

SKIN PENETRATION AND ANTIOXIDANT EFFECT OF COSMETO-TEXTILES WITH GALLIC ACID

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Abstract

In this work, the antioxidant gallic acid (GA) has been encapsulated in microspheres prepared with poly- ϵ -caprolactone (PCL) and incorporated into polyamide (PA) obtaining the cosmeto-textile. The topical application of the cosmeto-textile provides a reservoir effect in the skin delivery of GA. The close contact of the cosmeto-textile, containing microsphere-encapsulated GA (ME-GA), with the skin and their corresponding occlusion may be the main reasons that explain the crossing of active principle (GA) through the skin barrier, located in the stratum corneum, and its penetration into the different compartments of the skin, epidermis and dermis.

An *ex vivo* assessment was performed to evaluate the antioxidant effect of the ME-GA on the stratum corneum (SC) using the thiobarbituric acid reactive species (TBARS) test. The test is based on a non-invasive *ex vivo* methodology that evaluates lipid peroxides formed in the outermost layers of the SC from human volunteers after UV radiation to determine the effectiveness of an antioxidant. In this case, a ME-GA cosmeto-textile or ME-GA formulation were applied to the skin *in vivo* and lipid peroxidation (LPO) in the horny layer were determined after UV irradiation. This methodology may be used as a quality control tool to determine *ex vivo* the percentage of LPO inhibition on human SC for a variety of antioxidants that are topically applied, in this case GA.

Results show that LPO formation was inhibited in human SC when GA was applied directly or embedded in the cosmeto-textile, demonstrating the effectiveness of both applications. The percentage of LPO inhibition obtained after both topical applications was approximately 10% for the cosmeto-textile and 41% for the direct application of microspheres containing GA. This methodology could be used to determine the effectiveness of topically applied antioxidants encapsulated in cosmeto-textiles on human SC.

Keywords: gallic acid; microspheres; cosmeto-textiles; skin absorption; lipoperoxidation

1. INTRODUCTION

Biofunctional textiles are materials that exert a biological effect on human skin. Such textiles constitute the basis for the delivery system of cosmetic or pharmaceutical substances when the textiles come into contact with the skin[1]. Active substances are commonly incorporated into the vehicles, which may break when a garment rubs the skin, allowing for the release of the compounds directly to be absorbed by the skin. These cosmeto-textiles, with a slow released of the active compound into the skin, may help people with sensitive skin.

39 In fact, there are several textile products currently in the market that claim to have properties usually
40 found in cosmetics[2], such as moisturizing, slimming, energizing, refreshing, relaxing or vitalizing
41 properties, as well as UV protection[3-4] or simply fragrance. Thus, there is a real need to develop test
42 methods to verify the effectiveness and durability of these claimed properties[5].

43 The specific case of UV protection, sunscreen lotions, clothing and shade structures provide protection
44 from the deleterious effects of ultraviolet radiations (UV). The formation of reactive oxygen species
45 (ROS) with UV exposure and their effect on lipids has also been extensively studied[6]. ROS have been
46 implicated lipid oxidation [7], which can alter tissue structure via cross-linking, fragmentation, etc. ROS
47 was part of the damage processes in SC. Some studies[8] demonstrated a marked decrease in
48 intercellular delamination energy with increasing UV exposure indicating UV radiation causes a
49 significant decrease in cellular cohesion and thus an alteration of intercellular lipid or corneodesmosome
50 structure.

51 In case of cloths when UV hits the textile, different types of interactions occur depending upon the
52 substrate and its conditions. The UV protection by textile materials is a function of the chemical
53 characteristics, physico-chemical type of fibre, presence of UV absorbers, construction of fabric,
54 thickness, porosity, extension of the fabric, moisture content of the fabrics, colour and the finishing given
55 to the fabric. The UV transmitted through textile fabrics consists of the unchanged waves that pass
56 through the interstices of the fabric as well as scattered waves that have interacted with the fabrics.
57 Another part is absorbed when it penetrates the sample, and is converted into a different energy form.
58 The portion of radiation that travels through the fabric and reaches the skin is referred to as the
59 transmission component[9]. This portion of radiation could be one of the reasons to study the antioxidant
60 protection effect of a biofunctional textile prepared with an antioxidant active.

