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Article

Release of Vitamin B_{12} and Diclofenac Potassium from *N*,*N*-dimethylacrylamide-modified Arabic Gum Hydrogels - the Partition-Diffusion Model

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Recentemente, foi desenvolvido em nosso grupo de pesquisas um modelo que prevê 100% do perfil de liberação de soluto a partir de hidrogel. Este considera os efeitos de difusão e de partição e permite obter os valores da atividade de partição, α, e da constante cinética de liberação, k_R. Embora α e k_R sejam dependentes de fatores diversos, a hipótese de que a razão α/k_R não seja dependente do volume de fluido de intumescimento foi testada neste trabalho. Assim, foram determinados valores da razão α/k_R para vitamina B_{12} ($VitB_{12}$) e diclofenaco de potássio (DFK) que foram submetidos a ensaios de liberação em três temperaturas (25, 35 e 45 °C), sendo avaliados, em cada temperatura, os volumes 250, 350 e 450 mL. O hidrogel utilizado é baseado na goma arábica quimicamente modificada copolimerizada com *N*,*N*-dimetilacrilamida (AGm-DMAAm), na razão 60-40 (m/m) sendo o carregamento dos solutos feito durante o processo de gelificação. Foi verificado que a razão α/k_R não depende do volume do fluido de liberação para uma determinada temperatura. Mas a temperatura é um fator importante, influenciando fortemente o processo de liberação destes solutos. A variação da temperatura de 25 para 35 °C afeta mais a liberação de DFK do que a liberação de $VitB_{12}$. Isso foi atribuído a interações eletrostáticas entre os grupos ($R\text{-}COO^-$) contidos nesse soluto e a grupos positivamente carregados na matriz. Foram calculados os valores de tempo de meia vida de liberação, $t_{1/2}$, e a energia de ativação, Ea_R, do processo de liberação dos solutos.

Recently, a model predicting whole profile solute release from a hydrogel was developed. In such model, the partition activity, α, and release kinetic constant, k_R, can be obtained. Although α and k_R depend on many factors, the hypothesis that α/k_R ratio does not depend on external fluid volume was tested in the present study. Thus, α/k_R values for two distinct solutes, vitamin B_{12} ($VitB_{12}$) and diclofenac potassium (DFK), were obtained at 25, 35 and 45 °C using 250, 350 and 450 mL of external fluid. The hydrogel used in the experiments was obtained by the copolymerization of *N*,*N*-dimethylacrylamide-modified Arabic gum (AGm-DMAAm), at wt% ratio of 60-40. It was verified that α/k_R ratio is not volume dependent, at a certain temperature, but the temperature strongly influences the α/k_R ratio for both solutes. Changing temperature from 25 to 35 °C affected DFK release much more than $VitB_{12}$ release. This was attributed mainly to electrostatic interactions between ($R\text{-}COO^-$) from DFK and the positively charged groups in the GAm-DMAAm matrix. Additionally, the values for the half-life time release, $t_{1/2}$, equilibrium time release, t_{eq}, as well as the activation energy for releasing, Ea_R, were determined and discussed in light of the partition-diffusion model.

Keywords: Arabic gum hydrogels, partition-diffusion mathematic model, diclofenac potassium, vitamin B_{12}, partition coefficient, rate release constant

Introduction

Hydrogels are formed by natural and/or synthetic polymer chains entangled and crosslinked through chemical bonds of physical interactions. The tridimensional (3D) matrix of a hydrogel may present physical and chemical properties not observed in linear polymers. When in contact with water or aqueous solutions, the hydrogel has the ability to swell and to acquire a soft and elastic texture analogous to some animal tissues.[1-8] Often, in the 3D network of a hydrogel, pores of different sizes exist, with average values dependent on the amount of adsorbed water, the crosslinking density and network elasticity. The presence of pores allows the differential diffusion of different

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sized molecules through the network. Consequently, hydrogels are often used as devices for the controlled release of drugsfor different applications ranging from biomedical,[5,10,11] to agriculture for the delivery of plant nutrients.[9,12-14] In recent decades, a significant number of studies were carried out to assess the kinetic control of solute release from hydrogel matrices.[15-17] The majority of the published work in this field demonstrates that solute release from a hydrogel is strongly dependent on several factors, such as polymer composition, the geometry of the hydrogel matrix, the degree of swelling and solute-matrix,[18,19] among others. Under certain conditions, release occurs mainly through diffusion.[20] Consequently, the majority of the mathematic models reported in the literature are based in diffusion-controlled release. One of the most widely used models has been proposed by the Peppas´s research group.[21-24] This model, based on empirical grounds, allows the assessment of the release mechanism and, when a diffusion-controlled release occurs, a rate constant (or diffusion coefficient) can be computed; however, this model is limited to short-range release times; i.e., up to 60% of the cumulative drug release. Besides this limitation, most experiments involved initial and border conditions of non-steady state diffusion transport occurring in a stirred solution of limited volume.[25] Mass transport, consequently, should be described as a diffusion and partition process. Based on that, a mathematical model for solute release was proposed,[17] designated as the diffusion-partition model. The physical and chemical interactions between the solute and the hydrogel as well as solute-solvent interactions are considered in that model through the partition concept. The diffusion-partition model has been successfully used to model the release of DNA from PVA cryogels under different bulk conditions[26,27] and the release of bovine serum albumin (BSA) from thermosensitive hydrogels composed of alginate-Ca^{2+}/PNIPAAm.[28] The model has been shown to be effective for describing the whole release profile in different systems, and for providing reliable kinetic and equilibrium parameters.

