First International Congress of Forensic Sciences "Multidisciplinary Cooperation in Forensic Sciences: Current Approaches". Proceedings Book

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Polis Akademisi Yayınları: 87

1. Baskı, 2019, ANKARA

Polis Akademisi Yayınları: 87

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ISBN: 978-605-7822-28-4
1. Baskı, 2019, Ankara, 250 Adet

Tasarım: Yalçın AKTÜRK

Baskı:
Polis Akademisi Başkanlığı Basım ve Yayım Şube Müdürlüğü,
Fatih Sultan Mehmet Bulvarı No: 218 06200 Yenimahalle - Ankara

Polis Akademisi Yayınları Sertifika No: 45724
Polis Tarihi ve Kültürü Araştırma Merkezi
Polis Akademisi Başkanlığı, Necatibey Cad. No: 108,
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PREFACE

This congress proceedings volume contains the written versions of the contributions presented during the First International Congress of Forensic Sciences "Multidisciplinary Cooperation in Forensic Sciences: Current Approaches". The congress was held at J. W. Marriott Ankara on December 2nd, 2019.

Forensic sciences currently include many multidisciplinary research and studies on various themes, including crime, substance abuse, collection and examination of chemical, physical, and biological evidence and many other topics. Forensic sciences as a multidisciplinary field also collaborate with many related sciences such as physic, biology, chemistry, anthropology, statistic, psychology, sociology, law, and economy. Accordingly, one of the objectives of this congress was to underline the significance of multidisciplinary cooperation in forensic sciences in both academic research and applied settings. The congress encompassed a wide variety of topics including *criminalistics digital forensics and evidence of criminal law, forensic chemistry and forensic biology, forensic psychology and narcotics*. The comprehensive program consisted of twelve presentations and twenty-two posters in three sessions.

We believe that the congress was a good opportunity for participants coming from China, India, Spain, Finland, Azerbaijan, Serbia, and Turkey to present and discuss topics in their respective research areas.

We are looking forward to the next conference in 2020 and hope that these annual congresses will provide an international platform for discussing recent developments as well as sharing expertise and experiences in relation to forensic sciences.

We would like to thank all the participants for their contributions to the success of the congress program and for their contributions to this volume.

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"Multidisciplinary Cooperation In Forensic Sciences: Current Approaches"

December 2, 2019

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CHITOSAN-BASED MICROPOWDERS FOR THE DEVELOPMENT OF LATENT FİNGERMARKS – THE EFFECT OF BİOPOLYMER CONCENTRATİON ON POWDER PROPERTİES

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Abstract: Detection and enhancement of these marks nowadays is of a huge importance for law enforcement agencies in order to identify persons/offenders. Although commercial chemical and physical methods provide satisfying results on multiple surfaces (glass, wood, rubber surface, etc.), many surfaces still pose a great challenge (firearms, colored surfaces and fabrics, etc.), and not to mention their toxicity and detrimental effect to users. Thus, many research groups focus their efforts in constant improvement and development of already used methods and in preparation of new systems. Due to their specific properties, the polymeric materials, especially biopolymers, are drawing an incredible level of the attention, while experiencing the rapid growth in all areas of research and application. However, the application of these materials in the field of forensic science has not yet been sufficiently exploited and the scientific public is almost unaware of its potential in aforementioned forensic application. In this paper, conjugates based on chitosan were obtained by the precipitating method. The initial concentration of the biopolymer (biopolymer/crosslinker ration) was varied and their potential in detection and visualization of latent fingermarks was examined. The prepared micropowders were characterized by spectrophotometric and spectroscopy methods, while scanning electron microscopy confirmed the shape, size and uniformity of the obtained micropowders, as well as their ability to bind to the fingerprint residues. The results demonstrated that these bio-based micropreparations have potential for commercial development and could complement commercial powders in visualizing and enhancing latent fingermarks.

Keywords: Detection and Visualization of Latent Traces, (Bio)polymers, Chitosan, Forensic Sciences, Forensic Chemistry

Introduction

Fingerprints represent one of the main features of individuals used for distinction between and identification of suspects, victims, etc. in modern forensic science. These marks represent series of ridges and furrows and their random combinations (patterns) deposited from finger surfaces onto substrates, and are assumed to be unique for every person (Bumbrah, Sharma, & Jasuja, 2016; Mozayani & Noziglia, 2006).

In 1891 Ivan Vučetić was the first to classify fingerprints of the left and right hand by group and give them classification marks (along with Henry-Galton classification system), therefore being the pioneer in identifying offenders. He also practised to make the special classification marks for each form of papillary lines (the so-called decadactyloscopic formula), thus forming the first dactyloscopic collection. Shortly after, such a method, now known as dactyloscopy, became highly reliable for identification of persons in daily work of law inforcement agencies (Radmilović, 2008).

