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RAMAN IMAGING INVESTIGATION ON PAA-CLOTRIMAZOLE SYSTEM

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ABSTRACT. The dispersion of clotrimazole in the polyacrylic acid (PAA) matrix and the possible interaction between the drug and polymeric matrix were investigated by Raman imaging spectroscopy. The optical analysis shows an inhomogeneous distribution of the clotrimazole in the polymeric matrix. An area of 10x10 μm of the sample was scanned and a spectral image map of the most important bands was constructed. The imaging spectral map was constructed observing the intensity of the characteristic band 1045 cm^{-1} of clotrimazole respectively 1450 cm^{-1} of PAA, recorded for each point of the scanned area. The spectrum of the clotrimazole in the PAA matrix is essentially identical to the spectrum of pure clotrimazole. This indicates non interaction between polymeric matrix and clotrimazole.

Keywords: *PAA, clotrimazole, Raman spectroscopy*

INTRODUCTION

Systemic drug administration through the skin holds several advantages, such as the maintenance of constant drug levels in the blood, decrease of side effects and improvement of bioavailability by circumvention of hepatic first pass metabolism and increased patient compliance [1-4].

Hydrogels are usually macromolecular three dimensional networks of linear hydrophilic polymers capable of absorbing large amounts of water while remaining insoluble due to the presence of chemical or physical crosslinks [5–7]. Their relatively high water content is important in skin moisturization and elasticity, providing a better feel when applied to the skin, making them a good alternative to more conventional dosage forms such as creams, ointments and patches.

Chemical imaging is a methodology that allows automated measurements of samples through the combination of microscopes with vibrational (e.g. near-infrared and Raman) spectrometers to produce a 2D image of the components in a sample [12–16]. The final result of a chemical imaging experiment, be it mapping or true imaging, is a chemical image in which

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(ideally) all the components of the analyzed sample are identified based on their chemical composition and their spatial position in the sample [17]. This method allows the identification of chemical micro-structures that are not observed by bulk measurements of the sample. This can be particularly useful in pharmaceutical industry, where seemingly non-informative surfaces of tablets, powders, beads, and various other materials can be analyzed and a better understanding of the observed chemical images of the sample can be achieved [18-29].

Clotrimazole is a broad-spectrum antimycotic agent effective against pathogenic dermatophytes, yeasts and several species of Candida, Trichophyton, Microsporum, Epidermophyton and Malassezia [8]. The objective of the work presented here is to generate chemical images and characterize the pharmaceutical products which contain the clotrimazole integrated in PAA matrix. There are a number of reasons for studying this formulation by imaging approaches: first, it is useful to gain knowledge on whether the applied wet granulation process has been successful, or the granules are pure clotrimazole, and if any chemical interaction between the clotrimazole and the polymeric matrix (PAA) appears. Secondly, various physicochemical characteristics of the granules (such as dissolution) may be affected by their chemical structure (e.g. polymorphic or hydrate/solvate form) which will be determined by chemical imaging. Finally, the sizes of pure clotrimazole granules can be estimated; also this Raman technique can be a good method to verify the stability in time of the active substance integrated in polymeric matrix.

RESULTS AND DISCUSSION

For the beginning, each component of the PAA/Clotrimazole mixture was separately investigated by Raman spectroscopy. Figure 1 presents the two spectra of pure substance, which are included in the composition of the mixture.

The clotrimazole in powder state was introduced in the PAA gel, was stabilized and then was mixed to obtain a homogeneous distribution of active substance in the polymeric matrix. The optical microscopy analysis indicates a colloidal suspension of clotrimazole in the polymer matrix. The spectra obtained from different regions with different optical appearance are different. For example, curve A in figure 1 represents the spectrum of the PAA gel, and curve B represent the clotrimazole spectrum collected from an area with inhomogeneous optical appearance. These spectra were recorded in different regions of the same sample. On the other hand, if comparing the spectrum of the pure clotrimazole in powder state, with the spectrum recorded from the granules included in gel, they are identical. Therefore, the matrix of PAA did not affect the typical vibrational modes of the clotrimazole, figure 2. This result is very important for medical applications, showing that the active substance (clotrimazole) does not undergo any change in its properties when introduced into the polymeric matrix. Similar results were obtained for other concentrations of polymer.

