THE INFLUENCE OF TIME ON OXIDATION PROCESS OF FLUORESCEIN-CHITOSAN POWDER CONJUGATES USED TO DEVELOP LATENT FINGERPRINTS ON DIFFERENT SUBSTRATES

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Purpose

Detection and visualization of latent fingerprints is one of the main tasks for law enforcement agencies in order to identify a perpetrator of a criminal act. Fingerprints can often be found at the crime scene, when fingertips interact with objects and leave patterns specific for each individual (Mozayani & Noziglia, 2006; Daluz, 2019). Chemical and physical methods have been used for years to visualize fingerprints on different porous, semi-porous and non-porous substrates (Milašinović & Koturević, 2016; Daluz, 2019). Many commercial products are providing good results in everyday (forensic) application, while the choice of method depends on type, color and appearance of substrate, as well as the environmental conditions (Champod, Lennard, Margot, & Stoilovic, 2004; Mozayani & Noziglia, 2006; Bumbrah, Sharma, & Jasuja, 2016). Visualization of latent fingerprints on conventional surfaces is usually routine and easy, but many unconventional substrates such as firearms, skin, multicolored surfaces, etc. cause problem due to inadequate contrast between the papillary lines and background (Färber, Seul, Weisser, & Bohnert, 2010; Amata, Aprea, Chiuri, & Zampa, 2015; Barros & Stefani, 2019). Therefore, fluorescent formulations have been developed to overcome the lack of contrast with high fluorescence under alternative light sources (e. g. UV light) (Wang, et al., 2017; Chávez, Garcia, Oliva, & Diaz-Torres, 2021). Commercially available yellow and green fluorescent powders fluoresce when excited with light from UV up to blue (450 nm), while red, orange and pink fluorescent powders allow for a wider range of light, from UV up to green (530 nm) (BVDA – Fluorescent fingerprint powders, n.d.). Research groups are constantly trying to improve existing systems and to obtain even higher sensitivity and better contrast. A large number of powder substances contain natural or synthetic organic derivatives that exhibit fluorescence or phosphorescence when exposed to UV or laser light. Some of the organic substances that are used for years are acridine yellow and orange, coumarin 6, genipin, crystal violet, Nile blue, rhodamine B and 6G, etc. (Levinton-Shamuilov, Cohen, Azoury, Chaikovsky, & Almog, 2005; Araya-Hermosilla, et al., 2014; Vadivel, Nirmala, & Anbukumaran, 2021). Kumar et al. (2020) have prepared a novel aggregation-induced emission (AIE) based electron withdrawing moiety such as 2-(4-fluorophenyl)-3H-benzocoumarin, called Coumarin Fluorescent Tag (CFT). The investigation of latent fingerprints using mentioned system (in both solid and liquid state) showed immutable and consistent patterns due to the strong adsorption of CFT on

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the ridges of the fingerprint under visible and 365 nm UV light. The results showed that CFT can be used for detection and visualization of minutiae points and sweat pores of latent prints developed on non-porous substrates due to high photoluminescence properties (Kumar, Udayabhanu, Mahadevan, & Nagaraju, 2020). System based on similar emission phenomenon was proposed by (Li, et al., 2020). A novel composite combining the aggregation induced emission luminogens (AIEgens) salicylaldehyde azine (SAA) with montmorillonite (MMT) was used to develop latent fingerprints. The obtained results demonstrated high resolution and high quality of enhanced prints, due to the bright yellow emission of SAA and high absorptive capacity of MMT. Additionally, when compared with commercial powder, these composites showed higher match degree with control/reference fingerprint, which is a very promising result (Li, et al., 2020).

On the other hand, polymeric materials have drawn great interest from researchers due to their specific properties, particularly suitable for systems used in forensic examination of (latent) fingerprints (Milašinović N. , 2016; Chen, Kuo, Tsai, Ke, & Liao, 2016; Hejjaji, Smith, & Morris, 2017 (a); Hejjaji, Smith, & Morris, 2017 (b)). Some of our previous studies indicated great potential of chitosan- and dextran-based (bio)powders in visualizing latent prints. Fingerprints, previously kept in different storage conditions (humid and dry environment), were successfully developed on non-porous (glass) surface with prepared powders. Results showed that the prepared powders were uniform in size and shape, with adequate sensitivity and satisfying contrast in visualized fingerprint image (Vučković, Glođović, Radovanović, Janaćković, & Milašinović, 2020; Vučković, Dimitrijević, & Milašinović, 2020).

In this paper, chitosan-based powder systems conjugated with fluorescein and crosslinked with sodium-tripolyphosphate were obtained by simple ionotropic gelation process. Chitosan (Ch) was used due to its specific polycationic properties and its ability to bind to the fingerprint lipid residues by both electrostatic and lipophilic interactions. In solutions where the pH is below chitosan's pKa value, chitosan amino groups become protonated and could potentially interact with anions/polyanions, such as Na-TPP, but also with lipid residues from sweat (Hejjaji, Smith, & Morris, 2017 (b); Vučković, Glođović, Radovanović, Janaćković, & Milašinović, 2020). Additional conjugation with fluorescein was used to improve the sensitivity and contrast by fluorescence assisted by UV light. Prepared powders were used to visualize latent fingerprints deposited onto non-porous (glass), semi-porous (varnish paper) and porous (wood) surfaces, to enhance the quality of developed fingerprints by fluorescence assisted by UV light. The effect of time on oxidation of fluorescein in the conjugates used to develop traces was also investigated in order to determine whether the color change appears, as well as whether it affects the quality of developed fingerprints over time. The results showed good fluorescence under UV light and satisfying development of latent prints under visible light, indicating that prepared bio-based powder systems could complement some of the routinely used (fluorescent) systems, especially due to relatively low price and non-toxic properties.

Design/Methods/Approach

Materials

Medium molecular weight chitosan and itaconic acid (IA) were purchased from Sigma-Aldrich (USA), Na-TPP from Acros Organics (USA), and fluorescein was prepared according to procedure described by (Mohan, 2003). Distilled water was used for the preparation of all solutions. All materials were used without further treatment or purification.

Preparation of Fluorescein-Chitosan Powder Conjugates

The conjugates were prepared using different (w/w) amounts of chitosan and tripolyphosphate, 6/1 and 1/1 (respectively), because these conjugates demonstrated the best properties in our previous research (Vučković, Glođović, Radovanović, Janaćković, & Milašinović, 2020). Briefly, 0.2000 g of Ch, 0.1500 g of IA and 0.002 g of FL were dissolved in 100 ml of distilled water, and 0.0840 g of Na-TPP and 0.1500 g of IA were dissolved in 100 ml of distilled water, for one hour, at room temperature and low speed (~ 400 rpm). Afterward, the Ch-FL solution was added dropwise to the Na-TPP solution in ratios 6/1 and 1/1 (Ch-FL/Na-TPP), and then mixed for additional one hour, at the same conditions as already stated above. The particles were spontaneously formed due to ionotropic gelation of Ch-FL by Na-TPP. Afterward, mixtures were left at room temperature until complete solvent evaporation. The final conjugates were ground with pestle and mortar to fine powders and kept in desiccator until further use.

Characterization of the Fluorescein-Chitosan Powder Conjugates

FT-IR Analyses

Prepared fluorescein-chitosan powder conjugates were recorded in dry and solid state, using the *Bomem MB* 100 FT-IR spectrophotometer. Powders in amount of 1.5 mg were mixed and ground with 75 mg of potassium bromide and then compressed into pallets at pressure of 11 t for about a minute, using the *Graseby Specac* model: 15.011. The spectra were obtained in the wavenumber range between 4000 to 400 cm^{-1}, at 25 °C and at 4 cm^{-1} resolution spectra.

Optical Microscopy

The obtained (bio)powder conjugates were recorded with optical microscope *Leica FS C Comparison Macroscope*, equipped with the *Leica IM Matrox Meteor II Driver Software Module*. Powders were recorded in dry state, with and without backlighting. Prior to imaging under the microscope, latent fingerprints left on microscope glass slides were developed using prepared powder conjugates.

Development of Latent Fingerprints

In order to determine the performances of fluorescein-chitosan powder conjugates, one male donor, using only a right-hand thumb, deposited sebaceous/oily fingerprints onto non-porous, semi-porous and porous surfaces, i.e. glass, varnish paper and wood, respectively. The prints were then kept under laboratory (humid) conditions for a couple of minutes. That period allowed the traces to dry and reduce the residues, until the latent fingerprints were developed with synthetized powder conjugates 6/1 and 1/1, using BVDA Squirrel hair brush (BVDA, The Netherlands) (International Fingerprint Research Group, 2014).

Optical microscopy was additionally used in order to confirm binding of the fluorescein-chitosan powder conjugates and visualization of latent fingerprints on glass surface, that initially showed the best results. Therefore, sebaceous fingerprints were randomly deposited onto the glass microscopic slides (properly labeled) and left for a few minutes. After this period, fingerprints were visualized with

prepared 6/1 and 1/1 conjugates and recorded under the optical microscope to determine visualization capabilities and quality (International Fingerprint Research Group, 2014).

The influence of time on oxidation of fluorescein-chitosan powder conjugates was investigated under laboratory (humid) and desiccator (dry) conditions. Fingerprints developed on glass surface with conjugates 6/1 and 1/1 were kept under above-mentioned conditions and recorded again after 20 and 30 days, to observe potential color and appearance change due to possible oxidation progression. After the period of 30 days, powders were removed from fingerprints (using lancet) and analyzed again by FT-IR to observe possible changes/shifts of the initial spectra.

