In vitro evaluation of 5-Fluorouracil release by microspheres based on chitosan / Montmorillonite

Avaliação in vitro da libertação de 5-Fluorouracil por microesferas à base de quitosano / Montmorilonite

DOI:10.34117/bjdv6n12-565

Recebimento dos originais:17/11/2020
Aceitação para publicação:22/12/2020

Hanniman Denizard Cosme Barbosa
Doutorado
Instituição de atuação atual : Instituto Federal de Educação, Ciência e Tecnologia da Paraíba, IFPB
Endereço : Avenida Cabo Branco, 3106, apartamento 04, João Pessoa/PB
E-mail : denizard_jpa@hotmail.com

Bárbara Fernanda Figueiredo dos Santos
Mestrado
Instituição de atuação atual : Universidade Federal de Campina Grande, UFCG.
Endereço :Department of Materials Engineering, Federal University of Campina Grande, Campina Grande PB 58429-900, Brazil.
E-mail : nandasantos_babi@hotmail.com

Albaniza Alves Tavares
Doutorado
Instituição de atuação atual : Universidade Federal de Campina Grande, UFCG.
Endereço: Department of Materials Engineering, Federal University of Campina Grande, Campina Grande PB 58429-900, Brazil.
E-mail : albaniza.alves@gmail.com

Magno Duran Silva de Andrade
Graduação em Direito
Instituição de atuação atual: UFPB
Endereço: Avenida Sapé, 540, Manaíra, João Pessoa/PB; CEP 58038381
E-mail: magnoduran@gmail.com

Raony de Melo Ferreira
Especialista em Direito
Instituição de atuação atual: UFPB
Endereço: Rua Eliane Coutinho de Oliveira 14, Brisamar João Pessoa/PB, CEP: 58033-340
E-mail: raonydemelo@gmail.com

João Marçal Medeiros de Sousa
Graduação em curso
Instituição de atuação atual: UFPB
Endereço: R. Efigênio Barbosa da Silva, 206, apt. 103, CEP 58052-310, Jardim Cidade Universitária - João Pessoa (PB)
E-mail: joaomarcal489@gmail.com

Clayton Leite de Moura
Acadêmico de medicina desde 2016
Instituição de atuação atual: UFPB
Endereço completo: Rua Visconde de Taunay, 232, Diadema (SP), CEP: 09910-530
E-mail: claytonleitedemoura@gmail.com

Suédina Maria de Lima Silva
Pós-Doutorado
Instituição de atuação atual : Universidade Federal de Campina Grande, UFCG
Endereço: Department of Materials Engineering, Federal University of Campina Grande, Campina Grande PB 58429-900, Brazil.
E-mail : suedina.silva@ufcg.edu.br

ABSTRACT
5-fluorouracil (5-FU) is one of the most widely used chemotherapeutic compounds for cancer treatment and its rapid metabolization and non-uniform oral absorption are the limitations for its use as an oral chemotherapy. Therefore, this study was performed to evaluate the influence of a 5-fluorouracil (5-FU) nanocomposite microspheres with different diameters to test on a controlled release system in the gastrointestinal environment. 5-FU was incorporated into the chitosan/montmorillonite nanocomposite microspheres through the intercalation method. The microspheres containing the 5-FU were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and optical microscopy (OM). *In vitro* release fractions at different pHs (1.2, 7.4 and 10.0) were investigated by UV-vis spectroscopy. The release profile of 5-FU for the systems studied was adjusted through the Korsmeyer-Peppas kinetic model, and the results suggested that the mechanism of controlled release at pH 7.4 and 10 occurs by diffusion. In addition, the 5-FU microspheres diameter and roughness directly interfere with the release rate and the released fraction, since the F1 / F2 systems showed a difference in the released fraction of 5-FU of 7.97% and for the systems F3 / F4 the difference was 2.86%. The prepared F1, F2, F3 and F4 systems are suitable for delivery of 5-FU to the gastrointestinal environment in a controlled manner.

Keywords: Chitosan, 5-Fluorouracil, microspheres, controlled release.

RESUMO
O 5-fluorouracil (5-FU) é um dos compostos quimioterápicos mais utilizados para o tratamento do cancro e a sua rápida metabolização e absorção oral não uniforme são as limitações para a sua utilização como quimioterapia oral. Portanto, este estudo foi realizado para avaliar a influência de uma microesfera nanocomposta de 5-fluorouracil (5-FU) com diferentes diâmetros para testar num sistema de libertação controlada no ambiente gastrointestinal. O 5-FU foi incorporado nas microesferas de nanocompósitos quitosano/montmorillonite através do método de intercalação. As microesferas contendo o 5-FU caracterizavam-se pela difracção de raios X (XRD), microscopia electrónica de varrimento (SEM) e microscopia óptica (OM). As fracções de libertação in vitro a diferentes pHs (1,2, 7,4 e 10,0) foram investigadas por espectroscopia UV-vis. O perfil de libertação de 5-FU para os sistemas estudados foi ajustado através do modelo cinético de Korsmeyer-Peppas, e os resultados sugeriram que o mecanismo de libertação controlada a pH 7,4 e 10 ocorre por difusão. Além disso, o diâmetro e rugosidade das microesferas de 5-FU interferem directamente na taxa de libertação e na fracção libertada, uma vez que os sistemas F1 / F2 mostraram uma diferença na fracção libertada de 5-FU de 7,97% e para os sistemas F3 / F4 a diferença foi de 2,86%. Os sistemas F1, F2, F3 e F4 preparados são adequados para a entrega de 5-FU ao ambiente gastrointestinal de forma controlada.

Palavras-chave: Chitosano, 5-Fluorouracil, microesferas, libertação controlada.

1 INTRODUCTION

5-fluorouracil (5-FU) is one of the most widely used chemotherapeutic compound for cancer treatment, specifically for breast cancer (Monteiro et al., 2013, Jahani et al., 2017), gastric cancer (Kocar *et al.*, 2016; Xu e Tang, 2017; Zhang *et al.*, 2017), pancreatic (Kamisawa *et al.*, 2016; De Mestier *et al.*, 2017), colon-rectal (Dodov *et al.*, 2009; Jaferian *et al.*, 2016; Vargo *et al.*, 2016; Chandran *et al.*, 2017; Pretel *et al.*, 2017) and brain tumours (Lesniak e Brem, 2004; Sofis *et al.*, 2017; Sun *et al.*, 2017). One of the limitations of this chemotherapeutic is its rapid metabolization resulting in a biological half-life of only 10-20 min (Rejinold et al., 2011) and a non-uniform oral absorption (Joshi et al., 2009). Therefore, to obtain a therapeutic concentration of this drug in the blood, the amount of the drug is often increased and administration is given intravenously continuously, which increases the toxic side effects of 5-FU (Azhar and Olad, 2014). This form of administration usually occurs in hospitals due to associated technical aspects, and oral chemotherapy is an important step towards a more humanized treatment of patients: "Chemotherapy at home", which would bring comfort to the patient, since the treatment in hospitals usually leads to detachment from home (Feng et al., 2009, Tanday, 2014, Morris and Marshall-Lucette, 2017).

To minimize the toxic side effects of 5-FU, the encapsulation of this drug in polymeric matrices such as alginate (Arıca et al., 2002; Nagarwal, Ramesh et al., 2012; Lakkakula et al., 2017), polycaprolactone (Martini *et al.*, 1995; Salerno *et al.*, 2015; Salerno *et al.*, 2017), gelatin (Narayani e Rao, 1996; Huang *et al.*, 2009; Rajan *et al.*, 2013) and especially chitosan (Denkbaş *et al.*, 2000; Yan *et al.*, 2006; Zheng, Y. *et al.*, 2007; Arias, 2008; Zhang, 2008; Li *et al.*, 2009; Lin e Fu, 2009; Zhu *et al.*, 2009; Huang *et al.*, 2010; Ganguly *et al.*, 2011; Li *et al.*, 2011; Rejinold, N. S. *et al.*, 2011; Yan *et al.*, 2011; Anitha *et al.*, 2012; Cheng *et al.*, 2012; Lam *et al.*, 2012; Nagarwal, R. C. *et al.*, 2012; Tığlı Aydın e Pulat, 2012; Li *et al.*, 2013; Puga *et al.*, 2013; Anitha *et al.*, 2014; Fu *et al.*, 2014; Xu *et al.*, 2014; E.A.K *et al.*, 2016; Khoee *et al.*, 2017; M *et al.*, 2017) has been proposed.

