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THE APPLICATION OF CHITOSAN-BASED MICROPOWDERS IN DEVELOPMENT OF LATENT FINGERMARKS

Nikola Glođović
Nemanja Vučković, MSc

Nikola Milašinović, PhD[1]
University of Criminal Investigation and Police Studies, Belgrade, Serbia

Abstract: Latent fingermarks are common type of traces found at the crime scene. Therefore, their visualization and enhancement are widely used techniques in identification of offenders and accomplices by law enforcement agencies worldwide. Chemical and physical methods seemed to be reliable for that purpose and have now been used for years. Therefore, researchers focus their work in order to discover new methods for detection and visualization of latent fingermarks. In this regard, the usage of polymeric materials, especially biopolymers, has not yet been sufficiently tested, and scientific public is almost unaware of the potential of these materials in forensic science. In this paper, conjugates based on chitosan were obtained by the precipitating method and their application in detection and visualization of latent fingermarks was examined. The results demonstrated that this bio-based powder system could be potentially used as substitutes for commercially used powders in detecting and visualizing of latent fingermarks.

Keywords: Detection and Visualization of Latent Fingerprints, (Bio)polymers, Chitosan, Forensics

INTRODUCTION

Humans first spotted the presence of papillary lines several thousand years ago. The cave art was discovered in New Scotland and France with fingerprints and footprints dating as far as 15,000 years BC. **(Mozayani, Noziglia, 2006)**. During the rule of Hammurabi in the Babylon, fingermarks were used as cachet, that indicates their significance. Marcello Malpighi explained their occurance and classified them in 3 categories:

1 nikola.milasinovic@kpu.edu.rs

Whorl Ridge Patterns consists of almost concentric circles. Whorl patterns have been observed to most likely possess two deltas. These patterns account for 25% of all fingerprints. Loop Ridge patterns consists of ridges which will flow in one side, re-curve (loop-around), touch or pass through an imaginary line drawn from the delta to the core, and exits the pattern on the same side from which it entered. Loop pattern shape been observed to possess only one delta. These patterns account for 70% of all fingerprints. Arch Ridge Patterns consists of ridges flowing in one site and flowing out the opposite side of the print pattern. These patterns have been observed to possess no deltas. These patterns account for 5% of all fingerprints (Maltoni, Maio, Jain, Salil, 2009).

The pioneer in person identification using fingermarks was Ivan Vučetić, together with Francis Galton, in 1891. He introduced the practice of taking all ten fingerprints, and recording and classifying criminals based on the fingerprints of the left and right hand, as well as the special classification labels for each form of papillary line form, thus forming the first dactyloscopy collection. Soon, such a method, now known as dactyloscopy, has become very important for the reliable identification of persons in the daily work of the police and judiciary (Radmilović, 2008).

FINGERMARK ANATOMY

The skin is the largest human body organ that consists of three basic layers: epidermis, a protective layer consisting mainly of dead cells; dermis, an inner layer composed of living cells: sweat (eccrine and apocrine) and sebaceous glands, root of hair, collagen and elastic fibers, with blood vessels and nerves network (Choi, McDonagh, Maynard, & Roux, 2008); and hypoderm, a subcutaneous layer and consists mainly of fatty tissue (Champod, Lennard, Margot, & Stoilovic, 2004; Page, 2017; Sato, Kang, Saga, & Sato, 1989).

The papillary lines are found in the dermal layer and they increase the exchange of oxygen, nutrients and waste materials between the dermis and the epidermis. They are formed during embryonic development, and are genetically hereditary, but also depend on external factors of the environment. Their creation actually is a stochastic process, which depends on the position of the fetus at a given moment, the composition and density of the amniotic fluid, etc. (Champod, Lennard, Margot, & Stoilovic, 2004; Fingerprint, 2019; Weyermann, Roux, & Champod, 2011).