61 Gallic acid (GA) is a polyhydroxyphenolic compound present in leafy vegetables, fruits, and nuts[10-12].
62 GA exhibits variety of biological activities including antioxidant[13-14], anti-tumor[11, 15-17], anti-
63 inflammatory[18] and anti-bacterial[19-20]. Several line of evidence has shown that GA inhibits tumor cell
64 growth, migration and invasion in vitro[21]. GA has recently been applied in cosmeto-textiles as an active
65 component[22].

66 In this work, the GA was selected and incorporated into polyamide (PA) through microspheres prepared
67 from poly- ϵ -caprolactone (PCL). PCL is an aliphatic polyester having good chemical resistance to
68 solvents[23]. It is biodegradable and non-toxic to the human body[24]. And, it has the advantage of
69 controlling the release of the active principle over a period of several days to several weeks in contrast to
70 natural hydrophobic polymers, which have a shorter period of release[25]. Therefore PCL microspheres
71 have been used as a vehicle for textile application to study the absorption and desorption properties
72 thereof when incorporated into PA[22].

73 Polyamide (PA) was used as a textile fabric in this work to obtain biofunctional textiles because of their
74 comfort when in contact with the skin. PA has been used for a number of industrial, apparel, and medical
75 applications, such as wound sutures, artificial tendons, and medical packaging, due to its excellent wear
76 resistance, strength, toughness, elastic recovery, low initial modulus, appearance retention, ease of
77 coloration, and high resistance to rupture[26].

78 Using the *in vitro* methodology of percutaneous absorption, it is possible to detect the amount of the
79 active principle that penetrates each skin layer from a given cosmeto-textile[27]. It is reasonable to
80 assume that the reservoir capacity of a cosmeto-textile, close contact with the skin and the
81 corresponding skin occlusion may be the main factors that determine how an active principle (e.g., GA)
82 crosses the skin barrier, located in the stratum corneum, and penetrates the different compartments of
83 the skin. The *in vitro* skin delivery of PA containing ME-GA has previously been explored the results
84 obtained suggested that GA penetrates through the skin detecting at different skin layers (stratum
85 corneum, epidermis and dermis)[22].

86 The aim of this work was to assess the antioxidant efficacy of a cosmeto-textile containing microsphere-
87 encapsulated GA. Our study involves a topical antioxidant strategy to prevent damage to skin specially
88 the lipid fraction. The antioxidant capacity was determined using human volunteers after topical cosmeto-
89 textile application. An *ex vivo* assessment was performed to evaluate the protection effect on the stratum
90 corneum (SC) after cosmeto-textile application. The lipid peroxide formation was determined using the
91 thiobarbituric acid reactive species test (TBARS). This test is a non-invasive *ex vivo* method that uses
92 tape strips of the outermost layers of the SC from human volunteers to evaluate the effectiveness of an
93 antioxidant[28].

94 2. MATERIALS AND METHODS

95 2.1 Materials

96 The textile bandages used were knitted fabrics (plain stitch) of polyamide 78/68/1 (DeFiber, S.A., Spain).
97 Poly(vinyl alcohol) (PVA) (87–89% hydrolyzed, MW 31 000–50 000 Da) was used as a dispersant for
98 microsphere preparation; poly- ϵ -caprolactone (PCL) (MW 45 000 Da) was used as the microsphere
99 polymer. Both PVA and PCL were supplied by Sigma-Aldrich (Madrid, Spain). Gallic acid (GA), as the
100 active ingredient, was supplied by Sigma-Aldrich (Madrid, Spain). All chemicals used were of analytical
101 grade. Methanol (HPLC grade) and phosphoric acid were supplied by Merck (Darmstadt, Germany).