The use of chitosan to encapsulate the 5-FU drug is interesting because it comes from an abundant and low-cost renewable resource, and in addition is biocompatible, biodegradable, possess antioxidant, antimicrobial, anti-inflammatory and healing capacities and when in contact with human cells do not cause adverse reactions. Moreover, it can be degraded by enzymes widely found in the human body, generating non-toxic residues (Bagheri-Khoulenjani et al., 2009; Dash et al., 2011). However, in comparison with protein-based drugs (collagen, albumin, gelatin), the controlled release of anticancer drugs such as 5-FU, with low molecular mass, still presents certain limitations when encapsulated in polysaccharides such as chitosan. Due to the high degree of swelling of polysaccharides in general, followed by erosion and dissolution in aqueous medium and the swelling through hydration, with formation of a polymer network connected transversely (without dissolution), affects the release

time of drugs of low molar mass (Gupta et al., 2002, Aulton and Ortega, 2008, Yu et al., 2008, Bizerra e Silva, 2016). Therefore, the search for an efficient encapsulation to allow a slow release rate of 5-FU has motivated studies involving the preparation of chitosan / clay hybrids (Kevadiya et al., 2012; Azhar and Olad, 2014).

Montmorillonite (MMT) has been investigated for use in controlled release of 5-FU (Lin et al., 2002; Azhar and Olad, 2014), ibuprofen (Zheng, J. et al., 2007; Dziadkowiec et al., 2017 (Kevadiya et al., 2014), insulin (Kamari et al., 2017), ciprofloxacin (Kevadiya et al., 2014), olanzapine (Oliveira et al., 2017), gentamicin (Rapacz-Kmita *et al.*, 2017), venlafaxine (Jain and Datta, 2016) and irinotecan (Iliescu et al., 2014). All of these studies aim to delay the release of drugs into the gastric environment so that they can reach the intestinal mucosa and prolong the effects of the drug in the body.

Although 5-FU has been investigated for use in chitosan and montmorillonite systems, the preparation of microspheres with different diameters has not been investigated. Because the number of studies addressing the 5-FU encapsulation in the chitosan/clay hybrid is limited (Kevadiya et al., 2012, Azhar and Olad, 2014) and knowing the various benefits that can be achieved for controlling the release of 5-FU associating chitosan with montmorillonite (Yuan et al., 2010, Ennajih et al., 2012, Salcedo et al., 2012, Ahsan et al., 2017 and Jayrajsinh et al., 2017), the present study investigated the release of 5-FU *in vitro,* starting from microspheres with different chitosan / montmorillonite diameters under phosphate-buffered saline (PBS) solutions at pH 1.2, 7.4 and 10. The Korsmeyer-Peppas kinetic model was applied to elucidate the mechanism of drug release.

2 EXPERIMENTAL

2.1 MATERIALS

Chitosan, supplied as a powder by Polymar / Brazil, presented a deacetylation degree of 93.3%. Commercial sodium montmorillonite Cloisite® Na^+ (CL) was supplied as a powder, produced by Southem Clay Products, Texas / USA and purchased from Buntech, São Paulo / BR. This clay presents basal interplanar distance (d001) of 1.17 nm and density of 2.86 g / cm^3, according to technical standards. Glacial acetic acid (99.9% purity) and sodium hydroxide (99.9% purity), were both purchased from Vetec Química Fina. Sodium acetate trihydrate (99.5% purity) was supplied by Chemical Dynamics. Active substance 5-Fluorouracil (5-FU), molecular formula $C_4H_3FN_2O_2$, molar mass 130.1 $g.mol^{-1}$ and purity ≥ 99%, was supplied by Sigma-Aldrich and phosphate buffered saline (PBS) pH 1.2, 7.4 and 10 was acquired from Vetec Química fina.

2.2 METHODS

2.2.1 Encapsulation of 5-Fluorouracil

To obtain the 5-FU/MMT/chitosan microspheres, the solution of 4% (m/v) chitosan in 5% (v/v) acetic acid had the pH adjusted to 4.9 with addition of a hydroxide solution of sodium to 1 molar, under magnetic stirring at room temperature. Then, a dispersion of MMT was prepared at a concentration of 10% water with a stirrer / heater under a rotation of 400 rpm and 50 ± 0.5 °C for 30 min. This dispersion was added to the chitosan solution heated to 50 ± 0.5 ° C. The chitosan / MMT mixture was kept under mechanical stirring at 50 ± 0.5 ° C for 4 hours and 30 minutes.

For incorporation of the drug, 5-FU (150 mg) was dissolved in 18 mL of chitosan / MMT mixture, obtaining a mass ratio of 5-FU / MMT / chitosan of 1: 2: 4 as suggested by Huang et al. (2010). This mixture was used to obtain the microspheres by dripping in sodium hydroxide coagulant solution. For the dripping step, sodium acetate (600 mg) was added to the 5-FU / MMT / chitosan mixture obtaining a mass ratio of 1: 2: 4: 4 (5-FU / MMT / chitosan / acetate). This dispersion was dripped in 8% sodium hydroxide coagulant solution and kept under gentle stirring. For the incorporation of 5-FU into the chitosan-only microspheres, 5-FU (150 mg) was dissolved in 15 mL of chitosan solution (4%) with the addition of sodium acetate (600 mg), obtaining a 5-FU/chitosan/acetate ratio of 1:4:4. This solution was also dripped in 8% sodium hydroxide coagulant solution. The coagulant solution containing the microspheres was filtered on qualitative filter paper with pore opening of 14 μm, washed with distilled water to pH 7.5 and dried at 50 °C for 24 h. All microspheres were prepared with equipments developed in our laboratory, based on the work of Prado et al. (2010) and Nascimento et al. (2008). The nomenclature and composition of the microspheres are shown in Table 1.

Table 1: Composition of formulations and characterization of microspheres containing 5-FU.

Microspheres	5-FU (mg)	MMT (mg)	Chitosan (mg)	Acetate (mg)	EE (%)
F1	150	0	600	600	37.29
F2	150	0	600	600	29.37
F3	150	300	600	600	74.99
F4	150	300	600	600	64.96

5-FU – 5-Fluorouracil; MMT – Montmorilonita; EE – Efficiency of encapsulation

2.2.2 Scanning Electron Microscopy (SEM)

The morphology of the chitosan and chitosan/clay microspheres prepared in the absence and presence of 5-FU was observed in a scanning electron microscope (SEM). This analysis was conducted in a Shimadzu scanning electron microscope model SSY-550, 6000x, 30 KV, with gold coating.

2.2.3 Efficiency of encapsulation (EE)

The microspheres encapsulation efficiency (EE) with 5-FU was determined by UV-vis uptake of the microspheres wash water. To determine the amount of 5-FU released in the microspheres wash water, a calibration curve for the 5-FU was performed employing seven solutions of different concentrations, and the respective absorbance values were measured at λ_{max} 266 nm. The 5-FU EE in the systems was calculated according to Equation (1) (Papadimitriou et al., 2008):

$$EE\ (\%) = \frac{W_t - W_f}{W_t} * 100 \quad (1)$$

Where Wt represents the mass of 5-FU present in the microspheres; Wf represents the mass of 5-FU released during the washing process of the microspheres.

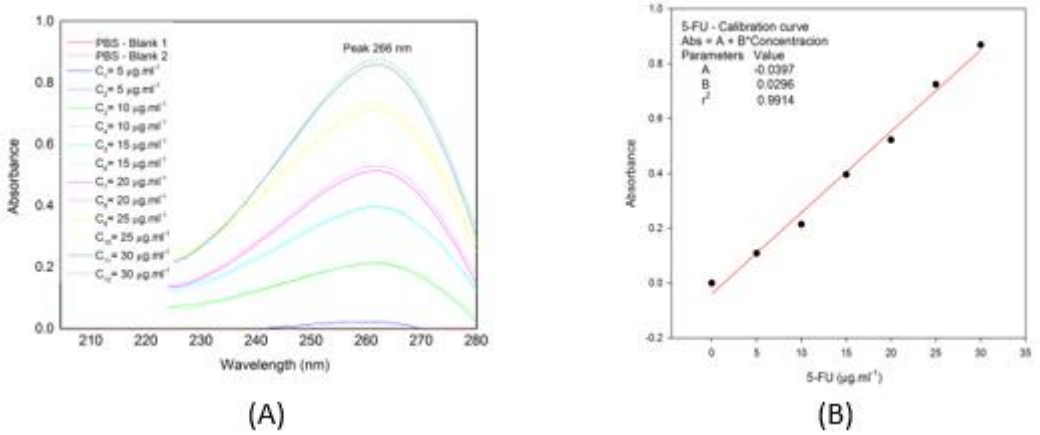
All measurements were performed in triplicate and the values were calculated according to Equations (2) and (3):

$$W_t = \frac{\text{Mass of washed microspheres}}{\text{Mass ratio}_{(5-FU)}} \quad (2)$$

$$W_f = V_{\text{wash up}} * C \quad (3)$$

The wash up volume ($V_{\text{wash up}}$) of the microspheres was 2200 mL and the concentration (C) was obtained through the UV-Vis calibration curve. The calibration curve was obtained from 7 concentration levels of 5-FU, equidistant and distributed between 0-30 $\mu g.mL^{-1}$. The absorption and calibration curves are represented below

Figure 1: UV-vis absorption curves of 5-FU in wash water (A) and 5-FU calibration curve in wash water (B).



