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ORIGINAL PAPER

Electrochemical visualization of latent fingerprints using polyphenazine dyes on brass cartridges

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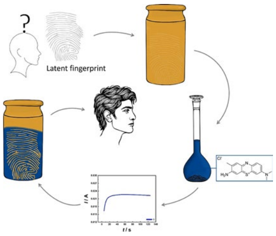
Abstract

This work is focused on the visualization of latent fingerprints left on unfired brass cartridges. Polymer films were prepared from 2 mM neutral red or 5 mM toluidine blue using two different electrochemical methods (cyclic voltammetry or chronoamperometry) with relatively short polymerization times. The conditions for the deposition of conductive polymers, poly(neutral red) and poly(toluidine blue), from a neutral medium (phosphate buffer with 0.1 M KNO_3 or 0.1 M KNO_3, respectively) were optimized to produce high-quality visualization of the remaining fingerprints on the brass substrates. The surface morphology and quality of the polymer films after the electrochemical deposition of both polyphenazine dyes were characterized by stereomicroscope. Phenazine dyes, which were used for the visualization of fingerprints, have been shown to provide different degrees of homogeneity in the deposited film. Furthermore, the dependence of the stability of the monomer solutions on their age, use, and storage conditions are discussed. Finally, a methodology is proposed for how to apply this technique of visualizing latent fingerprints with observed details of papillary lines in forensic practice.

Graphical abstract



Keywords Brass cartridge · Latent fingerprint · Dyes · Electrochemical polymerizations · Cyclic voltammetry · Material science

Introduction

Fingerprints, also known as dactyloscopic traces, are created by friction ridges placed on the palms and fingers and are used as basic evidence to identify people due to their uniqueness. Fingerprints can be found in visible, plastic, or latent forms. Latent fingerprints, which are invisible to the naked eye, are found most often at crime scenes [1, 2]. For their visualization, it is necessary to select a suitable dactyloscopic method.

Latent fingerprints are formed mainly by secretions from two types of glands and/or various contaminants, such as cosmetic products, soap residues, bacterial spores, or even

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chemical substances [1]. Eccrine sweat glands are located mainly on the papillary lines, whereas sebaceous sweat glands are found throughout the body (at the highest concentration on the scalp skin), except on the hands and feet. The sebaceous fingerprint is largely made up of free fatty acids and their esters. A substantial portion of both sebaceous and eccrine fingerprints consists of water, which can easily evaporate during fingerprint aging [1–4].

To date, there are only a few techniques that can successfully make fingerprints visible on metallic materials [5]. To choose the most appropriate method, it is necessary to consider, for example, the character of the trace, its assumed age, and the character of the material on which the trace is deposited [6]. At crime scenes, there are often smooth or rough non-porous surfaces on which fingerprints are applied; therefore, they are easily destroyed, especially if some time has elapsed since their deposition.

The recovery rate of fingerprints from firearms and ammunition is very low, so the cyanoacrylate fumigation process is frequently used to visualize them. However, the combination of cyanoacrylate fumigation together with the application of "Gun blue" and a fluorescent dye provides a better quality fingerprint [5, 6]. Both of these methods are unfavorable for the health of users; therefore, other suitable methods for visualizing fingerprint on these materials are being sought out. Recently, the use of conductive polymers has been offered as a possibility because, when a conductive polymer film is applied, its formation and resulting thickness can be easily regulated by setting the potential, concentration of the monomer solution, or the deposition time to achieve optimal results. With the help of conductive polymers, it is possible to effectively visualize even low-quality fingerprints on cartridges [7]. Suitable conductive polymers such as poly(3,4-ethylenedioxythiophene) (PEDOT) [7–9], polyaniline (PANI) [10], polypyrrole (PPy) [9], and poly(neutral red) (PNR) [11, 12] can be deposited on metal material either potentiostatically [7, 8] or by means of cyclic voltammetry (CV) [12]. From the study by Broncová et al., it is clear that CV can be used to visualize fingerprints and, moreover, that PNR can be applied to a brass substrate from a solution consisting of 0.1 M $NaNO_3$ in phosphate buffer with 2 mM neutral red (NR) [11]. Compared to the relatively lengthy polymerization of cyanoacrylate, both electrochemical methods are relatively simple and fast. The fingerprint can be visualized within four minutes [11, 12].