102 2.2 PCL-Microspheres

103 The solvent evaporation method was used to obtain microspheres by forming microemulsions ($w_1/o/w_2$
104 double emulsion)[22]. The preparation procedure was carried out twice to obtain a sufficient volume of
105 microspheres for all textile bandage applications.

106 Briefly, 20 ml of a 2.91% (w/w) dispersion of GA in water was added to 20 ml of 2.91% (w/w) PCL in
107 dichloromethane. A simple emulsion (w_1/o) was generated by mechanical agitation (ULTRA-TURRAX
108 T25, IKA) for 25 min at 24 000 rpm. This simple emulsion was then added to a continuous phase
109 consisting of 200 ml of an aqueous PVA solution (1.96% (w/w)) and was emulsified for 30 more minutes
110 at 20 000 rpm, resulting in a double emulsion ($w_1/o/w_2$). The method used was carried out at 4 °C [29].
111 The mixture was maintained under agitation at 400 rpm (20 h) at room temperature, leading to solvent
112 evaporation and consequently microsphere formation. The percentage of GA in the formulation is 0.49 %
113 (w/v) and its percentage related to microspheres without water was 11.60 %(w/w).

114 PCL microspheres of gallic acid (ME-GA) (mixture of both preparations) were applied to textiles (322 ± 1
115 cm^2 area) by bath exhaustion, with a bath ratio of 1/5 (1 g textile per 5 ml of treatment bath), at 50°C for
116 60 min with manual stirring performed every 10 min. To quantify the amount of product absorbed onto
117 the fabrics, the dry samples were weighed before and after 24 h of application under standard ambient
118 conditions ($23 \pm 2^\circ\text{C}$ and $55 \pm 5\%$ relative humidity).

119 The GA present in the formulations and absorbed onto the fabrics was extracted with methanol during 15
120 minutes under ultrasounds and quantified using a Hitachi-Merck HPLC equipped with an L-6000
121 Intelligent Pump, AS-4000 Autosampler and L-4250 UV-Vis Detector. The column used was a LiChrocart
122 250-4/Lichrosorb RP-18 (5 mm) (Darmstadt, Germany). The mobile phase was 90% water (with 0.7%
123 H_3PO_4)/10% methanol flowed at a rate of 1 ml/min. GA detection was conducted at 280 nm with a
124 retention time of 6.9 min. The area below the peak in the chromatogram was used to calculate the
125 concentration of GA using external standards that displayed linearity over a concentration range of 0.25
126 to 100 mg/ml. The equation yielding the regression line through the experimental values was ($\text{ABS} =$
127 $15967 \cdot [\text{GA}] + 4417.1$, $r^2 = 0.9999$). This analytical methodology was fully validated.

128

129 2.3 Size of PCL microspheres

130

131 The size distribution and polydispersity of the PCL microspheres were measured by dynamic light
132 scattering (DLS) (Zetasizer Nano ZS ZEN3600; Malvern Instruments Ltd., Malvern, Worcestershire, UK).
133 The non-invasive backscattering technology was used to minimize multiple scattering effects without the
134 need for sample dilution. The size distribution and polydispersity measurements were performed at room
135 temperature with polystyrol/polystyrene cells (Ref 67.754 Sarstedt). The scattered light was detected at
136 an angle of 173° . Each sample was measured in triplicate. The data were interpreted by considering the
137 size distribution by intensity. All data were collected and analyzed using the program DTS (dispersion
138 technology software) provided by Malvern Instruments Ltd.

139 2.4 Volunteers

140 The experimental protocol was conducted with 8 healthy Caucasian volunteers (all women) with
141 phototypes II, III and IV [30]. The mean age of the volunteers was 36.8 ± 13.6 years (range years) (Table
142 1). The volunteers refrained from using cosmetics, body oils, sunscreens or moisturizers on their arms 4
143 days prior to the study and during the study except for the 2 formulations selected. The participants were
144 given a detailed description of the study, and the corresponding written consent forms were obtained.
145 The study design was approved by the local ethics committee (IQAC-CSIC, Barcelona, Spain).