2.2.4 In vitro release study

The in vitro 5-FU release study was performed using 500 mg of the microspheres (F1, F2, F3 and F4) and 50 mL of PBS solution at different pH (1.2, 7.4 and 10) at 37 ± 0.5°C under constant

stirring at 100 rpm. After weighing, each sample was placed in a capped Erlenmeyer and the PBS solution was added to the different pHs: 1.2, 7.4 and 10. The 5-FU concentration released in the medium was determined from the absorbance spectra collected on a UV-vis spectrophotometer (Perkin Elmer model Lambda 35). To determine the concentration of released drug, a calibration curve was constructed for 5-FU employing six solutions of different concentrations and in triplicate. The λ_{max} values for 5-FU in the different buffer solutions (pH = 1.2, 7.4 and 10) were determined using the UV-vis spectrophotometer. The absorbance, λ_{max} and linear correlation values are shown in Table 2.

Table 2: 5-FU Absorbances (Abs) in PBS pH 1.2, 7.4 and 10.

5-FU ($\mu g.mL^{-1}$)	Abs 266 nm pH 1.2	Abs 266 nm pH 7.4	Abs 266 nm pH 10
PBS	-0.0027	**-0.0061**	0.0040
10	0.3820	**0.4613**	0.4716
20	0.8004	**0.9593**	0.9484
30	1.2372	**1.4599**	1.3911
40	1.6616	**2.0200**	1.7822
50	2.1644	**2.4639**	2.0582
r^2	0.9984	**0.9992**	0.9926

The concentration over time was determined by withdrawing 5 mL aliquots of the PBS release solution, in the range of 15 min to 1440 min (0.25-24 h), followed by replacement of the PBS solution. The concentration of the drug contained in the PBS aliquot was obtained by averaging the absorbance from two readings and applying the 5-FU calibration curve equation at the corresponding pH. The released fraction of 5-FU was calculated using Equation (4).

$$\text{Fraction released}_{(t)} = \frac{\text{Mass released}_{(t)}}{\text{Mass of the drug}} . 100 \quad (3)$$

For simulation of gastrointestinal tract conditions, the amount of 5-FU released from the microspheres was determined by withdrawing a volume of the delivery system at different times. To simulate stomach transit conditions aliquots were withdrawn up to two hours from the PBS pH 1.2 solution, from 2 to 6 hours from the PBS solution at pH 7.4 and at pH 10 from 6 to 24 hours, simulating the gastrointestinal transit conditions (Josué et al., 2000)

2.2.5 X-ray Diffraction (XRD)

Expansion of the clay basal spacing d_{001} in the 5-FU / chitosan / MMT system was indicated by X-ray diffraction with copper K_α radiation (λ = 1.5418 Å), 40 kV, 30 mA, scanning 2θ from 2 to 70 ° and scanning speed of 1°/ min.

2.2.6 Optical Microscopy

Optical microscopy (OM) of the microspheres was conducted in a microscope model Q734ZT series 059 - DP Instrumentos Científicos LTDA. The micrographs were obtained with a scale of 1: 2.04 (pix: μm) and were used to obtain the dimensional data of the microspheres.

3 RESULTS AND DISCUSSION

3.1 KINETICS OF RELEASE

The kinetics of 5-FU release from the microspheres was evaluated using the Korsmeyer et al. (1983) model. The values found for the 5-FU release profiles at pH 1.2, 7.4 and 10 are shown in Table 3. The n values indicate that at pH 10 the release of 5-FU from F1 and F2 occurred by an anomalous mechanism and at pH 7.4 the mechanism was through Fick diffusion. It is possible that the anomalous mechanism of dissolution has occurred as a result of the joint action of the diffusion and erosion processes of the polymeric matrix.

Table 3: Parameters of Korsmeyer-Peppas kinetic model of 5-FU release for various systems in PBS pH 1.2, 7.4 and 10.

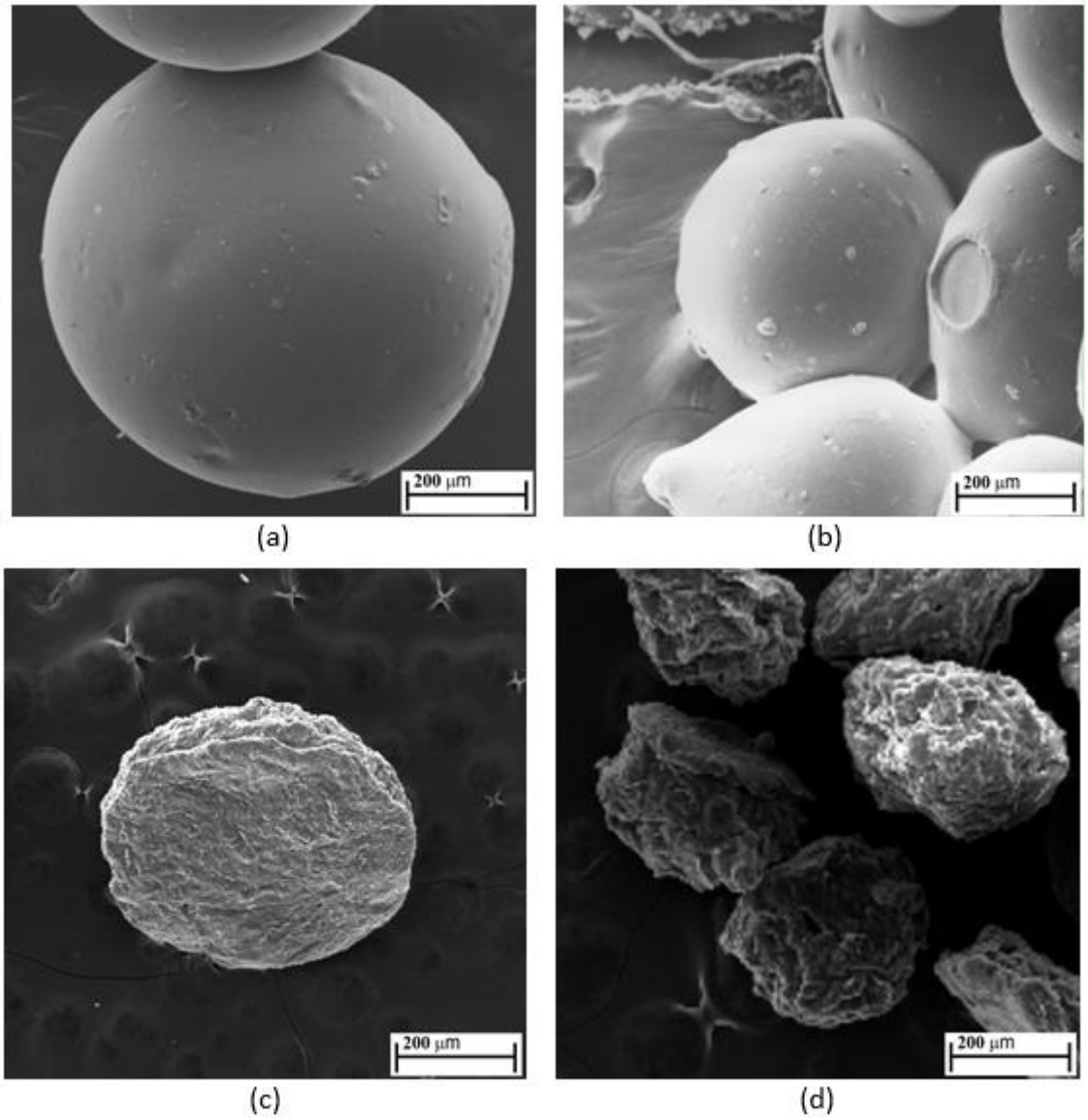
pH	Parameter	F1	F2	F3	F4
1.2	n	0.01	0.01	0.0025	0.0025
	r^2	0.9571	0.8286	0.8008	0.8288
7.4	n	0.01	0.12	0.4867	0.4492
	r^2	0.9315	0.8915	0.9845	0.8836
10	n	0.45	0.72	0.3762	0.4446
	r^2	0.9092	0.8999	0.9621	0.8828

The values of n presented in Table 3 indicate that the release of 5-FU at pH 10 from the F3 and F4 systems occurred by a Fick diffusion mechanism (Siepmann and Peppas, 2001). F3 at pH 7.4 showed a mechanism of release by diffusion of Fick and F4 presented an anomalous mechanism. However, the proximity of the exponent n with the anomalous transport range (0.45-0.89) indicates that there may be erosion in the polymeric matrix (Siepmann and Peppas, 2001).

3.2 SCANNING ELECTRON MICROSCOPY (SEM)

The morphologies of F1, F2, F3 and F4 microspheres were investigated by scanning electron microscopy. The microspheres images are represented in Figure 1. The SEM of the F1 and F2 microspheres present a seemingly smooth and continuous surface characterizing a denser morphology (Figure 1a-b). The SEMs of the F3 and F4 microspheres show an increase in the microspheres' surface roughness (Figure 1c-d), and this must be related to the loss of solvent during the drying process, since the microspheres F3 and F4 were prepared with the addition of the MMT dispersion, thus raising the proportion of the solvent.

Figure 2: SEM of the microspheres F1 (a), F2 (b), F3 (c) and F4 (d).