In our experiments, colored polymer films of PNR and poly(toluidine blue) (PTB) are applied on the working electrode (i.e., brass cartage) from the monomer solution [13]. Moreover, these polyphenazine dyes can also be used as electron mediators and have been shown to be promising sensitive layers for electrochemical sensors for the detection of various analytes [14]. Polyphenazine dyes can be electrochemically deposited on various metal substrates [15], which is advantageous for cartridges commonly made from metals such as copper, nickel, aluminum and their alloys (brass or steel) [1, 4, 16]. During the application, polymer films are deposited directly on the metal substrate between the papillary lines of the fingerprint; this procedure creates a so-called negative image, as described in the literature [2, 6, 8]. Due to the presence of sebum, the fingerprint acts as an insulating mask. The grease blocks the transfer of electrons between the electrode and the monomer solution. Greasy fingerprints on a metal substrate thus create local areas with high charge-transfer resistance, so the electropolymerization is inhibited and a layer of polymer cannot be formed there.

The goals of this work were to i) compare the quality of visualization latent fingerprints on unfired cartridges using both polyphenazine thin films, ii) evaluate the homogeneity of applied polymer films, and iii) characterize this procedure for future application on fired cartridges from forensic practice.

Results and discussion

CV or chronoamperometry (CA) are electrochemical methods suitable for the deposition of polyphenazine dyes on metal substrates. These methods enable close monitoring and control of the formation of the emerging polymer layer. Is expected in the future development of the method and, above all, the application of the method to fired cartridges, which is a key topic in forensic practice, are expected and this is a goal this research.

Visualization of fingerprint with electrochemical deposition of poly(neutral red)

Figure 1a shows the deposition processes of PNR on brass cartridges of the 0.45AUTO type. During PNR deposition, a cathodic peak around a potential of 0.07 V was observed in the first cycle. This peak shifted to a potential of 0.01 V as the number of cycles increased. The current apparently changed during polymerization, which indicated the formation of a polymer film on the brass substrate.

The shift of the cathodic peak occurring during PNR deposition varies for different brass cartridges, indicating changes in the redox behavior of a given substrate. Observed differences are due to the composition or structure of the brass surface. During PNR deposition on cartridges, the mean potential of cathodic peaks was 0.04 ± 0.01 V. The repetition of PNR deposition on brass cartridges of the same or different sizes is shown in Fig. 1b, where the effect of the cartridge size on the current response in CV is evident. Equally sized cartridges (A to C) exhibited the same current response for the 7th cycle

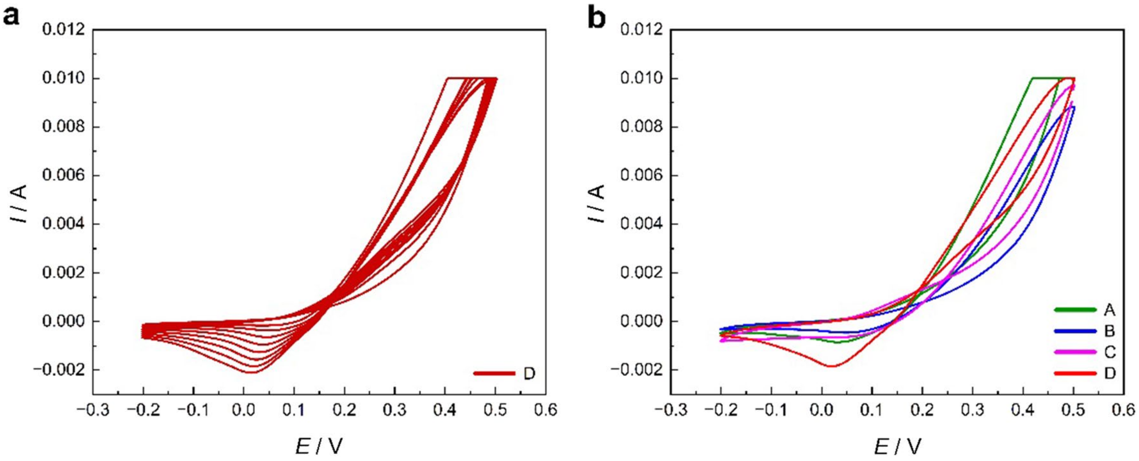


Fig. 1 Cyclic voltammogram of 2 mM NR in phosphate buffer with 0.1 M KNO_3 on cartridges with fresh fingerprints in the potential range from − 0.20 to 0.50 V (vs. Ag/AgCl) at a scan rate of 50 mV $s.^{-1}$; **a** whole deposition process (8 cycles, cartridge D) and **b** seventh cycle for 4 cartridges (A–D), compared deposition process on cartridges A–C (9 mm) and D (.45 AUTO)

of PNR deposition on their surface, while the larger cartridge (D) provided a much higher current signal.