146

Table1. Age and phototype of volunteers.

Volunteer	1	2	3	4	5	6	7	8	Mean \pm SD
Age	24	24	28	60	34	36	33	55	36.8 ± 13.6
Phototype	II	III	IV	II	IV	IV	IV	III	

147

148 2.5 *In vivo* cosmeto-textile and formulation application

149 The cosmeto-textiles (polyamide containing ME-GA) and control textiles (polyamide without antioxidant)
150 were applied on subjects' arms, maintaining close contact with the skin over a period of 4 days as a
151 bandage application.

152 Moreover, the formulation containing PCL microspheres of GA (ME-GA) was directly applied to the skin
153 of each volunteer. A skin area of 18 x 5 cm² was marked on the volunteers' forearms. Formulations were
154 applied by each volunteer twice a day over a period of 4 days on one forearm with a final total amount of
155 6 mL of ME-GA applied (327 µg/cm² of GA). The other forearm was used as a control without
156 antioxidant application.

157 **2.6 Tape stripping and lipid extraction**

158 The tape stripping of the SC of each forearm and arm was carried out on the 4th day in a conditioned
159 room at 25 ±1°C and 50 ± 2% relative humidity using D-Squame™ tape (φ= 22 mm, CuDerm, Dallas,
160 USA) previously pressed onto the skin with a roller and stripped in one quick move.

161 The weight of each tape was determined directly (Mettler Toledo Excellence XA105, Greifensee,
162 Switzerland) before and after stripping, and the weight of the SC removed was then determined. Then,
163 half of the strips were fixed in a glass plate and irradiated using a light source simulating UV solar
164 radiation (3.045 J/min cm², Suntest CPS, Atlas, USA). This radiation is double the maximum radiation
165 input in one day in June in Catalonia (1.50-1.84 J/min cm² equivalent to 21-26.5 MJ/m² per day [31]). The
166 UV exposure time was 60 min, yielding a UV irradiation intensity of 182.7 J/cm².

167 The SC lipids from a group of 3 strips were extracted with methanol (Merck, Darmstadt, Germany) with
168 sonicated in a Labsonic 1510 device (B. Braun, Melsungen, Germany) for 15 min.

169

170 **2.7 Determination of lipoperoxidation (LPO)**

171 Lipid peroxides (LPO species) were measured by the thiobarbituric acid (TBA) assay [28]. The
172 thiobarbituric acid-reactive species (TBARS) were quantified by spectrophotometry at 534 nm (Cary 300
173 Bio UV-Visible Spectrophotometer, Varian, USA). At low pH and elevated temperature, MDA readily
174 participates in a nucleophilic addition reaction with TBA, generating a red, fluorescent 1:2 MDA:TBA
175 adduct.

176 The results obtained were expressed as malonaldehyde bis(dimethyl acetal) equivalents (µM MDA)
177 using a standard curve for pure MDA-TBA complexes. The calibration curve was obtained by using
178 MDA (Sigma, St Louis, MO, USA) in different concentrations (0-40 µM). Additionally, negative and
179 positive controls were quantified using 0 and 100 µM MDA. The calibration curve was prepared on each
180 day of the experimental study. The general regression equation obtained from the experimental analysis
181 was $ABS = -0.0006 [MDA]^2 + 0.0751 [MDA] - 0.0137$ (correlation coefficient, $r^2 = 0.999$). The repeatability and
182 reproducibility of the analytical method was confirmed by the absorbance of the MDA positive control
183 (100 µM MDA). The mean value obtained for this MDA control (100 µM) was 2.3314 ± 0.0206 . The
184 results for each experimental analysis indicated good repeatability and intra-day precision with an
185 acceptable R.S.D. (<0.50%).

