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Detecting active ingredients of insect repellents and sunscreens topically in skin by Raman spectroscopy

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Abstract. We present the use of Raman spectroscopy for determination of functional characteristics of insect repellents and sunscreens by identifying the active ingredients of these products applied topically to the skin. Commercial formulations of insect repellents and sunscreens (SPF 15 and 30) were obtained, and Raman spectra were obtained from the formulations and from volunteers' skins with topical applications of such products compared to controls. The results indicated that, for insect repellents, the peaks at 527 and 1003 cm^{-1} were markers of the presence of the active ingredient diethyl toluamide in the skin, while for sunscreens, the peaks at 1177, 1288, and 1611 cm^{-1}, associated to octinoxate, benzophenone-3, and avobenzone, were markers of the presence of solar filters in the skin. The results suggested reliability in the use of Raman spectroscopy to identify the active ingredients of insect repellents and sunscreens topically applied on the skin; the applied methodology can be used to determine the functional characteristics of topical products with similar characteristics. [DOI: 10.1117/1.JBO.23.10.107003]

Keywords: Raman spectroscopy; sunscreen; insect repellent; *in vivo* detection; quality control.

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1 Introduction

Insect repellents and sunscreens are cosmetics that are practical means for environmental protection against risks brought by insects, such as mosquitoes or ticks, and solar radiation. Insect repellents are compounds that, when applied to skin, clothes, or surfaces, inhibit insect proximity. Their use reduces transmission of infectious diseases and resulting immunoallergic reactions from insect bites. Topic chemical repellents based on the active ingredient diethyl toluamide (DEET) are the most commonly used insect repellents worldwide.[1] Sunscreens are products intended to absorb solar radiation and to protect viable skin cells against potentially harmful ultraviolet radiation, such as sunburns and skin cancer. These products make use of solar filters, which are active ingredients that act by absorbing (organic and inorganic molecules) or by minimally reflecting/scattering (inorganic molecules and metallic oxides particles).[2,3] As with other cosmetics that also offer protective action against environmental risks, it is necessary to perform systematic assessments, which provide evidence showing that the attributed protective properties achieve the expected results.

The Brazilian Health Surveillance Agency (ANVISA) requires that manufacturers of insect repellents and sunscreens to present studies to attest product efficacy following methodologies acknowledged by the agency. For insect repellents, RDC 19/2013[4] adopts protocols from World Health Organization[5] and United States Environmental Protection Agency,[6] for sunscreens, RDC 30/2012[7] accepts test protocols from the United States Food and Drug Administration[8] and International Organization for Standardization (ISO).[9] These listed methodologies expose the individual to the aggressive environmental action (insects for repellents and solar radiation for sunscreens), comparing the effects between treated and untreated areas.

The experimental approaches adopted for repellents and sunscreens raise ethical issues related to the use of humans as research subjects. Each new formula requires new testing, resulting in routine exposure of several individuals. Besides ethical questions, several studies criticize these methods. Regarding insect repellents, Barnard[10] observed that these methodologies bring uncertainties which affect the precision of the results due to limitations imposed by issues such as test arrangement and insect appetite, among others. Other studies criticize assessment protocols based on insect behavior,[11–13] dealing with the challenges listed by Barnard but without proposing new solutions to mitigate such uncertainties.

Methodologies currently in use for determination of sun protection factor (SPF) are based on the cutaneous erythema produced by UVA and UVB radiation attenuation[14] and the UVA protection factor (UVAPF), which is based on the tanning reaction produced by UVA radiation onto skin, by measuring the persistent pigment darkening of the unprotected versus protected areas.[15] However, these methods may involve subjectivity, since the results depend on the interpretation, by a trained evaluator, of the erythema (for the SPF) or the darkening (for the UVAPF) produced on the skin.[16] Also, these methods are not able to detect the concentration or even the presence of the active ingredients onto the skin.

Currently, manufacturers of pharmaceutical products employ good manufacturing practices (GMPs) and process analytical technologies (PATs) to perform quality control during the production process.[17–19] Optical techniques such as near-infrared and Raman spectroscopy have been proposed as methods to obtain chemical information for real-time process monitoring and control for PAT technology as they supply the data from which relevant process and product information and conclusions are to be extracted.[20] Also, Raman spectroscopy has been used to detect the presence and bioavailability of pharmaceutical and

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natural active products applied topically onto the skin,[21,22] which may turn into a technique of choice for rapid identification and quantification of such products *in vivo* without sample preparation.

Raman spectroscopy is an optical technique based on the inelastic scattering of a monochromatic light (from a laser beam) when irradiating a polarizable molecule. On such molecules, such as organic ones, the molecular vibrations induce changes in the electronic cloud polarizability upon photon incidence, resulting in energy exchange between the molecular vibrational energy and the incident light due to the polarizability. The inelastic scattered light produces a characteristic spectrum (spectral signature or "molecular fingerprint"), with peaks at the same positions of the molecular vibration energy of the molecule.[23] Recent advances in Raman instrumentation can benefit from near-infrared excitation (1064 nm) and fiber optic Raman probes to perform real-time, fluorescenceless spectra collection.[24]

Raman spectroscopy has been used to assess the effectiveness of a variety of products for topical use such as moisturizing ingredient[25] and salicylic acid,[26] to detect the presence of endogenous or exogenous compounds present in the skin (epidermis, stratum corneum),[27,28] and to determine the biochemical composition of the skin aiming cancer diagnosis.[29] Topical cutaneous bioavailability of drugs and pharmaceutical products can be assessed by several techniques, including Raman spectroscopy.[22] Therefore, the Raman spectroscopy is suitable to identify the biochemical components present in the formulations and topically applied to the skin, with advantages including noninvasiveness, rapidness, nondestruction of the sample, and ability to obtain chemical information of the sample with molecular specificity. Such aspects may promote the development of a simple, reliable, and precise research protocol, aiming to determine the presence of the active ingredients of topically applied repellents and sunscreens. This approach would help in screening the presence of the active ingredient in the topical application, thus avoiding additional testing when the active ingredient under evaluation has already been proved to be effective and it is only important to know if the formulation is able to actually retain it.

