Permanent Make-Up (PMU) Inks Decolorization Using Plant Origin Materials

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Abstract: Permanent make-up (PMU) has become a very popular application over the last few years. The ingredients of PMU inks, used over the face area, are organic and inorganic substances very close to the chemical composition of tattoo inks. As the application rates increase, the demand for PMU removal rises. The aim of this study is to assess the decolorization of PMU inks using preparations originating from different plant sources. The leaves of Pelargonium zonale (PE) were extracted with water for 48 h. The Total Phenolic Content (TPC) of the extract was determined using the Folin–Ciocalteu technique reaching 201.34 ± 4.57 µg Gallic Acid Equivalents (GAE)/mL of extract. The antioxidant activity of the extract was 20.87 ± 0.36 µg of Trolox equivalents (TE)/mL and 3.56 ± 0.43 mg FeSO$_4$·7H$_2$O/mL of extract when assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) or ferric reducing antioxidant power (FRAP) assay respectively. The decolorization potential of PE leaf extract on five commercially available PMU inks of different hues was assessed by UV-Vis spectrophotometry in comparison to polyphenol oxidases enzyme (PPO). The results demonstrated higher absorption reduction that indicates decolorization potential for the inks that have mainly ferrous oxides as colorants.

Keywords: permanent make-up inks; PMU removal; polyphenol oxidase; Pelargonium zonale leaf extract

1. Introduction

Permanent Make-up (PMU) such as eyeliner, brow liner, and lip liner have become increasingly popular throughout the world. The popularity of PMU has increased the number of PMU artists all over the world and PMU applications but has also led to an increased demand for its removal. Laser removal is a method that cannot be used in every case of PMU application, as the face area is sensitive and laser-associated side effects may develop [1]. It is also an expensive procedure that needs repeated painful treatments to be completed. Consequently, PMU wearers prefer easier, faster and cheaper procedures for PMU removal. Many liquid products promising painless and fast PMU removal are commercially available [2,3].

PMU inks are liquid formulations that consist of a variety of components, with dyes or pigments being the main ingredients. The most common excipients that may be found in PMU inks are water, glycerol or ethanol. The inks cover a wide range of colors and shades and can be used on their own or carefully mixed to achieve the desired hue. They can be applied either manually or via specially designed instruments (PMU machines) [4].
PMU inks contain pigments but also binders, solvents, surfactants, preservatives and thickening agents. Surfactants adsorb to the surface of pigment agglomerates and decrease the surface tension of the solvent [5–7].

The main difference between PMU and tattoo inks is based on their particle size which is one of the main reasons that determines their duration. PMU inks consist of colorants with larger particles that, even if they are immobilized on the site of application by the immune system, last from a few months up to several years and the application needs to be repeated [8,9]. The colorants of tattoo inks have smaller particle sizes that cannot be broken down and are placed deeper into the dermis. Their application lasts for a lifetime, even if the color changes over the years due to many reasons such as tattoo artist experience, equipment type and quality, or sun exposure [9,10].

Although the PMU is very popular, there is also a high degree of dissatisfaction among the persons who have decided to alter a facial characteristic such as eyebrows, eyelashes, and lips, due to many low-quality applications. The correction of mistakes during the PMU application, such as wrong color choice, bad shape, or wrong site of application, is achieved by PMU removal approach.

It is a fact that PMU application to the face is a relatively easy procedure, but its removal is a difficult task. In order to remove unwanted PMU, the use of a laser can give good results over the eyebrows as the skin on this area is thick and can cope with the numerous laser treatments that are needed [11,12].

On the other hand, this technique cannot be used safely over the eyelids because the skin of this area is very thin and sensitive. Furthermore, due to their proximity, the eyelashes may be damaged by the laser. The skin of the lips is thicker and stronger but the results of treatments with Q-switched and picosecond lasers largely depend on the specific ink composition and previous repetitions of PMU application that creates multiple layers of colorants deposition. Especially for ferric oxide (Fe₂O₃) and titanium (IV) dioxide (TiO₂), laser irradiation may paradoxically cause a darkening as a result of oxidation–reduction reactions. In this case, multiple laser treatments are necessary to remove them. Ablative lasers, on the other hand, do not present these unwanted side-effects, but may pose the risk of scarring if not properly applied [12,13].

