Confocal Raman spectroscopy for the analysis of nail polish evidence

Maria López-López a,b, Joana Vaz c, Carmen García-Ruiz a,b,*

a University Institute of Research in Police Sciences, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain
b Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, Multipurpose Building of Chemistry, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain
c School of Science and Technology of the Health, University Lusófona of Humanities and Technologies, Campo Grande, 376, 1749-024 Lisbon, Portugal

1. Introduction

The analysis of paint evidence may provide useful information to assist in a crime reconstruction because small paint fragments may be transferred to an object or person in a crime. Then, the analysis of these evidence increase the information, for example, about the offender involved or the murder weapon. One example of the great utility of forensic paint analysis is in a car accident, especially when there are victims and the car involved in the accident escapes. The analysis of the paint stains or flakes may help to identify the car model or brand implied. Nail polishes are cosmetic paints that can also be susceptible of forensic analysis offering additional information of interest. In this case, evidence may be sourced from the rubbing of the nails against the different surfaces such as walls, papers, as well as other objects, causing unintentional loss of small nail polishes flakes or smudges.

The color of the nail polish is the first feature which could allow a quick identification of the sample. However, this is subjected to the perception and subjective interpretation of the forensic examiner and, additionally, two nail polishes of very similar color cannot be distinguished by eye [1]. Therefore, the chemical analysis of the nail polishes is performed to extract a great deal of information not subjected to analyst interpretation [1]. Chemically, nail polishes are complex samples made with different compounds according to brand, color and purpose of the product. Film forming agents (~15%) (e.g. nitrocellulose), thermoplastic resins (~7%) (e.g. tosylamide–formaldehyde), plasticizers (~7%) (e.g. camphor or dibutyl phthalate), solvents (~70%) (e.g. butyl stearate, acetate, alcohols, aliphatic and aromatic hydrocarbons), pigments (~0% to 1%) (e.g. calcium carbonate, zinc oxide, titanium dioxide, iron oxides), suspending agents (~1%) (e.g. urea, formaldehyde resin), or agents added to give shine (e.g. metal

Abstract

Nail polishes are cosmetic paints that may be susceptible of forensic analysis offering useful information to assist in a crime reconstruction. Although the nail polish appearance could allow a quick visual identification of the sample, this analysis is subjected to the perception and subjective interpretation of the forensic examiner. The chemical analysis of the nail polishes offers a great deal of information not subjected to analyst interpretation. Confocal Raman spectroscopy is a well-suited technique for the analysis of paints due to its non-invasive and non-destructive nature and its ability to supply information about the organic and inorganic components of the sample. In this work, 77 regular and gel nail polishes were analyzed with confocal Raman spectroscopy using two laser wavelengths (532 and 780 nm). The sample behavior under the two laser wavelengths and the differences in the spectra taken at different points of the sample were studied for each nail polish. Additionally, the spectra obtained for all the nail polishes were visually compared. The results concluded that the longer laser wavelength prevents sample burning and fluorescence effects; the similarity among the spectra collected within the sample is not directly related with the presence of glitter particles; and 64% of the samples analyzed showed a characteristic spectrum. Additionally, the use of confocal Raman spectroscopy for the forensic analysis of nail polishes evidence in the form of flakes or smudges on different surfaces was studied. The results showed that both types of evidence can be analyzed by the technique. Also, two non-invasive sampling methods for the collection of the evidence from the nails of the suspect or the victim were proposed: (i) to use acetone-soaked cotton swabs to remove the nail varnish; (ii) to scrape the nail polish from the nail with a blade. Both approaches, each exhibiting advantages and drawbacks in terms of transport and handling were appropriate for a further chemical analysis of the samples.

