Talanta 123 (2014) 135–139

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Confocal Raman spectroscopy to trace lipstick with their smudges on different surfaces

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ARTICLE INFO

Article history:
Received 11 November 2013
Received in revised form
4 February 2014
Accepted 10 February 2014
Available online 18 February 2014

Keywords:
Lipstick
Raman spectroscopy
lipstick smudges

ABSTRACT

Lipsticks are very popular cosmetic products that can be transferred by contact to different surfaces, being important forensic evidence with an intricate analysis if they are found in a crime scene. This study evaluates the use of confocal Raman microscopy at 780 nm excitation wavelength for the nondestructive identification of 49 lipsticks of different brands and colors, overcoming the lipstick fluorescence problem reported by previous works using other laser wavelengths. Although the lipsticks samples showed some fluorescence, this effect was not so intense to completely overwhelm the Raman spectra. Lipsticks smudges on twelve different surfaces commonly stained with these samples were also analyzed. In the case of the surfaces, some of them provided several bands to the smudge spectra compromising the identification of the lipstick. For these samples spectral subtraction of the interfering bands from the surface was performed. Finally, five different red lipsticks with very similar color were measured on different surfaces to evaluate the lipstick traceability with their smudges even on interfering surfaces. Although previous spectral subtraction was needed in some cases, all the smudged were linked to their corresponding lipsticks even when they are smeared on the interfering surfaces. As a consequence, confocal Raman microscopy using the 780 nm excitation laser is presented as a nondestructive powerful tool for the identification of these tricky samples.

1. Introduction

Lipstick is a cosmetic product containing wax, oil, and coloring agents as three main ingredients and some side ingredients as antioxidants, preservatives, and perfumes [1]. Since many women uses lipsticks on daily basis, lipstick smears can be found on various surfaces like cigarette butts, glasses, cups, kitchenware, cloths, etc. [2]. The samples stained with lipsticks can be found in a crime scene, and they may provide valuable forensic information on a potential suspect. As a consequence, the analysis of these samples could be done to trace the lipsticks smudges to the lipstick used.

A comprehensive revision of the techniques employed for examining lipsticks have evolved from simple optical methods [2] to modern analytical methods. Most of them focused on the determination of heavy metals in lipsticks. The lead concentration could be determined by graphite furnace Atomic Absorption Spectrometry (AAS) in alkaline solubilized samples [3], acid digested samples [4], and solid samples [5] or by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in acid digested samples [6]. There are also other works in the literature that, apart from lead, determine different elements present in lipsticks using other analytical techniques like Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) [7], Laser-Induced Breakdown Spectroscopy (LIBS) [7], Neutron Activation Analysis [8], and Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM-EDS) [9]. Also separation techniques such as Thin Layer Chromatography (TLC) [10], TLC combined with Gas Chromatography (GC) [11], Capillary Electrophoresis (CE) [12,13], High-Performance Liquid Chromatography (HPLC) [14], or GC coupled to Mass Spectrometry (GC–MS) [15] has been used for the analysis of lipsticks based on the dyes and other compounds previously extracted from the sample. However, although the information obtained by the above mentioned analytical methods is extremely useful for lipstick identification, they have the common drawback of a long sample preparation step that destroys the sample.

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http://dx.doi.org/10.1016/j.talanta.2014.02.025
0039-9140