The development of a technique suitable to measure the presence of topically applied insect repellents and sunscreens could help in obtaining information regarding the presence of the active ingredient in the skin *in vivo* as well as quantifying them, increasing the effectiveness of tests already carried out. Going in this direction, this work aims the use of Raman spectroscopy to: (a) evaluate the composition of insect repellents and sunscreens in formulations commercially available, by identifying the peaks of the most relevant active ingredients of these formulations; and (b) to determine the presence of these active ingredients when the formulations are topically applied to the skin of volunteers compared to a control site, in an effort to develop an optical methodology to assess the presence of these ingredients on skin.

2 Materials and Methods

2.1 *Selection of Volunteers*

This study was approved by the Ethics and Research Committee of Universidade Anhembi Morumbi (Process No. CAEE 69573917.9.0000.5492) in conformity with the Resolution 466/2012 of the Brazilian National Health Council from the Brazilian Ministry of Health.[30] Volunteers who agreed to participate signed an informed consent form.

A group of volunteers (nine women and five men) was enrolled for the repellent testing, and another group (seven women and seven men) was enrolled for the sunscreen testing. The skin phototype ranged from I to IV (phototype classification according to Fitzpatrick scale[31]). Inclusion criteria were healthy people, aged between 18 and 60 years, without any skin wound and absence of any known allergic reaction to the ingredients of the products. Exclusion criteria were allergic reaction to products during the experiment period and painful sensibility to the Raman laser beam.

2.2 *Samples*

The study used two samples of insect repellents of a market leading brand presented as spray and cream (identified as SPR and CR) and four samples of sunscreens, all presented as cream, of two market leading brands (identified as CEN and SD) with SPF of 15 and 30. The repellent products used in the study had a composition according to Table 1 and the sunscreen products had a composition according to Table 2.

Table 1 Composition of the insect repellent products used in the study per the information provided on the product labels. An asterisk (*) highlights the active ingredients.

	Presence in the formulation	
Insect repellent components	SPR	CR
DEET*	Yes (6.65%)	Yes (7.125%)
Aqua	No	Yes
Polyacrylic acid	No	Yes
Stearate-2	No	Yes
Stearyl alcohol glyceryl stearate	No	Yes
PEG-100 stearate	No	Yes
Methyl paraben	No	Yes
Triethanolamine	No	Yes
Parfum	Yes	Yes
Aloe barbadensis flower extract	Yes	Yes
Benzyl salicylate	Yes	Yes
Coumarin	Yes	Yes
Hydroxycitronellal	Yes	Yes
Limonene	Yes	Yes
Linalool	Yes	Yes
Butylphenyl methilpropianol	Yes	Yes
Alcohol benzyl methylpropional	No	Yes

Note: SPR, spray; CR, cream.

Table 2 Composition of the sunscreen products used in the study per the information provided on the product labels. An asterisk (*) highlights the active ingredients.

Sunscreen components	Presence in the formulation	
	Brand CEN (SPF 15 and 30)	Brand SD (SPF 15 and 30)
Ethylhexyl methoxycinnamate*	Yes	No
Benzophenone-3*	Yes	No
Octocrylene*	Yes	Yes
Ethylhexyl triazone*	No	Yes
Bis-octoxyphenol methoxyphenyl triazine*	Yes	Yes
Aqua	Yes	Yes
Titanium dioxide	Yes	Yes
Phenethyl benzoate	Yes	No
Isocetyl stearoyl stearate	Yes	No
Glyceryl stearate	Yes	No
Laureth-23	Yes	No
Diisopropyl adipate	Yes	No
Propylene glycol	Yes	No
PVP/eicosene copolymer	Yes	No
Cyclomethicone	Yes	No
Trilaureth-4 phosphate	Yes	No
Sodium polyacrylate	Yes	No
Decarboxy carnosine hydrocloride	Yes	No
Laureth-2	Yes	No
DMDM hydantoin	Yes	No
Methylparaben	Yes	No
Dimethicone	Yes	Yes
Tocopherol	Yes	No
Iodopropynyl butylcarbamate	Yes	No
Parfum	Yes	Yes
Daucus carota seed oil	Yes	No
Disodium EDTA	Yes	Yes
2-Bromo-2-nitropropane-1,3-diol	Yes	No
Hexyl cinnamal	Yes	No
D-Limonene	Yes	No
Linalool	Yes	No
C12-15 alkyl benzoate	No	Yes
Butylene glycol	No	Yes
Butyl methoxydibenzoylmethane	No	Yes
Potassium cetyl phosphate	No	Yes
Triacontanyl PVP	No	Yes
Aluminum starch octenylsuccinate	No	Yes
Silica	No	Yes
Benzyl alcohol	No	Yes
Phenoxyethanol	No	Yes
Triethanolamine	No	Yes
Cetyl palmitate	No	Yes
Tribehenin	No	Yes
Stearyl alcohol	No	Yes
Caprylyl methicone	No	Yes
Carbomer	No	Yes
Acrylates/C10-30 alkyl crosspolymer	No	Yes
Tocopheryl acetate	No	Yes

2.3 *Application of the Products on the Skin*

Initially, the sites where the products were applied were sanitized with an alcohol-soaked cloth (ethanol 95%) for removal of contaminants. For the insect repellents, volunteers received topical administration of spray and cream products on two circular sites of the anteromedial region of both forearms, each circular site with 25 mm in diameter, identified as SPR (spray) and CR (cream) [Fig. 1(a)]. A third circular site, located in the anteromedial region of left forearm and identified as CTR (control), was used as control and did not receive any product [Fig. 1(a)]. Spray insect repellent was applied by a single spray, 10 cm far from the volunteer's skin, at the SPR site. Cream repellent was applied throughout the marked area with the help of a microspatula, using a standard amount of cream (about 9.4 mg), on the site identified as CR located at the left forearm. The quantities of each product used in this study are compatible with recommendations from the repellent manufacturers as prescribed in the respective labels.

