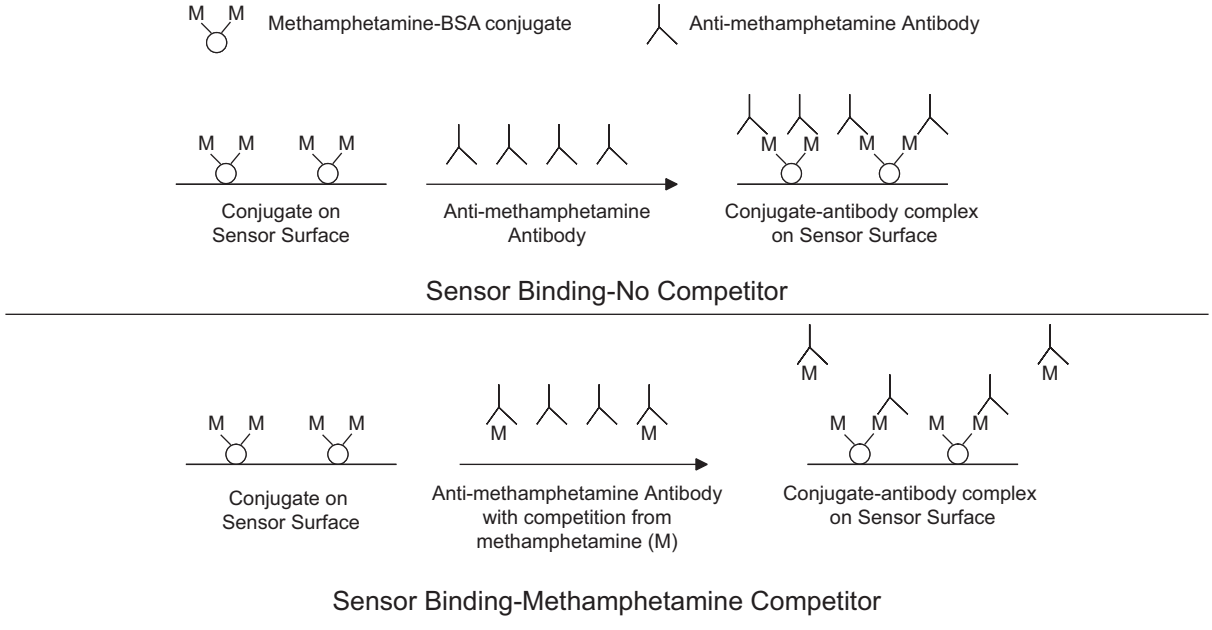

Figure 1. Surface plasmon resonance sensor methamphetamine competitive assay. The assay uses the competition of methamphetamine in solution for anti-methamphetamine antibodies that bind to the methamphetamine-BSA on the sensor surface