From the pictures obtained after the polymerization (Fig. 2), it is clear that the resulting film has a relatively coarse structure. The cartridges B and C could not be completely immersed in the monomer solution up to the edge because the crocodile clip (electric contact) could not be immersed. This problem will be solved in the future by a special holder with electrical contact for cartridge cases. Currently this holder is developed.

The PNR film was applied almost homogeneously but only in the spaces between the papillary lines. On one part of the cartridge, the PNR is applied only outside the fingerprint area, and on another cartridge, the PNR is also applied through the sebum. However, the fingerprints are of good quality and can be easily used for identification.

Visualization of fingerprint with electrochemical deposition of poly(toluidine blue)

Figure 3a shows the representative deposition of PTB on brass cartridges of type 9 mm with the CA method. The current first increased dramatically from 16 mA to approximately 23 mA and then fluctuated only slightly at this current value. The curves in Fig. 3b represent the repeated deposition of PTB on the surface of the same (9 mm cartridges E to G) and larger cartridges (0.45 AUTO, cartridge marked H), respectively. The outline of these curves is very similar to the curves of electrodeposition of PEDOT on cartridges

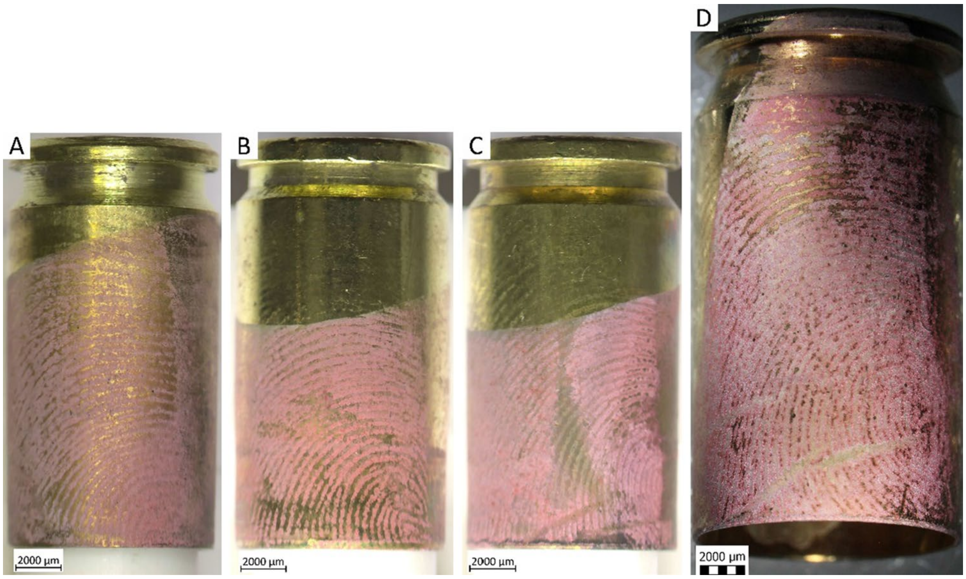


Fig. 2 Brass cartridge cases **A**, **B**, **C** (9 mm), and **D** (.45 AUTO) with a visualized latent fingerprint by PNR. Cases A to C were photographed by the stereoscopic microscope Zeiss and D with a Nikon