Sunscreen products were applied topically at the anteromedial region of each volunteer's right forearm. The application was performed on four circular sites with 25 mm diameter, identified with numbers from 1 to 4 [Fig. 1(b)]. In each region, a standard amount of sunscreen (9.4 mg) of the brands CEN

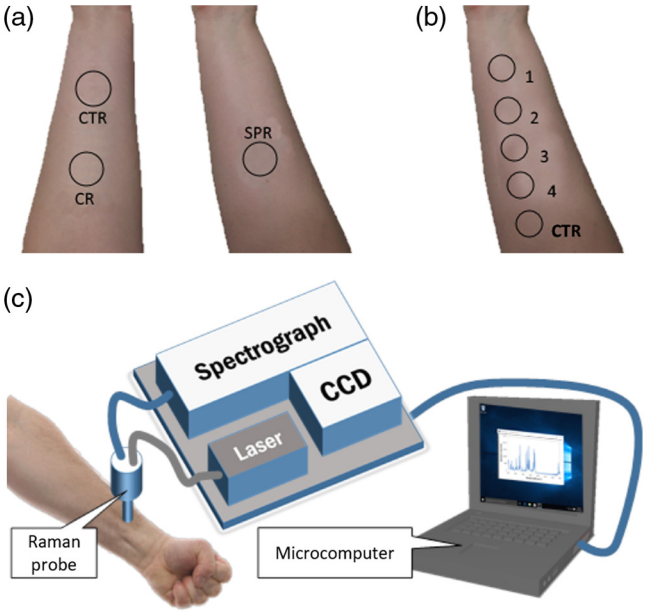

Fig. 1 (a) and (b) Forearm markings for repellent and sunscreen application in circular sites, respectively. (c) Schematic diagram of the dispersive Raman spectrometer used for spectral data acquisition. The Raman probe possesses a conical aluminum tip to promote the focal distance between the probe tip and the skin surface.

(SPF 15 and 30, sites 1 and 2) and SD (SPF 15 and 30, sites 3 and 4) was applied, with the help of a microspatula throughout the marked area. Site 5 was kept as a control region, identified as CTR [Fig. 1(b)]. The quantity of the sunscreen applied on the marked area was according to the recommendation by the ANVISA for SPF determination tests, corresponding to 2.0 mg/cm^2 (as preconized by ISO[9] and the European Cosmetic and Perfumery Association—COLIPA[32]). Volunteers waited for 30 min before Raman spectra acquisition.

2.4 *Collection of the Raman Spectra*

Initially, Raman spectra of the commercial products were acquired, placed on an aluminum sample holder, identified as REF_SPR and REF_CR (repellents spray and cream), and REF_CEN15, REF_CEN30, REF_SD15, and REF_SD30 (sunscreens of brands CEN and SD, with SPF 15 and 30, respectively). Then, spectra were acquired from each site shown in Fig. 1 in triplicate. Table 3 shows the number of spectra obtained in each site and the number of acquisitions per group. Some spectra were withdrawn due to very strong fluorescence background, particularly in the groups SD15 and SD30, which caused saturation of the detector in the Raman spectrometer and degradation of the Raman signal due to fluorescence shot noise.

Raman spectra of each region were acquired using a dispersive Raman spectrometer (model Dimension P-1, Lambda Solution Inc., Massachusetts), with a fiber optic Raman probe (model Vector Probe, Lambda Solutions Inc., Massachusetts) schematically presented in Fig. 1(c). The spectrometer uses a diode laser (830 nm, 350-mW max power) coupled to a Raman probe to irradiate the repellent/sunscreen sample or the skin. The probe captures the light scattered by the sample, which is dispersed by the spectrograph and directed to a high sensitivity, deep-cooled CCD camera connected to a notebook computer for acquisition and storage. Spectrometer

Table 3 Application sites, number of spectral acquisitions, and name of groups used in the study.

Application site (Fig. 1)		Number of spectra in each site[a]	Group name
Insect repellent (14 volunteers, 42 spectra)	SPR	42	SPR
	CR	39	CR
	CTR	42	CTR
Sunscreen (14 volunteers, 42 spectra)	1	42	CEN15
	2	37	CEN30
	3	33	SD15
	4	30	SD30
	CTR	42	CTR

Note: SPR, spray; CR, cream; CTR, control; CEN, brand 1; SD, brand 2.
[a]Some volunteers presented very strong fluorescence background after product's application and these spectra were withdrawn.

wavenumber calibration was checked through the Raman bands of naphthalene; the correction of the spectrometer's spectral response (intensity calibration) was applied at the time of spectrum storage. The Raman spectrometer has a resolution of about 4 cm^{-1} in the spectral range of 400 to 1800 cm^{-1}.

A conical aluminum tip was used at the probe's distal tip, to standardize the focal distance between the probe tip and skin, allowing for repeatability of excitation and collection geometry during spectra acquisition. Three spectra in each site area were acquired, each spectrum with acquisition time of 30 s and laser power on probe's tip set to 250 mW.

2.5 *Spectra Processing and Statistical Analysis*

Raman spectra from products placed on an aluminum sample holder and topically to skin were preprocessed the same way as detailed by Silveira et al.[33] First, high-intensity outliers from cosmic rays were manually removed; following, removal of the Raman background (most fluorescence emission) was done by fitting and subtracting a seventh-order polynomial to the baseline of each spectrum according to the procedure described by Lieber and Mahadevan-Jansen;[34] finally, in order to minimize errors derived from eventual subtle differences between measurement conditions (laser power, tissue absorption, artifact from probe movements, etc.) and to improve the comparison of the spectra from different sites, each spectrum was normalized by the area under the curve for discrete signals (1-norm).[35] After preprocessing, the mean spectrum of each group was calculated.

