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Comparison of different chemometric methods to extract chemical and physical information from Raman images of homogeneous and heterogeneous semi-solid pharmaceutical formulations

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ABSTRACT

In formulations of nanostructured lipid carriers, lipid solid dispersions and self-emulsifying drug delivery systems, it is common that a solid or semi-solid lipid excipient is mixed with a liquid solvent or liquid lipid. Even when the excipients are visually miscible upon melting, they might have microscopic non-homogeneities which could lead to instability over time and future phase separation. Raman mapping associated with chemometric methods can be useful to evaluate spatial distribution of compounds, however it has not been extensively applied to the formulations mentioned above. The aim of this work was to compare the outcomes of three different chemometric methods – principal components analysis (PCA), multivariate curve resolution with alternating least squares (MCR-ALS) and independent components analysis (ICA) – to study two systems of very different degrees of microscopic miscibility: cetyl palmitate + Transcutol© (heterogeneous) and polyethylene glycol 6000 (PEG 6000) + Tween 80© (homogeneous). These two samples were chosen due to large differences in spatial distribution of the compounds over the pixels which could require different approaches for data treatment. The three methods were compared regarding recovered concentrations (or scores), signals (or loadings) and the need for matrix augmentation to obtain reliable results. Results showed that PCA loadings were the mathematical differences of the spectra of pure compounds for both samples, and therefore only 'contrast images' could be generated. MCR and ICA provided signals that could be related to the chemical components, however MCR presented rotational ambiguities even for the very heterogeneous sample, a situation in which ICA performed better as a blind search method. For the homogeneous sample, both methods showed rank deficiency and therefore the use of a matrix augmentation was necessary. ICA and PCA allowed identifying physical modifications in the homogeneous semi-solid PEG 6000/Tween 80® sample over the time, probably due to the folding/unfolding of the crystalline chains of PEG 6000. Therefore, this work discusses the ability of the three chemometrics methods to extract information from Raman spectra in order to characterize the chemical, spatial and even physical aspects of semi-solid pharmaceutical formulations, which could be of much use for stability studies of different drug delivery systems.

1. Introduction

Modern pharmaceutical development has to deal with the fact that many of the recently discovered molecules with pharmacological activities are hydrophobic and therefore present low water solubility, which is responsible for their poor and erratic bioavailability. For these compounds to become commercially marketed products, special formulation strategies need to be developed such as solid-dispersions using hydrophilic carriers (Serajuddin, 1999), lipid-based formulations (LBF) (Pouton and Porter, 2008; Williams et al., 2013, Feeney et al., 2016), solid-lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), (Müller et al., 2000; Müller et al., 2011), to mention just a few.

In many of these formulations a solid or semi-solid excipient is melted and mixed with a liquid solvent, surfactant or co-surfactant in one step of the process. The manufacturing processes that use meltable excipients are nowadays preferred over solvent-based processes, due to

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the savings of time and costs, besides being ecologically more attractive. In the case of SLN and NLC formulations, the solid/semi-solid excipient is hydrophobic while in case of solid dispersions and self-emulsifying drug-delivery systems (SEDDS) formulations it is normally hydrophilic. The final mixture can be either solid, semi-solid or liquid depending on the relative amounts of the excipients. Visual inspection of miscibility during the mixing of the molten semi-solid and liquid excipients is normally carried out. However this procedure might not be sufficient to assure real miscibility and stability. Breitkreitz et al. showed that SEEDS formulations of atorvastatin calcium prepared with Gelucire© 44/14 and different solvents/co-surfactants that were visually homogeneous, presented very different miscibility behavior by Raman mapping (Breitkreitz et al., 2013). Even if excipients are miscible upon melting, after cooling they could present micro-heterogeneities. Such inhomogeneities (as agglomerates or channels of the liquid excipient inside the waxy solid matrix) could lead to instability over time and eventually phase separation. In this sense, it is important to detect this problem in the early steps of pharmaceutical development to avoid waste of time, work and resources.

Cetyl palmitate (hexadecyl hexadecanoate, CP) is one of most commonly used solid lipids for the preparation of NLC formulations (Anantachaisilp et al., 2010; Chantaburanan et al., 2017). In this kind of formulation, the mixing of the solid and liquid excipient leads to enhanced solubility of the active principle ingredient (API) by formation of an imperfect matrix structure (Saupe et al., 2006). The NLC is the second generation of solid lipid nanoparticles. In comparison to SLN, the improved drug upload capacity and physicochemical stability of NLC has been attributed to the incorporation of a liquid component into the inner part of the waxy solid matrix (Beloqui et al., 2016).

While many papers describe the use of differential scanning calorimetry (DSC), X-ray powder diffraction (XRD), atomic force microscopy and zeta potential measures (Saupe et al., 2006; Carbone et al., 2014; Karn-Orachai et al., 2014), only a few have used Raman imaging to evaluate the miscibility of excipients (Anantachaisilp et al., 2010; Karn-orachai et al., 2016; Suto et al., 2016). Recently we showed the miscibility of CP-based NLCs with Dhaykol 6040® and Capryol® using Raman mapping and classical least squares (CLS) where it was possible to observe the advantages of the use of multivariate methods (da Silva et al., 2017). However, CLS only works well in very limited situations (when Lambert-Beer's law is strictly followed and there are no interactions among the components). Therefore, exploring other chemometric tools should lead to a deeper understanding of this type of formulations.

