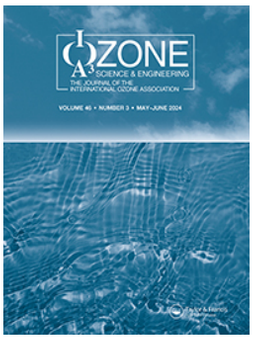

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Evaluation of the Ozone Effects on Human Hair Fiber: A Preliminary *In Vitro* Study

Pedro Augusto Laurindo Igreja Marrafa [a], Bianca Akemi Kawata [a,b], Hulair Braga Carneiro [c], Túlia de Souza Botelho [b], Henrique Cunha Carvalho [b,d], Cibele Rosana Ribeiro de Castro Lima [e], Fabio Roberto Passador [f], Carina Barros Mello [g], Adjaci Uchoa Fernandes [a,b], Adriana Barrinha Fernandes [a,b], and Carlos José de Lima [a,b]

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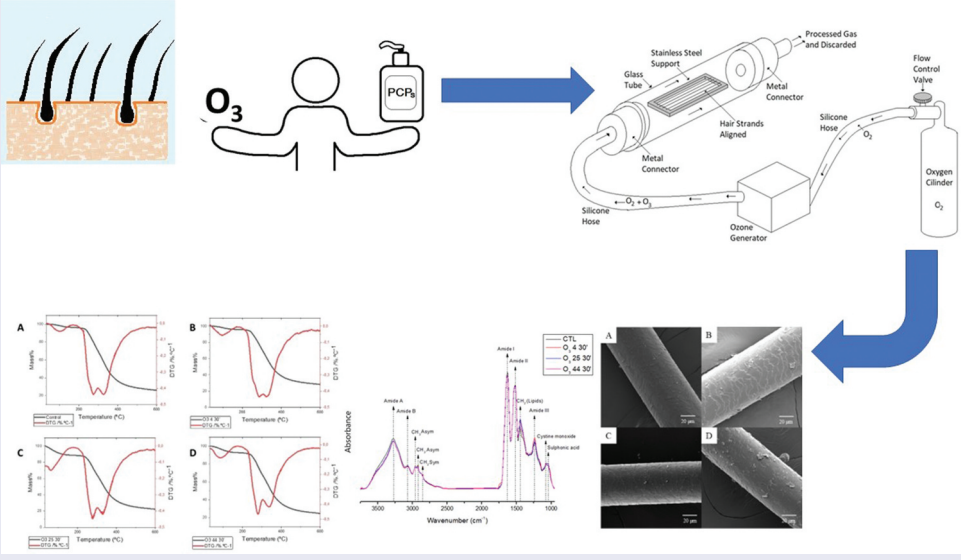
ABSTRACT

Research with ozone has shown potential for applicability, including use of ozone as an environmentally friendly alternative to personal care products (PCPs). However, for hair, there are few studies indicating the safety of using ozone gas, especially when in contact with hair fiber. The aim of this study was to characterize possible physicochemical changes that may occur in hair strands exposed to ozone gas. Scanning electron microscopy (SEM) and profilometry for surface analysis, Fourier transform infrared spectroscopy (FTIR) with attenuated total reflection (ATR) for chemical analysis, Thermogravimetry, and grazing incidence X-ray diffraction (GIXRD) for structural analysis were used. SEM and profilometry techniques showed evidence of changes in appearance of the hair fiber. While FTIR-ATR showed degradation of characteristic peaks on the hair infrared spectrum, with major changes at peaks 2920, 2851, 1633, 1235, 1075, and 1043 cm^{-1}, also associated with the cuticle and mainly related to the cell membrane complex (CMC). Thermogravimetry and GIXRD have shown the possible effect of ozone on human hair amino acids. The results obtained indicated that ozone gas applied on human hair showed oxidative action. Therefore, additional studies are required before discussing the replacement of PCPs by gas ozone.



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This article has been corrected with minor changes. These changes do not impact the academic content of the article.

Introduction

For humans, hair represents a structure that has long lost its functional importance during the evolution of the species, but whose value in emotional and social terms should not be underestimated (Velasco et al. 2009). Therefore, at present many studies on hair have been developed for identifying products and protocols that serve esthetics, maintenance, and preservation of the hair fiber (Lima et al. 2019).

The cuticle structure has a major influence on the permeation of substances into hair (Bhushan 2010; Robbins 1971). The cortex forms the middle layer of the hair fiber, is the largest component, and is responsible for mechanical resistance. It is composed of keratin macrofibrils aligned longitudinally along the fiber, in which the type, size, and quantity of randomly distributed melanin granules determine the hair color (Bhushan 2010; Robbins 1971; Velasco et al. 2009). Human hair consists of approximately 65 to 95% of its weight in proteins and the remainder is composed of water, pigments, lipids, and other components. Chemically, human hair is composed of about 80% keratin (Kaplin, Schwan and Zahn 1982; Wagner and Joekes 2007). The majority of changes in hair structure originate from the scalp, either from natural causes or from esthetic procedures. They result from the oxidation process caused by exposure to ultraviolet B rays (UVB) present in sunlight, discoloration, pigmentation, perming, and straightening processes (Trüeb 2015).

