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ORIGINAL ARTICLE

Determination of chemical components derived from 2% chlorhexidine gel degradation using gas chromatography-mass spectrometry

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Abstract

Objective. This study determined the chemical components derived from degradation of 2% chlorhexidine (CHX) gel and solution by using gas chromatography-mass spectrometry. ***Materials and methods.*** Three 2% CHX gels were used to identify the products of CHX gel degradation using gas chromatography-mass spectrometry. A solution of CHX was also evaluated to compare the degradation between gel and solution. Degradation was evaluated in four storage situations (on the worktable with light: on the worktable without light; in the Pasteur oven at 36.5°C without light; and in the refrigerator at 8°C without light). Measurements were made at four time points: initial analysis and 1, 3 and 6 months after. The conversion of CHX into para-chloroaniline in storage situations and in different periods was analyzed statistically using chi-square test ($\alpha = 5\%$). ***Results.*** The 2% CHX gel or solution had already degraded vial found within the period of validity, at all time points and for all storage conditions. The amount of para-chloroaniline (pCA) was directly proportional to time in the case of CHX solution, but not in CHX gel due to lack of homogeneity. CHX homogeneity in hydroxyethylcellulose gel was directly dependent on compounding mode. ***Conclusions.*** Degradation products, such as para-chloroaniline (pCA), orto- chloroaniline (oCA), meta-chloroaniline (mCA), reactive oxygen species (ROS) and organochlorines (ortho-chlorophenyl isocyanate and 2-amino-5-clorobenzonitrila) were found in 2% CHX gel and solution, regardless of storage conditions or time. In relationship to gel homogenization an alternative to produce 2% CHX gel and a new homogenization method have been developed.

Key Words: *chlorhexidine, para-chloroaniline, reactive oxygen species, chlorhexidine gel degradation, gas chromatography*

Introduction

Cleaning and shaping should reduce the number of bacteria in infected root canals [1,2]. Various irrigants have been recommended for cleaning and many are effective antibacterial agents according to different endodontic infection experimental models [1–4]. Chlorhexidine (CHX) has often been recommended as an antiseptic agent for routine dental plaque control [5,6], an endodontic irrigant when used in concentrations of 0.2–2% [2–7] or an intra-canal dressing when combined with calcium hydroxide [8].

CHX was developed in 1940 in the research laboratories of the Imperial Chemical Industries, in Macclesfield, England, to be used as an antiviral agent. As such, CHX was ineffective, but its antimicrobial properties were excellent. A strong base and very stable as a salt, CHX is a symmetrical detergent cationic molecule with two rings and two 4-chlorophenyl bi-biguanide groups connected by a central hexamethylene chain (1,1 '-Hexamethylene-bis-[5-(p-chlorophenyl) biguanide]). It belongs to the bis-biguanide class and is available in the form of acetate, hydrochloride or digluconate, its most frequent formulation [7,9–13]. Some of its characteristics are: molecular formula = $C_{22}H_{30}Cl_2N_{10}\ 2C_6H_{12}O_7$; molecular weight = 897.77; density = 1.06 g/cm^3; and boiling point = 134°C [13]. Its cationic nature promotes its connection with anionic compounds on bacterial surfaces (phosphate groups of the teichoic acid in Gram-positive and

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lipopolysaccharide in Gram-negative bacteria), which may affect its integrity. Potassium ions, which are small, are the first to appear when the cytoplasmic membrane is damaged. The change in cytoplasmic membrane permeability results in the precipitation of cytoplasmic proteins, which affects the cellular osmotic balance, interferes with metabolism, growth and cell division and inhibits membrane ATPase and the anaerobic process [5,6,10,14,15]. CHX does not detoxify endotoxins [16].

The antibacterial effectiveness of CHX has encouraged dentists to consider it as an alternative for the control of endodontic infections. However, the formation of toxic chemicals during its degradation [17–32], such as para-chloroaniline (pCA), 4-clorofenilureia, 4-clorofenilguanidina and 1-chloro-4-nitrobenzene, has raised concerns [17–19]. Degradation may be accelerated by the presence of light, higher temperatures and changes in pH [29,30].

The use of CHX in healthcare is widespread and consolidated. However, studies should investigate the potential risks of its use because of the production of toxic by-products during its decomposition. This study used gas chromatography-mass spectrometry to determine the chemical components derived from the degradation of 2% CHX gel under four storage conditions and at different time points.

Materials and methods

Chemical reagents

The research grade reagents used in this study were: para-chloroaniline (Sigma-Aldrich®, WGK, Steinheim, Germany); 20% chlorhexidine digluconate (Sigma-Aldrich Corp., N. C9394–25 ml, CAS N. 18472–51–0 and L. 0001440607, Steinheim am Albuch, Germany); hydroxyethylcellulose (Natrosol, PharmaSpecial®, São Paulo, Brazil); acetonitrile (Solusorb®, JT. Baker, Phillipsburg, NJ); deionized water (Quimis®, Campinas, Brazil); propylene glycol (Synth®, Diadema, Brazil); and methylparaben (Synth®).