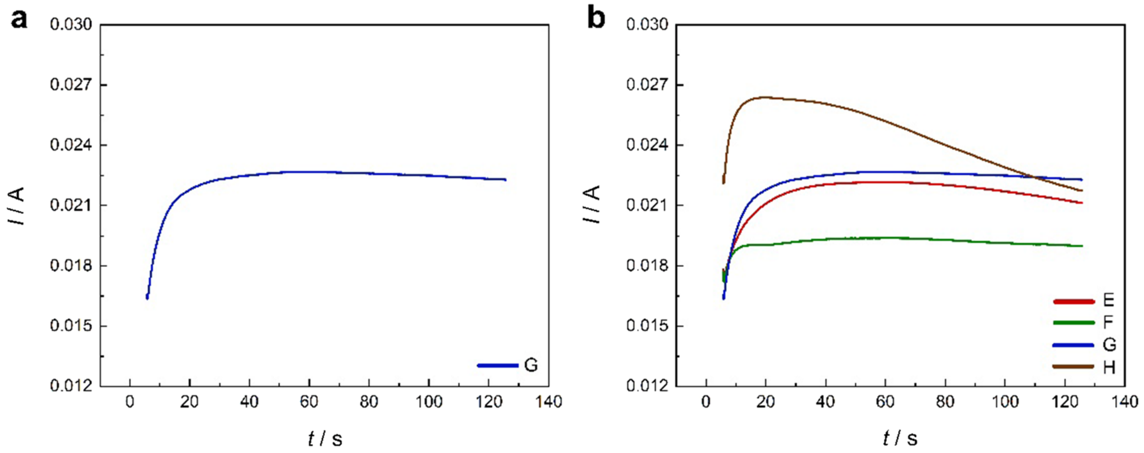


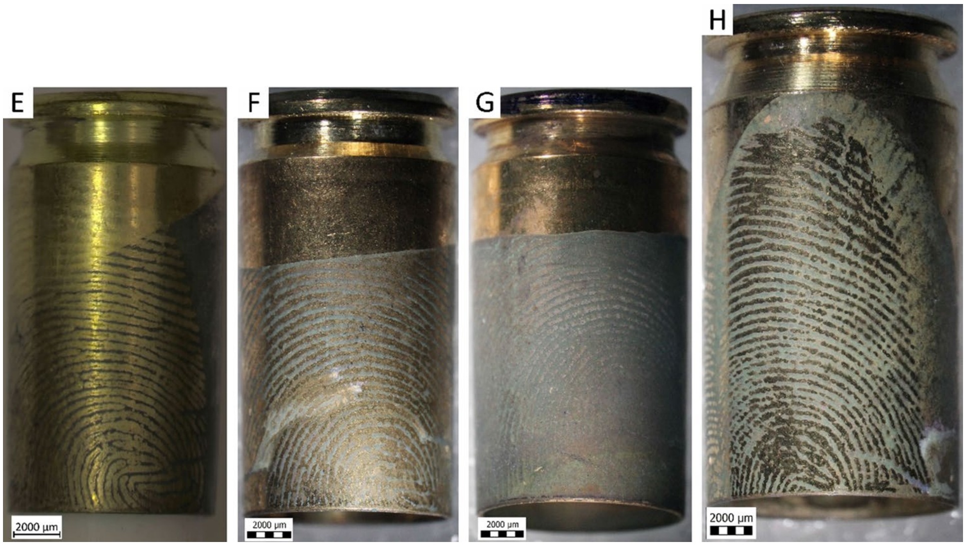
Fig. 3 Chronoamperogram of 5 mM toluidine blue 0.1 M KNO_3 on cartridges with a fresh fingerprint at a potential of 0.50 V (vs. Ag/AgCl) for 120 s; **a** representative deposition process (cartridge H) and **b** comparison of the deposition process on cartridges E–G (9 mm) and H (.45 AUTO)

presented by Costa et al. [7]. During the electrolytic process, the brass is partially dissolved, and at the same time, the PTB polymer layer is deposited on the brass surface. The dissolution of the brass surface, however, does not occur throughout the deposition but only at the beginning, causing stronger anchoring of the polymer film on the surface.

To avoid over-layering of the fingerprint by the polymer layer, as observed by Costa et al. [7] at the potentiostatic deposition of PEDOT, the thickness of the deposited polymer layer should not be significantly greater than the thickness of the fingerprint grease. The thickness of the fingerprint ranges from 0.14 to 2.0 µm [10] and is influenced by the amount of the matrix that forms it. It depends on what makes up it is matrix, whether it is eccrine, sebaceous sweat or a combination of them, these parameters being individual for each individual person [2, 10, 11]. The thickness of the resulting film was regulated either by varying the potential within a certain interval (for the CV experiments) or by applying a constant potential and varying the deposition time of the film.