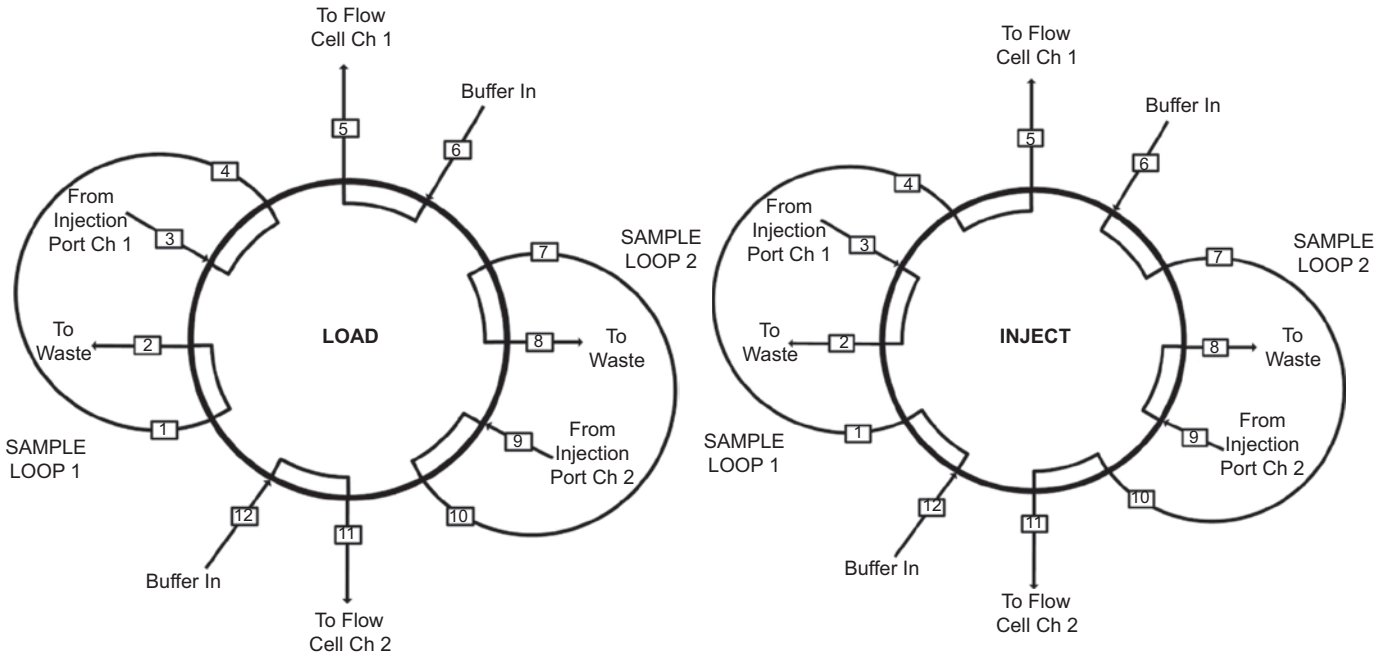

Figure 2. Schematic diagram of 12 port sampling valve in the load and inject positions. This allows samples to be load into the two sampling loops which are then injected simultaneously into channels 1 and 2 of the flow cell containing the SPR sensor.

injected into the flow cell based on the injection time and the constant syringe pump flow rate. The software provides the ability to do reproducible injections at set time intervals and a separate software package allows for analysis of the data.

Immobilization of methamphetamine-BSA protein on the sensor surface

The protein was immobilized on the sensor surface using the procedure recommended by the manufacturer using EDC-NHS activation of COOH on the sensor surface. The following is a brief description of the process. The buffer flow rate was 25 µl/min for the immobilization. Fifty microliters of 100 mM HCl was injected into both channels to clean the surfaces. A 0.4 M solution of EDC in water was mixed with an equal volume of 0.1 M NHS in water and the resulting mixture was diluted 1/200 in water. Twenty-five microliters of the diluted EDC-NHS mixture was immediately injected into the measurement channel to activate the surface COOH. Then 175 µl of 10 µg/ml methamphetamine-BSA protein in 10 mM sodium acetate buffer (pH 5) was injected into the measurement channel so that the protein covalently bound to the surface through the activated COOH which react with free amino (NH) groups on the protein. Fifty microliters of 1 M ethanolamine (pH 8) was injected into both channels to react with any leftover reactive COOH and finally 25 µl of 10 mM H_3PO_4 was injected into both channels.

Response of the sensor

The buffer flow rate was 25 µl/min for the response curve and 50 µl of 1/500 dilution of the anti-methamphetamine antibody in running buffer was injected into both channels. The signal was allowed to decay for 8 min after the injection and then the surface was regenerated by injecting 25 µl of 10 mM H_3PO_4 into both channels.