The use of ozone (O_3) as a disinfectant has increased in recent years due to its highly effective bactericidal properties (Marson et al. 2016; Moccia et al. 2020; Oliveira et al. 2023; Taba et al. 2023). In addition to its anti-inflammatory and healing effects, ozone represents a potential for development in the cosmetic industry due to its oxidative effects, as it is an environmentally friendly alternative. Some studies have even investigated the possibility of using ozonated water as a substitute for personal care products (PCP) (Grechkanyova et al. 2018; Peng et al. 2022; Zeng and Lu 2018), since other researches have previously shown some level of environmental pollution from PCP in water (Sun, Ren and Ni 2020; Tijani et al. 2016; Wardrop et al. 2016). The few studies that have addressed the oxidative effects of ozone gas on human hair fibers have, however, focused only on surface analysis of the effect of ozone on human hair (Pandrangi and Morrison GC 2008; Peng et al. 2022). Therefore, the aim of the present study was to evaluate the biochemical changes that ozone gas induces on human hair fibers *in vitro* using SEM and profilometry techniques for morphological analysis. In addition, FTIR, thermogravimetry, and GIXRD were used to enable understanding of the possible chemical and structural changes after the exposure of human hair to different doses of ozone gas.

Material and methods

The human hair samples used consisted of Caucasian untreated hair (Perucaria TM, Brazil), which was divided into 12 cm-long strands weighing approximately 2 g, washed with a 10% Sodium Laureth Sulfate solution (SLES) (BianquimicaTM, Brazil) and then separated strand by strand on a nylon net for a wig cap.

Sample ozonation procedures

The washed samples, stapled to the nylon net, were fixed on a rectangular piece of stainless steel in the shape of a 1 mm thick frame, placed in a glass tube. On the plate, the specimens were separated strand by strand and a piece of stainless steel was attached to each end of the glass tube. One of the sides was fitted with a silicone tube positioned so that the gas flowed to the cuticle positioning in the opposite direction, while the other side was connected to an aspirator fitted with another silicone tube. In this way, all the excess ozone gas was processed and discarded to the external environment (Figure 1). Three different dosages of ozone gas (4, 25, and 44 mg/L concentration) were applied for 30 min. We used a corona-discharge ozone generator (MS3G, Medical Systems Ltda, Brazil), supplied with a 0.125 L/min oxygen (O_2) flow rate at the inlet.

Applied ozone dosage

For each test performed with ozone gas, 8 hair strands (each was 12 cm long) with a total length of 96 cm were used. When the hair diameter was measured through an optical microscope, an mean value of 0.07 mm was obtained, so the total surface area of the hair sample width was approximately 21.1×10^{-5} m^2 .

Considering the O_2 cylinder flow rate of 0.125 L/min, the O_3 generator, previously calibrated with a UV photometric analyzer (BMT 964 ST, BMT Messtechnik GmbH, Germany), provided ozone concentrations of 4, 25, and 44 mg/L at the reactor outlet. These ozone dosages were chosen to simulate the effect of continuous application of ozone, as the goal was to replace daily-use products in the future. Thus, the ozone feed rates were 0.5, 3.1, and 5.5 mg/min. This resulted in applied ozone dosages of 70, 440, and 780 g/m^2 (Van Leeuwen 2015).

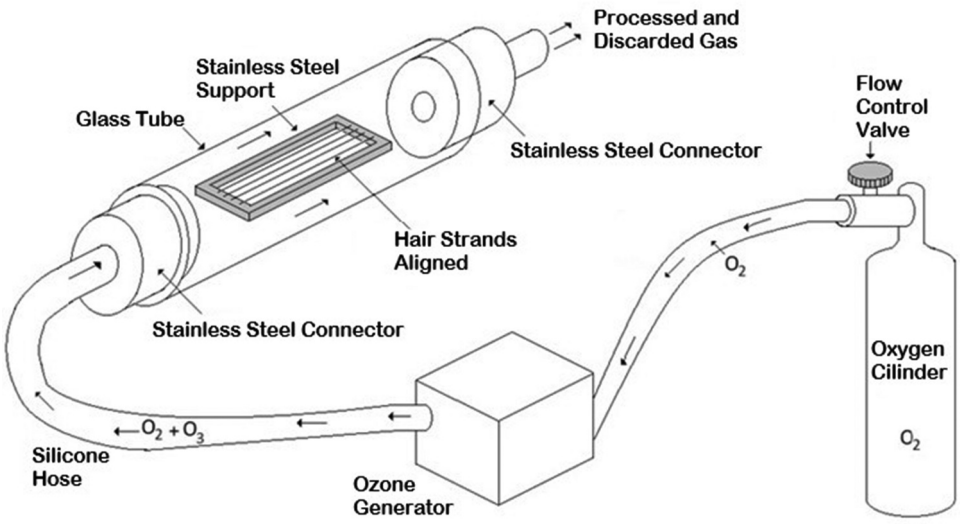

Figure 1. Schematic representation of the assembly for exposure of the hair fiber to the ozone gas. The system was elaborated considering a fluid-dynamic situation.

Scanning electron microscopy (SEM)

The hair samples were fixed on metal stubs with a double-sided carbon adhesive strip. Then, the samples were sputter-coated with a thin film of gold by using a Denton Vacuum Desk II metallizer (USA). The micrographs were obtained with a SEM, model VEGA 3 LMU (Tescan, Germany), with a 5 kV beam voltage and a secondary electron (SE) detector.

Profilometry

The fiber surface relief was analyzed by using the Alpha-Step D-500 equipment from KLA Instruments (USA). A conical diamond probe with a spherical tip and a 2 µm radius was used, with the cone forming an angle of 60°. Scanning was performed at 0.25 mm intervals at a speed of 0.01 mm/s and a force of 0.3 mN applied to the probe.

Infrared spectroscopy

For infrared spectroscopy, the attenuated total reflection (ATR) technique was used with the Frontier infrared spectrometer (FT-IR/FIR) (Perkin Elmer, USA). This system worked with an average of 16 scans and a time of 2 seconds per scan and covered a spectral range from 450 to 4000 cm^{-1}, the resolution was set to 4 cm^{-1}. Origin Pro software (v. 8.5) was used to plot the spectra.