186 Briefly, a 0.5 ml volume of SC lipid extracts and standard MDA solutions was added to aliquots (1 ml) of
187 a solution made up with 0.4% TBA (Sigma, St Louis, MO, USA) and 15% trichloroacetic acid (TCA)
188 (Merck, Darmstadt, Germany) in 100 ml of HCl solution (0.25 M). The mixture was incubated for 1 h in a
189 boiling water bath. Fresh TBA /TCA stock solution was prepared on each day of analysis.

190 Following the experimental procedure[28], the amount of LPO formed from unprotected skin (placebo)
191 and the amount of LPO formed from protected skin (antioxidant application) were calculated based on
192 the absorbance values obtained for the SC extraction samples by MDA determination. Therefore, the
193 percentage of LPO inhibition (% LPO inhib.) was determined from the difference in the amount of LPO
194 formed between the SC placebos and SC antioxidant samples.

195 **2.8 Statistics**

196 Global results were calculated as medians with their 0.25 and 0.75 percentiles (0.25; 0.75). The Mann-
197 Whitney test was applied for group comparisons. The software used was STATGRAPHICS plus 5.
198 Significant differences in the mean values were evaluated by an F-test. A *p* value below 0.05 was
199 considered significant.

200 **3. RESULTS AND DISCUSSION**

201

202 Formulations containing PCL microspheres were prepared using a double emulsion method, as
203 described in the Materials and Methods section. The diameter of the particles in the ME-GA mixture was
204 measured with Dynamic Light Scattering (DLS) and results indicated the presence of microspheres with
205 a homogeneous size distribution of 2692 nm with a polydispersity index of 0.437. Moreover, the amount
206 of GA present in the PCL microsphere formulation was determined by HPLC using an external
207 calibration curve. The amount of GA in the ME-GA formulation was $0.49 \pm 0.01\%$ (w/v).

208

209 The performance of biofunctional textiles with release properties could be affected by a number of
210 factors, such as biocompatibility, biostability, biodegradability, drug delivery efficiency, drug quantity,
211 system design and control in terms of dose, rate and time of development[32]. Thus, it is very important
212 to establish the precise amount of active agents present in a textile before its use as a textile drug
213 delivery system. In this study, PCL microspheres were applied by bath exhaustion, as discussed in the
214 'Materials and Methods' section. Textiles were submitted to a bath impregnation formulation that
215 contained ME-GA. Table 2 shows the weights of the PA bandages before and after application. The final
216 percentages of dry product incorporated into the fabrics were calculated based on the weight difference
217 between the initial dry fabrics and the dry fabrics after the bath exhaustion process. The median amount
218 of dry product applied was $11.59 \pm 0.65\%$ over weight of fiber (owf). Taking into account the percentage of
219 GA in PCL microspheres without water (11.60% (w/w)), the mean of the treated area of fabric (322 cm²)
220 and the mass increase of the fabric (0.9124 g), the mean amount of GA/cm² in the textile calculated
221 accounts for $350 \pm 17 \mu\text{g GA/cm}^2$.

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Table 2. Weights of PA bandage before and after ME-GA application by bath exhaustion and the total dry product on the textile after water evaporation

Volunteer	PA WEIGHT after 24h at 23°C and 55%Hr			Dry Product
	Bandage before ME-GA application (g)	Bandage after ME-GA application (g)	Mass Increased (g)	% owf of ME-GA
1	8.3385	9.2989	0.9604	11.52
2	5.6969	6.3860	0.6891	12.09
3	8.3429	9.3572	1.0143	12.06
4	8.4450	9.4432	0.9982	11.82
5	8.2804	9.2498	0.9694	11.71
6	8.1517	8.9948	0.8431	10.34
Mean	7.8759	8.7883	0.9124	11.59

228
229
230

231 In addition, a piece of treated textile was used to extract GA with methanol using an ultrasound bath to
232 quantify GA by HPLC. The amount of GA incorporated into the textiles was determined to be $376.63 \pm$
233 $19.93 \mu\text{g GA/cm}^2$ fabric. The theoretical value of approximately $350 \mu\text{g GA/cm}^2$ on PA obtained from
234 mass increase of fabric confirms the equivalence of the two methodologies in determining the amount of
235 GA in the textiles. In addition, this result indicated that it was substantively between the textile and active
236 agent, which could play an important role during the application process.

