



5th International Electronic Conference on Medicinal Chemistry

1-30 November 2019

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by



pharmaceuticals

Chemical imaging in pharmaceuticals for formulation analysis, quality control, and monitoring of formulation interactions with biological tissue

Ievgeniia Iermak ^{1*}, Ana Paula da Silva ², Cristina Kurachi ¹, Vanderlei Salvador
Bagnato ¹, Natalia Mayumi Inada ¹

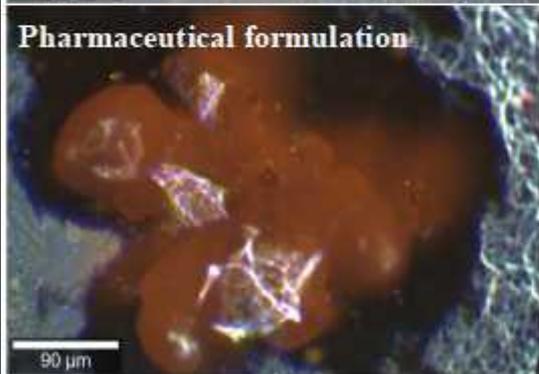
¹ São Carlos Institute of Physics, University of São Paulo, Trabalhador Sao-carlense Av., 400, São Carlos, SP 13566-590, Brazil;

² PDT Pharma Ind. Com. Pharmaceutical Products LTDA, Marechal Deodoro da Fonseca Str., 67, Cravinhos, SP 14140-000, Brazil.

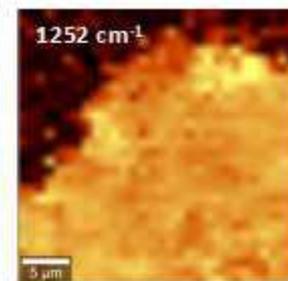
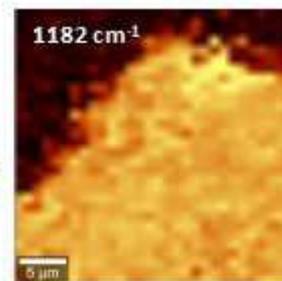
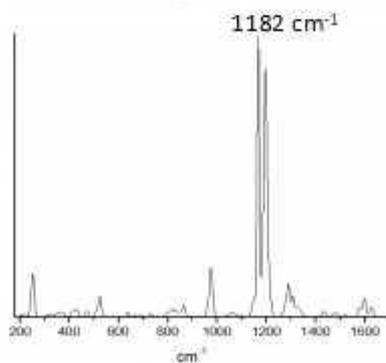
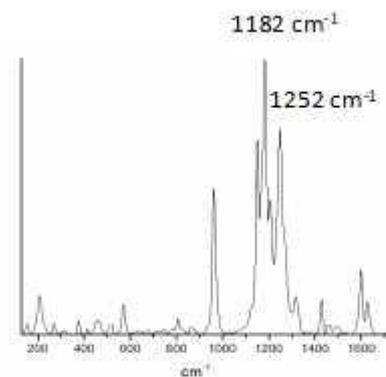
* Corresponding author: ievgeniia.iermak@gmail.com



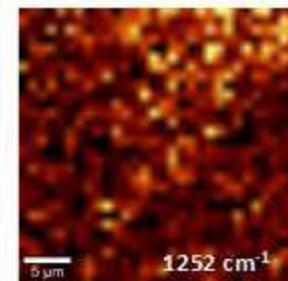
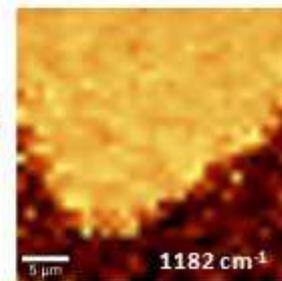
Chemical imaging in pharmaceuticals for formulation analysis, quality control, and monitoring of formulation interactions with biological tissue



Spectroscopical analysis



Chemical imaging of pharmaceutical formulation



5th International Electronic Conference
on Medicinal Chemistry
1-30 November 2019

sponsors:



pharmaceuticals

Abstract: Raman microspectroscopy is an elegant and efficient tool for chemical analysis of the samples since it provides information about the structure, conformation, and changes of molecules both in pharmaceutical formulation and in the biological tissue. It is especially useful for samples containing water since water absorption is not dominating the Raman spectrum, as it happens in the case of infrared spectroscopy. Analysis of several formulations for onychomycosis treatment is shown as the example of Raman microspectroscopy application in pharmaceuticals. Differences between the formulations are shown to be linked to their efficiency in the infection treatment. Comparison of Raman microspectroscopy as the tool to analyze pharmaceutical formulations, their efficiency and stability is made in relation to the other spectroscopical and microscopical methods and the advantages and disadvantages of several techniques are discussed. The application of the results obtained in this study is demonstrated on further improvement of the formulation in development.