* Corresponding author at: Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, Multipurpose Building of Chemistry, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain.
Tel.: + 34 91 885 64 31.
E-mail address: carmen.gruiz@uah.es (C. García-Ruiz).

http://dx.doi.org/10.1016/j.talanta.2015.02.003
0039-9140 © 2015 Elsevier B.V. All rights reserved.
oxide, aluminum and plastic foil) are present in the nail polishes formulation [2–4]. Probably due to this complex nature, only a very few works dealing with the forensic analysis of nail polishes were published in the scientific literature in the last decade. Laser Desorption Mass Spectrometry (LDMS) has been used to analyze nail polishes yielding some unexpected inorganic materials [2]. The comparison of the positive and negative ion LDMS spectra of three fingernail polishes from the same manufacturer suggested that a forensic mass spectral database could be useful for a quick search to find a match. Secondary Ion Mass Spectrometry (SIMS) was also used for nail polishes characterization [5]. However, the method proposed required the use of multivariate analysis techniques. The use of a portable Energy Dispersive X-ray fluorescence spectrometer (EDXRF) and principal component analysis (PCA) was also used to classify five brands of nail polishes samples using filter paper as surface support in a quick, in-situ and no preparation way [6]. However, although the results obtained with the above mentioned techniques are very promising, the methods proposed often require sample preparation, reagents, skilled operators that can lead with a difficult data interpretation, chemometrics knowledge, or background subtraction. Additionally, the limited sample size usually submitted to the forensic laboratories and sample preservation requirements mandate a selection of the methodology sequence to maximize the discriminating power of the analytical scheme using first non-destructive methods.

Raman spectroscopy is a well-suited technique for the analysis of paints due to its non-invasive and non-destructive nature. These features are desired in the forensic analysis because, if necessary, the not altered sample can be subsequently analyzed by other analytical technique. Also, Raman spectroscopy provides information about the pigments, binders, and additives present in the paint sample [7]. Additionally, if the system includes a confocal microscope it enables the operator visualizing and focusing on small samples, to obtain high spatial resolution, or to provide optical section of the samples without physical section [8,9]. Due to its advantages, confocal Raman spectroscopy has been used in the analysis of various types of paint samples such as inks in questioned documents [10], automobile paints [11,12], or painting pigments [13,14], among others. However, the generation of fluorescence and burning effects are the main drawbacks of the Raman spectroscopy technique. These two undesired effects can hinder the spectra collection, making necessary to select an appropriate laser wavelength [8,15].

This paper aims to assess the utility of confocal Raman spectroscopy for the analysis of nail polishes evidence found in a crime scene. For this, it pursues (i) to study the use of two different lasers wavelengths for the analysis of regular and gel nail polishes of different colors to overcome fluorescence and burning effects, (ii) to investigate the spectral differences within the same sample as well as among all the samples used to create better understanding of the Raman analysis of the nail polishes, (iii) to assess the use of the Raman technique for the analysis of nail polishes as forensic evidence and, (iv) to propose different approaches for the non-invasive collection of the evidence from the nails of the suspect or the victim to help the potential analyst not only in the analyst interpretation but also in the difficult task of an appropriate sample collection.

2. Materials and methods

2.1. Samples

56 Regular nail varnishes and 21 gel nail varnishes of different brands and colors were analyzed (Tables 1 and 2). The regular nail varnishes were applied on aluminum foil and left to dry for two days. The samples of gel nail polishes were applied on aluminum foil and a second layer of a special varnish (sample 77, Table 2) was then applied on top of the first layer to adhere the nail polish layers to the nails. Then, the gel nail polish samples were placed under a UV lamp for approximately 3 min to dry. Three different regular nail varnishes (samples 8, 14 and 25, Table 1) were also applied on the nails of a volunteer and on plastic fake nails and left to dry for two days. The painted nail fragments were cut before being placed under the microscope to be analyzed. The volunteer left, after two days of drying, nail varnish marks in office paper, wood with white melamine finish and plaster. Then, two approaches for collecting nail polishes evidence were used. First, nail varnishes flakes were obtained from the nails of the volunteer by scraping the nail with a blade. Also, acetone-soaked cotton swabs were used to remove the nail varnishes from the volunteer nails. The substrates with nail polish smudges, the nail varnishes smudges flakes and the cotton swabs were placed under the Raman microscope and measured.