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1.1. Fingerprint structure

The skin, as the largest human organ, consists of three basic layers: epidermis, surface (outer) layer consisting mainly of dead cells, has a protective function; 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, consists mainly of fatty tissue (Champod, Lennard, Margot, & Stoilovic, 2004; 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. These papillae are formed on the outer layer of the skin, by stacking series of furrows, lumps and bulges inside the dermis. On the basis of fingerprint anatomy, fingerprints can be classified into four main types (arch, left loop, right loop and whorl) and three additional types (damaged, complex and amputated). All fingerprints (except the arches) also contain a core, which represents the central part of the print, and a delta, which is the place of first branching within the print. Also, the papillary lines are not smooth curved lines and they form many details, which can be classified into three characteristic groups: beginning or ending; branching or joining; and exceptional forms. All of these details, called minutiae, represent disruptions and changes in the flow of papillary lines, and contain important information to distinguish prints and to reliably identify a person (Champod, Lennard, Margot, & Stoilovic, 2004; Jojić, Babić, & Đurović, 2014; Mitrović, 1998; Weyermann, Roux, & Champod, 2011). Some of the minutiae points are ridge ending, ridge dot, island, lake, bridge, bifurcation, trifurcation, etc. and few of them are shown in Figure 1.

Figure 1. Basic fingerprint characteristics and some minutiae.

Source: http://syhrl.blogspot.com/2012/04/036-fyp-minutiae.html.

The papillary line prints are nowadays one of the most important physical evidence in forensic science and can often be found at the crime scene. Three distinct types of fingerprint that could be recovered are: patent (positive and negative), plastic (three-dimensional image of traces) and latent. Visible traces can be fixed, photographed, lifted and then analyzed in the laboratory to determine a person's identity (Champod, Lennard, Margot, & Stoilovic, 2004).

From a forensic aspect, the most important ones are latent fingerprints, since such prints are commonly found, but hardly detectable at the crime scene – they are present, but also imperceptible. Latent fingermarks contain sweat, sebum and other substances secreted by the sweat glands. Sweat consists of water (> 98%), salts, minerals, organic acids, urea, sugar, etc., while sebum contains fats,

glycerides, esters, etc. In order to manipulate with and analyze such traces, these must be visualized first, which is why different optical, physical and chemical methods have to be employed. After applying these methods and developing traces, they can be further analyzed and compared with those found in dactyloscopic collection databases in order to identify suspects, victims, etc. (Färber, Seul, Weisser, & Bohnert, 2010; Trapecar & Balazic, 2007).

1.2. Methods for visualization of latent fingermarks

There are many methods used to develop latent prints, but they can be divided into three main categories: optical, physical and chemical methods. The fingermark is visible as long as there is sufficient contrast between the mark and the substrate, and sometimes it may be necessary to use colored, oblique or UV lighting to observe weakly visible marks. Typical application of optical methods refers to utilization of UV light, e.g. when luminol solution is applied on bloodstains (in order to detect potential luminescence) (Champod, Lennard, Margot, & Stoilovic, 2004).

On the other hand, chemical methods are based on chemical reactions between chemical species and latent fingerprint residues, and these are used on different porous, semi-porous and non-porous surfaces. Commonly used chemical methods are: ninhydrin, silver nitrate, cyanoacrylate and iodine fuming. Their main advantage is chemical reaction and production of steady complexes, with satisfying development of fingerprint residues. However, many of these methods are toxic and harmful to human health and the environment, and must be carefully applied. Additionally, even though chemical methods provide stable fingerprint complexes, such prints often cannot be further examined (Almog, Hirshfeld, & Klug, 1982; Bumbrah, Sharma, & Jasuja, 2016; Champod, Lennard, Margot, & Stoilovic, 2004; Datta, Lee, Ramotowski, & Gaensslen, 2001; Milašinović & Koturević, 2016).

In regard to this, scientists have developed physical methods in order to overcome some of the aforementioned problems. Physical methods include physical interactions, i.e. the physical binding of certain substances (usually powders and dyes) to specific components in the latent trace, and are generally applied on non-porous and rarely on semi-porous surfaces. On the basis of chemical constitution, the fingerprint dusting formulations can be classified into regular (classic), metallic, luminescent and thermoplastic powders. These powder substances can be applied on different (usually non-porous) surfaces, and the type of powder that will be used depends on the color, structure and other characteristics of substrate itself. Physical methods are less aggressive and detrimental, when compared to chemical methods, but they also have some disadvantages. Sometimes, an inappropriate brush handling can ruin a fingerprint and it cannot be recovered afterwards. Moreover, some powders can overfill a fingerprint, and some are, more or less, harmful when inhaled, due to their toxicity (e.g. metallic powders, which contain iron and/or other metal traces) (Bumbrah, Sharma, & Jasuja, 2016; Datta, Lee, Ramotowski, & Gaensslen, 2001; Haque, Westland, Milligan, & Kerr, 1989; Levinton-Shamuilov, Cohen, Azoury, Chaikovsky, & Almog, 2005; Milašinović & Koturević, 2016; Sodhi & Kaur, 2001). In order to overcome the last mentioned disadvantage, many researchers are fostering the development of new detection and enhancement systems, and some novel methods encompass the utilization of various (bio)polymers (Milašinović, 2016).

1.3. Polymer materials: classification, properties and utilization

Polymers are organic or inorganic compounds consisting of molecules with large molecular weights (macromolecules), defined with multiple repetition of constituent units (basic motifs) in the

molecular chain, which are interconnected (most often) with covalent bonds. They can be classified by origin, chemical composition, macromolecule binding method, polymerization mechanism, processing properties, fields of application, etc. According to their source, polymers are divided into three main 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, etc.). Additionally, 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 little or no harmful effects (Van de Velde & Kiekens, 2002). Biopolymers (chitosan in particular) have various technological and industrial applications, but their exploitation in forensic applications, especially in development of latent fingermarks, has not been studied enough so far.