3.3 OPTICAL MICROSCOPY (OM)

The F1 and F2 microspheres presented larger dimensional values than F3 and F4 (Table 4), because these microspheres preserved the round shape after the drying process. The data demonstrate that F3 and F4 suffered a reduction in volume and surface area. According to Sinha et al. (2004), this modification may be related to the chitosan/solvent ratio, because the solution used to obtain the F3 and F4 microspheres had a 20% higher solvent proportion due to MMT dispersion, in agreement with the data obtained in the SEM.

Figure 3: Images obtained with OM from the 5-FU microspheres.

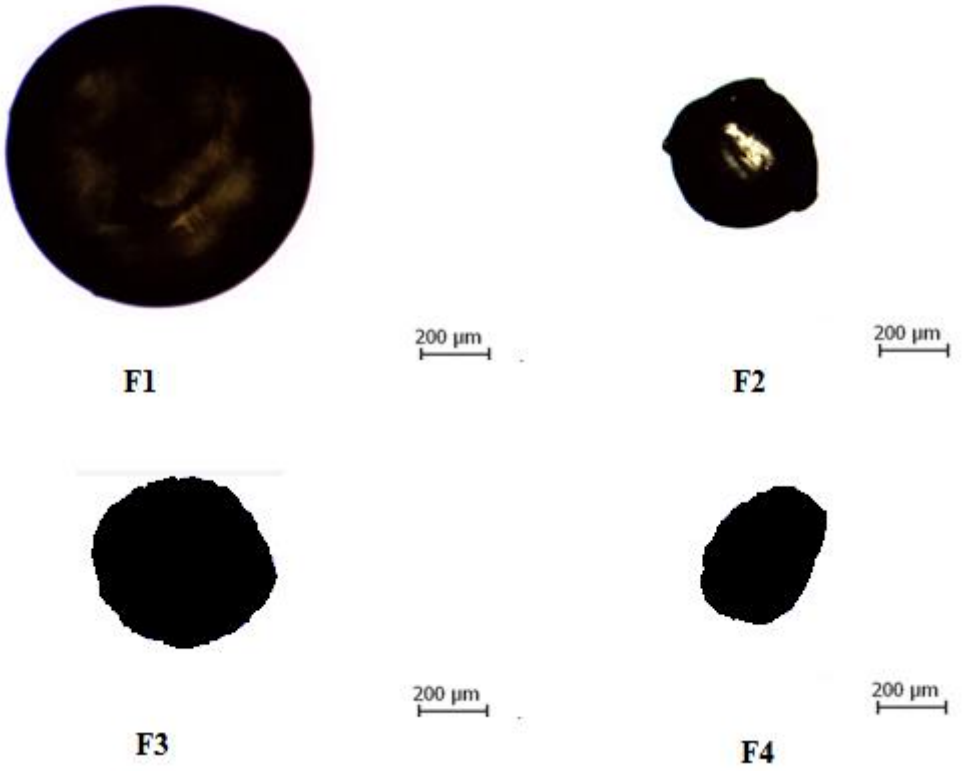


Table 4: Dimensional data of 5-FU microspheres.

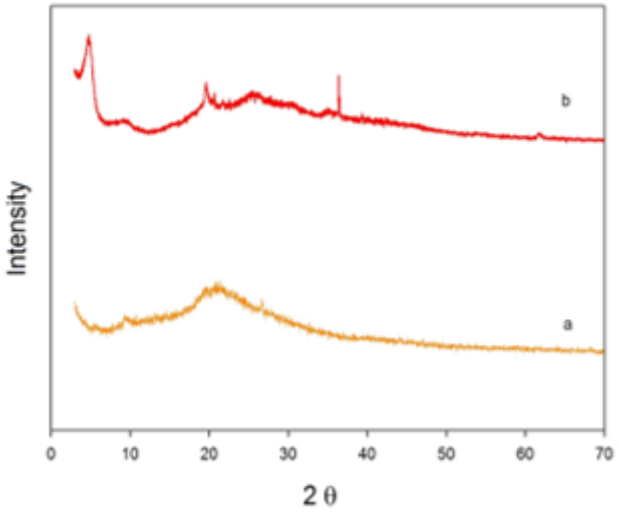
Microspheres	Equivalent diameter (mm)	Volume (mm^3)	Surface area ($mm^2.g^{-1}$)
F1	0.836 ± 0.042	0.310 ± 0.043	1428.0 ±227.9
F2	0.451 ± 0.014	0.048 ± 0.004	2138.9 ± 302.5
F3	0.560 ± 0.023	0.037±0.001	2172.9 ± 198.9
F4	0.413±0.015	0.013±0.001	2475.9 ± 84.8

3.4 XRD

X-ray diffraction is a technique that is used to identify the crystalline phase and the structural properties of solid materials. The X-ray diffraction patterns of chitosan and 5-FU / chitosan / MMT are shown in Figure 4. The XRD pattern of chitosan shows two characteristics diffraction peaks at 10° and 20° (Fig. 4a) (Lizardi-Mendoza et al., 2016). The pure MMT exhibits a characteristic peak at 2θ = 7.13°, which corresponds to a basal spacing of 1.24 nm (Olad, 2011). For the nanocomposite 5-FU / chitosan / MMT (Fig. 4b), the characteristic peak related to mineral clay (2θ = 7,13 °), was shifted to a smaller degree (2θ = 4.87 °), corresponding to a basal interplanar distance (d_{001}), calculated on the basis of the Bragg equation, of 1.81 nm. This indicates that intercalated nanocomposites were obtained and that possibly chitosan and 5-FU bilayers were positioned between the montmorillonite layers. This interaction is favored by electrostatic interaction of groups ($-NH_3^+$) in the second-layer with acetate ions of the chitosan solution, making the sites accessible for anion exchange (Tan et al., 2008).

Considering that the silicate layer thickness is 0.96 nm (Azhar and Olad, 2014), chitosan and 5-FU expanded the intercalation space by 0.85 nm, indicating the intercalation of 5-FU in the interspace and the confirmation of the 5-FU / chitosan / MMT intercalated nanocomposite.

Figure 4: X-ray diffraction (a) chitosan, (b) 5-FU/chitosan/MMT.



3.5 ENCAPSULATION EFFICIENCY (EE)

The drug encapsulation process was performed during the production of chitosan (F1 and F2) and chitosan / clay (F3 and F4) microspheres, with 5-FU being dissolved in the chitosan solution and subsequently dripped into the coagulant solution. In this way, the drug can be entrapped in the polymer matrix. The encapsulation efficiencies ranged from 29.37% to 74.99% (Table 1). Previous work reports 5-FU encapsulation efficiencies between 28-66% (Yang and Hon, 2009) and 29-69% (Aydin and Pulat, 2012). In this study, the results showed a good correlation with previous studies.

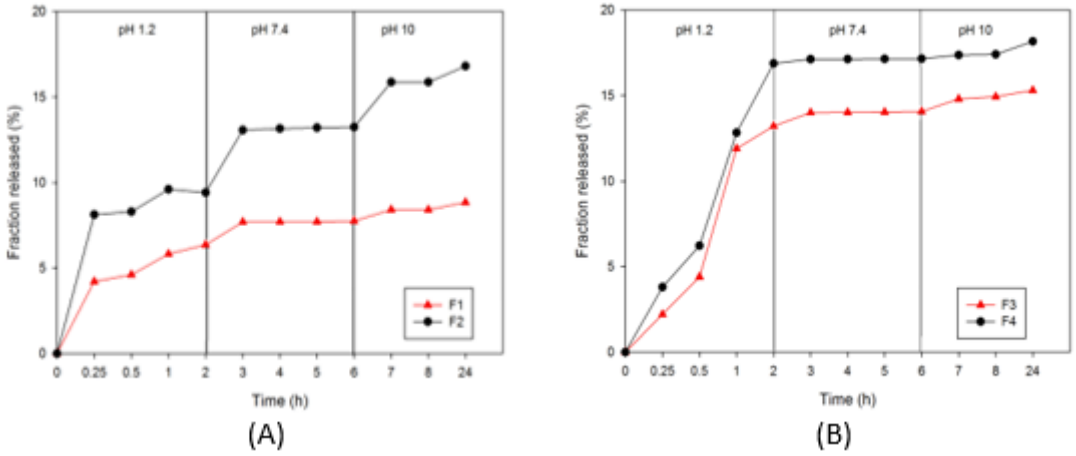
The presence of MMT affected the 5-FU encapsulation, verified by the 5-FU by UV-Vis quantification in the wash water of the microspheres. The wash water of the microspheres F1; F2; F3 and F4 presented levels of 19.79; 21.15; 7.07 and 8.89 $\mu g.mL^{-1}$ of 5-FU, showing that the presence of MMT reduces the loss of 5-FU during the washing process, corroborating with the data on formation of intercalated nanocomposites, with 5- FU interspersed between the MMT layers.