Using the recent literature related to Raman spectra of chemical components, the peaks corresponding to spectra of the active ingredients listed on repellent and sunscreen product labels (Tables 1 and 2, respectively) were identified within the spectra of the undiluted products. Then, the peaks of these ingredients were identified on the spectra of the skin that received the products compared to the spectra of untreated skin (control). These peaks were evaluated to verify if there was a statistically significant difference between the peak intensities of the sites that

received the products compared to controls. The statistical analysis consisted of the Kolmogorov–Smirnov test to verify data normality and one-way analysis of variance (ANOVA) test followed by Tukey–Kramer *post hoc* test. The null hypothesis for the ANOVA was the equality in the mean of the peaks in the groups without (control) and with products, and the significance level to reject the null hypothesis was considered to be 5% ($p < 0.05$).[36]

3 Results and Discussion

Results are organized to reproduce the order in which the data acquisition and interpretation occurred, according to the methodology presented. First, the spectra of undiluted products placed on an aluminum sample holder are presented followed by a table showing the relationship between the active ingredients listed in Tables 1 and 2 and the Raman peaks shown in the literature. Next, the spectra of the skin with the products applied and the untreated skin (control) are presented highlighting the peaks of the active ingredients identified in the literature. Finally, the intensities of the peaks referred to the active ingredients in the skin with products applied and control are presented along with the results of the significance level (ANOVA and Tukey p-value) between the groups of skin with products and untreated skin (control).

3.1 *Insect Repellent*

3.1.1 *Raman spectra and most significative peaks*

Figure 2 shows the Raman spectra of repellent products in spray and cream (undiluted products, groups CR, and SPR) placed on an aluminum sample holder, with the peaks at 526, 690, 1003, 1295, 1458, and 1608 cm^{-1} present in both formulations. Table 4 lists the active ingredients indicated on the product's description label, the Raman peaks of the active ingredients found in the literature, and the indication whether these peaks are the present or not in the spectra of the products. Figure 2 shows peaks that match those of active ingredient DEET present in the repellent formulation according to Table 4.

Figure 3 presents the mean Raman spectra of the skin from volunteers with the applied repellent products in spray (SPR) and cream (CR) as well as the mean spectra of the skin without repellent (CTR). The Raman peaks labeled in Fig. 3(a) correspond to the most relevant peaks of the active ingredient DEET. In fact, all these peaks are in positions close and overlapped to the peaks of the CTR skin. Figures 4(b)–4(d) magnify the peaks at 526, 690, and 1003 cm^{-1}: at 526 cm^{-1} there is an overlap with the skin's Raman peak at around 540 cm^{-1}, attributed to the stretching vibration of the S–S disulphide bridge in skin proteins (actin, collagen, and elastin);[28] at 690 cm^{-1} the DEET peak is in a valley between the skin's Raman peaks at 640 and 720 cm^{-1}, attributed to proteins (C–C twisting of phenylalanine and tyrosine and C–S stretching of the proteins, respectively, the last one presenting contribution from the C–N vibration at 720 cm^{-1} from the choline of phospholipids);[29] and at 1003 cm^{-1} there is an overlap with the skin's peak at 1004 cm^{-1} from proteins (aromatic ring vibration of phenylalanine and tyrosine).

3.1.2 *Statistical analysis of the peaks of repellent's active ingredient identified on the skin*

Figure 4 shows the mean intensity and standard deviation of the Raman peaks related to DEET and observed in the groups SPR, CR, and CTR in Figs. 4(b)–4(d). The results of the ANOVA and Tukey *post hoc* tests applied to these intensities of the three groups are also shown. As the peaks at 527 and 1003 cm^{-1} presented statistically significant difference of the SPR and CR versus CTR, these peaks indicate the presence of DEET on the volunteer's skin.

In the region of the peaks at 527 and 1003 cm^{-1} [Figs. 3(b) and 3(d)], where the skin peaks (proteins peaks at 540 and 1004 cm^{-1}) overlap with the peaks of DEET, the ANOVA identified the presence of the DEET in the SPR and CR groups.

3.2 *Sunscreen*

3.2.1 *Raman spectra and most significative peaks*

Figure 5 shows the Raman spectra of the sunscreen products (undiluted) of the brands CEN (SPF 15 and 30) and SD (SPF 15 and 30) placed on aluminum sample holder, with the peaks at 1003, 1177, 1288, 1310, 1564, 1605, and 1611 cm^{-1}. The correspondence of the observed peaks with the active ingredients identified in the product's description (label) is indicated in Table 5. The spectra of products show peaks in the same positions but differ in the intensity of the peaks. These differences are related to different concentrations of the active ingredients between the products of the two brands

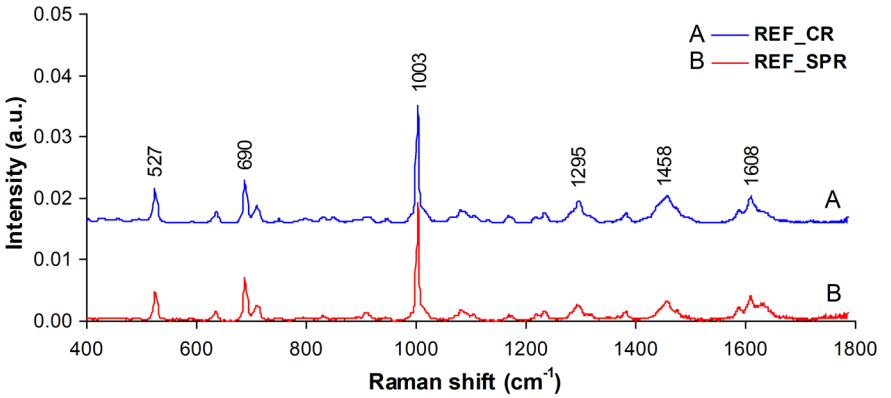

Fig. 2 Raman spectra of repellents in cream (REF_CR) and spray (REF_SPR) formulations. The labeled peaks match the ones of the active ingredient DEET as presented in the reference spectrum of *N, N*-diethyl-m-toluamide in *Chemical Book*.[37]

Table 4 Active ingredient present in the product's description (label) of the repellents, characteristic Raman peaks of the active ingredient as identified in the literature and the presence on these peaks in the sample's spectra.