Statistical analysis

The analysis was performed with the Instat software (v. 3.0, GraphPad Software Inc., San Diego CA, USA) using parametric one-way-ANOVA, nonparametric comparison (Kruskal-Wallis test with posttest) and Dunn Test in order to compare all groups. The Principal Component Analysis (PCA) were used with the aim of identifying the Groups in which these spectral differences were statistically significant ($p < 0.05$).

Grazing incidence X-ray diffraction (GIXRD)

The crystalline phases of each sample were studied by X-ray diffraction using a PANalytical Empyrean instrument, with copper $K\alpha_1$ radiation ($\lambda = 1.5406$ Å) excited at a voltage of 40 kV and an electric current of 40 mA. Seeman-Bohlin geometry was used, with a scattering angle from 5° to 70° and a velocity of 0.001 °/sec.

Thermogravimetric analysis (TGA)

The TGA technique was used to evaluate the stability of the hair fiber, obtained as a function of mass loss when exposed to temperature variations. A thermogravimetric analyzer, model TG 209 F1 (Netzsch, Germany), was used. All tests were performed with the following parameters: alumina crucible, N_2 gas purge, purge gas range of 20/2000 mg, a heating rate of 20 °C/min, final temperature of 600 °C, and sample mass of 30 mg.

Results and discussion

SEM

The SEM technique was used to evaluate the effect of direct exposure of hair fiber to ozone gas without interference from other contact agents. Figure 2 shows the microscopic images of control group and ozonated hair

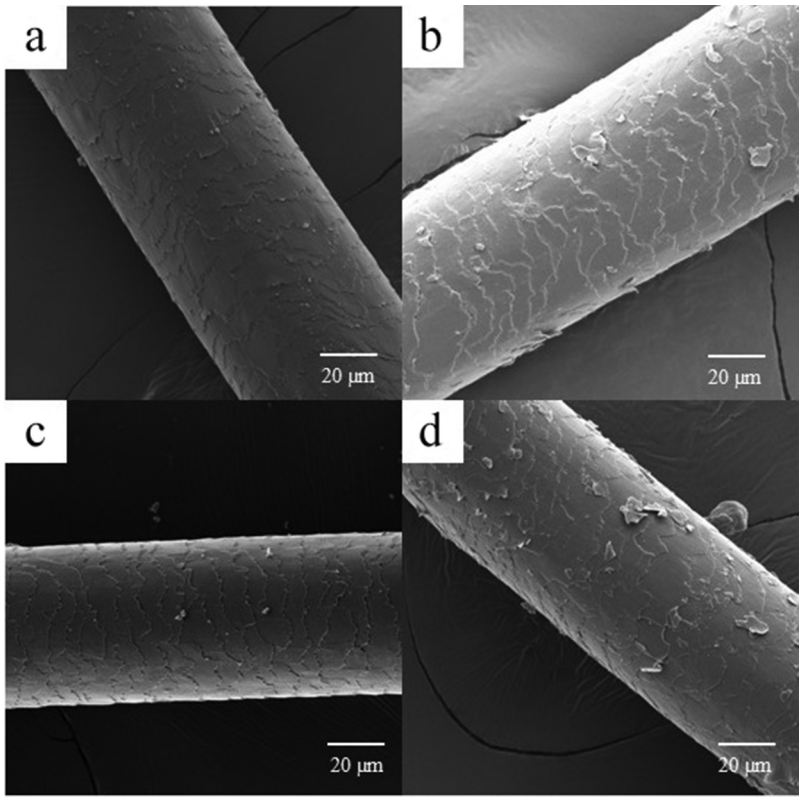

Figure 2. SEM images of hair fibers exposed to ozone gas. Figure a shows a control hair sample; figures B, C, and D show the hair samples exposed to ozone concentrations of 4, 25, and 44 mg/L, respectively, for 30 minutes.

fibers. Figure 2a shows a portion of the control sample It is possible to observe the natural wear of the strand after exposure to agents present in the environment, as the cuticle layers remained fixed in their original shape and were oriented to overlap in a shape similar to the scales along the length of the hair fiber (Kaliyadan et al. 2016; Richena and Rezende 2016; Wei, Bhushan and Torgerson 2005).

From Figures 2b and d, it is possible to see that some cuticle "scales" were lifted along the hair fiber. This change was irregular and random, scattered throughout the entire surface of the hair strand. However, when the images were compared, more elevation of the cuticle could be visualized in the bottom of Figure 2D. Whereas the first figure Figure 2(a) showed more visible changes in the image, indicating less detachment of the cuticle. It is important to highlight that the relationship between the hair cuticle lifting caused by dynamic contact of the gas on the hair surface was negligible since the gas flowed through the glass tube at a calibrated flow rate of 0.125 L/min and the glass tube cross-section area was 3.8 cm^2. Therefore, the gas in this glass tube was in a laminar fluid dynamics regime with an average velocity of 5.5 mm/s, as it resulted in a low Reynolds number (~9). This led to the dynamic contact of the gas with the hair strands being unable to lift the cuticle, which was consistent with the results by SEM.

Profilometry

Using the profilometer, an average distance of about 8 µm between the tips of the cuticular cells was found for all hair samples. However, despite the similar mean value, a higher number of inter-tip distances of more than 10 µm were detected in the ozonated samples, as shown in Figure 3.

However, it is possible to perform arithmetic mean roughness analysis, in which the arithmetic mean of the absolute values of the profile height deviations within the length (L) evaluation is recorded and measured from the centerline. Ra = (|Z1| + |Z2| + |Z3|. . .| ZN |)/N. which made it possible to check this, despite the small, almost irrelevant variation of the values, as seen in Table 1.