3.6 SIMULATION OF GASTROINTESTINAL RELEASE

Figure 4 shows two release steps in all of the systems, the first with fast release (burst) in the first two hours and another long phase (lag) release until the end of the test. Usually abrupt release occurs in a short period of time compared to the entire release process (Yang et al., 2016). The high initial release of 5-FU should have occurred mainly by dissolving the 5-FU present on the surface of the systems and the second release phase must have involved diffusion, according to the kinetic release model.

At pH 1.2, the release of 5-FU occurred during the time interval from 0 to 2 hours, with release of 6.35%, 9.40%, 13.21% and 16.87% for the microspheres F1, F2, F3 and F4, respectively. At pH 7.4 it was observed that there was 5-FU release of 7.74%, 13.23%, 14.06% and 17.15% from 2 to 6 hours. Finally at pH 10 and in the time interval of 6 to 24 hours, the release of 5-FU was 8.83%, 16.80%, 15.30% and 18.16%.

Figure 5: *In vitro* simulation of gastrointestinal release of 5-FU from the microspheres without MMT (A) and with MMT (B).



Legenda: Fraction released – Time – mudra as virgulas para pontos no eixo X e os pHs

We could observe that there was higher release of 5-FU in acid medium, because the amino groups of chitosan are protonated (-$NH3^{+}$) easily in acid medium (Gierszewska and Ostrowska-Czubenko, 2016). The release data of the microspheres during 2 hours in the gastric environment confirm the permeability of the microspheres to PBS at pH 1.2. Comparatively, the F3 and F4 systems showed greater release in the gastric environment, probably associated with the montmorillonite lamellae that were exposed to water; the water molecules are adsorbed on the surface of the layers, which are then separated from each other. Another aspect that may have contributed to the higher release of 5-FU is the roughness of the surface of F3 and F4 systems, as can be observed in Figure 1c-d, since an increased roughness leads to a faster drug release (Kalosakas and Martini, 2015). Higher roughness favors interlamellar swelling, and leads to increased surface area. This increase may have favored diffusion of 5-FU, as it is accompanied by increased erosion and diffusion front (Lopes et al., 2005; Lee et al., 2012).

At neutral (7.4) and alkaline pH (10) the released fraction of 5-FU was lower, evidencing a greater stability of the polymer matrix. As pH increases, the protonation of chitosan decreases, which reduces the degree of swelling. At pH close to neutrality, there is a balance between the charges, which promotes maximum interaction between the ionizable groups, providing greater stability to the matrix (Mendes et al., 2011). This stability reduces the number of protonated amino groups in chitosan,

affecting the swelling and release properties of 5-FU by the microspheres. At pH 10 most amino groups were deprotonated, indicating that the release of 5-FU in this medium should depend on the swelling characteristics of the clay and the solubility of 5-FU in the alkaline medium (Azhar and Olad, 2014). The released fraction of 5-FU was higher in F3 and F4 systems, and this characteristic must be related to the increase of the surface area and the size of the microspheres, since the release is influenced directly by particle size and surface area (Fangueiro et al., 2012). The increased release in F3 and F4 formulations should show significant improvements in the absorption and bioavailability properties of 5-FU *in vivo* (Shegokar and Muller, 2010; Moschwitzer, 2013).

Differences between the released fractions of 5-FU at the end of the test (F1 / F2 = 2.86%, F3 / F4 = 7.97%) are probably related to the increase in the surface area and the presence of MMT. In the F1 / F2 system, there was an increase in the surface area from 1428.0 to 2138.9 $mm^2.g^{-1}$ and in the F3 / F4 system from 2172.9 to 2475.9 $mm^2.g^{-1}$. The magnitude of area increase of the systems justifies the increase of the mass released, since the increase of the surface area of the microspheres increases the released mass of 5-FU. This occurs because the mass flow (J) is a relation between the total mass (m) that goes through an area (A) per unit of time (t), according to Equation (6) (Livi, 2000; Lee and Yeo, 2015).

$$J = \frac{m}{A.t} \qquad (6)$$

The results revealed that at pH 7.4 and pH 10, 5-FU is slowly released by the MMT-containing microspheres as compared to the release profile of the MMT-free microspheres. Probably the release difference should be related to the 5-FU intercalation in the silicate layers, resulting in smaller diffusion within the matrix, delaying and suppressing 5-FU release (Azhar and Olad, 2014). Molecular dynamics simulations agree with these experimental observations and suggest that pH variation significantly affects the conformation and solubility of chitosan, as well as the release of drugs by MMT, interfering with the drug release process (Silva *et al.*, 2011; Madeleine-Perdrillat *et al.*, 2016; Sanchis *et al.*, 2017). Therefore, 5-FU release occurs faster at pH 1.2 for all systems studied and slower at pH 7.4 and 10, as shown in Figure 5. This suggests that the systems studied can be used for delivery of 5-FU in the gastrointestinal environment, with predominance of release in the gastric environment.

4 CONCLUSIONS

In this study, 5-FU was incorporated into MMT through the intercalation method to obtain chitosan-based microspheres. The microspheres with chitosan nanocomposites (F3 and F4) presented controlled release characteristics, with a significant reduction in the release rate, mainly at pH 7.4 and

10. The encapsulation efficiency was higher in the nanocomposite systems, due to the interaction between the biopolymer, 5-FU and MMT. The release profiles of 5-FU containing microspheres showed high correlation with the Korsmeyer-Peppas kinetic model. The release of 5-FU in nanocomposite systems showed controlled release characteristics in the gastrointestinal environment. Reducing the diameter of the microspheres increases the released fraction of 5-FU, which can significantly increase absorption and bioavailability in the gastrointestinal environment.

REFERENCES

AHSAN, S. M. et al. Chitosan as biomaterial in drug delivery and tissue engineering. **International Journal of Biological Macromolecules**, 2017/09/01/ 2017. ISSN 0141-8130. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0141813017318846 >.

ANITHA, A. et al. 5-flourouracil loaded N, O-carboxymethyl chitosan nanoparticles as an anticancer nanomedicine for breast cancer. **Journal of biomedical nanotechnology,** v. 8, n. 1, p. 29-42, 2012. ISSN 1550-7033.

ANITHA, A. et al. In vitro combinatorial anticancer effects of 5-fluorouracil and curcumin loaded N,O-carboxymethyl chitosan nanoparticles toward colon cancer and in vivo pharmacokinetic studies. **European Journal of Pharmaceutics and Biopharmaceutics,** v. 88, n. 1, p. 238-251, 9// 2014. ISSN 0939-6411. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0939641114001349 >.

ARIAS, J. L. Novel Strategies to Improve the Anticancer Action of 5-Fluorouracil by Using Drug Delivery Systems. **Molecules,** v. 13, n. 10, p. 2340-2369, 2008. ISSN 1420-3049.

ARıCA, B. et al. 5-Fluorouracil encapsulated alginate beads for the treatment of breast cancer. **International journal of pharmaceutics,** v. 242, n. 1, p. 267-269, 2002. ISSN 0378-5173.

AULTON, M. E.; ORTEGA, G. G. **Delineamento de formas farmacêuticas**. Artmed, 2008. ISBN 853630152X.

AYDIN, R.; PULAT, M. 5-Fluorouracil encapsulated chitosan nanoparticles for pH-stimulated drug delivery: evaluation of controlled release kinetics. **Journal of Nanomaterials,** v. 2012, p. 42, 2012. ISSN 1687-4110. Disponível em: < http://downloads.hindawi.com/journals/jnm/2012/313961.pdf >.

AZHAR, F. F.; OLAD, A. A study on sustained release formulations for oral delivery of 5-fluorouracil based on alginate–chitosan/montmorillonite nanocomposite systems. **Applied Clay Science,** v. 101, p. 288-296, 2014/11/01/ 2014. ISSN 0169-1317. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0169131714003354 >.

BIZERRA, A.; SILVA, V. SISTEMAS DE LIBERAÇÃO CONTROLADA: Mecanismos e aplicações. **Revista Saúde e Meio Ambiente,** v. 3, n. 2, p. 1-12, 2016. ISSN 2447-8822.

CHANDRAN, S. P. et al. Nano drug delivery strategy of 5-fluorouracil for the treatment of colorectal cancer. **Journal of Cancer Research and Practice,** v. 4, n. 2, p. 45-48, 2017/06/01/ 2017. ISSN 2311-3006. Disponível em: < http://www.sciencedirect.com/science/article/pii/S2311300616301847 >.

CHEN, X.-X. et al. Ficus virens proanthocyanidins induced apoptosis in breast cancer cells concomitantly ameliorated 5-fluorouracil induced intestinal mucositis in rats. **Food and Chemical Toxicology,** v. 110, n. Supplement C, p. 49-61, 2017/12/01/ 2017. ISSN 0278-6915. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0278691517306051 >.

CHENG, M. et al. Synthesis of galactosylated chitosan/5-fluorouracil nanoparticles and its characteristics, in vitro and in vivo release studies. **J Biomed Mater Res B Appl Biomater,** v. 100, n. 8, p. 2035-43, Nov 2012. ISSN 1552-4981 (Electronic)
1552-4973 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22865703 >.

