

Review

Sample Preparation of Cosmetic Products for the Determination of Heavy Metals

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Abstract: The sample preparation of a cosmetic specimen in Cosmetic Science for the purpose of determining the analytical composition of heavy and toxic metals such as lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) is of particular importance due to the difficulty of handling the sample. There are two main methods of sample preparation. The first method is the wet digestion of the sample with strong acids such as H_2SO_4 , HNO_3 , HF, and HNO_3/HCl (1:3) and the combination of a strong acid with H_2O_2 . Liquid digestion of the sample under the influence of strong acids damages the organic material of the sample and converts the carbon into carbon dioxide. The contained metals are oxidized to the highest oxidizing step and converted to soluble salts. A problem with this method is the loss of metals during digestion because it occurs at high temperatures as well as the decrease in the concentration of the residual acid. The second method of preparation is the wet liquid digestion of the sample with strong acids in a microwave oven in a closed vessel. The acids that are used are mainly HNO_3 or mixtures of acids such as HNO_3-HCl and $HNO_3-H_2SO_4$. When the sample in the acid's solvent is exposed to microwave energy, it can reach temperatures substantially above the boiling temperature of the acid solution. The result is the decomposition of the organic material, the oxidation of the metals, and their conversion to soluble nitrates. The advantages of using microwaves are the ability to control the temperature, pressure, and loss of metals and, thus, avoid erroneous measurement results. Simultaneously with the above, extraction methods have been, for almost a decade, very effective complementary processes that we can use to enrich a sample of a cosmetic product. Liquid-liquid dispersion micro-extraction (DLLME) and solid phase extraction (SPE) are the two main methods used in sample preparation and are usually applied after the digestion process.

Keywords: chemical analysis; spectroscopy; formulation/stability; color cosmetics; wet digestion; dry digestion



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1. Introduction

This work is based on information obtained with the assistance of the ScienceDirect database and using keywords that refer to methods of pretreatment and the analysis of metals in cosmetic products. About eighty articles were evaluated in this search and the results of forty-four of them are presented.

In the international literature over the last twenty years, a significant number of articles have been presented that refer to methods of analysis of cosmetic products. Siti Zulaikha R. et al. [1], Zhixiong Zhong and Gongke Li [2], and Foster Mesko [3] have cited in their respective review articles a series of analytical methods for the different categories of compounds, i.e., preservatives, antioxidants, perfumes, and phthalates, found in a cosmetic product including heavy metals. This report focuses on providing an overview of the methods of pretreatment of cosmetic samples with the sole aim of identifying the heavy metals in them.

Metals have been found as contaminants in a range of cosmetic products, including eye shadow, whitening toothpaste, nail polish, sunscreen, foundation, lipstick, and hair

colors [4–6]. Heavy metals that pollute the natural environment are absorbed by plants and may exist in seed oils and other parts of the plants that are to be used as raw materials of cosmetics products [7].

While some metals are contaminants due to the route of the chemical synthesis of cosmetic ingredients, others serve as additives or colorants because of their specific properties. For instance, titanium dioxide has been used in the cosmetics industry as a “white pigment” and a “sunscreen agent”. Chromium (III) is used in a very small number of products as a colorant, and iron oxides are common colorants in eye shadows and blushes. Some aluminum compounds are colorants in lip glosses, lipsticks, and nail polishes. Some color additives may be contaminated by heavy metals, such as D&C Red 6, which can be contaminated by arsenic, lead, and mercury [8]. Annex II of the Cosmetics Regulation (EC) No 1223/2009 lists the heavy metals and their derivatives that are prohibited, while Annexes III, IV, V, and VI list the metals that can be used at a maximum concentration in the cosmetic products.

Three of the most reliable methods for the detection and analytical control of heavy metals are Flame Atomic Absorption Spectroscopy (FAAS), Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) [9]. The advantages of the aforementioned analytical methods include speed, good repeatability, and automatization. Before applying the appropriate analytical method, the cosmetic product undergoes some necessary pretreatments in order to release the heavy metals from the excipients and convert them to a high oxidizing state. Therefore, some processing methodologies have been developed for the isolation of heavy metals from cosmetic products prior to spectroscopic analysis.

In this way, an increase in the solubility of metals in aqueous solutions can be achieved and these metals can be measured by atomic spectroscopy (AS).

The number of samples plays a role in the success and reliability of the selected method of chemical analysis. The homogeneity of the sample is also critical. Heterogeneous samples of cosmetic products, e.g., emulsions and microemulsions (lyophilic colloids), have to be homogenized before the application of the analytical method. After sampling or homogenization if it is needed, an appropriate amount of sample is obtained and subjected to a solubilization process. The solubilization process to be followed depends on the type of component to be determined. For the solubilization of inorganic compounds such as minerals and trace elements, various procedures are selected. The most applicable methods are dry digestion, wet digestion, solid phase microextraction, and chelation solvent extraction.