Nevertheless, although slight, changes were evident in SEM images, in the same way as the number of valleys larger than 10 µm increased in the profilometry analyses. This may indicate the effect of ozone on the hair cuticle since the distance between adjacent cells of the hair cuticle was 6–7 µm (Sauermann et al. 1988).

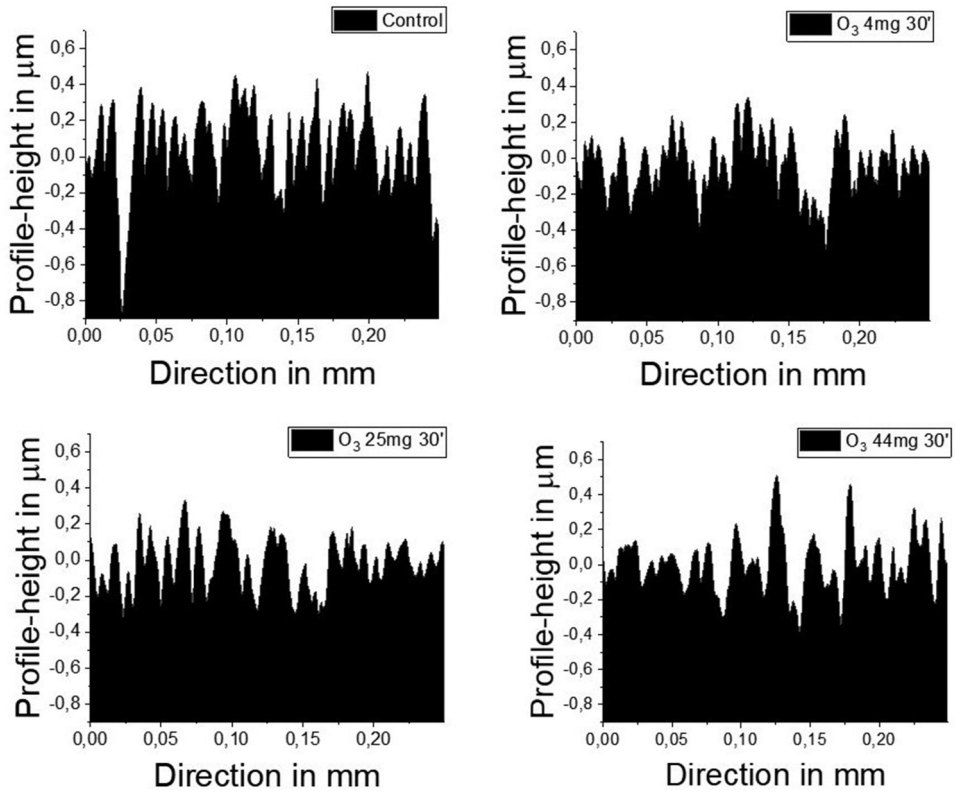

Figure 3. The surface profiles of human hair samples (0.25 mm dimension scan).

Table 1. Average Roughness (Ra) of hair fibers scanned by profilometry.

	Control	O_3 4 mg/L 30′	O_3 25 mg/L 30′	O_3 44 mg/L 30′
Mean	0.154 ± 0.017	0.116 ± 0.002	0.149 ± 0.002	0.148 ± 0.019

FTIR-UATR

Figure 4 shows the FTIR-UATR spectrum of control and ozonated the hair samples. The FTIR spectra show that the intensity of bands 3278, 3073, 2958, 2920, 1633, 1518, and 1235 cm^{-1} gradually decreased, which may be related to the oxidation process of the substances represented by these IR peaks.

Based on the literature on infrared spectroscopy of human hair, the spectra obtained could be divided into five regions. Table 2 shows the relationship between the peaks found in the spectra obtained and characteristics of the chemical composition of the capillary fiber they represent (Lima et al. 2019; Lyman and Schofield 2008).

The first region, in the range of 4000 to 3200 cm^{-1}, represented the presence of water and the carboxylic group in human hair, thus, this region was responsible for pointing out the character of hydrogen bonds and carboxylic acids. In the second region, located between 3200 and 1400 cm^{-1}, the bands for the functional groups of the hair fiber were observed, being hydrogen stretches, stretching vibrations of fatty acids, and asymmetric stretching of lipids and proteins. Among the bands evaluated, seven were within the second region of the hair spectrum, which was related to hydrogen stretches, fatty acid stretch vibration, and asymmetric stretching of lipids and proteins. The bands 2920 and 2851 cm^{-1}, were related to the stretching and bending modes of CH_2 and CH_3 of lipids and methyl groups, suffered degradation in their peaks, meanwhile, the band 1451 cm^{-1}, which was also correlated to lipids, had increased (Lima et al. 2019; Mujeeb and Zafar 2017; Zhang et al. 2020).

This data probably indicated that ozone may oxidize part of the hair cuticle, leading to the appearance of cavities, and thus influencing the generation of subproducts from the lipids present in the cuticle. In other studies it has been suggested that ozone immediately reacts with polyunsaturated fatty acids, similar to CMC present in the skin, thereby producing reactive oxygen species (ROS) and lipopeptides (LOP) (Lima et al. 2019; Pandrangi and Morrison GC 2008; Travagli et al. 2010; Valacchi, Fortino and Bocci 2005).