Active ingredient	Presence in label		Characteristic Raman peaks	
	SPR	CR	From the literature (cm^{-1})	From Fig. 2 (cm^{-1})
DEET	Yes	Yes	527, 690, 712, 1003, 1293, 1457, 1472, and 1608[37]	526, 690, 1003, 1295, 1458, and 1608

Note: CR, cream; SPR, spray.

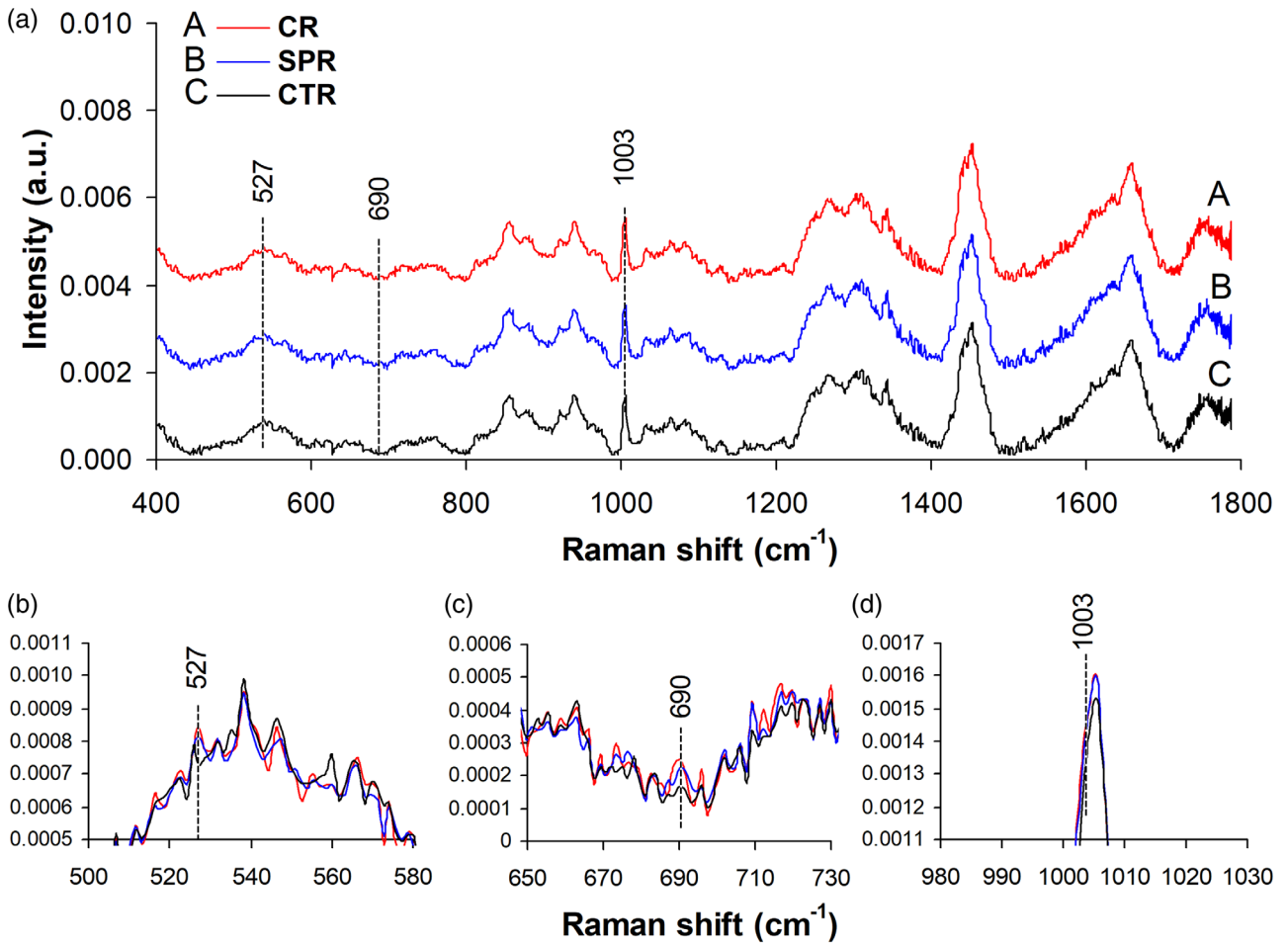

Fig. 3 (a) Mean Raman spectra of the skin from volunteers with the applied repellent products in spray (SPR) and cream (CR) and the skin without repellent (CTR). Plots of the peaks at (b) 527 cm^{-1}, (c) 690 cm^{-1}, and (d) 1003 cm^{-1} to allow the observation of the overlap of DEET peaks on SPR and CR with the peaks of skin from controls (CTR).

and SPFs. Also, some peaks are present in one brand and absent in another.

Figure 6 shows the mean Raman spectra of the groups with sunscreen products applied on the skin: CEN15, CEN30, SD15, and SD30 and the control group CTR. The labeled Raman peaks in Fig. 6(a): at 1003, 1177, 1288, and 1611 cm^{-1}, are those identified in the products that correspond to the active ingredients as listed in Table 5. As occurred with the repellents, some of the peaks of the active ingredients overlap with the peaks of the CTR skin. Figures 6(b)–6(f) magnify the peaks at 1003, 1177, 1288, 1564, and 1611 cm^{-1}, respectively. The spectra shown in Fig. 6(a) do not show the peak at 1564 cm^{-1}, related to octocrylene; instead, Fig. 6(e) shows that there is an overlap of this peak with the peak from skin at around 1562 cm^{-1}, associated to nucleic acids, proteins, and hemoglobin.[29] In addition, the proximity to the high-intensity peak in 1611 cm^{-1}, which is associated to octinoxate and avobenzone, with the low intensity peaks from skin at 1613 cm^{-1}, assigned to nucleic acids, proteins, amino acids (phenylalanine, tyrosine, and tryptophan), and hemoglobin,[29] allows the identification of this peak and it is used as reference for the identification of both octinoxate and avobenzone.