As an alternative to the laser method of PMU removal, many commercially available tattoo removal products have been developed so far mainly by companies that produce PMU inks.

The removal procedure is the same as the PMU application, but instead of PMU colors, the PMU remover is applied [13,14].

Many of the PMU removers contain plant origin extracts with decolorization capacity. Commercial PMU decolorization products that have been used in recent years usually contain small percentages of zinc oxide, magnesium oxide, calcium oxide, triethanolamine, n-propanol, benzoic acid, glycolic acid, citric acid, malic acid, and other substances that could create scars after the removal procedure [15].

Plant extracts with natural decolorization capacity are the latest trend in PMU removal. Today, a wide range of PMU inks or decolorization products are available. In some cases, they are based solely on plant extracts or claiming to be “totally organic”, a marketing-driven rather than chemical nature characterization [13–16]. Unfortunately, because these products are not under cosmetics legislation, the reference of the list of ingredients on the product’s label is not mandatory. Very little information is available about the ingredients or the purity of these products. This fact poses a considerable risk on consumers’ health, as their application is performed intradermally [2,17,18]. According to the new legislation in European Union that was set in action after 4 January 2022, the use of more than 4000 hazardous chemicals in tattoo and permanent makeup inks will be limited. Several substances or groups of substances used in tattoo or PMU inks such as certain azo dyes, carcinogenic aromatic amines, polycyclic aromatic hydrocarbons, metals, methanol, skin sensitizers and preservatives are allowed only at maximum concentration limits [19–21].
Similar restrictions are set for substances that, in the Cosmetic Products Regulation (EC) No 1223/2009, are included in Annex II (substances prohibited in cosmetic products) and in Annex IV as prohibited to be used in products applied on mucus membranes or in the eye or allowed only in rinse-off products and other conditions, such as for purity, listed in Annex IV [22].

In this study, the decolorization ability of polyphenol oxidase (PPO) and of *Pelargonium zonale* leaf extract (PE) were investigated against commercially available PMU inks. PE’s characteristics, such as its phenolic content and antioxidant activity, that may affect its action in PMU decolorization were investigated. Its decolorization potential was assessed in vitro on commercially available PMU using PPO as control [23,24].

*Pelargonium* belongs to the family *Geraniaceae*, a genus of about 250 species of perennial tiny shrubs with aromatic leaves, native to South Africa. *Pelargonium zonale* is cultivated as an ornamental, perennial, shrubby, pubescent plant with rounded-cordate leaves and zygomorphic flowers with a sweet-balsamic odor. *Pelargonium* sp. is well-known for its distinctive aroma, which is found in perfumery, cosmetics, and aromatherapy goods. Traditional medicine made use of leaves, tubers, and roots. According to phytochemical research, *Pelargonium* species are rich in phenolic compounds such flavonoids, tannins, phenolic acids, and coumarins and have antioxidant, antibacterial, immunomodulatory, topical hemostatic, and anti-sickling properties [25].

The components (colorants and additives) or even the impurities that are present in tattoo inks may generate singlet oxygen in presence of UV light [26], while in absence of light the aggregation of tattoo ink particles may induce the production of reactive oxygen species (ROS) [27]. Antioxidants such as phenolic compounds have an effect on the protection of the tattooed skin area skin during the decolorization process from ROS [28].

2. Experimental

2.1. PMU Inks Materials and Methods

Five PMU colorants for different facial applications like lips (I), eyebrows (II, III, V) and eyelids (IV), were bought from local suppliers. Their ingredients were recorded, and further information was collected from CosIng, ECHA and NICNAS (Table 1) [29–32].

