Determination of Time since Deposition of Fingerprints via Colorimetric Assays
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ABSTRACT: Past investigations involving fingerprints have revolved heavily around the image of the fingerprint—including the minutiae, scarring, and other distinguishing features—to visually find a match to its originator. Recently, it has been proven that the biochemical composition can be used to determine originator attributes, such as sex, via chemical and enzymatic cascades. While this provides pertinent information about the originator’s identity, it is not the only piece of information that can be provided. This research was designed with three goals in mind: (1) identify how long it would take before an aged female fingerprint could no longer be differentiated from a male fingerprint, (2) identify a correlation between the data collected and a specific time since deposition (TSD) time point, and (3) identify if a specific amino acid could be contributing to the decreasing response seen for the aging fingerprints. Using ultraviolet–visible (UV–vis) spectroscopy, aged fingerprints were evaluated over the course of 12 weeks via three chemical assays previously used for fingerprint analysis—the ninhydrin assay, the Bradford assay, and the Sakaguchi assay. As fingerprints age, the conditions they are exposed to cause the biochemical composition to decompose. As this occurs, there is less available to be detected by analytical means. This results in a less intense color production and, thus, a lower measured absorbance. The results displayed here afforded the ability to conclude that all three goals set forth for this research were accomplished—a female fingerprint can be differentiated from a male fingerprint for at least 12 weeks, UV–vis data collected from aged fingerprints can be correlated to a TSD range but not necessarily a specific time point, and the decomposition of at least a single amino acid can afford the ability to estimate the TSD of the fingerprint.

INTRODUCTION
Over the past 5 years, the research conducted by our group has focused on ascertaining information about the person who may have left a fingerprint behind at a crime scene, specifically identifying the sex of the originator. The primary purpose of these investigations was to obtain information to assist law enforcement with identifying their suspect in cases where there is no DNA, a smudged or partial fingerprint (since a picture would be difficult to obtain in these instances), or if there is simply no match in CODIS or AFIS. Throughout these investigations, only freshly deposited fingerprint samples were analyzed to minimize potential variation. However, the identity of the person of interest is not the only pertinent information that is needed.

There are many instances where a person of interest has been identified, but they claim that they were there in the past—explaining the presence of their fingerprints—not at the time when the crime was committed. Without knowing exactly how long the fingerprint has been at the scene of the crime, there is no concrete, scientific evidence that this may not be true. This concept of fingerprint aging, or the time since deposition (TSD), has been the focus of ongoing research since the mid-1900s. Initially, the age of a fingerprint was inferred by following several processes including, but not limited to, a comparative examination of the crime scene fingerprints with those of the person of interest which were placed under experimental conditions mimicking those of the crime scene. However, an expert interpreting this information would require knowledge of the surface properties from which the fingerprint was taken, specifically how these properties would affect the initial fingerprint formation and the influence of the surface on the aging process of the fingerprint’s biochemical components. Globally, the influence of the surface has been studied in detail, and it has been determined that fingerprint aging involves both changes to the biochemical components in the fingerprint residue and to the image itself. As expected, the rate of aging largely depends on the qualitative and quantitative compositions of the residue, the surface on which a fingerprint is found, and, perhaps most importantly, on environmental conditions. Utilizing this