The papillary line prints today are one of the most important evidence in the forensic practice, because these fingerprints/impressions can often be found at the crime scene. The four essential properties of the papillary lines are: immutability, individuality, classification (according to basic forms) and portability (Dactyloscopy, 2019).

However, to distinguish the prints, the most significant features are minutiae points, such are ending of papillary line, dot, island, lake, bridge, bifurcation, trifurcation, etc., representing interruptions of papillary lines, contain the information necessary for reliable identification. (Champod, Lennard, Margot, & Stoilovic, 2004; Јојић, Бабић, & Ђуровић, 2014). In general, traces in the criminalistics, and thus fingerprint marks, may be visible and latent (invisible). Visible fingermark traces are divided into positive, negative and embossed (three-dimensional). These traces are easier to work with compared to latent, especially because it misses two, maybe the most difficult steps – locating and visualizing the trace. Conventional methods daily used by law enforcement worldwide can be categorized in 3 groups-Optical, Physical and Chemical methods.

Chemical methods are based on chemical reactions between chemical compound and a fingerprint trace on various porous, semiporous and non-porous surfaces. Physical methods include physical interactions, the physical binding of certain (usually powdered) substances to certain components from the latent trace, and are generally applied to non-porous and rarely semiporous surfaces (Bumbrah, Sharma, & Jasu, 2016; Champod, Lennard, Margot, Stoilovic, 2004; Knowles, 2001; Milašinović & Koturević, 2016). Depending on the physical interaction, humidity, background contrast, various powders are used in the practical applications. The powder is applied to a latent trace sample or subject using a brush (ordinary or magnetic, depending on the type of powder used), whereby the powder particles are physically attached to the components of the sweat deposited from the fingermark, which results in the visualization (appearance) of the entire image of the left trace. Afterwards, traces of papillary lines can be fixed, excluded and analyzed in the laboratory (Bumbrah, Sharma, & Jasu, 2016; Champod, Lennard, Margot, Stoilovic, 2004; Milašinović & Koturević, 2016; Sodhi & Kaur, 2001).

Ordinary (classical) powder substances consist of polymeric resin (adhesive agent) and colored substance (contributes to the contrast). Adhesive system binds to the sweat and oily components present in the fingermark. The use of metal powders is widespread, and their basic advantage over organic powders is a longer lifetime. A large number of powdered substances contain natural or synthetic organic derivatives that show fluorescence or phosphorescence when exposed to UV or laser light. The advantage of these powders is the application on multicolored surfaces, where the problem is the contrast, and can also be used for poorly visible traces (Horswell, 2004; Pounds, Grigg, & Mongkolaussavaratana, 1990).

In addition to the fact that powder substances are very often used in everyday forensic work, they also have certain disadvantages. It often happens that, due to improper handling of the brush, irreversible destruction of the trace occurs; powder can sometimes "overfill" the prints and thus make the identification and analysis of minutiae impossible; many commercial powders are harmful and toxic, and inhalation can affect the health of the applicant/user. In order to overcome the last-mentioned disadvantage, researchers are developing new systems for the

visualization of latent traces based on the application of (bio)polymeric materials (Milašinović N., 2016).

POLYMER MATERIALS: CLASSIFICATION, FEATURES AND UTILIZATION

Polymers are organic or inorganic compounds consisting of molecules with large molecular masses (macromolecules), and by multiple repetition of constituent units (basic motifs) in the molecular chain, which are interconnected (most often) with covalent bonds. Polymers with a small number of basic motifs in which the degree of polymerization is between 2 and 20 are called oligomers (Jovanović & Đonlagić, 2004; Plavšić, 1996). According to the source they come from, polymers are divided into three groups: natural, synthetic and semi-synthetic polymers (Jovanović & Đonlagić, 2004).