Keywords: Raman microspectroscopy; drug delivery; topical pharmaceutical formulation



Introduction

What is Raman Microspectroscopy?

- *Non-destructive chemical analysis* technique which provides detailed information about chemical structure, phase and polymorphy, crystallinity and molecular interactions
- In combination with mapping (or imaging) Raman systems, it is possible to generate images based on the sample's Raman spectrum. These images show *distribution of individual chemical components, polymorphs and phases, and variation in crystallinity*



Introduction

Raman effect

Types of vibrational motions of the molecule:

- Bending motion between three atoms connected by two bonds
- Stretching motion between two bonded atoms
- Out-of-plane deformation modes that change an otherwise planar structure into a non-planar one



Introduction

Raman effect

The spectrum depends on:

- masses of the atoms in the molecule
- strength of their chemical bonds
- atomic arrangement

Raman shift in wave numbers (cm^{-1}):

$$\bar{\nu} = \frac{1}{\lambda_{\text{incident}}} - \frac{1}{\lambda_{\text{scattered}}}$$



Introduction

Advantages of Raman microscopy

- In contrast to IR not sensitive to water, so *in vivo* measurements of biological samples is possible
- In contrast to fluorescence spectroscopy, it is possible to obtain information about the molecule conformation
- Non-destructive
- Raman spectra contains “fingerprint” of the molecule
- No labeling of the sample is necessary
- Imaging and mapping components in the sample is possible



Introduction

What information do we get from Raman microscopy?

- Information about chemical structure and identity
- Content uniformity and component distribution
- Contamination and impurity identification
- Reaction monitoring
- Drug interactions with the tissue



Introduction

Present study

- Curcumin is a component obtained from *Curcuma Longa* rhizome and has been studied as a potential photosensitizing compound for the inactivation of microorganisms
- Onychomycosis are fungal infections that affect the nail plate, leading to thickening, hardening, discoloration and disintegration of the nail
- Two different topical formulations (hydrophilic gel and water/oil cream) containing curcumin were developed for onychomycosis treatment by the Photodynamic Therapy (PDT) technique
- In PDT, a photosensitizing compound is activated by light and, in the presence of oxygen, leads to several reactive oxygen species (ROS) production, inactivating microorganisms