Substances tested

This study analyzed a 2% CHX solution (Sigma-Aldrich®) and three 2% CHX gels: chlorhexidine gel (Maquira, Brazil); chlorhexidine gel Uniararas (Uniararas School of Pharmacy, Araras, Brazil); Sigma-Aldrich® chlorhexidine gel (Steinheim) prepared in the laboratory before the experiment (LAGRO, FORP, USP, São Paulo, Brazil).

Preparation of 2% CHX gel in the research laboratory before experiment

For the preparation of the 2% CHX gel, we placed 2 mL of 20% CHX (Sigma-Aldrich®) into a 50-mL beaker, added 18 g of gel (Natrosol), stirred with a glass rod for 3 min, fractionated it into Eppendorf tubes with and without tape protection and stored them under different experimental conditions.

Preparation of solution of 2% CHX digluconate (Sigma-Aldrich®)

For the preparation of the 2% CHX digluconate solution, we placed 2 mL of 20% CHX (Sigma-Aldrich®) into a 50-mL beaker, added 18 mL of distilled deionized water stirred with a glass rod for 3 min, divided it into Eppendorf tubes with and without tape protection and stored them under different experimental conditions.

Conventional compounding of 2% CHX gel in School of Pharmacy

Conventional compounding of CHX gel in pharmacies often follows the steps described here: liquid products are weighed and mixed in a beaker with water at 70°C. Next, 4 g of Natrosol are added little by little, the mixture is stirred at regular intervals and then allowed to rest; the resulting product is a gel. CHX is added after gel preparation and mixed by hand to obtain an apparently homogenous CHX gel.

2% CHX gel homogeneity

A pilot study indicated that gels, both commercial brands (Chlorhexidine Gel®, Maquira, Paraná, Brazil) and those compounded in pharmacies (chlorhexidine gel Uniararas, Uniararas School of Pharmacy, Araras, Brazil) were not homogeneous. Therefore, an alternative to produce 2% CHX gel and a new homogenization method have been developed.

Analysis of CHX gel homogeneity

The homogeneity of gels produced conventionally or according to the method suggested here was analyzed in aliquots of ~ 0.1 g of each gel using 1.7 mL plastic tubes (Eppendorf®). CHX contained in each aliquot was extracted and the sample was filtered using 0.5 mL of acetonotrile (ACN) and a microfilter coupled to an organic phase. We added 1.5 mL of ACN to that solution, stirred it with a glass rod, removed 0.5 mL of the homogeneous solution, placed it in a 5-mL volumetric flask and completed the volume with ACN. A comparative analysis using UV-VIS spectrophotometry defined how to handle CHX gel: the optimal wavelength for our experiment was 253 nanometers.

Analysis of storage conditions at four time points

We defined four storage conditions: on the worktable with light; on the worktable without light; in a Pasteur

Table I. Concentrations (%) used for the pCA calibration curve.

Percentage of conversion	Number of molecules
0.5%	1.11×10^{-7}
1.5%	3.34×10^{-7}
2.5%	5.57×10^{-7}
3.5%	7.80×10^{-7}
6.5%	1.45×10^{-6}
10%	2.23×10^{-6}

oven at 36.5°C without light; and in the refrigerator at 8°C without light. The four times points were defined as time of initial analysis and 1, 3 and 6 months after that.

All samples were placed in 1.7-mL plastic tubes (Eppendorf®) containing 1 g of gel and 1 mL of CHX solution. For the conditions without light, the tubes were completely sealed with black tape (3M, Campinas, Brazil) and stored according to the conditions described above. The total number of samples distributed into the various storage conditions and time points was 52.

Preparation of standard pCA solution

To prepare the standard pCA solutions, we put 2 mg of pCA (Sigma-Aldrich®) into a 20-mL beaker and added 5 mL of ACN. The solution was stirred with a glass rod for 3 min and placed in vials for gas chromatography-mass spectrometry (GC-MS).

Extraction method for gas chromatography-mass spectrometry (GC-MS)

After the pre-defined time intervals, 0.5 mL of ACN was added to the samples and the Eppendorf® tubes were placed in the magnetic stirrer for 2 min. The volume obtained was transferred to a 20-mL beaker, 1.5 mL of CAN was added to it, and the solution was stirred with a glass rod for 1 min. A microsyringe was used to aspirate the volume obtained and, after the needle was placed back, to transfer it to a 0.45-μm filter for organic solvents. After filtration, the solution was placed in a vial for GC-MS.