Natural polymers or biopolymers produced by living organisms are divided into three basic groups: polynucleotides (DNA, RNA, etc.), polypeptides (collagen, gelatin, etc.) and polysaccharides (chitosan, alginate, dextran, xanthan gum). In addition, biopolymers are produced from renewable sources, and their most important properties are biodegradability and non-toxicity, allowing them easy decomposition by various microorganisms, while, when in contact with the human organism, show no harmful effects (Van de Velde & Kiekens, 2002). Biopolymers have various applications in the industry, but their utilization in the forensic science, more specifically in detection of latent fingermarks, has not been sufficiently explored yet. In this paper, chitosan-based conjugates were synthetized and characterized in order to achieve fine powder structure and applied in detection and visualization of latent fingermarks.

Chitosan (Ch) is a cationic heteropolysaccharide, consisting of β-(1→4)-linked units of deacetylated D-glucosamine (deacetyl units) and N-acetyl-D-glucosamine (acetylated units), and its chemical structure is shown in Figure 1 (left). It is obtained by partial deacetylation of chitin obtained from crab or shrimp shells using alkaline substances. The ratio of free amino groups, or degree of deacetylation, in commercially available chitosans is generally over 60%. According to the molecular weight, expressed in units of daltons (Da), it is divided into chitosan with small (<150 kDa), medium (150-700 kDa) and large molecular weights (700-1000 kDa) (Čalija, Milić , Krajisnik, & Racic, 2013). From a criminal-forensic aspect, the fact that chitosans of large molecular weights are not dissolved in water (Budinski-Simendić, and others, 2014) is a very important fact, since fingerprints consist of sweat, containing over 98% water. Chitosan can potentially bind (usually with the addition of some components) to the residues present in the print, such as salts, fats, organic acids, etc.

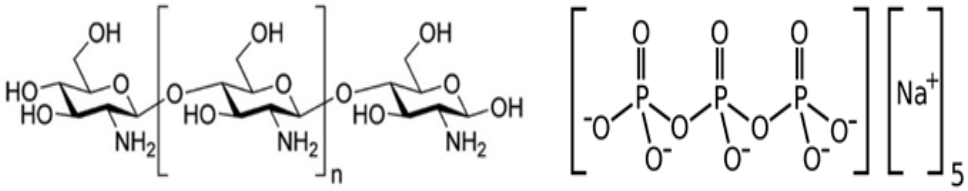

***Figure 1**. Chemical structure of Chitosan (left) and chemical structure of TPP (right)*

Sources: *Mahapatro & Singh, 2011, Sodium triphosphate, 2018*

Bearing in mind all of the above-mentioned chitosan characteristics and the ability of systems based on chitosan to bind to the sweat fingerprint residues using electrostatic and lipophilic interactions, in this paper the chitosan with medium molecular weight was used for synthesis of the conjugate. The pH value of the dissolving solution was adjusted to allow protonation of amino groups present in chitosan and to enable the potential interaction with negatively charged portions of sodium salt of the polyphosphate penta-anion (Sodium Tripolyphosphate (TPP), Figure 1 (right), but also with lipid residues present in sweat (Hejjaji, Smith, Morris, 2017 (b); Il Dueik & Morris, 2013; Il'ina & Varlamov, 2005; Morris, Castile, Smith, Adams, & Harding, 2011). In addition, the viscosity, zeta potential, size and swelling properties of the prepared conjugates may have an impact on the mucoadhesion/bioadhesion of the chitosan-TPP microparticles to a specific surface, and therefore, affect their application in the detection and development of latent fingerprints (Hejjaji, Smith, & Morris, 2017 (b); Il Dueik & Morris, 2013).

In this paper, chitosan-based conjugates were prepared by the precipitation method, using TPP as a crosslinking agent. Synthetized conjugates have shown satisfactory binding ability to the sweat and/or lipid residues present in latent fingermarks.

MATERIALS AND METHODS

Materials

Chitosan medium molecular weight (M~243kDa) was obtained from Sigma-Aldrich (Germany), and TPP from Across Organics (USA). Viscosity measurements were monitored using a Brookfield DV-E viscometer with a S00 spindle at a speed of 60 rpm. For the preparation of buffer solutions, distilled water was used. Acetate buffers of a specific pH values were prepared by dissolving sodium acetate and acetic acid in distilled water. Buffer solutions were then used to dissolve chitosan flakes and TPP powder. All components were used without any purification or treatment.

