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TECHNICAL NOTE

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A Microfluidic Device for Presumptive Testing of Controlled Substances*

ABSTRACT: A simple microfluidic device (MFD) has been developed to perform multiple color and crystal tests for controlled substance analysis. The MFD method uses less sample and reagents and generates less waste than traditional spot plate methods while performing several tests simultaneously. This methodology provides significantly more analytical information for a single sample analysis. The current generation device is the size of a microscope slide with four analytical channels: one for microcrystal tests and three for color tests. The optimized devices were subjected to a rigorous validation study using comparative replicate analyses and several operators. Target analytes were methamphetamine, amphetamine, cocaine, and oxycodone and color test reagents used were the Marquis, Simon, and cobalt thiocyanate. For the crystal tests, platinic chloride was used. The validation study showed the MFD's limits of detection to be in the picogram range. Positive tests results were observed in complex mixtures in which the controlled substance was present at concentrations of 5–10% (w/w). The microcrystal reagents showed greater sensitivity than color test reagents when used in the device. Reagent use and waste generation using the devices was 95% less that that used and generated using the traditional methods. The device performance was also shown to be operator independent.

KEYWORDS: forensic science, microfluidic devices, drug analysis, controlled substances, microcrystal tests, presumptive tests, color tests

Micro-total analytical systems (μTAS), also known as "lab-on-a-chip" devices, microfluidic devices (MFDs), or simply "chips," have been suggested for uses in forensic toxicology and DNA typing (1–4). Due to the complexity and cost, these devices are likely years away from general forensic application; however, MFDs can be quickly adapted to less ambitious tasks such as solid dose analysis of controlled substances. Analytical results can be obtained in a few seconds, requiring less volume of reagent and sample compared to spot tests. This in turn reduces the cost of testing while providing more analytical information when compared to current presumptive testing methods.

Presumptive testing for controlled substances utilizes color tests and in some cases, microcrystal tests. Occasionally color tests are employed in the field, but mostly both are limited to laboratory use. Detection is based on visual observation of a color change or in the case of the crystal tests, visual identification using microscopy and crystal morphology. The color tests are done in a spot plate and the crystal tests on a microscope slide. In a sense, these tests are sequential, with each test requiring a separate aliquot of the sample. The more tests that are performed, the more sample is consumed and waste generated. One of the advantages of using an MFD as applied here is the ability to perform several simultaneous tests in parallel on the same sample aliquot, consuming less overall sample. Simultaneous testing increases the discrimination power and confidence in the identification of the controlled substance, even though the identification is still considered presumptive. The addition of a crystal test will be useful for laboratory applications of the devices.

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Four generations of devices were constructed and tested in this study. Devices were initially made of poly-dimethylsiloxane (PDMS) but subsequent generations used glass. Each generation was tested and modified to optimize channel structure and dimensions for both the color and crystal tests. Because detection for this device is based on visual observation and simple microscopy, the size of the device was limited to that of standard microscope slides. The final design allowed for three color tests and one crystal test to be performed simultaneously.

Given their frequency in casework, cocaine, methamphetamine, amphetamine, and oxycodone were targeted in the present study. Pure samples, along with mixtures containing common diluents were tested. Experiments employing variable drug concentration, reagent volume, and channel dimensions confirmed that reactions inside MFDs accurately and reliably reproduce traditional bench methods in less time while consuming *c.* 95% less sample and reagents. The MFD designs evaluated here used simple vacuum-driven flow generated by a syringe interface.

Materials and Methods

Materials

Cocaine, methamphetamine, amphetamine, oxycodone, lidocaine (all as hydrochloride salts), cobalt thiocyanate, sodium carbonate, sulfuric acid, platinic chloride, formaldehyde, dextrose, aspirin, starch, and microscope slides were purchased from Sigma Aldrich (St. Louis, MO). Sodium nitroprusside and caffeine were purchased from Fluka (St. Louis, MO). All chemicals were analytical grade or better. Large glass slides (2" × 4") were purchased from Protea BioScience (Morgantown, WV). Marquis, Simon, cobalt thiocyanate, and platinic chloride reagents were made in-house, and allowed to equilibrate 72 h (5,6). These reagents were chosen because of their selectivity and sensitivity toward the controlled substance drug families of phenethylamines, tropane alkaloids, and opiate alkaloids regularly encountered in casework.