237

238 After analytical determination, microspheres of GA were applied in vivo. Cosmeto-textiles containing ME-
239 GA were fixed on one arm of 6 volunteers, and the ME-GA formulation (concentration of 0.49% w/v) was
240 directly applied on one forearm of 6 volunteers; both applications were maintained for 4 days. The GA
241 applied in skin was of $377 \mu\text{g/cm}^2$ for cosmeto-textile and $327 \mu\text{g/cm}^2$ for formulation. The outermost
242 layers of SC were then obtained using tape as described in the Materials and Methods. The results
243 obtained for the mean weight of SC for both applications were similar. The mean weights of the SC
244 obtained were 0.83 ± 0.11 and $0.76 \pm 0.17 \text{ mg/3 tape strips}$ for the cosmeto-textile and direct application
245 modes, respectively.

246 Half of the SC layers in the strips (from unprotected and protected skin) obtained by each application
247 mode were irradiated with an intensity of 182.7 J/cm^2 . LPO compounds were then extracted from a group
248 of 3 strips. The LPO values and the percentage of LPO inhibition due to the application of the cosmeto-
249 textiles and the formulation were determined for all volunteers.

250 The LPO results obtained in μM from unprotected and protected skin are shown in Table 3, as well as
251 the percentages of LPO inhibition (%). Figure 1 presents the medians of the results obtained from
252 unprotected and protected skin when ME-GA was applied via a cosmeto-textile or directly.

253

254 Table 3. LPO values obtained from unprotected and protected skin with ME-GA for each volunteer when
 255 cosmeto-textiles and formulation were applied.

Application	Volunteer	LPO (μM)			% LPO median (percentil 0.25; 0.75)
		Unprotected skin	Protected skin	% LPO inhib.	
ME-GA Cosmeto- Textiles	1	0.7899	0.7245	8.28	10.12 (8.68; 15.73)
	2	0.5891	0.4860	17.51	
	3	0.9059	0.8120	10.37	
	4	0.1820	0.1640	9.87	
	5	0.2967	0.1904	35.81	
	6	0.5205	0.4939	5.11	
ME-GA formulation	3	0.8223	0.3310	59.75	41.45 (34.43; 52.97)
	4	0.0470	0.0304	35.19	
	5	0.2517	0.1140	54.72	
	6	0.3155	0.2077	34.17	
	7	0.3803	0.1989	47.71	
	8	0.0963	0.0956	0.77	

256

257 As shown, the amounts of LPO obtained from unprotected skin were higher than those obtained from
 258 protected skin using ME-GA via each application mode (Figure 1). These results demonstrate a lower
 259 extent of formation of lipid peroxides in the SC of skin protected after UV irradiation when the antioxidant
 260 was used. The amounts of LPO obtained for each volunteer were highly variable due to the effects of
 261 several variables such as the amount of GA delivered into skin, the different skin types of the volunteers,
 262 and the amount of SC present in the samples. Therefore, even the results are not statistically significant
 263 for any of the applications there is an appreciable difference in the amounts of LPO obtained from
 264 unprotected and protected skin.

265

266 Despite the variation in the percentage of LPO inhibition, the results indicate a clear inhibition of LPO for
 267 both ME-GA applications. It appears that the ME-GA used has a protective effect on human SC against
 268 lipid peroxidation, with the percentage of LPO inhibition reaching 10.12% LPO (8.68; 15.73) for the
 269 cosmeto-textile application and 41.45% LPO (34.43; 52.97) for the direct application of the ME-GA
 270 formulation.