**Table 1.** Colorant ingredients of the tested PMU inks.

| CI     | Annex IV # | Ingredient Name | CAS            | Chemical Structure | Oxidation Number | T.D. Lash Pink I | T.D. Sunset II | S20 N.C. III | Dark Brown IV | S10 V |
|--------|-------------|-----------------|----------------|-------------------|------------------|------------------|----------------|--------------|--------------|-------|
| CI 77791 | 143         | Titanium dioxide | 13463-67-7     | TiO₂              | Ti(IV)           | ✓                | ✓              | ✓            | ✓            | ✓     |
| CI 56110 | N/A         | Pigment Red 254  | 84632-65-5     | N/A               | N/A              | ✓                | ✓              | ✓            | ✓            | ✓     |
| CI 12466 | N/A         | Pigment red 269  | 67990-05-0     | N/A               | N/A              | ✓                | ✓              | ✓            | ✓            | ✓     |
| CI 77491 | IV/135      | Red Iron Oxide   | 1309-37-1/1317-61-9/1345-27-3/52357-70-7/1345-25-1 | Fe₂O₃             | Fe (III)         | ✓                | ✓              | ✓            | ✓            | ✓     |
| CI 77288 | IV/129      | Chromium Oxide Green | 1308-38-9   | Cr₂O₃             | Cr (III)         | ✓                | ✓              | ✓            | ✓            | ✓     |
2.2. Preparation of PE

For the preparation of PE, 10 g of fresh leaves were mixed with 100 mL distilled water and left for 48 h at 20 °C. The mixture was filtered through filter paper and kept at 4 °C until further use.

Determination of Total Phenolic Content (TPC)

The Folin–Ciocalteu technique was used to determine the total phenolic content of PE [33]. Briefly, in a 96-well plate, we mixed 20 μL of appropriate dilutions of the sample, with 180 μL pure water, 20 μL Folin–Ciocalteu reagent, and 20 μL of Sodium Carbonate (13.75%), and the mixture was incubated for 30 min at room temperature in the dark.

After the incubation period, the absorbance was measured at 750 nm using a UV/vis microplate reader (Sunrise, Tecan Austria). Appropriate concentrations of gallic acid (20–200 μg/mL) were used as a positive control. Utilizing the regression relationship between the concentration of gallic acid and absorbance, a standard calibration curve was created, and the findings were presented as μg of Gallic Acid Equivalents (GAE)/mL of extract. 

$$y = 0.0035x - 0.005; R^2 = 0.9944$$

2.3. Antioxidant Activity

Two separate tests were used to determine the antioxidant activity of the aqueous extract: Scavenging Activity on DPPH Free Radical and the Ferric-reducing antioxidant power (FRAP) assay.

2.3.1. DPPH Method

The extract’s free radical scavenging activity was assessed using the DPPH technique with certain modifications. A total of 5 μL of appropriate dilutions of extract (or standard) was mixed with 195 μL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, in a 96-well plate. Trolox was used as a positive control in a concentration range of 1925–500 μg/mL [34]. The absorbance at 540 nm was measured after a 30 min incubation period far from light, at room temperature.

Using the following equation, the DPPH scavenging effect was determined as the percentage of DPPH decolorization: 

$$\% \text{DPPH Scavenging activity} = ((A \text{ blank} – A \text{ sample})/A \text{ blank}) \times 100,$$

where A is absorbance. The results were expressed as Trolox equivalents (TE)/mL of the extract, using the regression relationship between the
concentration of Trolox and the absorbance at 540 nm; \( y = -0.0384x + 0.9083 \), \( R^2 = 0.9905 \).

In our experiment, the concentration of Trolox that scavenges the DPPH free radical at a percentage of 50% (IC50) was 12.17 \( \mu \text{g/mL} \) (calculated using GraphPad Prism 6, GraphPad Software, CA, USA) and is in accordance with the literature [35,36].

2.3.2. Ferric Reducing Antioxidant Power (FRAP) Assay

With minor adjustments, the sample’s ability to reduce Fe3+ was measured according to the protocol published by Benzie IF, Strain JJ, 1996 [37]. Before each experiment, 300 mM acetate buffer pH = 3.6, 40 mM HCl, 2,4,6-Tripyridyl-s-triazine (TPTZ) 10 mM and an Iron (III) chloride hexahydrate (FeCl3 $$\cdot$$ 6 H2O) 20 mM were freshly prepared.