2.2. Instrumentation

A Thermo Scientific DXR Raman confocal microscope (Waltham, MA) equipped with 532 and 780 nm lasers was used. The nail varnishes on the aluminum foil were first visualized under the DXR Raman confocal microscope. For the Raman measurements, gratings of 900 and 400 lines/mm were used for the 532 and 780 nm lasers, respectively. 50 μm Slit aperture and 50 × magnifications were used for both excitations wavelengths. The laser power on sample ranged from a few to 10.0 mW depending on the sample and the laser. The wavenumber rage measured was 150–2500 cm⁻1. Five different points were analyzed from each sample to check the sample homogeneity. All the spectra were fluorescence corrected (polynomial of order 6) using the Thermo Scientific Omnic for dispersive Raman 8.3.103 software.

3. Results and discussion

3.1. Raman spectra of nail polishes

Regular and gel nail polishes with different color applied on aluminum foil to facilitate the sample preparation, visualization and measurement were analyzed using the 532 and 780 nm lasers in order to select the laser excitation wavelength that could overcome fluorescence and burning effects. The 77 samples measured were classified in four groups according to the results obtained at 532 and 780 nm. Tables 1 and 2 summarize the results obtained for the regular and gel nail polishes, respectively. In the first group there are those nail polishes (28 samples, 36.4% of the samples) that showed the same spectra with both lasers, not showing burning or fluorescent effects. The second group represent the 49.4% of the samples (38 samples) and is constituted by nail polishes that only show spectrum at 780 nm. The unsuccessful results obtained at 532 nm for this second group of nail polishes may be attributed to fluorescence or burning effects. The third group (6 samples, 7.8% of the samples) is composed of blue or green nail polishes that show different spectra at 532 and 780 nm. As an example, the Raman spectra of Fashion Studio Astor 228 First Act (sample 1, Table 1) at 532 and 780 nm are depicted in Fig. 1. The observed differences may be attributed to the Prussian blue pigment which according to the literature presents the most intense bands at about 2154, 2102, 538 and 278 cm⁻1 and is easily burned at 532 nm [16,17]. The fourth group (5 samples, 6.5% of the samples) is composed of nail polishes that due to burning
(brown and blacks samples) or fluorescence effects (blue samples) did not show Raman spectra at 532 or 780 nm.

3.2. Study of the differences in the spectra taken at different points of the sample

Searching for differences between questioned and known samples is the basic thrust of forensic paint analysis and comparison. However, differences in physical or chemical features can exist even in samples that are known to be from the same source. Therefore, a forensic paint examiner goal is to assess the significance of any observed differences to conclude if an analysis suggests that the paint samples could have a common origin [7]. In this regard, the chemical sample homogeneity is an important parameter to consider. For this, the samples appearance observed under the microscope and the spectral differences at five points of the sample was studied. The results obtained were summarized in Tables 1 and 2. Regarding to the appearance of the nail polishes,
Fig. 1. Spectra of the nail polish sample 1 (Table 1) using two laser wavelengths. a Laser at 532 nm, 2.0 mW, 50 μm slit aperture and 50 x magnifications; b laser at 780 nm, 5.0 mW, 50 μm slit aperture and 50 x magnifications. Spectral acquisition times: (a) and (b) 10 s × 5 acquisitions.

Fig. 2 depicts the visual images under the microscope and the Raman spectra of four regular polishes. Two of them (one matte and one glitter) showed identical spectral at the five points measured. Fig. 2a and c) the 5 spectra collected of one of the nail polishes are similar while in the second sample of each type of nail polishes the characteristic spectra of the Inocos transparent top coat nail polish was observed. Fig. 3 depicts a Raman depth profiling performed on the gel nail polish sample Sb nail Classic line color 065 (sample 57) covered by a second layer of Inocos transparent top coat nail polish (sample 77). As can be seen, as we went deeper into the sample the bands at about 1450 and 1724 cm⁻¹ coming from the gel polish topcoat decrease while the bands at about 679, 1142, 1340 and 1527 cm⁻¹ from the layer underneath increase. This fact highlights the importance of acquiring spectra at different focus points to minimize the risk of identifying the samples only by the topcoat.
Fig. 2. Visual images and Raman spectra (five different points) of the regular matte nail polishes (Table 2) (a) sample 12 and (b) sample 48 and the glossy nail polishes (c) sample 16 and (d) sample 27. Raman conditions: laser at 780 nm, laser power, 8.0 mW, 50 μm slit aperture, 50 × magnifications and 10 s × 5 acquisitions.
3.3. Raman spectral discrimination of nail polishes