Based on the above, the aim of this review is to present and classify the methods for the pretreatment of cosmetic products with regard to heavy metals as well as to describe new pretreatment methods. The new methods and combinations of older and more recent methods are usually faster, more reliable, and of a lower cost.

2. Methodologies for the Preparation of Cosmetic Products

2.1. Wet Digestion Methods with Heat and Microwaves

The wet method is mainly applied to such cosmetics products as powders, oils, soaps, and emulsions. The term wet digestion [10,11] includes all those methods by which the organic materials of a cosmetic sample, with the exception of metals, are destroyed. The degradation process is accomplished using an acid solution. The commonly used acids are nitric acid (HNO₃), sulfuric acid (H₂SO₄), and mixtures of acids.

Mesco et al. [12] used a mixture of nitric acid and hydrofluoric acid (HNO₃/HF) for the determination of cadmium (Cd) and lead (Pb) in lipsticks. For the digestion, a mixture of HNO₃ (6 mL) and HF (2 mL) was used for each sample. The samples were placed in polytetrafluorethylene (PTFE-TFM) autoclave shells of microwave digestion equipment. The program was adjusted to 750 Watts for 10 min and 0 Watts for 20 min. After cooling, the samples were diluted with distilled water and the quantity analysis of Cd and Pd was performed by ICP-MS. The results of the analysis were from <3 to 31 ng g⁻¹ Cd and from

271 to 2250 ng g⁻¹ Pb. Additionally, the combination of an acid with hydrogen peroxide (H₂O₂) increases the oxidizing capacity of the acid. The determination of heavy metals in soaps and skin-lightening cosmetics is performed by pretreatment using mixtures of nitric acid and hydrogen peroxide (HNO₃/H₂O₂). Endah et al. [13] reported the determination of palladium (Pd) in cosmetics soaps using a mixture of HNO₃ and H₂O₂ for the sample digestion. Ten samples of different soaps were digested by a mixture of HNO₃ 65% *w/w* (15 mL) and H₂O₂ 30% *w/w* (15 mL) by heating in open glass flasks at 100 °C until a clear solution was formed. After the sample's destruction, the mixtures were diluted, homogenized, and filtered. The quantitative analysis was performed by AAS. The amounts of Pd ranged from 6.4 µg g⁻¹ to 22.4 µg g⁻¹.

Another mixture of nitric acid, hydrofluoric acid, and hydrogen peroxide (HNO₃/HF/H₂O₂) for the digestion of skin-whitening cosmetics and heavy metals determination was presented by Alqadami et al. [14]. According to the method, fifteen skin-whitening cosmetics were digested using microwave digestion equipment. The samples were transferred to a microwave-assisted digestion Teflon vessel followed by the addition of HNO₃ (5 mL), H₂O₂ (2 mL), and HF (1 mL). The heating program was set to 195 °C and the power/time setting (450/10, 0/2, 300/10, 350/15) was adjusted. Afterwards, each sample mixture was filtered and diluted by deionized water. The quantitative analysis was performed by ICP-AES. Bismuth (Bi) was detected in five samples and the obtained amounts ranged from below the LOD to 7097 µg g⁻¹. In comparison with the amount of Cadmium (Cd), it was detected in six cosmetics and the amounts varied between 0.20 and 0.6 µg g⁻¹. In nine samples, the amount of Cd was below the LOD. Mercury (Hg) was detected in ten samples in an amount that ranged between 0.70 and 2700 µg g⁻¹. In five samples, Hg was below the LOD. Lead (Pb) was detected in 11 of the samples and the concentration ranged from 1.2 to 143 µg g⁻¹. In four samples, Pb was detected at a concentration below the LOD. Titanium (Ti) was detected in all fifteen samples and the concentration ranged from 2.0 to 1650 µg g⁻¹. Arsenic (As) was detected in seven analyzed samples, and the amounts varied from below the LOD to 12.30 µg g⁻¹.

The wet digestion process takes place in special devices known as microwave digesters. The cosmetic sample is placed in the digester with the acid or the mixture of acids and possibly H₂O₂. Then, in order to achieve the quantitative decomposition and destruction of the organic materials, the digesters are placed in autoclaves (shells) made of Teflon (TFH), where they are then heated with the help of microwaves [15,16]. The increase in the temperature results in an increase in the pressure inside the autoclave system and therefore an increase in the reactivity of the acids. At this point, it should be emphasized that during the digestion process the maximum degree of carbon removal from the organic material must be achieved, because residual carbon can cause interference during the analysis of the sample. The lower the residual levels of carbon in the final solution, the greater the accuracy and repeatability of the analysis [17].

