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Differentiation of red lipsticks using the attenuated total reflection technique supported by two chemometric methods

Marta Gładysz, Małgorzata Król*, Paweł Kościelniak

Laboratory for Forensic Chemistry, Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Kraków, Gronostajowa 2, 30-387 Kraków, Poland

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ABSTRACT

The main challenge for the identification and differentiation of lipsticks for forensic purposes is the fact that they have a similar chemical composition — in particular, samples of the same hue. The objective of the presented research was to solve this problem using a nondestructive analytical technique — attenuated total reflection spectroscopy (ATR). 38 red lipsticks produced by 20 different manufacturers were examined in optimized experimental conditions. To facilitate discrimination and provide more extensive analyses of the obtained data, two chemometric techniques: principal component analysis and cluster analysis were used. Ultimately, nine groups of investigated lipstick samples with comparable chemical compositions were differentiated. Moreover, lipstick smears on six different interfering surfaces were analyzed and spectral subtraction was performed in order to identify individual samples. The established approach succeeded in identifying the index number and the manufacturer of the samples by using an in-lab built ATR spectra library. The developed method demonstrates a great potential for the differentiation and identification of red lipsticks with a very similar hue. It also seems to have good prospects for future application in forensic science investigations.

1. Introduction

Lipstick residues and smears may be significant forensic evidence in investigations, in particular in cases of rape, sexual assault, murder and burglary. They may be found left on a variety of surfaces — for instance, tissue paper, cigarette butts, glasses, drinking cups, clothing etc., and constitute indirect evidence linking a suspect with the victim or crime scene [1–4]. Nevertheless, identification of lipstick samples and differentiation between them can be a major challenge, because the majority of lipsticks have a similar composition. They are composed of three main ingredients: oils (typically 40–70%) — a mixture of castor, vegetable, mineral or lanolin oil; waxes (approximately 15%) – especially beeswax or carnauba; and colouring agents (roughly 8%) – organic dyes such as erythrosine, amaranth, rhodamine, tartrazine, derivatives of fluorescein, and inorganic pigments, particularly titanium dioxide and iron oxides. Other constituents occurring in smaller quantities are antioxidant materials and perfumes [3,4].

There have been many publications regarding lipstick examination, reporting results from simple optical [5] to modern analytical methods (i.e. CE [6], HPLC [7,8], UHPLC–MS [9], GC–MS [10], ICP-OES [11], ICP–MS [12], AAS [11,13], LIBS [14,15], XRF [16]). However, most of them have focused on (aspects of) the negative impact of the lipstick components on humans and the environment. Among other things, the content of azo [6], xanthene [7,17] or other organic colorants [8,9], as well as hazardous nitrosamines [10] and heavy metals (e.g. Pb) [11–16,18–20] in lipsticks have been studied. In the case of forensic lipstick examination, the most common technique is nondestructive Raman spectroscopy [2,3,21,22]. It should be pointed out here, however, that in every mentioned article, Raman spectroscopy has been accompanied by problems associated with fluorescence. An *in-situ* method of surface enhanced resonance Raman scattering (SERRS) detection of the colorants in lipstick smears on both glass and cotton surfaces was reported [23], but a surfactant was required to obtain SERRS spectra of the dyes and pigments in such waxy samples. Moreover, the technique of neutron activation analysis (NAA) – a nondestructive and highly sensitive but also very time-consuming and expensive method – was employed to study the presence of trace elements in samples of lipsticks [24].

* Corresponding author at: Laboratory for Forensic Chemistry, Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Kraków, Gronostajowa 2, 30-387 Kraków, Poland.
E-mail address: krolm@chemia.uj.edu.pl (M. Król).

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Another technique described in the literature used to differentiate lipstick is TLC [1,4,25]. However, although the information obtained by the TLC analytical methods was usually sufficient for lipstick differentiation from the forensic point of view, these methods have the important drawback of destructive sample preparation.

One of the techniques, which – to the best of our knowledge – has not yet been applied to lipstick examination is attenuated total reflection spectroscopy (ATR). Two significant advantages of ATR, namely that the sample is not destroyed and no sample preparation is required, have led to this technique being widely employed in forensic science investigations [26–29]. Consequently, the aim of the presented research was to develop the first ATR-based procedure enabling differentiation of red lipsticks. Additionally, chemometric methods such as principal component analysis (PCA) and cluster analysis (CA) were applied in order to facilitate discrimination and provide more extensive analyses of the obtained data. Lastly, the in-lab built ATR spectra library, enabling identification of all investigated samples, was tested by analyzing traces of random lipsticks on six different, potentially interfering surfaces.