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information, many of the original investigations into determining the age of the fingerprint were centered on monitoring the visual changes in fingerprint images such as the dulling of the residue, loss of tackiness, narrowing of the fingerprints, and loss of the continuity of the fingerprints, among others. In recent years, however, significant progress has been made regarding establishing a reliable and accurate method for determining fingerprint TSD—especially with respect to using more than just the fingerprint image. One such study aimed at using fluorescence spectroscopy to monitor the expected protein and lipid oxidation reactions within aging fingerprints. Another group at the National Institute of Standards and Technology (NIST) also determined the age of a fingerprint by studying the degree to which fatty acids—specifically palmitic acid—in the fingerprint’s ridges had migrated down into the valleys via time-of-flight secondary ion imaging mass spectrometry (TOF-SIMS) analysis. An important feature of this study is that it does not depend on circumstantial chemical changes within the fingerprint; rather it depends on molecular weight and well-studied models of molecular diffusion. However, the fact that this imaging mass spectrometer is not field-deployable is largely disadvantageous. In 2017, specific biochemical components within fingerprints were identified and monitored via Raman spectroscopy to evaluate the age of fingerprints when left in ambient light versus no light. Light conditions appeared to have a significant impact on decay rates of specific Raman bands for squalene, unsaturated fatty acids, and carotenoids. The proteins, however, demonstrated more stability. The greatest advantage of this approach is the nondestructive quality of Raman spectroscopy. The aforementioned studies are just a few examples of the research that has been conducted over the past century with respect to fingerprint TSD, with many more focusing on the fading of the overall image over time. Additionally, the development of a comprehensive analytical approach for determining the TSD of a fingerprint has yet to be practically applied in the field. As such, the focus of this research shifted from fresh fingerprints for sex identification to establishing a reliable and accurate way to determine fingerprint TSD. This study was also designed to support the sex identification studies by determining precisely how long the differentiation between a fresh male and an aged female fingerprint could be made. However, as reported in the previous publications, male fingerprints contain nearly half of the amino acid concentrations compared to female fingerprints. As a result, the color change of the reactions is significantly less, resulting in a lower absorbance value that, over time, would result in no response from the chemical systems. Because of this, only female fingerprints were used in this research as they produce a high enough absorbance value to be able to adequately see the decreasing color change both visibly and spectrophotometrically. In the past decade, the development and use of on-site colorimetric assays has been a rapidly expanding market. The fields in which these methodologies are used span from biomedical to environmental to forensics largely due to their speed, versatility, and ease of use, many of which have potential to be paired with smart device imaging applications. For these reasons, three chemical assays that generate three distinct colorimetric readouts were chosen for this investigation. Additionally, as indicated in the 2017 Raman spectroscopy study, there are specific compounds within a fingerprint that are decomposing faster than others under certain conditions; the assays chosen for these experiments presented here aid in the determination of what exactly is decomposing over time within a fingerprint due to the fact that the target analyte—amino acids—decreases in quantity from 21 to 1. In the case of the ninhydrin assay, 21 free amino acids known to be present in fingerprints were targets. However, given that the response of the assay is the summation of responses for each amino acid, it is nearly impossible to determine which amino acids are decomposing. This led to the use of the Bradford assay, which targets a small subgroup of protein-bound amino acids. The use of this assay would provide insight into whether it was the amino acids themselves causing the decrease in response to indicate aging, or if proteins were contributing to the aging response. Finally, the Sakaguchi assay was exploited because it targets arginine, which is present in the group of 21 free amino acids targeted by the ninhydrin assay as well as the small subgroup of protein-bound amino acids targeted by the Bradford assay. The Sakaguchi assay would ultimately indicate whether a single amino acid alone could indicate aging or if there were additional contributing factors.