Gas chromatography was conducted in the temperature-programming mode with a DB-5MS column (25 m × 0.25 mm × 0.25 μm). The initial column temperature was 85°C for 5 min; it was then increased linearly at a rate of 10°C/min to 280°C and held at this temperature for 10 min. The temperature of the injection port was 250°C and the GC/MS interface was maintained at 150°C. The helium carrier gas flow rate was 1.0 mL/min. To standardize the method, a pCA calibration curve was defined by preparing six pCA solutions in ACN at concentrations ranging from 0.5–10% and the calculations were made for the number of CHX mols in 1 g of gel (2–2.23% × 10^{-5} mol), considered to be 100% of the possible conversion of CHX into pCA (1 g of gel–0.02 g (2.23 × 10^{-5} mol)—100% CHX conversion). Based on that value, the pCA calibration curve was built using the values in Table I, which shows that the concentrations were similar for the chromatograms, the gels and the solution analyzed (Figure 1).

This step was performed to quantify results. Figure 1A shows the peak response obtained with the six pCA solutions. The calibration curve was defined according to the peak area of pCA concentration in their chromatograms. Figure 1B shows the pCA calibration curve.

The conversion of CHX gels into para-chloroaniline in storage situations and in different periods was analyzed statistically using a chi-square test (α = 5%) (SPSS Inc., Windows 19.0 Chicago, IL).

Results

Figure 2A shows the results of UV-VIS spectrophotometry for the 2% CHX gel prepared conventionally.

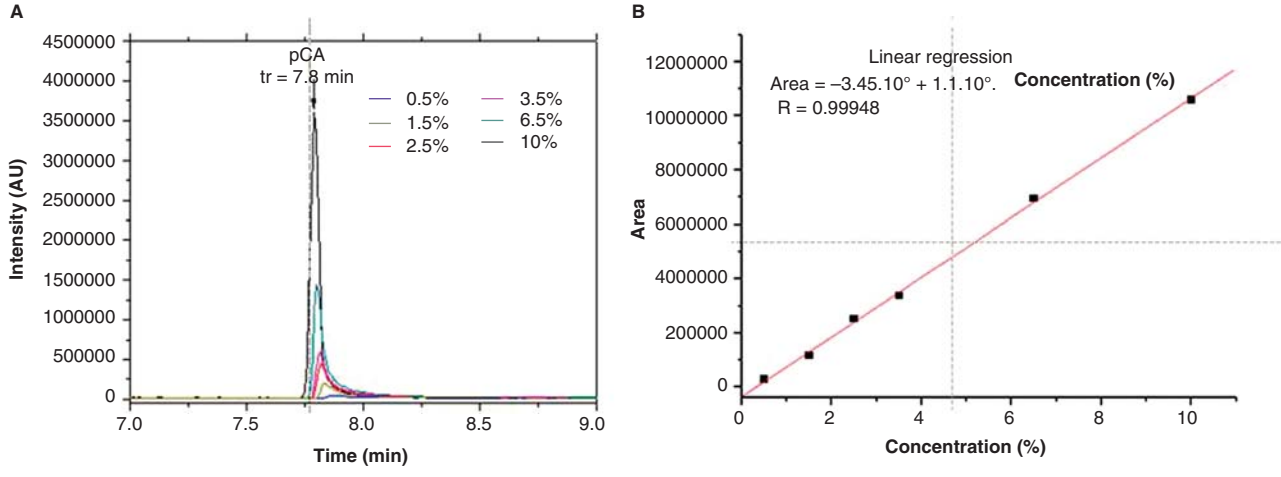

Figure 1. (A) Chromatogram used for pCA calibration curve. (B) Linear regression equation to quantify the percentage of CHX conversion into pCA.