TABLE 1—*Samples tested.*

% (w/w)	Substances	%	Substances
100	Methamphetamine	100	Oxycodone
50/50	Methamphetamine/caffeine	50/50	Oxycodone/lidocaine
10/90	Methamphetamine/dextrose	25/75	Oxycodone/dextrose
5/15/80	Methamphetamine/caffeine/starch	5/35/60	Oxycodone/lidocaine/starch
100	Amphetamine	100	Cocaine
50/50	Amphetamine/starch	50/50	Cocaine/lidocaine
25/75	Amphetamine/dextrose	15/85	Cocaine/aspirin
5/20/75	Amphetamine/starch/dextrose	10/25/65	Cocaine/caffeine/lidocaine

Four sample concentrations and mixtures were prepared for analyses using traditional and microfluidic device methods. Diluents were selected to mimic casework. The diluents used and their proportions based on weight percent (wt % or w/w%) can be found in Table 1.

Instruments

A Leica DMLP polarizing microscope (Leica Microsystems U.S.A., Bannockburn, IL) was used to view and image drug microcrystals. Micrographs were taken of the crystals using QICAM QIMAGING Fast 1394 camera using Image Pro Plus® software (Silver Springs, MD). A Craic DMRX UV/Vis micro-spectrophotometer microscope (San Dimas, CA) was used to observe color change, and record visible reflectance spectra from each of the color tests. Photographs of the color tests were taken using a PixiLink camera using PixiLink software (Vancouver, BC, Canada).

Spot Plate Color Tests

For comparative purposes, color tests were performed in spot plates using typical lab procedures (5–7). Approximately 2–5 mg drug sample was placed on a well plate and dissolved in water. A drop of color reagent was then added to the well. The resulting mixtures were examined using a visible microspectrophotometer at a total magnification of 150× and documented using photomicrographs. Three different color reagents (Marquis, Simon and cobalt thiocyanate) were applied to each drug mixture shown in Table 1 and each test was performed ten times to parallel the experiments performed using the optimized microfluidic device. Results are presented in Table 2.

Standard (ASTM) Microcrystal Test

For each drug concentration, crystal testing was performed using American Society for Testing and Materials (ASTM) methods (5,6,8–10). Approximately 2–5 mg of the drug sample was placed on a microscope slide, and dissolved in D.I. water. A drop of the crystallizing reagent was also placed on the slide, and a glass rod was used to join the two drops in a narrow channel. The resulting crystals were examined using the polarizing microscope at a total magnification of 100×. Photomicrographs were taken for later comparison with crystals formed in the device. As with the color reagents, 10 replicate analyses were performed in parallel with testing in the microfluidic device with results presented in Table 2.

Optimization of Microfluidic Devices

The MFDs evolved through four generations, each incorporating changes based on experimental findings. The first generation was made of poly-dimethylsiloxane (PDMS) as shown in Figure 1. The PDMS proved difficult to clean and problems arose re-sealing it to the glass substrate after multiple uses. Subsequent generations of devices were made using glass slides. Larger slides were used initially (2" × 4") instead of traditional microscope slides (1" × 3"). This allowed for multiple channels to be etched on each chip to test different parameters, such as channel length, width, depth, reagent-sample introduction, and applied pressure on the syringe.