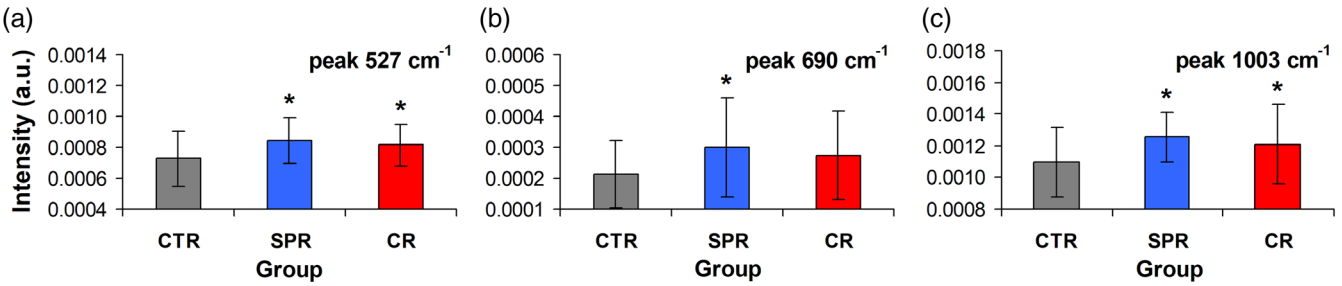

Fig. 4 Plot of the mean intensity and standard deviation of the Raman peaks of the active ingredient DEET found in the spectra of the skin of the groups CTR, SPR, and CR: (a) peak 527 cm^{-1}, (b) peak 690 cm^{-1}, and (c) peak 1003 cm^{-1}. The symbol "*" indicates a statistically significant difference between the groups SPR versus CTR and CR versus CTR ($p < 0.05$, ANOVA, and Tukey *post hoc* tests).

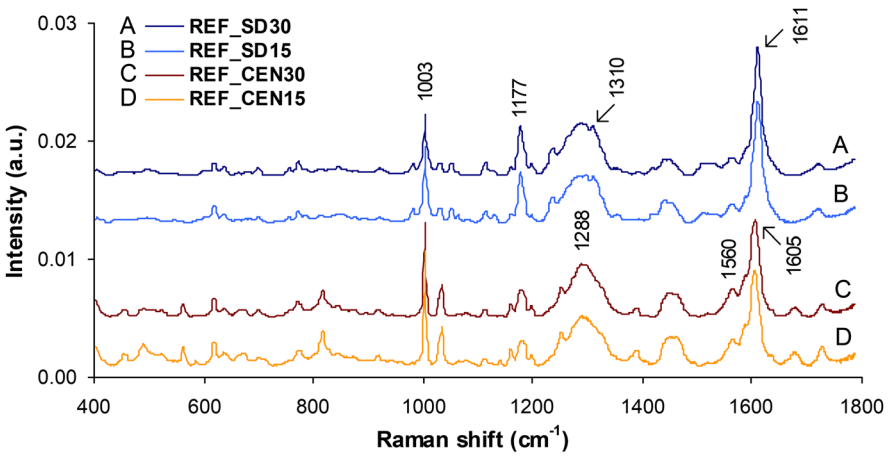

Fig. 5 Raman spectra of sunscreen products of the two bands CEN and SD in two different SPFs (SPF 15 and 30). The labeled peaks represent the most significative peaks.

Table 5 Active ingredients present in the product's description (label) of the sunscreens, characteristic Raman peaks of the active ingredients as identified in the literature.

Active ingredient	Presence in label		Characteristic Raman peaks	
	CEN15 and CEN30	SD15 and SD30	From literature (cm^{-1})	From Fig. 5 (cm^{-1})
Ethylhexyl methoxycinnamate (octinoxate)	Yes	No	1170 and 1613[38]	1177 and 1605
Benzophenone-3	Yes	No	1000 and 1280[39]	1003 and 1288
Octocrylene	Yes	Yes	1560[40]	1564
Bis-octoxyphenol methoxyphenyl triazine	Yes	Yes	*	—
Ethylhexyl triazone	No	Yes	*	—
Butyl methoxydibenzoylmethane (avobenzone)	No	Yes	1605[41]	1611

*Compounds with spectra not found in the literature

Concerning the peak at 1003 cm^{-1}, there is an overlap with the peak at 1004 cm^{-1}, attributed to proteins (aromatic ring vibration of phenylalanine and tyrosine),[29] as occurred with the repellents.

3.2.2 *Statistical analysis of the peaks of sunscreen's active ingredients identified on the skin*

Figure 7 shows the mean intensity and standard deviation of the Raman peaks related to the active ingredients of sunscreens in the skin spectra in the groups CEN15, CEN30, SD15, SD30, and CTR. The results of ANOVA and Tukey *post hoc* tests applied to these intensities of the five groups are also shown. As the peaks at 1003 and 1288 cm^{-1} and peaks 1177 and 1611 cm^{-1} presented a statistically significant difference of the CEN15 versus CTR and CEN30 versus CTR, it is possible to state that these peaks indicate the presence of the sunscreen CEN applied to volunteer's skin due to the peaks of the active ingredients benzophenone-3 (related to peaks 1003 and 1288 cm^{-1} according to Table 5) and octinoxate (related to peaks at 1177 and 1605 cm^{-1} according to Table 5). Similarly, as the peak at 1611 cm^{-1} presented a statistically significant difference of the SD15 and SD30 versus CTR, this peak indicates the presence of the sunscreen SD due to the peak of avobenzone (Table 5).