The spectrum also showed changes in the band at 1633 cm^{-1}, which corresponds to β-leaf, the dominant conformation of the cuticle structure, possibly corroborating the images obtained by SEM (Lima et al. 2019; Lyman and Schofield 2008). In general,

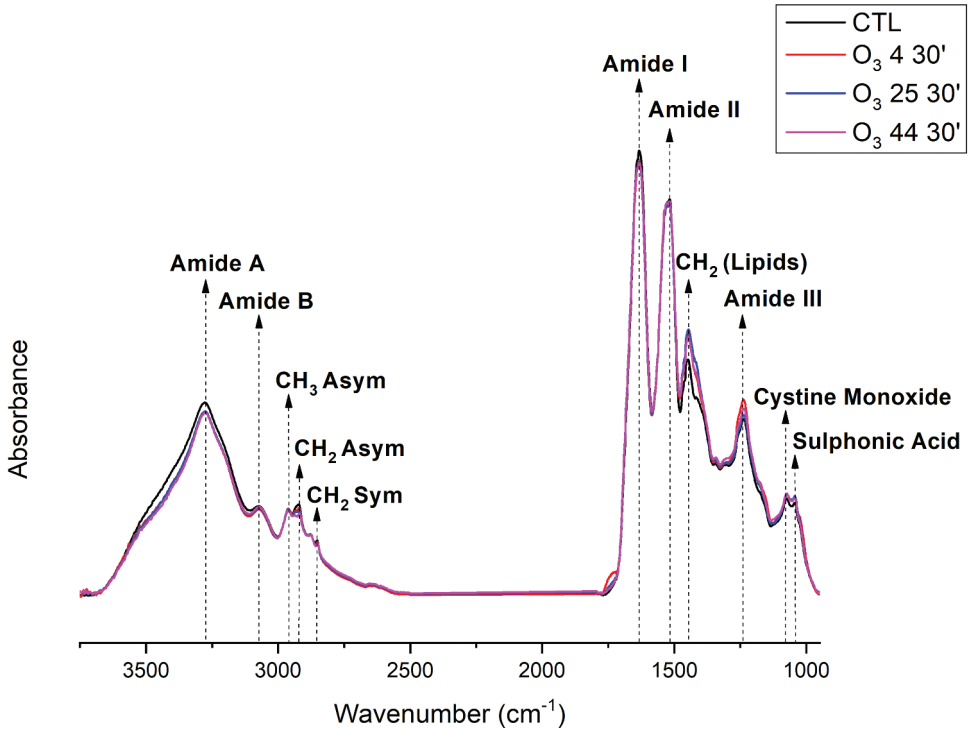

Figure 4. Standard FTIR-ATR spectra from 950 to 3750 cm^{-1} comparing samples of control and ozonated at dosages of 4, 25, and 44 mg/L for 30 minutes.

Table 2. Assignment of the main bands of spectra in samples of Caucasian untreated hair. Adapted from Lima et al. (2019).

Functional Group	Wavenumber (cm^{-1})
Amide A	3278
Amide B	3073
CH_3 Asym	2958
CH_2 Asym	2920
CH_2 Sym	2851
Amide I	1633
Amide II	1518
CH_2 (Lipids)	1451
Amide III	1235
Cystine Monoxide	1075
Sulphonic Acid	1043
NO_2	874
N-H_2	802

peaks in the 1700 1480 cm^{-1} spectral region are sensitive to changes in the secondary structural conformation of the hair protein. They can be useful for identifying alterations caused by chemical processes in hair, which may be related to ozone interaction to cysteine (Cys), due to high reactivity of ozone with sulfur-containing amino acids as methionine, and its rapid reactions with Cys (SH) and Cys (S) (Sharma and Graham 2010). It was also possible to observe that excessive exposure to ozone may have a certain limit on changes caused in the FTIR spectra of hair after reaching a certain level, in which the changes begin to stabilize, as can be seen at band 1633 cm^{-1}. Moreover, the increase at band 1235 cm^{-1}Invalid Date NaN, NaNbe interpreted as the formation of byproducts from the interaction of ozone with amino acids from band 1633 cm−1, since both bands are related to human hair amino acids (Mujeeb and Zafar 2017; Zhang et al. 2020).

Figure 5 presents the PCA variables (Principal Components PCs – intensities and Scores – spectral variables) extracted from the dataset. The first two Scores are depicted in Figure 5, resembling the FTIR spectra of human hair compounds. Score 1 is related to the general features of human hair, while Score 2 is associated with residual effects of exposure to ozone, primarily attributed to changes in the stretching and bending modes of CH_2 and CH3 groups in lipids and methyl groups (2920 and 2851 cm^{-1}) and amide I (1633 cm^{-1}).

Analysis of the intensity of the PCs, shown in Figure 5, revealed that ozonated groups exhibited lower intensity for PC1 compared with the control, but without any significant difference. Concerning PC2, the ozonated samples were characterized by a positive peak at 1451 cm^{-1} and negative peaks at 2920, 2851, and 1633 cm^{-1} in Score 2, with statistically significant differences between the groups control and O_3 44 30' (* p = 0.0165). This suggested a potential confirmation of the oxidative effect of ozone on human hair lipids, primarily manifesting as the degradation of peaks correlated with CMC and an increase in the 1633 cm^{-1} band (Lima et al.