Competitive assay for methamphetamine

The assay uses the competition of methamphetamine in solution for anti-methamphetamine antibodies that bind to the methamphetamine-BSA on the sensor surface, as shown in Figure 1. To perform the competitive assay, a fixed concentration of methamphetamine antibody is added to different concentrations of methamphetamine in solution. In our assay, a volume of the methamphetamine solution in running buffer was added to an equal volume of 1/250 dilution of the methamphetamine antibody in running buffer and the resulting solution was incubated for 30 min at room temperature. Fifty microliters of the incubated solution was then injected into both channels and the response determined at running buffer flow rate of 25 µl/min. After a decay time of 4 min, the surface was regenerated by injecting 25 µl of 10 mM H_3PO_4 into both channels. The response from the assay was measured at the maximum of the binding curve at the end of the injection. The response vs methamphetamine concentration was fitted with a four parameter logistic function using SigmaPlot (Systat Software, Inc., San Jose, CA). The four parameter logistic function is commonly used to fit data from immunoassays.

Surface sampling of methamphetamine

Various amounts of methamphetamine (0–250 ng) were spiked onto the smooth side of 4”×4” (10 cm × 10 cm = 100 cm^2) ceramic tiles by pipetting a solution of methamphetamine in methanol at various

concentrations and letting it dry for 1 h. The ceramic tiles were chosen because they have a smooth surface of the proper area (100 cm^2) and we used them in evaluating the lateral flow assay mentioned in the introduction. The methamphetamine on the surface was sampled by wetting a cotton swab with running buffer and wiping the surface carefully in a regular pattern such that the entire surface was wiped. The surface was first wiped in a vertical direction (W pattern), then in a horizontal direction (Z pattern), and finally again in a vertical direction. The swab was then put into 1 ml of running buffer to extract the methamphetamine from the swab.

Results

Response of the sensor

A typical response curve of the sensor is shown in Figure 3. The buffer flow rate was 25 µl/min and 50 µl of 1/500 dilution of the anti-methamphetamine antibody in running buffer was injected into both channels Figure 3 shows the difference in response from the measurement channel and the reference channel. The response increases during the time the antibody is injected due to binding of the antibody to methamphetamine-BSA on the sensor surface of measurement channel and after the injection the signal decays slowly as the antibody dissociates from the surface. The slow decay curve indicated tight binding of the antibody to the methamphetamine-BSA on the sensor surface.

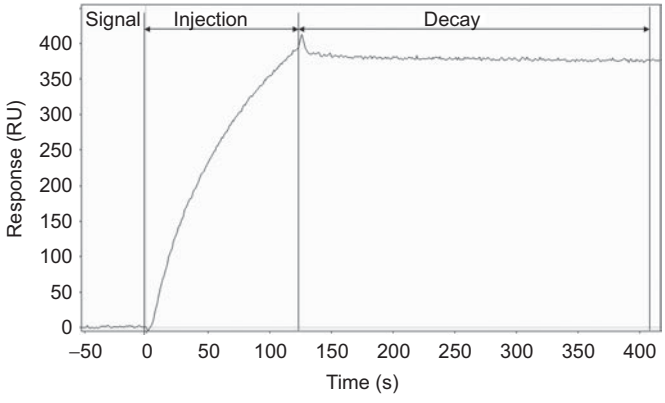

Figure 3. Sensor response was determined at a buffer flow rate of 25 µl/min with a 50 µl injection of a 1/500 dilution of the anti-methamphetamine antibody. RU-resonance units or response units with 1 RU equivalent to 1 µ refractive index unit, which represents ~ 1 pg of protein/mm^2.

Response of the assay

Figure 4A shows the response of the assay for a range of methamphetamine concentrations. The maximum signal from the binding was determined at each concentration and $\%B/B_0$ was calculated, where B is the signal at a given concentration and B_0 is the signal at 0 concentration. $\%B/B_0$ against concentration is plotted in Figure 4B along with error bars (showing the standard deviation of B/B_0) determined for this data which represent the average of three runs done on three separate days. The response of the assay was tested from 0–250 ng/ml of methamphetamine and a four parameter logistic fit of the data resulted in a fit with R^2 of 0.991. Figure 5 shows the observed vs expected values based on the four parameter logistic fit. A linear fit of this data gave a slope close to 1 with a R^2 also close to 1, indicating an excellent fit to the data. A $\%B/B_0$ of 90% is typically used as the limit of detection in immunoassays, and this gave a detection limit of 8.6 ng/ml for this data.