DE MESTIER, L.; CROS, J.; HAMMEL, P. 5-Fluorouracil biomarkers in pancreatic neuroendocrine tumours. **Pancreatology,** v. 17, n. 4, p. 527-528, 2017/07/01/ 2017. ISSN 1424-3903. Disponível em: < http://www.sciencedirect.com/science/article/pii/S142439031730501X >.

DENKBAŞ, E.; SEYYAL, M.; PIŞKIN, E. Implantable 5-fluorouracil loaded chitosan scaffolds prepared by wet spinning. **Journal of membrane Science,** v. 172, n. 1, p. 33-38, 2000. ISSN 0376-7388.

DODOV, M. G. et al. Wheat germ agglutinin-conjugated chitosan–Ca–alginate microparticles for local colon delivery of 5-FU: Development and in vitro characterization. **International journal of pharmaceutics,** v. 381, n. 2, p. 166-175, 2009. ISSN 0378-5173.

DZIADKOWIEC, J. et al. Preparation, characterization and application in controlled release of Ibuprofen-loaded Guar Gum/Montmorillonite Bionanocomposites. **Applied Clay Science,** v. 135, n. Supplement C, p. 52-63, 2017/01/01/ 2017. ISSN 0169-1317. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0169131716303611 >.

E.A.K, N. et al. A comparative study of 5-Fluorouracil release from chitosan/silver and chitosan/silver/MWCNT nanocomposites and their cytotoxicity towards MCF-7. **Materials Science and Engineering: C,** v. 66, n. Supplement C, p. 244-250, 2016/09/01/ 2016. ISSN 0928-4931. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0928493116303885 >.

ENNAJIH, H. et al. Chitosan–montmorillonite bio-based aerogel hybrid microspheres. **Microporous and Mesoporous Materials,** v. 152, n. Supplement C, p. 208-213, 2012/04/01/ 2012. ISSN 1387-1811. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1387181111005580 >.

FANGUEIRO, J. F. et al. Desenvolvimento, produção e caracterização de nanocristais de fármacos pouco solúveis. **Química Nova,** v. 35, p. 1848-1853, 2012. ISSN 0100-4042. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-40422012000900025&nrm=iso >.

FENG, S.-S. et al. Poly (lactide)–vitamin E derivative/montmorillonite nanoparticle formulations for the oral delivery of Docetaxel. **Biomaterials,** v. 30, n. 19, p. 3297-3306, 2009. ISSN 0142-9612.

FU, D.-J. et al. Preparation and characterization of mPEG grafted chitosan micelles as 5-fluorouracil carriers for effective anti-tumor activity. **Chinese Chemical Letters,** v. 25, n. 11, p. 1435-1440, 11// 2014. ISSN 1001-8417. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1001841714003040 >.

GANGULY, K.; AMINABHAVI, T. M.; KULKARNI, A. R. Colon Targeting of 5-Fluorouracil Using Polyethylene Glycol Cross-linked Chitosan Microspheres Enteric Coated with Cellulose Acetate Phthalate. **Industrial & Engineering Chemistry Research,** v. 50, n. 21, p. 11797-11807, 2011. ISSN 0888-5885
1520-5045.

GIERSZEWSKA, M.; OSTROWSKA-CZUBENKO, J. Chitosan-based membranes with different ionic crosslinking density for pharmaceutical and industrial applications. **Carbohydrate Polymers,** v. 153, n. Supplement C, p. 501-511, 2016/11/20/ 2016. ISSN 0144-8617. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0144861716309249 >.

GUPTA, P.; VERMANI, K.; GARG, S. Hydrogels: from controlled release to pH-responsive drug delivery. **Drug discovery today,** v. 7, n. 10, p. 569-579, 2002. ISSN 1359-6446.

HUANG, K.-S. et al. Microfluidic controlling monodisperse microdroplet for 5-fluorouracil loaded genipin-gelatin microcapsules. **Journal of Controlled Release,** v. 137, n. 1, p. 15-19, 2009/07/01/ 2009. ISSN 0168-3659. Disponível em: < http://www.sciencedirect.com/science/article/pii/S016836590900128X >.

HUANG, L. et al. Preparation of chitosan/chondroitin sulfate complex microcapsules and application in controlled release of 5-fluorouracil. **Carbohydrate Polymers,** v. 80, n. 1, p. 168-173, 2010. ISSN 01448617.

ILIESCU, R. I. et al. Montmorillonite–alginate nanocomposite as a drug delivery system – incorporation and in vitro release of irinotecan. **International Journal of Pharmaceutics,** v. 463, n. 2, p. 184-192, 2014/03/25/ 2014. ISSN 0378-5173. Disponível em: < http://www.sciencedirect.com/science/article/pii/S037851731300776X >.

JAFERIAN, S.; NEGAHDARI, B.; EATEMADI, A. Colon cancer targeting using conjugates biomaterial 5-flurouracil. **Biomedicine & Pharmacotherapy,** v. 84, n. Supplement C, p. 780-788, 2016/12/01/ 2016. ISSN 0753-3322. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0753332216314111 >.

JAHANI, M. et al. l-arginine alters the effect of 5-fluorouracil on breast cancer cells in favor of apoptosis. **Biomedicine & Pharmacotherapy,** v. 88, n. Supplement C, p. 114-123, 2017/04/01/ 2017. ISSN 0753-3322. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0753332216325707 >.

JAIN, S.; DATTA, M. Montmorillonite-alginate microspheres as a delivery vehicle for oral extended release of Venlafaxine hydrochloride. **Journal of Drug Delivery Science and Technology,** v. 33, n. Supplement C, p. 149-156, 2016/06/01/ 2016. ISSN 1773-2247. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1773224716301447 >.

JAYRAJSINH, S. et al. Montmorillonite nanoclay as a multifaceted drug-delivery carrier: A review. **Journal of Drug Delivery Science and Technology,** v. 39, n. Supplement C, p. 200-209, 2017/06/01/ 2017. ISSN 1773-2247. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1773224717301004 >.

JOSHI, G. V. et al. Montmorillonite as a drug delivery system: intercalation and in vitro release of timolol maleate. **International Journal of Pharmaceutics,** v. 374, n. 1, p. 53-57, 2009. ISSN 0378-5173.

JOSUÉ, A. et al. Liberação controlada da eosina impregnada em microesferas de copolímero de quitosana e poli (ácido acrílico). **Polímeros: Ciência e tecnologia,** v. 10, n. 3, p. 116-121, 2000. ISSN 1678-5169.

KALOSAKAS, G.; MARTINI, D. Drug release from slabs and the effects of surface roughness. **International Journal of Pharmaceutics,** v. 496, n. 2, p. 291-298, 2015/12/30/ 2015. ISSN 0378-5173. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0378517315302866 >.

KAMARI, Y.; GHIACI, P.; GHIACI, M. Study on montmorillonite/insulin/TiO2 hybrid nanocomposite as a new oral drug-delivery system. **Materials Science and Engineering: C,** v. 75, n. Supplement C, p. 822-828, 2017/06/01/ 2017. ISSN 0928-4931. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0928493116320586 >.

KAMISAWA, T. et al. Pancreatic cancer. **The Lancet,** v. 388, n. 10039, p. 73-85, 2016/07/02/ 2016. ISSN 0140-6736. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0140673616001410 >.

KEVADIYA, B. D. et al. Layered inorganic nanocomposites: a promising carrier for 5-fluorouracil (5-FU). **Eur J Pharm Biopharm,** v. 81, n. 1, p. 91-101, May 2012. ISSN 1873-3441 (Electronic)
0939-6411 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22269936 >.

KEVADIYA, B. D. et al. Biodegradable gelatin–ciprofloxacin–montmorillonite composite hydrogels for controlled drug release and wound dressing application. **Colloids and Surfaces B: Biointerfaces,** v. 122, n. Supplement C, p. 175-183, 2014/10/01/ 2014. ISSN 0927-7765. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0927776514003440 >.

KHOEE, S.; SAADATINIA, A.; BAFKARY, R. Ultrasound-assisted synthesis of pH-responsive nanovector based on PEG/chitosan coated magnetite nanoparticles for 5-FU delivery. **Ultrasonics Sonochemistry,** v. 39, n. Supplement C, p. 144-152, 2017/11/01/ 2017. ISSN 1350-4177. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1350417717301888 >.

KOCAR, M. et al. Adjuvant chemoradiotherapy combined with cisplatin, 5-fluorouracil and folinic acid for locally advanced gastric cancer. **Journal of Oncological Science,** v. 2, n. 1, p. 12-15, 2016/04/01/ 2016. ISSN 2452-3364. Disponível em: < http://www.sciencedirect.com/science/article/pii/S245233641630019X >.

KORSMEYER, R. W. et al. Mechanisms of solute release from porous hydrophilic polymers. **International journal of Pharmaceutics,** v. 15, n. 1, p. 25-35, 1983. ISSN 0378-5173.