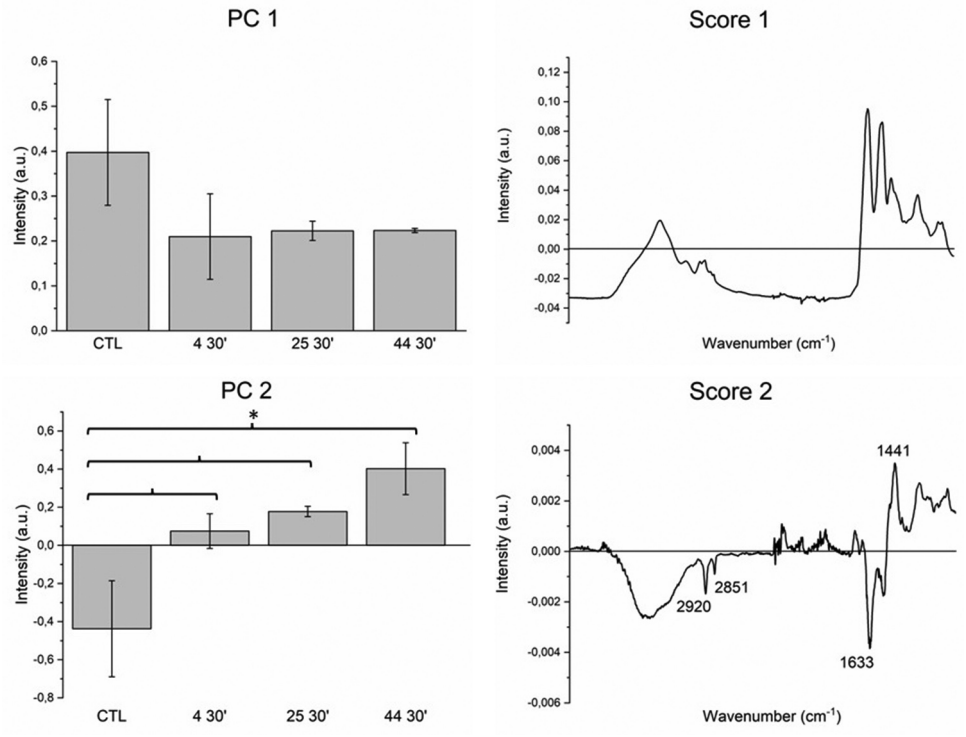

Figure 5. Plotting of variables of the principal components (PC1, PC2, Score1 and Score2), showing the intensity of each loading and the main features related to the constituents of human hair. PC2 showed statistically significant differences between the groups control and O_3 44 30′ ($p < 0.05$ *).

2019; Pandrangi and Morrison GC 2008; Travagli et al. 2010; Valacchi, Fortino and Bocci 2005).

An example of changes that are usually found due to products formed by oxidation of human hair is the increase of peaks 1075 and 1043 cm^{-1}, corresponding to cystine monoxide (R-SO-S-R) and sulfonic acid (R-S(=O)₂-OH), respectively (Lima et al. 2019; Robbins 1971; Zhang et al. 2020). This type of alteration could also be seen in the FTIR spectra of ozonated hair samples. The increase in these peaks in the spectra evaluated was evidence that the process of ozone oxidation was the formation of products from the reaction of ozone with sulfur-containing amino acids since these peaks are parameters for hair fibers that have undergone oxidative processes (Sharma and Graham 2010).

TGA

The TGA curves (Figure 6) illustrated the thermal behavior of the hair samples. Based on the TGA results, three main events with a mass loss of hair samples could be seen, indicating their thermal profile: first the elimination of water (dehydration); second, keratin decomposition, and third, elimination of the carbonaceous material formed (Lima et al. 2019).

Analyzing Figure 6 and Table 3 of TGA curves of the four samples, considering sample (A) as a basis for comparison because it was a control hair sample, it can be seen that samples (C) and (D) showed higher water loss values in Event 1, and then showed less mass loss in the 2nd and 3rd events, while sample (B) showed values closer to those of untreated hair but with some variation, as can be seen in Table 3.

The first and second mass loss events show that the first two groups had similar values, while the other two groups showed higher loss values in the first event and lower loss values in the second event. The decrease in the mass loss in the second event pointed to a disorganization of the keratin structure, which may be correlated with the action of ozone gas on its amino acids (Éhen et al. 2004; Monteiro, Maciel and Longo 2005; Sharma and Graham 2010).

The increase in mass loss in the first event may represent the formation of aqueous byproducts from oxidation of the keratin, but no study had indicated the direct formation of water from the oxidation of proteins or lipids. However, some studies have suggested the formation of hydrogen trioxide (HOOOH) from the interaction of ozone gas with saturated organic compounds, in which HOOOH would be a metastable intermediate, resulting from reactive oxygen species (ROS) that would form H_2O and O_2 (Cerkovnik and Plesničar 2013; Kanofsky and Sima 1991).

The increase in mass loss in the first event may also have been a dehydration process caused by heating

