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Journal of Dermatology & Cosmetology

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In-vivo study using Raman Spectroscopy to estimate the effect of fairness creams on skin

Abstract

Serious scientific Research in the field of cosmetics has speared headed the growth of cosmetic industry, which adds undeniable credibility to the products. Many a time, a clinically simple, non-invasive dermatological technique is required to study the effect of the products in real time. Raman spectroscopy can be employed in his regard as it is a versatile technique which can be employed to study the nature and effects of these cosmetic products. In this short communication, we are reporting the study on the effect of fairness creams available in the market for the general Indian populace using Raman spectroscopy.

Volume 2 Issue 5 - 2018

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Received: March 27, 2018 | **Published:** September 21, 2018

Introduction

Driven by medical, pharmaceutical, and cosmetic research, a large number of methods have been developed to obtain information about the presence and concentration of molecular compounds in the skin. Many of these methods are invasive, which require material to be removed from the skin and analyzed *in vitro*.[1] They alter the system under investigation either by extraction of compounds from the skin or by physical disruption of cell layers. In a number of studies extraction techniques- are employed to determine concentration levels of free amino acids in the stratum corneum.[1] Alternatively, Raman spectroscopy is a versatile technique which can be effectively employed to study biomolecules without any sample preparation. It can provide information on important molecules, natural moisturizing factors as well as exogenous molecules such as glycerol delivered from skin care products.[2] Raman spectroscopy can directly measure the water content from the skin surface down to the upper epidermis with high depth resolution.[3] Moreover, it produces a more absolute measurement value than other methods. The objective of this study is to measure the dermal changes caused by the commercially available fairness creams (in Indian market) *in vivo* using Raman spectroscopy and their effectiveness in reducing the melanin content in the skin.

Materials and methods

Study population includes twelve different subjects in the age group of 20-30 years and a written consent of the subjects. To evaluate the change, if present, before and after the application of five different creams, an attempt has been made using Raman spectroscopy. In this regard, the spectra were acquired at zero hour of the application of the cream and after one hour. Since Raman spectroscopic studies are non-invasive, the study can be repeated on the same skin area. Hence the effects of the molecules can be studied as a function of time.

Experimental methods

The spectroscopic study was carried out on a dedicated confocal Raman micro spectrometer. Raman spectra of the skin of forearm by using a sapphire ball lens fiber probe. A laser beam from a diode laser of fixed wavelength 784.12nm was used. Light that is scattered by the tissue is collected by the same optical fiber connected to the spectrograph. The instrument was calibrated to the 520.7cm^{-1} peak of silica. The integration time for every Raman spectrum was set to 20sec and every window was scanned twice to subtract cosmic showers. The spectra were acquired over a wave number region of 600 to 1800cm^{-1}. This wave number region includes amide modes, protein and lipid Raman signature. The power at the sample was 26±0.5mW for all the experiments performed.[4]

Every Raman instrument has a unique spectral responsiveness, to correct this; an intensity calibration factor was applied to every raw spectrum to obtain accurate and reproducible results. Then the spectra were baseline corrected using 5th order polynomial, followed by spectral smoothening with Savityzy-Golay filter. All the data analyses mentioned above were performed using Labspec[5] software provided by Horiba Jobin Yvon. The averaged Raman spectra of the control area and the different regions the creams are applied at zero hour and at the first hour are shown in (Figure 1A) (Figure 1B) respectively. Table 1 following table gives the major peaks that are present in the control area and the respective peak assignments.