The FRAP reagent was prepared by mixing 75 mL acetate buffer with 15 mL TPTZ solution and 15 mL of FeCl3 $$\cdot$$ 6H2O solution. FeSO4$$\times$$7H2O was used as a standard in a concentration range from 0.1–0.5 mM. In a 96-well polystyrene microplate, 60 \( \mu \text{L} \) of adequately diluted sample/standard/blank, 56 \( \mu \text{L} \) of acetate buffer, and 180 \( \mu \text{L} \) of FRAP reagent were added, mixed, and incubated for 10 min far from light. At the end of the incubation period, the absorbance was measured at 594 nm using UV/vis microplate reader (Sunrise, Tecan Austria). The results were expressed as mg FeSO4$$\times$$7H2O/mL of extract and were calculated using the FeSO4$$\times$$7H2O standard curve; \( y = 3.9026x - 0.0042 \), \( R^2 = 0.9973 \).

2.4. Monitoring the Decolorization Potential by Spectrophotometry

The decolorization potential of PE and PPO was assessed using spectrophotometry [23].

Commercially available PPO isolated from mushroom (Agaricus bisporus) was used as control in homogenized 0.1 M potassium phosphate buffer [23,38]. The absorption spectrum of each ink was monitored by UV-Vis spectrophotometry. Solutions of 10 mg/mL in deionized water were prepared for each ink. Their absorption spectrum was obtained scanning then at a range of 200–400 nm in a UV-1800 UV-Vis Spectrophotometer (SHIMADZU, Kyoto, Japan). Aqueous solutions (10 mg/mL) were prepared for each ink. PPO solution in water in concentration 20 \( \mu \text{L} \) of each colorant diluted in 100 \( \mu \text{L} \) H2O. PMU ink solutions were mixed with PE or PPO solution in 1/10 (v/v) ratio and incubated for 60 min at 37 °C. The absorption spectrum of each mixture was obtained by ultraviolet-visible (UV/Vis) spectrophotometry (UV-1800 UV-Vis Spectrophotometer, SHIMADZU, Kyoto, Japan). The decolorization potential of each medium was assessed by calculating the mean change percentage of absorption (A) maximum in the visible range (400–800 nm) of each colorant before and after incubation with each decolorization medium.

3. Results and Discussion

3.1. PMU Composition

The detailed information on the colorants that were mentioned on the labels of the five PMU inks of this study are summarized in Table 1. The PMU inks contained nine colorants in different combinations. They were referred by their CI indexes a system of colorant classification adopted by the Society of Dyers and Colourists and the American Association of Textile Chemists and Colorists that classifies the colorants according to their chemical structure (Table 2) [21,39,40]. The colorants were mainly metal oxides. TiO2 was present in all PMU inks, as it is used for hue brightening due to its white color and optical scattering properties.

Apart from PMU I, ferrous oxides in several oxidative numbers (Fe(II), Fe(III)] was used in all other inks). The inks III and V, that were for use on the eyebrows, contained Cr (III) oxide, while ink IV, which was used for eyelids, contained carbon black. Three PMU inks (I, II, V) contained organic molecules as colorants. Specifically, inks I and II contained CI 56110, ink I had also CI 12466 in its formula, and ink V contained CI 56300.
Table 2. Color index (CI) system of classification of colorants.

| Type of Colorants       | CI Numbers       | Chemical Structure                                                                 |
|-------------------------|-----------------|-------------------------------------------------------------------------------------|
| Organic molecules       | 11000–19999     | Azo dyes                                                                            |
|                         | 20000–39999     | Diazo, Triazo, Polyazo and Azolic dyes                                              |
|                         | 40000–74999     | stilbenes, diarylmethanes, triarylmethanes, xanthenes, acridine, quinolones, methines, thiazoles, indamines, indophenols, azines, oxazines, thiazines, aminoketones, thiazoles, indamines, indophenols, azines, oxazines, thiazines, aminoketones, anthraquinones, indigoids and phthalocyanines |
| Naturally occurring dyes| 75000–75999     | Animal, Curcuminoid and Plant dyes                                                  |
| Oxidation bases         | 76000–76999     | Not applicable for tattoo and PMU                                                   |
| Inorganic pigments      | 77000–77999     | e.g., iron oxide pigments, chromium(III) oxide, Titanium dioxide, Zinc oxide          |

