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Article type : Technical Note Benzoic acid derivatives as luminescent sublimation dyes in cyanoacrylate fuming of latent fingerprints Christopher Azaldegui BS^{1,2}; Guadalupe Aguilar BS^{3,4}; Sarahi Enriquez BS^{1,5}; Chris Madonna BS^{1,6}; Casie Parish Fisher PhD³; Raychelle Burks PhD^{1,7} ¹Department of Chemistry, School of Natural Sciences, St. Edward's University, Austin, TX, USA. ²Program in Chemical Biology, Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA ³Department of Forensic Science, School of Natural Sciences, St. Edward's University, Austin, TX, USA ⁴Travis County Medical Examiner's Office, Austin, TX 78724, USA ⁵Endeavor DNA, El Paso, TX, USA ⁶North Texas Fire Academy, Rockwall, TX, USA ⁷Department of Chemistry, College of Arts and Science, American University, Washington, DC, USA Corresponding author: Raychelle Burks PhD Email: burks@american.edu Funding for this work was provided by the Departments of Chemistry and Forensic Science, St. Edward's University and the National Science Foundation Grant No. 1608754. This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the <u>Version of Record</u>. Please cite this article as <u>doi</u>:

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Abstract

Development of latent prints employing cyanoacrylate ester (CA) can be a multi-step process including CA fuming and subsequent fluorescent staining to produce fingerprints of sufficient contrast for comparison work. To enable a single step CA fuming - staining process, a selection of fluorophores have been developed as sublimation dyes in CA fuming. A greater array of such luminescent sublimation dyes would allow users greater flexibility in selecting a particular dye - CA combination to best suit their processing needs. Toward this end, six benzoic acid derivatives were evaluated for use as luminescent sublimation dyes under elementary CA fuming conditions using a single non-porous surface type and an inexpensive handheld UV lamp for excitation. Two benzoic acid derivatives, 2-hydroxybenzoic acid (salicylic acid) and 2-aminobenzoic acid (anthranilic acid), were identified as new potential luminescent sublimation dyes with stained fingerprints excited at 254 nm. The fluorescence intensity and stability of prints produced via the sublimation of CA with 2-hydroxybenzoic acid and 2-aminobenzoic acid were evaluated over approximately six weeks using image and statistical analysis.

KEYWORDS

latent fingerprint, cyanoacrylate fuming, sublimation, luminescent, image analysis, benzoic acid derivatives

Highlights

- One step luminescent CA fuming is a desirable alternative to multistep fuming and staining methods.
- Six benzoic acid derivatives were investigated for use as sublimation dyes in CA fuming.
- Image analysis was used to evaluate fluorescence intensity and stability of processed fingerprints.
- Two benzoic acid derivatives were identified as new potential sublimation dyes in luminescent CA fuming.

Introduction

The use of fingerprints to distinguish individuals is one of the oldest and most useful tools in forensic identification (1,2). Due to the variability in composition of latent fingerprints, along with the numerous types of surface on which latent fingerprints are deposited, numerous optical, physical, and chemical processes have been developed in order to visualize latent fingerprint residue (3). In the past 50 years, one of the most significant latent fingerprint processing techniques has been cyanoacrylate ester (CA) fuming (4). CA fuming results in the colorless sweat secretion of a latent fingerprint being coated in white polymer fibers. While this polymer coating is typically easier to see than the unprocessed latent fingerprint, a secondary stain might be needed to add contrast to aid an examiner when evaluating fingerprints on certain surfaces. In many cases, CA fuming is part of a multi-step process which can be time intensive and laborious. Products such as PolyCyano UV, Lumicyano[™], and PECA Multiband have been developed to reduce the multi-step fuming and staining process to a single step via sublimation of select luminescent dyes during CA fuming (5–8). Seeking to expand the repertoire of single-step luminescent CA techniques, we explored the use of benzoic acid derivatives as potential luminescent sublimation dyes.