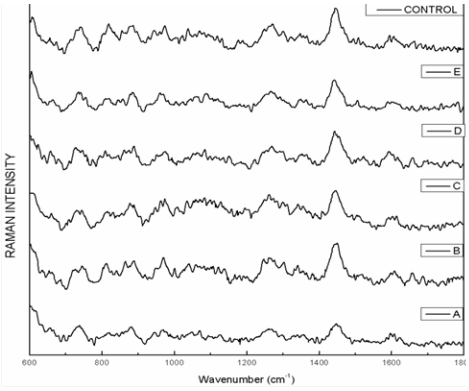
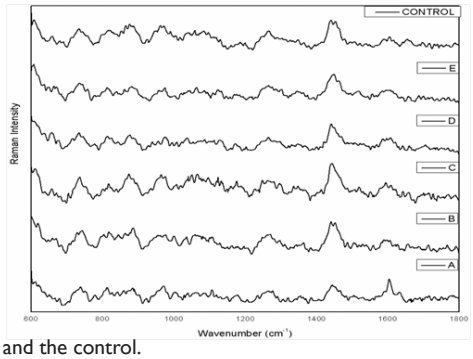


Figure 1A Averaged spectral profile of the skin at zero hour after cream



application and the control.

Figure 1B Averaged spectral profile of the skin at first hour after cream application and the control.

Table 1 Normalized intensity of the peak at 1058cm-1

Wave number cm^{-1}	Assignment
876	C-C stretch, hydroxyproline
931	CH_3 rocking, collagen proline
951	4-hydroxy proline
963	Apatite
1004	Aromatic ring
1032	Proline
1062	Stretching (C-C) skeletal trans conformation lipid
1123	Glycogen
1158	Cartenoid
1176	Tyrosine, phenyalanine
1200	Hydroxyproline, tyrosine
1250	Amide III
1265	C-H plane in deformation
1319	CH_3CH_2 twisting
1343	CH_3CH_2 wag
1385	Deformational vibration (CH_3) symm
1445	CH_3CH_2 deformation in collagen
1652	Deformational vibration (C=0) amide I alpha-helix
1661	Amide I

Results

Spectral signature at zero hour

The application of cream A resulted in the presence of peak at 1606cm^{-1} and, a peak at 1105cm^{-1}. Further, broad peaks at 1380 and 1580cm^{-1} were also observed in control. Cysteic acid, at 1045cm^{-1}, increased with bleaching, all the creams showed bleaching effect as there was increase in this peak intensity. There is a decreased intensity at 853 and 825cm^{-1} peak on application of all the creams. The bands are diminished at 1004cm^{-1} when creams D and E are applied. When cream B is applied, Raman shifts in 876cm^{-1} to 890cm^{-1} and there is a presence of band present at 1363cm^{-1} and a vibration band at 1343cm^{-1} is also seen when creams C, D and E were applied.

Spectral signature at first hour

After one hour, the averaged spectra of all five creams applied on the surface of the skin along with the control are shown in Figure 1B. A sharp peak at 1606cm^{-1} was observed at zero hour of application of cream A and this peak was absent after an hour. Similarly the peak due to lipids at 1105cm^{-1} was also absent. The peaks due to melanin at 1380 and 1580cm^{-1} which were prominent in control, was found to be decreased where the creams A, B and C were applied even after an hour of application.

Statistical analysis

Principal component analysis was carried out for the two sets of data, viz., immediately after the application of the cream and after one hour of application. Both the sets yielded only two Principal Components (PC) that was statistically relevant. For these 2 PCs, ANOVA was carried out to test the significance of these extracted components. In both the cases the PC 2 yielded a better statistical significance than PC1. Supplementary Figures 1 & 2 show the score plots for both the data sets. After one hour of application of the cream, it can be inferred from the plot that the PC scores of the control and the cream applied regions are nearly the same. Spectral signature for different skin creams: The consistent peak at 1058cm^{-1} is found to be shifted to 1045cm^{-1} on application of the cream on the skin. Table 2 gives the prominent peaks and their peak assignments for the different creams and Table1 gives the normalized intensity of the peak at 1058cm^{-1}.