Spiked tiles

Table 1 and Figure 6 give the recovery from spiked tiles. The tile spiked with 0 methamphetamine gave a response close to 0 and other spiked tiles gave recoveries from 38–86%. The recovery was fit with a linear trend line indicating the sampling method gave a response proportional to the

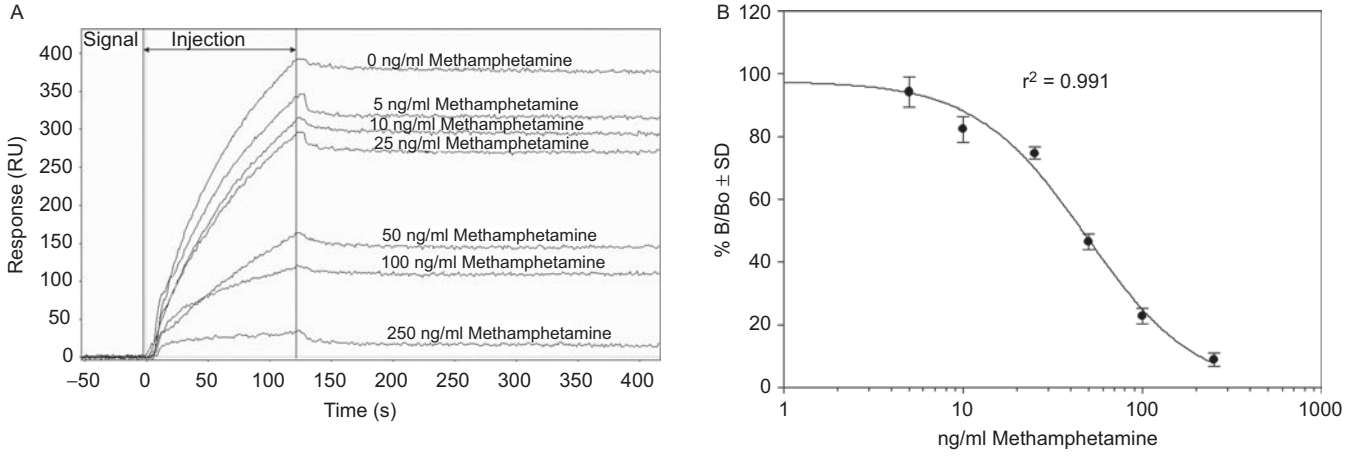

Figure 4. (A)Assay response was determined by mixing a volume of a 1/250 dilution of the anti-methamphetamine antibody with an equal volume of a methamphetamine standard at the indicated concentration and determining the sensor response of the mixture after 30 min incubation. (B) Assay response plotted against methamphetamine concentration- error bars show the standard deviation for this data which represent the average of three runs done on three separate days.

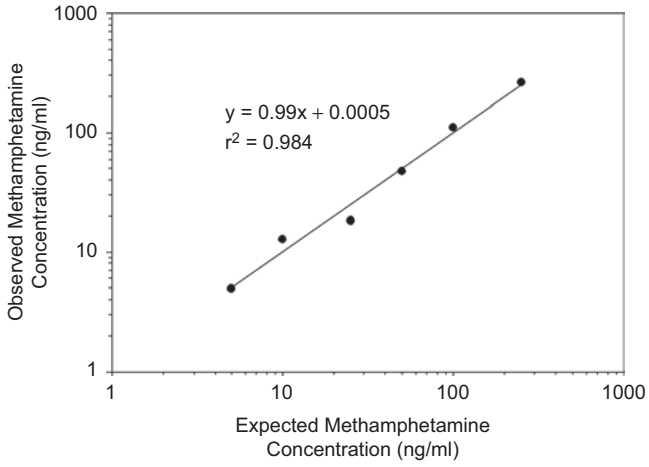

Figure 5. Observed vs expected concentration for four parameter logistic fit of standard curve.