Benzoic acid derivatives were selected for investigation for two reasons. First, we observed that both the luminescent dyes p-dimethylaminobenzaldehyde (4) and 3-chloro-6-ethoxy-tetrazine (9) are disubstituted aromatic six-member rings. Second, we were familiar with the fluorescence behavior of 2-hydroxybenzoic acid (10), more popularly known as salicylic acid, which shares the structural features of the aforementioned luminescent dyes. Appearing blue (11–14), emission of 2-hydroxybenzoic acid (2-HBA) and its anions' has been determined to be approximately 400 nm for the dianion, 408 nm for the monoanion, and 450 nm for the neutral acid, with major absorbance bands centering around 234 nm and 300 nm (11,15,16). Depending on the analysis, excitation wavelengths used to observe the fluorescence of 2-HBA and/or its anions have ranged along the aforementioned absorbance bands (10–22).

We expanded our luminescent sublimation dye candidate list to include other benzoic acid derivatives based on our targeted review of relevant literature on benzoic acid derivatives' fluorescence dynamics (10–30), ease of availability, and affordability. Included in this list is the popular fluorescent probe 2-aminobenzoic acid (2-ABA), also known as anthranillic acid (28,31). Like 2-HBA, excitation of 2-ABA results in emission of blue light (28,32,33). The dominant absorbance bands of 2-ABA are red-shifted relative to 2-HBA, with peak maximums centered at 247 nm and 336 nm (28). Other selected benzoic acid derivatives selected for this proof-of-concept work either had, or were expected to have, absorbance bands near 2-HBA and 2-ABA due to shared structural features. In addition to 2-HBA and 2-ABA, our luminescent sublimation dye candidate list included 2-methoxybenzoic acid (2-MBA), 4-hydroxybenzoic acid (4-HBA), 4-aminobenzoic acid (4-ABA), and 4-fluorosulfonylbenzoic acid (4-FSBA) as well as the parent compound benzoic acid (BA). Structures are shown in Table 1.

Beyond their promising luminescent properties, the sublimation temperatures of select BA derivatives further encouraged our study. Having a sublimation temperature similar to heat-accelerated CA fuming conditions, along with preferentially staining CA, are the two basic challenges to developing new sublimation dyes (6,34,35). In heat-accelerated CA fuming, temperatures of less than 200 °C are used to avoid CA thermal degradation, with 120 °C a popular choice (36). The reported sublimation temperatures (T_{sub}) of BA, 2-HBA, 2-ABA, 2-MBA, 4-HBA, and 4-ABA are less than 200 °C (37,38), with each T_{sub} being near commonly employed CA fuming temperatures. These T_{sub} values are included in Table 1.

For our proof-of-concept study, we contrained ourselves to the use of an elementary CA fuming set-up, a simple handheld UV lamp (254 nm and 365 nm) as an excitation source, and a camera without speciality filters to support use of any newly developed single-step luminescent CA techniques by analysts working with constrained budgets and/or limited equipment access. As our study involved screening several candidates, a single surface type was employed in this

first-ever investigation of BA derivatives as possible luminescent sublimation dyes in CA fuming. Of the candidates shown in Table 1, two show promise for use in single-step luminescent CA fuming: 2-HBA and 2-ABA. The fluorescence intensity and stability of these stains were monitored over a period of approximately six weeks using image analysis via an in-house authored PythonTM script to extract normalized fluorescence intensity data from collected images, followed by statistical analysis of this data.