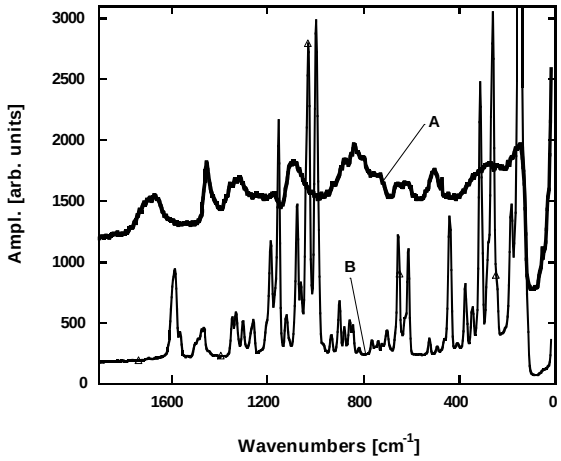


Figure 1. Raman spectra of pure PAA (A) and pure clotrimazole (B)

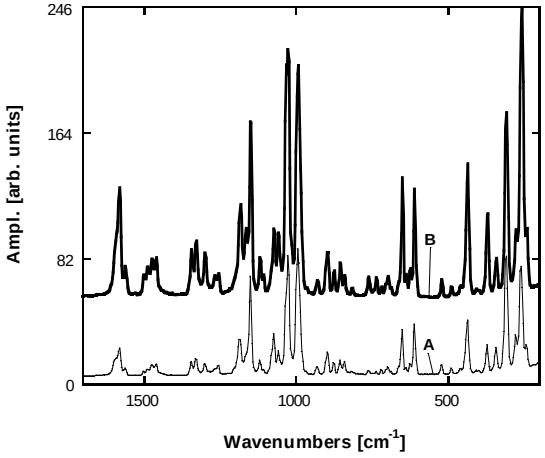


Figure 2. Raman spectra of pure Clotrimazol (B) and Clotrimazol granules embedded in PAA gel matrix (A).

Figure 3 shows the Raman spectrum of the stabilized gel with TEA at 1%concentration, the Raman spectrum of the same gel at the same concentration, after drying, and the Raman spectrum of the powder state of PAA. These show that the polymeric matrix hydration of the PAA is a reversible process, as water did not bring significant changes in the polymeric gel after drying. The bands in the Raman spectrum of powder state PAA are found exactly at the same positions as in the Raman spectrum for dry gel of PAA. The only significant change is the band at 846 cm^{-1}, band assigned by Bardet et *al*, as stretching vibration of carboxyl group of neighboring C-C bond [30].

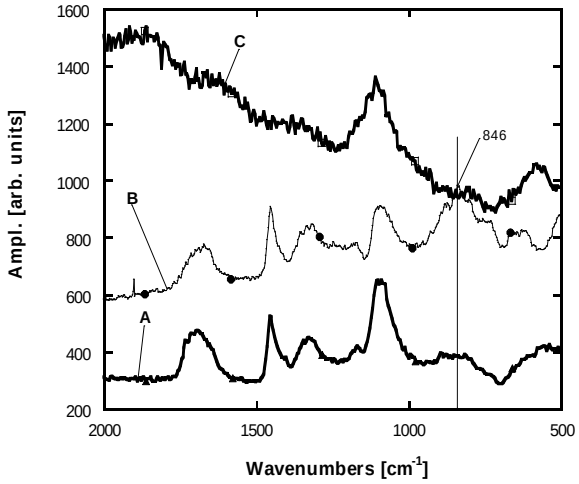


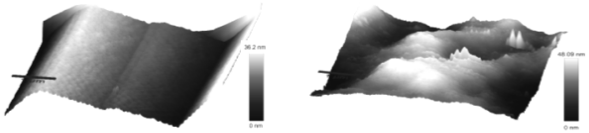
Figure 3. Raman spectra recorded on samples before and after drying PAA gels. After the first drying gel (A), pure PAA (B) and Raman spectrum recorded from same PAA gel stabilized with TEA (C)