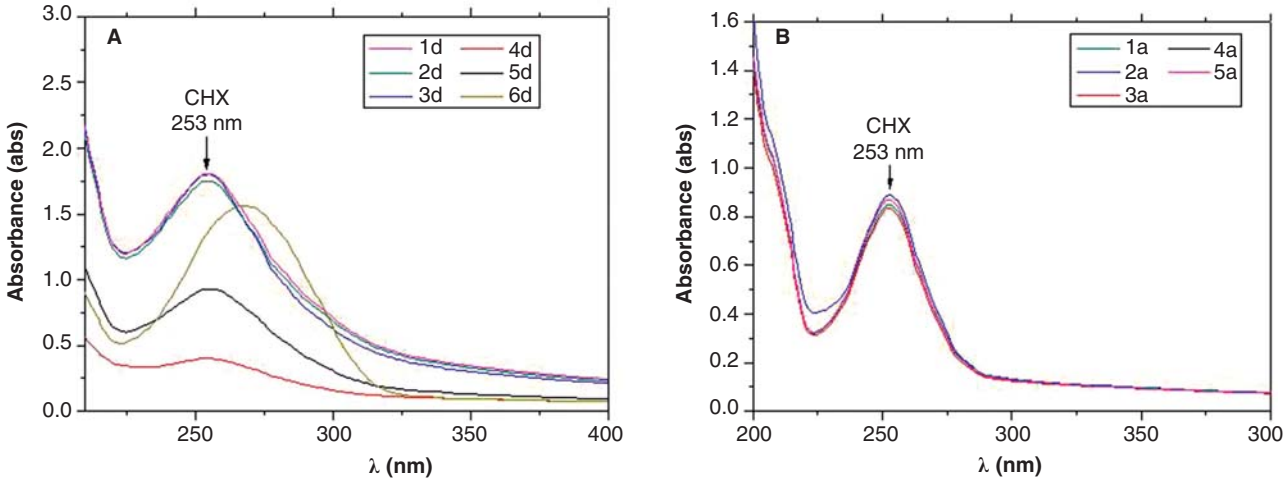

Figure 2. (A) UV-VIS spectrophotometry: gel extractions of 2% CHX produced conventionally. (B) UV-VIS spectrophotometry: gel extraction of 2% CHX produced using the method suggested.

Peak responses were not uniform. CHX analyzed using UV-VIS spectrophotometry had an ideal peak wavelength at 253 nm (after six analyses). Responses were irregular before extractions. Absorbance had different levels of concentration, which confirmed that the commercial brand and the conventionally compounded CHX gels were not homogeneous. Figure 2B shows the results of UV-VIS spectrophotometry for the 2% CHX gel compounded in the laboratory using the method suggested here. Peak responses were regular for the five samples extracted and analyzed. The peaks corresponded to the front wavelength and the absorbance, which indicated that CHX was a homogeneous gel in this case. The differences in methods used to prepare CHX gels may explain the different results and pCA percentages in the conditions analyzed independently.

All chromatographic analyses of CHX gel Uniaraas detected pCA and the product which is more toxic was held quantifying the conversion of CHX into para-chloroaniline (pCA), which was calculated using the equation of the pCA curve (Figure 1B). CHX degradation into pCA was found in all storage conditions and at all time-points. This suggests that this degradation is an intrinsic factor of CHX, regardless of the conditions under analysis (Table II). This verified significant differences in conversion (%) into para-chloroaniline in storage situations and in different periods ($p = 0.002$). Table III shows the results of pCA conversion into CHX calculated using the equation of the pCA calibration curve. The degradation of CHX gel (commercial brand) into pCA was also found in all storage conditions and time points, which also suggests that this degradation is an intrinsic factor of CHX, regardless of the condition under analysis. It did not identify significant differences in conversion (%) into para-chloroaniline in storage conditions and in periods evaluated ($p = 0.342$).

CHX-gel compounded in the laboratory

The chromatographic analysis at the first time point showed CHX gel (Sigma-Aldrich®) extraction (Figure 3A), as indicated by arrow 1. A retention time (RT) peak of 6.5 min for the generation of reactive oxygen species (ROS) (m/z = 153, 125, 63 and 50) suggested the presence of ortho-chlorophenyl isocyanate. The peak indicated by arrow 2, at RT 7.8 min, corresponded to pCA (m/z = 127, 65 and 45). The arrow at RT 8.2 min indicates the presence of a pCA isomer, meta- or ortho-chloroaniline (m/z = 127, 65 and 45) and at RT

Table II. Conversion (%) of CHX into para-chloroaniline (CHX gel, Uniararas).

	Initial analysis of *pCA*, 1.11%			
Storage situation	1 month	3 months	6 months	p^*
on the work table with light	0.84%	1.22%	1.27%	0.002
on the work table without light	0.93%	1.16%	0.98%	
in Pasteur oven at 36.5°C without light	1.18%	0.84%	0.92%	
in the refrigerator at 8°C without light	0.95%	0.99%	0.76%	

*Chi-square.

Table III. Conversion (%) of CHX into para-chloroaniline (CHX gel, commercial brand).

	Initial analysis of pCA, 1.07%			
Storage situation	1 month	3 months	6 months	*p**
on the worktable with light	1.15%	1.03%	1.14%	0.342
on the worktable without light	1.00%	1.00%	1.43%	
in Pasteur oven at 36.5°C without light	0.93%	0.89%	1.01%	
in the refrigerator at 8°C without light	0.67%	0.63%	0.61%	

*Chi-square.

13.9 min, the fifth generation of ROS (m/z = 149, 105, 65 and 50), not detected in our experiment chemically. In Figure 3A, RT 14.4 min has another peak of another ROS (m/z = 152, 125 and 63), which corresponds to the presence of 2-amino-5-clorobenzonitrile, an organochlorine. Chromatographic analysis of CHX gel (Sigma-Aldrich®) at the first time point revealed five products of CHX oxidation: ortho-chlorophenyl isocyanate; pCA; an isomer, either ortho or meta-chloroaniline; a chemically unidentified ROS; and 2-amino-5-clorobenzonitrila.