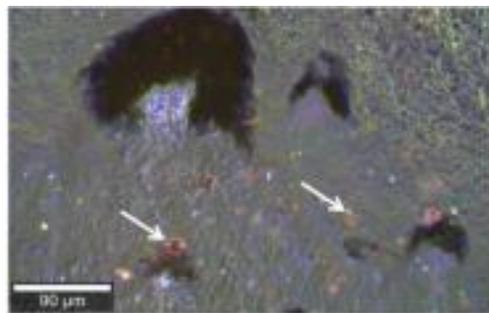


Results and discussion

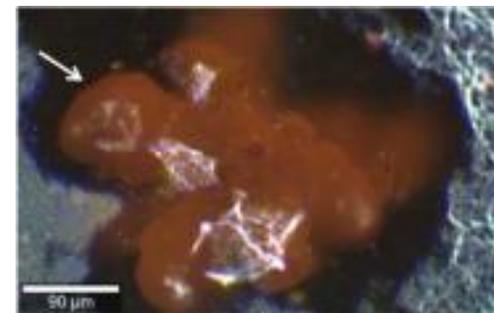
Micrographs of formulations



cream



gel



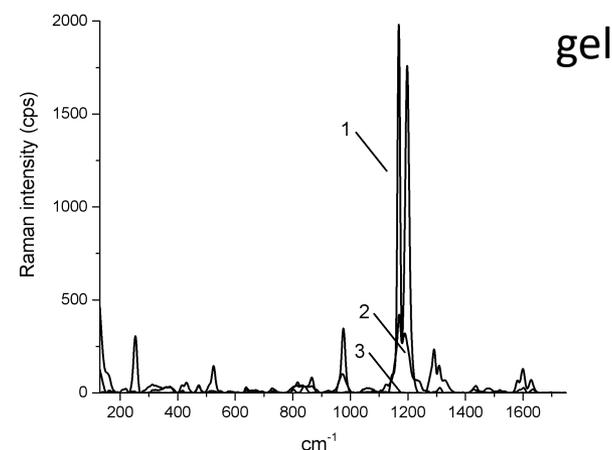
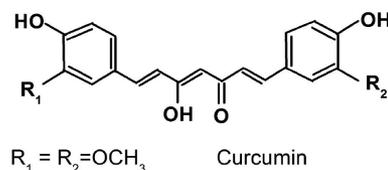
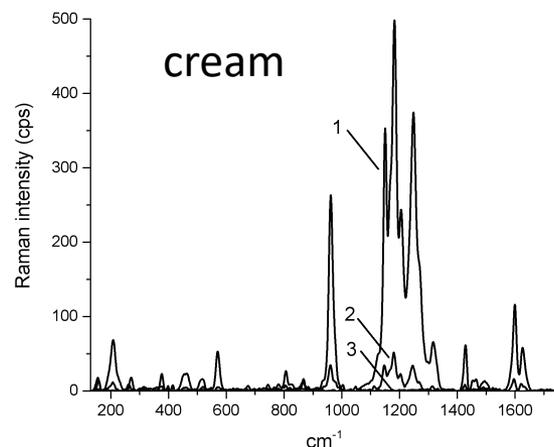
gel

- curcumin is present in the form of particles, distributed around the base
- the base itself contains curcumin
- gel contains both small and large pieces of curcumin



Results and discussion

Raman spectra

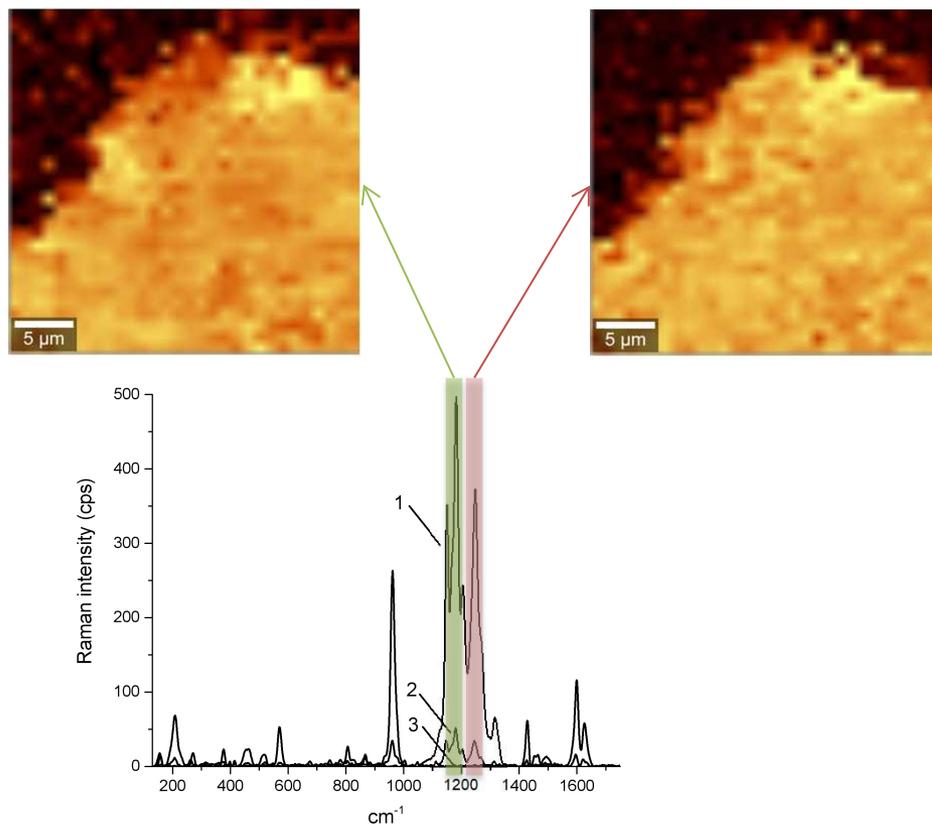


- base has much weaker spectra intensity
- some Raman bands are absent in a spectra of the gel
- the bands in a gel show 2-5 cm⁻¹ shifts in a different particles, meanwhile in a cream they are always in the same position