This band does not disappear, but the Raman intensity of signal due the drying effect is greatly reduced because the carboxyl group was stabilized with TEA. The medium intensity band assigned to stretching vibration of C = O bond by Bardet et *al*, moves from 1683 cm^{-1} to 1688 cm^{-1} without change of intensity. This shift is due to the same effect, neutralizing the carboxyl group, which includes this C = O bond [22]. The spectrum of the stabilized gel with TEA of PAA has a Raman band intense presented only at 1105 cm^{-1}, assigned to the stretching vibration of C-CH_2 bond. This indicates that the level of dissolution did not influence the stability of the chain C - C bonds of acrylic acid monomer, figure 3. [30]. Following hydration and drying repeated processes, the PAA matrix remains stable, the hydration of these gels proved to be a reversible process.

Further investigations were carried out on dry PAA gels using AFM, analyzing the effects of the hydration-drying repeated process. The image AFM analysis shows that increasing of the polymer concentration, the roughness of the surface of PAA gel also increases. This takes the form of homogeneity in the gel polymer when the polymer concentration is higher. However, the increasing polymer concentration leads to increase of the sizes inhomogeneous zone. At 0.5% of PAA gel, in figure 4. b) more numerous homogeneous areas appear, while for the concentration of 1% an increase in surface roughness is observed, caused by the merger of inhomogeneous areas, figure 4 b).

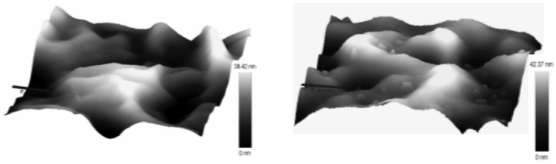
Following the hydration and drying at 1% the roughness is increased due the emergence of distinct areas on a much smaller surface than the previous situation, figure 5. After the first and second drying such heterogeneous areas are not observed on the surface, but after the third cycle of hydration and drying, the surface has a pronounced roughness in the dry gel. A possible explanation

for this could be the presence of a specific division of conglomeration PAA gel, after several repeated processes of hydration and drying.



a b

Figure 4. AFM images of 0.5% (a) and 1% (b) PAA gel after the first drying



a b

Figure 5. AFM of the 0.5% PAA gel after the second (a) and third (b) drying

Global Raman imaging

A (white) light microscopy composite image of a typical sample prior to mapping or imaging analysis is shown in Figure 7. This situation is repeated in a number of samples that were imaged, leading to conclusion that the large granules can very easily be prepared while the smaller granules of the analyzed formulation tend to stay closely agglomerated.

The method of investigation by Raman spectroscopy of clotrimazole granules can be optimized to identify the exact area they occupy in the polymeric matrix. This method involves scanning a predetermined area of the dry gel and step by step, recording the spectrum of this area. With the help of a map performed with the Witec 3.0 software the exact area that contains clotrimazole is obtained. A combination of Raman band at 1454 cm^{-1}, specific dry to PAA gel, and the band 1045 cm^{-1} specific to clotrimazole gel is presented in figure 7. These typical bands of the two phases were extracted from Raman spectra, figure 6. The bands employed for the Raman map of figure 7 are marked by gray arrows in figure 6. Zone B is the area denoted by the Raman spectra of clotrimazole and zone A is the map denoted by the Raman spectrum of specific dry PAA gel. Because there are no other distinct areas of interface between clotrimazole and the gel polymer, one can say that the two distinct components of this system do not interact chemically between each other.