Chitosan (Ch) is a cationic heteropolysaccharide, consisting of β-(1→4)-linked units of deacetylated *D*-glucosamine (deacetylated units) and *N*-acetyl-*D*-glucosamine (acetylated units). It is produced by partial deacetylation of chitin (the second most abundant natural polymer after cellulose, prepared by processing crab or shrimp shells). The ratio of free amino groups or degree of deacetylation, in commercially available chitosan 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 high molecular weight (700-1000 kDa) (Čalija, Milić, Krajišnik, & Račić, 2013). From a forensic aspect, the important fact is that chitosan of a higher molecular weight does not dissolve in water, since fingerprints consist of sweat, containing over 98% of water. Hence, chitosan (usually with the addition of some substances) can potentially bind to the residues present in fingerprints, such are salts, fats, organic acids, etc. (Budinski-Simendić et al, 2014; Marguerite, 2006).

Considering all of the chitosan characteristics mentioned above, as well as the ability of chitosan-based systems to bind to the sweat fingerprint residues with both electrostatic and lipophilic interactions, in this paper medium molecular weight chitosan was used for conjugates' synthesis. The chitosan-based conjugates in form of powders were prepared by the precipitating method, using TPP as a crosslinking agent. Synthetized conjugates were then applied in detection and visualization of latent fingermarks and demonstrated the ability to bind to the sweat and lipid residues present in the latent fingerprint.

2. Materials and methods

2.1. Materials

Medium molecular weight chitosan ($M_w \sim 243$ kDa) was purchased from Sigma-Aldrich (Germany) and TPP from Acros Organics (USA). Distilled water was used for all buffer solutions preparation. Acetate buffers of various pHs were prepared by dissolving sodium acetate and acetic acid in distilled water, in order to obtain buffer solution of desired pH value. Buffer solutions were used to dissolve chitosan flakes and TPP powder. All materials were used without any purification or treatment.

2.2. Preparation of chitosan-based conjugates

In order to optimize various parameters for conjugate synthesis, the following parameters were varied: the mass and concentration of the components, and the pH of the initial solutions. Briefly, chitosan solutions were prepared by dissolving 0.2000 g of chitosan flakes in 100 mL of acetate

buffer, in order to obtain 0.20 w/v % chitosan solution. The pH value of 4.32 (enough below chitosan pK_a value) enables protonation of chitosan amino groups, while at the same time accommodates the pH of the sweat (~4.5) (Ali & Yosipovitch, 2013). TPP solutions were obtained by dissolving 0.0840 g of TPP powder in 100 mL of acetate buffer, in order to produce 0.084 w/v % TPP solution. In order to prepare the appropriate volume of the final solution, the modified experimental procedures described by Morris et al. (Hejjaji, Smith, & Morris, 2017 (b); Il Dueik & Morris, 2013) were applied by adding dropwise the chitosan solution to TPP solution in different ratios: 6/1, 4/1, 1/1, 1/4 and 1/6 (Ch/TPP), and the effect of components concentration on conjugates' properties was examined. The samples were stirred at low speed and at room temperature using a magnetic stirrer, and the microparticles were spontaneously formed due to ionic crosslinking of chitosan by TPP. Prepared microparticle suspensions were left at 37 °C, until complete solvent evaporation, and final conjugates were ground with pestle and mortar to fine powders and kept in desiccator until further use.

3. Characterization of the obtained conjugates

3.1. UV-VIS Spectrophotometry

The molar concentration of initial chitosan and TPP solutions, as well as the concentration of various samples solutions (prior to precipitation, and in different stages), were determined using Ultraviolet-visible spectrophotometer Shimadzu UV-1800. The sample solutions were scanned in a full range of wavelength (190-1100 nm) in order to determine the absorbance and maximum absorption (λ_{max}) using acetate buffer (pH 4.32) as the initial probe solution.

3.2. FT-IR Analysis

The samples of synthetized conjugates were recorded in solid state, using the Bomem MB 100 FT-IR spectrophotometer. Samples in amount of 1.5 mg were mixed and ground with 75 mg of potassium bromide and then compressed into pallets at a 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.

3.3 Optical microscopy

The powdered samples were recorded with optical microscope Leica FS C Comparison Macroscope, equipped with The Leica IM Matrox Meteor II Driver Software Module. Samples were tested in dry state, with and without backlight. Prior to imaging under the microscope, latent fingerprints left on microscope glass slides were developed using prepared powder samples.

3.4. SEM Analysis

SEM analysis of prepared conjugates was performed using electron microscope Tescan Mira3 XMU (Cranberry Township, USA). Powdered samples were recorded in dry state. Before the analysis, powdered samples were lyophilized and frozen in liquid nitrogen. Subsequently, prepared samples were coated using gold/platinum alloy (15/85) under vacuum using Polaron SC502 sputter coater.