The combination of wet digestion and the use of microwaves allows for the degradation of the excipients of the cosmetic product and the dissolution of the metals. In practice, this is achieved by taking a small amount of sample and a small amount of each strong acid, while the process is carried out at a higher speed to avoid partial or total loss of volatile compounds of heavy metals [18]. The digestion can be done safely since the microwave digester generally has:

1. a safety system to automatically stop the microwave transmission when the door is not properly closed or opened during operation or, finally, when it detects a leak in a sample container;
2. a microwave cavity made of stainless steel coated with a suitable PTFE (PFA) material resistant to the use of acids and organic solvents; and
3. a gas exhaust system whose surface is made of materials resistant to corrosion by acids and organic solvents.

In addition, the shells are resistant to a maximum pressure of 7 bar and temperatures up to 200 °C. The high-purity synthetic material (Teflon) from which the shells are made

is suitable for the use of solutions of strong acids and organic solvents, while their safe enclosure is ensured as well as the possibility of automatically decreasing the pressure when it exceeds the safety limit. After the procedure, the clear solution of the resulting sample is diluted with distilled water in a volumetric flask to become the final solution of unknown concentration.

2.1.1. The Action of Acids

The action of acids on the dissolution of metals is essentially a redox reaction in which the metal is oxidized to a cation and the acidic hydrogen is reduced to elemental hydrogen that is released (Figure 1). In this way, only the elements that are more electronegative than hydrogen are solubilized according to the typical redox potential. The solubilization of metal compounds found in cosmetic products is a combination of neutralization and redox reactions for the oxidation of the hydrocarbon organic matter $(CH_2)_n$.



Figure 1. The action of acids on the organic matter and the dissolution of metals.

Dissolution with Nitric Acid (HNO_3)

One of the main reasons that makes nitric acid (HNO_3) preferred in the digestion of samples is its simultaneous function as an acid and as an oxidant (Figure 2). As a strong acid (Reaction 1), it can dissolve inorganic metal oxides contained in cosmetic samples, and as an oxidant (Reaction 2) it can oxidize zero-valence metals and convert them into the soluble ionic form as shown in the following reactions.

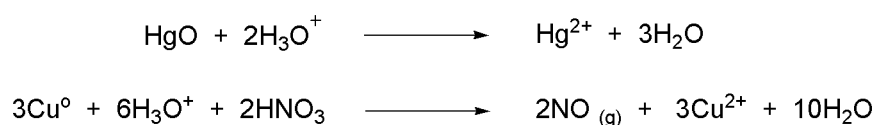


Figure 2. Simultaneous function of nitric acid as an oxidant and as a strong acid.

Another advantage of using nitric acid is the fact that its salts are soluble in water. In addition, nitrate ions (NO_3^-) constitute a desirable substrate in atomic absorption spectrometry (AAS) and in the ICP-OES technique.

Dissolution with Nitric Acid (HNO_3) and Hydrogen Peroxide (H_2O_2)

Nitric acid (HNO_3) dissolves completely in water and oxidizes all metals except for the noble ones. It is used for the dissolution of inorganic and organic samples, although in most cases the decomposition of organic matter is not satisfactory. Metal nitrates are soluble in water and are used to prepare standard solutions. Commercially concentrated nitric acid contains 65%–69% HNO_3 . The content of evaporating nitric acid is more than 69%. When it contains more than 97.5% HNO_3 , it is called white nitric acid, while the red vapor contains more than 86% HNO_3 and 6%–15% nitrogen oxides. Concentrated nitric acid is unstable. Light and heating leads to the degradation of the acid, giving molecular oxygen O_2 , water (H_2O), and nitrogen dioxide (NO_2) (Figure 3). The NO_2 is responsible for the deep brown color of the acid solutions.



Figure 3. Decomposition of nitric acid.

Hydrogen peroxide (H_2O_2) is a potent oxidizing agent and its role in redox reactions depends on the relative oxidizing/reducing strength of the other reactor and the acidity of the reaction mixture (Figure 4). According to Nernst's equation, the oxidizing power

(E_0 : redox potential in Volts) of an oxidizing agent depends additionally on the pH of the solution. The oxidizing activity of H_2O_2 in acidic solutions is $E_0 = + 1.776$ Volts, while in alkaline solutions it is $E_0 = + 0.878$ Volts [19].



Figure 4. Oxidation effect and redox potential of hydrogen peroxide.