CONCLUSIONS

The results presented here illustrate that chemical imaging is very useful for determining the chemical nature of the pharmaceutical granules. Raman applied mapping and imaging techniques clearly recognize the granules

consisting only of the major excipient (PAA) or the clotrimazole. The majority of the granules are found to be mixtures of clotrimazole and PAA gel. In addition,

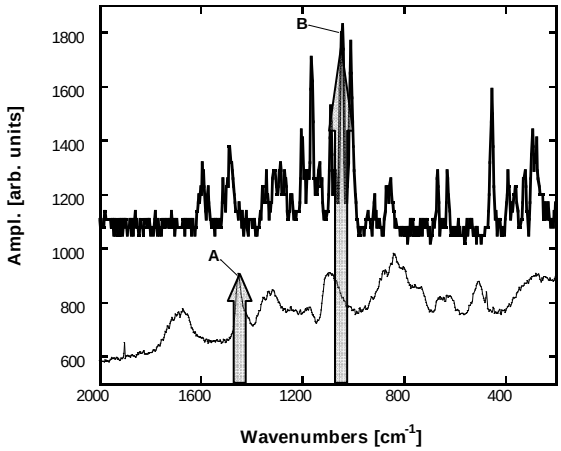


Figure 6. Raman spectra collected from two specific spots on the sample.

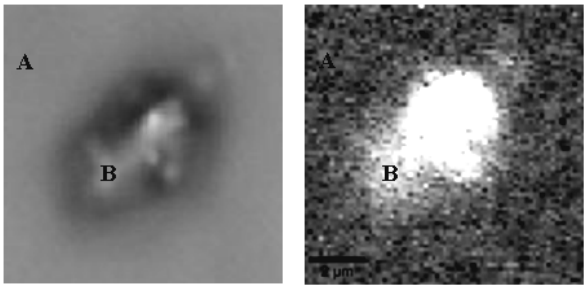


Figure 7. Optical image of the sample (left) and combined Raman map obtained by reconstructing the spatial distribution of the 1450 cm^{-1} (PAA - zone A) and 1045 cm^{-1} (Clotrimazole - zone B) bands.

it should be mentioned that smaller granules or particles (less than 10 μm) are also clearly visualized and can be chemically imaged. However, the information content is much higher in chemical mapping and imaging measurements. This makes them indispensable in preliminary experiments for determining the nature of the granules. An important observation concerns the stability of the topical mixture, insofar as clotrimazole immersed in the polymeric matrix does not have any chemical interaction.

EXPERIMENTAL SECTION

Materials: polyacrillic acid (PAA 940, B.F. Goodrich) and clotrimazole (Beijing Double Crane, Pharmaceutical Co. LTD, China).

Methods: Preparation of mucoadhesive gels was made by mixing the aqueous dispersion of the clotrimazole. The dispersions of PAA were neutralized with triethanolamine and the concentration of PAA was 1,5% in gel. Clotrimazole was incorporated by suspending it in a ratio of 1% in the gel.

Raman analyses: The dried granules were analyzed as obtained. No sample preparation of any sort was applied. Relatively large granules (dimensions of least 5-10 μm) were obtained. The samples also contained small granules which were not the focus of this study. For this reason no sampling methodology was developed to separate these particles. The complex product was deposed on thin films on microscope glass plates for the measurements [31]. The samples were investigated by Raman spectroscopy using a confocal Raman microscope (Witec CRM 200), at room temperature. Raman spectra were excited with 100mW of 633 nm light produced by a He-Ne laser and were recorded in backscattering geometry. No sample damage was detected in the experiments.

The AFM images were recorded with an Alpha 300 AFM module attached to the same Witec CRM 200 system. AFM scanning was performed by "tapping" mode, on an area of 5x5 μm. An average area of 10 μm × 10 μm was typically imaged. Since the granules were randomly distributed onto the microscope slide, the total imaging area was chosen to cover only one granule in a reasonable experimental time, typically less than 10 minutes. The imaged area was therefore slightly different from one experiment to another. All the Raman images were preceded by the acquisition of white light images of the sample across the area selected for imaging. Fortunately, the regions of strong and non-overlapping Raman bands for PAA and clotrimazole were found to be next to each other in the 400–1600 cm^{-1}region. This spectral region was employed for all the Raman measurements.

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