Ciência e Agrotecnologia
SCIENCE AND AGROTECHNOLOGY
Ciência e Agrotecnologia, 45:e003221, 2021
http://dx.doi.org/10.1590/1413-7054202145003221
Food Science and Technology
eISSN 1981-1829

Oregano essential oil encapsulation following the complex coacervation method: Influence of temperature, ionic strength, and pH on the release kinetics in aqueous medium

Encapsulação de óleo essencial de orégano pelo método de coacervação complexa: Influência da temperatura, força iônica e pH na cinética de liberação em meio aquoso

Vítor Mathias Muneratto[1*], Thais Cristina Benatti Gallo[1], Vania Regina Nicoletti[1]

[1]Universidade Estadual Paulista "Júlio de Mesquita Filho"/UNESP, Instituto de Biociências Letras e Ciências Exatas de São José do Rio Preto/IBILCE, Departamento de Engenharia e Tecnologia de Alimentos, São José do Rio Preto, SP, Brasil
Corresponding author: vitor.mathiasm@gmail.com
Received in June 20, 2021 and approved in August 20, 2021

ABSTRACT

Oregano essential oil (OEO) exhibits antimicrobial and antioxidant properties. The bioactive compounds of OEO are volatile. Thus, encapsulation helps in protecting the activity of the compound when exposed to harmful conditions such as high (or low) temperature, light, and oxygen. Encapsulation also helps to improve the dispersibility of the compound in an aqueous medium, facilitating its application in formulated food products. We studied the release kinetics of OEO microencapsulated by gelatin/gum. Arabic complex coacervation was assessed, focusing on the influence of the physicochemical properties of the aqueous release medium (temperature (4, 25, and 30 °C), ionic strength (0, 0.5, 1.0, and 1.5 M), and pH (3.8, 4.2, and 4.8)). Their capacity to act as release triggers was also investigated. High OEO release rates were recorded in the media with high ionic strengths (74% release in 5 h), high pH (78% release in 7 h), and low temperature (71% release in 7 h). In media at a temperature above 30 °C and pH 3.8, the coacervated structure was disintegrated. A centered face experimental design (CFD) based on 17 samples was constructed, and an empirical model was developed to predict the maximum release conditions. The highest percentage of oil (reaching up to approximately 85%) is released over longer periods of time (7 h on an average) without damaging the integrity of the microcapsule. Peppas' model showed the best fitting for all release profiles, with $R^2 > 0.86$ and relative average residual error < 6%, indicating the domain of Fickian diffusion during OEO release.

Index terms: Microencapsulation; mass transfer; diffusion; gelatin; gum Arabic.

RESUMO

Óleo essencial de orégano (OEO) apresenta propriedades antimicrobianas e antioxidantes. Os compostos bioativos do OEO são voláteis. Portanto, a encapsulação ajuda a proteger a atividade dos compostos quando expostos a condições danosas, como alta (ou baixa) temperatura, luz e oxigênio. A encapsulação também auxilia a melhorar a dispersibilidade do componente em meios aquosos, facilitando sua aplicação em produtos alimentícios formulados. Nós estudamos a cinética de liberação do OEO microencapsulado por coacervação complexa entre gelatina e goma arábica, focando a influência das propriedades físico-químicas do meio aquoso de liberação (temperatura (4, 25 e 30 °C), força iônica (0, 0.5, 1.0 e 1.5 M) e pH (3.8, 4.2 e 4.8)). Suas capacidades de agirem como gatilhos de liberação também foram investigadas. Altas taxas de OEO liberado ocorreram nos meios com elevada força iônica (74% liberado em 5 h), alto pH (78% liberado em 7 h) e baixa temperatura (71% liberado em 7 h). Em meios com temperatura acima de 30 °C e pH 3.8, a estrutura coacervada foi desintegrada. Um delineamento de faces centradas (DFC) baseado em 17 amostras foi construído e um modelo empírico desenvolvido para prever as condições de máxima liberação. A maior porcentagem de óleo (atingindo até 85%) é liberada em longos períodos de tempo (em média 7 h) sem causar danos às microcápsulas. O modelo de Peppas mostrou o melhor ajuste para todos perfis de liberação, com $R^2 > 0,86$ e erro residual médio < 6%, indicando o domínio da difusão de Fick na liberação de OEO.

Termos para indexação: Microencapsulação; transferência de massa; difusão; gelatina; goma arábica.

INTRODUCTION

The use of synthetic additives in food is being discouraged from obtaining healthy food. Hence, natural additives are being researched. Essential oils are characterized as oily aromatic liquids extracted from plants, flowers, seeds, tree barks, roots, and sprouts (Burt et al., 2004; Calo et al., 2015; Asbahani et al., 2015). They are a suitable choice as nature additives as they exhibit a range of beneficial properties (antioxidant, insecticide,

antiviral, antimicrobial, and antifungal). These properties can be attributed to the presence of intrinsic bioactive and volatile compounds. Essential oils have been widely used in the production of cosmetics, food, and pharmaceuticals. Mint, thyme, cinnamon, eucalyptus, rosemary, oregano, and citronella are some of the most famous components (Rodríguez et al., 2016; Castro et al., 2017).

Oregano essential oil (OEO) is composed of over thirty compounds, such as terpenes and phenols. 78 to 82% of OEO is composed of phenolic compounds (carvacrol and thymol) that determine the antioxidant and antimicrobial characteristics of OEO. These compounds dictate the effectiveness of OEO in controlling microbial growth as they depolarize the plasmatic membrane of bacteria cells, causing their death (Bakry et al., 2016). Carvacrol and thymol can inhibit the growth of food poisoning bacteria (Friedman, 2014; Bagamboula et al., 2004; Burt et al., 2007; Friedman et al., 2007).

In spite of its important functional properties, OEO is a complex mixture of volatile and chemically unstable compounds, the stability of which can be affected under conditions of high temperatures and in the presence of oxygen and light (Hijo et al., 2014). Furthermore, due to the natural hydrophobicity of essential oils, the organic compounds present in food matrices can interact with OEO and reduce the antimicrobial effect and action time (Bakry et al., 2016; Bhargava et al., 2015). Microencapsulation can potentially help in overcoming these limitations. Microencapsulation can protect the volatile compounds from the effect of the external environment. Thus, an extended time of release can be achieved (De Prisco et al., 2016; Bakry et al., 2016; Dias et al., 2015; Martins et al., 2009; 2011a, b).