Findings

Fluorescein-chitosan powder conjugates were used to visualize latent fingerprints deposited onto non-porous (glass), semi-porous (varnish paper) and porous (wood) surface. Deposited sebaceous fingerprints were developed with prepared conjugates 6/1 and 1/1, using BVDA Squirrel hair brush. Both powders were applied on multiple number of fingerprints on each substrate, to evaluate the quality and reproducibility of their application. The best results were obtained with the sebaceous fingerprints deposited onto the glass surface and developed with conjugate 6/1. The results obtained on varnish paper and wood substrate were poor, with obvious "blurring" of the fingerprints, since more fingerprint residues (and powder particles) retained in the porous structure of these substrates.

Figure 1 shows sebaceous fingerprints of one donor, developed with prepared conjugates 6/1 (Figure 1, 1)) and 1/1 (Figure 1, 2)) on glass, varnish paper and wood surface. Latent fingerprints left on varnish paper and wood surface were visualized and then lifted (with BVDA black Gellifters) to obtain adequate contrast for recording and comparison. The prints were then photographed under visible light using a 64 MP camera (aperture $f/1.8$, pixel size 0.8 μm). Fingerprints developed on glass substrate were recorded with assistance of black background surface for appropriate contrast.

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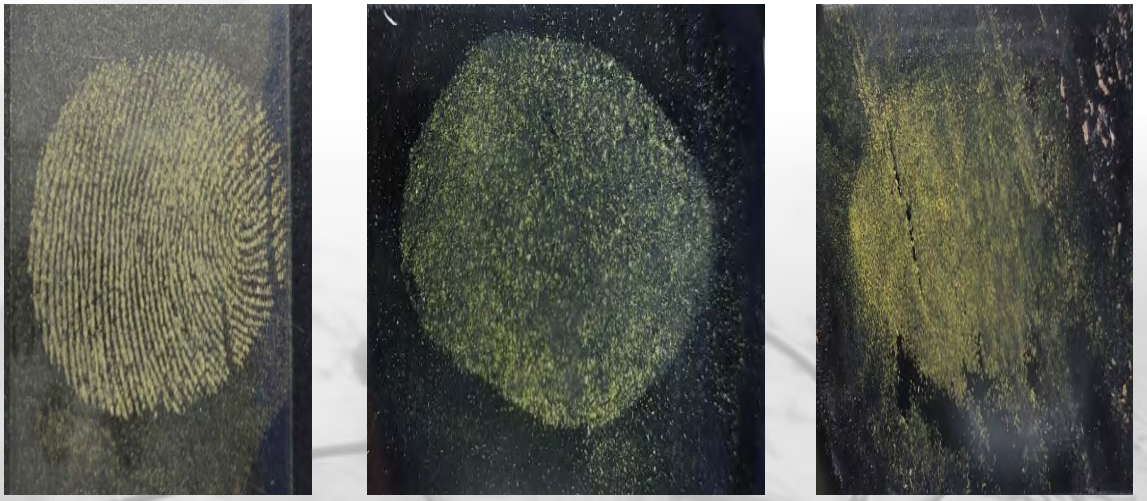

a) b) c)

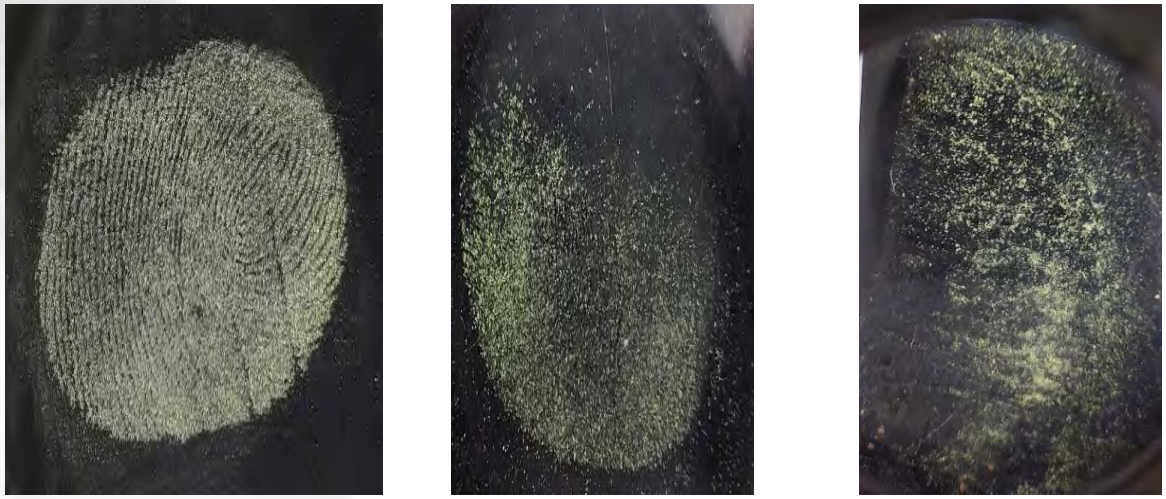

Figure 1 *Sebaceous fingerprints developed on: a) glass, b) varnish paper and c) wood surface, using prepared fluorescein-chitosan powder conjugates: 1) 6/1 and 2) 1/1, and recorded under visible light. Fingerprints developed on varnish paper and wood surface were visualized and then lifted with BVDA black Gellifters, while prints developed on glass substrate were recorded with assistance of black background surface to obtain adequate contrast for recording and comparison.*

When observing fingerprints developed on different substrates, it is obvious that the best results were obtained on glass (non-porous) substrate, because smooth/flat glass surface enabled easy binding of the powder particles to the latent fingerprint residues, making visible fingerprint basic pattern and minutiae points, i.e. fingerprint level I and II features, respectively (Figure 1, a)). On the other hand, fingerprint contour was visible on the prints developed on varnish paper, but without specific fingerprint characteristics, while prints developed on wood surface did not contain adequate contrast for analysis. Therefore, fingerprints visualized on semi-porous and porous substrate were transferred to black Gellifters with aim to provide better contrast, but the results were poor in this case, as well (Figure 1, b) and c)). This may be associated with porosity of paper and wood surface, where the majority of fingerprint residues is reduced, thus disabling binding of the powder and visualization of complete fingerprint image. Furthermore, when comparing fingerprints developed with different conjugates, results indicated that conjugate 6/1 enables better detection and enhancement of latent fingerprints. Powder conjugate 6/1 developed the prints with continuous papillary line flow, enabling good contrast and visualization of fingerprint level I and II features (Figure 1, 1), a)). On the other hand, conjugate 1/1 showed good results, but with obvious "overpowdering" of fingerprints, and consequently the disruption of the line flow, probably due to a larger size of the particles, when compared to conjugate 6/1.

Figure 2 represents the same fingerprints as Figure 1, but recorded under UV light in longwave mode (375 nm). Contrary to recording under visible light, prints left on varnish paper and wood surface could be photographed using alternative light source – UV light (without lifting).

The results showed in Figure 2 confirmed the presumptions observed in Figure 1. The fine/smooth structure of glass surface enabled the visualization of complete fingerprint images (Figure 2, a)), while fingerprints developed on varnish paper and wood surface were of poor quality, even without basic fingerprint patterns (Figure 2, b) and c)). However, the results obtained under UV light are promising and such fingerprints could be visualized with adhesive films based on polymeric materials with fluo-

rescein. Based on these initial results, glass surface was used to thoroughly investigate the application performances, as well to evaluate the influence of time on oxidation process of fluorescein-chitosan powder conjugates.