2.2. Dry Ashing Method

Ash is the inorganic residue remaining after the heating of the sample in the presence of oxidizing agents and provides an indication of the total amount of minerals within a sample. Dry digestion or dry ashing is the heating of the sample in a controlled atmosphere with the aim of quantitative decomposition of the organic matter and conversion of the inorganic components into ash [20] as described in the scheme shown in Figure 5, whereas the heavy metals are converted by the atmospheric oxygen into metal oxides (Figure 6).



Figure 5. Oxidation of organic matter by the dry ashing method.

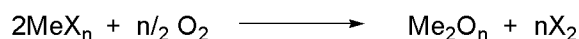


Figure 6. Transformation of metals into metal oxides by dry ashing digestion.

Dry ashing is followed by solubilization of the solid residue, usually under acidic conditions, as presented in Figure 7, and the various trace elements in the resulting solution are determined by atomic spectroscopy (AS).



Figure 7. Solubilization of metal oxides and metal ions with nitric acid.

The parameters that determine the final process of the methodology are: (1) the nature and the weight of the sample; (2) the components to be determined; (3) the temperature of the reaction; (4) the heating time; and (5) the pan for thermal analysis. The temperature should be neither too high nor too low. At low temperatures, there is a possibility of incomplete incineration. At very high temperatures, there is a risk of loss of heavy metals. In both cases, the quantification of the heavy metals is not possible.

Temperatures in the range of 500 ± 50 °C are used in many cases of digestion of cosmetic samples that are placed in a high muffle furnace. During dry digestion, water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in the air to carbon dioxide (CO_2), water (H_2O), and molecular nitrogen (N_2). On the other hand, the use of temperatures below 400 °C leads to incomplete oxidation of the sample in conventional ovens. In that case, auxiliary reagents, e.g., acids, are needed.

Dry ashing is particularly suitable for digesting cosmetic products rich in oils and wax.

Brandão et al. [21] reported the determination of lead (Pb) in a lip liner, an oil-absorbent powder, a mascara, a concealer, lipsticks, a lip gloss, and a foundation. The sample of each cosmetic was placed into a crucible and ashed in a muffle furnace at 600 °C for 1 h. The residue was cooled and digested on a hot plate at 100 °C by the addition of 15 mL of HNO_3 99% w/w. After filtration, the mixture was diluted with 2 mL of HNO_3 and deionized water. The quantitative analysis of Pb was performed by Flame Atomic

Absorption Spectrometry. The amount of Pb in the lip liner was $29.0 \pm 9.2 \mu\text{g g}^{-1}$, that in the concealer was $7.4 \pm 1.3 \mu\text{g g}^{-1}$, that in the mascara was $15.8 \pm 0.2 \mu\text{g g}^{-1}$, and that in the oil-absorbent powder was $17.3 \pm 2.9 \mu\text{g g}^{-1}$. The levels of Pb in the lipsticks, lip gloss, and foundation ranged from not detected to $73.1 \pm 5.2 \text{mg g}^{-1}$, from 4.7 to $11.7 \pm 2.8 \mu\text{g g}^{-1}$, and from 7.8 to $32.9 \pm 1.4 \mu\text{g g}^{-1}$, respectively.

In Attard et al.'s study [22], the use of auxiliary reagents ($\text{HNO}_3/\text{H}_2\text{O}_2$) for the dry ashing of nine toothpaste samples is reported. The determination of silver (Ag), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), tin (Sn), zinc (Zn), iron (Fe), and manganese (Mn) was performed by a Microwave Plasma-Atomic Emission Spectrometer (MP-AES). The dry digestion of the toothpaste samples was achieved by HNO_3 5% *w/w* (5 mL) and H_2O_2 34.5% *w/w* at 80–90 °C. Following digestion, the samples were ashed in a muffle furnace for 6 h at 500 °C. After ashing, the samples were dissolved in 5% HNO_3 . The residues were filtered and the filtrates were diluted with deionized water. The Ag was found to range between 2.0 and $5.23 \mu\text{g g}^{-1}$. The amount of Cr in all samples was between 0.28 and $7.35 \mu\text{g g}^{-1}$. The Cu ranged between 0.73 and $3.68 \mu\text{g g}^{-1}$, and the Ni ranged between 0.43 and $2.54 \mu\text{g g}^{-1}$. The range of Pb was from 2.23 to $12.04 \mu\text{g g}^{-1}$. The Sn and Zn ranged between 82.99 and $178.3 \mu\text{g g}^{-1}$ and between 0.0 and $2417 \mu\text{g g}^{-1}$, respectively. The values of Fe and Mn were determined to lie between 1.76 and $17.68 \mu\text{g g}^{-1}$ and between 0.20 and $2.07 \mu\text{g g}^{-1}$, respectively. Finally, Cd and Hg were not detected in all nine toothpaste samples.