**RESULTS**

Fingerprint TSD. The TSD analysis was designed for 84 days (12 weeks) of experiments using the ninhydrin, Bradford, and Sakaguchi assays, 70 fingerprints each. The fingerprints were collected on a sheet of polyethylene film (PEF) and left fully exposed on a laboratory bench at 21°C. This environment provided authentic conditions to those expected at a crime scene, in the sense that they were exposed to everyday particles from people walking by as well as light conditions that were not predetermined. As previously mentioned, the only nonauthentic aspect is the thermostat-controlled condition—although it could be considered authentic for samples found inside a thermostat-controlled house. Figure 1 depicts the results of the (A) ninhydrin, (B) Bradford, and (C) Sakaguchi assays following 84 days (12 weeks) of fingerprint aging. “Day 0” indicates the samples that were collected and analyzed immediately to provide the control responses. Remarkably, all three chemical assays were determined to be capable of differentiating between an aged female fingerprint and a fresh male fingerprint for up to 84 days. At the end of this study, the aged female samples were beginning to provide responses close to that of a fresh male sample but could still possibly make this differentiation with time points past the 84 days. Unexpectedly, there was an interesting trend seen with respect to time-point fluctuations seen across the three assays. The ninhydrin assay (Figure 1A), which targets the largest number of amino acids, continued to have significant fluctuations among each time point with no consistent decreasing trend seen. However, the Bradford assay (Figure 1B), which targets a small group of amino acids, showed only minimal fluctuation across each time point. Although it is difficult to pinpoint the specific reason for this, the likely cause is due to the fact that the Bradford assay targets amino acids connected to proteins, as opposed to free amino acids in the body, which may have provided some extra stability. Finally, the Sakaguchi assay (Figure 1C), which only targets one amino acid (arginine), displayed a combination of two previous results. There was substantial fluctuation in...
response—a finding that was inconsistent with the results of the sex identification studies, where the Sakaguchi assay presented the least amount of error and fluctuation—up to the 7-day time point, at which time, there is a shift to a clear decreasing trend for the remainder of the time points. The final goal was to determine if a single amino acid could be used for identifying TSD. Based on the above results from the Sakaguchi assay, a single amino acid provides enough response for a substantial amount of time to be used to determine TSD of a fingerprint. Ultimately, however, neither the Bradford assay nor the Sakaguchi assay would be beneficial in determining a specific time point, but rather could be used to establish a range of TSD.

DISCUSSION

Previous research into fingerprint composition analysis has solely focused on utilizing freshly deposited fingerprint samples, which were collected, extracted, and analyzed immediately. The information garnered from these processes has only been correlated to the sex of the fingerprint originator. However, there is much more information that law enforcement is interested in and it was believed that fingerprints had the potential to provide such information, in addition to the originator’s sex. With that in mind, the topic of the most recent investigation was TSD (time since deposition) or age of the fingerprint. This is especially important to law enforcement because they often have suspects who claim to have been at the location of a crime days or weeks before, but never at the actual time the crime was committed. Given that fingerprints are composed of biological compounds that naturally decompose over time, the next logical step for our group was to continue to target these compounds with respect to their decomposition. This research was threefold in its purpose. First, the goal was to determine how long the differentiation between an aged female fingerprint and fresh male fingerprint could be made. The second purpose was to determine if it was possible to directly correlate a specific time point for the age of the fingerprint. The final goal was to find what exactly in the fingerprint is decomposing to provide the decreasing response seen for the aging fingerprints.

With respect to the research presented here, the conditions of the experiments were as close to authentic situations as possible. However, the volunteer, collection surface, and lab space temperature were kept constant. These steps were taken to ensure that the best possible responses were obtained as this was the pilot investigation for the fingerprint TSD concept. By keeping as many variables as possible constant, we were able to minimize potential variations and maximize the amount of sample obtained. The ultimate goal of this concept aims at establishing a direct correlation between temperature and TSD. With this information, law enforcement would be able to enter a crime scene, record the temperature, 21 °C in the case of this research, and then conduct the aging analysis of the fingerprint. Once the data are obtained, they can match the data with the recorded temperature to determine the actual TSD of the fingerprint using a system designed like a compatibility chart. Current additional TSD studies being conducted by our group involve aging on nonideal surfaces and in varying temperatures that more closely mimic those found at real crime scenes. Additionally, fingerprints collected from multiple volunteers are being explored.