Complex coacervation is a method of microencapsulation that involves the use of polymers of opposite charges. These polymers build a strong coating matrix (high-temperature conditions can affect the stability of the volatile compounds present in essential oils). It is important that complex coacervation be carried out under ideal conditions. The process should be carried out under conditions of optimal wall materials, temperature and pH. Under the optimal conditions, microcapsules with high content of inner oil (up to 99 % of efficiency) and high stability can be produced. A simple and low-cost process (Bakry et al., 2016; Martins et al., 2012; Sharkawy et al., 2017) can be followed for the fabrication of the microcapsules. The most commonly used wall materials for complex coacervation are gelatin (a cationic protein at pH below its isoelectric point) and gum Arabic (an anionic arabinose–galactose polysaccharide complex that remains in its negatively charged state under a wide pH range) (Marfil et al., 2018).

Three parameters determine the occurrence of complex coacervation: pH, temperature, and ionic strength. Temperature influences the process of protein ionization by changing the component (side chain, carboxylic group, and amines) charges. The charge of the components influences the generation of the complex coacervation microcapsules as they are only be generated when the wall materials have opposite charges. The two other parameters influence polymer interactions (electrostatic attraction inducing complex formation). The protein/polysaccharide ratio significantly influences the complex coacervation process and dictates the complex formation intensity (Priftis; Laugel; Tirrell, 2012; Schmitt et al., 1998). These physicochemical properties significantly influence the production of proper coacervated microcapsules. They also affect the stability of the encapsulating matrix and, as a consequence, the release of the core material. We studied the release kinetics of OEO encapsulated in gelatin/gum Arabic (via complex coacervation) in an aqueous medium. We focused on the effects of temperature, ionic strength, and pH on the oil release and the morphology of the microcapsules during the release assays to understand the release mechanism.

MATERIAL AND METHODS

Material

Commercially available OEO (Laszlo Aromatologia, Brazil) was used as the core material. Type "B" gelatin (LS Chemicals, Brazil) and pure Arabic gum (Química Comtemporânea Ltda., Brazil) were used as wall materials. Analytical grade chemicals and deionized water were used in all the assays.

Production of microcapsules

Microcapsules were produced following the process of complex coacervation using gum Arabic and gelatin as wall materials. Each solution consisted of gum Arabic (1%) and gelatin (1%, g wall material/100 g solution). The solutions were prepared by dissolving and homogenizing these materials in deionized water under conditions of magnetic stirring (at 50 ºC). OEO (1%, g OEO/100 g solution) was added to the gelatin solution, and the mixture was homogenized at 13500 rpm for 5 min (IKA T25- Digital Ultra Turrax, Staufen, Germany). Following the addition of oil, the primary emulsion was added to the gum Arabic solution to obtain the final solution (with a 1:1 polymer ratio) at 50 ºC. The solution was prepared under conditions of continuous magnetic stirring. The pH was adjusted to 4.0 ± 0.2 using glacial acetic acid. A digital pH

meter (Tecnal, TEC-3MP, Brazil) was used to determine the pH of the solutions. The system was immersed into an ice bath (under conditions of constant stirring) until the temperature was 10 ± 0.5 ºC. Lastly, the system was covered with tinfoil to protect it from the effects of light. It was kept refrigerated at 4 ºC for 24 h for the complete sedimentation of the produced microcapsules. The sediments were separated following the process of sieving (38 µm). After separation, the microcapsules were washed with deionized water.

Structural and morphological characterization

An optical microscope (Olympus CX31) equipped with an SC30 photographic camera, managed by AnalySIS getIT software, was used to study the morphological and structural characteristics of the microcapsules produced following the process of complex coacervation during the assays.

Retention capacity

The encapsulation efficiency of OEO was evaluated by studying the retention capacity (RC). The process described by Marfil (2018) was modified to conduct the experiments. Aliquots of microcapsules (2 g) were mixed with the citrate–phosphate buffer solution (5 mL) at pH 8.0 ± 0.1 to break the encapsulation matrix. Hexane (5 mL) was added to extract the oil. The flasks containing the samples were vortexed for 2 min. The organic phase was removed using a micropipette and placed in a tube. Hexane (5 mL) was added to the samples and the procedure was repeated six times. At the end of the process, the absorbance was recorded at 275 nm to determine the total oil content in the microcapsules with reference to a standard curve. The retention capacity (*RC*) was calculated based on Equation (1):

$$\mathrm{RC}(\%) = \frac{\text{total OEO content in microcapsules}}{\text{OEO initial content in the emulsion}} \times 100 \quad (1)$$

In vitro release kinetics

The OEO release kinetics was assessed in distinct aqueous media by varying the release medium parameters: ionic strength (0 to 1.5 M), pH (3.8 up to 4.8), and temperature (between 4 and 30 ºC) (Table 1). The compositions of the different liquid media investigated were different from the basic composition (distilled water containing 1.35% soybean oil (mL oil/100 mL solution) and 0.1% Tween 80 (mL Tween/100 mL solution)).

Table 1: Physicochemical properties of the aqueous media used to study OEO release kinetics.

Medium	Ionic strength (M)	pH	Temperature (ºC)
IS0.0	0	4.2	25
IS0.5	0.5		
IS1.0	1		
IS1.5	1.5		
pH3.8	0.5	3.8	25
pH4.8		4.8	
T04	0.5	4.2	4
T30			30

The released OEO was quantified following the method proposed by Hosseini et al. (2013). The reported method was slightly modified. Aliquots of 20 g of microcapsules were added to flasks containing 20 mL of the desired liquid medium. The mixture was subjected to magnetic stirring in a thermostatic shaking water bath. Nine sets of samples were used for each release assay. At predetermined times, a sample (7 mL) of the solution contained in one of the nine flasks was withdrawn for quantification of the released oil. The spectrophotometry technique was used for characterization. The absorbance at 275 nm was recorded. The OEO concentration was calculated based on standard curves generated for each specific medium. The percentage of oil released (Q) was calculated from Equation (2). The calculation was based on the quantity of released OEO at each time (C_t) and the total amount of oil microencapsulated in the sample (C_0). The equation is represented as follows:

$$Q(\%) = \frac{C_t}{C_0} \times 100 \quad (2)$$

To model the release kinetics, the average curves for OEO liberation, based on the experimentally determined release profiles, were adjusted to four mathematical models: Zero Order, First Order, Higuchi, and Peppas (Jiménez-Alvarado et al., 2009). The corresponding equations are presented in Table 2.