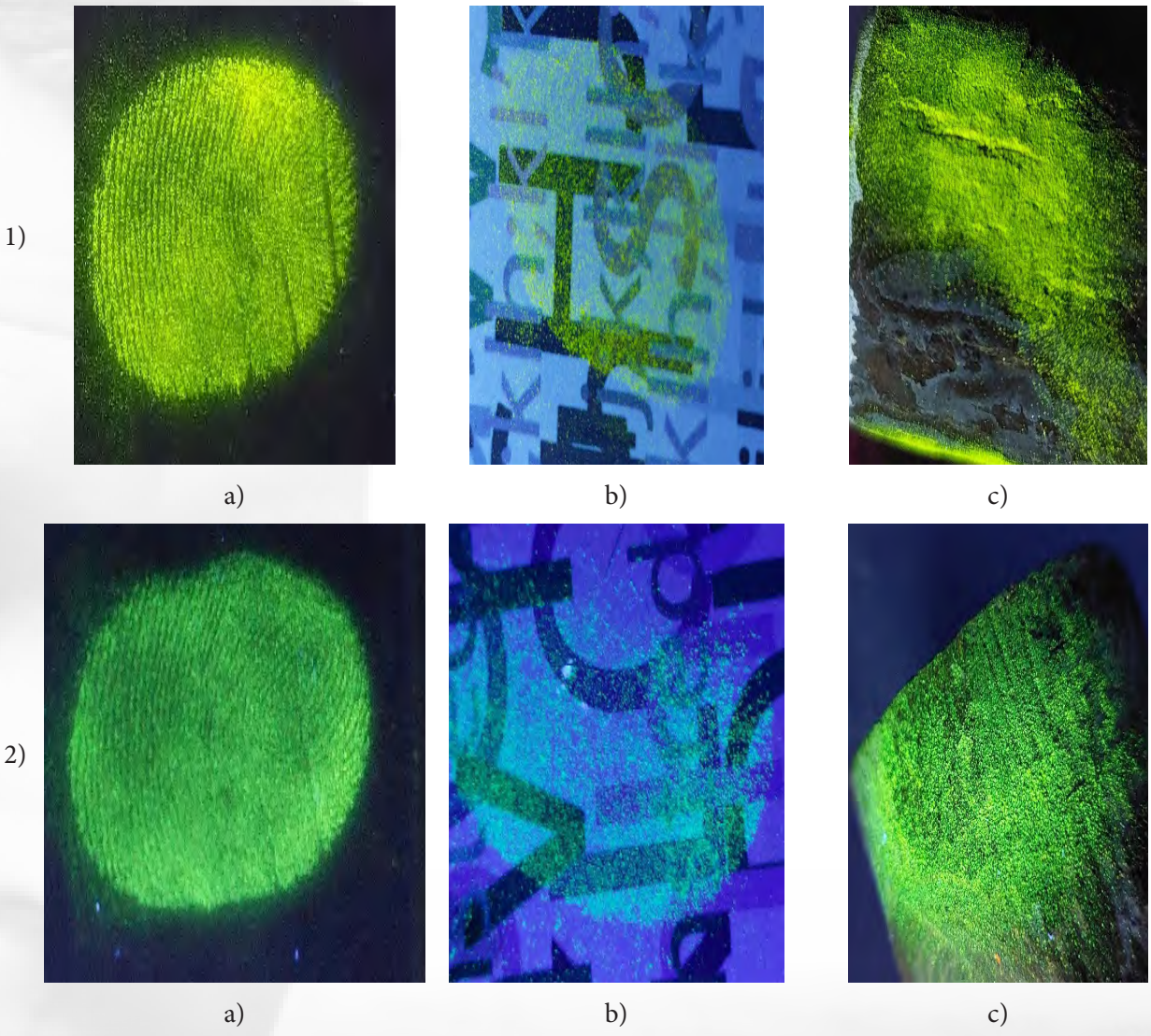

Figure 2 *Sebaceous fingerprints developed on: a) glass, b) varnish paper and c) wood surface, using prepared fluorescein-chitosan powder conjugates: 1) 6/1 and 2) 1/1, and recorded under UV light (375 nm).*

FT-IR Analyses

FT-IR analyses were performed to determine interactions between components of prepared conjugates, i.e. interactions due to ionotropic gelation process, as well to evaluate the potential impact of time on oxidation of fluorescein-chitosan conjugates. Figure 3 shows spectra of pure chitosan and fluorescein, as well as conjugates 1/1 and 6/1. In spectra of pure Ch, as well as conjugates 1/1 and 6/1, a characteristic strong and wide absorption band around 3500–3400 cm^{-1} indicates the overlapped stretching and bending of –OH and $–NH_2$ groups (Milašinović, et al., 2016). However, this band is stronger in spectrum of pure Ch when compared to spectra of prepared conjugates, probably due to

a larger number of free amino and hydroxyl groups (these groups are occupied in spectra of prepared conjugates as a consequence of ionotropic gelation and conjugation) (Vučković, Glođović, Radovanović, Janaćković, & Milašinović, 2020). The small shoulder peaks around 2920 cm^{-1} and 2870 cm^{-1} are present in the spectra of pure Ch, as well as in the spectra of both prepared conjugates, and can be related to the stretching of C–H groups (Ali, Rajendran, & Joshi, 2011). The spectrum of pure Ch (Fig. 3, a) has a characteristic bands amide bond: specifically at 1655 cm^{-1} related to the stretching of the C=O bonds within the $–CONH_2$ group (amide I band), at 1596 cm^{-1} due to the stretching of the N–H bond of $–NH_2$ (amide II band) and band at 1379 cm^{-1} corresponding to amide III band (Prlainović N. Ž., et al., 2013; Prlainović N. Ž., et al., 2016). Small shoulder peak around 1320 cm^{-1} can be associated with the deformation of O–H bonds of –CH–OH, while peak around 1070 cm^{-1} may be due to stretching of C–O bonds of Ch chains (Vučković, Glođović, Radovanović, Janaćković, & Milašinović, 2020).

Fluorescein also has some specific bands and peaks. Strong and sharp peak around 1580 cm^{-1} is related to skeletal C–C stretching containing conjugated carbonyl band, and asymmetric carboxylate stretching in xanthene ring. The peak around 1465 cm^{-1} can be associated with skeletal C–C stretching conjugated with symmetric COO^- stretching in xanthene ring (Wang, Roitberg, Meuse, & Gaigalas, 2001; Pirillo, Cornaglia, Ferreira, & Rueda, 2008). Medium intensity bands around 1385 cm^{-1} and 1310 cm^{-1} may be connected with phenoxide ion stretching conjugated with stretching in xanthene ring, and aromatic skeletal C–C stretching in xanthene ring, respectively. Two sharp bands around 1210 cm^{-1} and 1115 cm^{-1} can be related with C–O–C stretching of xanthene ring, and in plane bending of aromatic C–H bonds, respectively (Wang, Roitberg, Meuse, & Gaigalas, 2001; Pirillo, Cornaglia, Ferreira, & Rueda, 2008).

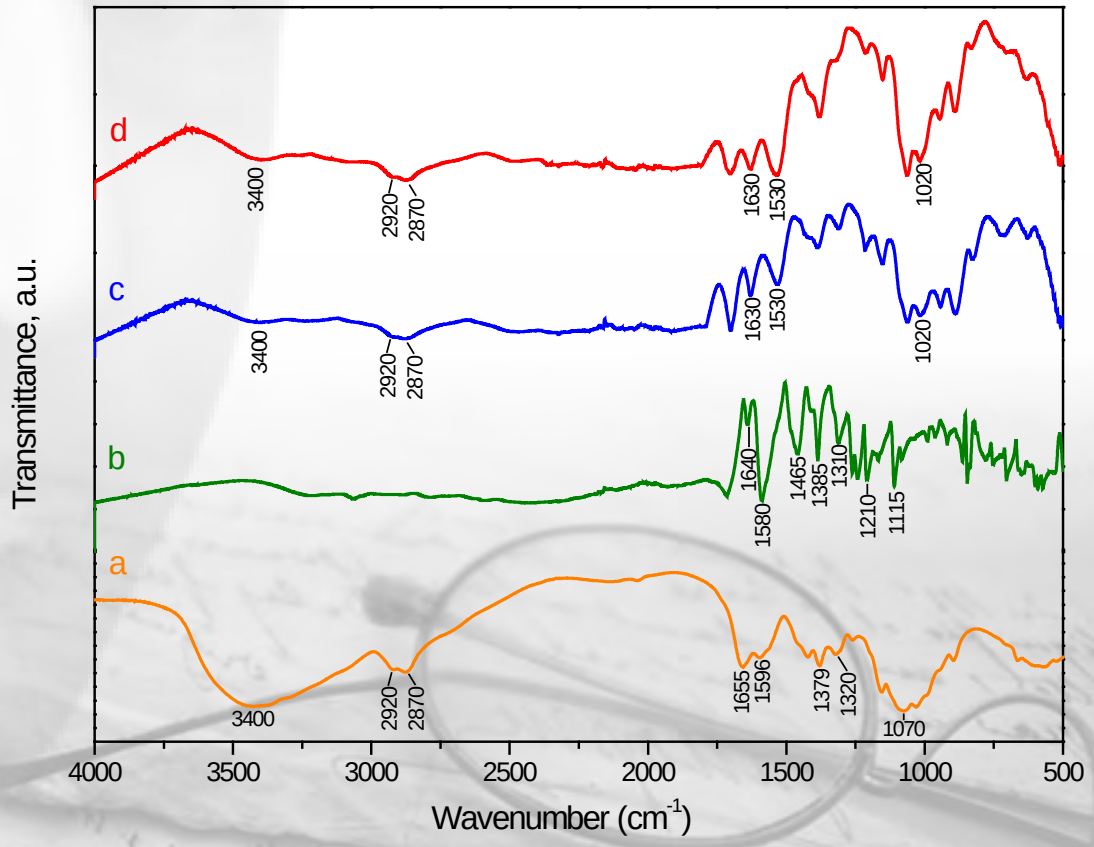

Figure 3 *FT-IR spectra of: a) pure Ch, b) pure FL, c) 1/1 and d) 6/1.*

When observing spectra of conjugates 1/1 and 6/1, some bands related to chitosan chain interactions can be spotted. The peak around 1530 cm^{-1} indicates the deformation of the N–H bond (amide band II in chitosan) (Hejjaji, Smith, & Morris, 2017 (a)). The peak at 1380 cm^{-1} could be associated with deformation of the O–H bond at –CH–OH (Wang & Liu, 2014), while the appearance of the absorption peak at 1155 cm^{-1} could be due to the swinging of NH_3^+ (Vučković, Glođović, Radovanović, Janaćković, & Milašinović, 2020).

Broad band around 1655 cm^{-1} at spectrum of pure Ch and weak band around 1640 cm^{-1} at spectrum of pure fluorescein may have resulted in the formation on new medium band around 1630 cm^{-1} at spectra of prepared conjugates, and can be associated with antisymmetric deformation vibrations of the NH_3^+ groups (Žalnėravičius, Paškevičius, Mažeika, & Jagminas, 2018). Additionally, a sharper band around 1530 cm^{-1} at spectra of prepared conjugates may be attributed to the vibration of C=O originating from amide groups, indicating the formation of fluorescein-chitosan conjugates. The band at approximately 1020 cm^{-1} is related to the bending vibrations of the pyranose ring of Ch (Saldías, et al., 2018).

FT-IR analyses were also performed with samples that were kept for 30 days in different storage conditions (humid and dry environment), in order to determine whether the humidity and/or sunlight would affect the structure of prepared conjugates. After 30 days, the powders were removed from fingerprints using scalpel, and again analyzed by FT-IR to compare potential changes/shifts in spectra. Figure 4 shows samples of prepared conjugates 1/1 and 6/1, as well as samples of these conjugates kept under different storage (humid and dry) conditions (*note*: the amount of sample 1/1 kept under dry conditions was insufficient for FT-IR analysis).