Figure 3B shows the chromatographic results after 1 month of storage under different conditions: with light, without light, in an oven at 36.5°C and in a refrigerator at 8°C. The peaks indicated by arrows 1, 2, 3, 5 and 6 correspond to the same products detected at the first time point: ROS (ortho-chlorophenyl isocyanate) at RT 6.5 min, PCA at RT 7.8 min, ortho- or meta-chloroaniline (oCA, mCA) at RT 8.2 min, unidentified ROS at RT 13.9 min and ROS (2-amino-5-clorobenzonitrila) at RT 14.4 min. A new peak, indicated by arrow 4, at RT 12.5 min

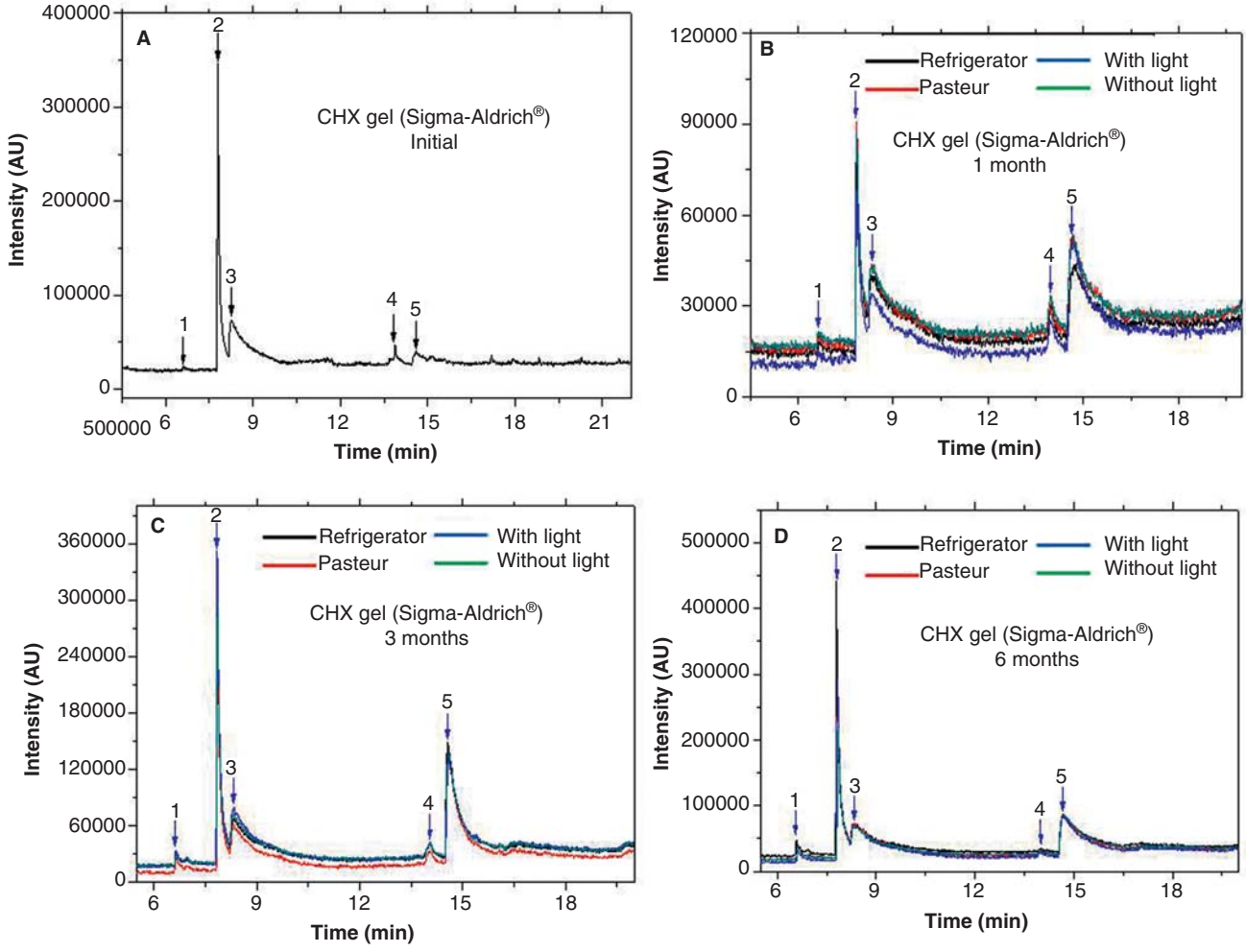

Figure 3. Chromatogram at initial time point (A), 1 (B), 3 (C) and 6 months (D) after CHX gel extraction (Sigma-Aldrich®).