3.5. Development of latent fingermarks

In order to determine the functionality and capability of prepared powders, three different donors deposited sebaceous and dry fingerprints onto a glass (non-porous), rubber (semi-porous) and paper (porous) surface. According to the guidelines proposed by the International Fingerprint Research Group (IFRG), ”natural“, sebaceous (oily) and dry fingerprints (not specifically modified) were deposited on aforementioned surfaces using technical scale, in order to simulate real conditions and determine the pressure on surfaces (force accommodated to 100-150 g, per fingerprint), and the prints were then left under laboratory (humid) conditions for a period of approximately 24 hours. That period allowed the traces to dry and reduce the residues, by the time the latent fingerprints were visualized by applying powders with BVDA Squirrel hair brush (BVDA, The Netherlands).

IFRG recommends the implementation of 4 phases in order to assess detection and visualization techniques and new systems:

- Phase 1 (*Pilot Studies*);
- Phase 2 (*Optimisation & Comparison*);
- Phase 3 (*Validation*);
- Phase 4 (*Operational Evaluation & Casework Trials*).

By setting up the experimental procedures as stated above, Phase 1 (of 4) was satisfied, in order to determine the functionality of prepared powders (International Fingerprint Research Group (IFRG), 2014).

4. Results and discussion

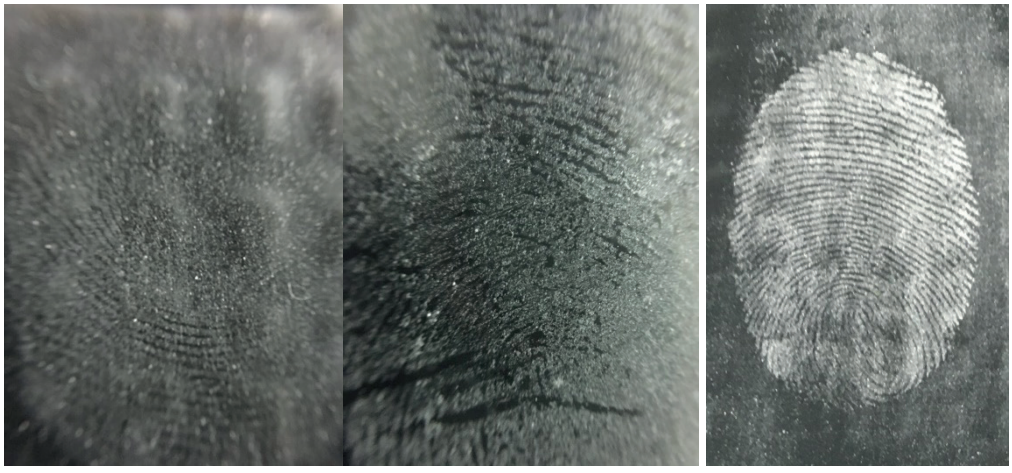
The obtained conjugates were prepared in solutions at pH bellow chitosan's pK_a value (6.5), where chitosan amino groups were protonated and these NH_3^+ groups could potentially interact with anions or polyanions, such as sodium tripolyphosphate (TPP), but also with lipid and sweat residues present in latent fingerprints (Hejjaji, Smith, & Morris, 2017 (b); Il Dueik & Morris, 2013; Il'ina & Varlamov, 2005; Morris, Castile, Smith, Adams, & Harding, 2011). A chemical structure of TPP allows the crosslinking of chitosan chains by binding O^- anions from phosphate groups to the protonated amino groups of chitosan (Hejjaji, Smith, & Morris, 2017 (b); Il Dueik & Morris, 2013).

The synthetized powders were labeled as S(Ch/TPP), where "S" refers to the sample, "Ch" to the concentration of chitosan, and "TPP" to the concentration of sodium tripolyphosphate, used to prepare the desired conjugates. By varying the concentrations of conjugates' components, the following powders were obtained: S(6/1), S(4/1), S(1/1), S(1/4) and S(1/6), where the samples S(6/1) and S(1/1) showed the best results in terms of developing latent fingermarks and were thoroughly examined.

Obtained powders were tested on paper, rubber and glass surface. Powder samples were applied on deposited fingerprints over 20 times on each of the surfaces, in order to determine the possibility of their application, i.e. the capability to achieve desired visualization results. The best results are accomplished with the sebaceous fingermarks deposited on the glass and rubber surface, and developed using the powder S(6/1). On the other hand, when applied on the white paper surface, due to yellowish powder appearance, it was not possible to achieve satisfying contrast between the fingermark and the background.

Figure 2 shows the oily fingerprints (two different donors) on a rubber (semi-porous) surface,

developed by S(1/1) and S(6/1) powders and commercial BVDA Magnetic silver powder, as a control powder. The prints were then photographed using a magnifying glass (magnification ×5) with a 12 MP camera (aperture *f*/2.2, pixel size 1.22 μm). On both prints developed with synthetized powders, there are relatively visible (although partial) basic types of papillary line patterns (whorls), and a solid contrast between the papillae trace and the interpapillary space was evident, with slightly less visible minutiae. Furthermore, the powder smearing and "overfilling" of the prints is noticeable, which may be due to the characteristics of the substrate itself (creases, bulges and indentations), which also contributed to the development of partial rather than complete fingerprint image. By comparing the two prints, it is evident that the powder S(6/1) binds better to the fingerprint residues (Figure 2, b)) than S(1/1) (Figure 2, a)), potentially due to the presence of finer microparticle structure and higher content of chitosan, with a lower degree of crosslinking. Smearing was also detected during the development of the latent prints using BVDA magnetic silver powder, but with well perceptible papillary lines and solid contrast (Figure 2, c). By comparing the prints developed with the synthetized powders, with the prints developed with the magnetic powder, it may be concluded that the powder S(6/1) shows solid results and makes visible the individual minutiae, while the powder S(1/1) poorly demonstrates the visualization of the papillae traces.