Furthermore, a greasy fingerprint has been successfully visualized with PTB on the cartridges multiple times (Fig. 4). It can be seen from the images that the resulting film is very fine, the thickness of the film is in the range of several tens of micrometers, yet the characteristic ridges of the fingerprint can be found. It is evident that PTB compared with PNR, is more homogeneously applied and only in the space between the papillary lines and not on the fingerprint. The print is visualized in very good quality and can be used for possible identification.

Fig. 4 Brass cartridge cases E, F, G (9 mm), and H (.45 AUTO) with a visualized latent fingerprint by PTB. Cases E was photographed by stereoscopic microscope Zeiss and F–H with a Nikon



Comparison of polyphenazine dyes deposition

Part of this study was devoted to a comparison of the quality of the created PNR and PTB polymer films. Although NR and toluidine blue (TB) differ in their structure in only one atom (N versus S), the behavior of these substances during deposition is vastly different, and it is necessary to take this into account.

Age and stability of the monomer solutions before deposition of polyphenazine dyes

First, we examined the usability of the NR and TB monomer solutions. To ensure the stability of the monomer solution, it is necessary to keep the solution in the refrigerator before each use and re-homogenize it in an ultrasonic bath to ensure the perfect dissolution of any monomer residues.

We observed that the age of the NR monomer solution can have a positive effect on the quality of the visualized fingerprints. The freshly prepared solution (in the day of the experiment) tended to form an inhomogeneous polymer film with larger clusters of particles (Fig. 5a). On the other hand, storing the solution for a longer period of time in the refrigerator enabled the creation of a more homogeneous and better quality polymer film. These findings suggest that the time variability and reuse of the supporting electrolyte also play a key role in the resulting quality of the electrochemical fingerprint visualization. On the contrary, the age of the TB monomer solution does not have such a visible effect as in the case of NR. The freshly prepared solution produced the same homogeneous PTB forms as the older solution (Fig. 5b).

The experiments revealed that the NR solution can be effectively used for electrochemical polymerization up to several times (maximum five times) compared to TB (three times). With further repeated use, the thickness of the polymer layer gradually decreased, and this resulted in a quality deterioration of fingerprint visualization.

During PTB deposition, turbidity was observed in the monomer solution, indicating excessive dissolution of the brass cartridges. On the contrary, no such turbidity was observed in the solution after the application of PNR.

The choice of supporting electrolyte for PNR and PTB the deposition was different. In the case of PNR, a phosphate buffer with 0.1 M KNO_3 was used. The pH of the solution at the end of the PNR deposition remained the same as that before polymerization. On the other hand, the supporting electrolyte for PTB polymerization consisted of only 0.1 M KNO_3 and produced a blue gelatinous compound at the bottom of the electrochemical cell after PTB deposition. The pH value of this supporting electrolyte was measured before and after the application of PTB and was 5.81 and 9.33, respectively. Thus, the electrochemical experiment increases the pH of the non-buffered solution. It is related to the formation of $Cu(OH)_2$, as described in experiments with PEDOT [7].

Second-level details of the fingerprint on cartridge cases after the application of polyphenazine films

When optimizing the electrochemical deposition, it was necessary to "soften" the polymerization conditions to eliminate brass dissolution to a minimum by choosing a narrower potential range for CV or setting lower potential values for CA. The resulting polymer film was more homogeneous and brighter in color, as shown in Fig. 6. In the visualized fingerprint on the cartridges, the second-level details found (1–26) are marked with numbers, name of the details are mentioned in Table 1. Some of them can be observed with the naked eye, for example, terminations, bifurcation, and eyelets. The assignment of numbers to the individual details was made using the DactyScope Pro digital dactyloscopic comparison