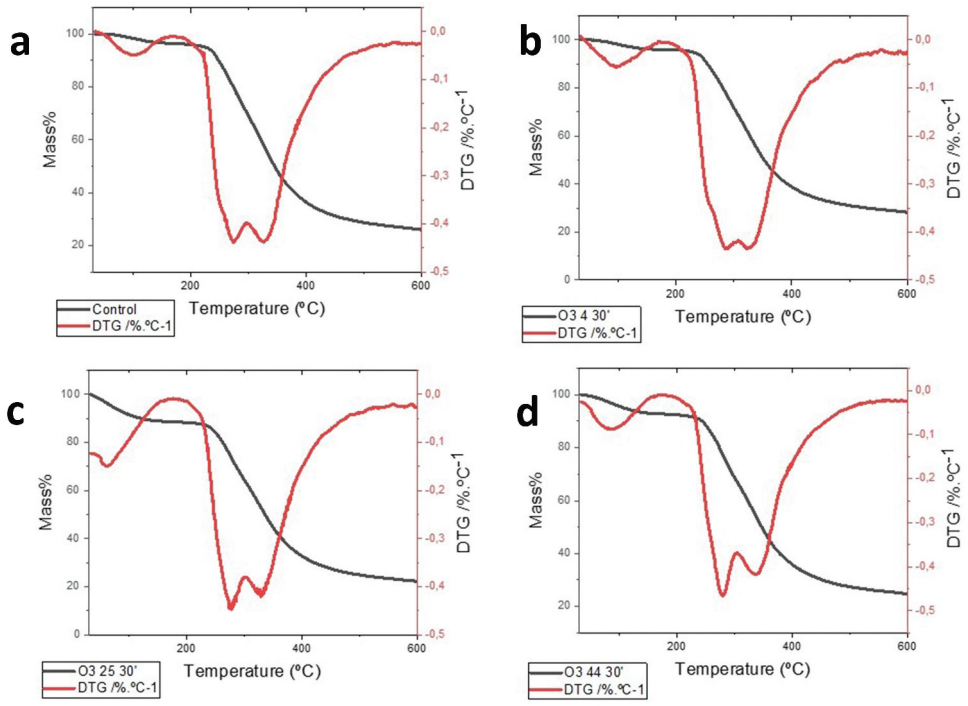

Figure 6. TGA curves were obtained in an open Al_2O_3 crucible at 20 °C/min under a dynamic nitrogen atmosphere of control and ozonated hair samples.

Table 3. Thermogravimetry mass loss values of the Control and ozonated human hair fiber.

	Control	O_3 4 mg/L 30′	O_3 25 mg/L 30′	O_3 44 mg/L 30′
Event 1	4.12	4.42	13.44	9.17
Event 2	50.35	50.57	47.15	47.35
Event 3	19.53	16.83	17.32	18.88

from the ozonated samples. Ozone is known to react with some of the main amino acids of human hair, such as cysteine, methionine, tyrosine, and histidine, resulting in the formation of cysteine, sulfone, 2-Amino-3-(3,4-dioxo-cyclohexa-1,5-dienyl)-propionic acid and 2-Amino-N-formylureido-succinamic acid, respectively (Cataldo 2003 Kanofsky and Sima 1990; Sharma and Graham 2010). However, some previous studies have suggested the thermal stability of cysteine present in human hair, indicating that cystine possibly starts to decompose at 250 °C (Milczarek, Zielinski, and Garcia 1992; Monteiro, Maciel and Longo 2005). Thus, due to the thermogravimetric analysis, the exposure of these products to heat may have caused dehydration, which may corroborate the finding of increase in mass loss in the first event and decrease in the second event. Based on the studies about amino acids dehydration due to exposure to high temperatures, it is possible to elaborate a mechanistic proposal (Figure 7) of how the amino acids interact with the ozone gas; and its byproducts become more susceptible to starting their decomposition process by dehydrating at temperatures below those found in the literature for the original decomposition/dehydration of amino acids (Kanofsky and Sima 1990; Cataldo 2003; Li et al. 2006; Rodante, Marrosu and Catalani 1992; Sharma and Graham 2010).

GIXRD

By the X-ray diffraction technique, it was possible to note the shape of a predominantly amorphous structure of human hair. There were only two crystal structures present on the hair fiber: α-helix and β-sheet. Both structures showed a peak at $2\theta = 9^{o}$, which corresponded to a d-spacing value of 0.98 nm. Moreover, the αhelix and β-sheet had the highest intensity peak at $2\theta = 17.8$ and 19^{o}, respectively, corresponding to d-spacing values of 0.51 and 0.47 nm. Nevertheless, as a consequence of overlapping of the αhelix and β-sheet signals at 17.8 and 19^{o}, both of them could not be unambiguously assigned (Idris et al. 2013; Zhang et al. 2015). Figure 8 shows the XRD curves of control and ozonated hair fibers. The peaks were slightly displaced about the theoretical values because due to the sample format, it was not possible to keep the surface analyzed at completely the same height as that of the plane of the sample holder surface. When the sample is not completely aligned with the sample holder surface (deeper or higher), the peaks shift to the left or right.

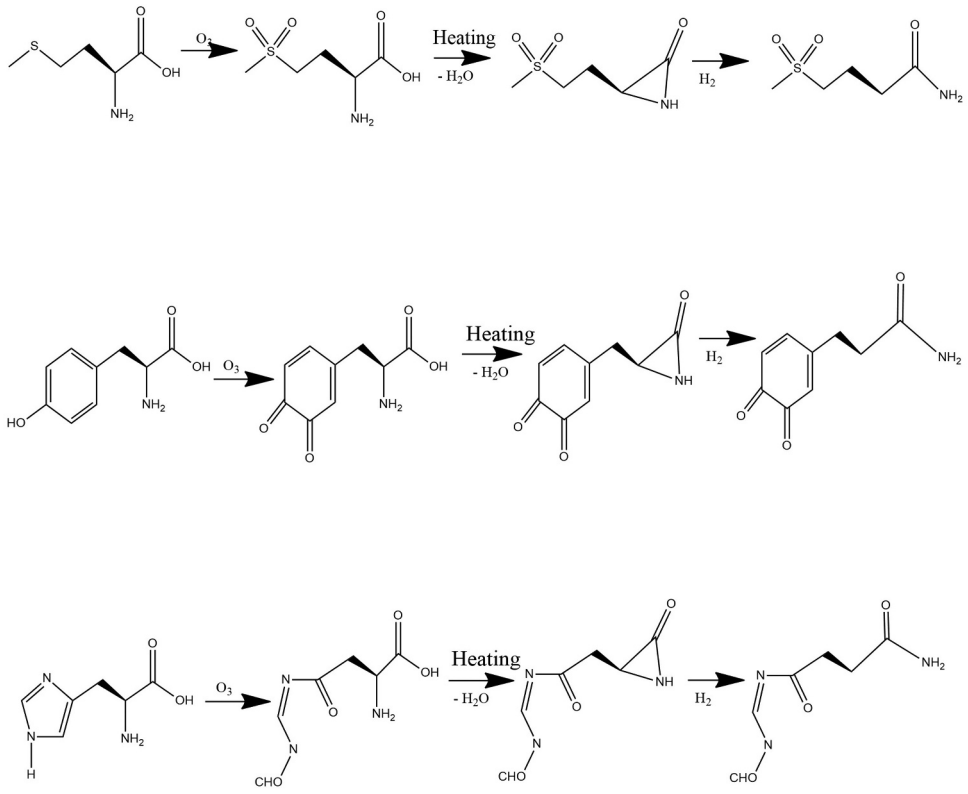

Figure 7. Diagram of the mechanistic proposal of byproduct dehydration after ozone reactions with methionine, tyrosine, and histidine.