271 Similar amounts of SC were retrieved on the 3 strips: 0.79 ± 0.15 mg/3 tape strips. The percentage of
 272 LPO inhibition was calculated based on the total amount of lipid peroxides on SC treated or not treated
 273 with antioxidant. The results of LPO inhibition (%/mg of SC) obtained were 11.28%/mg (8.09; 35.58) for
 274 the ME-GA cosmeto-textiles and 43.65%/mg (14.37; 53.38) for the ME-GA formulation.

275 TBARS assay is a convenient and simple method which has been frequently used as a standard
 276 biomarker of LPO in vivo with human plasma and urine. LPO is the major reaction taking place under
 277 oxidative stress and assumed to play an important role in the pathogenesis and progression of many
 278 diseases[33]. This paper reports a non-invasive ex vivo method to detect LPO in human skin using tape

279 strips of the outmost layers of SC from human volunteers. This strategy permits evaluate the
280 effectiveness of topical treatment with antioxidant against oxidative stress caused by UV exposure.

281 In a previous study[22], a specific *in vitro* percutaneous absorption methodology was designed to
282 demonstrate the delivery of an encapsulated component from a textile to the different skin layers
283 (stratum corneum, epidermis or dermis). The percutaneous absorption results obtained are shown in
284 Figure 2.

285

286 The percutaneous absorption results indicate that the skin penetration of GA released from PCL
287 microspheres that were applied directly to the skin was higher than that observed when GA was
288 embedded within the cosmeto-textiles. In all cases, the highest amount of GA that penetrated the skin
289 was observed in the superficial layer (SC, stratum corneum), where the amount was 5 to 10 times higher
290 than that in the epidermis or the dermis. In conclusion, an interesting reservoir effect may have been
291 promoted when biofunctional textiles were used.

292 Results concerning skin delivery to the different skin layers (stratum corneum, epidermis and dermis)
293 showed that ME-GA incorporated into the cosmeto-textiles displayed high GA retention. This retention
294 capacity of cosmeto-textiles was deduced by the lower amount of antioxidant found in the inner skin
295 layers compared to that measured after the direct application of ME-GA. This suggests a slower but
296 continuous release of antioxidant to the skin when ME-GA cosmeto-textile is applied. The high amount of
297 GA in the SC layer for both applications can be related to the extensive LPO inhibition observed after UV
298 irradiation of the tape strips containing SC. In addition, a stronger protective effect of the ME-GA
299 formulation was observed compared to the ME-GA cosmeto-textiles. It may also be due to the different
300 amounts of GA found in the SC for the two different types of applications. The cosmeto-textile presents
301 two diffusion phases, first this active compound must diffuse from the fabric to the skin surface when the
302 cosmeto-textile is topically applied. The second phase is the penetration of the compound at the different
303 skin layers. The low release of GA from the cosmeto-textiles may suggest a slow but continuous release
304 of the antioxidant to the skin.

305 Although the content of antioxidant present in the skin was lower for the cosmeto-textile mode of
306 application than for the direct mode of application, it is important to note that the amount released
307 demonstrated sufficient capacity to combat lipid peroxidation in human SC. The use of cosmeto-textiles
308 allowed for a reservoir effect with a prolonged and controlled dose of the antioxidant such that the
309 corresponding UV protection was also prolonged.

310 4. CONCLUSIONS

311 This study explored the antioxidant efficacy of a cosmeto-textile containing ME-GA and a formulation
312 containing gallic acid in microspheres. The effectiveness of the antioxidant via the ME-GA cosmeto-
313 textile and direct application routes was determined by a non-invasive *ex vivo* method with tape strips of
314 the outermost layers of the SC of human volunteers. The antioxidant was applied to prevent lipid
315 peroxidation in the horny layer after UV irradiation.

316 The results indicate that LPO was inhibited in human SC when GA was applied, demonstrating the
317 effectiveness of both applications. The capacity of each application route to reduce LPO in the outermost

318 layers of the skin was confirmed. The percentage of LPO inhibition obtained after each topical
319 application was approximately 10% for the cosmeto-textile route and 41% for the direct route.