Materials and methods

Materials, equipment, and software

All luminescent sublimation candidates (hereafter "candidate") were purchased from various chemical supply companies, such as Sigma-Aldrich (St. Louis, MO), at technical grade or better and were used as received without further purification. Cyanoacrylate ethyl ester, as the product Stick Fast™ CA-THIN from TMI Products (Peachtree City, GA), was purchased from the manufacturer via Amazon. Similarly purchased were aluminum large weighing boats from Heathrow Scientific (Vernon Hills, IL). Hefty^(R) Party Cups and fuming chamber supplies (Aqua Culture 10-gallon glass aquariums, Husky^(R) plastic drop cloths, and 3M CommandTM small wire hooks) were purchased from local Walmart stores. A six watt UVGL-55 combination short (254 nm) and long (365 nm) wavelength handheld UV lamp from UVP (Upland, CA) was used for excitation. The open source programming language PythonTM, along with the Open Source Computer Vision Library (OpenCV), were used for image analysis and data visualizations. Microsoft Excel^(R) 2016 and MiniTab^(R) 19 was used for statistical analysis. The use of standard lab equipment and glassware, along with office supplies, are described within their relevant subsections below.

Safety

Safety data sheets for all redyes were reviewed and the appropriate safety precautions were enacted. All latent print processing activities were conducted in fume hoods. Standard safety protocols regarding the use of UV light sources were followed.

Standalone fluorescence assessment

Solid samples of each candidate were visually assessed for fluorescence upon excitation with our UV handheld lamp at both 254 nm and 365 nm. Cyanoacrylate ethyl ester (hereafter "CA"), along with all mixtures of CA and a candidate (hereafter "CA + candidate" or "CA + <candidate abbreviation>"), were also evaluated. Visual observations were recorded.

Latent fingerprint sample preparation

For our study of selected candidates, the single surface selected were the inexpensive plastic Hefty^(R) Party Cups, which have a glossy red exterior and a matte white interior. The white surface was the focus of our study. Cups were cut to produce four sections of approximately equal size. Three sections, each to contain two latent fingerprints on the white side, were designated for use with each CA or CA + candidate mixtures, being labeled accordingly. The fuming date was also marked on each section. An elongated paperclip was punched through the top of each cup section for suspension from a hook affixed to the roof of the fuming tank. Letters A - F were used to designate each latent fingerprint location, with two letters per section. A latent fingerprint from a single donor was deposited adjacent to each letter, resulting in a set of six latent fingerprints (A - F) for development using CA or a CA + candidate mixture each. Depletion of the latent print deposits was not a variable for this proof-of-concept work, which is focused on the feasibility of selected benzoic acid derivatives as luminescent sublimation dyes in CA fuming. To minimize the role of depletion in our current work, the donor deposited the prints for a single candidate's processing set, then waited 48 hours to deposit another set of prints. This timing was adopted to help curtail secretion depletion that could occur if all prints were deposited at once. Regarding donor participation, approval by the governing ethical oversight body was secured.

Fuming

Three fuming chambers were constructed as detailed elsewhere (1,3,35). Briefly, glass aquarium tanks and heavy-duty plastic drop cloths were used to construct enclosed fuming chambers. CommandTM hooks were attached to the glass ceiling of each chamber for hanging cup sections. Within each chamber, a standard lab hot plate set to approximately 130 °C was used to heat both (1) CA or a CA + candidate mixtures for fuming and (2) deionized water in a

50 mL beaker. To initiate fuming, an aluminum weigh boat containing CA or CA + candidate mixture was placed on a chamber's hot plate and the chamber was guickly sealed. Preliminary fuming work established five drops of CA (~0.1600 g) and a processing time of 8 minutes resulted in suitable fingerprint development while avoiding overdevelopment. An approximate CA:candidate weight ratio of 2:1 was used for all CA + candidate fuming runs. After weighing out the required amount of candidate, five drops of CA were added and the components mixed. The weigh boat would then be placed in the chamber to initiate fuming. To expedite processing, candidates amounts can be pre-weighed into boats, covered with Parafilm^(R) (Amcor; Zurich, Switzerland) and stored. CA would then be added directly prior to a processing run. Between fuming runs of different CA + candidate mixtures, the chambers were vigorously cleaned with standard glassware soap, rinsed with deionized water and acetone, and allowed to dry overnight prior to further use. Before samples were developed using a CA + candidate mixture, a CA only reference run (i.e. a positive control) was done using latent fingerprint samples prepared as detailed above. This allowed us to verify that CA fuming conditions were suitable latent fingerprint processing and that no fluorescent candidates were contaminating any subsequent processing runs.