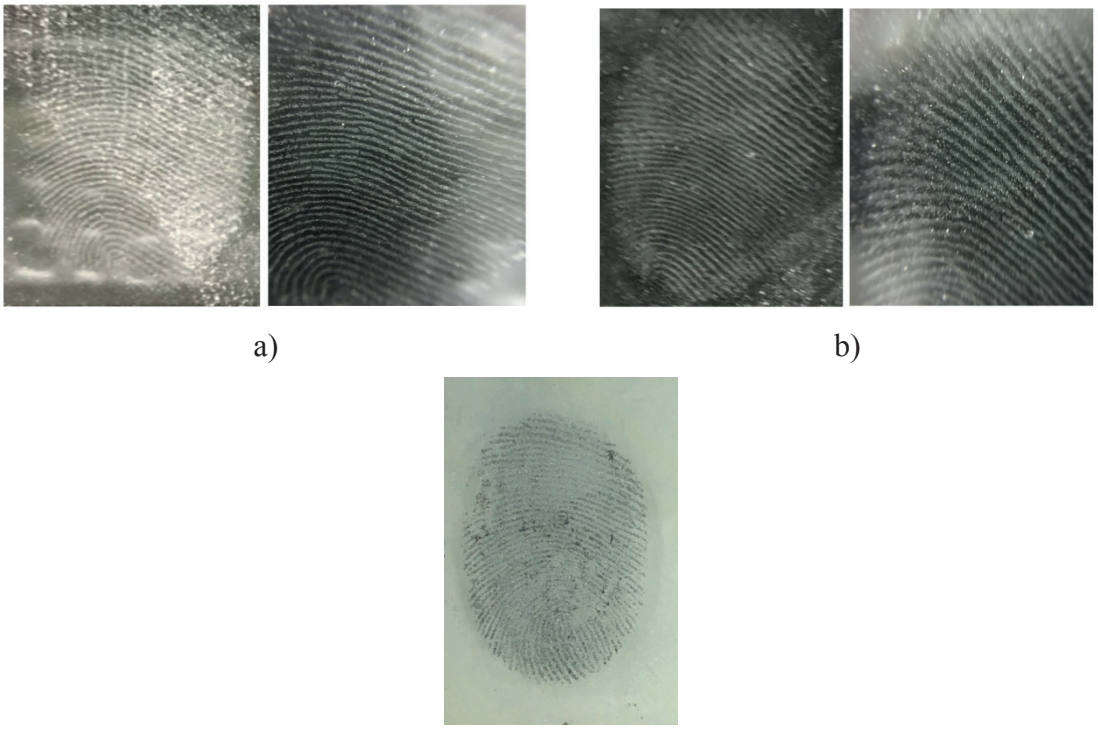
a) b) c)

Figure 2. Fingerprints developed on a rubber surface, using following powders: a) S(1/1); b) S(6/1) and c) BVDA Magnetic silver powder.

Figure 3 shows the sebaceous marks (two different donors) on a glass (non-porous) surface, developed by S(1/1) and S(6/1) powders and commercial BVDA Magnetic silver powder (control sample). Compared to the prints developed on the rubber surface, it is evident that both prints visualized by the synthetized powders on the glass surface show clearly visible basic types of papillary line patterns (right loop), an excellent contrast between the papillae trace and the background, along with papillary lines, interpapillary space and minutiae.

Furthermore, due to the smooth structure of the glass surface, the powders showed very good interaction with the papillae traces, showing no smearing, which was evident with the traces in Figure 2. Powder sample S(6/1) binds better to the fingerprint residues, enabling papillae continuity along the entire fingerprint image (Figure 3, b)), which is less perceptible comparing to fingerprints developed with S(1/1), where more frequent interruptions of papillary line flow (Figure 3, a)) were noticeable. This may be due to the finer particle structure and, again, higher content of chitosan in sample S(6/1), or

a larger number of protonated amino groups that can interact with the fingerprint residues. Fingerprints developed with magnetic powder showed clearly visible fingerprint pattern (right loop), good contrast, along with well perceptible papillae and minutiae (Figure 3, c)). By comparing the synthetized samples with BVDA Magnetic silver powder, it is evident that prepared powders showed excellent interaction with fingerprint residues and equally good results as commercially available powder.



a) b)

c)

Figure 3. Fingerprints developed on a glass surface, using following powders: a) S(1/1); b) S(6/1) and c) BVDA Magnetic silver powder.

The development of fingermarks on the paper (porous) surface did not provide satisfying results, so these results were not shown. Additionally, despite the good results achieved by enhancing the prints on the rubber surface, the best results were accomplished on a glass surface, where all the essential (identifying and individual) characteristics of the prints were clearly visible.