The spectra of all the samples were visually compared to check the differences among their Raman spectra. The results obtained were summarized in Tables 1 and 2. 46 of 72 samples (64% of the samples) showed a characteristic spectrum while 26 did not. The samples that show the same spectra were grouped obtaining 8 different groups (labeled from A to H in Tables 1 and 2). Each group shows the same spectrum for all the nail polishes that make up that set. The groups A, B, C and H are composed of nail polishes samples that differ in appearance (the presence of glitter particles or because of the color) from the others belonging to the same group, making that a final visual identification and distinction from the others can be made. The common spectrum obtained for several nail polishes, even among samples with very different color, may be due to the common presence of an unknown compound in a high concentration or very Raman active that overwhelms the signals of the other compounds present in the nail polish formulation. The samples present in groups D and E are regular matte polishes that show the same color (deep red). Although these samples cannot be visually distinguished, their Raman spectra make a certain distinction between the samples. Most of the white and transparent samples analyzed were grouped in F and G groups. Two of four samples from group F can be visually distinguished due to the differences in color (mud and transparent samples) while the other two whites cannot. Group G presents two white samples and two transparent, which makes possible subdivide the group in two. The visual differentiation based on the visual nail polishes appearance (clear differences in color changes or glitter presence) after the chemical analysis by Raman spectroscopy increases the percentage of distinction (86% of the samples shows characteristic spectral and visual features). Additionally, it should be noticed that although 10 samples cannot be distinguished, each of them only show similarity with another sample, resulting in five groups of two samples each.

3.4. Analytical approaches for forensic evidence

Nail polishes evidence are usually fragments of painted nails (including artificial nails) or nail polishes smudges. For this reason, both types of samples were prepared to evaluate the use of confocal Raman spectroscopy for their analysis. Firstly, the Raman spectra of nail polishes on nails and plastic fake were acquired at 780 nm. The nails show characteristics bands at about 510, 1003, 1319, 1449, 1644 and 1880 cm$^{-1}$ [18,19] while plastic fake nails show bands (e.g. 1602, 1450, 1183, 1155, 1032 and 1001 cm$^{-1}$) that are characteristic of polyvinyl chloride plastic [20]. Then, three nail polishes were applied on both types of nails, and the Raman spectra were collected when they were dried. Fig. 4 depicts the Raman spectra of a nail varnish (Essence color & go 107 naughty and pink, sample 8 in Table 1) on a nail and a plastic fake nail showing that when the nail varnish is applied on both surfaces, and the laser is focused on the nail varnish layer, the spectra only showed spectral bands coming from this second layer.

However, it is important to consider non-invasive methods for collecting physical evidence. For this reason, two sampling methods that do not require cutting the nails of the victim or anyone suspected of being implicated in the crime were evaluated. The first method consisted on swabbing the nail with acetone to remove the nail polish while the second one was to scrape the nail polish from the nail with a blade. Fig. 5 shows the Raman spectra of sample 14 (Essence color & go 113 do you speak love?). As can be seen, in both cases a good spectrum of the nail polish can be obtained, presenting each method its own advantages and disadvantages. Thus, in the case of the nail polish flakes obtained by scraping the nail with a blade, the samples obtained are easy to focus and do not show interfering bands. Nevertheless, due to the small size of the nail polish flakes these evidence are difficult to handle and transport. On the other hand, the cotton swab used to collect the nail polish required a careful focusing on the nail polish present on the cotton fibers, and also, the cotton fibers gives additional bands and fluorescence to the nail polish Raman spectrum. However, the use of cotton swab to remove the nail polish from the nail is an approach extremely easy to perform, and also, facilitates handling and transportation.