corresponds to methylparaben (m/z = 152, 121, 65 and 45). This response pattern was repeated for all storage conditions after 1 month. The peak of the red line indicated by arrows 2 and 6 increased as CHX oxidation increased due to the higher temperatures. At 1 month, the results for all storage conditions showed all the products found at the first time point plus methylparaben. In Figures 3C and D, which correspond to 3 and 6 months, chromatography also showed the peaks and products generated at 1 month and the greater pCA peak intensity was found for storage with light and in the refrigerator. As pCA was detected in all chromatographic analyses of this gel, the product which is more toxic was held, quantifying the conversion of CHX into pCA. This was calculated using the equation of the pCA calibration curve (Figure 1B) and results are shown in Table IV. Significant differences in conversion (%) into para-chloroaniline in storage circumstances and in periods were observed (p = 0.018). The degradation of the CHX gel prepared in the laboratory (Sigma-Aldrich®) into pCA was found in all storage conditions and at all time points. This suggests that this degradation is an intrinsic factor of CHX, regardless of the conditions under analysis.

2% CHX solution (Sigma-Aldrich®)

The chromatographic analysis at the first time point showed 2% CHX solution (Sigma-Aldrich®) extraction (Figure 4A), as indicated by arrow 1. RT 7.8 min indicates the detection of pCA (m/z = 127, 65 and 45). At RT 8.2 min, another peak, indicated by arrow 2, indicates the presence of an isomer of pCA, either meta- or ortho-chloroaniline (m/z = 127, 65 and 45). The peak at RT 13.9 min, indicated by arrow 3 in Figure 4A, confirms the presence of an unidentified ROS (m/z = 149, 105, 65 and 51). At RT 14.4 min, the peak indicated by arrow 4 indicates the generation of ROS (m/z = 152, 125 and 63); in this case, 2-amino-5-clorobenzonitrile, an organochlorine. Chromatography at the first time point showed two types of ROS (one unidentified and the other, 2-amino-5-clorobenzonitrile) and pCA and its isomers, oCA or mCA.

Figure 4B shows the results at 1 month in all storage conditions. The peaks indicated by arrows 2, 3, 4 and 5 correspond to the same products found in the analysis of CHX solution at the first time point: pCA at RT 7.8 min, ortho- or meta-chloroaniline at RT 8.2 min, ROS at RT 13.9 min and ROS and 2-amino-5-clorobenzonitrila at RT 14.4 min. A new peak was detected at RT 6.5 min (arrow 1), which corresponded to a new generation of ROS (m/z = 153, 125, 63 and 50) and demonstrated the presence of ortho-chlorophenyl isocyanate. This response pattern was repeated for all storage conditions after 1 month. The peak of the red line indicated by arrows 2, 3, 4 and 5 increased and formed a new peak as CHX oxidation increased due to the higher temperatures. At 1 month, the results for all storage conditions showed all the products found at the first time point plus a new ROS.

Figures 4C and D, which correspond to 3 and 6 months, show that chromatography detected the same peaks and products generated at 1 month, but the greatest pCA peak intensity was found for storage with light and in the refrigerator. As pCA was detected in all chromatographic analyses of this gel, the product which is more toxic was held quantifying the conversion of CHX into pCA. This was calculated using the equation of the pCA calibration curve (Figure 1B) and results are shown in Table V. CHX degradation into pCA was found for all storage conditions and at all time points, which suggests that this degradation is an intrinsic factor of CHX. However, the percentage of pCA found in the samples increases over time, which indicates that the amount of pCA in samples of CHX solution is directly proportional to time.

CHX solution or gel degrades into toxic products regardless of storage conditions. Data about the conversion of CHX solution into pCA are shown in Table V. Significant differences weres not found in conversion (%) into para-chloroaniline in storages circumstances and in periods (p = 0.051).

Discussion

Regardless of storage conditions, the following degradation products of 2% CHX gel and solution were

Table IV. Conversion (%) of CHX into para-chloroaniline (CHX gel prepared in laboratory, Sigma-Aldrich®).

	Initial analysis of pCA, 1.53%			
Storage situation	1 month	3 months	6 months	p*
on the worktable with light	1.01%	1.37%	1.11%	0.018
on the worktable without light	0.88%	1.30%	1.09%	
in Pasteur oven at 36.5°C without light	1.31%	1.14%	1.00%	
in the refrigerator at 8°C without light	0.96%	1.25%	1.29%	

*Chi-square.