Reagent chambers on the fluidic device held *c.* 1 mL of reagent, which was more than sufficient to conduct all validation testing. Sample introduction was accomplished by first placing a homogenized portion of the sample in the sample inlet well of the device. A solution of 0.5 M hydrochloric acid and methanol in a 1:1 (v/v)

TABLE 2—*Color and crystal tests in spot plates and microscope slides as per ASTM methods. For each, n = 10. + indicates replicates gave positive reaction, – indicates no reaction for the replicates.*

% w/w	Substances	Marquis	Simon	CoSCN	Crystal
100	Methamphetamine	+	+	–	+
50/50	Methamphetamine/caffeine	+	+	–	+
10/90	Methamphetamine/dextrose	+	+	–	+
5/15/80	Methamphetamine/caffeine/starch	–	–	–	+
100	Amphetamine	+	–	–	+
50/50	Amphetamine/starch	+	–	–	+
25/75	Amphetamine/dextrose	+	–	–	+
5/20/75	Amphetamine/starch/dextrose	–	–	–	+
100	Oxycodone	+	–	–	+
50/50	Oxycodone/lidocaine	+	–	–	+
25/75	Oxycodone/dextrose	+	–	–	+
5/35/60	Oxycodone/lidocaine/starch	–	–	–	+
100	Cocaine	–	–	+	+
50/50	Cocaine/lidocaine	–	–	+	+
15/85	Cocaine/aspirin	–	–	+	+
10/25/65	Cocaine/caffeine/lidocaine	–	–	–	+

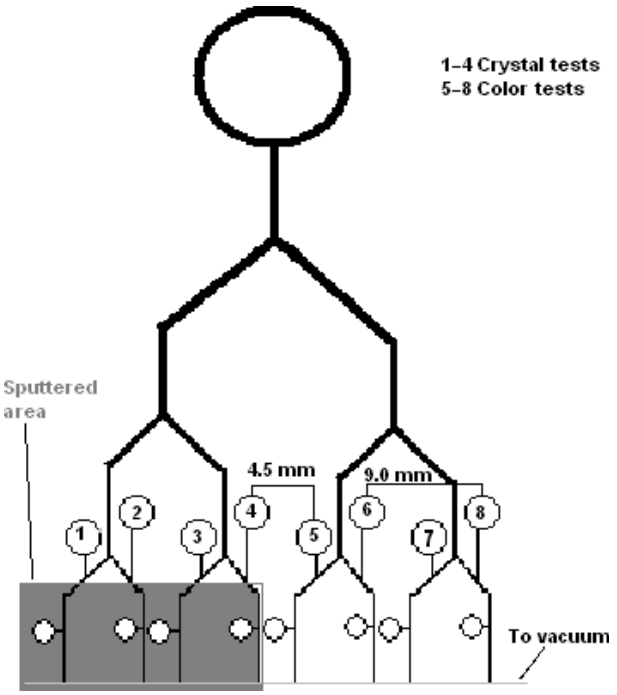

FIG. 1—*Schematic of the original design for the microfluidic device made in PDMS. The number of channels was reduced in subsequent generations. While never validated, this design was used as proof-of-concept for miniaturization of the color and crystal tests.*

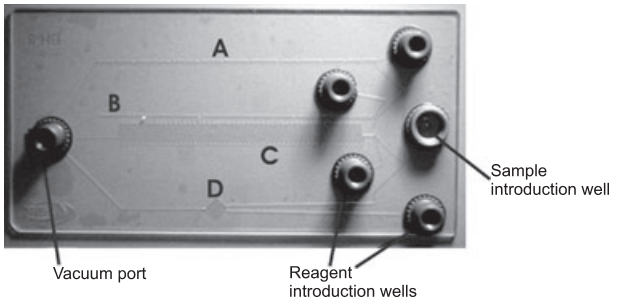

FIG. 2—*Microfluidic devices made on larger slides (2" × 4") tested different dimensions and channels to optimize mixing. Three channels (A–C) tested for color reactions and one channel for crystal reactions (D).*

ratio was added to the well and mixed. A 1.0 mL syringe was used to create a vacuum at the end of the chip, drawing the sample solution and reagents through the channels.