Even though all inorganic colorants were permitted for use in cosmetics, none of these three colorants were mentioned in Annex IV of the cosmetic regulation EU 1223/2009 [22]. The uses declared in ECHA of CI 56110 (Pigment Red 254) and CI 12466 (Pigment Red 269) are in coating products, inks, toners, and polymers. They can also be found in machine washing liquids, automotive care products, paints and coating, fragrances and air fresheners, cooling liquids in refrigerators, oil-based electric heaters, hydraulic liquids in automotive suspension, lubricants in motor oil, and break fluids. [31] Furthermore, CI 12466 is listed in Appendix 13 of Regulation (EU) 2020/2081 with concentration limit 0.1% by weight. [21] CI 56300 (Quinophthalone Yellow-Organic Pigment Yellow 138) can be found in coating products, fillers, putties, plasters, modelling clay, and polymers [30].

3.2. Total Phenolic Content and Antioxidant Capacity of the PE Extract

At a concentration of 100 mg fresh plant material/mL, the DPPH scavenging activity reached 89.30% suggesting that it could act as a free radical inhibitor or scavenger. In detail, the results of DPPH scavenging activity, FRAP assay along with the total phenolic content are presented in Table 3. In an earlier study among four Pelargonium species, P. zonale leaves had the highest antioxidant activity and the highest polyphenol content (catechin and epicatechin derivatives, cyanidol derivatives, delphinidin-3-O-rutinoside, quercetin glycosides, luteolin, kaempferol, and caffeic acid) [41].

Table 3. Total phenolic content and antioxidant capacity of the PE extract.

| Total Phenolic Content (µg/mL) | DPPH Scavenging (µg Trolox Equivalent/mL) | FRAP Assay (mg FeSO₄×7H₂O/mL) |
|-------------------------------|------------------------------------------|-------------------------------|
| 201.34 ± 4.57                 | 20.87 ± 0.36                             | 3.56 ± 0.43                  |

3.3. Decolorization Potential of PE

The UV absorption spectrum of each ink was either decreased, remained unaltered, or even increased in some cases without presenting any shift of the absorption maxima or new absorption, at least at the visible range (400–700 nm), implying that there was chemical alteration of the existing molecules (Figure 1).

In particular, ink I showed a change of absorption of −15.87% ± 2.22% or 3.74% ± 1.97% respectively (p < 0.05). In Ink II the absorption was reduced by both decolorization media (PE or PPO) by −11.40% ± 0.06% or −15.55% ± 0.00% (p < 0.05). The absorption of ink III was significantly reduced after incubation with PE compared to PPO by −80.40% ± 1.42% and −68.94% ± 1.60% respectively, (p < 0.05). PE did not present a decolorization potential for Ink IV, as absorption was increased by 48.29% ± 2.17% after incubation with PE. On the contrary, the absorption after incubation with PPO was decreased by −33.76% ± 7.89%, indicating a better decolorization potential. Ink V absorption was...
reduced by both decolorization media (PE: $-64.15\% \pm 0.90\%$, PPO: $-39.97\% \pm 0.34\%$) but PE showed a better potential ($p < 0.05$) (Figure 2).

Figure 1. Absorption spectra of the aqueous solution of each ink (1–5) after mixing them with PE (A) or PPO (B) and incubation at 37 °C for 60 min.

The results indicate that the best decolorization potential of PE and PPO was achieved on PMU inks that mainly contained inorganic colorants (inks III, V). On the contrary, the decolorization action on organic colorants (inks I, II) appeared weak.
The percentage of change of the aqueous solution of each ink after mixing them with PE (■) or PPO (■) and incubation at 37 °C for 60 min (p < 0.05).

4. Conclusions

The decolorization potential of PE on PMU inks seems to depend on their colorant composition. Tattoo inks that consisted of inorganic material, mainly of ferrous oxides, presented maximum depigmentation potential compared to tattoo inks that contained organic ingredients. The antioxidant potential of the plant extract used may diminish the harmful effects of POS and contribute to the overall skin treatment during the decolorization procedure. The use of plant-originated material as PMU decolorization agents is of great interest as a safe alternative to laser treatments and might represents a “new” step in the field of PMU removal.

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