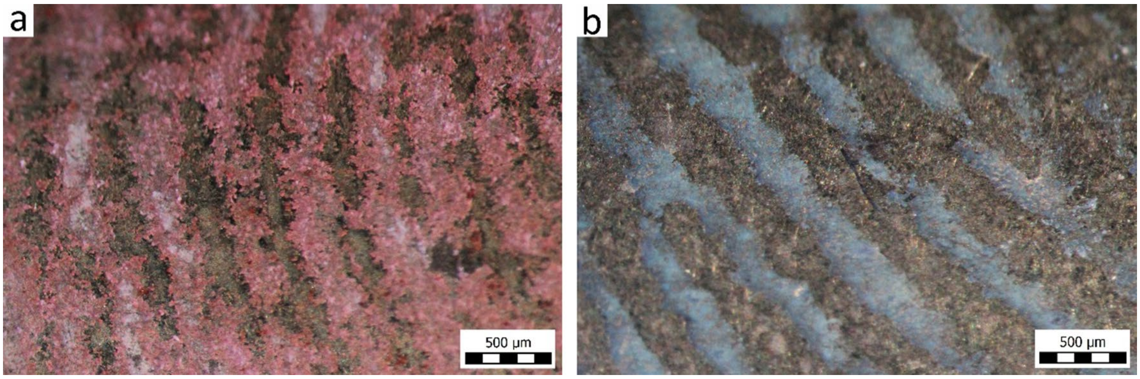


Fig. 5 Homogeneity of deposited polyphenazine layers on brass cartridges with fingerprint; **a** PNR prepared from 15 days old solution and **b** PTB prepared from fresh solution (images taken using a Nikon stereoscopic microscope)

device and the LUCIA Forensic program. The dactyloscopy workplaces at expert workplaces of the Police of the Czech Republic are equipped with this device and software.

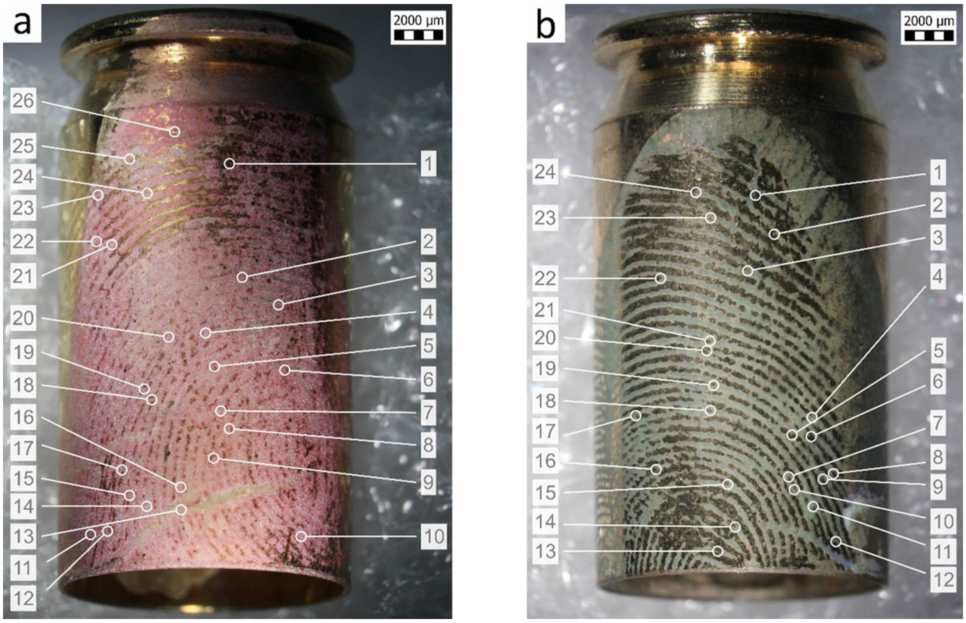


Fig. 6 Unfired brass cartridges after deposition of **a** PNR on cartridge D and **b** PTB on cartridge H with a visualized grease fingerprint and associated second-level details (images taken with a Nikon stereoscopic microscope)

Table 1 Identification of second-level details [2] of the fingerprint (in Fig. 6)

Second-level details of the fingerprint		PNR (Fig. 6a)	PTB (Fig. 6b)
Ridge ending		1, 2, 3, 4, 8, 9, 10, 11, 12, 13, 17, 18, 19, 20, 22, 25	2, 3, 4, 8, 9, 11, 13, 14, 15, 16, 18, 19, 21, 22, 23, 24
Bifurcation		5, 6, 7, 14, 15, 16, 21, 23, 24, 26	1, 5, 6, 12, 17, 20
Island (short ridge)			7, 10