Raman spectroscopy is a noncontact, nondestructive technique able to provide immediate and useful information about the identity of the sample requiring minimal or no sample preparation [16]. Moreover, confocal Raman microscopy has the ability to obtain the Raman spectra of a certain substance avoiding or reducing interference from the material around and underneath. Due to the clear advantages of the Raman spectroscopy for the nondestructive analysis of lipsticks, a few articles are referred in the literature about the use of this technique for the analysis of these samples. Rodger et al. [17] measured the Raman spectra of six red lipsticks at 514.5 nm. The authors reported that the fluorescence was very high at this wavelength and hence, the sample spectra of the samples could not be measured. To quench the fluorescence they applied a silver colloid. In the work of Salihoglu and Went [18] they used a longer wavelength (632.8 nm) to measure the Raman spectra of 69 different lipsticks. The authors reported a substantial reduction of the fluorescence effect at the selected wavelength but the 10% of the samples still remained too fluorescent to obtain the Raman spectra. Very recently, the same authors studied the use of confocal Raman spectroscopy for the differentiation of lipstick smears on different surfaces. They used both 473 and 633 nm excitation wavelengths to obtain the Raman spectra of four lipsticks smears on seven different textile fibers [19]. The best results were reported at 473 nm due to the intense fluorescence interference from fibers using the 633 nm wavelength. In fact, only less than the half of the spectra was obtained at 633 nm. As well, the authors analyzed the same four lipstick samples on cigarette butts and on tissues but only with the 633 nm laser. In the case of the cigarette butts, only one spectrum of the four lipsticks was unable to be collected. However, the smears on tissue were identified with minimum interference. Then, the spectra of ten different lipsticks obtained at 633 nm were properly classified using Principal Components Analysis (PCA) and the k-Nearest Neighbors (kNN) classifier. Additionally, they obtained the same good results using the same wavelength to classify the smears of two lipsticks on four different fibers.

In the light of the previous mentioned articles, and to overcome the fluorescence problems reported by them, the present article was focused to use of a longer laser excitation wavelength (780 nm) for the Raman collection of 49 lipsticks of different brands and colors and their smudges on 12 different surfaces. The use of spectral subtraction as a tool to overcome the significant challenge toward tracing the lipstick smudges on interfering substrates was also assessed. Finally, the possibility to identify and trace five different red lipsticks with their smudges even on interfering surfaces was studied.

2. Materials and methods

2.1. Samples

Forty-nine different lipsticks of different brands and colors were analyzed (Table 1). Five different red lipsticks (Gisèle Denis Richissime 3 Rouge Pur, L´Oréal Perfection Les Beux Arts 201, L´Oréal Color Riche 106 Real Red, Margaret Astor 158 Grosella, and Vitamol) were applied on the lips of a volunteer, who left lipstick marks in twelve different surfaces: crystal glass, brown glass bottle, green glass bottle, tissue (100% cellulose), cigarette, paper cup, white T-shirt (100% cotton), blue T-shirt (56% cotton and 44% viscose), labcoat (67% polyester and 33% cotton), metal fork, transparent plastic cup, and white plastic cup.

2.2. Instrumentation

A Thermo Scientific DXR Raman microscope (Waltham, MA), with 400 lines per mm grating, 780 nm excitation wavelength, and 50 μm slit aperture was used. The laser power on sample was 10.0 mW. The wavenumber range measured ranged from 100 to 2500 cm^{-1}. Ten different areas were analyzed from each lipstick to check the homogeneity of the sample at 10× and 50× magnification objective lens. Finally, the microscope was set to 10× magnification for the neat substrates and lipsticks samples, and 20× for the lipstick smudges and 20 spectra of 10 s were recorded for all samples. All the spectra were fluorescence corrected (polynomial of order 5) and normalized using the Thermo Scientific Omnic for dispersive Raman 8.3.103 software. Spectral subtraction was also performed with the Omnic software.

Table 1
Lipsticks used in the study.