The first and second generation designs displayed laminar flow characteristics in the channels where reagents and sample mixed. Although color changes could still be seen, the reaction zone was in a diffuse thin band at the interface of the two solutions rather than a concentrated zone of intense color. To study methods to improve mixing, various channel patterns were etched into a third generation device (Fig. 2). Five replicate analyses were conducted for each of three (A-C, Fig. 2) channels. Channel design A provided the best results (Fig. 3*a* as an example) based on color intensity, reproducibility and sensitivity, while channel C, a linear channel with sinusoidal curvature, failed to yield any observable color change. This result was attributed to the channel's overall length being too long relative to the applied driving force delivered by the syringe.

The fourth channel, D, was designated for crystal testing. Early chip designs were hampered when crystals were washed away from the zone of formation. To address this, a frit was inserted into the channel after the mixing chamber to prevent the crystals from being washed away (Fig. 3*b*). At higher concentration of the drug sample, crystals will accumulate against the frit in a tight pack. However, a sufficient number of distinct crystals remain isolated in the crystal chamber and are easily recognized. As the concentration of the drug decreases, crystal accumulation by the frit decreased, alleviating this problem. The next and final generation of devices were reduced in size to 1" × 3" (standard microscopy slides) using channel A's bulbous shape for the three color channels. The frit used for the crystal channel was decreased further in size; thus, the only dimension of the channels that changed when reduced to the smaller chips was channel length.

Validation Procedures

The smaller final design was subjected to a rigorous validation protocol to determine the limits of detection using the samples listed in Table 1. Parallel testing was performed using traditional spot plate and microcrystal methods as previously described. Reproducibility and robustness were also explored using replicate testing by six different operators, ranging from experienced to novice. One operator was one of the authors (Hanes), two were undergraduate chemistry majors with experience using simple presumptive and crystal tests, and the remaining three were volunteers with no

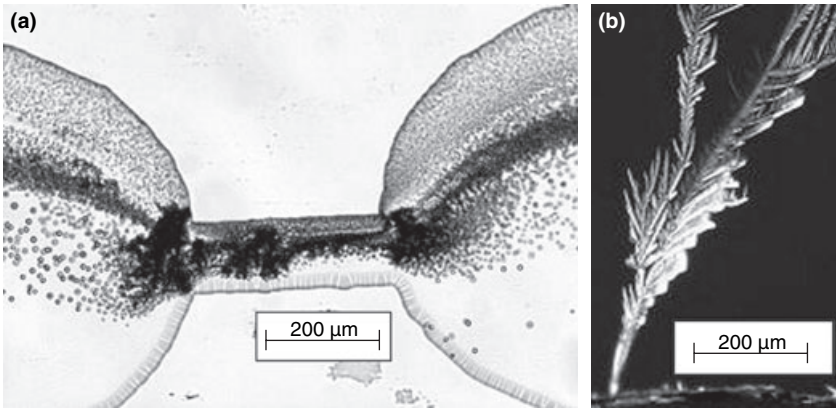

FIG. 3—*(a) Channel A (Fig. 2) containing cocaine hydrochloride and cobalt thiocyanate. The granules formed in the center are the deep blue particles characteristic of a positive reaction. (b) Channel D shows a positive crystal growth from the reaction of cocaine hydrochloride and platinic chloride.*

experience. All operators were briefed on how to use the device and then left to work alone. Thus, the results obtained were from investigators independent of assistance and at different levels of scientific expertise. This mimics the situation that would be expected in field applications.

Six replicates were performed for each test and applied to each of the mixtures shown in Table 1. After each test, the microfluidic device was washed with 10 mL of 0.5 M hydrochloric acid and blank tests were performed using deionized water. If positive results were found, the channels were washed with 5 mL of methanol, allowed to stand for 10 min and rinsed again with 5 mL of 0.5 M hydrochloric acid. This process was repeated until the channels were verified as clean. Given the small number of prototype devices fabricated, it was necessary to perform this rinsing cycle to insure that there was no carry-over. In actual deployment, the chips are designed to be disposed of after a single use, eliminating any potential carry-over. The housing in which the chip is mounted, which is the more costly of the two assemblies, would be reused.