The Ch/TPP conjugate formation mechanism was examined using UV-Vis spectrophotometry and FT-IR analysis, while the morphology of the particles was characterized by SEM analysis and optical microscopy. UV analysis was performed to determine concentrations of initial chitosan and TPP solutions, as well as solutions obtained after crosslinking chitosan chains with TPP. The obtained results (data not shown) in terms of the position of peak at 306.6 cm^{-1} and its decrease in absorbance value from 1.221 to 0.778, may address the larger crosslinking of chitosan in the sample S(6/1) compared to the S(1/1).

4.1. FT-IR analysis

FT-IR analysis was performed to confirm the formation of conjugates, i.e. to confirm chitosan crosslinking by TPP in the ionotropic gelation process. Figure 4 shows the spectra of pure TPP and pure chitosan, as well as the spectra of the S(1/1) and S(6/1) samples. In the spectrum of pure TPP

(Figure 4, spectrum 1), a characteristic band at 1215 cm^{-1} may be indicating the stretching of P=O bonds (Dudhani & Kosaraju, 2010). The band at 1145 cm^{-1} may be due to symmetrical and asymmetric stretching of PO_2 groups, while the bar at 1093 cm^{-1} could indicate symmetric and asymmetric stretching of PO_3 groups. The peak at 903 cm^{-1} is most likely related to the asymmetric stretching of the P–O–P bonds (Hejjaji, Smith, & Morris, 2017 (a)).

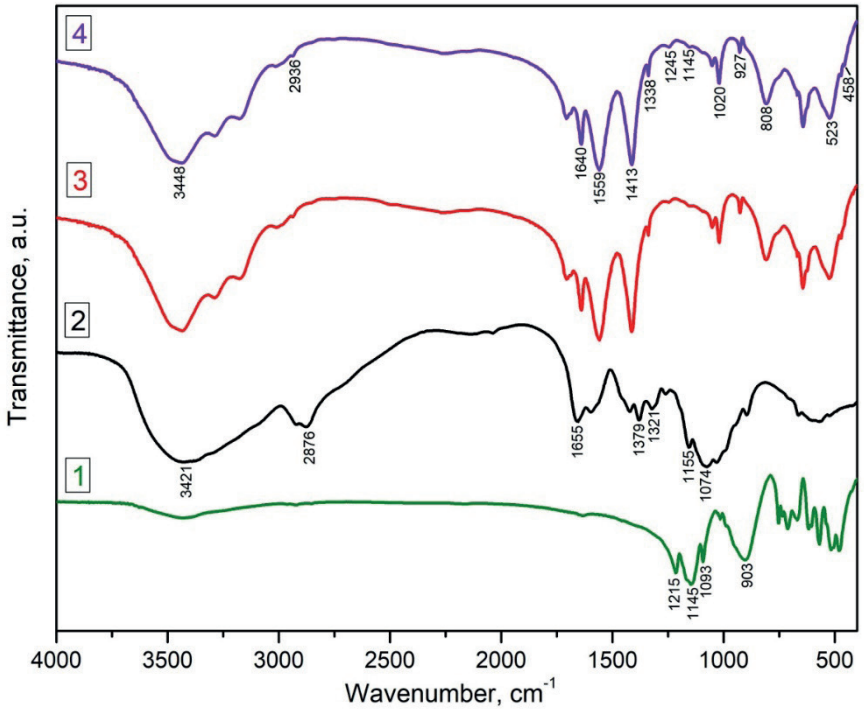


Figure 4. FT-IR spectra: 1) pure TPP; 2) pure chitosan; 3) S(1/1) and 4) S(6/1).

Chitosan possesses several groups that can form hydrogen bonds. The spectrum of pure chitosan (Figure 4, spectrum 2) shows a characteristic strong and wide absorption band at 3421 cm^{-1}, which originates from the overlapping of stretching and bending of the –OH and $–NH_2$ groups (Milašinović, Čalija, Vidović, Crevar Sakač, Vujić, & Knežević-Jugović, 2016). The band at 2876 cm^{-1} indicates the stretching of the C–H bonds (Alia, Rajendran, & Joshi, 2011).

Furthermore, the peak at 1655 cm^{-1} may be related to the stretching of the C=O bonds within the $–CONH_2$ group (amide I band), while the peak at 1596 cm^{-1} may be due to the stretching of the N–H bond at $–NH_2$ (amide II band) (Wang & Liu, 2014). The peaks at 1379 cm^{-1} and 1321 cm^{-1} indicate deformation of the O–H bonds at $–CH_2–OH$ and –CH–OH, while the peak at 1074 cm^{-1} can be associated with the stretching of the S–O bonds (Hejjaji, Smith, & Morris, 2017 (a); Wang & Liu, 2014).

In both spectra of the prepared samples (Figure 4, spectra 3 and 4), when compared to the spectrum of pure chitosan, shifting of the absorption band from 3421 cm^{-1} to 3448 cm^{-1} may be due to the increased number of hydrogen bonds formation (Wu, Yang, Wang, Hu, & Fu, 2005). The peak at 1655 cm^{-1} disappears and the new one occurs at 1640 cm^{-1}, which is probably related to the antisymmetric deformation vibrations of the N–H bonds of the NH_3^+ group (Žalnėravičius, Paškevičius, Mažeika, & Jagminas, 2018). Furthermore, shifting of the peak from 1596 cm^{-1} to 1559 cm^{-1} indicates the formation of a bond between ammonium and phosphate ions. In other words, substitution of one hydrogen atom of the chitosan amino group with the phosphate group of the polyphosphate penta-

anion, produces the NH_3^+–PO^- bond, indicating that the amino group is the only reactive chitosan functional group (Hejjaji, Smith, & Morris, 2017 (a)). Additionally, the appearance of the absorption band at 1145 cm^{-1} may be due to the rocking of NH_3^+ (Roddick-Lanzilotta, Connor, & McQuillan, 1998). Finally, the peak shifting from 1215 cm^{-1} (in the spectrum of pure TPP) to 1245 cm^{-1} may be due to the stretching of the P=O bonds, which additionally confirms the binding of ammonium and phosphate ions, i.e. the formation of conjugates (Qi & Xu, 2004).