Lipstick	Color
Agatha Ruiz de la Prada	Pink
Avon Petal	Pink
Avon Oxford wine	Purple
Avon Instant Mocha	Pink
Avon Bronze Treasue	Brown
Avon Perfect Peach	Brown
Avon Rosewood Glaze	Pink
Clinique Long Last Lipstick 91 Perfect Beige	Pink
Clinique Long Last Lipstick FJ Merlot	Brown
Dékade 55	Brown
Deliplus 28	Red
Diane Moore 37 Extra Mate	Red
Diane Moore	Brown
Dr. Pierre Ricaud 19326 Transparent café	Brown
Gisèle Denis Richissime 3 Rouge Pur	Red
H&M Berry	Red
H&M Kiss and Tell Red	Red
Kiko Ultra glossy stylo 803	Pink
Kiko Smart Lipstick 904	Pink
Kiko Smart Lipstick 84	Pink
Kiko Smart Lipstick 10	Brown
Kiko Smart Lipstick 912	Pink
Kiko Smart Lipstick 909	Red
Lancome Rouge in Love 201	Red
L´Oréal Color Riche 106 Real Red	Red
L´Oréal Color persist 618	Brown
L´Oréal Perfection Les Beux Arts 201	Red
Maite Díaz	Brown
Margaret Astor 158 Grosella	Red
Margaret Astor Soft Sensation 416 Dangerous Beige	Brown
Margaret Astor Soft Sensation Double Excellence 025	Pink
Margaret Astor 493 Soft Sensation	Red
Margaret Astor 086 Silver Sensation	Red
Margaret Astor 495 Soft Sensation	Red
Margaret Astor 488 Soft Sensation	Red
Maybelline 488 Soft Sensation	Brown
Maybelline Color Sensational 527 Lady Red	Red
Maybelline 140 Tiger Eyes	Brown
Newline 2010 381	Brown
Oriflame 12128 Cabaret Red	Red
Orlane 02	Pink
Rimmel Rich Moisture 290	Pink
Tiqwa 20	Brown
Tous 03 grape	Brown
Tous 04 cherry	Red
Vera Cristal	Brown
Vitamol	Red
Wynie Paris 05	Red
Yves Saint Laurent 134 Cinnamon Velvet	Brown

2.3. Statistical spectra

Thermo Scientific Omnic software was used to calculate the average (arithmetic mean of the *Y* values for each data point) and variance (standard deviation of the *Y* values for each data point) spectra of the normalized spectra. Relative Standard Deviations (RSD) were calculated according to the formula $RSD\% = 100s/\overline{x}$, where s is the standard deviation and $\overline{x}$ the mean of the data.

3. Results and discussion

The Raman spectra of the 49 lipsticks of different brands and colors (Table 1) were directly measured by placing the samples under the microscope. Although almost all the samples showed some fluorescence at 780 nm, in any of the cases the fluorescence was so intense to completely overwhelm the Raman spectra. Furthermore, the fluorescence artifact was easily removed by employing a baseline correction (polynomial of order 5) that reduces the effect on the baseline curvature. Thereby, the Raman spectra of the 49 lipsticks were obtained. The visual inspection of the spectra was performed and approximately thirty different groups not related with the brand or color of the samples were observed. This finding suggests that the lipstick spectra studied are quite distinctive of each lipstick. Additionally, eight samples were analyzed at 10 different points using the 10× and 50× magnifications to assess the sample inhomogeneity problem described by Salahioglu and Went [18]. The average and the variance spectra of each sample were calculated for the eight samples. RSD ranging