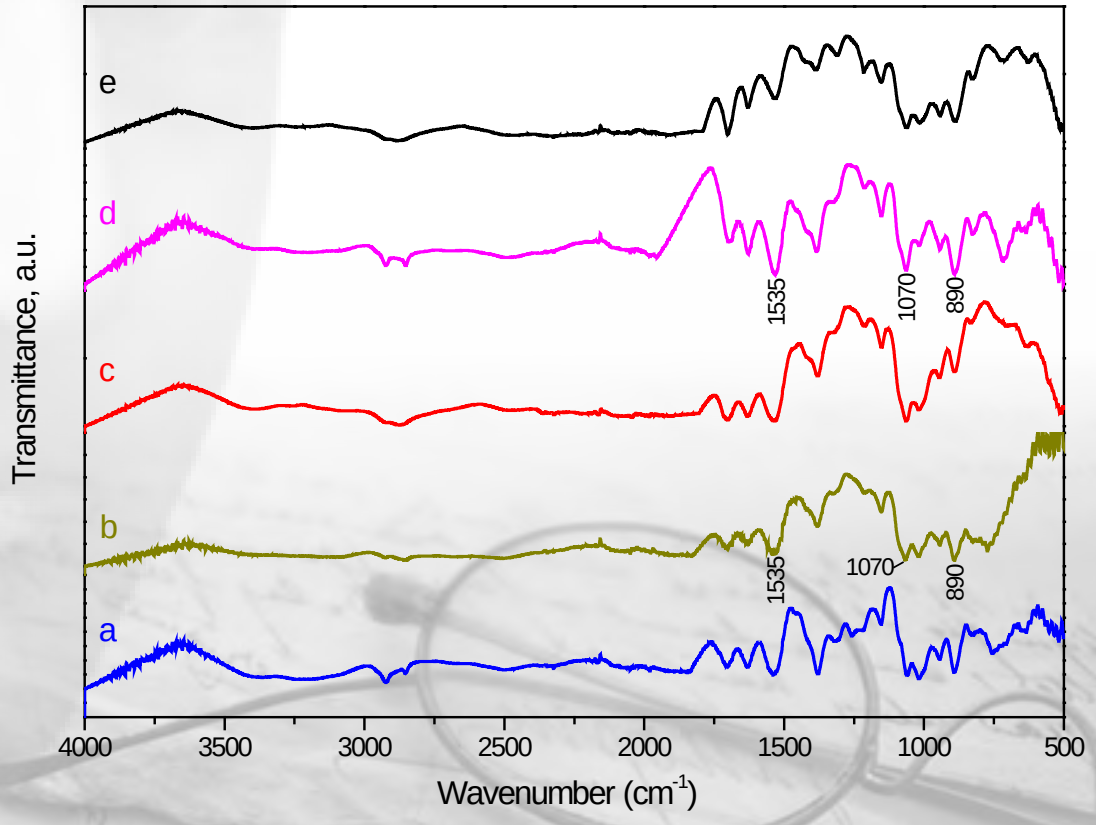

Figure 4 *FT-IR spectra of conjugates: a) 1/1 (initial), b) 1/1 (kept under humid conditions), c) 6/1 (initial), d) 6/1 (kept under humid conditions) and e) 6/1 (kept under dry conditions).*

When comparing spectra in Figure 4, the change in some peaks' intensity is noticeable between the initial spectra and the spectra of samples kept under different storage conditions. The decrease (for sample 1/1) and increase (for sample 6/1) in intensity of peaks around 1535 cm^{-1}, 1070 cm^{-1}, and 890 cm^{-1}, at spectra kept under humid conditions (Figure 4, b) and d)) when compared to initial spectra (Figure 4, a) and c)), respectively, indicate that humid environments have influenced the structure of prepared fluorescein-chitosan powder conjugates. This is often a problem when preserving powder conjugates before/after the application (SDS – LP Dual Use Fingerprint Powder, 2013). On the other hand, when observing the sample kept under dry conditions, the changes were minor (maybe due to the sunlight), so it is appropriate to protect powders from the influence of moisture and radiation, and storage them in dark, cool and dry place (SDS – LP Dual Use Fingerprint Powder, 2013).

Optical Microscopy

The Structure of Powder Conjugates

Optical microscopy was used to approximate the size and uniformity of prepared powder conjugates. Figure 5 shows the images of prepared powder conjugates, recorded with optical microscope *Leica FS C Comparison Macroscope*, equipped with the *Leica IM Matrox Meteor II Driver Software Module*, using magnification ×15, without backlighting (Figure 5, a)), with bright-field (Figure 5, b)) and dark-field contrast technique (Figure 5, c)). Different backlighting modes were used to obtain adequate contrast, to observe both amassed and thin layers of powders. Since the best results were achieved on a non-porous (glass) surface, the same was used for further research. Powder samples were deposited onto microscopic glass slides to approximate the size and compare the uniformity of their particles.

When comparing prepared powder conjugates recorded with different backlighting modes, it was determined that conjugate 6/1 had yellowish appearance, with small and uniform particles that do not stick to the glass substrate (they disperse easily), while conjugate 1/1 had greenish appearance, with also small and relatively uniform particles, but with noticeable spiking to the glass surface. This observation is related to the initial results. The "blurring" of the fingerprints developed with conjugate 1/1 was noticeable, probably due to a larger dimeter of particles and increased adhesion to the substrate.

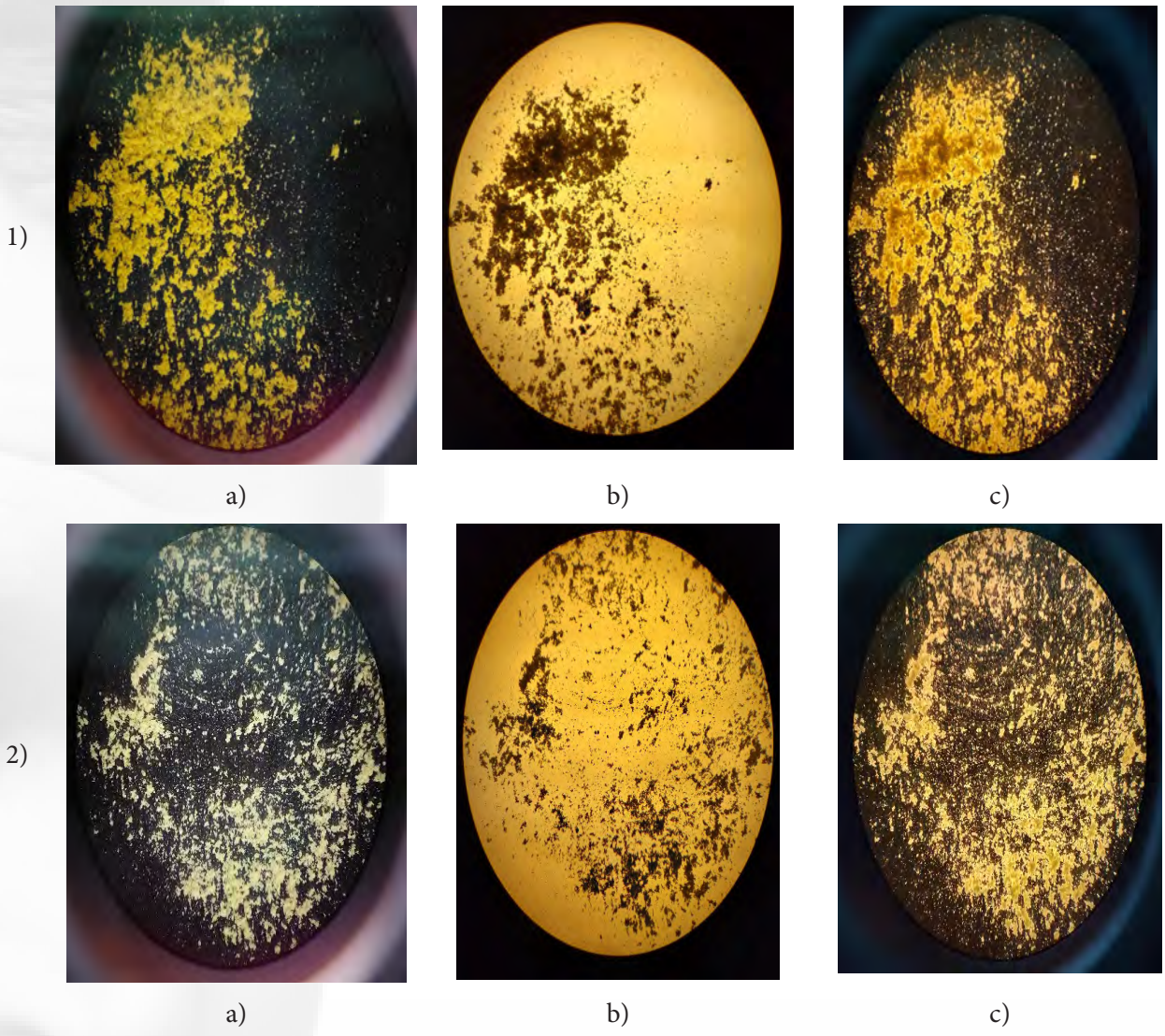

Figure 5 *Microscopic images of prepared powder conjugates: 1) 6/1 and 2) 1/1, recorded: a) without backlighting, b) with bright-field contrast technique and c) with dark-field contrast technique, using optical microscope (magnification ×15).*

Testing of Powder Conjugates

In order to confirm the initial results of application, as well as to determine whether the time could influence the fluorescein-chitosan conjugates appearance and color, prepared powder conjugates were used to visualize latent fingerprints deposited onto glass substrate. Following the Guidelines proposed by the International Fingerprint Research Group (IFRG) (International Fingerprint Research Group, 2014), sebaceous fingerprints were deposited onto microscopic glass slides using a technical scale (force applied to accommodate 100-150 g) and left for a few minutes. After this period, fingerprints were developed with prepared conjugates 1/1 and 6/1. The developed fingerprints were then photographed with optical microscope *Leica FS C Comparison Macroscope*, equipped with the *Leica IM Matrox Meteor II Driver Software Module*, without backlighting (Figure 6, a)), with bright-field (Figure 6, b)) and dark-field contrast technique (Figure 6, c)), using the magnification ×7.5. Different backlighting modes were used to obtain adequate contrast, to observe both level I and II features of fingerprints.