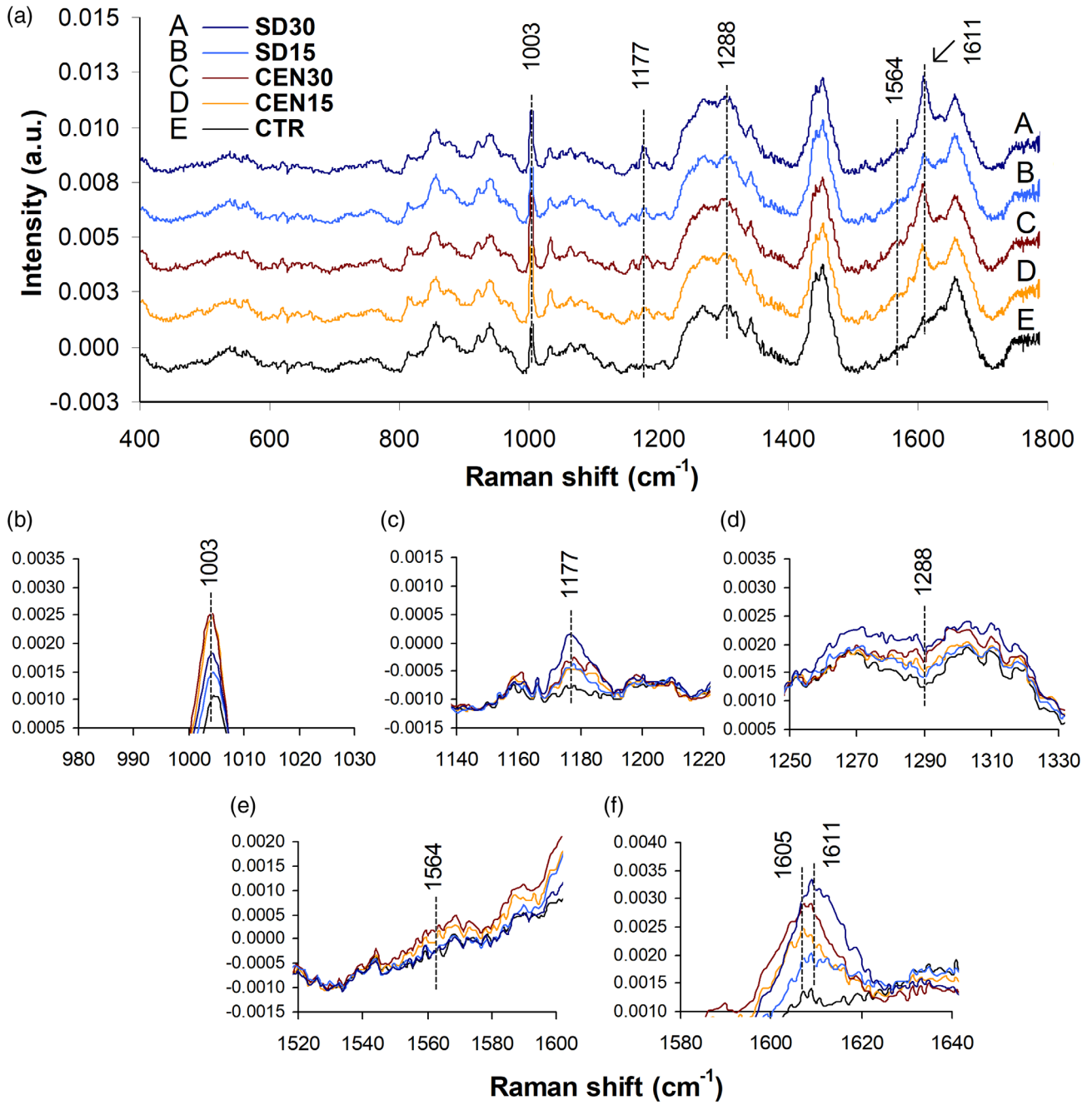

Fig. 6 (a) Mean Raman spectra of the skin from volunteers with applied sunscreen products (CEN15, CEN30, SD15, and SD30) and from control (CTR). The plots of the peaks at (b) 1003 cm^{-1}, (c) 1177 cm^{-1}, (d) 1288 cm^{-1}, (e) 1564 cm^{-1}, and (f) 1605/1611 cm^{-1} allow the observation of the overlap of the peaks from CTR, CEN15, CEN30, SD15, and SD30 with the peaks of CTR skin.

3.3 *Identification of the Active Ingredients Using Raman Spectroscopy*

This work is the first to show the presence of the active ingredients of insect repellent and sunscreen products topically applied to the skin. Chrit et al.[25] investigated the effects of active ingredients for skin hydration using confocal Raman, showing that Raman spectroscopy was capable to show the hydration enhancement effect brought by the active ingredients. Mélot et al.[42] investigated the effect of compounds used as helpers on retinol transportation through skin layers with confocal Raman, showing that Raman spectroscopy was effective to measure efficiency of formulation on transporting of desired molecules through the skin.

The use of the peaks from DEET at 527 and 1003 cm^{-1} permits us to detect the presence of insect repellents applied topically to the skin, besides the existing overlap with the Raman peaks of the skin. In fact, the differences in the positions of these Raman bands from the positions of the bands found in the skin, at 540 cm^{-1} (attributed to disulphide bridge of proteins) and at 1004 cm^{-1} (attributed to the aromatic ring vibration of phenylalanine and tyrosine), makes possible the identification of these peaks in skin of nontreated and treated volunteers (SPR and CR versus CTR) with statistically significant differences. The other