Discussion

Spectral signature at zero hour

The presence of peak at 1606cm^{-1} which may be attributed to phenylalanine[4] and peak at 1105cm^{-1} which may be due to C-C stretch lipid.[5] The broad peaks due to melanin at 1380 and 1580cm^{-1} which is seen in control is decreased on application of cream C alone.[6] The rest of the creams do not show any changes to the melanin content at the zero hour. Cysteic acid, at 1045cm^{-1}, increased with bleaching, all the creams showed bleaching effect as there was increase in this peak intensity.[7] When there is a bleaching effect, the level of tyrosine decreases and is seen in the decreased intensity at 853 and 825cm^{-1} peaks on application of all the creams.[7] The bands diminished at 1004cm^{-1} are considered to be due to aromatic ring, when creams D and E applied may due to the bleaching effect of these creams. The Raman shift observed on applying cream B from 876cm^{-1} (C-C stretch hydroxyproline) to 890cm^{-1} (structural protein) and the band present at 1363cm^{-1} are due to tryptophan.[8,9] The vibration band at 1343$cm^{-1}$is considered to be due to CH_3CH_2 wag, when creams C, D and E were applied.[10]

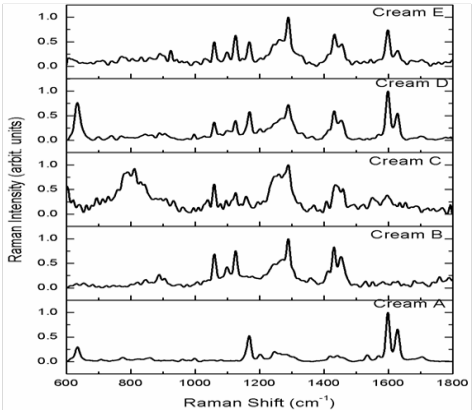
Spectral signature at first hour

The intensity at 1045cm^{-1} (Cysteic acid) was found to be still higher even after one hour of the application of the creams B, D and E. The level of tyrosine was found to be decreased when creams B, D and E were applied. The process of bleaching leads to lower levels of tyrosine.

Spectral signature for different skin creams

Citation: Daniel A, Savarimuthu WP, Gowripal Y, et al. *In-vivo* study using Raman Spectroscopy to estimate the effect of fairness creams on skin. *J Dermat Cosmetol* . 2018;2(5):57–59.
DOI: 10.15406/jdc.2018.02.00085

The cysteic acid in the creams is found as zwitterion cysteine tagged with Ag nanoparticles.[11] Hence there is a consistent peak at 1058cm^{-1}. This peak is shifted to 1045cm^{-1} on application of the cream on the skin which may be attributed to cystein oxidized to cysteic acid hence causing bleaching effect. From Table 2 which gives the prominent peaks and their peak assignments for the different creams. It is observed that, though all the creams exhibit bleaching effect when applied on the skin, Cream A does not exhibit the peak due to cysteine



with nano particles peak at 1058cm^{-1} as shown in Supplementary Table 1 (Figure 1C).

Figure 1C Spectra of raw cream.

Table 2 Prominent peaks and their peak assignments for the different creams.

Wave number (cm^{-1})	Peak assignments	Creams
635	Au nano particles	A,D
1016	tryptophan	B,C,D,E
1100	PO_2 stretch protein	B,C,D,E
1129	C-C stretch in lipid	B,C,D,E
1171	γ (CC)	A,C,D,E
1290	amide III C-N	B,C,D,E
1431	CH_2 bending and wagging	B,C,D,E
1456	CH_3 bending in elastin	B,C,D,E
1600	Au nano particles	A,C,D,E
1058	zwitterions cysteine with Ag nanoparticles	A,B,C,D,E

Conclusion

In conclusion, this pilot study reveals that every cream lightens the skin by bleaching it. This bleaching effect of the skin can be attributed to the oxidation of cystein to cysteic acid. This effect is a short term effect. It was observed that, as the time progresses from the time of application, the skin does not exhibit the bleaching effect as the PC scores plot shows. Furthermore, the study has to be carried for the longer duration of continued application.

Acknowledgements

None.

Conflict of interest

Author declares that there is no conflict of interest.

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