4.2. Optical microscopy

4.2.1. The appearance of powdered samples

Figure 5 shows images of prepared powders taken by Leica FS C Comparison Macroscope, equipped with The Leica IM Matrox Meteor II Driver Software Module, using magnification ×75 and two contrast techniques (backlighting). Since the best results were obtained on a non-porous (glass) surface, the same surface was used for further analyses. Powder samples were deposited on microscopic glass slides in the form of a fine (thinner) and amassed (thicker) layer, in order to compare the uniformity of the particles.

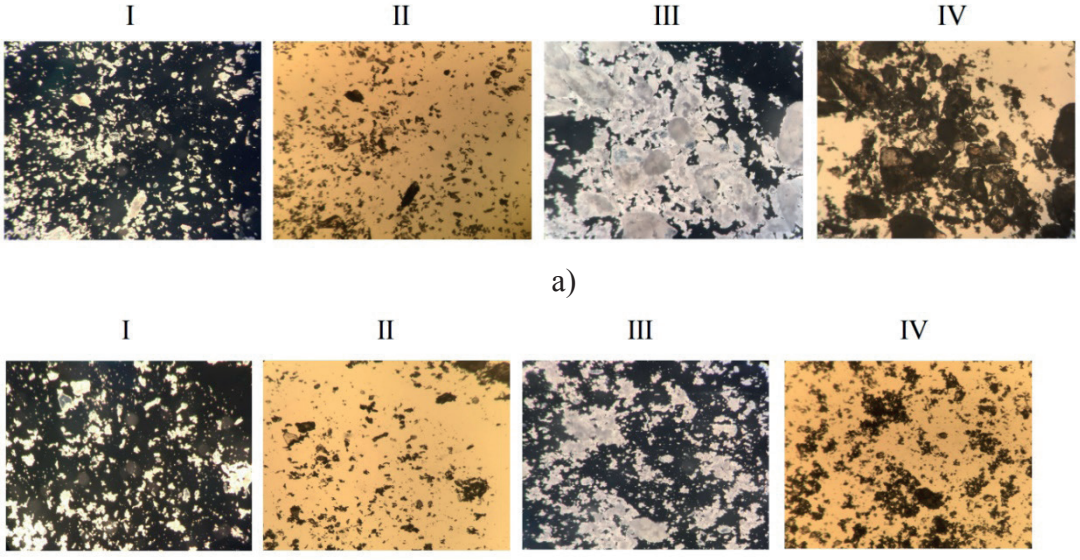
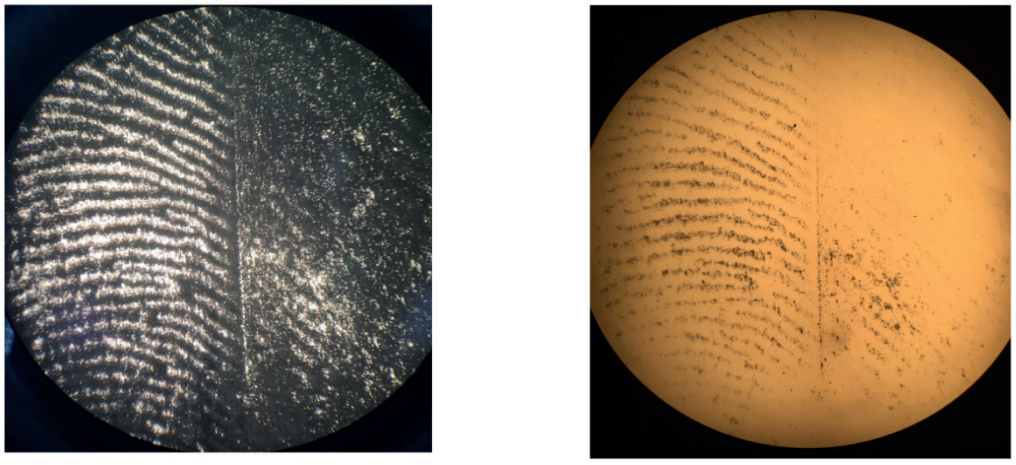


Figure 5. Microscopic images of powder samples, deposited on microscopic slides and recorded with optical microscope (magnification ×75): a) sample S(1/1) and b) sample S(6/1); Numbers I and III show images taken with dark-field contrast technique and II and IV represent images taken in bright-field contrast technique.

By comparing the thin layers of the powdered samples (Figure 5, I and II), it is evident that the sample S(6/1) contains more uniform and smaller diameter particles, which were easy to apply and do not stick to the microscope slides (dry and clean), while at the same time were facilely homogenized during the preparation. When observing the powder samples deposited in the thicker layer, the above-mentioned characteristics are even more obvious (Figure 5, III and IV). Dark-field and bright-field contrast techniques were used to obtain the best contrast between samples and background, which enabled even the smallest particles to be perceptible.