After visualization and recording, fingerprints developed on glass slides were kept under laboratory (humid) and desiccator (dry) environment to evaluate the time influence on oxidation of fluorescein in prepared powders. Developed fingerprints were kept under mentioned conditions, and recorded again after 20 and 30 days, in order to observe potential changes in appearance and/or color of powder conjugates. For practical reasons, only immediately recorded fingerprints (day 0) were shown in the Manuscript, while detailed results are represented in the Supplementary Material.

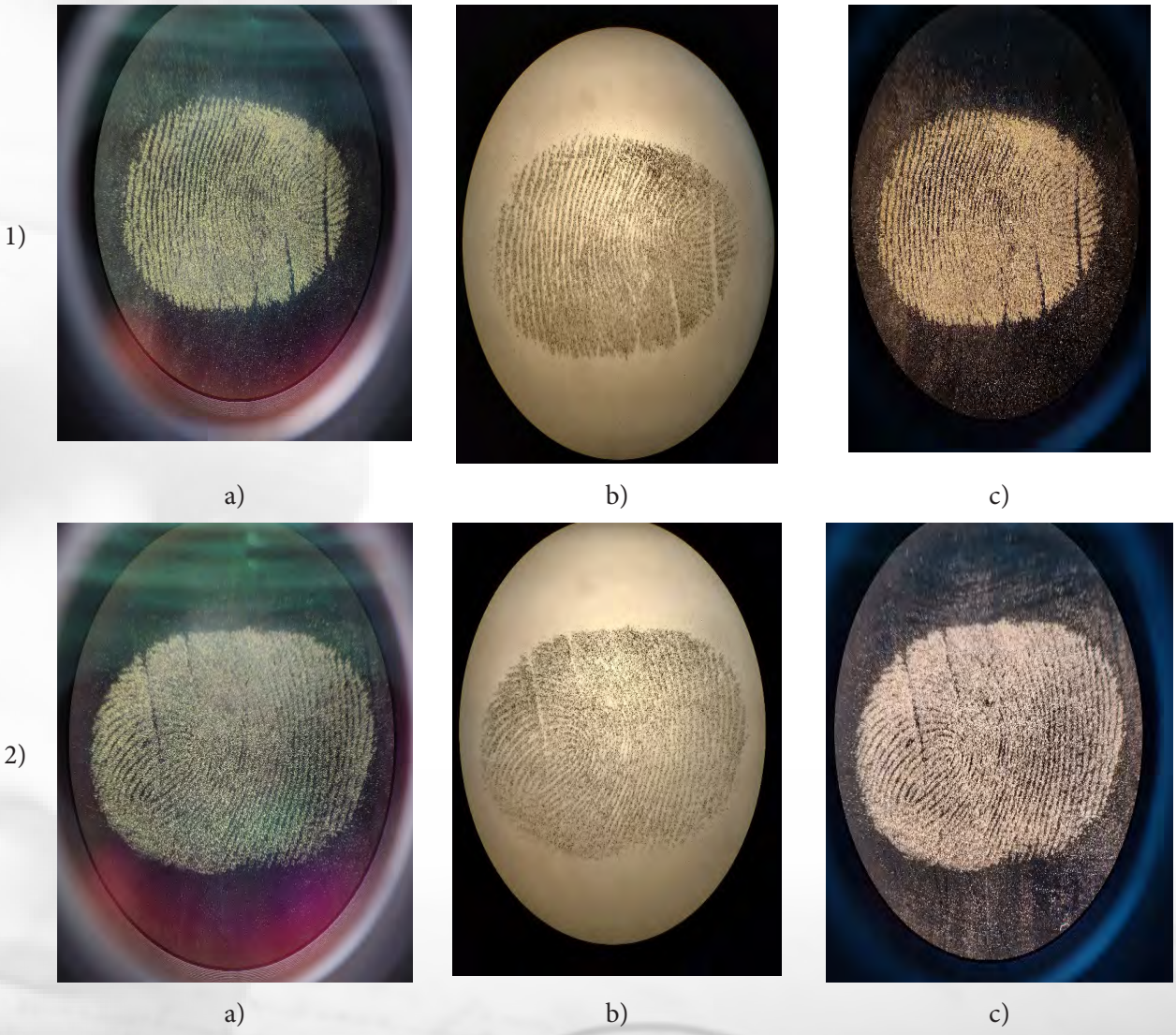

Figure 6 *Sebaceous fingerprints on glass substrate developed with prepared powder conjugates: 1) 6/1 and 2) 1/1, recorded immediately after visualization: a) without backlighting, b) with bright-field contrast technique and c) with dark-field contrast technique, using optical microscope (magnification ×7.5).*

Following the results that were obtained by initial testing on glass substrate (Figure 1, a)), the results obtained by optical microscopy confirmed those presumptions. It was determined that conjugate 6/1 enabled development of the papillary lines with their continuous flow and basic fingerprint pattern, making visible minutiae points as well (i. e. level I and II features of fingerprints). On the other hand, noticeable "overpowdering" of the interpapillary space occurred when conjugate 1/1 was applied, thus making disruptions in the flow of papillary lines (Gürbüz, Özmen Monkul, İpeksaç, Gürtekin Seden, & Erol, 2015).

Moreover, Figures S1–S6 presented in Supplementary Material document show developed (sebaceous) fingerprints stored under humid and dry environment, recorded immediately after visualization, and after 20 and 30 days, in order to evaluate the impact of time on oxidation of fluorescein present in prepared conjugates. Results indicated that moisture contributed to the change of powders' structure by creating agglomerates of the particles. Therefore, powder conjugates should be kept under dry conditions (thoroughly explained in Supplementary Material document).

Originality/Value

In this paper, two fluorescein-chitosan powder conjugates were obtained by simple ionotropic gelation process. Chitosan (Ch) was used due to its non-toxic and specific polycationic properties, while fluorescein was used due to its ability to fluoresce under UV light, with aim to increase sensitivity and quality of visualized fingerprints. Prepared powder conjugates were tested on glass, varnish paper and wood surface, and initial results showed that the best visualization of fingerprints is obtained on glass (non-porous) surface, with conjugate 6/1. These results were confirmed by optical microscopy, while FT-IR analyses demonstrated the formation of conjugates. Afterward, the influence of time on oxidation process of fluorescein-chitosan powders was investigated by optical microscopy and FT-IR. Developed fingerprints were stored under humid and dry conditions and reevaluated after 20 and 30 days. Results indicated that humid environment contributes to the change in structure of prepared conjugates, particularly noticeable in FT-IR spectra. Therefore, fingerprint powders should be kept in dry, cool and dark place (protected from moisture, sunlight and high temperature). On the other hand, conjugate 6/1 showed better performances than conjugate 1/1, enabling continuous papillary line flow and developing complete fingerprint images, with level I and II features (which are necessary for reliable identification of individual). Additionally, prepared conjugates are cheap, non-toxic, easy applicable and persistent (during the testing period). Based on all obtained results, prepared powder conjugates could be used as a supplement for routinely used systems on non-porous surfaces. Additional research should be directed to improvement of powders for application on multicolored and other problematic substrates, with the aim to increase sensitivity, fluorescence and quality of developed fingerprints.

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Supplementary Material

The Guidelines proposed by the International Fingerprint Research Group (IFRG) were followed to evaluate the performances of prepared powder conjugates for visualization of latent fingerprints (International Fingerprint Research Group, 2014). Therefore, one male donor deposited the sebaceous right-hand thumb prints onto non-porous (glass) substrate using a technical scale (force accommodated to 100–150 g, per fingerprint), to simulate manipulation with objects under real conditions. Fingerprints were left for a couple of minutes, after which they were developed using prepared conjugates 6/1 and 1/1. Subsequently, fingerprints were recorded with *Leica FS C Comparison Macroscope*, equipped with the *Leica IM1000 Matrox Meteor II Driver Software Module*, using magnification ×7.5, without backlighting (Figures S1-S6, a)), with bright-field (Figures S1-S6, b)) and dark-field contrast technique (Figure S1-S6, c)). Different backlighting modes were used to obtain adequate contrast, to observe both level I and II features of fingerprints. Additionally, after visualization and recording fingerprints developed on glass slides were kept under laboratory (humid) and desiccator (dry) environment to evaluate the time influence on oxidation of prepared powders. Developed fingerprints were kept for 20 and 30 days, and after each period they were photographed using mentioned optical microscope, to observe potential changes in appearance and/or color of prepared powder conjugates.

Developed Sebaceous Fingerprints – Day 0 (Recorded Immediately After Development)

Sebaceous fingerprints of one male donor developed and recorded immediately after deposition, with prepared conjugates 6/1 (Figure S1, 1)) and 1/1 (Figure S1, 2)). Figure S1 shows fingerprints developed on glass (non-porous) surface, recorded without backlighting (Figure S1, a)), with bright-field (Figure S1, b)) and dark-field (Figure S1, c)) contrast technique. Afterward, these fingerprints were kept under laboratory (humid) conditions, and recorded again after 20 and 30 days.

