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# Polymer/clay nanocomposite films as active packaging material: Modeling of antimicrobial release

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ABSTRACT

An integral combination of design of experiments (DoE), partial least squares regression (PLSR) and response surface methodology (RSM) was applied to model the release of carvacrol into a food simulant from an antimicrobial material based on low-density polyethylene/organically-modified montmorillonite (LDPE/OMM) nanocomposite films. Using a D-optimal design, one qualitative-multi-level *x*-variable and two quantitative *x*-variables of the nanocomposite formulation were studied: type of OMM, concentration of OMM and concentration of a compatibilizer agent. Eight *y*-responses were simultaneously modeled using PLSR, including four kinetic/diffusion properties of carvacrol release toward food simulant (initial rate of release, overall kinetic rate constant, diffusion coefficient and diffusion rate constant), and four packaging properties (Young's modulus, intercalation, decomposition and melting temperature). An ANOVA-validated ($p < 0.05$) PLS model was obtained with $R^2 = 0.923$ and $Q^2 = 0.453$, modeling the all *x*-variables with all *y*-responses simultaneously. A formulation that maximizes packaging properties and minimizes carvacrol release was calculated by using RSM.



## 1. Introduction

Food trade in a globalized market poses several challenges, including how to prolong shelf life in fresh food while maintaining quality and freshness during long transport and storage time periods. Inhibition of foodborne microbial growth has become vital for reducing loss of fresh food. Antimicrobial active packaging is a recent concept in food technology based on incorporating an antimicrobial compound inside the packaging material or using a packaging material with inherent antimicrobial properties to reduce or inhibit microbial growth [1]. Among the many desired properties of an antimicrobial packaging material, the controlled release of the antimicrobial agent is critical. When this release occurs too quickly, the minimum inhibition concentration is not sustained for long periods of time. When this release occurs too slowly, a sufficient concentration of the antimicrobial agent at an early stage is not achieved, and spoilage is not controlled [2].

The use of synthetic chemicals as antimicrobial agents on food has been restricted due to their potential acute toxic effects and increasing consumer interest toward natural compounds (*i.e.* green compounds). Essential oils and their components, which primary consist of terpenoids and phenolic compounds, *e.g.*, carvacrol, are categorized as GRAS (generally

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recognized as safe) compounds by the US FDA and therefore these compounds have been used as food additives at low concentrations [3]. Essential oils have also been incorporated into different materials to develop antimicrobial active food packaging *e.g.*, carvacrol and thymol on polypropylene films [3], thyme and oregano essential oils on soy edible films [4], and carvacrol on HDPE [5] among others.

Active packaging research has been focused on the development of polymer/clay nanocomposites as a packaging matrix. Improved mechanical, thermal, rheological and barrier properties have been reported on intercalated nanocomposite matrices compared to neat organic polymers [6]. Nanoclay is spread on the polymer matrix resulting in a homogeneous layer dispersion creating "tortuous pathways" (see Fig. 1), which difficult the diffusion of molecules throughout the matrix. This approach provides a potential controlled release mechanism for antimicrobial drugs [7].

Homogeneous dispersion of nanoclay in organic polymer is not easily achieved due to the hydrophilicity of clay. Organically-modified nanoclays are used to increase the affinity between the clay and the polymer, thus increasing the interlayer distance and the lipophilicity of the nanoclay [8]. Several reports have indicated that organically-modified nanoclays (often organically-modified montmorillonite) are potential controlled release materials for drugs, and drug–clay interactions depending on the chemical nature of the organically-modified nanoclay have been recently considered and studied as a strategy for drug release modeling [8–10]. Compatibilizer agents are also used to improve intercalation of nanocomposites by increasing the interlayer distance of nanoclays, which favors the penetration of the polymer into clay galleries. These compatibilizer agents are based on maleic anhydride grafted copolymers, and high degrees of intercalation have been achieved on nanocomposites when these agents are used on the formulation [11].

The development of controlled release in antimicrobial packaging is limited, and the efforts to control the release kinetics of antimicrobials have been focused on changing the structure of the matrices [12–14]. Studies of the controlled release of drugs in packaging materials are often carried out with a univariate approach, and parallel analysis of the packaging properties of these materials is frequently required [15–17]. The multiple variables involved in antimicrobial controlled release modeling, such as the composition or structure of the polymer matrix, can result in substantial changes in the mechanical, thermal, rheological and barrier properties of the final product. Therefore, determining the optimal matrix formulation for modeling antimicrobial controlled release as well as maintaining optimal packaging properties is a complex challenge. To solve the classical problem of how to model several properties in a material when several variables exert influence over them in a complex way, the design of experiments (DoE) and multivariate calibration are appropriate approaches.

Response surface methodology (RSM) is a set of statistical techniques that can fit a mathematical equation to experimental data to predict the behavior of the studied system. From the experimental data, an empirical model is obtained in the form of a polynomial function that describes the experimental area studied [18]. It is necessary to carry out a correct set of experiments that provide consistent data in the experimental region that is studied. DoE is based on the systematic variation of variables by changing all of their levels simultaneously with the optimum number of experiments, which reduces the time, materials, reagents and overall cost. This set of experiments is defined by a matrix, which is composed of a different combination of the variable levels [19]. Among the different types of DoE, D-optimal design is especially useful when the design has multi-level qualitative variables or a high number of variables. It consists of a computer-generated DoE that choose the best set of experiments from a pool of theoretically possible designs. D-optimal design allows for a smaller number of experiments to be performed to tackle qualitative or categorical variables with more than two levels when they are combined with quantitative variables in the same experiment. This design finds those experiments that have the best spread and a balanced distribution of the number of runs for each level of a qualitative variable. The application of D-optimal design has been reported in different areas including engineering [20], kinetic chemical reactions [21], chemical synthesis [22], food technology [23], analytical chemistry [24] and clinical use [25].

A measured experimental response ($y$) is dependent of the experimental variables ($x$) according to $y = f(x)$. If a second-order interaction model describes the function, this model is represented by the polynomial shown in Eq. (1)

$$y = b_0 \sum_{i=1}^{k} b_i x_i + \sum_{1 \leqslant i \leqslant j}^{k} b_{ij} x_i x_j + \varepsilon \tag{1}$$

where $y$ is the measured response, $b_0$ is the constant term, $b_i$ are the unknown regression coefficients of the variables $x_i$ that must be determined, and $\varepsilon$ is the residual. In matrix notation, Eq. (1) can be represented as

$$\mathbf{Y} = \mathbf{XB} + \mathbf{E} \tag{2}$$

where $\mathbf{Y}$ is the matrix of all responses, $\mathbf{X}$ is the matrix of all variables, $\mathbf{B}$ is the vector of regression coefficients, and $\mathbf{E}$ is the matrix error. To obtain vector $\mathbf{B}$, which contains the regression coefficients ($b$), with the lowest $\varepsilon$, Eq. (2) can be solved using a least squares method resulting in

$$\mathbf{b} = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{Y} \tag{3}$$

where $\mathbf{X}'$ is the transpose of $\mathbf{X}$. The model accuracy can be measured by a variance-covariance matrix $\mathbf{V}(\mathbf{b})$ as expressed in Eq. (4)

$$\mathbf{V}(\mathbf{b}) = \sigma^2(\mathbf{X}'\mathbf{X})^{-1} \tag{4}$$