Results and discussion

Chemical imaging of the cream

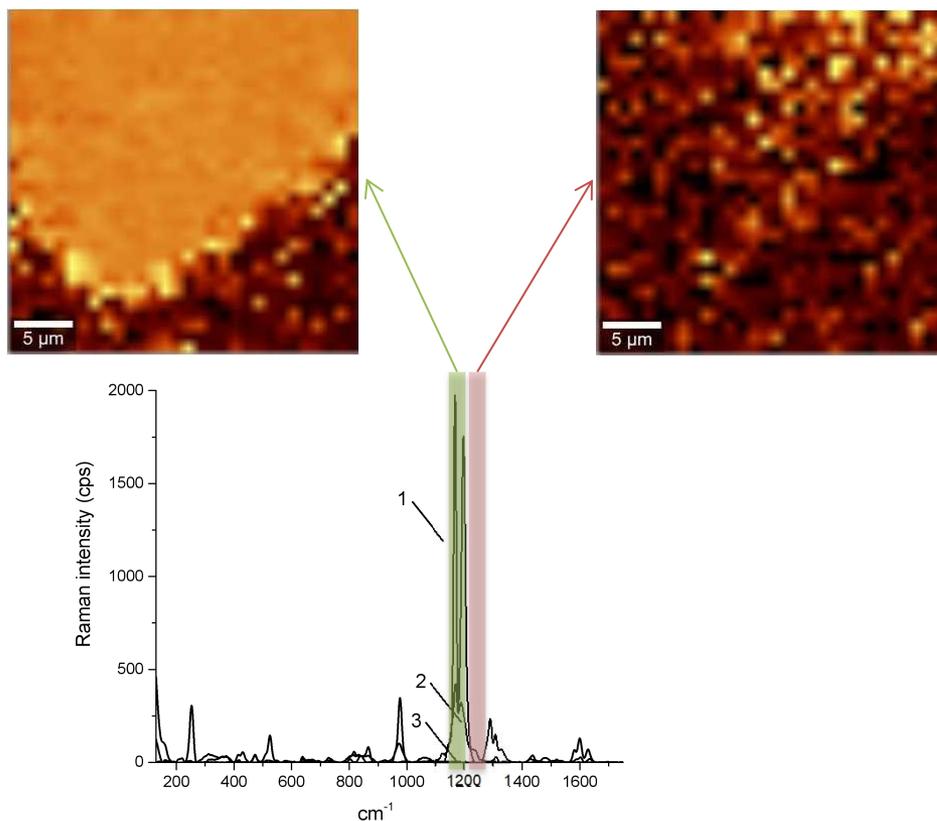


- The peak at 1182 cm⁻¹ is always present (shows the presence of curcumin in the cream)
- In the cream the number of pixels containing the 1252 cm⁻¹ peak is approximately the same as the number of pixels containing the 1182 cm⁻¹ peak



Results and discussion

Chemical imaging of the gel



- The peak at 1182 cm⁻¹ is always present (shows the presence of curcumin in the gel)
- In the gel the number of pixels containing the 1252 cm⁻¹ peak is much lower than the number of pixels containing the 1182 cm⁻¹ peak