4.2.2. Testing of the powdered samples

In order to determine the application capability of synthetized powders, following the procedures proposed by the International Fingerprint Research Group, fingerprints were deposited on microscope slides using a technical scale (force applied to accommodate 100-150 g, per fingerprint) and left for a few minutes (with the aim at comparing synthetized powders and confirming previous results). After this period, the fingerprints were separated into halves using a thin glass barrier, and then two different powdered samples were used for their development – the sample S(6/1) was applied on the left and S(1/1) on the right barrier side, using BVDA Squirrel hair brush. Afterwards, fingerprints were photographed using the aforementioned optical microscope, with dark-field (Figure 6, a)) and bright-field (Figure 6, b)) techniques, with the magnification ×15. The application of the powders to the fingerprints and their capture was repeated several times, with only one of the results shown here, for practical reasons.



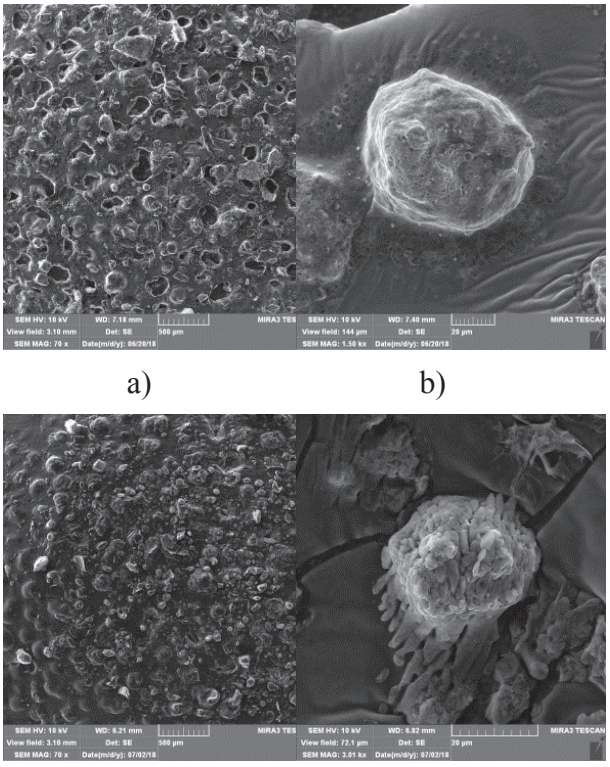
a) b)

Figure 6. Fingerprints developed using powders S(6/1) (left side of the images) and S(1/1) (right side of the images), then photographed using optical microscope (magnification ×15), with: a) dark-field and b) bright-field contrast techniques.

It is evident that the powder sample S(6/1) showed better adhesion and binding to the fingerprint residues, making visible papillary lines (and minutiae), with excellent contrast between the papillae and the background (Figure 6, a) and b) – left side of the images). On the other hand, using powder sample S(1/1) it is obvious that the visualization of papillary lines was not satisfying, due to the poor interaction with fingerprint residues (Figure 6, a) and b) – right side of the images).

4.3. SEM analysis of prepared systems

SEM analysis was performed in order to determine microparticle morphology and surface fineness, their size and uniformity. Figure 7 shows SEM micrographs of powdered samples S(1/1) and S(6/1), at different magnifications.



a) b)

c) d)

Figure 7. SEM micrographs of prepared powder samples: a) S(1/1) (×70); b) S(1/1) (×1500); c) S(6/1) (×70); d) S(6/1) (×3001).

SEM analyses confirmed that the particles of powder sample S(1/1) have a rough surface and are less uniform (Figure 7, a)), compared to the particles of S(6/1) (Figure 7, c)), which was previously assumed. Particles of sample S(6/1) are more uniform and finer, which is additionally confirmed by their smaller diameter size (Figure 7, d)), compared to particles of sample S(1/1) (Figure 7, b)) (Hejjaji, Smith. & Morris, 2017 (a)). As expected, better binding of finer powder S(6/1) was observed, due to the much smaller diameter of the powder particles. It was also very likely that a larger initial concentration of chitosan initiated the higher protonation of amino groups and thus instigated the interaction with lipid residues, resulting in better enhancement of latent fingerprints.

Conclusions

In this paper, chitosan-based conjugates, obtained by precipitating method and further crosslinked with sodium tripolyphosphate in ionotropic gelation process, were synthetized and characterized in order to determine capability of their application in detecting and visualizing latent fingermarks. Additionally, the concentration of biopolymer/crosslinking agent (Ch/TPP) was varied to evaluate its effect on powder performances. Based on the achieved results, the best powder performances were obtained with samples S(6/1), having smaller and more uniform particles in size, possessing better adhesion properties to the fingerprints residues due to a larger number of protonated amino groups, when compared to other prepared samples, resulting in better development of fingerprints. On the other hand, as many other dusting formulations, these conjugates are easily handled and applicable, requiring no prior knowledge and the method itself is non-destructive, avoiding irreversible loss of traces. Hence, this novel bio-based powder system could potentially supplement and/or replace some of the commercial methods in detecting and visualizing latent fingermarks.

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