SYNTHESIS OF THE CHITOSAN-BASED CONJUGATE

In order to optimize the various parameters for conjugate synthesis, the following parameters were varied: the mass and concentration of the constituents, and the pH of the initial solutions. In brief, chitosan solutions were prepared by dissolving 0.2000 g of chitosan flakes in 100 cm^3 of acetate buffer (pH adjusted to 4.32 allowing the protonation of its amino groups (Hejjaji, Smith, & Morris, 2017 (b) which approximately corresponds to the pH of the sweat (~ 4.5)) in order to obtain 0.20 % of chitosan solution. . TPP solutions were obtained by dissolving 0.0840 g of TPP powder in 100 cm^3 of acetate buffer. In order to obtain the appropriate volume of the final solution, the experimental procedures according to the Hejjaji et al. (Hejjaji, Smith, & Morris, 2017 (b)) was modified, and the chitosan solution was added to the solution of TPP in different volume ratios: 6/1, 4/1, 1/1, 1/4 and 1/6 (first and second number correspond to the amount of Ch and TPP used). During the synthesis of the conjugates, the samples were mixed at low speeds and at room temperature using a magnetic stirrer, and the microparticles were spontaneously formed due to ionic cross-linkage of chitosan by TPP. Prepared microparticle suspensions were left in the incubator at 37 °C until complete evaporation of the solvent, and then the synthesized conjugates were powdered using pestle and mortar. The resulting powders of the conjugate were then tested on different surfaces, such as: glass (non-porous), rubber (semi-porous) and paper (porous) surface.

CHARACTERIZATION OF PREPARED CONJUGATES

UV-Vis spectrophotometry

Molar concentration of the initial chitosan and TPP solutions, as well as the concentration of the sample solution (prior to precipitation, and in different phases), were determined using the UV/Vis spectrophotometer Shimadzu UV-1800. Samples were scanned at full wavelength range (190-1100 nm) to determine absorbance and maximum absorption (λ_{max}), using acetate buffer (pH 4.32) as the initial probe solution.

FT-IR analysis

The samples of conjugate powder, in solid state, were recorded using the Bomem MB 100 FT-IR spectrophotometer. Samples of 1.5 mg were mixed and ground with 75 mg of potassium bromide and then compressed into discs at pressure of 11 t for one minute using the Graseby Specac model: 15.011. The spectra were obtained in the wavelength range from 4000 to 400 cm^{-1}, at 25 °C and with a spectral resolution of 4 cm^{-1}.

Optical microscopy

The powder samples were recorded using the Leica FS C Comparison Macroscope optical microscope, equipped with the Leica IM Matrox Meteor II modulation software. Samples were tested in dry condition, with and without backlighting. Before imaging under the microscope, latent fingerprint left on microscopic slides were enhanced using prepared sample conjugates.

DETECTION AND VISUALIZATION OF FINGERMARKS

In order to determine the functionality and possibilities of realistic, everyday use of the prepared powders, fingerprints of three donors were deposited on glass (non-porous), rubber (semi-porous) and paper (porous) surface. Following the guidelines proposed by the International Fingerprint Research Group (IFRG), "natural", oily fingerprints were deposited on the tested surfaces using a technical scale in order to simulate real conditions and to determine the pressure level on the substrate (force accommodated to 100-150 g), and the prints were then left in laboratory (humid) conditions for a period of approximately 24 hours. This period allowed the traces to dry and reduce the amount of residues until the latent fingerprints were enhanced using powder samples.