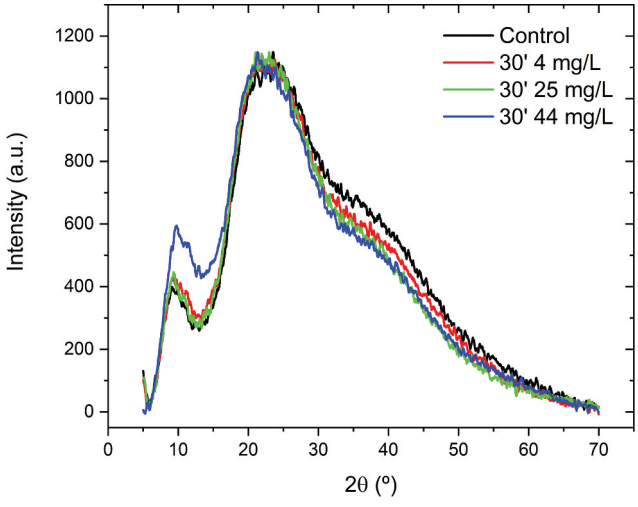

Figure 8. XRD pattern obtained from control and ozonated human hair samples.

From Figure 8 it is possible to observe a slight increase in the peak at 9º when the ozonated samples were compared with the control. The largest difference was shown in the sample exposed for 30 min to an ozone concentration of 44 mg/L. To provide a better illustration of this increase, the relative intensity of the two peaks (I_1/I_2) was calculated, as shown in Figure 9.

Ozone trace as an alternative to PCPs

A study recently developed by Peng et al. (2022), suggested trace ozone as an alternative to PCPs in personal care activities, with the aim of reducing the emission of organic matter, total nitrogen (TN), and total phosphorus (TP), respectively. However, there is no analysis of the effects of continuous use of ozone on the skin appendages, which could have cumulative effects such as those seen resulting from environmental and chemical products (Cavagnino et al. 2022; Trüeb 2015). In this sense, this study used high ozone dosages to simulate the effect of cumulative stress on hair fibers exposed to ozone, however, as shown in the results, even with the oxidative effect caused by ozone gas on hair fiber, it was necessary to use elevated dosages to observe this change.

At present, new studies are being conducted by our research group to allow a greater understanding of the effects of ozone gas on the hair fiber. For this purpose, some tests are being carried out, such as verifying the variability of the strength, elasticity modulus, and combability of the human hair, under the application of different ozone gas dosages, with the aim of finding the toxicological safe dosages.

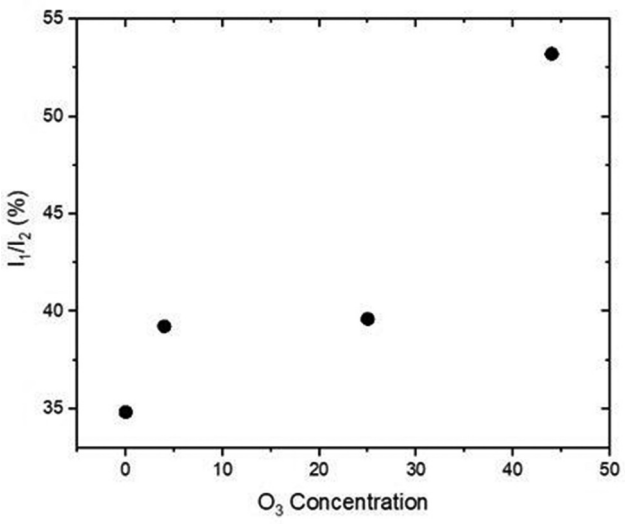

Figure 9. The relative intensity between peaks at 9 and 21° as a function of the ozone concentration in the human hair samples.

Conclusion

Ozone has shown potential for use as a possible alternative to replace PCPs. In this study, the effects of ozone gas on human hair fibers were investigated, according to the biochemical and morphological changes. In an *in vitro* study, it was observed that the high doses of ozone applied to the hair fiber caused structural and biochemical changes. The FTIR analysis showed degradation and an increase in peaks at 2920 and 1451 cm^{-1}, respectively, which could be associated with the interaction of ozone with CMC's lipids, as confirmed by the PCA. An increase observed in the peak at 1235 cm^{-1} could also be related to the oxidative action of ozone on hair amino acids, by originating new amino acids as byproducts. The same process could be associated with the variation in mass loss observed by thermogravimetric analysis. New studies are still necessary to enable understanding of the action of ozone on the crystalline hair structure, which showed a slight change by GIXRD analysis. Therefore, further studies must be conducted with hair fibers of different ethnicities, in addition to *in vivo* protocols, to confirm and deepen the analysis of biochemical changes observed. The aims should be to verify the safe taxological limits for hair before replacement of PCPs by trace ozone, and promote ongoing discussions in relation to the topic.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data that support the findings are available on request from the corresponding author, Marrafa PALI. The data are not publicly available due to their containing information from doctorate thesis.

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