2.3. New Extraction Methods

2.3.1. Chelation Solvent Extraction Methods for Separation of Metal Ions

Recent research studies refer to the extraction of heavy metals for the purpose of assessing substrates such as soil and seawater. The tendency of heavy metals to react with organic nucleophilic reagents is attributed to their d orbitals, which act as electron acceptors, resulting in chelate compounds. The advantage of chelates is their high stability and lipophilicity. Their high lipophilicity is due to the organic ligand, which makes the metals more fat-soluble and therefore extractable from an aqueous environment by organic solvents.

The research study [23] reports the formation of a chelate with sodium diethyldithiocarbamate (NADDC), which acts as a ligand. The resulting fat-soluble chelating complexes with Ni(II), Co(II), Cu(II), Se(IV), and Cr(VI), which were isolated by solid phase extraction (SPE) and determined by HPLC-UV.

Another study [24] reported the extraction of the heavy metals Pb(II), Cu(II), Cd(II), Zn(II), Ni(II), and Hg(II) using different ligands, such as L-5-glutamyl-L-cysteinylglycine (GCG), ethylenediamine tetra acetic acid (EDTA), and triphosphonic acid (NTTA).

The HPLC-UV technique was used for the separation and quality control of similar chelates formed by ethylenediamine-N,N-bis(2-hydroxyphenylacetic acid) (EDDHA) and diethylenetriamine penta acetic acid (DTPA) with Cu(II) [25].

Another scientific publication relates to the use of SPE to analyze the heavy metals Pb(II), Ba(II), Mn(II), Cr(III), Ni(II), Cu(II), Hg(II), Zn(II), Fe(III), and Co(II) after the formation of chelates with pyrrolidinedithiocarbamate (PDC). The chelates were chromatographed and detected by HPLC-UV [26].

The formation of complexes between metals and suitable chelating agents can be considered an interesting approach to sample preparation. In this direction, Asadi and his colleagues introduced in 2006 the method of dispersive liquid-liquid microextraction (DLLME) [27]. The most important advantages of this method are its speed, ease of use, and generally low cost of application.

For these reasons, the method has been adopted and modified by various research teams in order to be used, *inter alia*, for the determination of metals in cosmetic products.

Nageswara Rao and Nageswara Rao [28] used ammonium pyrrolidine dithiocarbamate (ADPC) to complex Pb ions in a toothpaste sample. The sample was first digested with nitric acid and hydrogen peroxide and then the lead ions were complexed with ADPC

and extracted with chloroform. The final determination was made by Atomic Absorption Spectrometry and the results were not satisfactory compared with the other methods of Pb determination presented in the article, which are based solely on the digestion of the sample. The causes of the error were identified to be the limited stability of the Pb complex in the organic solvent and the incomplete mode of extraction.

In 2011, Chen and colleagues used the DLLME method in order to determine the presence of mercury species in liquid cosmetic samples [29]. They used APDC as a chelating agent and methanol as a disperser solvent. The organic solvent (extraction solvent) was replaced with an ionic liquid and 1-hexyl-3-methylimidazoliumhexafluorophosphate (C6MIM)(PF6) showed the best extraction properties. By replacing the toxic organic solvent, the method becomes environmentally friendly, and the ionic liquid is characterized by excellent properties, such as high volatility, satisfactory chemical and thermal stability, and good solubility in many organic solvents. This method (IL-DLLME) was combined with high-performance liquid chromatography–inductively coupled plasma mass spectrophotometry (HPLC-ICP-MS) and, after optimization of the experimental conditions, the authors presented a simple, fast, and sensitive procedure for determination of mercury species in liquid cosmetic products.

The DLLME technique was used by Fattahi et al. to determine the lead in lipstick and hair dyes [30]. The method was conjugated with the solidification of floating organic drop (SFO) method. According to the procedure, the sample is initially treated using microwave-assisted acid digestion. The lead ions are chelated with diethyldithiophosphoric acid (DDTP), while 1-undecanol is used as the extraction solvent and acetone is used as the disperser solvent. Lead is complexed with DDTP and extracted into 1-undecanol in the form of drops floating in the aqueous solvent. After cooling in an ice bath, the droplets solidify on the surface and are then transferred to a different vial from which a sample is taken for the quantitative determination of the lead by the graphite furnace atomic absorption (GFAAS) method. The final results showed that, in addition to the satisfactory performance of the process of extraction of lead from the sample, the whole of the applied method provides satisfactory results in terms of the linearity of the method in the examined concentration range.