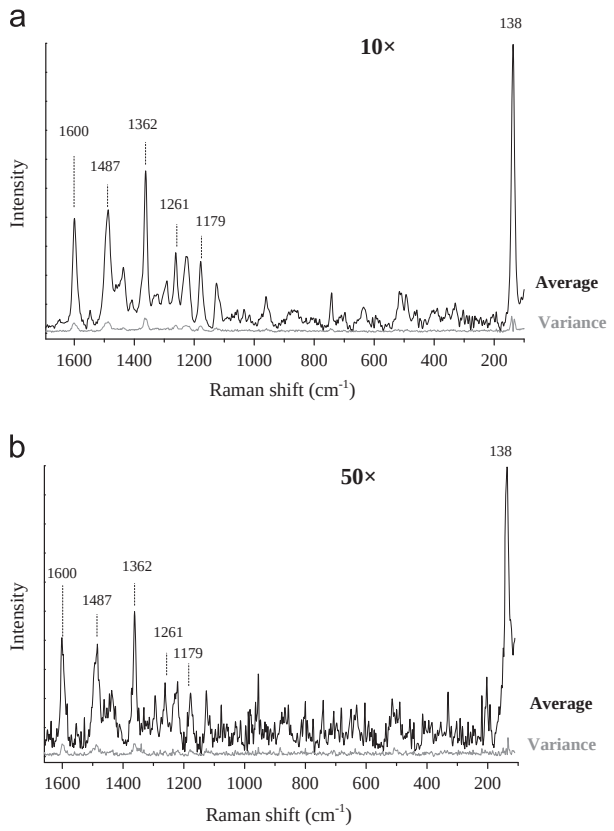

Fig. 1. Average (black line) and variance (gray line) Raman spectra of 10 spectra collected at different points of Margaret Astor 488 Soft Sensation lipstick using 10× (a) and 50× magnification objective lens (b). Raman conditions: laser at 780 nm, 10.0 mW, 50 μm slit aperture. Spectral acquisition times: 10× magnification, 1 s × 20 acquisitions; 50× magnification, 100 s × 3 acquisitions. Several bands are labeled for clarity.

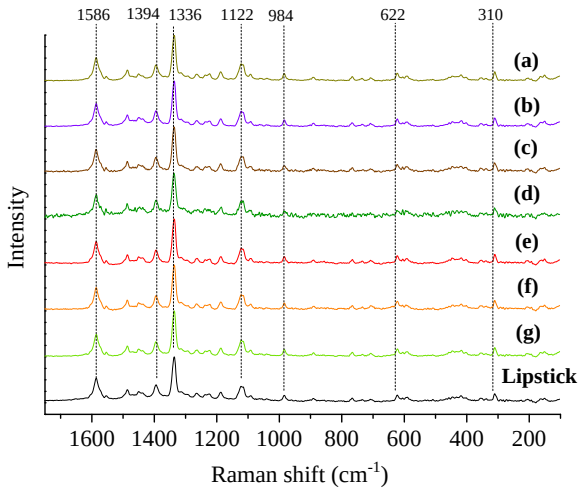

Fig. 2. Comparison among Raman spectrum of a lipstick (bottom) and their respective lipstick smudges on seven different non-interfering surfaces. Smudges of Loreal Perfection Les Beux Arts 201 on (a) tissue, (b) crystal glass, (c) brown glass bottle, (d) green glass bottle, (e) metal fork, (f) transparent plastic cup, and (g) white T-shirt. Raman conditions as in Fig. 1. 10× and 20× magnification objective lens for lipstick and lipstick smudges, respectively. Spectral acquisition times: 10 s × 20 acquisitions. Several bands are labeled for clarity.

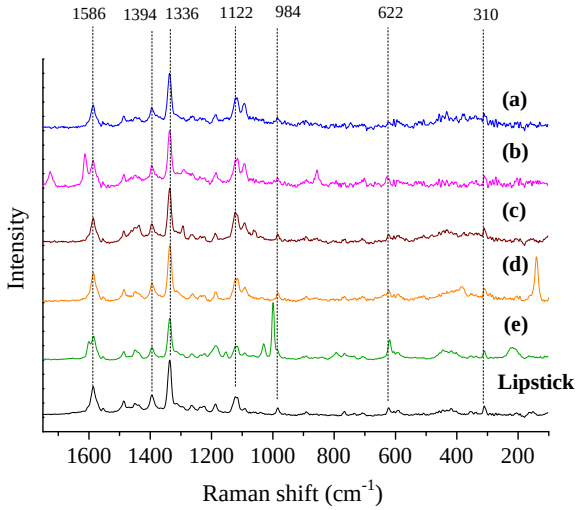

Fig. 3. Comparison among Raman spectrum of a lipstick (bottom) and their respective lipstick smudges on five different interfering surfaces. Smudges of Loreal Perfection Les Beux Arts 201 on (a) blue T-shirt, (b) labcoat, (c) paper cup, (d) cigarette butt, and (e) white plastic cup. Raman conditions as in Fig. 2. Several bands are labeled for clarity.