RESULTS AND DISCUSSION

The Ch/TPP conjugate formation mechanism was tested using UV/Vis spectrophotometry and FT-IR analysis, while the morphology of the particles was characterized by SEM analysis and optical microscopy. The synthesized powders are labeled as S(Ch/TPP), where "Ch" refers to the chitosan concentration, and "TPP" to the concentration of tripolyphosphate (relative to chitosan) used to prepare the desired conjugate.

The powders were tested on paper, rubber and glass surfaces. Powders were applied on deposited fingerprints over 20 times on each of the surfaces, in order to determine the possibility of their application, or the ability to achieve desired visualization results. The best results are achieved with the "oily" fingermarks, using the powder S(6/1), left on the glass and rubber surface. On a white paper surface, due to yellowish powder appearance, it was not possible to achieve a satisfactory contrast between the fingermark and the background.

Figure 2 shows fingerprints (two different donors) on the rubber (semi-porous) surface, developed with S(6/1) powder. The basic forms of papillary lines (circular) are clearly visible, a solid contrast between the papilloma trail and the intermediate space, with slightly less visible minutiae. Furthermore, there is evident powder "overfilling" of the prints, which can be a consequence of the characteristics of the surface itself (folds, protrusions and depressions). Figure 2 also

shows good adhesion of the powder to the surface, due to the fine particles appearance.

Figure 2: *Fingermarks developed on rubber surface with S(6/1)*

Figure 3 shows "oily" fingermark on glass surface developed with S(6/1). In comparison to fingermark developed on the rubber surface, it showed noticeably higher quality, and basic form of papillary lines are clearly visible (the right loop). The great contrast was achieved between papillary lines and background, and minutiae are even more obvious.

Figure 3. *Fingermarks developed on glass surface with S(6/1). Right image shows the same marks but with slightly higher magnifications*

Moreover, due to the smooth structure of the glass surface, the powders showed a very good interaction with the papillary lines, without the print "overfilling", which was observed on the fingermarks in Figure 2. The powdered sample

showed good adhesion, and therefore continuity of papilla traces compared to rubber surface.

The development of fingermarks on the paper (porous) surface did not show satisfactory results, due to which these results were not shown. Also, despite the solid results achieved by enhancing the prints on the rubber surface, the best results were achieved on a glass surface, where all the essential (identification and individual) characteristics of the prints are clearly visible.

UV/VIS SPECTROPHOTOMETRY SOLUTION ANALYSIS BEFORE AND AFTER CROSSLINKING

UV analysis was carried out in order to determine the concentration of initial chitosan and TPP solutions, as well as solutions obtained after chitosan was crosslinked by TPP. Samples labeled P1 and P2 refer to the initial chitosan and TPP solutions, while those labeled P3 and P4 refer to synthetic conjugates, i.e. samples S(1/1) and S(6/1), respectively. All samples were analyzed in triplicate and the mean values of their spectra are shown in Figure 4.

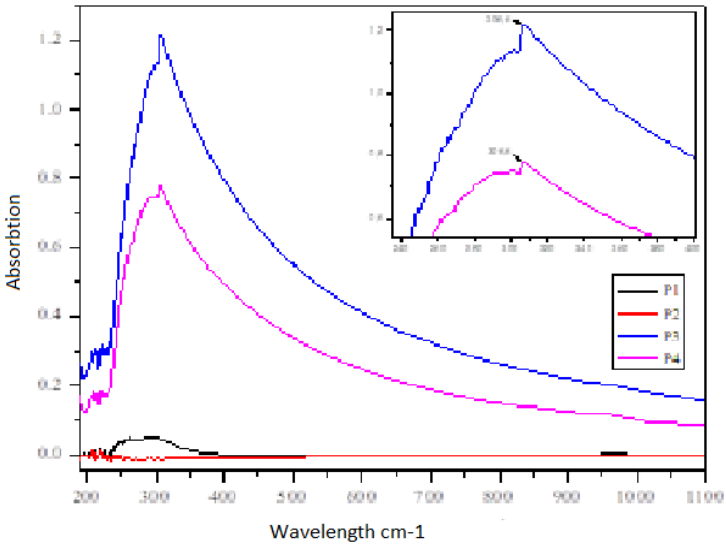

Figure 4: *UV spectra of solution samples: P1- initial solution of chitosan; P2- initial solution of TPP; P3- solution of S(1/1) and P4- solution of S(6/1)*