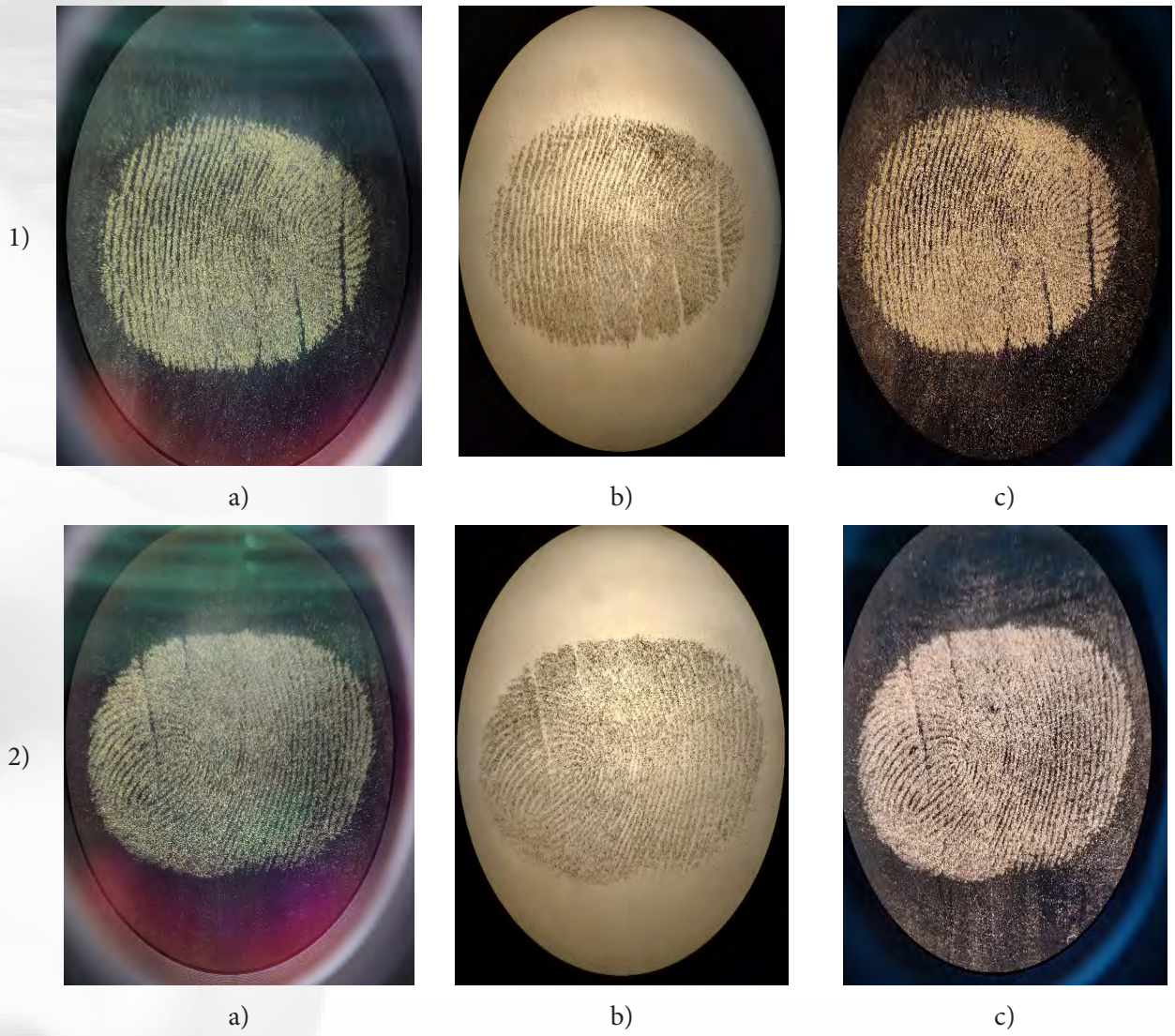

Figure S1 *Sebaceous fingerprints on glass substrate developed with prepared powder conjugates: 1) 6/1 and 2) 1/1, recorded: a) without backlighting, b) with bright-field contrast technique and c) with dark-field contrast technique, using optical microscope (magnification ×7.5).*

Developed Sebaceous Fingerprints – Day 20 (Kept Under Humid Conditions, Recorded After 20 Days)

Sebaceous fingerprints of one male donor, developed immediately after deposition with prepared conjugates 6/1 (Figure S2, 1)) and 1/1 (Figure S2, 2)), kept under humid (laboratory) conditions and recorded after 20 days. Figure S2 shows fingerprints developed on glass (non-porous) surface, recorded without backlighting (Figure S2, a)), with bright-field (Figure S2, b)) and dark-field (Figure S2, c)) contrast technique.

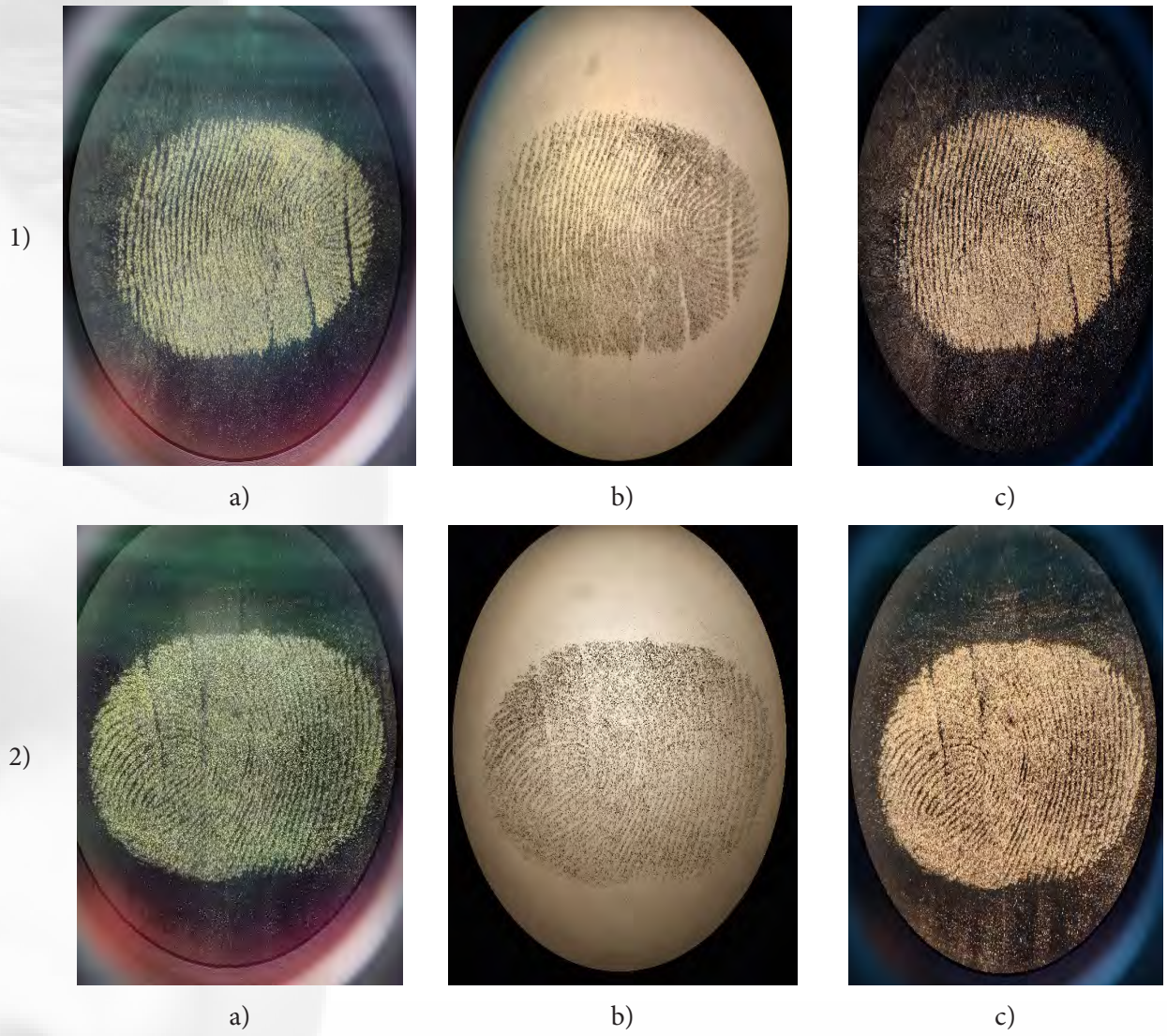

Figure S2 *Sebaceous fingerprints on glass substrate developed with prepared powder conjugates: 1) 6/1 and 2) 1/1, kept under humid conditions for 20 days and recorded: a) without backlighting, b) with bright-field contrast technique and c) with dark-field contrast technique, using optical microscope (magnification ×7.5).*

Developed Sebaceous Fingerprints – Day 30 (Kept Under Humid Conditions, Recorded After 30 Days)

Sebaceous fingerprints of one male donor, developed immediately after deposition with prepared conjugates 6/1 (Figure S3, 1)) and 1/1 (Figure S3, 2)), kept under humid (laboratory) conditions and recorded after 30 days. Figure S3 shows fingerprints developed on glass (non-porous) surface, recorded without backlighting (Figure S3, a)), with bright-field (Figure S3, b)) and dark-field (Figure S3, c)) contrast technique.