Table 1. Average ng methamphetamine recovered vs ng methamphetamine spiked onto tile.

ng spiked	Avg ng recovered	*n*	CV
0	1.3	1	
10	6.1	3	40.7
25	9.8	3	24.2
50	32.7	3	21.6
100	85.9	3	8.3
250	158.7	2	7.0
500	200.6	1	

n = number of repeat measurements on different dates; CV = coefficient of variation of measurement = 100* standard deviation/average.

methamphetamine on the surface even though it was not 100% efficient in sampling the methamphetamine. As shown in Table 1, more measurements were done from 25–100 ng/cm^2 since this is the range of most interest in assessing surface contamination. A tile spiked with 25 ng of methamphetamine gave a recovery of 9.8 ng, which is over the limit of detection, so methamphetamine contamination on the tile can be detected at that level.

Discussion

The presence of methamphetamine residues on surfaces from illicit drug manufacturing is a widespread problem. Many states provide procedures for identification and clean-up of contaminated facilities (State of California 2005; Minnesota Department of Health 2007). Congress has written a law which calls for the development of field methods to assess methamphetamine contamination (110th Congress of the United States 2007). Analytical techniques such as LC-MS (Data Chem Laboratories, 2004) provide an assessment of contamination but cannot provide input into clean-up in a timely manner. A field readable lateral flow device (MethChek™ 50, SKC Inc., Eighty Four, PA) developed by our group can be used with methamphetamine wipe samples in the field but does not provide quantitative results. Therefore, a direct

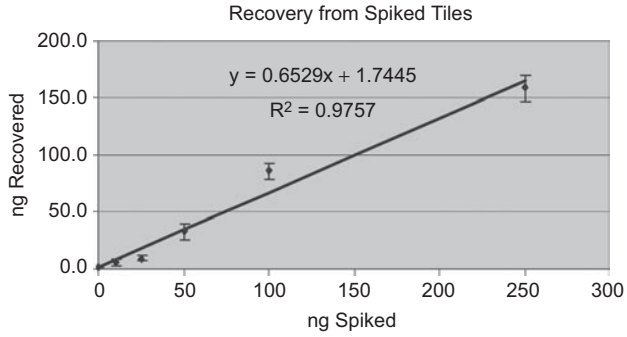

Figure 6. Mass of methamphetamine recovered from tiles spiked with methamphetamine

reading instrumental technique is needed to quantitatively assess the contamination of surfaces with methamphetamine in the field. The SPR-based methamphetamine measurement made by the SensiQ Discovery was able to detect surface contamination by methamphetamine at 25 ng on a 100 cm^2 surface area. Since the limit to verify clean-up of a contaminated area is 50 ng/100 cm^2, this technique should be suitable to verify that the analyte has been removed from a suspected methamphetamine-contaminated surface.

The device is a compact instrument that can be easily transported to various locations. Since its sensitivity is adequate for the intended measurements, it is a candidate for use as a field instrument for methamphetamine contamination. However, several issues would have to be explored before its successful application. The injection technique, which is versatile since it allows injections of varying volumes, is more suitable for the laboratory environment and would make field measurements tedious. However, the authors believe the instrument's liquid handling could be modified for field measurements. The syringe pump may also have to be replaced by another method of obtaining constant liquid flow, since the pump requires line power and is somewhat delicate. Other issues might include ruggedness and reliability. At the present time, the instrument requires an experienced operator to be sure the instrument is operating properly. Also the effects of environmental parameters such as temperature and humidity would have to be explored to define conditions wherein the instrument could be successfully used.

Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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