Time trial

All CA + candidate mixtures were assessed for latent fingerprint development and fluorescence under both normal laboratory lighting (white light) and upon excitation using both wavelengths of the aforementioned UV lamp. All fingerprints were exposed to UV light for several seconds at most to note light emission or the lack thereof. Those CA + candidate mixtures that resulted in fluorescently stained CA developed prints, along with a CA reference set (i.e. positive control set) A - F, were moved forward for longer term monitoring to assess the intensity and stability of fluorescence over a period of approximately six weeks using image analysis as detailed in the next subsection. To the samples moving forward to the time trial, a label with a scale was placed adjacent to each developed print. To this label the candidate abbreviation, latent fingerprint letter A - F, and fuming date were added. This additional labeling provided additional identification for each processed print over the course of the time trial.

Image collection and analysis

Over the course of the time trial, developed fingerprints were photographed using a Nikon D90 DSLR digital camera with a Kodak FK4520 +10 close-up macro lens. Samples were evaluated under normal lab lighting conditions and under illumination with our UV lamp. During the time trial, all photography set-up was uniform and the UV lamp was placed to the left of fingerprints. Exposure to UV light was 1-2 minutes at most. All fingerprints were photographed over a 45 day period every other day for the first three weeks, then every four days for the remaining time, which resulted in a total of 15 monitoring days. Image files were saved as JPEG Fine files and transferred to a restricted access drive each day images were captured. The images collected were solely for the evaluation of fluorescence intensity and stability. We did not employ image collection or analysis to produce sub-items for individualization purposes.

Image analysis was done using an in-house authored Python[™] script, which employs OpenCV. This script converts a color image into a grayscale image. Next, the grayscale image is processed using an adaptive thresholding to generate a pure black-and-white (binary) image. Adaptive thresholding, with its use of regional threshold values, takes into account varying illumination conditions of an image (39). The adaptive thresholding algorithm compares each grayscale image pixel value to a threshold value determined for the pixel's region of the image. If the pixel value is below the threshold value, the pixel's value is reassigned to black. Pixel values above the threshold value are reassigned to white. These reassignments yield a binary image with white pixels corresponding to fluorescence (F) and black pixels to no fluorescence (NF). With the generated binary images of processed fingerprints, white pixels would correspond to fluorescently stained prints and the black pixels to a non-stained background. For a binary image, our Python script calculates its white (F) and black (NF) pixel counts, along with the ratio F/NF. This ratio provides us with a scaled fluorescence intensity value for each image. Our script automates analysis of all images in a designated directory (folder), producing a single CSV file storing the F, NF, and F/NF value for each image file processed. This script also saves all grayscale and binary images produced in so-named folders. Our Python[™] script is provided in SI as both a TXT and PY file titled "SI CA-IMGAnalysis-Adaptive". The TXT and PY files are identical in content, with the PY file able to be used directly within Python[™] or a Python[™] interpreter. We hope providing our Python[™] script will enable analysts and researchers to further employ image analysis in the development and evaluation of fluorescent stains.

Statistical analysis

For each CA and CA + candidate F/NF data set (n = 6 per monitoring day), the average and standard deviation values for was calculated using Excel. To explore variation within and between CA and CA + candidate F/NF data sets, repeated measurement analysis of variance (RM-ANOVA) at a 95% confidence level followed by Dunnett's test (40) was completed using Minitab.