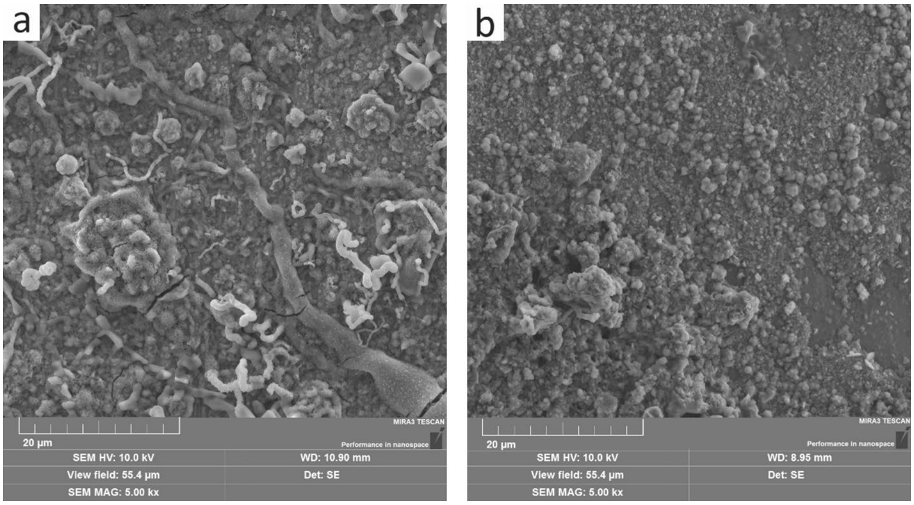


Fig. 7 Scanning electron microscopy images of the morphology of polyphenazine films taken in the mode of secondary electrons of **a** PNR and **b** PTB

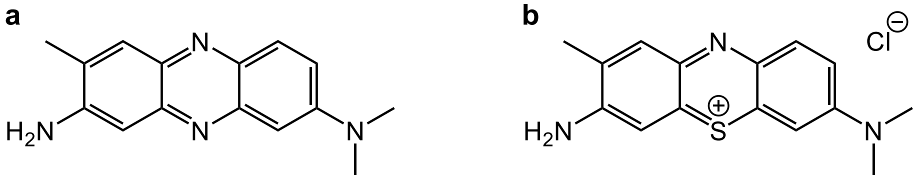


Fig. 8 Structures of the monomers: **a** neutral red and **b** toluidine blue

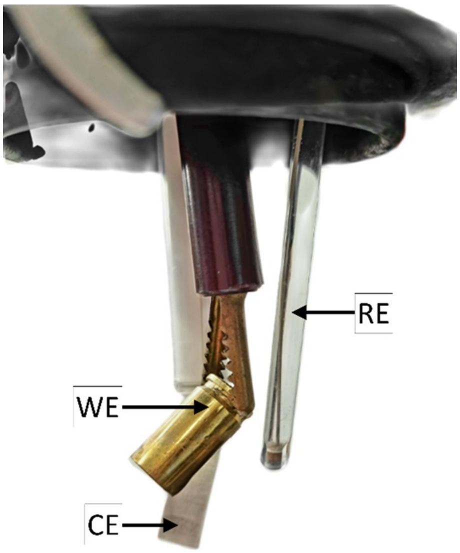


Fig. 9 Electrode arrangement: working electrode (WE) – 9 mm cartridge, counter electrode (CE)—large-area Pt electrode, reference electrode (RE)—saturated Ag/AgCl

Morphology of deposited polyphenazine films

The morphology of the polymer films, fingerprint and interface were determined using SEM. It is clear from Fig. 7 that PNR forms fibers a diameter of about several units of micrometers (the fibers reached lengths of over 82 µm), while PTB forms rather shorter oval/granular structures (the size of the granules was around 12 µm). Both polymer films cover the brass surface without any defects.

From CV and SEM, it was found that the size of the cartridges (used 9 mm and AUTO.45) does not affect neither the quality of visualization of the fingerprint nor the homogeneity of the films.

Conclusion

This proposal of a simple fingerprint visualization method based on the electrochemical deposition of poly(neutral red) and poly(toluidine blue) from a neutral environment, where the damage to the genetic information is minimized, could facilitate the fingerprinting of cartridges in practice. The parameters of both visualization methods (base electrolyte, potential range, number of cycles or applied potential, and deposition time) were gradually optimized until the imprint was sufficiently visible.