LAKKAKULA, J. R.; MATSHAYA, T.; KRAUSE, R. W. M. Cationic cyclodextrin/alginate chitosan nanoflowers as 5-fluorouracil drug delivery system. **Materials Science and Engineering: C,** v. 70, n. Part 1, p. 169-177, 2017/01/01/ 2017. ISSN 0928-4931. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0928493116310220 >.

LAM, P. L. et al. Development of hydrocortisone succinic acid/and 5-fluorouracil/chitosan microcapsules for oral and topical drug deliveries. **Bioorg Med Chem Lett,** v. 22, n. 9, p. 3213-8, May 1 2012. ISSN 1464-3405 (Electronic)
0960-894X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22460032 >.

LEE, H.-S. et al. Chitosan adsorption on hydroxyapatite and its role in preventing acid erosion. **Journal of Colloid and Interface Science,** v. 385, n. 1, p. 235-243, 2012/11/01/ 2012. ISSN 0021-9797. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0021979712007205 >.

LEE, J. H.; YEO, Y. Controlled drug release from pharmaceutical nanocarriers. **Chemical Engineering Science,** v. 125, n. Supplement C, p. 75-84, 2015/03/24/ 2015. ISSN 0009-2509. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0009250914004710 >.

LESNIAK, M. S.; BREM, H. Targeted therapy for brain tumours. **Nature Reviews Drug Discovery,** v. 3, n. 6, p. 499-508, 2004. ISSN 1474-1776.

LI, G. et al. Thermo- and pH-sensitive ionic-crosslinked hollow spheres from chitosan-based graft copolymer for 5-fluorouracil release. **Int J Biol Macromol,** v. 55, p. 69-74, Apr 2013. ISSN 1879-0003 (Electronic)

0141-8130 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23313823 >.

LI, H.-P.; WANG, Z.-D.; YU, T. Microwave irradiation synthesis of novel ramose chitosan-based-5-fluorouracil and research on its in vitro release. **Medicinal Chemistry Research,** v. 20, n. 1, p. 67-73, 2009. ISSN 1054-2523
1554-8120.

LI, P. et al. Development of chitosan nanoparticles as drug delivery systems for 5-fluorouracil and leucovorin blends. **Carbohydrate Polymers,** v. 85, n. 3, p. 698-704, 2011. ISSN 01448617.

LIN, C. C.; FU, C. H. Controlled release study of 5-fluorouracil-loaded chitosan/polyethylene glycol microparticles. **Drug Deliv,** v. 16, n. 5, p. 274-9, Jul 2009. ISSN 1521-0464 (Electronic)
1071-7544 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19538009 >.

LIN, F.-H. et al. A study of purified montmorillonite intercalated with 5-fluorouracil as drug carrier. **Biomaterials,** v. 23, n. 9, p. 1981-1987, 2002. ISSN 0142-9612.

LIVI, C. P. **Fundamentos de Fenômenos de Transporte: Um Texto Para Cursos Básicos**. Grupo Gen-LTC, 2000. ISBN 8521620586.

LIZARDI-MENDOZA, J.; ARGÜELLES MONAL, W. M.; GOYCOOLEA VALENCIA, F. M. Chapter 1 - Chemical Characteristics and Functional Properties of Chitosan A2 - Bautista-Baños, Silvia. In: ROMANAZZI, G. e JIMÉNEZ-APARICIO, A. (Ed.). **Chitosan in the Preservation of Agricultural Commodities**. San Diego: Academic Press, 2016. p.3-31. ISBN 978-0-12-802735-6.

LOPES, C. M.; LOBO, J. M. S.; COSTA, P. Formas farmacêuticas de liberação modificada: polímeros hidrofílicos. **Revista Brasileira de Ciências Farmacêuticas,** v. 41, p. 143-154, 2005. ISSN 1516-9332. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-93322005000200003&nrm=iso >.

M, G. A. et al. Synthesis and characterization of cystamine conjugated chitosan-SS-mPEG based 5-Fluorouracil loaded polymeric nanoparticles for redox responsive drug release. **European Journal of Pharmaceutical Sciences**, 2017/11/01/ 2017. ISSN 0928-0987. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0928098717305882 >.

MADELEINE-PERDRILLAT, C. et al. Effect of hydration on molecular dynamics and structure in chitosan films. **Food Hydrocolloids,** v. 61, n. Supplement C, p. 57-65, 2016/12/01/ 2016. ISSN 0268-005X. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0268005X16301710 >.

MARTINI, L. G.; COLLETT, J. H.; ATTWOOD, D. The release of 5-fluorouracil from a swellable matrix of a triblock copolymer of ε-caprolactone and ethylene oxide. **Pharmaceutical research,** v. 12, n. 11, p. 1786-1790, 1995. ISSN 0724-8741.

MENDES, A. A. et al. Aplicação de quitosana como suporte para a imobilização de enzimas de interesse industrial. **Química Nova,** v. 34, n. 5, p. 831-840, 2011.

MONTEIRO, D. L. M. et al. Câncer de mama na gravidez e quimioterapia: revisão sistemática. **Revista da Associação Médica Brasileira,** v. 59, n. 2, p. 174-180, 2013/03/01/ 2013. ISSN 0104-4230. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0104423013000067 >.

MORRIS, M.; MARSHALL-LUCETTE, S. The Experience of Myeloma Caregivers During Home-Based Oral Chemotherapy Treatment: A Qualitative Study. **Seminars in Oncology Nursing,** v. 33, n. 3, p. 362-371, 2017/08/01/ 2017. ISSN 0749-2081. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0749208117300451 >.

MOSCHWITZER, J. P. Drug nanocrystals in the commercial pharmaceutical development process. **Int J Pharm,** v. 453, n. 1, p. 142-56, Aug 30 2013. ISSN 1873-3476 (Electronic)
0378-5173 (Linking).

NAGARWAL, R. C.; KUMAR, R.; PANDIT, J. K. Chitosan coated sodium alginate-chitosan nanoparticles loaded with 5-FU for ocular delivery: in vitro characterization and in vivo study in rabbit eye. **Eur J Pharm Sci,** v. 47, n. 4, p. 678-85, Nov 20 2012. ISSN 1879-0720 (Electronic)
0928-0987 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22922098 >.

NAGARWAL, R. C.; KUMAR, R.; PANDIT, J. K. Chitosan coated sodium alginate–chitosan nanoparticles loaded with 5-FU for ocular delivery: In vitro characterization and in vivo study in rabbit eye. **European Journal of Pharmaceutical Sciences,** v. 47, n. 4, p. 678-685, 2012/11/20/ 2012. ISSN 0928-0987. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0928098712003065 >.

NARAYANI, R.; RAO, K. P. Gelatin microsphere cocktails of different sizes for the controlled release of anticancer drugs. **International journal of pharmaceutics,** v. 143, n. 2, p. 255-258, 1996. ISSN 0378-5173.

NASCIMENTO, R. F. et al. Um sistema simples para preparação de microesferas de quitosana. **Química Nova,** v. 31, p. 3, 2008. Disponível em: < http://www.quimicanova.sbq.org.br/imagebank/pdf/Vol31No1_160_27-NT06369.pdf >.

OLAD, A. Polymer/clay nanocomposites. In: (Ed.). **Advances in diverse industrial applications of nanocomposites**: InTech, 2011.

OLIVEIRA, A. S.; ALCÂNTARA, A. C. S.; PERGHER, S. B. C. Bionanocomposite systems based on montmorillonite and biopolymers for the controlled release of olanzapine. **Materials Science and Engineering: C,** v. 75, n. Supplement C, p. 1250-1258, 2017/06/01/ 2017. ISSN 0928-4931. Disponível em: < http://www.sciencedirect.com/science/article/pii/S092849311631685X >.

PAPADIMITRIOU, S. et al. Chitosan nanoparticles loaded with dorzolamide and pramipexole. **Carbohydrate Polymers,** v. 73, n. 1, p. 44-54, 7/4/ 2008. ISSN 0144-8617. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0144861707005528 >.

PARK, J. K. et al. Controlled release of donepezil intercalated in smectite clays. **International Journal of Pharmaceutics,** v. 359, n. 1, p. 198-204, 2008. ISSN 0378-5173.

PRADO, A. et al. Sistema de baixo custo para produção de microesferas de quitosana. **Analytica,** v. 44, p. 5, 2010. Disponível em: < http://sec.sbq.org.br/cdrom/32ra/resumos/T2146-1.pdf >.

PRETEL, E. et al. Development of biomedical 5-fluorouracil nanoplatforms for colon cancer chemotherapy: Influence of process and formulation parameters. **International Journal of Pharmaceutics,** v. 530, n. 1, p. 155-164, 2017/09/15/ 2017. ISSN 0378-5173. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0378517317306634 >.

PUGA, A. M. et al. Pectin-coated chitosan microgels crosslinked on superhydrophobic surfaces for 5-fluorouracil encapsulation. **Carbohydrate Polymers,** v. 98, n. 1, p. 331-340, 10/15/ 2013. ISSN 0144-8617. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0144861713005912 >.