The amount of drug sample reaching each reaction zone was determined to be *c.* 5 μg. This estimate arose by previous experiments using 1 mg of sample and monitoring flow rate, the size of the entrance hole and the volume of the channel. The use of 1–3 mg was selected as being comparable to the amount of sample typically swabbed using a Q-tip, also determined experimentally.

Results and Discussion

Oxycodone was used as a substitute for heroin (and representative of the opiate class) due to the cost of analytical-grade heroin. Results of the spot plate tests are summarized in Table 2 and compare with the same tests performed in the microfluidic device reported in Table 3. The results presented in Table 2 come as no surprise; all replicate analyses yield the same results with reagent-specific behaviors and loss of sensitivity at low concentration limits. The crystal tests proved more sensitive than the color tests, a pattern that was repeated in the microfluidic device testing. Only two mixtures, complex combinations of amphetamine/starch/dextrose and oxycodone/lidocaine/starch failed to yield crystals in the microfluidic devices across all operators. While this test did allow for estimating the limit of detection for the devices, the chemical explanation for the failure of crystal growth under these conditions was not further explored here.

The concentrations needed for positive color tests varied from 10% to 25% w/w or *c.* 50–125 pg of the drug. This compares favorably with reported detection limits of color tests performed in spot plates which range from 5 to 100 μg (3). Note that in this reference, the lowest observable and repeatable concentration was multiplied by a factor of 10 to obtain the reported LOD. One of the advantages of using small confined channels is to concentrate the color change to a reduced area, making it easier to see even without magnification. To further confirm that color changes observed in the chip were the same as seen in spot plate methods, visible spectra were recorded for color change reactions obtained by both methods, an example of which is shown in Fig. 4.

Further examination of the results shown in Table 2 (traditional methods) to Table 3 (MFD methods), reveal identical results when drugs are present in higher concentrations. As concentration decreases, differences in results are apparent. For example, with one dilute methamphetamine (methamphetamine/caffeine/starch 5/15/80%w/w), the appearance of a positive blue using the Simon test in the MFD contrasts with the failure of the same test using spot plate methods. This result is likely due to smaller volumes, increased contact, and increased localized concentrations, but further studies of this were not undertaken in the present work. In the 10/90 wt% methamphetamine/dextrose sample, all the results coincided with traditional method results.

Amphetamine and oxycodone both yielded the same results using the MFD or spot plates. However, at the lowest concentrations, both amphetamine and oxycodone mixtures failed to produce positive crystal formation inside the MFD. This loss of sensitivity likely is a result of decreased concentration rather than the type of diluents used. Cocaine results proved to be most similar between the spot plate and MFD techniques, providing similar results at the lowest level of concentration.

Conclusion and Future Work

The goal of this research was to create a MFD device capable of performing simultaneous presumptive tests for field and lab testing of controlled substances. The final MFD allowed for three color tests and one crystal test to be completed in less than 15 sec using less than 1 mg sample for all four tests. The microfluidic protocol used significantly less reagents and produced far less waste than the same sequence of tests using spot plate and ASTM methods. If these devices were mass produced and disposable, a significant cost savings for lab and field applications could be realized. In the lab, use of the devices would provide greater analytical information per

TABLE 3—*Microfluidic device results. Number of positives reported out of six trials with six different operators.*

%	Substances	Marquis	Simon	CoSCN	Crystal
100	Methamphetamine	6	6	0	6
50/50	Methamphetamine/caffeine	6	6	0	6
10/90	Methamphetamine/dextrose	6	5	0	6
5/15/80	Methamphetamine/caffeine/starch	0	1	0	6
100	Amphetamine	6	0	0	6
50/50	Amphetamine/starch	6	0	0	6
25/75	Amphetamine/dextrose	6	0	0	6
5/20/75	Amphetamine/starch/dextrose	0	0	0	0
100	Oxycodone	6	0	0	6
50/50	Oxycodone/lidocaine	6	0	0	6
25/75	Oxycodone/dextrose	5	0	0	6
5/35/60	Oxycodone/lidocaine/starch	0	0	0	0
100	Cocaine	0	0	6	6
50/50	Cocaine/lidocaine	0	0	6	6
15/85	Cocaine/aspirin	0	0	5	6
10/25/65	Cocaine/caffeine/lidocaine	0	0	0	4