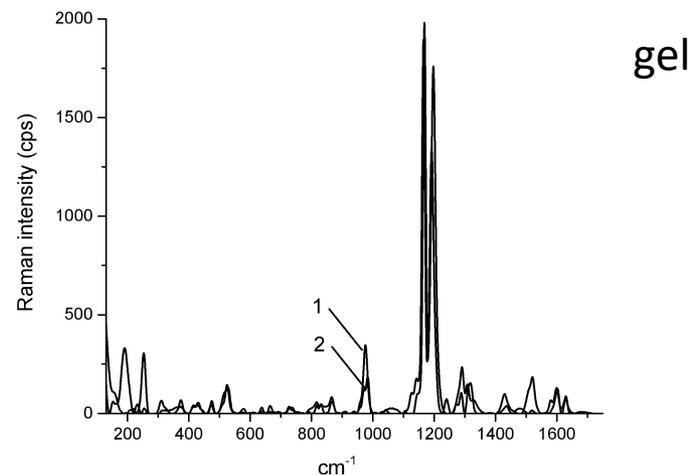
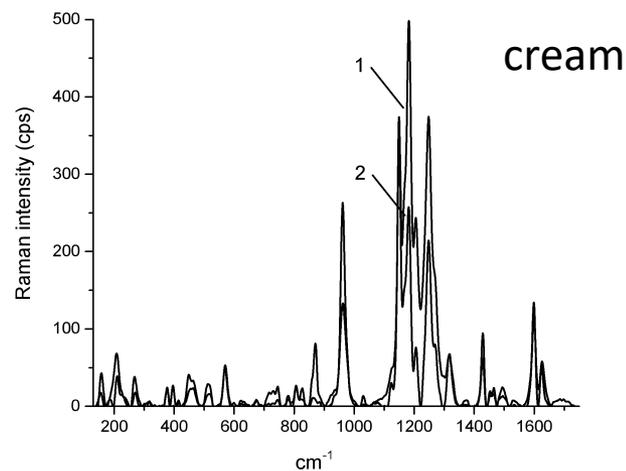
Results and discussion

- In the cream, all aggregates contain curcumin in the same conformation
- In the gel there are two types of aggregates, with some of them not showing the peak in Raman spectra at 1252 cm^{-1}
- Raman peak at 1252 cm^{-1} corresponds to the enol form of the curcumin molecule, which is typically present in solutions
- Keto form is typical one for the crystal state of curcumin
- In the gel not all curcumin is dissolved, and some is still in a form of undissolved crystals



Results and discussion

Formulations stability analysis



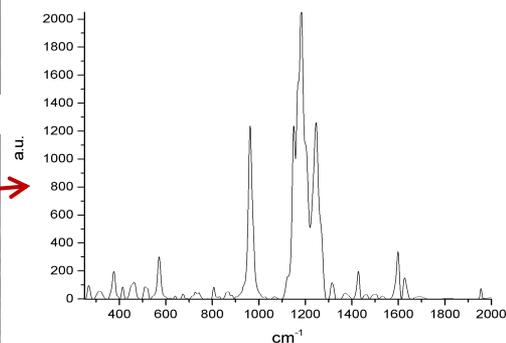
- Formulations irradiated with the blue light of the same wavelength and intensity for the same amount of time as used in PDT treatment
- Curcumin is not bleached substantially, the spectra of the particles remained unchanged



Results and discussion

Formulations interaction with tissue analysis

- The nail surface after the PDT with cream appeared to be of even color and showed identical Raman spectra in all the parts of the nail plate
- The nail surface after PDT with the gel showed patterns of two different colors and presence of the non-bleached curcumin particles, confirmed by the Raman spectra
- Undissolved curcumin in the gel is not delivered efficiently to the nail plate



Understanding the behavior of the active molecule within the pharmaceutical formulation helps for a better clinical outcome in the treatment and could help the pharmaceutical companies to improve formulations while maintaining the efficacy and safety of the active substance



Results and discussion

Further steps in formulations development

- New formulations for onychomycosis treatment containing curcumin are under development
- The conformation of curcumin in the new formulations is under control by Raman microspectroscopy
- The ability of formulations base to dissolve curcumin is controlled by the intensity of Raman spectra of curcumin in the base



Conclusions

- With Raman microspectroscopy it is possible to characterise pharmaceutical formulations and their active components
- Studies of the formulation stability are possible
- Raman analysis is non-destructive and possible *in vivo*
- No special preparation of the sample is necessary
- Analysis of the molecular mechanisms of the treatment is possible

We are open for collaborations!

Let's connect:

ievgeniia.iermak@gmail.com



Acknowledgments

This research was funded by grant from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP/CEPOF Grant No. 2013/07276-1). It was supported by a CNPq fellowship (INCT Basic Optics and Applied to Life Sciences Grant No. 465360/2014-9) and Coordination for the Improvement of Higher Education Personnel – CAPES. APS was supported by a CNPq fellowship (grant No. 381132/2010-2).