A year later, Daşbaşı and his colleagues presented the application of DLLME in the detection of bismuth (Bi) in a number of samples, including hair dyes [31]. Initially, a sample is treated with nitric and perchloric acid. Then, using crystal violet as a counterpart ion for the anions of bismuth tetrachloride (BiCl₄⁻), ethanol as the dispersing solvent, and chloroform as the extraction solvent, the Bi(III) ions are extracted from the aqueous solution. After centrifugation, a sample is taken from the chloroform layer, which contains the complexed ions, to determine the bismuth concentration using atomic flame absorption spectrometry (FAAS).

Sacmaci and Sacmaci, in two research studies, used DLLME to determine the concentration of cadmium and nickel ions in a number of cosmetic products. In their first study, the samples included lipstick, moisturizers, lotion, and make-up products [32]. After the completion of the digestion process, the sample was treated using methanol and chloroform as the disperser and the extraction solvent, respectively, and methyl-5-[(Z)-pyridin-4-yl-diazenylquinolin-8-ol (MPQ) was used as a chelating reagent. The pH was adjusted to 8 using NH₃/NH₄Cl. In their second study, the determination of nickel in lipstick, lip gloss, hand creams, and shaving foams is presented [33]. The methodology used was the same as in their first study, but as a chelating agent they used 5-[(Z)-isoxazol-3-yl-diazenyl]-2-methyl-quinolin-8-ol (MMD). MMD was shown to be a powerful and selective means of binding Ni ions. In the above-described procedures, the digestions were done by treating each sample with nitric acid (HNO₃) and hydrogen peroxide (H₂O₂) and the determination of the metal ions was achieved by flame atomic absorption spectrometry (FAAS).

An alternative method of processing and enriching a sample is the cloud point extraction method (CPE), which was introduced by Watanabe and colleagues in 1972 [34]. The method is characterized as a very simple, economical, and environmentally friendly process.

The theoretical principle of the method, which over the years has changed and evolved significantly, is based on the use of aqueous solutions of surfactants that show turbidity if a factor such as temperature or pressure changes or a substance is added. In the process of an analysis, this is usually achieved by increasing the temperature. The observed turbidity is mainly due to the micelle formation of surfactants. As a result, two phases are created: the surfactant-rich phase, which contains the analyte; and the aqueous phase. The final separation of these two phases is accelerated by centrifugation. It should be noted that in the determination of metals the use of chelates helps in the binding of the analytes and in the transport of the corresponding complexes within the micelles.

In this context, Altunai and Gurcan [35] used microwave-assisted cloud point extraction (UA-CPE) to determine the tin (Sn), lead (Pb), and cadmium (Cd) metal ions in cosmetic products. They used Tween 60 (polyethyloxysorbitanmonostearate) as the surfactant, which has non-ionic properties, and the complexation was done through Victoria Pure Blue BO (VBO+), which forms stable complexes with most metals depending on the pH. In this case, the pH of the solution was adjusted to 8.5, where the metals are in anionic and neutral forms ($\text{Pb}(\text{OH})_3^-$, $\text{Sn}(\text{OH})_3^-$, $\text{Cd}(\text{OH})_2$, or/and $\text{Cd}(\text{OH})_3^-$). At the same time, cetylpyridinium chloride (CPC) was used as a cationic surfactant to enhance the sensitivity of the method but also as a counterpart ion in the complex formation stage. CPC can also help transport the complexes inside the micelle. During the above-described preparation of the sample, after digestion with acids the pH was raised to 8.5 followed by the addition of CPC, Tween 60, and VPB+. After sonication for 60 min, the solution became cloudy and the formed complexes were extracted in the Tween 60 phase (the micelle phase). The phase separation was achieved by centrifugation and the final determination was done by atomic flame absorption spectrometry (FAAS). The authors of the article present an analysis of the conditions that affect the effectiveness of the method, concluding that it is a simple and effective analytical method. The authors also applied the method to a number of cosmetic products, such as shampoo, conditioner, hair dyes, lipstick, nail polish, skin cleansers, foundation, eyeliner, gel, and powder. The validation of the method was performed using two certified reference materials. Results on linearity, detection limits, and relative standard deviations were compared with similar results from other methods used for the determination of the above analytes reported in the literature. From this comparison, the proposed procedure showed a lower or comparable detection limit, good precision, and a high sensitivity enhancement factor. It should also be mentioned that the method has a low cost and the chemicals have (relatively) low toxicity. The experimental results for the determination of the mean metal contents, arranged in decreasing order, showed that: for hair dye, nail polish, and skin cleanser, $\text{Cd}(\text{II}) > \text{Pb}(\text{II}) > \text{Sn}(\text{II})$; for shampoo, foundation, gel, and lipstick, $\text{Pb}(\text{II}) > \text{Sn}(\text{II}) > \text{Cd}(\text{II})$; for powder and eyeliner, $\text{Cd}(\text{II}) > \text{Sn}(\text{II}) > \text{Pb}(\text{II})$; and for conditioner, $\text{Sn}(\text{II}) > \text{Cd}(\text{II}) > \text{Pb}(\text{II})$.