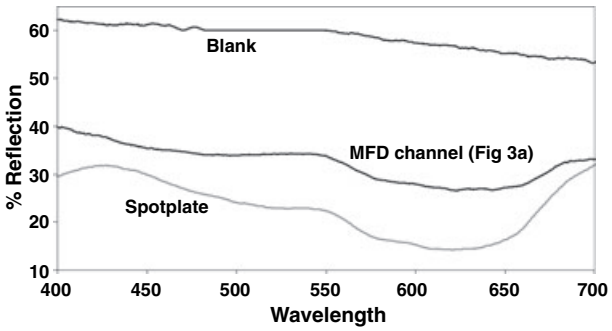

FIG. 4—*Comparison of visible spectra for the reaction product of cocaine and cobalt thiocyanate in a spot plate well and in the microfluidic device (Fig. 3a). The blank spectrum was of the reagent only acquired in the microfluidic device.*

single sample aliquot consumed. Additional testing would include other substances such as MDMA or ecstasy.

The next generation of device has been constructed and includes a simple solid phase extraction channel. The goal is to create a device that can be used in the field with a portable ATR microspectrophotometer to obtain an IR spectrum from a dried sample from the extraction channel (11). Such a device would provide identification for a significant number of typical samples. Additional changes will enhance the laboratory and field applicability of the device.

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References

1. Moorthy J, Mensing G, Kim D, Mohany S, Eddington DT, Tepp WH, et al. Microfluidic tectonics platform: a colorimetric, disposable botulinum toxin enzyme–linked immunosorbent assay system. Electrophoresis 2004;25:1705–13.
2. Smith W. Forensic sample analysis on a microchip. Anal Chem 2004;76(7):117A.
3. Olofsson J, Pihl J, Sinclair J, Sahlin E, Karlsson M, Orwar O. A microfluidics approach to the problem of creating separate solution environments accessible from macroscopic volumes. Anal Chem 2004;76(17):4968–76.
4. Reichmuth D, Shepodd T, Kirby B. On-chip high-pressure picoliter injector for pressure-driven flow through porous media. Anal Chem 2004;76(17):5063–8.
5. O'Neal C, Crouch D, Fatah A. Validation of twelve chemical spot tests for the detection of drugs of abuse. Forensic Sci Int 2000;109(3):189–201.
6. American Society for Testing and Standards. Standard guide for microcrystal testing in the forensic analysis of cocaine. West Conshohocken, PA: American Society for Testing and Materials, 1998;E1968–98:1–2.
7. Jeffrey W. Colour tests. In: Moffat AC, Osselton MD, Widdop B, editors. Clarke's analysis of drugs and poisons, 3rd ed. 2003;1:279–300.
8. American Society for Testing and Standards. Standard guide for microcrystal testing in the forensic analysis of methamphetamine and amphetamine. West Conshohocken, PA: American Society for Testing and Materials, 2001:1–2.
9. American Society for Testing and Standards. Standard guide for microcrystal testing in the forensic analysis of phencyclidine and its analogues. West Conshohocken, PA: American Society for Testing and Materials, 2001;E 2125–01:1–3.
10. Balulescu G, Constantinescu T. Thin layer chromatography combined with microreactions for the identification of alkaloids in mixtures. Anal Chem 1975;47(13):2155–60.
11. Wielbo D. Tebbett I. The use of microcrystal tests in conjunction with Fourier transform infra red spectroscopy for the rapid identification of street drugs. J Forensic Sci 1992;37(4):1134–48.

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