The data corresponding to OEO release over time was fitted using the non-linear regression method. The fitting quality was evaluated based on the coefficient of determination (R^2) and the sum of relative residuals (E) calculated from Equation (7), where V_{pred} is the predicted value, V_{exp} is the experimental value, and N denotes the number of experimental data.

Table 2: Mathematical models and their respective equations.

Model	Equation	
Zero Order	$Q = K_0(t) + Q_0$	(3)
First Order	$lnQ = K_1(t) + lnQ_0$	(4)
Higuchi	$Q = K_H(t^{1/2}) + Q_0$	(5)
Peppas	$Q = k(t^n)$	(6)

*Q: cumulative percentage of liberated material in time t; Q_0: initial cumulative percentage of encapsulated material; K_0, K_1, K_H: constant representing the models; k: structural and geometrical constants of the matrix; n: diffusional exponent.

$$E = \frac{I}{N}\sum_{i=1}^{i=N}\left(\frac{\left|V_{pred,i} - V_{exp,i}\right|}{V_{exp,i}}\right) \quad (7)$$

Statistical analysis

The results obtained from the triplicate determinations were used for variance analysis, and the average values were used for the Tukey test at 5% of probability using Minitab 18.

Experimental design

The results from the first set of experimental assays are listed in Table 1. The second series of assays were carried out to complete a centered face design (CFD) to analyze the effects of the three independent variables (pH, temperature, and ionic strength) on the percentage of OEO release. In total, 17 samples were analyzed (Table 3). The results were used to obtain an empirical model for the prediction of the maximum release conditions.

The following polynomial model (Equation 8) was used to fit the data:

$$y = \beta_0 + \beta_1 \cdot x_1 + \beta_2 \cdot x_2 + \beta_3 \cdot x_3 + \beta_{11} \cdot x_1^2 + \beta_{22} \cdot x_2^2 + \beta_{33} \cdot x_3^2 + \beta_{12} \cdot x_1 \cdot x_2 + \beta_{13} \cdot x_1 \cdot x_3 + \beta_{23} + x_2 \cdot x_3, \quad (8)$$

where y is the response variable, x_1 denotes the pH, x_2 denotes the temperature, x_3 represents the ionic strength, and β_i and β_{ij} are the fitted coefficients.

The results were used to conduct the ANOVA (analysis of variance) tests and the fitted model was

Table 3: OEO release assays conducted in different aqueous mediums identified by coded variables and the real values corresponding to the centered face design (CFD) and final percentage of the oil release.

Samples	x_1 (pH)	x_2 (Temperature)	x_3 (Ionic strength)	y (%Release)*
1	-1 (3.8)	-1 (4)	-1 (0.5)	37.0
2	1 (4.8)	-1 (4)	-1 (0.5)	70.3
3	-1 (3.8)	1 (46)	-1 (0.5)	0.0**
4	1 (4.8)	1 (46)	-1 (0.5)	0.0**
5	-1 (3.8)	-1 (4)	1 (1.5)	58.7
6	1 (4.8)	-1 (4)	1 (1.5)	83.1
7	-1 (3.8)	1 (46)	1 (1.5)	0.0**
8	1 (4.8)	1 (46)	1 (1.5)	0.0**
9	-1 (3.8)	0 (25)	0 (1.0)	66.8
10	1 (4.8)	0 (25)	0 (1.0)	73.6
11	0 (4.3)	-1 (4)	0 (1.0)	79.8
12	0 (4.3)	1 (46)	0 (1.0)	0.0**
13	0 (4.3)	0 (25)	-1 (0.5)	59.6
14	0 (4.3)	0 (25)	1 (1.5)	74.0
15	0 (4.3)	0 (25)	0 (1.0)	69.4
16	0 (4.3)	0 (25)	0 (1.0)	71.7
17	0 (4.3)	0 (25)	0 (1.0)	72.2

* % Release (y) values were defined as the released percentage of OEO after 7 h. ** As the objective of the optimization model is to ensure oil release over extended periods with a higher release, y = 0 refers to samples for which the microcapsules got disintegrated after the first 10 min of agitation (as they were subjected to conditions of medium to high temperatures).

subjected to lack of fit, coefficient of determination, and coefficient of regression tests. PROTIMIZA (Rodrigues; Iemma, 2014) was used to analyze the data. For the fitted model, the coefficient of determination (R^2) obtained was satisfactory (98,87%), the coefficient of regression was significant, and the lack of fit was not significant, showing good accuracy and fitting of the model.

RESULTS AND DISCUSSION

Microencapsulation retention capacity

The microcapsules produced following the process of complex coacervation, with the core/wall material rate of 1:2 (w/w), presented a retention capacity of 64.4 ± 0.3%, which was slightly lower than the desirable values. Bastos et al. (2020) studied the microencapsulation of volatile essential oil (black pepper) following the process of complex coacervation in the presence of beta-lactoglobulin and sodium alginate. They reported that the ratio of loaded oil to theoretical oil content ranged between 20 and 85%, depending on the core/wall material rate.

Similar results were reported by Rungwasantisuk and Raibhu (2020) for the microencapsulation of lavender essential oil in gelatin/gum Arabic complex coacervates. It was reported that the encapsulation efficiency increased (from 80 to 84%) with increasing amounts of essential oil. The efficiency increased till the gelatin/gum Arabic coacervate retained the capacity to accommodate the oil. In the absence of the wall, the efficiency increased from 67 to 76%.

Prata and Grosso (2015) studied the influence of the oil phase in the process of complex coacervation. It was observed that the essential oils presented higher encapsulation efficiencies and stability than others, such as mineral and vegetable oils. The higher efficiency could be attributed to the hydrophilic compounds that could act as surfactants.

Influence of ionic strength, pH, and temperature on the OEO release kinetics

The release profiles of OEO in the aqueous medium were significantly affected by the different physicochemical conditions. High and rapid initial release, a phenomenon known as "burst release", was the common characteristic observed in all the media. These release percentages increased from 30 to 40% within the first 10 min. This could be attributed to the presence of OEO in the outer and inner surfaces (Figure 1) of the microcapsules, as discussed by Gallo et al. (2020).

The release of OEO from the microcapsules added to the medium in the absence of NaCl exhibited a random variation and non-sequential increasing results (Figure 2). As the ionic strength increased, the nature changed. The highest repeatability and small mean deviations were achieved with the medium with the maximum NaCl concentration when the concentration was proportional to the percentage of oil released. The maximum percentage of released OEO (74%) was observed in the medium containing 1.5 NaCl, and the minimum release was achieved in the medium not containing NaCl (56%).