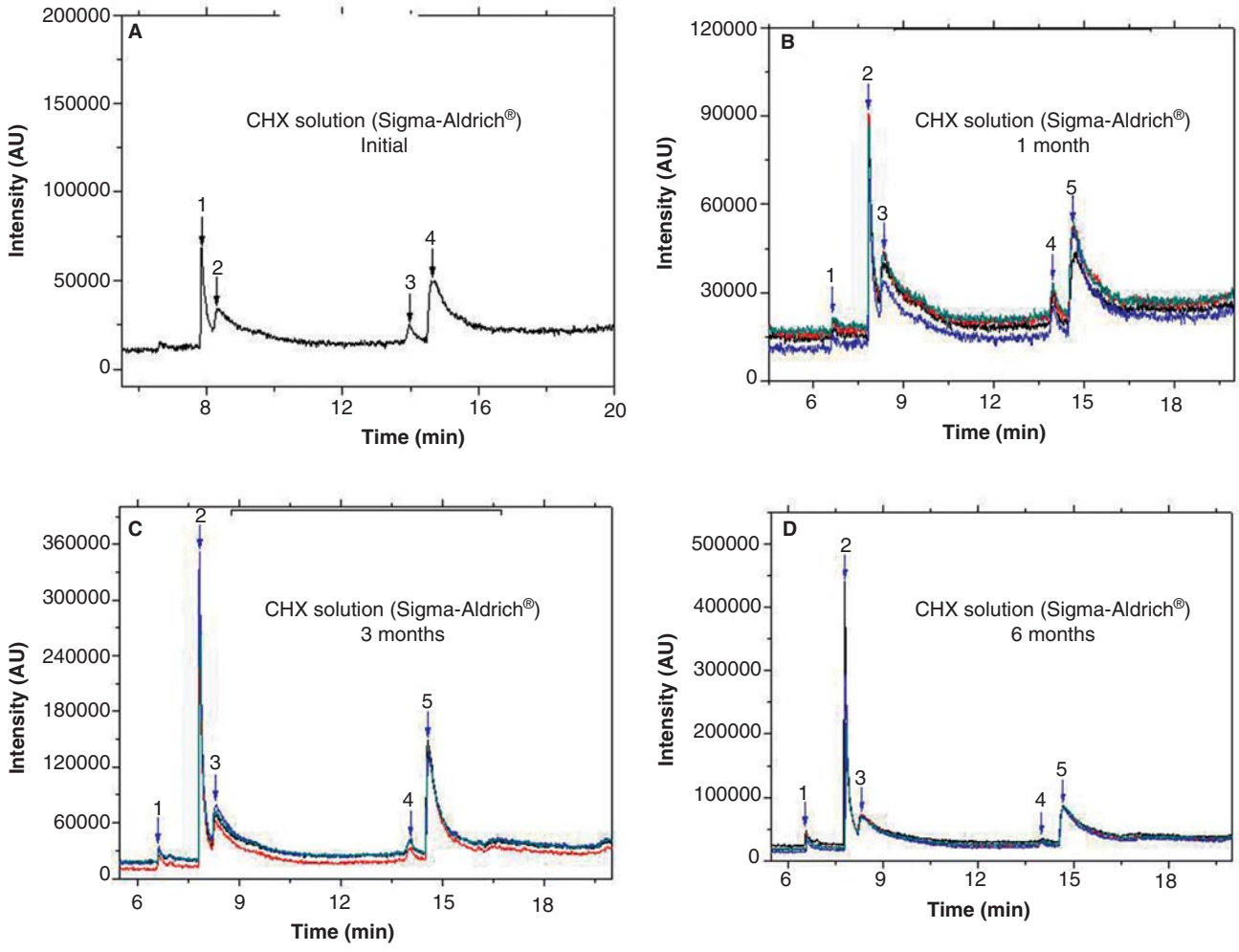

Figure 4. Chromatogram at initial time point (A), 1 (B), 3 (C) and 6 months (D) after CHX solution extraction (Sigma-Aldrich®).

found: pCA, oCA or MCA (pCA isomers), ROS and organochlorines (ortho-chlorophenyl isocyanate and 2-amino-5-clorobenzonitrila). The 2% CHX gel and solution had already degraded vial found in commercially available within the period of validity, at all time points and under all storage conditions. The amount of pCA found in CHX solution was directly proportional to time of analysis, which was not detected in the commercial brand or the pharmacy-compounded CHX due to lack of homogeneity. CHX gel (Uniraras) and CHX prepared in the laboratory showed significant differences in conversion (%) into para-chloroaniline between storage situations and periods. The homogeneity of CHX hydroxyethylcellulose gel is directly dependent on compounding mode.

GC-MS proved to be effective in identifying pCA alone or in 2% CHX gel or solution samples. Furthermore, the task of detailing peaks derived from the degradation of CHX is in agreement with

Table V. Conversion (%) of CHX into para-chloroaniline (CHX solution, Sigma-Aldrich®).