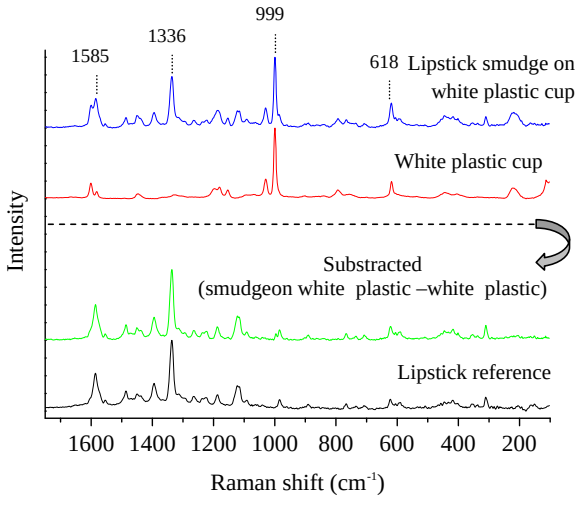

Fig. 4. Smudge of lipstick Loreal Perfection Les Beux Arts 201 on a white plastic cup illustrating spectral subtraction. From top to bottom: Raman spectra of lipstick smudge on white plastic cup, white plastic cup, subtraction difference of lipstick on white plastic cup - white plastic cup (white plastic cup spectrum scaled by a factor of 0.5 prior to subtraction), lipstick reference. Raman conditions and spectral acquisition times as in Fig. 1. 10× and 20× magnification objective lens for reference spectra (lipstick and white plastic cup) and for lipstick smudge, respectively. Several bands are labeled for clarity.

from 1–10% were obtained for five samples, RSD values of ~20% were calculated for two samples, and a RSD of 30% was obtained for one lipstick. This means that the lipstick inhomogeneity greatly differs among the samples depending on their composition. Fig. 1 depicts the average and variance Raman spectrum obtained for one red lipstick at ten different points using the 10× and 50× magnifications. It shows that the variance at 10× and 50× magnifications is quite similar but the *S*/*N* ratio is better with the lower magnification objective, additionally requiring less acquisition time. Thus, the lower magnification objective possible for each sample was employed.

Lipsticks smudges were then measured using 780 nm excitation wavelength on 12 different surfaces commonly stained with these samples. The first purpose of this second study was to assess the possibility of analyzing lipstick smudges on different surfaces by Raman spectroscopy without using the silver colloid proposed by Rodger et al. [17]. The second aim was to find a wavelength that works at all the possible surfaces where lipsticks smudges could be found. Figs. 2 and 3 compare the Raman spectrum of a lipstick with their respective lipstick smudges spectra. The objects employed as surfaces were tissue, crystal glass, brown glass bottle, green glass bottle, metal fork, transparent plastic cup, white T-shirt, blue T-shirt, labcoat, paper cup, cigarette butt, and white plastic cup. As can be seen, some of the surfaces did not interfere with the lipstick spectrum (Fig. 2), but others (blue T-shirt, labcoat, paper cup, cigarette butt, and white plastic cup) (Fig. 3) provided several bands to the lipstick spectrum compromising the identification of the sample. Spectral subtraction is a pre-processing method applied in cases where sample spectra have to be cleansed from spectral contribution of unwanted compounds [20]. A few examples of the application of this method with a forensic purpose could be the fiber interference subtraction from drugs-of-abuse particles on clothing [21] or the subtraction of different surfaces (glass, tile, cotton, and denim) to obtain the spectra of blood stains [22]. In the present work, for the lipstick smudges with interfering bands, spectral subtraction of the surface was