2.3.2. Solid Phase Microextraction as a Tool for Trace Element Determination

SPME is a new sample preparation technique. The method has the advantage that it is not necessary to use organic solvents for the extraction and acquisition of organic compounds as required by conventional extraction using a two-phase water/organic solvent system. SPME [36] is also applied in cosmetology as a pretreatment method for the isolation of organic compounds, such as preservatives (parabens), UV filters, and dyes, from other components. After their isolation, qualitative and quantitative determination are possible with instrumental analysis methods such as HPLC-UV and LC-MS.

For heavy metals, solid-phase extraction methods with carbon nanotubes, Fe_3O_4 (a material with magnetic properties), and amalgamation have been reported. In the case of metals in cosmetics, Algadami et al. [37] presented a new, highly sensitive method used to detect arsenic, cadmium, bismuth, arsenic, lead, and titanium in whitening creams. The

method consists of the initial digestion of the samples with acids, which takes place with the help of microwaves, and the channeling of a solution containing the above product of digestion into a microcolumn. The microcolumn is made of solid-phase multi-coated carbon nanotubes, which bind metal ions to finally create an enriched sample. The retained ions are eluted with nitric acid and the analysis is completed using ICP-OES. The article also presents an analysis of the parameters that may affect the results of the method, such as pH, flow rate, and elution conditions.

In the same year, Embrahizaadech et al. [38] presented a modified form of the solid-phase extraction method used to quantify Pb and Cd in lipsticks and eye shadows. The method is based on the use of Fe₃O₄ nanoparticles, which are characterized by magnetic properties. The nanoparticles were superficially modified by the introduction of 2,6-diaminopyridine, which binds to the solid support by means of a suitable linker. 2,6-diamino-pyridine has the ability to bind the lead and cadmium ions that are formed after digestion of the original sample with strong acids. The Fe₃O₄ nanoparticles are separated from the solution by magnetization and, after treatment, which ultimately involves the effect of a small volume of a diluted acid solution (HCl in the presence of thiourea), the analytes are removed from the substrate. The final quantitative determination is achieved using FAAS. The above-described SPE method using material with magnetic properties (M-SPE) is characterized by high repeatability and a high recovery rate, while the interference of metals such as alkalis, alkaline earths, and transition elements seems to be negligible.

To conclude the presentation of the processes by which we can quantify a metal, we will dwell on the special case of mercury (Hg). Given that Hg is a metal that can pass into the gaseous state relatively easily, Saadat-zadeh and his colleagues presented a process by which we can achieve the immediate determination of Hg in a cosmetic product [39]. The test was performed on a total of 72 samples, including lipstick, mascaras, eye shadows, and eyeliner. A Milestone DMA-80 Direct Mercury Analyzer was used in the process and the sample without any further treatment was initially subjected to thermal decomposition. The Hg vapors were then dispersed and selectively bound by gold particles to form amalgams. Finally, after a sufficient amount of oxygen had been introduced into the system to remove all residual gases or by-products of decomposition, the system was heated again to re-vaporize the mercury, which was quantified by FAAS. It is essentially a process similar to that of SPE except that the component to be bound and analyzed is in the gas phase. The whole procedure is based on the protocol of method 7473 of the U.S. EPA [40].

The solid-phase microextraction (SPME) method also includes gel fiber extraction. The technique is based on the distribution of volatile components between the sample and the solid static phase (polymeric material), which is coated with the silica gel (fused silica gel) of the SPME device. A key parameter of the success of the extraction is the choice of gel fiber. The fiber can be coated with polar material or non-polar material [41]. The choice of fiber depends on the polarity of the extracted organic compound. Polar organic compounds are strongly adsorbed by the polar-material-coated silica gel fiber. Single organic compounds are strongly adsorbed by the non-polar-material-coated silica gel fiber. The adsorbed organic compound in the gel fiber can then be determined by an analytical method, such as GC, GC-MS, HPLC, or LC-MS [42,43], and with the corresponding detector, such as an Ionization Detector (ID), UV, an Electrochemical Detector (ECD), a Refractive Index Detector (RID), AAS, or ICP. The method is selected based on the physicochemical properties of the substance, such as the solubility, volatility, ionization, absorption–emission of radiation, change in redox potential, and change in refractive index. The SPME technique is used for liquid, gaseous, and solid samples and isolates volatile and semi-volatile components.