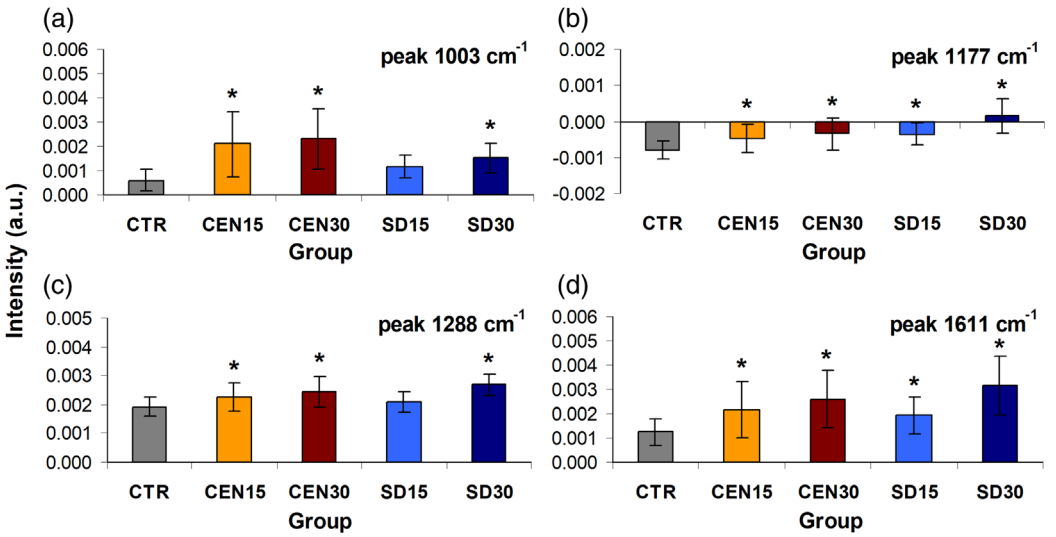

Fig. 7 Plot of the mean intensity and standard deviation of the Raman peaks of the active ingredients of the sunscreen products found in the spectra of the skin: (a) peak 1003 cm^{-1}, (b) peak 1177 cm^{-1}, (c) peak 1288 cm^{-1}, and (d) peak 1611 cm^{-1}. The symbol "*" indicates a statistically significant difference between the groups CEN15, CEN30, SD15, and SD30 versus CTR when indicated ($p < 0.05$, ANOVA, and Tukey *post hoc* tests).

peaks of the DEET are not significant due to their low intensity and overlap with the peaks of the skin.

An interesting application of this study is the detection of possible degradation of DEET under sunlight exposure, since Bório et al.[43] demonstrated that peaks at 524 and 1003 cm^{-1} from DEET are unstable when the repellent is irradiated by ultraviolet light (UVA and UVB, 7.0 mW/cm^2 for 8 h), which could potentially reduce its topical effectiveness after prolonged sun exposure. The study can also be applied in assessing the required amount of product to promote the desired repellent effectiveness since the Raman spectrum can detect the presence of the active ingredient quantitatively through its peak intensity.

The assessment of the active ingredients in the spectra of sunscreens indicated that Raman spectroscopy was able to identify the differences in the composition of the products under test. The presence of the peaks at 1003 and 1288 cm^{-1} (related to benzophenone-3) and peaks at 1177 and 1605 cm^{-1} (related to octinoxate) for the CEN brand, and the peak at 1611 cm^{-1} (related to avobenzone) for the SD brand, evidenced of the presence of related active ingredients in these products, thus suggesting the capability of discriminative (qualitative) and quantitative analyses of Raman spectroscopy. This capability is also demonstrated when the products are applied topically to the skin, including identifying the brand.

An interesting finding of the study was the difference between the spectra of sunscreens comparing the two different SPFs of the same brand. This difference is clear in the nonnormalized spectra of undiluted products (not shown). Comparing the intensity of the undiluted products, it is possible to check that the higher SPF results in higher intensities of peaks related to the active ingredients of the formula, since the brands keep the same formulation, changing only the concentration.

Raman spectroscopy provides qualitative information regarding the composition of the sample under analysis as well as quantitative information related to the presence of the compound, which can be useful to manufacturers to detect and quantify the active ingredients of pharmaceutical formulations topically applied to the skin and may be used for quality control during the production process, thus attending GMP and PAT technology processes.[17–19] As the manufacturers know the concentration of the active ingredients and excipients of the formulation, it is possible to observe the presence of each active ingredient in the skin of an individual following an application protocol, or even to perform tests for degradation due to environmental conditions or simulated situations of use.

The Raman spectra showed peaks of the active ingredients that can be used to detect and identify the presence of insect repellents and sunscreens topically applied to the skin. Raman spectroscopy is a noninvasive and nondestructive technique that can be applied to measure the composition of the samples *in situ* and *in vivo* and has shown a reliable and quick means for the identification of these topical products in the skin, helping the identification of these compounds in protocols of efficacy evaluation. Finally, the reason for the high fluorescence background in some volunteers after product application could be related to interactions of the product with the stratum corneum, which could be exploited in a future study.

4 Conclusion

In this work, Raman spectroscopy has been used to detect the active ingredients of insect repellent products in two formulations (spray and cream) and sunscreen products of two brands in two SPFs. The Raman spectra of undiluted products presented the peaks of the active ingredients [DEET for repellents and octinoxate and benzophenone-3 (CEN brand) and avobenzone (SD brand) for sunscreens]. The skin of volunteers topically treated with repellents and sunscreens (9.4 mg, 2.0 mg/cm^2) showed peaks referred to the active ingredients compared to controls. Statistical analysis applied to the peaks of the active ingredients showed significant differences in the intensities of the peaks at 527 and 1003 cm^{-1} for the repellents and 1003 and 1288 cm^{-1} (related to benzophenone-3), 1177 and 1605 cm^{-1} (related to octinoxate) for the CEN brand sunscreens and 1611 cm^{-1} (related to avobenzone) for the SD brand, compared to controls, which could be used as markers of the presence of these topical products in the volunteer's skin.

The methodology based on Raman spectroscopy can be used to evaluate the effectiveness of topical products that depends on the presence of the active ingredients in the skin such as repellents and sunscreens.

Disclosures

All authors declare no conflict of interest.

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