Finally, the volunteer was asked to leave small nail polishes marks on three different surfaces. The objects employed as surfaces were office paper, white melamine coated wood, and plaster. Fig. 6 compares the Raman spectrum of a nail polish (Flormar nail enamel 323, sample 25 in Table 1) with their respective nail polish
Fig. 5. Comparison among Raman spectrum of (a) nail varnishes flakes of sample 14 (Table 1), (b) cotton swab used to collect sample 14 from nails, (c) cotton swab and (d) sample 14. Raman conditions: laser at 780 nm, 50 μm slit aperture and 50 × magnifications. Laser power: (a), (b) and (d) 8.0 mW and (c) 5.0 mW. Spectral acquisition times: (a), (d), and (e) 10 s × 5 acquisitions, and (b) 10 s × 10 acquisitions.

Fig. 6. Comparison among Raman spectrum of the sample 25 (Table 1) (bottom) and their respective smudges spectra on three different surfaces. (a) Sample 25 smudge on wood with white melamine finish, (b) white melamine coated wood, (c) sample 25 smudge on plaster wall, (d) plaster, (e) sample 25 smudge on paper, (f) paper and (g) nail polish. Raman conditions: laser at 780 nm, 50 μm slit aperture and 50 × magnifications. Laser power: (a), (b) and (f) 10.0 mW, and (c–e) and (g) 5.0 mW. Spectral acquisition times: (a), (c) and (e) 10 s × 10 acquisitions, and (b), (d), (f) and (g) 10 s × 5 acquisitions.

smudges and the substrates spectra. As can be seen, the melamine coated wood provided two bands at about 449 and 610 cm⁻¹ to the nail polish spectrum that could compromise the sample identification. However, the other substrates used did not interfere in the collection of the nail polishes marks spectra.

4. Conclusions

The Raman spectra obtained for different regular and gel nail polishes collected at two different laser wavelengths (532 and 780 nm) demonstrate that the half of the samples only shows spectra when they were analyzed at 780 nm due to burning or fluorescence effects at 532 nm. This fact may lead to conclude that longer laser wavelengths are more appropriate for the analysis of nail polishes. It should also be noted that some nail polish samples showed a different spectra at each laser wavelength. This effect was clearly observed for blue nail polishes that present the Prussian Blue pigment in their formulation. Regarding to the spectra taken at different points within the same sample, most of the samples showed the same spectra. However, it is always advisable to collect several spectra at different points (5 spectra) to ensure that the spectra taken are representative of the sample. On the other hand, the Raman spectrum of a nail polish sample should nevertheless be treated cautiously for identification purposes because some nail polishes showed the same spectra although they present different appearance (in terms of color or glitter particles presence). This can be due to the common presence of compound(s) in a high concentration or very Raman active that overwhelms the rest of compounds signals.

Nail polishes evidence (nail polishes fragments or smudges) can also be susceptible of analysis by confocal Raman avoiding any type of sample preparation. The Raman spectrum of nail polishes on nail, or plastic fake nails, can be easily acquired by placing under the Raman microscope small nail fragments. However, to collect the sample from the nails of the suspect or the victim in an effective and non-invasive way, two approaches can be used: (i) to swipe the nail with acetone to remove the nail polish and (ii) to scrape the nail polish from the nail with a blade. Although both sampling approaches can be used, the first one facilitates sample handling and transport but requires better sample focusing and offers additional cotton bands to the spectra acquired, while the second one is easier to focus and the Raman spectra obtained to do not show interfering bands. The Raman spectra of the nail polishes smudges, on the other hand, can be collected after carefully focusing the sample. Nevertheless, due to the thin film of nail polish present on the substrate it is possible to find interfering bands coming from the substrate. Also, it should be stressed that smudges produced by nails painted with different layers of nail polishes or with nails polishes with many sparkling particles may make the interpretation difficult.

Although the results obtained in this study demonstrate that the use of Raman spectroscopy could give solution to a greater number of cases regarding nail polishes, further research should be performed in this field. The use of chemometric methods (e.g., PCA, pure classification methods such as Knn or LDA, or class-modeling methodologies like SIMCA or PLS-DA) will provide more objective criteria for decision making, and therefore they should be explored in the near future. Additionally, it should be highlighted that the results present here should be checked on larger group of samples and the influence of the thickness of the layer (s) applied should be investigated further.

References