The large number of ions produced during the dissociation of NaCl can interfere with the electrostatic interactions generated by the microcapsule structure. The interference weakens the encapsulant matrix and increases the amount of oil released (Priftis et al., 2012). It is interesting to observe that the microcapsules were not destroyed, though they exhibited irregular sinuous shapes in the 1.5 NaCl medium (Figure 1 A). This indicated wall wakening under conditions of high salt content. Such an observation is not made in the media with lower concentrations of NaCl.

The effects of pH can be observed in Figure 2. The maximum release rates were observed when the microcapsules were immersed in the medium of pH 4.8. High release values under the predetermined times (78 % in 7 hours) were observed under these conditions. Lower release rates were observed in media of pH 4.2 (59 % in 7 hours). Intermediate rates were observed in media of pH 3.8. The medium with pH 3.8 exhibited an atypical event: the microcapsules were destroyed during agitation (240–300 min; Figures 1 D1 and D2). It is important to highlight that the complex coacervation microcapsules were produced at pH 4.0. The only medium studied at a lower pH was the medium at pH 3.8.

The possible trigger mechanism may be explained based on the zeta potential values for gum Arabic and gelatin presented by Cruz et al. (2016) and Aziz et al. (2016): at pH 3.8, the difference in charge density between the wall materials is 19 mV, whereas at pH 4.0 it is 22 mV and at pH 4.8 it is 15 mV. Thus, the maximum charge difference is observed at pH 4.0 (hence, it is the optimal pH for the production of the stable microcapsules). The charge difference observed at pH 3.8 was smaller than that observed at pH 4.0. The destruction of the microcapsules could be attributed to the charge difference, prolonged stirring times, and the presence of salt in the medium. The minimum difference was observed at pH 4.8, resulting in the weakening of the complex and increased release values. However, the microcapsules were not destroyed under these conditions.

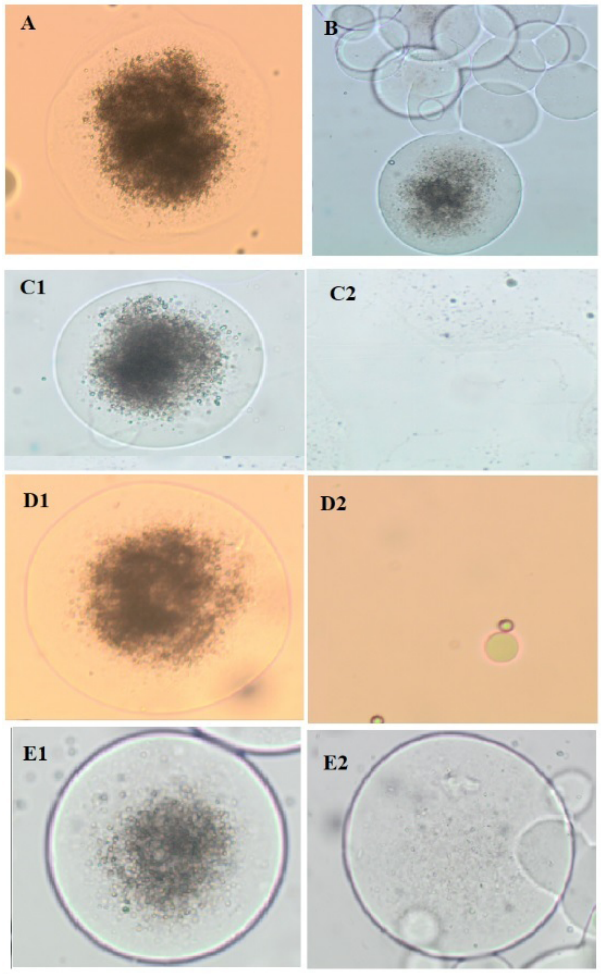

Figure 1: Microscopic images of the microcapsules in contact with different aqueous media: (A) 1.5 M ionic strength; (B) 4 ºC after agglomeration; (C1 and C2) 30°C before and after disintegration, respectively; (D1 and D2) pH 3.8 before and after disintegration, respectively; (E1 and E2) Oil released thorough microcapsules, after 1 and 7 hours, respectively.

High OEO release values were observed when the temperatures were at 4 and 30 ºC. The minimum release was observed at room temperature. Although the microcapsules were stored at 30 °C, the release was not observed after 3 h (Figures 1 C1 and C2). Such observations were not made for capsules stored at 4 and 25 °C. Based on these results, it was hypothesized that the temperature decrease results in the higher stability of the microcapsules and increased OEO release rate (a steady profile with a clear increasing behavior). The increase in temperature also resulted in the chaotic behavior at 25 ºC and the disintegration of the microcapsules at 30 ºC (attributable to prolonged exposure to high-temperature conditions). In addition, it is important to highlight the unique event that took place at 4 ºC following prolonged treatment periods (6 and 7 h). Under these conditions, the microcapsules started to agglomerate (Figure 1B). This reveals that besides affecting the liberation rates, the decrease in temperature can also influence the spatial distribution of the capsules. Priftis et al. (2012) reported that the decrease in temperature could be related to an intensification in the polymer interactions, causing the microcapsules to approach each other. This result agreed well with the results obtained by us.

Mathematical modeling of the release profiles

Following the experimental determination of the OEO release profiles, selected mathematical models (Zero Order, First Order, Higuchi, and Peppas) (Table 2) were adjusted to the average data of each aqueous medium. The Zero and First Order models did not present suitable fitting values and large errors (E) between 60 and 90% were obtained. The results obtained using the Higuchi and Peppas models are presented in Table 4. Comparison of the statistical parameters led to the conclusion that the Peppas model was the most appropriate to describe OEO release from the complex coacervated microcapsules. The highest determination coefficient ($R^2 > 0.86$) and lowest residual error ($E < 6\%$) was obtained using this model. The corresponding fitting curves and parameters are shown in Figure 2 and Table 5.

Peppas model is based on two parameters: k, a geometrical and structural constant, and n, the release exponent, which is directly related to the release mechanism. In the case of spherical microcapsules (considered in the present study), for $n \leq 0.43$, the dominating mechanism can be explained by Fickian diffusion (Rodríguez et al., 2016; Siepmann; Peppas, 2001). This was also validated by the microscopic images showing oil release through the walls of the undamaged microcapsule (Figures 1 E1 and E2). Therefore, OEO diffuses through the capsule walls (due to concentration gradient) into the aqueous medium in which the microcapsules are immersed.