Quantitative extraction of toxic metals, such as Hg(II), Pb(IV), Cd(II), and Sn(IV), can be achieved by the gel fiber method after the organometallic compounds are first modified. The gel fiber coating material should have lipophilic properties so that organometallic molecules that are lipophilic can be extracted. The Grignard reaction is suitable for the alkylation of metal ions (Figure 8).

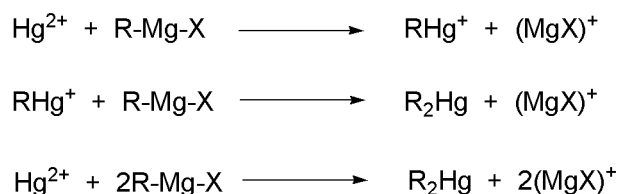


Figure 8. Alkylation of mercury ions by the Grignard reaction.

The choice of analytical method depends on the physicochemical properties of the organometallic compound. The fact that organometallic compounds are very volatile at high temperatures makes them detectable by gas chromatography, and techniques that can be applied include SPME-GC-ID and SPME-GC-MS. In addition, the lipophilic nature of these compounds makes them soluble in the lipophilic organic elution systems used in liquid chromatography. In this case, a method that combines SPME and LC can be applied.

A relevant scientific study of this technique refers to the determination of heavy metals, such as Hg, Sn, and Pb, in biological materials as well as in environmental samples, such as seawater, river water, petroleum, and fish, for the purpose of their qualitative and quantitative determination [44]. The method has also been proposed for the determination of the reported metals in cosmetic products. The technique is based on the pretreatment of the sample by gentle digestion or pretreatment in an aqueous environment. The Hg(II), Sn(IV), and Pb(IV) ions contained in the sample are then converted with alkylating reagents to organometallic compounds. Then, the organic metal derivatives are isolated by the solid-phase microextraction technique (SPME). The high solubility and strong adsorption of organometallic compounds in the gel fiber (static phase) are the main advantages of the method. The organometallic compounds that were adsorbed into the gel fiber are then introduced into the GC injector and, due to their high temperature and volatility, are released and enter the analysis system. The analytical techniques used for the determination are GC, GC-MS, and GC-coupled atomic absorption (GC-AAS) or GC-coupled plasma emission (GC-ICP). As a summary Table 1, lists the extraction methods for the analysis of heavy metals in cosmetics products.

Table 1. Extraction methods for the analysis of heavy metals in cosmetics products.

Extraction Method	Analysis Method	Metals								Cosmetic Products			
		As	Bi	Cd	Hg	Ni	Pb	Pd	Sn		Ti		
Simple Extraction	FAAS										V	Toothpaste [28]	
IL-DLLME	HPLC-ICP-MS				V								Hand moisturizing lotion, skin refreshers [29]
	GFAAS										V		Hair dyes, lipsticks [30]
	FAAS			V									Hair dyes [31]
DLLME	FAAS			V		V							Lipstick, lotion, make-up products, moisturizing cream [32]
	FAAS					V							Hand cream, lip gloss, lipstick, shaving foams [33]
UA-CPE	FAAS			V					V	V			Conditioner, eyeliner, gel, hair dyes, lipstick, nail polish, powder, shampoo, skin cleanser [35]
SPME (on carbon nanotubes)	ICP-OES	V	V	V	V		V					V	Whitening creams [37]
M-SPE (with Fe ₃ O ₄)	FAAS			V				V					Lipstick, eyeshadow [38]
Amalgamation	FAAS				V								Eyebrow pencils, eyeshadows, lipsticks, mascaras [39]

3. Conclusions

The digestion process is by far the most important method for the pretreatment of a sample. In the case of the analysis of metals in cosmetic products, in the review article presented by Zulaikha et al. [1], the determination is made through the process of digestion. Over the last decade, however, extraction methods have also provided a significant alternative and can be used as a tool for the successful pretreatment of the sample.

Other alternative methods are currently under development. Our group is currently working to develop a method for the analysis of cosmetics based on the transformation of metals into organometallic compounds, a pretreatment process that is already used on other substrates. For cosmetic products, it is important to investigate the development of this method with the aim of reducing the cost of analysis, increasing the speed, and simplifying the quantification. In this direction, GC and GC-MS could be complementary analytical techniques to AAS and ICP.

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