	Initial analysis of pCA, 0.68%			
Storage situation	1 month	3 months	6 months	$p^{\star}$
on the worktable with light	0.68%	1.75%	1.71%	0.051
on the worktable without light	0.76%	1.50%	1.50%	
in Pasteur oven at 36.5°C without light	0.79%	1.48%	1.62%	
in the refrigerator at 8°C without light	0.74%	1.53%	2.21%	

*Chi-square.

previous studies [20,24,25], which reported similar results. In gas chromatography-mass spectrometry, samples are bombarded by electrons and broken ions and generate positive and negative radicals. The difference in the mass/charge ratio of ions generated will separate them [33]. The by-products found in the spectra are derived from CHX decomposition due to the instability of the molecule and not due to the ionization technique [34].

In this study, pCA and an isomer (ortho- or meta-chloroaniline) were detected in all samples analyzed. The response peaks, compared to the pCA peak in the standard solution, confirmed the presence of pCA, results that are similar to those reported in other studies [20,24,25].

The characteristic peak of methylparaben (a preservative used in the samples) at RT 12.5 min was detected in all samples, except in the analysis of 2% CHX solution (Sigma-Aldrich®), which was prepared without the addition of a preservative. In the analysis of the commercial brand of chlorhexidine gel at the first time point, this peak was not detected, although this substance was listed in the product's formulation. This fact may be attributed to the lack of gel homogeneity.

There was no discrepancy in the conversion of the CHX into pCA in any gel, regardless of storage conditions (with and without light, in oven at 36.5°C or in refrigerator at 8°C). However, significant increases were observed in the production of ROS and isomers (oCA, mCA) in function of time in the conditions under analysis. These products have pro-oxidative properties capable of promoting changes in DNA [21,22].

The CHX solution prepared in the laboratory using 20% CHX solution (Sigma-Aldrich®) showed a gradual increase of pCA, in contrast with the results found in the analyses of the gels, which suggests that the gel may be able to hinder the decomposition of CHX into pCA. This decreased rate of CHX oxidation may be attributed to the steric hindrance of the solid particles in the colloidal dispersion of the gel, something that does not occur in an aqueous solution in which the particles are homogeneously dissolved. The lack of steric hindrance in our solution might have been the cause of the increase in the rate of CHX oxidation. Gel is a colloidal dispersion in which the dispersant is a solid and the liquid is dispersed, whereas a solution is a colloidal dispersion in which the dispersant is a liquid and the solid is dispersed.

In addition to pCA and its isomers, all samples showed formation of two ROS (organochlorines: ortho-chlorophenyl isocyanate and 2-amino-5-clorobenzonitrila). These products are highly toxic and may cause cell changes [13,17,18,20–22,24]. The amount of pCA found in gel samples was too small to confirm whether time, heat or presence of light increases CHX oxidation. At 6 months, there were smaller percentages of pCA than at the other time points. This difference may be explained by the formation or increase of by-products of the pCA structure, that is, an increase in the amount of isomers (oCa or mCA) or an increase of ROS, which was not uniform. Therefore, this study found that gels were not homogeneous.

The formation of pCA was more gradual and uniform when analyzed in the solution. The degradation of CHX into pCA proved to be an intrinsic factor of CHX, independent of external factors. The analysis of the CHX solution (Sigma-Aldrich®) confirmed that pCA generation is directly proportional to time. Five products of CHX degradation were detected: ortho-chlorophenyl isocyanate; pCA; oCa or mCA; 2-amino-5-clorobenzonitrila; and an unidentified ROS. The conventionally-compounded CHX gel was not homogeneous, but the method described above for gel preparation was effective in promoting CHX homogeneity. This suggests that gel homogeneity is dependent on compounding mode.

New studies should be conducted to analyze the genotoxic potential of CHX and its byproducts and the tissue damage that they may promote. Risks and benefits of the indication of this substance should be carefully evaluated. However, although the broad antibacterial effect of CHX has been proven, its clinical application should be reviewed if the analysis of cytotoxicity and genotoxicity suggests damage to DNA. Palarettia et al. [35] found that the presence of metalloporphyrin in the reaction system substantially increases the formation of pCA (up to 58%) as a CHX metabolite. These findings provide evidence of the possible *in vivo* formation of pCA as a metabolite of CHX, mediated by cytochrome P450 metabolism, which might increase the systemic risk to the health of patients treated with 2% CHX solution or pastes produced by mixing 2% CHX solution with calcium hydroxide. The formation of chloroaniline from all isomers of chloronitrobenzene was also mediated by cytochrome P450 metabolism.

Conclusions

Degradation products, such as *para*-chloroaniline (pCA), *orto*-chloroaniline (oCA), *meta*-chloroaniline (mCA), reactive oxygen species (ROS) and organochlorines (ortho-chlorophenyl isocyanate and 2-amino-5-clorobenzonitrila) were found in 2% CHX gel and solution, regardless of storage conditions or time. In relationship of gel homogenization an alternative to produce 2% CHX gel and a new homogenization method have been developed.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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