There were no significant differences between the k values, as they were in the range of 23 – 33 min^{-n}. It is noteworthy that the samples at pH 3.8 and T30 (destroyed due to the extreme conditions of the aqueous solutions) presented low k values. The maximum n value was recorded for the T30 sample. Thus, it can be inferred that the more intense the physicochemical parameters of the medium are, the more pronounced influence they shall exert on the mechanism that affects the release kinetics.

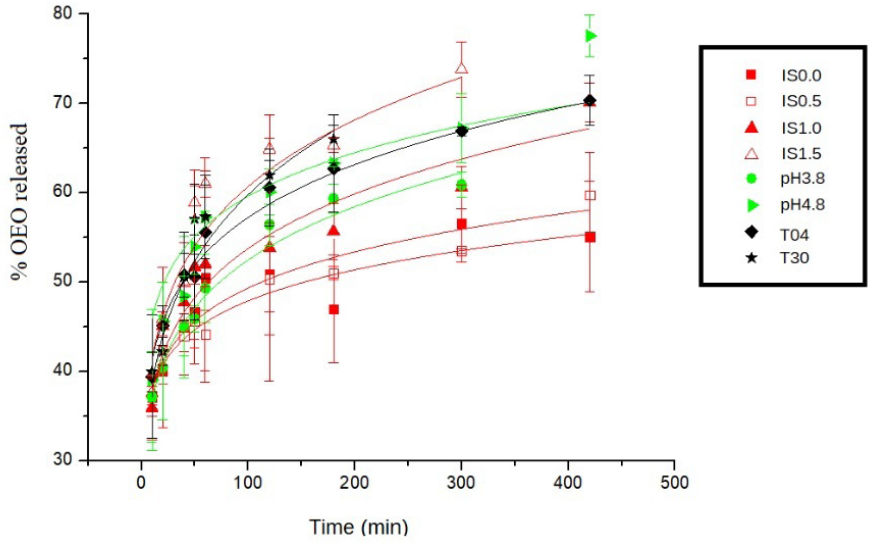

Figure 2: OEO release profiles in different aqueous media and solid curves representing the fitted Peppas model.

Table 4: Determination coefficient (R^2) and relative mean residual error (E) values.

Samples	Higuchi		Peppas	
	R^2	*E* (%)	R^2	*E* (%)
IS0.0	0.879	39.5	0.896	3.9
IS0.5	0.761	38.8	0.989	2.5
IS1.0	0.627	35.4	0.935	4.8
IS1.5	0.693	29.9	0.934	4.9
pH3.8	0.625	33.6	0.944	2.2
pH4.8	0.661	32.1	0.868	5.9
T04	0.459	37.2	0.995	2.1
T30	0.809	26.2	0.967	3.4

Unagolla e Jayasuriya (2018) studied the release kinetics of encapsulated vancomycin using mathematical models such as the Peppas power-law model. The maximum *k* values reported by them could be correlated to the higher liberation rate. We also observed that the most stable medium with the maximum release rate (IS=1.0, pH=4.8, and T=04) also presented the maximum *k* values. The medium IS0.0 exhibited a high *k* value of 32.89 ± 5.05. However, due to its instability, a high standard deviation between the replicates was observed. This could explain the unusually high *k* value observed in a low liberation rate medium.

Table 5: Fitting parameters for the Peppas model (Equation 6) adjusted to OEO release in different aqueous media.

Samples	pH	Temperature (°C)	Ionic Strength (mol.L^{-1})	Peppas model parameters	
				k (min^{-n})	n
IS0.0	4.2	25	0	32.89 ± 5.05^{abc}*	0.088 ± 0.029^{b}
IS0.5			0.5	30.61 ± 0.48^{be}	0.103 ± 0.002^{b}
IS1.0			1	33.16 ± 0.34^{ad}	0.100 ± 0.013^{b}
IS1.5			1.5	27.27 ± 1.35^{bc}	0.154 ± 0.028^{ab}
pH3.8	3.8	25	0.5	23.30 ± 2.23^{c}	0.141 ± 0.053^{ab}
pH4.8	4.8			32.21 ± 5.52^{cde}	0.163 ± 0.041^{ab}
T04	4.2	4	0.5	30.42 ± 1.33^{bd}	0.149 ± 0.027^{ab}
T30		30		24.13 ± 1.65^{c}	0.185 ± 0.030^{a}

*Values in the same column with different letters are significantly different ($p < 0.05$).

Empirical model for predicting OEO release and experimental validation

The results for the response variable, i.e., the final percentage of oil release, corresponding to a CFD (Table 3), were analyzed using the regression analysis method and the obtained empirical model (Equation 8). The corresponding coefficients (Table 6) were used for the construction of the response surfaces presented in Figure 3.

The aim was to achieve the maximum OEO release over an extended period of time. It is possible to identify the combinations of pH, temperature, and ionic strength under which the optimum or poor extended-release of OEO can be achieved (Figure 2). Four assays were carried out to validate these results. The physicochemical properties of the aqueous medium corresponded to two different areas in the contour graphics: two assays in the optimum conditions and two assays in the poor region. The first area maintains the same goal of the modeling process, including media with high controlled oil release rates over a prolonged period. The second one involves media with low oil liberation percentage, including those conditions under which the microcapsules disintegrate, releasing oil in a short span of time. This allows fast volatilization. The selected media are presented in Table 7.

Table 6: Regression coefficients presented in Equation 8, used for predicting the final OEO % release (*y*).

Coefficients	Values
β_0	71.47
β_1	6.45
β_{11}	n.s.
β_2	-32.89
β_{22}	-33.39
β_3	4,89
β_{33}	-6.49
β_{12}	-7.21
β_{13}	n.s.
β_{23}	-4.31
Model	$Y = 71.47 + 6.45\,x_1 - 32.89\,x_2 - 33.39\,x_2^2 + 4.89\,x_3 - 6.49\,x_3^2 - 7.21\,x_1\,x_2 - 4.31\,x_2\,x_3$

*n.s. = non significant (p>0.05). ** *y* = % release; x_1 = pH; x_2 = temperature; x_3 = ionic strength.

