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Evaluation of the microscopic distribution of florfenicol in feed pellets for salmon by Fourier Transform infrared imaging and multivariate analysis

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ABSTRACT

Fourier Transform infrared imaging and multivariate analysis were used to identify, at the microscopic level, the presence of florfenicol (FF), a heavily-used antibiotic in the salmon industry, supplied to fishes in feed pellets for the treatment of salmonid rickettsial septicemia (SRS). The FF distribution was evaluated using Principal Component Analysis (PCA) and Augmented Multivariate Curve Resolution with Alternating Least Squares (augmented MCR-ALS) on the spectra obtained from images with pixel sizes of 6.25 μm × 6.25 μm and 1.56 μm × 1.56 μm, in different zones of feed pellets. Since the concentration of the drug was 3.44 mg FF/g pellet, this is the first report showing the powerful ability of the used of spectroscopic techniques and multivariate analysis, especially the augmented MCR-ALS, to describe the FF distribution in both the surface and inner parts of feed pellets at low concentration, in a complex matrix and at the microscopic level. The results allow monitoring the incorporation of the drug into the feed pellets.

1. Introduction

In the salmon industry, the use of food pellets with drugs is a common practice to ensure all fish receive treatment against different diseases which affect their healthy growth. The most devastating disease is salmonid rickettsial septicemia (SRS), caused by *Piscirickettsia salmonis*, which kills millions of salmon and produces millions of losses. To fight both this disease and others, florfenicol (FF) is the main antibiotic included in pellets to feed salmons and to achieve optimal health through proper lifestyle and nutrition [1–4].

As it is known, it is important that a pharmaceutical tablet has a homogeneous distribution of its components to maintain its pharmacokinetic properties [5,6]. The main technique used in the pharmaceutical industry for the development of new products, quality control, and determination of bioequivalence is the dissolution test [7], which cannot give information concerning a drug or excipient distributions in tablets. This is a problem when it is necessary to compare whether two batches of tablets made at different times have equal compound distributions, how different the compound distributions are when different processes are used to create the same tablets or how the distributions of compounds in tablets change when the excipients change slightly in proportion to their prices, which occurs very frequently in the case of feed pellets, etc.

During the last decade, hyperspectral imaging obtained with infrared spectroscopy together with chemometric techniques has been increasingly used in the pharmaceutical industry to obtain additional chemical information of pharmaceutical products. A very good example is the work of Hiroki Hifumi et al., 2016, wherein the ability of mid infrared imaging (commonly known as Fourier Transform infrared imaging or FTIR imaging) to determine *in situ* characteristics of different polymer based films was demonstrated. That work provided information on the distributions of each component in the different films, the rate of water ingress, the dissolution behavior of each component and the interactions of drug with the excipients [8]. Some other examples of the use of this technique in the pharmaceutical industry include the study of the stability of pharmaceutical solid dosage forms, the effects of moisture and pressure on tablet compaction, the effects of modifying the pH microenvironment on the dissolution of ibuprofen from

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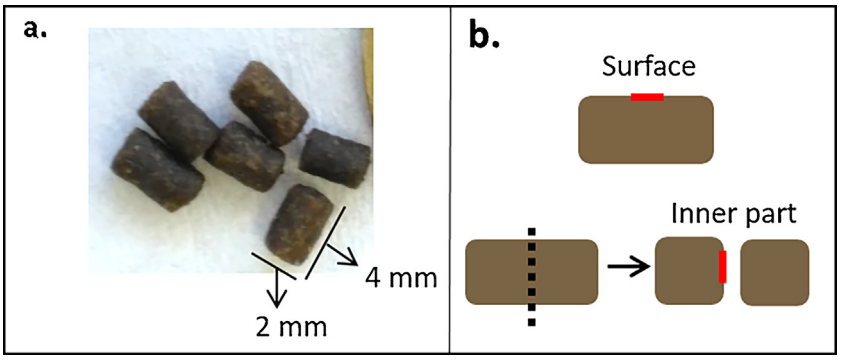

Fig. 1. Description of feed pellet containing FF. a) Feed pellet dimensions; b) Zones of pellets where hyperspectral images were obtained (red lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hydroxypropyl methyl cellulose (HPMC) matrices, studies on tablet dissolution and drug release, among others [9–19].