Samples P3 and P4 coincide at a peak position of 306.6 cm^{-1}, which may refer to chitosan crosslinked by TPP and derivation of the conjugate. At the same peak value (306.6 cm^{-1}), a decrease in the absorbance value from 1.221 (P3) to 0.778 (P4) may be the result of a larger chitosan crosslinking in the sample S(6/1) (P4). S(1/1) was used as the reference sample.

FT-IR ANALYSIS

The FT-IR analysis was carried out with the aim at confirming the formation of the conjugate, in the course of ionotropic gelation. Figure 5shows the spectra of pure TPP and samples S(1/1) and S(6/1). In the spectrum of a pure TPP (Figure 5, spectrum 1), characteristic peak at 1215 cm^{-1} is observed, indicating the stretching of P=O bonds (Dudhani & Kosaraju, 2010). The peak at 1145 cm^{-1} could be due to the symmetric and asymmetric stretching of the PO_2 group, while the peak at 1093 cm^{-1} could indicate the symmetric and asymmetric stretching of PO_3 groups. Peak at 903 cm^{-1}, may be associated with asymmetric stretching of P-O-P bonds (Hejjaji, Smith, & Morris, 2017 (a)).

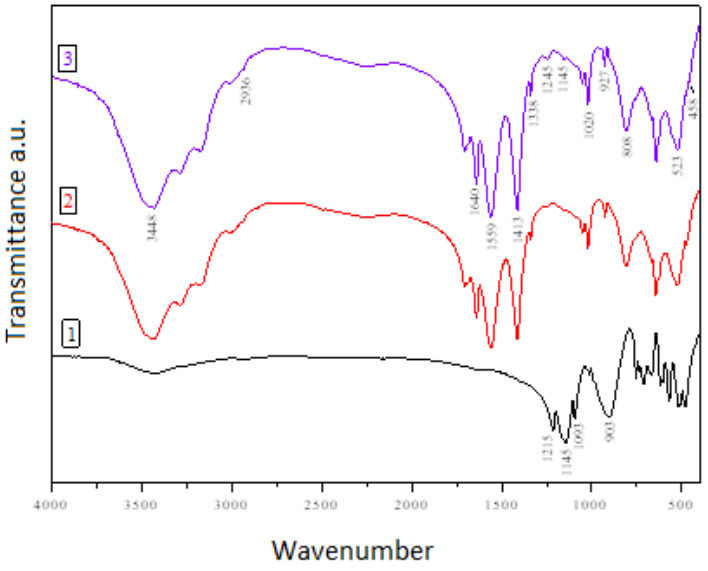

Figure 5: *FT-IR spectrum 1) pure TPP; 2) U (1/1); 3) U (6/1)*

On both spectra samples (Figure 5, spectra 2 and 3), a characteristic strong and wide absorption peak at 3448 cm^{-1} might indicate the overlapping stretching and bending of -OH and $-NH_2$ groups (Milašinović, Čalija, Vidović, Crevar Sakač, Vujić & Knežević-Jugović, 2016). The appearance of the peak at 1640 cm^{-1} could be the result of antisymmetric deformation vibrations of the NH_3^+ group of chitosan (Žalnėravičius, Paškevičius, Mažeika, & Jagminas, 2018), while the peak at 1559 cm^{-1} indicates the deformation of the N-H bond (amide band II in chitosan) (Hejjaji, Smith, & Morris, 2017 (a)). The peak at 1338 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 1145 cm^{-1} could be due to the swinging of NH_3^+ (Roddick- Lanzilotta, Connor, McQuillan, 1998).