Results and discussion

Pre-fuming observations

During the standalone fluorescence assessment of the candidates, no fluorescence was observed for any candidates upon excitation at 365 nm. Only 2-HBA and 2-ABA were observed to fluoresce upon excitation at 254 nm. Considering the relevant literature (10–30), it is likely that fluorescence was not observed for other candidates because either any induced emission at the excitation wavelengths of 254 nm and 365 nm are not perceptible by human eyes or these wavelengths were not suitable for excitation. While only 2-HBA and 2-ABA were observed to fluoresce, all CA + candidate mixtures were evaluated for possible fluorescence to probe the possible effect of CA on observable fluorescence of each candidate at our excitation wavelengths. The general phenomenons of solvent and pH dependent fluorescence are well known (23). The effect of pH of a range of aqueous solvents on the intensity and stability of the fluorescence of all three positional isomers of aminobenzoic acid has been characterized (24-26), with a similar work done for 2-HBA (16), 2-ABA (27), and BA (41). To our knowledge, the effect of CA on the fluorescence of our candidates has not been investigated for CA as a monomeric solution, polymer medium, or polymeric medium deposited on latent fingerprints. As such, all CA + candidate mixtures were evaluated for fluorescence upon mixing and post-fuming at the aforementioned wavelengths. Upon mixing, only CA + 2-HBA and CA + 2-ABA were observed to fluoresce upon excitation at 254 nm. No fluorescence was observed for any CA + candidate mixtures upon excitation at 365 nm.

Post-fuming observations

When observed under standard laboratory lighting without excitation, we observed that each set of CA + candidate developed fingerprints A - F were similar in appearance to the CA only

reference set A - F. Only CA + 2-HBA and CA + 2-ABA resulted in fluorescently stained fingerprint sets A - F upon excitation at 254 nm. Upon excitation at 356 nm, no fluorescence was observed for any of the CA + candidate sets of developed fingerprints. To investigate the fluorescence intensity of CA + 2-HBA and CA + 2-ABA developed fingerprints over several weeks, latent fingerprint sets were processed and observed for 45 days as detailed in Materials and Methods. Also included in our time trial was CA as a reference set and CA + 2-MBA set. CA served as a standard fuming check, while both CA and CA + 2-MBA served as non-observablefluorescence reference sets. While other CA + candidate mixtures could have also served the latter purpose, 2-MBA shares substituent position on the parent benzoic acid with 2-HBA and 2-ABA.

Like the hydroxyl substituent of 2-HBA and the amine substituent of 2-ABA, the methoxy substituent of 2-MBA is in the ortho position. When excited at a range of select wavelengths, including 254 nm, 2-HBA and 2-ABA both emit visible light (16,27–30). In 2-HBA and 2-ABA, as well as compounds of similar structure, the ortho positioning between substituents seems to enable intramolecular hydrogen bonding which affects absorbance, fluorescence, and spectral shifts (16,27–30). Though 2-MBA shares this ortho positioning, its lambda max in aqueous solutions has been noted as 296 nm for the neutral acid and 280 nm for the monoanion, with emission for the former observed at 365 nm and the later at 340 nm (16). Though our standalone fluorescence assessment revealed no fluorescence for CA + MBA, we included this combination in our time trial given 2-MBA's shared ortho positioning with 2-ABA and 2-HBA and to probe possible longer-term solvent effects. Time trial data from this range of CA + candidate mixtures also provides valuable information for our future study of the fluorescent stain deposition and sorption mechanics and the mechanisms of fluorescence for successful sublimation dyes candidates.

Time trial work with CA (i.e. positive control), CA + 2-HBA, CA + 2-ABA, and CA + 2-MBA fingerprint sets mirrored our earlier screening work with only CA + 2-HBA and CA + 2-ABA resulting fluorescently stained fingerprint sets. Each developed print A - F for both CA + 2-HBA and CA + 2-ABA were observed to fluoresce upon excitation at 254 nm over the course of the time trial, with no notable changes to fluorescence catalogued by observers over the course of the time trial. None of the CA or CA + 2-MBA treated fingerprints were observed to fluoresce upon excitation over the course of the time trial. None of the CA or CA + 2-MBA treated fingerprints were observed to fluoresce upon excitation over the course of the time trial. Our visual assessments of processed prints noted a distinct difference between non-fluorescent developed prints and fluorescent developed prints. Fig. 1 shows representative fluorescently stained fingerprints for (e) CA + 2-HBA and (g)

CA + 2-ABA under UV irradiation at 254 nm, with (a) CA and (c) CA + 2-MBA included for comparison. All time trial images are provided within the zip folder titled "Time trial images" at our Open Science Framework (OSF) project available at DOI <u>10.17605/OSF.IO/RU3ZH</u>.