2. Experimental

The solutions (isopropanol and methanol) used throughout the experiments were supplied by Sigma-Aldrich (Germany). 38 red lipsticks (for 5 randomly selected 5 items of the same index number were bought) of a very similar hue representing 20 different manufacturers were either purchased in local shops or donated by *Inglot Sp. z o.o.* (cosmetics company). Detailed information about all of them is presented in Table 1.

The experiments were carried out using a Thermo Nicolet iS50 FTIR (Thermo Fisher Scientific Co, Waltham, MA, USA) with a Smart Orbit micro-ATR accessory. All spectra were collected from 650 to 4000 cm^{-1} by putting small amounts of a sample (approximately 10 mg) on the ZnSe crystal and carefully pressing it with an ATR pressure tower. After each measurement, the surface of the crystal was cleaned with 50% v/v isopropanol and then with methanol in order to avoid contamination. A new background was collected prior to the analysis of each new sample.

Processing of the obtained spectra was carried out using Thermo Electron's OMNIC 9 software. All statistical analyses were performed using Statistica 12.5 PL software (StatSoft, Tulsa, OK, USA) and OriginPro 2017 software (OriginLab Corporation, Northampton, MA, USA).

3. Applied chemometric/computational methods

3.1. Principal component analysis

PCA is a well-known multivariate statistical method enabling reduction of the dimensionality of the original dataset by creating a linear combination of variables called principal components (PCs). However, the determination of the optimal number of components that should be chosen is a crucial step. Inappropriate estimation of PCs could lead to loss of information. The Scree test, Kaiser's stopping rule and the percentage of cumulative variance are the most frequently used strategies [30], and these were applied in this study. Furthermore, PCs are a very useful tool for demonstrating the relationships between analyzed samples.

3.2. Cluster analysis

In CA, samples are assembled in high dimensional space. At the beginning, each sample constitutes its own separate cluster, and then two objects, which are the closest to each other, are combined. This procedure is accomplished repeatedly until all samples are arranged into one cluster. It is worth pointing out that several methods for measuring similarity and combining clusters are available. Their choice depends on the investigated samples and this should be evaluated experimentally [31]. In this study, Euclidean distances were calculated and Ward's method was employed. Furthermore, in the literature, over 20 different rules for stopping an obtained dendrogram are known. In this article, Mojena's stopping rule was considered to be the most efficient.

According to Mojena's stopping rule, one should select the number of groups where the following inequality (Eq. (1)) is fulfilled

$$d_{i+1} > \overline{d} + ks_d \quad (1)$$

where d_0,d_n, d_{n-1} are distances corresponding to n, n – 1, ... 1 clusters. The terms $\overline{d}$, s_d, and k are the mean, the standard deviation of the d values, and a constant, respectively [32]. As stated by Milligan and Cooper [33], the value of k should be 1.25.

3.3. Correlation method

Correlation coefficients (CC) were computed as Pearson correlation coefficients by means of Thermo Scientific OMNIC software. It seems important to note that the used algorithm additionally included elimination of the effects of baseline variation.

4. Results and discussion

Firstly, the parameters of the experiments were optimized. It is known that increasing the number of scans reduces the signal-to-noise level of data, and enables one to distinguish small peaks from noise, and that lower resolution can have an effect on the differentiation of peaks. Therefore, 16, 20 and 60 scans, and resolutions of 4 and 2 cm^{-1} were tested. Despite the fact that only slight differences were observed between registered spectra, 20 scans per second with 4 cm^{-1} spectral resolution for both background and samples were considered to be optimal.

The homogeneity (intra-variability) of lipsticks was investigated by analyzing spectra obtained from three different parts of several randomly selected lipstick samples (L3, L10, L14, L21, L31, L32, L34 and L36) during one day. As demonstrated in exemplary Fig. 1a presenting the results obtained for three lipsticks, no visual differences were found on the spectra. Consequently, it was considered that the samples were homogeneous and their composition was stable during one day.

Additionally, during three days the inter-variability of lipstick was examined using 5 of the previously selected lipsticks. The similarity of the spectra was calculated as correlation coefficient (L10: 99.90 ± 0.04; L31: 99.91 ± 0.04; L32: 99.94 ± 0.01; L34: 99.83 ± 0.06; L36: 99.92 ± 006). The presented results provided the basis for claiming that the analyzed samples of the lipstick exhibited almost unchanged chemical composition also within a few days. Moreover, both above-mentioned experiments proved the high intra- and inter-repeatability of the proposed ATR-FTIR method.

Finally, the variation of chemical composition of lipstick samples within the same series was also investigated during one day by analyzing five items (a–e) of five samples (L10, L31, L32, L34 and L36) from the same manufacturer with the same index number. As it can be seen in exemplary Fig. 1b — the obtained spectra for samples L36 a–e almost completely overlapped. Therefore, it was ascertained that the chemical composition of analyzed lipsticks that were both produced by the same