OPTICAL MICROSCOPY

The structure of powdered samples

Figure 8 shows the images of prepared powdered samples, taken by Leica IM1000 microscope, equipped with the Matrox Meteor II modulation software, using magnification ×75 and various contrast techniques (backlighting). Since the best results were achieved on a non-porous (glass) surface, the same was used for further research. Powdered samples were deposited on microscopic slides in the form of a gossamer (thinner) and coarse (thicker) layer, in order to compare the uniformity of the particles. By comparing the thin layers of the powdered samples (Figure 6, labels I and II), it is evident that the sample S(6/1) possesses uniform and small diameter particles which were easy to apply and do not stick to the microscope slides (dry and clean), while at the same time were more easily triturated during the preparation. In the case of the powdered samples deposited in the thick layer, the above characteristics are even more apparent (Figures 6, III and IV). The dark-field and bright-field techniques were used to achieve the best possible contrast between samples and backgrounds, which empowered the most small-scaled particles to be spotted.

Figure 6: *Microscopic images of powdered samples applied on microscopic slides (magnification ×75); I and III represent images taken in dark-field technique; II and IV represent images taken in bright-field technique contrast.*

TESTING OF THE POWDERED SAMPLES

In order to determine the possibility of application and usability of synthetized powder, following the procedures proposed by the International Fingerprint Research Group, fingerprints were deposited on microscopic slides using a technical scale (force applied to accommodate 100-150 g) and left for a few minutes. After this period, the fingermarks were separated into halves using a thin glass barrier, and then two different powdered samples were used for their visualization, the sample S(6/1) was applied on the left and S(1/1) on the right barrier side, as a reference sample. Then, fingermarks were taken using an optical microscope, with dark-field technique (Figure 7, a)) and light-field (Figure 7, b)), with the ×15 magnification. For practical reasons only selected results were shown.

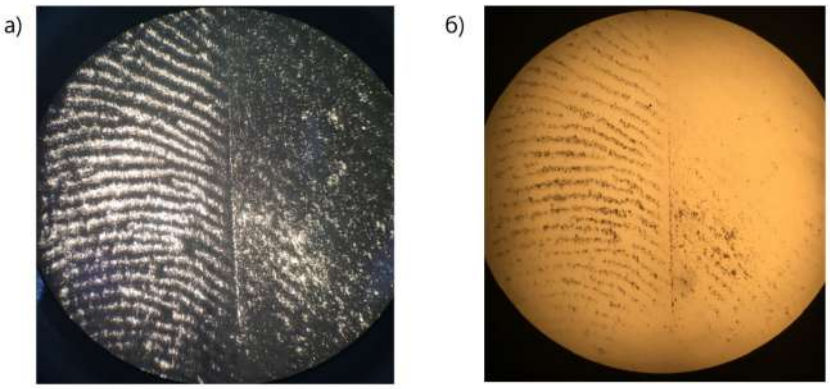

Figure 7: *Fingermarks visualized with S(6/1) (left side of both images) and S(1/1) (the right side of both images), recorded using optical microscope (magnification ×15) with dark-field (a)) and bright-field (b)) techniques.*

It is evident that the powder sample S(6/1) showed better adhesion to the fingerprint residues, making visible papillary lines (and minutiae), with excellent contrast between the papilla and the background (Figure 7, a)). On the other hand, with the application of the powdered sample S(1/1) it was evident that no good interaction with of the powder to the fingerprint residues was achieved.

CONCLUSION

In this paper work, chitosan-based conjugates obtained by the precipitating methods and further ionotropic crosslinking using sodium tripolyphosphate as the crosslinking agent were prepared and characterized, and the possibility of these conjugates' application for the detection and enhancement of latent fingerprint traces was examined. Based on the achieved results prepared samples were small and uniform in size, possess good adhesion properties to the sweat residues left on non-porous and semi-porous surfaces, were easy to handle and had good potential to be applied as substitutes for commercially used powders in forensic science fingerprint enhancement applications.

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