During experiments after optimizing the deposition conditions of polyphenazine dyes using both CV and CA, considerable reproducibility was identified in the sebaceous fingerprint visualization process. Repeatable high-quality visualization was achieved even when the cartridge was replaced with a larger cartridge.

The morphology and structure of the modified surfaces were studied and it has been proved that the quality of visualization depends on the homogeneity of the polymer layer. In summary, it can be stated that the polymer film prepared from the toluidine blue solution was more homogeneous compared to the thin layer from neutral red. This depends on the nature of the monomer solution or the deposition technique and it will be the subject of further investigation.

Materials and methods

All commercial chemicals were used as received without further purification. NR, TB, whose structures are depicted in Fig. 8 H_2SO_4, KH_2PO_4, $Na_2HPO_4 \cdot 12H_2O$, KNO_3, acetone and ethanol were obtained from Lachema, Lachner,

Table 2 Conditions for the deposition of polyphenazine dyes on brass cartridges with applied fingerprints

Monomer	NR	TB
Deposition technique	CV	CA
Applied polymer	PNR	PTB
Parameters	Potential Range = −0.2–0.5 V	Applied potential = 0.5 V
	Scan rate = 50 mV s^{-1}	Time interval = 10 ms
	Number of cycles = 8	Time of applied potential = 120 s

and Penta (Czech Republic), respectively. Phosphate buffer (pH = 7, UCT Prague) consisted of KH_2PO_4 (3.39 g/dm^3) and $Na_2HPO_4 \cdot 12H_2O$ (8.95 g/dm^3). Solutions of monomer dyes were prepared i) in pH = 7 phosphate buffer with 0.1 M KNO_3 for 2 mM NR and ii) in 0.1 M KNO_3 for 5 mM TB. All solutions were prepared by dissolving substances in redistilled water (UCT Prague).

The visualization of sebaceous fingerprints was conducted on unfired caliber cartridges: 9 mm Luger and 0.45 AUTO. The Criminalistics Institute Prague, Police of the Czech Republic, provided disassembled cartridges for the experiments (the unfired cartridge was deprived of the projectile, the powder charge was removed, and the primer remained intact and was left in the cartridge). Dimensions of cartridges used: 9 mm Luger also from Sellier & Bellot (standard cartridge length without a bullet and its diameter is: 19.15 mm and 9.01 mm) and 0.45 AUTO from the same company (standard cartridge length without a bullet and its diameter is: 23.72 mm and 11.43 mm).

The cartridges were first chemically cleaned by soaking them in a sequence of solutions: redistilled water, acetone, warm soapy water, and ethanol. Subsequently, a greasy fingerprint was applied to the dry substrate by i) washing the hands with warm soapy water, ii) allowing them to dry freely, iii) rubbing the right thumb against the bridge of the nose and forehead due to the formation of a greasy film, and iv) with a slight pressure for period of 1–2 s fingerprint was applied, according to the Beresford procedure [17]. An anonymous donor imprint was applied to all substrates. All visualized fingerprints were published with the consent of the donor.

Electrochemical measurements were performed with a PGSTAT-12 Autolab potentiostat/galvanostat (Eco-Chemie, The Netherlands). The electrode cell was composed of a saturated Ag/AgCl reference electrode, a Pt large-area electrode as an auxiliary electrode, and brass cartridges as working electrodes, which were clamped with alligator clips (Fig. 9).

The deposition of polyphenazine dyes was carried out under the conditions listed in Table 2, which were gradually optimized for the cartridges. After finishing the polymerization, the substrates were rinsed first in monomer-free base electrolyte and subsequently in redistilled water and allowed to air dry under laboratory conditions. Regular maintenance of the electrode system (cleaning) is required after every second to third use with sulfuric acid as the supporting electrolyte and cyclic voltammetry to guarantee the reproducibility and quality of the results.

The visualized fingerprints were photographically documented using a Stemi 508 trino stereoscopic microscope equipped with an AxioCam 208 color camera (Zeiss, Germany), an SMZ1500 stereoscopic microscope complemented by a Canon 1100D digital SLR camera (Nikon, Japan) and a Mira 3 LMH scanning electron microscope (SEM) (Tescan, Czech Republic).

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