Based on the data displayed here, the first question of how long an aged female fingerprint could be differentiated from a fresh male fingerprint was universally addressed. It was determined that all three chemical assays—the ninhydrin, Bradford, and Sakaguchi assays—could make this differentiation up to 84 days (12 weeks) at a constant temperature of 21 °C. The second goal of determining if any of these assays could pinpoint a specific TSD was not as explicit. The ninhydrin assay, while capable of differentiating between fresh male and aged female fingerprints for the duration of the experiment, did not demonstrate a clear decreasing trend over that time. This indicated that it may not be possible to monitor the TSD with this assay. However, there is a possibility that the consistency in response is because multiple amino acids contribute to the overall response. To address this question,
the Bradford assay was employed since it targets a small subset of amino acids. As seen above, this assay demonstrated a clear decreasing trend, indicating that it was a better fit for determining TSD, compared to the ninhydrin assay. These results brought about an additional question—what is really contributing to the response given that both assays target amino acids, the difference being that the ninhydrin assay interacts with free amino acids while the Bradford assay interacts with those that are protein-bound. As a result, a final chemical assay was employed—the Sakaguchi assay—for the detection of arginine. This assay was particularly beneficial because arginine is an amino acid that is targeted by both the ninhydrin and Bradford assays as well. Ultimately, this analysis displayed a combination of the two previous results—strong intensity of response with minor fluctuations for the first 7 days, followed by a clear decreasing trend for the remainder of the time points. These results clearly indicated that fingerprint TSD was possible to be determined using a single amino acid, which slowly decomposes over time. It is important to note that while the Bradford and Sakaguchi assays displayed a decreasing trend, they are not necessarily sensitive enough to establish a specific TSD. However, the establishment of a TSD range is certainly possible. While these determinations were successful for female fingerprints, it is acknowledged that the same results are unlikely to be accomplished for male fingerprints due to the low optical response that is generated from a fresh fingerprint even when taken from more authentic surfaces.\(^1,3,4\) Additionally, while these three investigations ended after 12 weeks, all three assays have the potential to continue to make the differentiation between male and female fingerprints as well as provide an approximate TSD at time points surpassing the ones presented here given the gradual trend observed. The ability to conduct TSD investigations using only one analyte—in this case, arginine—affords the opportunity to be able to determine a specific originator attribute as well as the TSD of the fingerprint itself, a concept that would provide the greatest foundation to the onsite methodology our group aims to develop for law enforcement.

## CONCLUSIONS

The success of these preliminary investigations has opened the door to many additional long-term experiments that are currently being conducted by our group with the hope of providing more detailed information regarding the TSD of authentic fingerprints. These investigations include determining the effects of environmental conditions such as temperature, humidity, and light on fingerprint TSD. Additionally, the ultimate goal of this research is to transition the chemical assays to on-site methodologies by applying the assays directly to the fingerprint and generating a colorimetric image. The use of ninhydrin for imaging fingerprints has been well documented\(^19−21\) and frequently utilized by law enforcement. The other two methods have not yet been utilized for fingerprint imaging but hold great potential as well. The colorimetric response on the fingerprint image would then be photographed by an application on a smart device that allows for the quantification of the color output. An application of this nature is currently being explored by our group.\(^13\)

## EXPERIMENTAL SECTION

### Ethics Statement. The Institutional Review Board, Office of Pre-Award and Compliance at the University at Albany fully approved the experimental protocols demonstrated in this manuscript. These protocols were carried out in accordance with the office’s requirement of obtaining informed consent, in the form of a signature from each volunteer, acknowledging that they are aware of the procedure that will take place, any risks or benefits that may accompany the study, as well as acknowledging that they will not receive any payment for their participation. Informed consent from all volunteers who participated in this research study was obtained.

### Materials. The following enzymes and organic/inorganic chemicals were purchased from Sigma-Aldrich: premade ninhydrin solution (consisting of 2% ninhydrin, hydridantin in dimethyl sulfoxide (DMSO) and lithium acetate buffer pH 5.2), Bradford reagent (consisting of Coomassie Brilliant Blue G-250 dye, methanol, and phosphoric acid), \(\alpha\)-naphthol, sodium hydroxide (NaOH), 200 proof ethanol (EtOH), bromine (Br₂, ACS reagent), urea. Hydrolichloric acid (HCl), manufactured by EMD Millipore were purchased from Fisher Scientific. Additionally, polyethylene film or PEF (plastic wrap) was purchased from Price Chopper. Water used in all of the experiments was ultrapure (18.2 MΩ cm) water from PURELAB Flex, an ELGA water purification system.

### Instrumentation and Measurements. A Molecular Devices UV/vis spectrophotometer/plate reader (SpectraMax NanoDrop 384) was used to take optical measurements of the samples. Spectrum settings were used with the following ranges: 450−630 nm for the ninhydrin assay,\(^7\) 530−650 nm for the Bradford assay,\(^1\) and 400−600 nm for the Sakaguchi assay.\(^3\) All experiments utilized 96-well microtiter polystyrene (PS, Thermo Scientific) well plates along with polyethylene microcentrifuge tubes from VWR.