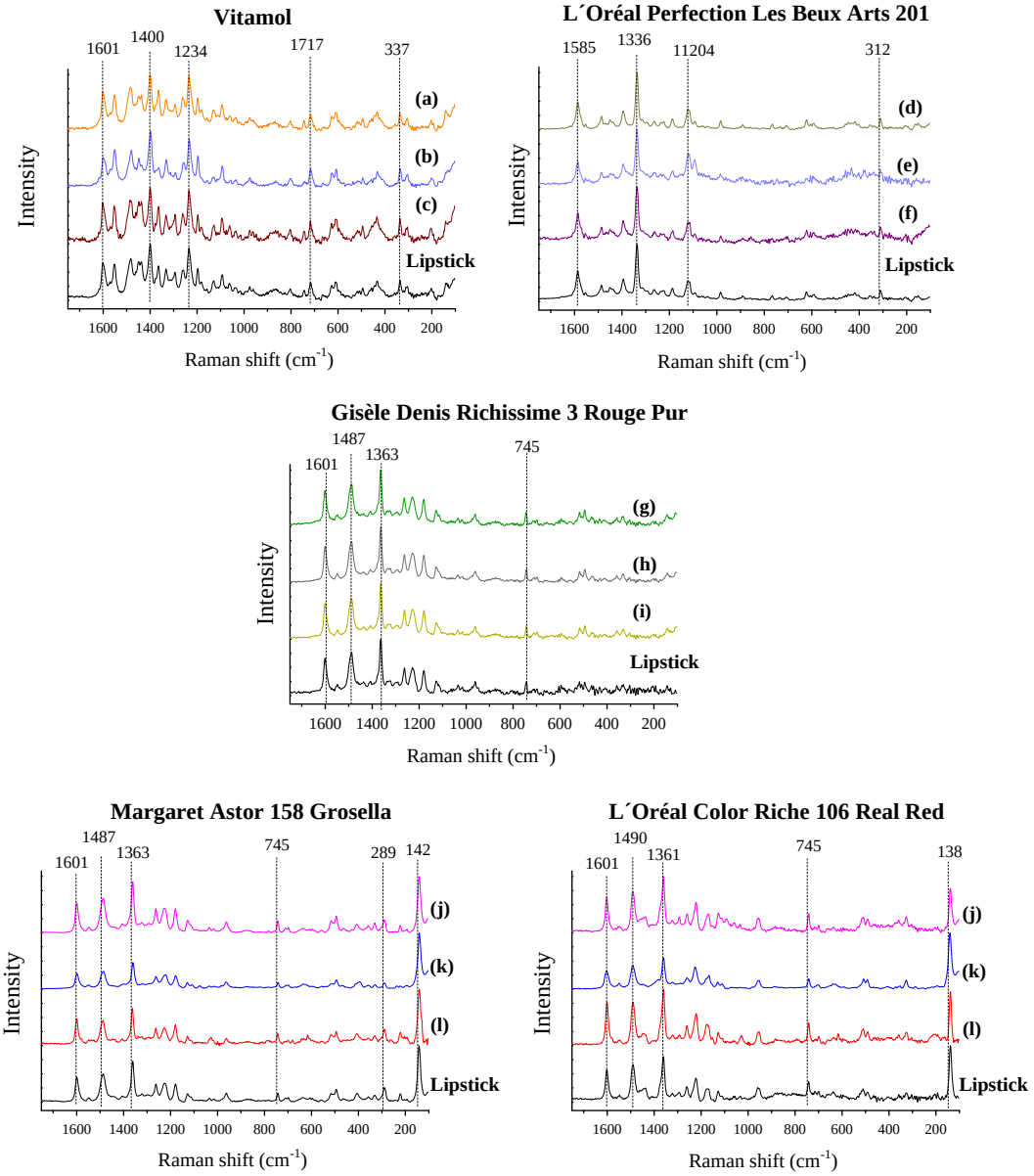

Fig. 5. Comparison among Raman spectrum of five different red lipsticks (Vitamol, Gisèle Denis Richissime 3 Rouge, L´Oréal Perfection Les Beux Arts 201, Margaret Astor 158 Grosella, and L´Oréal Color Riche 106 Real Red) and their smudges on different surfaces: (a) tissue, (b) crystal glass, (c) brown glass bottle, (d) white T-shirt, (e) blue T-shirt, (f) labcoat, (g) green glass bottle, (h) metal fork, (i) transparent plastic cup, (j) paper cup, (k) cigarette butt, and (l) white plastic cup. The surfaces that provided additional bands to the lipstick spectra were subtracted. Raman conditions as in Fig. 2. Several bands are labeled for clarity.

performed. As an example, Fig. 4 depicts the spectral subtraction of the white plastic from the lipstick smudge. The lipstick smudge spectrum was first obtained, showing a contribution from the lipstick and the white plastic. Then, a spectrum from another point of the white plastic cup where there is no lipstick presence was recorded. This second spectrum has only bands from the white plastic that according to the literature can be identified as polystyrene [23]. Finally, the second spectrum was subtracted from the first spectrum, resulting in a spectrum almost purely composed of bands from the lipstick.