320 This finding is in accord with the results obtained from a percutaneous absorption study in which PCL
321 microspheres containing GA and the textiles were impregnated with the same formulation. Percutaneous
322 absorption data indicate a high level of skin penetration for GA that was directly applied within PCL
323 microspheres. The high extent of LPO inhibition measured is proportional to the amount of GA measured
324 in the outermost layers of the skin.

325 The LPO methodology could be used to verify the antioxidant capacity of encapsulated substances
326 transferred into human skin by cosmeto-textiles that can deliver specific doses of active ingredients. The
327 incorporation of antioxidants into cosmeto-textiles allows for the natural photoprotection capacity of the
328 skin to be enhanced when such textiles are used for prolonged application periods.

329

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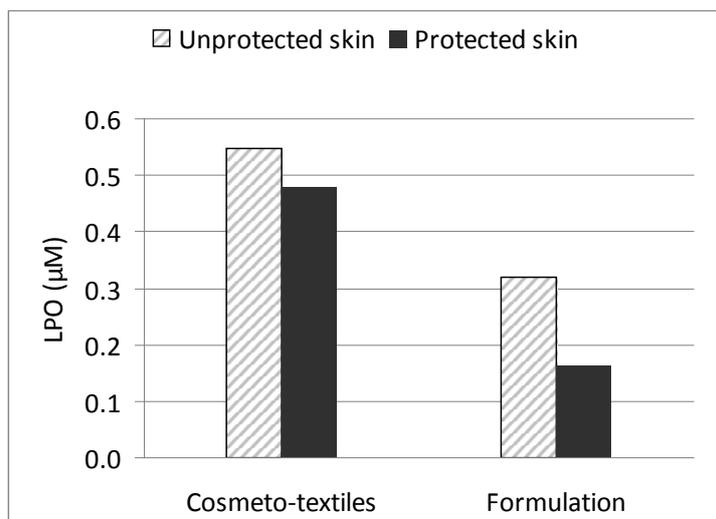


Figure 1. LPO values obtained from unprotected and protected skin with ME-GA cosmeto-textiles and ME-GA formulation ($p>0.05$).

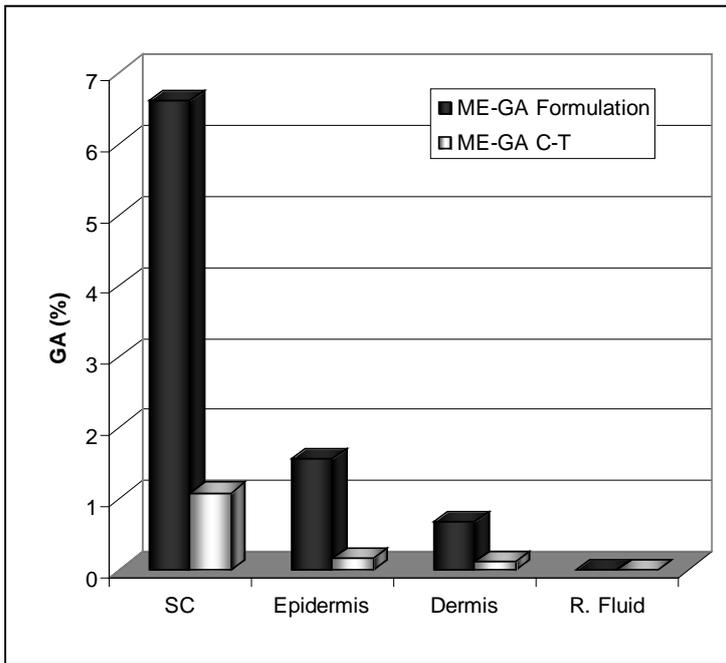


Figure 2. *In vitro* percutaneous absorption of GA from PCL-microspheres and from biofunctional textiles (PA). (SC: stratum corneum; E: epidermis; D: dermis; R. Fluid: receptor fluid)