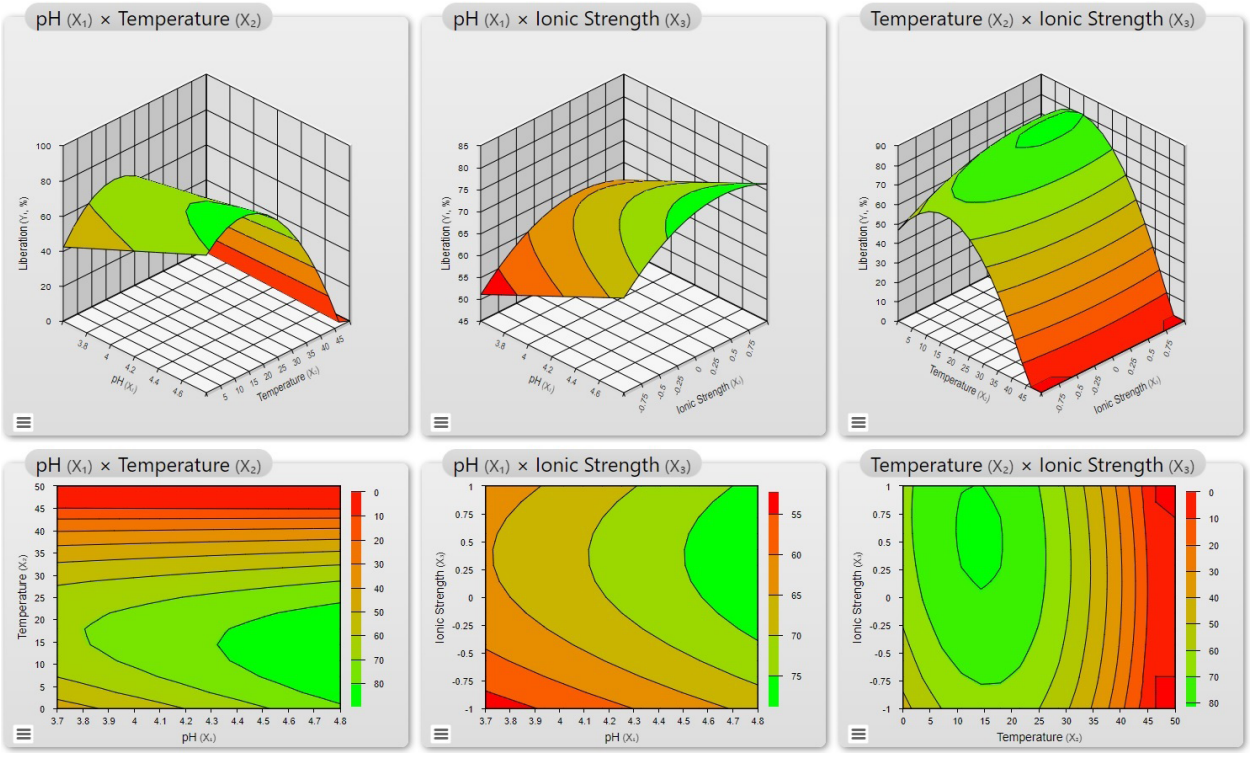

Figure 3: Response surfaces and contour graphics representing the effects of pH, temperature, and ionic strength on the final OEO % release.

Table 7: Selected assays in the optimized (OM) and poor (PM) conditions for maximizing OEO release over an extended period.

Medium	Ionic Strength (M)	pH	Temperature (ºC)
OM1	1.20	4.6	25
OM2	1.35	4.3	4
PM1	0.40	4.3	4
PM2	0.60	4.0	25

In the new set of experimental assays, the characteristic burst release phenomenon was also observed. The OEO release was in the range of 32.4% to 37.54% during the first 10 min for the four samples. This indicated that the phenomenon was independent of the medium properties, confirming that it is directly connected to the amount of oil present near the surface of the microcapsule.

Following the initial burst release, the OM1 assay showed a rapid increase in the liberation rates (Figure 5). The rate was 56% in the first hour and increased to 65% after 3 hours. After 7 h, the rate was 78%. It is important to compare the last experimental result with the result predicted by the model, which is 76.21 ± 2.26 %. The model showed good accuracy.

Similar results were obtained following the OM2 test. The percentages of release were approximately 50%, 67%, and 76% after 1, 3, and 7 h, respectively (Figure 5). The model predicted a total liberation percentage of 74.23 ± 4.21% after 7 h of the experiment. The result was in good agreement with the experimental result.

In contrast, PM1 showed lower release rates (as predicted by the model), reaching 51% after 1 h, 58% after 3 h, and 63% in 7 h (Figure 5). The model estimated a value of 58.11 ± 4.73 %.

Low PM2 liberation values were also obtained. The release was 50%, 56%, and approximately 59% after 1, 3, and 7 h, respectively (Figure 5). The predicted oil liberation value was 59.53 ± 3.04%.

The microscopic images in Figure 4 illustrate the oil releasing process over time. The dark regions indicate OEO. It is possible to observe high liberation percentages in OM1 and OM2. PM1 and PM2 demonstrate significantly lower rates, represented by the darkened areas. It is noteworthy that all microcapsules remained intact during the controlled liberation of the encapsulated bioactive compounds.

Further experiments were conducted following the preliminary tests. The Peppas model could be used to obtain a higher coefficient of determination (R^2) and smaller errors (E) (Table 8). Significant differences between k and n values were recorded while comparing the OM1 and OM2 assays with PM1 and PM2. The n values of the optimized media were approximately twice the values obtained for the poor area, reflecting the higher slopes of the release profiles. The k values for the optimized area were smaller. The small values could be attributed to the good regularity in liberation. Finally, the dominant liberation mechanism was confirmed to follow the Fickian diffusion method.