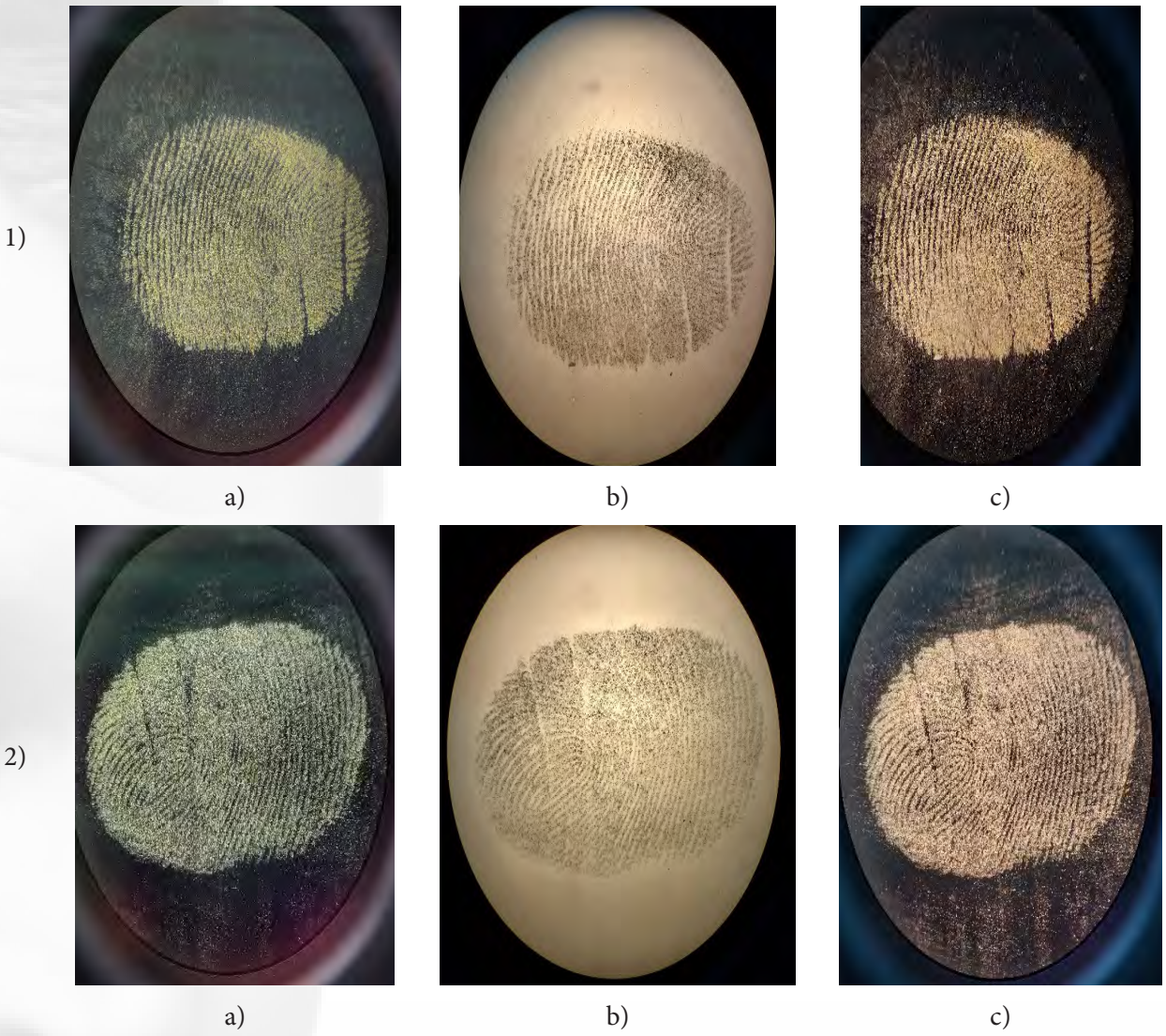

Figure S3 *Sebaceous fingerprints on glass substrate developed with prepared powder conjugates: 1) 6/1 and 2) 1/1, kept under humid conditions for 30 days and recorded: a) without backlighting, b) with bright-field contrast technique and c) with dark-field contrast technique, using optical microscope (magnification ×7.5).*

Developed Sebaceous Fingerprints – Day 0 (Recorded Immediately After Development)

Sebaceous fingerprints of one male donor developed and recorded immediately after deposition, with prepared conjugates 6/1 (Figure S4, 1)) and 1/1 (Figure S4, 2)). Figure S4 shows fingerprints developed on glass (non-porous) surface, recorded without backlighting (Figure S4, a)), with bright-field (Figure S4, b)) and dark-field (Figure S4, c)) contrast technique. Afterward, these fingerprints were kept under desiccator (dry) conditions, and recorded again after 20 and 30 days.

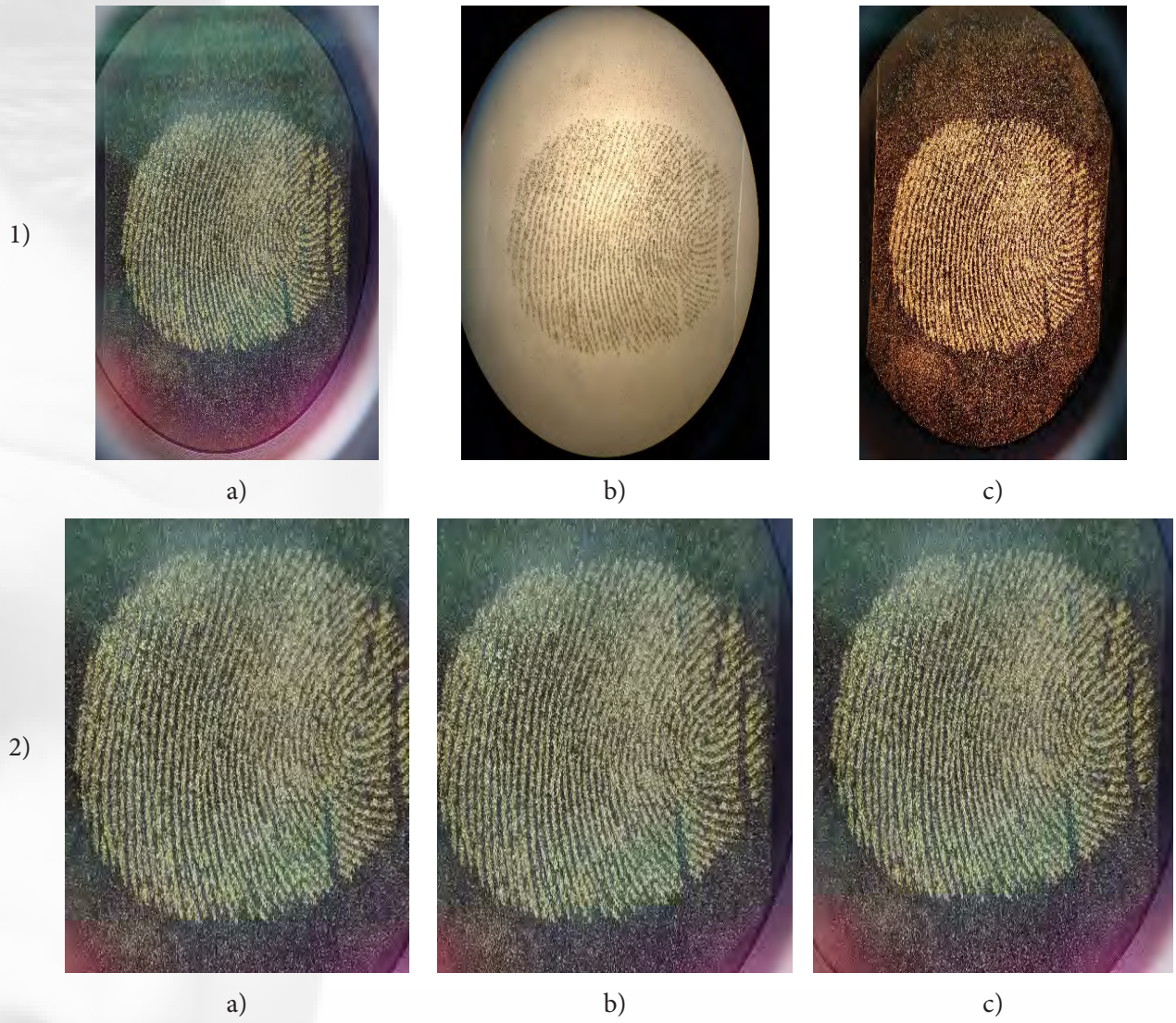

Figure S4 *Sebaceous fingerprints on glass substrate developed with prepared powder conjugates: 1) 6/1 and 2) 1/1, recorded: a) without backlighting, b) with bright-field contrast technique and c) with dark-field contrast technique, using optical microscope (magnification ×7.5).*

Developed Sebaceous Fingerprints – Day 20 (Kept Under Dry Conditions, Recorded After 20 Days)

Sebaceous fingerprints of one male donor, developed immediately after deposition with prepared conjugates 6/1 (Figure S5, 1)) and 1/1 (Figure S5, 2)), kept under dry (desiccator) conditions and recorded after 20 days. Figure S5 shows fingerprints developed on glass (non-porous) surface, recorded without backlighting (Figure S5, a)), with bright-field (Figure S5, b)) and dark-field (Figure S5, c)) contrast technique.