### Ninhydrin Chemical Assay. The ninhydrin assay was the first chemical assay used in 2016 for sex identification from real fingerprints.\(^2\) In this reaction, shown in Scheme 1, ninhydrin reacts with \(\alpha\)-amino acids present in the fingerprint extract to generate hydridantin, aldehydes, ammonia, and carbon dioxide.\(^23\) Any remaining ninhydrin then condenses in the presence of ammonia and hydridantin to produce diketohydridyldiene-diketohydrindamine (DYDA), which creates the intense blue-purple color known as Ruhemann’s purple.\(^7\) Ultimately, this production of color can be measured via UV−vis spectroscopy. This publication utilized only freshly

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**Scheme 1. Ninhydrin Chemical Assay for the Analysis of Amino Acid Content in Both Mimicked and Authentic Fingerprint Content. Ruhemann’s Purple Is Also Known as DYDA (Diketohydrindylidene-Diketohydrindamine)**
collected fingerprint samples to minimize potential variation that would result in samples that had been left exposed to environmental conditions.

To simplify the chemical assay for future use by law enforcement, since the ultimate goal is to have these systems in a field-deployable concept, a premade ninhydrin solution was purchased from Sigma-Aldrich.24 As previously mentioned in the Materials Section, this solution consisted of 2% ninhydrin and hydriodantin in DMSO along with lithium acetate buffer pH 5.2. After several optimization steps, the final ratio of reagents was determined to be 1:1:1 (equal volumes of 100 μL) of ninhydrin solution, fingerprint sample, and 18.2 MΩ-cm water. For this assay, the maximum wavelength (λ_{max}) for the expected color is 570 nm.24

Bradford Chemical Assay. The second chemical assay that was used was the Bradford assay, published in 2017 for sex identification.1 Again, all samples for the original publication exploited real fingerprints that were freshly collected at the time of analysis. For the research presented in this manuscript utilizing aged fingerprints, the assay remained the same as the previous publication since a premade Bradford reagent was already being utilized. The reaction, Scheme 2, is initiated when the Bradford reagent interacts with the basic and aromatic amino acids—arginine, histidine, lysine, phenylalanine, tyrosine, and tryptophan—present in the fingerprint sample. The rate of production and the intensity of this color are proportional to the overall concentration of all six amino acids in the sample. To carry out the assay, 150 μL of the commercially available Bradford Reagent was combined with 50 μL of ultrapure 18.2 MΩ-cm water and 100 μL of the extracted fingerprint sample. For the Bradford assay, λ_{max} of the colored product was 595 nm.25-30

Sakaguchi Chemical Assay. The final chemical assay utilized for sex identification using freshly deposited authentic fingerprints was the Sakaguchi assay, published most recently in 2018.1 This assay, depicted in Scheme 3, targets only arginine. Here, α-naphthol and sodium hypobromite react with the guanidine group under alkaline conditions31 and, once complete, creates a visibly distinct, red-colored complex.

The previously published protocol for the Sakaguchi assay was optimized for use with this aging experiment. This protocol utilized prechilled solutions of 1.05% NaOH and 0.146 mM α-naphthol, which were added to 100 μL of the extracted fingerprint samples contained in microcentrifuge tubes in an ice bath. This mixture was briefly vortexed and placed back in the ice bath for 5 min. In the meantime, 2 mM sodium hypobromite was placed in the wells where the final reaction would occur. After the ice bath incubation, the contents of the microcentrifuge tubes were placed in separate wells. Using a multichannel pipette, the total contents of the bottom wells were transferred into the wells containing sodium hypobromite. Upon interaction, a slightly visible pink color appeared within a few seconds. Immediately following the appearance of color, 6.24% urea was used to stabilize the color so that it did not dissipate before the spectrophotometric measurements could be taken. The λ_{max} value for this reaction is 500 nm.21,32 The most critical aspect of this assay was that all solutions needed to be prechilled and kept on ice for the duration of the experiment. This also includes the microcentrifuge tubes that were used for the initial reaction. This temperature only deviated during the spectrum runs, which were at 37 °C.