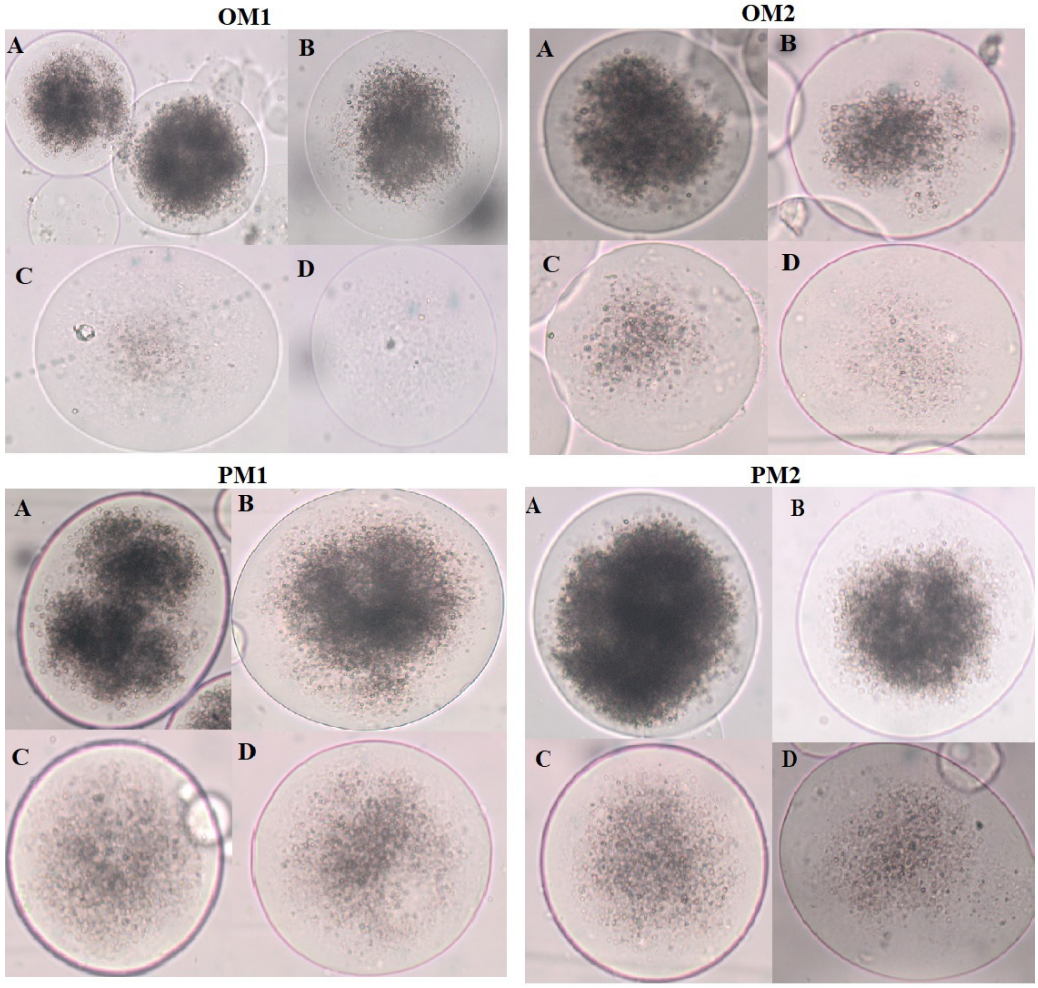

Figure 4: Microscopic images of the microcapsules in contact with different aqueous media at different times: (A) 10 min, (B) 60 min, (C) 240 min, and (D) 420 min.

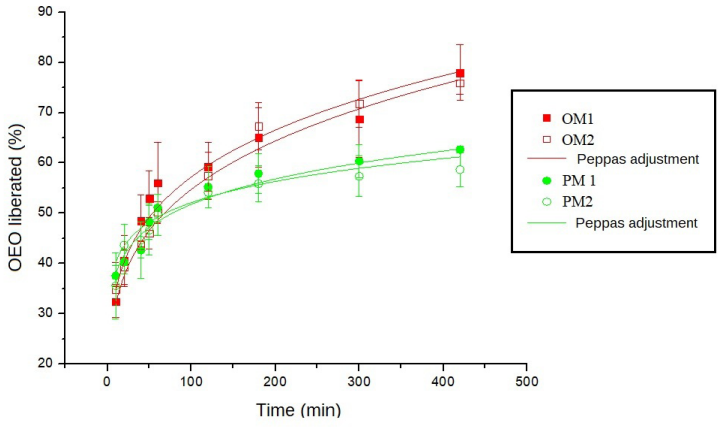

Figure 5: OEO release profiles for validation assays in different aqueous media; solid curves represent the fitted Peppas model.

Table 8: Fitting parameters for the Peppas model for the liberation curves.

Medium	Peppas adjustment		Model parameters	
	R^2	E (%)	k (min^{-n})	n
OM1	0.97	3.71	21.02 ± 1.30[b]	0.22 ± 0.013[b]
OM2	0.99	3.06	18.66 ± 0.69[b]	0.23 ± 0.008[b]
PM1	0.99	3.09	27.88 ± 0.98[a]	0.13 ± 0.006[a]
PM2	0.89	3.61	31.11 ± 2.14[a]	0.11 ± 0.014[a]

*Values in the same column with different letters are significantly different ($p < 0.05$).

CONCLUSIONS

It was observed that the physicochemical parameters influenced the OEO liberation kinetics. Increasing ionic strength resulted in increased OEO release over time as the presence of a large number of ions in the solution weakens the electrostatic interactions that form the basis of the integrity of the encapsulating matrix. The zeta potential difference between the biopolymers that constitute the encapsulating matrix influences encapsulation. Media with pH 4.8 showed higher release percentages. Under conditions of more acidic pH, the microcapsules disintegrated. A decrease in the temperature results in higher release stability and particle agglomeration. The microcapsules disintegrated under conditions of high temperature. CFD resulted in a model capable of predicting the best aqueous medium parameters to achieve controlled OEO liberation rates. The model could be validated by conducting additional experimental assays under conditions of maximum and minimum release. Conditions for maximum oil liberation could be determined. The mathematical model that best described the release profiles was the Peppas model, and the fitting results showed that the dominating mass transfer mechanism for OEO release could be explained by Fickian diffusion.

ACKNOWLEDGMENT

This work was supported by FAPESP (grant number 2019/05085-0) and CNPq (grant number 423066/2016-1). The authors also thank Laszlo Aromatologia (Belo Horizonte, Brazil) for helping with the experiments involving OEO.

AUTHOR CONTRIBUTION

Conceptual Idea: Nicoletti, V. R.; Gallo, T. C. B.; Muneratto, V. M. Methodology design: Nicoletti, V. R.; Gallo, T. C. B.; Muneratto, V. M. Data collection: Muneratto, V. M.; Data analysis and interpretation: Nicoletti, V. R. Muneratto, V. M. and Writing and editing: Nicoletti, V. R.; Gallo, T. C. B. Muneratto, V.M.