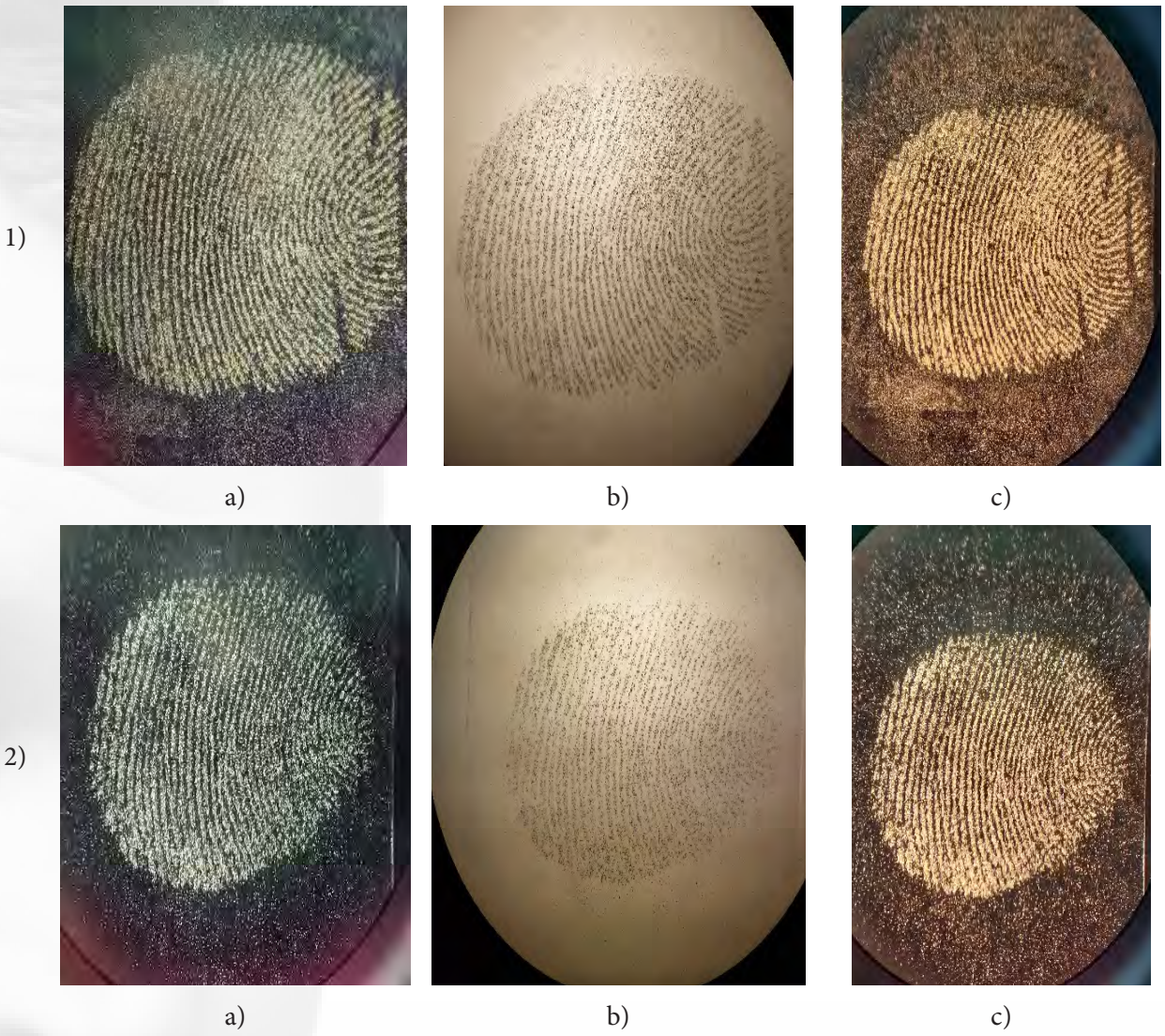

Figure S5 *Sebaceous fingerprints on glass substrate developed with prepared powder conjugates: 1) 6/1 and 2) 1/1, kept under dry conditions for 20 days and recorded: a) without backlighting, b) with bright-field contrast technique and c) with dark-field contrast technique, using optical microscope (magnification ×7.5).*

Developed Sebaceous Fingerprints – Day 30 (Kept Under Dry Conditions, Recorded After 30 Days)

Sebaceous fingerprints of one male donor, developed immediately after deposition with prepared conjugates 6/1 (Figure S6, 1)) and 1/1 (Figure S6, 2)), kept under dry (desiccator) conditions and recorded after 30 days. Figure S6 shows fingerprints developed on glass (non-porous) surface, recorded without backlighting (Figure S6, a)), with bright-field (Figure S6, b)) and dark-field (Figure S6, c)) contrast technique.

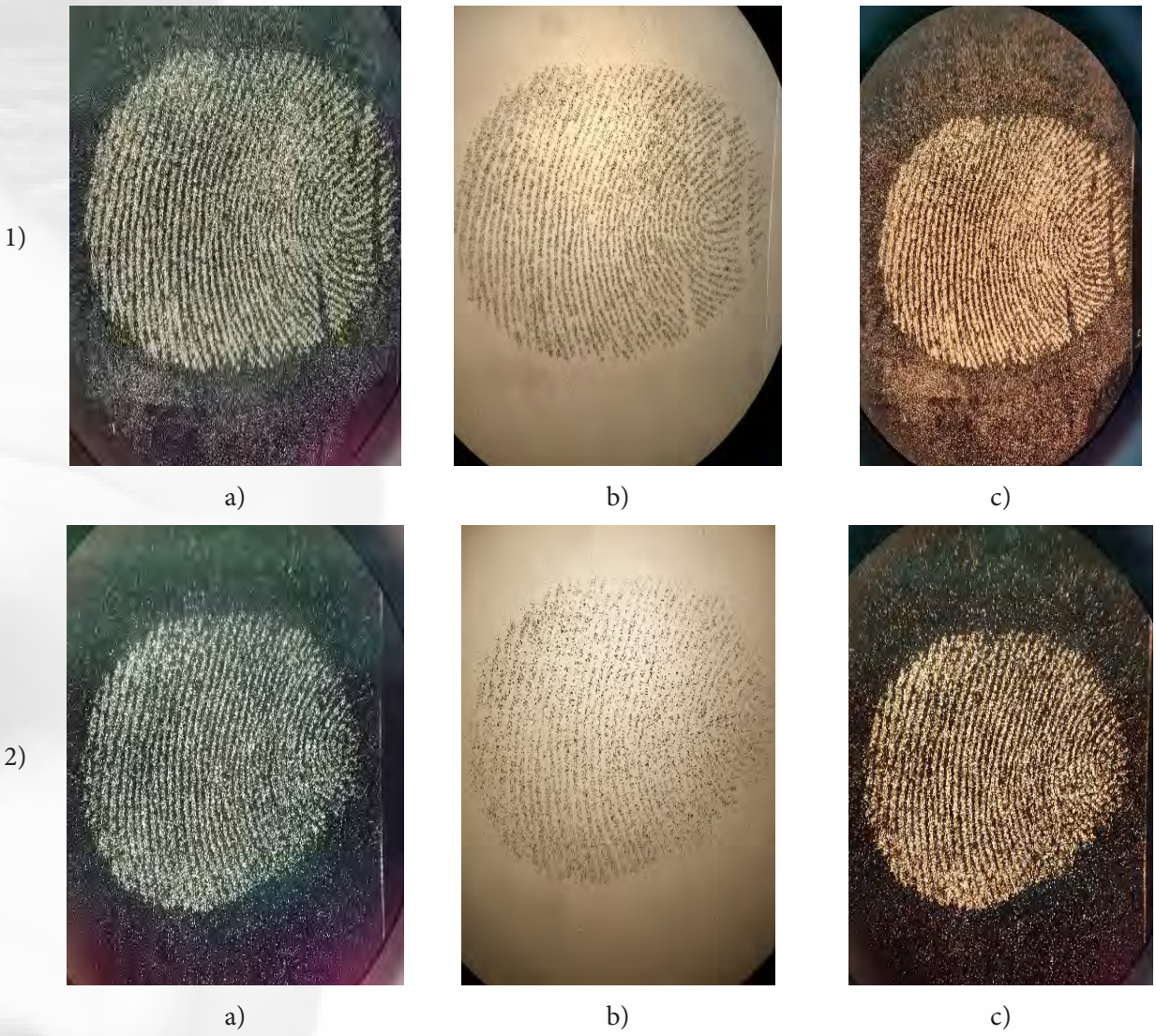

Figure S6 *Sebaceous fingerprints on glass substrate developed with prepared powder conjugates: 1) 6/1 and 2) 1/1, kept under dry conditions for 30 days and recorded: a) without backlighting, b) with bright-field contrast technique and c) with dark-field contrast technique, using optical microscope (magnification ×7.5).*

Originality/Value

According to the results from previous research (Vučković, Glođović, Radovanović, Janaćković, & Milašinović, 2020), humid environment is often contributing to better adhesion (binding) of the powders to the fingerprint residues. However, the impact of moisture on powder preservation is completely opposite. As confirmed by FT-IR analysis, developed fingerprints that were kept under humid conditions showed potential change in structure, unlike those kept under dry conditions (with only minor shifts). Moisture can affect the powders by creating agglomerates of the particles, which afterward cannot adhere well to the (latent) fingerprint residues. We assume that structure change occurred due to the moisture and sunlight for fingerprints kept under humid conditions, while sunlight only slightly affected the appearance of fingerprints kept under dry conditions. Therefore, under humid

conditions, fingerprints became "blurred" over the time, with slight disruption in the papillary line flow. Additionally, although the researchers were very careful while manipulating the samples, there is a possibility that accidental (minor) changes were made to the fingerprints, which contributed this conclusion to be drawn.

When observing fingerprints from Figure S1-S6 under UV light, there were no obvious differences in fluorescence over the period of time (from initial period to 30 days), under both storage conditions and for both prepared conjugates, so these results are not shown in this paper.

Comparison of Fingerprints and Final Conclusions

As confirmed by initial testing and optical microscopy, the best results were obtained with sebaceous fingerprints deposited onto glass (non-porous) substrate and developed with conjugate 6/1. Flat glass surface contributed to the far better binding of powders to fingerprint residues. At last, when comparing the influence of time on oxidation of fluorescein-based powders, it was determined that dry (desiccator) conditions are more suitable, so powders should be stored in dry (but also dark and cool) place (SDS – LP Dual Use Fingerprint Powder, 2013).

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