Polyethylene glycol (PEG) is a hydrophilic polymer largely used in pharmaceutical development to enhance the solubility of poorly water-soluble drugs (Gullapalli and Mazzitelli, 2015; Van Duong and Van den Mooter, 2016). Depending on its molecular weight, PEG can vary from a clear liquid (200–600 g/mol) to a white solid wax (> 1000 g/mol). Above 20,000 g/mol they are called polyethylene oxides (PEOs). PEG has a safety profile for human use and its availability at low cost makes it a very interesting excipient for pharmaceutical development. Although this polymer enhances the wettability of poorly water-soluble drugs, it is not capable of preventing precipitation upon dilution in aqueous medium by itself and, for this reason, it is common to associate it with another component, normally a lipid or a surfactant. Solid polyethylene glycol has a semi-crystalline structure, forming lamellae with chains either fully extended or folded – once or twice – depending on the crystallization conditions (Verheyen et al., 2002). Unga et al. studied binary mixtures of PEG with twelve liquid lipids to understand their influence on PEG crystallization by DSC and XRD. According to their results, the properties of incorporated lipids govern the crystallization behavior of PEG, changing the ratio of folded/unfolded chains of this excipient (Unga et al., 2010).

PEG is frequently associated with polysorbate 80 (Tween®), a non-ionic surfactant. It is suggested that when PEG and Tween 80® are mixed, the surfactant is miscible only in the amorphous part of PEG (Morris et al., 1992). Also, it has been described that the miscibility of the two compounds increases with the molecular weight of PEG (Tejwani et al., 2000). The PEG/Tween 80© system has demonstrated increased bioavailability for many drugs (Dannenfelser et al., 2004, Joshi et al., 2004) generating stable formulations. Nevertheless, a solid dispersion of saquinavir mesylate using PEG 4000 and Tween 80® was reported to be unstable over time. The authors attributed this instability to modifications in the degree of crystalline order/disorder of the carrier over the time (Caon et al., 2013).

Raman mapping generates chemical and spatial information, and it has been used for the evaluation of several different delivery systems (Gordon and McGoverin 2011; Smith et al., 2015). However, very few studies focus on the use of this technique for the development of semi-solid formulations for oral delivery (Breitkreitz et al., 2013; Sacré et al., 2015). The use of chemometric methods associated with this technique ensures that relevant information is extracted from the samples. Chemometric methods that do not require a calibration sample set in order to extract information are of particular interest, such as principal components analysis (PCA) and curve resolution methods, for example MCR-ALS (multivariate curve resolution with alternating least squares) and independent components analysis (ICA). While the first two are more common in literature, ICA is a recent method, not widely exploited in pharmaceutical development up to the moment. Using "*ICA and pharmaceutical*" as topics in Web of Science, 32 publications were found and only 2 using Raman imaging (Boiret et al., 2014; Lin et al., 2012). To the best of our knowledge, this is the first time that ICA is used on semi-solid formulations. It is important to highlight that even though these methods do not require a calibration sample set, data from a very homogeneous sample can present rank deficiency issues due to the lack of variability in the pixels (Lohumi et al., 2017; Tauler et al., 1995), therefore it becomes necessary to include samples with different concentration of the components, generating what is called an augmented matrix.

The aim of this work is to compare the outcomes of three different chemometric methods: principal components analysis (PCA), multivariate curve resolution with alternating least squares (MCR-ALS) and independent components analysis (ICA) to study two systems of very different degrees of microscopic miscibility: cetyl palmitate + Transcutol© (heterogeneous) and PEG 6000 + Tween 80© (homogeneous). We discuss the ability of these chemometric methods to describe the miscibility and physical stability of two very different semi-solid formulations, based on Raman mapping data. It is described how the degree of heterogeneity (pixel-to-pixel variability) affects each method and their ability to detect physical changes that could lead to instabilities even in very homogeneous semi-solid formulations. A brief description of each method is provided in the Section 2.4.

2. Materials and methods

2.1. Materials

Cetyl Palmitate was purchased by Dhaymers Química Fina (Brazil), Transcutol® was kindly donated by Gattefossé (France), polyethylene glycol 6000 and Tween 80® were purchased from Synth (Brazil). The structure of each excipient is shown in Fig. S1.

2.2. Sample preparation and Raman mapping

The samples were prepared by heating 10 °C above the melting point of CP (54° C) and PEG 6000 (58–63 °C) and the liquid excipient (Transcutol© or Tween 80©) was added under stirring until a visually homogeneous mixture was obtained. The sample CP/Transcutol® was prepared to have the bulk concentration of 70% w/w of CP and 30% w/w of Transcutol®. Nine samples of PEG 6000 and Tween 80® were prepared, varying the proportions from 10 to 90% (w/w) (Table S1). The samples were cooled to room temperature in an aluminum cell and