REFERENCES

ASBAHANI, A. E. et al. Essential oils: From extraction to encapsulation. International Journal of Pharmaceutics, 483(1-2):220-243, 2015.

AZIZ, F. R. A. et al. Microencapsulation of citronella oil by complex coacervation using chitosangelatin (b) system: Operating design, preparation and characterization. MATEC Web of Conferences, 69:04002, 2016.

BAGAMBOULA, C. F. et al. Inhibitory effects of spices and herbs toward Shigella sonnei and S. flexneri. Food Microbiology, 21(1):33-42, 2001.

BAKRY, A. M. et al. Microencapsulation of oils: A comprehensive review of benefits, techniques, and applications. Comprehensive Reviews in Food Science and Food Safety, 15(1):143-182. 2016.

BASTOS, L. P. H. et al. Complex coacervates of beta-lactoglobulin/sodium alginate for the microencapsulation of black pepper (*Piper nigrum* L.) essential oil: Simulated gastrointestinal conditions and modeling release kinetics. International Journal of Biological Macromolecules, 160:861-870, 2020.

BHARGAVA, K. et al. Application of an oregano oil nanoemulsion to the control of foodborne bacteria on fresh lettuce. Food Microbiology, 47:69-73, 2015.

BURT, S. Essential oils: Their antibacterial properties and potential applications in foods - A review. International Journal of Food Microbiology, 94(3):223-253, 2004.

BURT, S. et al. Inhibition of *Salmonella enterica* serotype enteritidis on agar and raw chicken by carvacrol vapor. International Journal of Food Microbiology, 119(3):346-350, 2007.

CALO, J. R. et al. Essential oils as antimicrobials in food systems - A review. Food Control, 54:111-119, 2015.

CASTRO, M. A. A. et al. β-Cyclodextrin inclusion complexes containing clove (*Eugenia caryophyllata*) and Mexican oregano (*Lippia berlandieri*) essential oils: Preparation, physicochemical and antimicrobial characterization. Food Packaging and Shelf Life, 14:91-101, 2017.

CRUZ, R. C. et al. Electrical impedance spectroscopy for monitoring the gum Arabic-chitosan complexation process in bulk solution. Colloids and Surfaces A-Physicochemical and Engineering Aspects, 495:125-135, 2016.

DE PRISCO, A.; MAURIELLO, G. Probiocation of foods: A focus on microencapsulation tool. Trends in Food Science & Technology, 48:27-39, 2016.

DIAS, M. I. et al. Microencapsulation of bioactives for food applications. Food & Function, 6(4):1035-1052, 2015.

FRIEDMAN, M. Chemistry and multibeneficial bioactivities of carvacrol (4-Isopropyl-2- methylphenol), a component of essential oils produced by aromatic plants and spices. Journal of Agricultural and Food Chemistry, 62(31):7652-7570, 2014.

FRIEDMAN, M. et al. Recipes for antimicrobial wine marinades against *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*. Journal of Food Science, 76(6):M207-M213, 2007.

GALLO, T. C. B. et al. Oregano essential oil encapsulated in alginate beads: Release kinetics as affected by electrostatic interaction with whey proteins and freeze-drying. Journal of Food Processing and Preservation, 44(12):e14947, 2020.

HIJO, A. C. T. et al. Physical and thermal properties of oregano (*Origanum vulgare* L.) essential oil microparticles. Journal of Food Process Engineering, 38(1):1-10, 2014.

HOSSEINI, S. F. et al. Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: Preparation, characterization and in vitro release study. Carbohydrate Polymers, 95(1):50-56, 2013.

JIMÉNEZ-ALVARADO, R. et al. Ferrous bisglycinate content and release in W1/O/W2 multiple emulsions stabilized by protein-polysaccharide complexes. Food Hydrocolloids, 23(8):2425-2433, 2009.

MARFIL, P. H. M. et al. Production and characterization of palm oil microcapsules obtained by complex coacervation in gelatin/gum Arabic. Journal of Food Process Engineering, 41(4):e12673, 2018.

MARTINS, I. M. et al. Microencapsulation of thyme oil by coacervation. Journal of Microencapsulation, 26(8):667-675, 2009.

MARTINS, I. M. et al. Polylactide-based thyme oil microcapsules production: Evaluation of surfactants. Industrial & Engineering Chemistry Research, 50(2):898-904, 2011a.

MARTINS, I. M. et al. Release of thyme oil from polylactide microcapsules. Industrial & Engineering Chemistry Research, 50(24):13752-13761, 2011b.

MARTINS, I. M. et al. Release study of thymol and p-cymene from polylactide microcapsules. Industrial & Engineering Chemistry Research, 51(35):11565-11571, 2012.

PRATA, A. S.; GROSSO, C. R. F. Influence of the oil phase on the microencapsulation by complex coacervation. Journal of the American Oil Chemists Society, 92(7):1063-1072, 2015.

PRIFTIS, D.; LAUGEL, N.; TIRREL, M. Thermodynamic characterization of polypeptide complex coacervation. Langmuir, 28(45):15947-15957, 2012.

RODRÍGUEZ, J. et al. Current encapsulation strategies for bioactive oils: From alimentary to pharmaceutical perspectives. Food Research International, 83:96-101, 2016.

RODRIGUES, M. I.; IEMMA, A. F. Planejamento de experimentos e otimização de processos. 3.ed. Campinas: Editora Cárita, 2014. 358p.

RUNGWASANTISUK, A.; RAIBHU, S. Application of encapsulating lavender essential oil in gelatin/gum-arabic complex coacervate and varnish screen-printing in making fragrant gift-wrapping paper. Progress in Organic Coatings, 149:105924, 2020.

SCHMITT, C. et al. Structure and technofunctional properties of protein-polysaccharide complexes: A review. Critical reviews in food science and nutrition, 38(8):689-753, 1998.

SHARKAWY, A. et al. Aroma-loaded microcapsules with antibacterial activity for eco-friendly textile application: Synthesis, characterization, release and green grafting. Industrial & Engineering Chemistry Research, 56(19):5516-5526, 2017.

SIEPMANN, J.; PEPPAS, N. A. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Advanced Drug Delivery Reviews, 48(2-3):139-157, 2001.

UNAGOLLA, J. M.; JAYASURIYA, A. C. Drug transport mechanism and in vitro release kinetics of vancomycin encapsulated chitosan-alginate polyelectrolyte microparticles as a controlled drug delivery system. European Journal of Pharmaceutical Sciences, 114:199-209, 2018.