Aging Protocol. To begin this pilot study into the age of a fingerprint, 70 fingerprints—5 fingerprints for 14 different time points—were collected on PEF for each chemical assay and left exposed to age on a lab bench. These samples were collected from a single female volunteer to limit possible variations in responses. It is acknowledged that there is some variation from person to person as seen in previous publications,2-4 and this effect in conjunction with fingerprint TSD will be assessed in the future. Likewise, the PEF was the only surface used for these analyses to limit the variation expected from different surfaces during this preliminary investigation into TSD using these chemical assays. Furthermore, with respect to limiting variation in responses, the fingerprints were aged in a thermostat-controlled lab space at a constant temperature of 21 °C throughout the duration of the experiment. The effect of temperature and other environmental conditions on the aging of authentic fingerprints will be intentionally and explicitly investigated in future research conducted by our group.

The time frame of fingerprint TSD included fresh fingerprints (Day 0), along with a set of five “aged” fingerprints each day for 7 days, followed by a set of five “aged” fingerprints once a week on days 14, 21, and 28. The study concluded with...
analyses of five “aged” fingerprints after 42, 63, and 84 days. Additionally, a set of five fresh male fingerprints were collected on PEF and were extracted for immediate analysis. As previously mentioned, the chemical assays utilized were modified versions of the 2016 ninhydrin and 2018 Sakaguchi chemical assays as well as the original 2017 Bradford assay.

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

(1) Huynh, C.; Brunelle, E.; Halámková, L.; Agudelo, J.; Halámek, J. Forensic identification of gender from fingerprints. Anal. Chem. 2015, 87, 11531–11536.

(2) Brunelle, E.; Huynh, C.; Le, A.-M.; Halámková, L.; Agudelo, J.; Halámek, J. New horizons for ninhydrin: Colorimetric determination of gender from fingerprints. Anal. Chem. 2016, 88, 2413–2420.

(3) Brunelle, E.; Le, A.-M.; Huynh, C.; Wingfield, K.; Halámková, L.; Agudelo, J.; Halámek, J. Coomassie brilliant blue G-250 dye: an application for forensic fingerprint analysis. Anal. Chem. 2017, 89, 4314–4319.

(4) Brunelle, E.; Huynh, C.; Alin, E.; Eldridge, M.; Le, A.-M.; Halámková, L.; Halámek, J. Fingerprint analysis: moving toward multiattribute determination via individual markers. Anal. Chem. 2018, 90, 980–987.

(5) Baniuk, K. Determination of age of fingerprints. Forensic Sci. Int. 1990, 46, 133–137.

(6) van Dam, A.; Schwarz, J. C. V.; de Vos, J.; Siebes, M.; Sijen, T.; van Leeuwen, T. G.; Aalders, M. C. G.; Lambrechts, S. A. G. Oxidation monitoring by fluorescence spectroscopy reveals the age of fingerprints. Angew. Chem., Int. Ed. 2014, 53, 6272–6275.

(7) Muramoto, S.; Sisco, E. Infrared spectroscopy and spectroscopic imaging in forensic science. Anal. Chem. 2015, 87, 3035–3038.

(8) Widener, A. Putting An Age On Fingerprints. Chem. Eng. News 2015, 93, 4.

(9) National Institute of Standards and Technology, “Who, What, WHEN: Determining the Age of Fingerprints”, 2018.

(10) Andersson, P. O.; Lejon, C.; Mikaelsson, T.; Landström, L. Towards fingerprint dating: a Raman spectroscopy proof-of-concept study. ChemistryOpen 2017, 6, 706–709.

(11) O’Hagan, A.; Green, S. Crime scene to court: a study on fingerprint marking. Forensic Res. Criminal. Int. J. 2018, 6, 491–503.