Hyperspectral images are cubes of numerical information containing two axes, *x* and *y*, of spatial information, and a third side, *z*, spectral information. In the case of FTIR imaging, *z* contains the values of absorbance, transmittance, or reflectance obtained from each pixel, of a mid-infrared range of wavenumbers. To find which pixels describe the distribution of a certain compound contained in this cube of information, a selection of the wavenumber characteristic of each compound needed to reproduce false color images can be performed. This produces good results when the number of components in the samples is low and the bands of each component are well differentiated. However, in the case of systems with a higher number of chemical components, and a high occurrence of overlapping bands, such as the case described in this work, the selectivity of FTIR imaging can be improved by the use of multivariate techniques [5,20]. Principal Component Analysis (PCA) and Multivariate Curve Resolution with Alternating Least Squares (MCR-ALS) are very useful bilinear algorithms able to describe component distributions in images, and their mathematical theory is already widely described the in literature [12,13,21–23]. Before the application of bilinear methods, the hypercube is unfolded in a matrix (**X**) with only one spatial dimension, the product of x^*y pixels and one spectral dimension, *z*. PCA is an exploratory algorithm used to obtain some idea, in its first instance, of the chemical components that could be contained in the images. This method derives new variables, called principal components (PC's), as linear combinations of the original variables, reducing the data dimension via the decomposition of the matrix **X** into two matrices, scores (**T**) and loadings (**L**), according to Eq. (1), in order to explain the maximum variance of the initial matrix [22].

$$\boldsymbol{X} = \boldsymbol{T}\boldsymbol{L}^{\boldsymbol{T}} + \boldsymbol{E} \quad (1)$$

where E, is the residual error in the reconstruction of **X,** with a certain number of PC's.

In the case of compound distributions in images, scores contains information of how each pixel is related to each PC, while loadings show the importances of wavenumbers to these relations. By definition, PC1 accounts for the highest explained variance; thus, this PC can describe the main component of an image. For example, in a given sample, if the loading of PC1 is very similar to the spectrum of the active ingredient or is very influenced by the bands of this spectrum, PC1 probably corresponds to the active ingredient, and its scores map will show its distribution. The results obtained by PCA depend, like almost every chemometric techniques, on the type of data used to create the model, and a PCA model is not always able to well describe the compound distribution.

MCR-ALS is an algorithm based on PCA that is used to decide how many components should be considered. This algorithm consists of decomposing a matrix (**D**) into the product of two matrices: relative concentrations (**C**) and pure spectra (**S**), according to Eq. (2):

$$\boldsymbol{D} = \boldsymbol{C}\boldsymbol{S}^{\boldsymbol{T}} + \boldsymbol{E} \quad (2)$$

The greatest difference from PCA is that it is possible to restrict ALS in order to find results with chemical meanings according to what we are looking for [24,25]. In FTIR imaging, ALS is restricted to obtain concentrations and spectra with positive values. In general, both of these techniques have shown their power to describe the distribution of active principles and/or different excipients in the pharmaceutical industry [21,26–28].

In this work, we developed PCA and MCR-ALS models to study the microscopical distribution of FF in feed pellets supplied to salmon, using hyperspectral images obtained with FTIR imaging and the Attenuated Total Reflectance (ATR). The analytical conditions of the spatial resolutions, such as the pixel sizes in the acquisition of hyperspectral images as well as the two mentioned chemometric methods, were compared to find which conditions are more appropriate for the study of the distribution of FF in the pellets. In addition, these analyses were performed on different zones of the pellets, in order to determine information regarding the distribution of FF in the pellets before they are supplied to salmon cage cultures. This work aims to demonstrate whether it is possible to identify FF at the microscopic level in the pellets using the proposed techniques, even when considering that the drug is contained in a very complex matrix (in the presence of proteins, fish oil, flour, minerals, vitamins and others) with multiple active components in the infrared region and at low concentrations.

2. Materials and methods

2.1. FF standard and feed pellets for fishes

The FF standard was provided by Cargill Chile Ltda. Comparison between the spectrum of this standard (explained below) and the FF spectrum reported by Sadeghi et al. [29] shows that the two are almost identical.

Fish pellets with included FF (3.44 mg FF/g pellet) were obtained from Cargill Chile Ltda. The fish feed pellets have different sizes according to fish size, called "calibers"; in this case, caliber 50 is the size of pellet to feed very small salmon. Fig. 1 shows the fish feed pellet used in this work with dimensions of caliber 50 pellets (Fig. 1a).

2.2. Acquisition of hyperspectral images

Hyperspectral images were obtained with a Perkin Elmer spotlight 400 FTIR system in ATR mode, under the following conditions: spectral range of 4000–748 cm^{-1}, spectral resolution of 8 cm^{-1} and