RAJAN, M. et al. Hyaluronidase enzyme core-5-fluorouracil-loaded chitosan-PEG-gelatin polymer nanocomposites as targeted and controlled drug delivery vehicles. **International Journal of Pharmaceutics,** v. 453, n. 2, p. 514-522, 2013/09/10/ 2013. ISSN 0378-5173. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0378517313005395 >.

RAPACZ-KMITA, A. et al. Characterisation, in vitro release study, and antibacterial activity of montmorillonite-gentamicin complex material. **Materials Science and Engineering: C,** v. 70, n. Part 1, p. 471-478, 2017/01/01/ 2017. ISSN 0928-4931. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0928493116312917 >.

REJINOLD et al. Biodegradable and thermo-sensitive chitosan-g-poly (N-vinylcaprolactam) nanoparticles as a 5-fluorouracil carrier. **Carbohydrate polymers,** v. 83, n. 2, p. 776-786, 2011. ISSN 0144-8617.

REJINOLD, N. S. et al. Biodegradable and thermo-sensitive chitosan-g-poly(N-vinylcaprolactam) nanoparticles as a 5-fluorouracil carrier. **Carbohydrate Polymers,** v. 83, n. 2, p. 776-786, 2011. ISSN 01448617.

SALCEDO, I. et al. In vitro biocompatibility and mucoadhesion of montmorillonite chitosan nanocomposite: A new drug delivery. **Applied Clay Science,** v. 55, n. Supplement C, p. 131-137, 2012/01/01/ 2012. ISSN 0169-1317. Disponível em: < http://www.sciencedirect.com/science/article/pii/S016913171100370X >.

SALERNO, A.; DOMINGO, C.; SAURINA, J. PCL foamed scaffolds loaded with 5-fluorouracil anti-cancer drug prepared by an eco-friendly route. **Materials Science and Engineering: C,** v. 75, n. Supplement C, p. 1191-1197, 2017/06/01/ 2017. ISSN 0928-4931. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0928493116316952 >.

SALERNO, A.; SAURINA, J.; DOMINGO, C. Supercritical CO2 foamed polycaprolactone scaffolds for controlled delivery of 5-fluorouracil, nicotinamide and triflusal. **International Journal of Pharmaceutics,** v. 496, n. 2, p. 654-663, 2015/12/30/ 2015. ISSN 0378-5173. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0378517315303598 >.

SANCHIS, M. J. et al. Molecular dynamics of carrageenan composites reinforced with Cloisite Na+ montmorillonite nanoclay. **Carbohydrate Polymers,** v. 176, n. Supplement C, p. 117-126, 2017/11/15/ 2017. ISSN 0144-8617. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0144861717308780 >.

SHEGOKAR, R.; MULLER, R. H. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. **Int J Pharm,** v. 399, n. 1-2, p. 129-39, Oct 31 2010. ISSN 1873-3476 (Electronic)
0378-5173 (Linking).

SIEPMANN, J.; PEPPAS, N. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). **Advanced drug delivery reviews,** v. 48, n. 2, p. 139-157, 2001. ISSN 0169-409X.

SILVA, K. C. C. et al. Estudo do efeito do pH e da força iônica em filamentos de quitosana via simulação por dinâmica molecular. **Sociedade Brasileira de Química**, 2011.

SINHA, V. R. et al. Chitosan microspheres as a potential carrier for drugs. **International Journal of Pharmaceutics,** v. 274, n. 1–2, p. 1-33, 4/15/ 2004. ISSN 0378-5173. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0378517304000225 >.

SOFIS, M. J. et al. KU32 prevents 5-fluorouracil induced cognitive impairment. **Behavioural Brain Research,** v. 329, n. Supplement C, p. 186-190, 2017/06/30/ 2017. ISSN 0166-4328. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0166432817303145 >.

SUN, C. et al. Noninvasive nanoparticle strategies for brain tumor targeting. **Nanomedicine: Nanotechnology, Biology and Medicine,** v. 13, n. 8, p. 2605-2621, 2017/11/01/ 2017. ISSN 1549-9634. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1549963417301399 >.

TAN, W. et al. A novel method to prepare chitosan/montmorillonite nanocomposites in the presence of hydroxy-aluminum oligomeric cations. **Composites Science and Technology,** v. 68, n. 14, p. 2917-2921, 2008/11/01/ 2008. ISSN 0266-3538. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0266353807004058 >.

TANDAY, S. Palliative chemotherapy leads to more deaths away from home. **The Lancet Oncology,** v. 15, n. 4, p. e157, 2014/04/01/ 2014. ISSN 1470-2045. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1470204514701111 >.

TıĞLı AYDıN, R. S.; PULAT, M. 5-Fluorouracil Encapsulated Chitosan Nanoparticles for pH-Stimulated Drug Delivery: Evaluation of Controlled Release Kinetics. **Journal of Nanomaterials,** v. 2012, p. 1-10, 2012. ISSN 1687-4110
1687-4129.

VARGO, C. A. et al. Successful Completion of Adjuvant Chemotherapy in a Patient With Colon Cancer Experiencing 5-Fluorouracil–Induced Cardiac Vasospasm. **Clinical Colorectal Cancer,** v. 15, n. 2, p. e61-e63, 2016/06/01/ 2016. ISSN 1533-0028. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1533002815001437 >.

XU, G.-Y.; TANG, X.-J. Troxerutin (TXN) potentiated 5-Fluorouracil (5-Fu) treatment of human gastric cancer through suppressing STAT3/NF-κB and Bcl-2 signaling pathways. **Biomedicine & Pharmacotherapy,** v. 92, n. Supplement C, p. 95-107, 2017/08/01/ 2017. ISSN 0753-3322. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0753332217306285 >.

XU, Y. et al. Oxidation and pH responsive nanoparticles based on ferrocene-modified chitosan oligosaccharide for 5-fluorouracil delivery. **Carbohydrate Polymers,** v. 114, n. 0, p. 27-35, 12/19/ 2014. ISSN 0144-8617. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0144861714007541 >.

YAN, C. et al. Nanoparticles of 5-fluorouracil (5-FU) loaded N-succinyl-chitosan (Suc-Chi) for cancer chemotherapy: preparation, characterization--in-vitro drug release and anti-tumour activity. **J Pharm Pharmacol,** v. 58, n. 9, p. 1177-81, Sep 2006. ISSN 0022-3573 (Print)
0022-3573 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16945175 >.

YAN, S. et al. Layer-by-layer assembly of poly(L-glutamic acid)/chitosan microcapsules for high loading and sustained release of 5-fluorouracil. **Eur J Pharm Biopharm,** v. 78, n. 3, p. 336-45, Aug 2011. ISSN 1873-3441 (Electronic)
0939-6411 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21195174 >.

YANG, H. C.; HON, M. H. The effect of the molecular weight of chitosan nanoparticles and its application on drug delivery. **Microchemical Journal,** v. 92, n. 1, p. 87-91, 2009. ISSN 0026-265X.

YANG, J.-H. et al. Drug–clay nanohybrids as sustained delivery systems. **Applied Clay Science,** v. 130, n. Supplement C, p. 20-32, 2016/09/01/ 2016. ISSN 0169-1317. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0169131716300229 >.

YU, C.-Y. et al. Sustained release of antineoplastic drugs from chitosan-reinforced alginate microparticle drug delivery systems. **International journal of pharmaceutics,** v. 357, n. 1, p. 15-21, 2008. ISSN 0378-5173.

YUAN, Q. et al. Controlled and extended drug release behavior of chitosan-based nanoparticle carrier. **Acta biomaterialia,** v. 6, n. 3, p. 1140-1148, 2010. ISSN 1742-7061.

ZHANG, D.-Y. Preparation of chitosan-polyaspartic acid-5-fluorouracil nanoparticles and its anti-carcinoma effect on tumor growth in nude mice. **World Journal of Gastroenterology,** v. 14, n. 22, p. 3554, 2008. ISSN 1007-9327.

ZHANG, L. et al. Combination of NRP1-mediated iRGD with 5-fluorouracil suppresses proliferation, migration and invasion of gastric cancer cells. **Biomedicine & Pharmacotherapy,** v. 93, n. Supplement C, p. 1136-1143, 2017/09/01/ 2017. ISSN 0753-3322. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0753332217325507 >.

ZHENG, J. et al. Study on ibuprofen/montmorillonite intercalation composites as drug release system. **Applied clay science,** v. 36, n. 4, p. 297-301, 2007. ISSN 0169-1317.

ZHENG, Y. et al. Nanoparticles based on the complex of chitosan and polyaspartic acid sodium salt: preparation, characterization and the use for 5-fluorouracil delivery. **Eur J Pharm Biopharm,** v. 67, n. 3, p. 621-31, Nov 2007. ISSN 0939-6411 (Print)
0939-6411 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17533123 >.

ZHU, L. et al. Chitosan-coated magnetic nanoparticles as carriers of 5-fluorouracil: preparation, characterization and cytotoxicity studies. **Colloids Surf B Biointerfaces,** v. 68, n. 1, p. 1-6, Jan 1 2009. ISSN 1873-4367 (Electronic)
0927-7765 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19013060 >.