(12) Lin, H.; Wang, X.; Lv, W.; Li, F.; et al. Dopamine-Based Paper Analytical Device for Truly Equipment-Free and Naked-Eye Biosensing Based on the Target-Initiated Catalyzed Oxidation. ACS Appl. Mater. Interfaces 2019, 11, 36469–36475.

(13) Hair, M. E.; Gerkmann, R.; Mathis, A. I.; Halámková, L.; Halámek, J. Noninvasive Concept for Optical Ethanol Sensing on the Skin Surface with Camera-Based Quantification. Anal. Chem. 2019, 91, 15860–15865.

(14) Xu, H.; Wu, D.; Li, C.-Q.; Lu, Z.; Liao, X.-Y.; Huang, J.; Wu, Z.-S. Label-free colorimetric detection of cancer related gene based on two-step amplification of molecular machine. Biosens. Bioelectron. 2017, 90, 314–320.

(15) Chen, W.; Fang, X.; Li, H.; Cao, H.; Kong, J. A simple paper-based colorimetric device for rapid mercury (II) assay. Sci. Rep. 2016, 6, No. 31948.

(16) Kang, S.-M.; Jang, S.-C.; Kim, G. Y.; Lee, C.-S.; Huh, Y. S.; Roh, C. A rapid in situ colorimetric assay for cobalt detection by the naked eye. Sensors 2016, 16, 626.

(17) Huynh, C.; Brunelle, E.; Agudelo, J.; Halámek, J. Bioaffinity-based assay for the sensitive detection and discrimination of sweat aimed at forensic applications. Talanta 2017, 170, 210–214.

(18) Chen, C.-A.; Wang, P.-W.; Yen, Y.-C.; Lin, H.-L.; Fan, Y.-C.; Wu, S.-M.; Chen, C.-F. Fast analysis of ketamine using a colorimetric immunosensor assay on a paper-based analytical device. Sens. Actuators B 2019, 282, 251–258.

(19) Exline, D. L.; Wallace, C.; Roux, C.; Lennard, C.; Nelson, M. P.; Treado, P. J. Forensic applications of chemical imaging: latent fingerprint detection using visible absorption and luminescence. J. Forensic Sci. 2003, 48, 1047–1053.

(20) Champod, C.; Lennard, C.; Margot, P.; Stoilovic, M. Fingerprints and Other Ridge Skin Impressions; CRC Press: Boca Raton, FL, 2004.

(21) Yamashita, B.; French, M. Fingerprint Sourcebook; Rockville, MD, 2010.

(22) Moore, S.; Stein, W. H. Photometric ninhydrin method for use in the chromatography of amino acids. J. Biol. Chem. 1948, 176, 367–388.

(23) Ruhemann, S. XCLII. —Triketohydridene hydrate. Part IV. Hydrindantin and its analogues. J. Chem. Soc., Trans. 1911, 99, 1306–1486.

(24) https://www.sigmaaldrich.com/catalog/product/sigma/n7285?lang=enion=US.

(25) Wu, Y.; Hussain, M.; Fassihi, R. Development of a simple analytical methodology for determination of glucosamine release from modified release matrix tablets. J. Pharm. Biomed. Anal. 2005, 38, 263–269.

(26) Friedman, M. Applications of the ninhydrin reaction for analysis of amino acids, peptides, and proteins to agricultural and biomedical sciences. J. Agric. Food Chem. 2004, 52, 385–406.

(27) http://www.bio-rad.com/webroot/web/pdf/literature/4110065A.pdf.

(28) https://www.thermofisher.com/order/catalog/product/20279.

(29) Compton, S. J.; Jones, C. G. Mechanism of dye response and interference in the Bradford protein assay. Anal. Chem. 2015, 87, 1047–1053.

(30) http://www.bio-rad.com/featured/en/bradford-assay.html.

(31) Weber, C. J. A modification of Sakaguchi’s reaction for the quantitative determination of arginine. J. Biol. Chem. 1930, 86, 217–222.

(32) Sakaguchi, S. A new color reaction for the estimation of protein and arginine. J. Biochem. 1925, 5, 25–31.