Image and statistical analysis

As described in Materials and Methods, our Python[™] script processes a color image to a blackand-white binary image via adaptive thresholding yielding a scaled fluorescence intensity (F/NF), where the binary image's white pixels correspond to fluorescence (F) and its black pixels correspond to no fluorescence (NF). For the collected images (a), (c), (e) and (g) in Fig. 1, their corresponding binary images are shown in (b), (d), (f), and (h). All grayscale and binary images produced via script processing of collected color images are also within the zip folder titled "Time trial images" at our Open Science Framework (OSF) project available at DOI <u>10.17605/OSF.IO/RU3ZH</u>. Evaluating F/NF data allowed us to monitor normalized fluorescence intensity over time for each of the latent fingerprints processed, which enabled us to examine the stability of fluorescence over time.

Fig. 2 shows the average F/NF for CA, CA + 2-HBA, CA + 2-ABA, and CA + 2-MBA processed fingerprints over the 45-day time trial. For CA and time trial CA + candidate mixtures, each monitoring day's average F/NF value was determined by averaging the individual F/NF values for each treatment's six processed fingerprints A - F. Time trial F, N, F/NF, and average F/NF data is provided in the SI file "SI_Image Analysis Data". As seen in the main graph in Fig. 2, the average F/NF values (solid lines) for candidates 2-HBA and 2-ABA dwarf the average F/NF values for CA and CA + 2-MBA. The Fig. 2 inset graph shows the average F/NF values for CA and CA + 2-MBA. The Fig. 2 inset graph shows the average F/NF values for CA and CA + 2-MBA. The Fig. 2 inset graph shows the average F/NF values for CA and CA + 2-MBA treated fingerprints are orders of magnitude smaller, in line with the results shown in Fig. 1. The shaded area encompassing each average (solid) line illustrates the range of F/NF values for each processed fingerprint A - F for the designated treatments. Such a range is expected given the variability in (1) CA polymerization and deposition processes, (2) fluorescent stain deposition and sorption processes, and/or (3) fluorescence quantum yield.

Though we visually noted a distinct difference between non-fluorescent developed fingerprint sets (CA and CA + 2-MBA) and fluorescent developed fingerprint sets (CA + 2-HBA and CA + 2-ABA), we also explored CA and CA + candidate F/NF data sets using RM-ANOVA at a 95% confidence level followed by Dunnett's test. Results of this analysis are provided in the SI file

"SI_RM-ANOVA and Dunnett analysis". This analysis allowed us to explore variance within a fingerprint set A - F and between sets. RM-ANOVA and Dunnett's test results All CA + 2-MBA processed prints A - F were statistically indistinguishable from all CA processed prints A - F. For CA + 2-HBA, processed prints A, B, D and F being statistically distinguishable from the CA set, while C and E were statistically indistinguishable. For CA + 2-ABA, processed prints B - F being statistically distinguishable from the CA set, while C and E were statistically indistinguishable. For CA + 2-ABA, processed prints B - F being statistically distinguishable from the CA set, while A was statistically indistinguishable. As stated earlier, such variation is to be expected given the various processes involved. Overall, CA + 2-HBA and CA + 2-ABA processing resulted in fluorescently stained fingerprints via a single-step fuming process with fluorescence observed over the duration of our time trial. Both 2-HBA and 2-ABA are available from major chemical suppliers such Sigma Aldrich and affordably priced at less than \$0.50 per gram. Our preliminary results indicate that both 2-HBA and 2-ABA can function as sublimation dyes in a single-step luminescent CA fuming.