The possibility to identify and trace the lipsticks with their smudges even on interfering surfaces was also evaluated by comparing five different red lipsticks with very similar color and their smudges on the twelve surfaces above mentioned. The surfaces that provided additional bands to the lipstick spectra were subtracted. Fig. 5 depicts the results obtained for several spectra obtained. The Raman spectra of the lipsticks Vitamol and L´Oréal Perfection Les Beux Arts 201 and their smudges were very different to the other three lipsticks, allowing to trace these lipsticks with their corresponding smudges. Gisèle Denis Richissime 3 Rouge lipstick and their smudges spectra showed several bands that were also present in the spectra of Margaret Astor 158 Grosella and L´Oréal Color Riche 106 Real Red. However, Gisèle Denis Richissime 3 Rouge lipstick and their smudges did not present band at about 142 cm^{-1} present in the Raman spectrum of Margaret Astor 158 Grosella or the band at about 138 cm^{-1} present in the spectrum of L´Oréal Color Riche 106 Real Red, allowing the identification of the mentioned lipstick and their smudges. Margaret Astor 158 Grosella and L´Oréal Color Riche 106 Real Red showed a very similar spectrum. Fig. 5 compares the spectra obtained for both lipsticks on the same surfaces to discard any possible surface interference. As can be seen, the two lipsticks spectra are practically only differentiated by the presence of a band at about 289 cm^{-1} in the Margaret Astor 158 Grosella and a small change in the position and intensity of the most intense band in both types of spectra (present at about 142 cm^{-1} in the Margaret Astor 158 Grosella spectrum and at about 138 cm^{-1} in the L´Oréal Color Riche 106 Real Red spectrum).

4. Conclusions

The Raman spectra of 49 lipsticks were obtained at 780 nm. At this wavelength the fluorescence drawback of some samples can be easily overcome by a simple baseline correction not compromising the quality of the spectra. The same good results were obtained for the lipsticks smudges on twelve different surfaces commonly stained with these samples (tissue, crystal glass, brown glass bottle, green glass bottle, metal fork, transparent plastic cup, white T-shirt, blue T-shirt, labcoat, paper cup, cigarette butt, and white plastic cup). Some of these surfaces interfered with the lipstick spectrum providing additional bands that require, in some cases, their removal. The spectral subtraction by measuring the spectra of the lipstick on the surface and a surface spectrum, and subsequently subtracting them, resulted in an isolated spectrum of the lipstick. Although the interfering band removal can be performed more elegantly by multivariate curve resolution, the approach performed in this work does not require chemometric knowledge. However, it should be highlighted that the proposed subtraction approach could lead to different results depending on the analyst and care must be exercised when performing subtraction. Raman spectral subtraction is not simply a one to one subtraction of two spectra and new or negative bands can appear that may not represent the true spectrum of the sample. Additionally, there is no real need of subtraction if the analyst is comparing different lipstick smudges on the same surface since the interferent is constant. However, spectral subtraction assists when the comparison of lipstick smudges on different interfering surfaces is performed.

The Raman spectra of five different red lipsticks on 12 different surfaces were compared to evaluate if the lipsticks can be linked to their smudges by Raman spectroscopy even on interfering surfaces. For those smudges that presented additional bands from the surfaces, the mentioned spectral subtraction was previously applied. As was expected, since the spectra were quite distinctive of each lipstick, the visual inspection of the spectra allowed the differentiation of the five lipsticks smudges even on interfering surfaces. These findings confirm that confocal Raman spectroscopy is a successful tool that can be applied quickly and in a non-destructive way for the traceability of lipsticks smudges even when they are on interfering substrates.

Finally, pure classification methods (e.g. Knn or LDA) or, even better, a class-modeling methodology (e.g. SIMCA or PLS-DA) could be used for an identification of lipsticks and smudges that not depends on the analyst.

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