Limitations

The array of latent fingerprint development techniques in use reflects the variety of factors analysts must consider when processing items including, but not limited to: (a) the type of latent print residue, (b) surface type, texture, and condition, and (c) other types of forensic processing to be done on the item or collected sub-items (3). Each latent fingerprint development technique has limitations that restrict its use and/or dictate its place within a particular order of evidence processing. Though the work presented herein is primarily proof-of-concept focused on the feasibility of BA derivatives as sublimation dyes in CA fuming, the limitations of the use of 2-HBA and 2-ABA must be discussed. We employed a single donor and a single surface, plus utilized a fingerprint deposition timeline to mitigate depletion. We recognize these experimental conditions likely restrict the application of the sublimation techniques presented within, but limited variables are routine in proof-of-concept work. Our group plans to more expansively probe the use of 2-HBA, 2-ABA, 2-MBA, and other BA derivatives as luminescent sublimation dyes and hope the demonstrated potential of BA derivatives spurs work external to our group.

Excitation at 254 nm enabled the observation of blue fluorescence of 2-HBA and 2-ABA. Prolonged exposure to UV light in the UVA (wavelength 320-420 nm), UVB (wavelength 280-320 nm), and specifically UVC (wavelength 200-280 nm) can cause DNA degradation that renders relevant biological specimens unsuitable for forensic DNA analysis (42–46). Processed prints evaluated during the course of this work were exposed for seconds for visual observations to 1 - 2 minutes (at most) during the time trial portion to capture an image. If subsequent DNA recovery is to be done from an item, it has been advised that the damage UV light, particularly UVC, can cause be taken into account when using shortwave UV to detect and capture latent fingerprints (46). As with other latent fingerprint processing techniques used in conjugations with DNA analysis, it is essential that the correct processing sequence be employed to - as stated in the second volume of the U.K. Home Office Fingerprint Source Book - "maximise evidential opportunities" (46).

Conclusion

Two new sublimation dyes, 2-HBA and 2-ABA, were identified and preliminary characterized for use during CA fuming. The low cost of 2-HBA and 2-ABA, their straightforward integration into an elementary CA fuming scheme, and only a handheld UV lamp required for excitation support their inclusion into the library of luminescent sublimation dyes. Our current and future work is focused on addressing the limitations discussed in the previous section, as well as testing additional benzoic acid derivatives, exploring the use of alternative light sources and filters, probing the mechanics fluorescent stain sorption, exploring stain fluorescence mechanisms in the CA vapor phase, and elucidating each sublimation dye's fluorescence quantum yield toward our goal of increasing the repertoire of sublimation dyes. While more expansive studies will contribute a broader view, the potential of benzoic acid derivatives to serve as sublimation dyes under elementary CA fuming conditions is encouraging.

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TABLE 1 The structures and sublimation temperatures (T_{sub}) of the co-sublimation candidates explored. The T_{sub} values for BA, 2-HBA, 2-MBA, and 4-HBA are from (37), 2-ABA and 4-ABA are from (38). Our review of literature did not reveal a reported T_{sub} for 4-FSBA.

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Figure Legends

FIGURE 1 Latent fingerprints treated by (a - b) CA, (c - d) CA + 2-MBA, (e - f) CA + 2-HBA, (g - h) CA + 2-ABA. Images a, c, e, and g are representative color images captured during the time trial paired with their Python script generated binary images b, d, f, and h. All time trial images are provided within the zip folder titled "Time trial images" at our Open Science Framework (OSF) project available at DOI <u>10.17605/OSF.IO/RU3ZH</u>.

FIGURE 2 Normalized fluorescence intensity, F/NF, over the 45-day monitoring time for CA + 2-HBA and CA + 2-ABA with the inset graph highlighting the CA and CA + 2-MBA. Each point is the average F/NF for latent fingerprints A - F for each fuming treatment, thus the solid line represents the average F/NF for CA, CA + 2-HBA, CA + 2-ABA, and CA + 2-MBA processed fingerprints. Shaded areas encompassing each solid line graphically represents the range of F/NF values for each fuming treatment's processed fingerprints A - F.

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