In the diffusion partition-model, the partition activity, α, characterizes the physical chemical affinity of the solute for both phases (3D matrix and external fluid), according to equation:[17]

$$\alpha = \frac{F_{max}}{1 - F_{max}} \quad (1)$$

where F_R and F_{max} are the fraction of the released solute at time t and at equilibrium, respectively, being the F_R calculated through the equation

$$F_R = \frac{C_{R,t}}{C_0} \quad (2)$$

where $C_{R,t}$ is the concentration of the solute released, at time t, and C_0 is the initial concentration of solute inside the hydrogel matrix. The value of α expresses the physical and chemical affinities of the solute for the hydrogel matrix and the external fluid. According to Reis *et al.*,[17] the diffusion of a solute from a hydrogel into the external fluid occurs even when $\alpha > 0$. Also, a higher α value indicates a higher affinity of the solute for the external fluid.

In the presence of a partition, i.e., if the solubility of the solute in the 3D hydrogel matrix and the external fluid is different, the mass transport of the solute into or out of hydrogel will arise in such a way that:

$$\frac{dC_{R,t}}{dt} = k_R(C_0 - C_{R,t}) - k_A(C_{R,0} - C_{A,t}) \quad (3)$$

where $C_{R,0}$ and $C_{R,t}$ are the concentration of the released solute at $t = 0$ and at time t, respectively, C_0 is the initial concentration of solute in the hydrogel and $C_{A,t}$ is the concentration of absorbed solute at a specific time t. k_R and k_A are the kinetic constants of release and of absorption, respectively. Considering solute release as a reversible process, and from the kinetic law equation (equation 3), and taking into account the considerations of the model,[17] the release kinetics of a solute can be determined using the following equation:

$$F_R = F_{max}(1 - e^{-(k_R/F_{max})t}) \quad (4)$$

where F_R is the fraction of released solute at time t, $F_{max} = C_{R,max}/C_0$, and $C_{R,max}$ is the maximum concentration of solute in solution after release from the hydrogel.

In a similar way, if release occurs through second order reversible kinetics, the following equation can be derived:

$$F_R = \frac{F_{max}\left(e^{2(k_R/\alpha)t} - 1\right)}{1 - 2F_{max} + e^{2(k_R/\alpha)t}} \quad (5)$$

This equation can be used to predict F_R for a given release time if the values of k_R and F_{max} are known.

F_R values, at any release time, depend on k_R and F_{max} and, according to equation 1, the latter is also dependent on the α parameter. Thus, F_R values are also dependent on temperature, pressure, pH, ionic strength, hydrogel geometry, etc. Another important practical parameter is the volume of the external fluid. Despite the robustness of this model to describe the release profile for different systems,[17,26-28] the effect of the latter variable has not yet been checked and, consequently, its effect on α and k_R remains unclear; in other words, we are interested in determining if α and k_R are extensive properties. If so,

the following hypothesis arises: assuming that α and k_R are equally affected by the change in the volume of the external fluid, the ratio α/k_R should remain constant and, consequently, such a ratio can be considered as an intensive property considering the volume of the external fluid. In order to test this hypothesis, assays on the release of vitamin B_{12} ($VitB_{12}$) and diclofenac potassium (DFK) from hydrogels were carried out in this work using three different volumes of the external fluid (250, 350 and 450 mL) at three temperatures (25, 35 and 45 °C). $VitB_{12}$ is an essential vitamin used for treating anemia, memory loss, etc., due to $VitB_{12}$ deficiency in the blood. DFK is a non-steroidal anti-inflammatory drug with analgesic and antipyretic action. These two water soluble model drugs were chosen for their different molecular weight and steric hindrance that may affect permeation through the gel network.

The hydrogels used in this work, designated as AGm-DMAAm, are based on chemically modified Arabic gum (AGm) copolymerized with *N*,*N*-dimethylacrylamide (DMAAm).

Beyond the evaluation of the effect of the external fluid volume on the α/k_R ratio, the release of $VitB_{12}$ and DFK from AGm-DMAAm was characterized by half-life time values, $t_{1/2}$, and the activation energy for release, Ea_R . This latter parameter was computed from the dependence of k_R on the temperature. The aim of this work was to shed light on the release of both drugs from Arabic gum-based hydrogels, and to further validate the partition-diffusion model by establishing appropriate experimental border conditions allowing the extension of this model to a larger set of release systems. It is worth highlighting that such an evaluation has not been yet published and represents an easier alternative to other approaches for assessing non-steady state conditions of a limited volume.

Experimental

Materials

Arabic gum (AG, CAS: 9000-01-5) was purchased from Company - Sudan, and glycidyl methacrylate (GMA, 97%, CAS: 106-91-20) was purchased from Fluka Analytical, USA. Ethyl alcohol (99.5%, CAS 60-17-5), hydrochloric acid (HCl, 36.5-38%, CAS: 7647-010), potassium chloride (KCl, 99%, CAS: 7447-40-7) and potassium phosphate monobasic (KH_2PO_4, 98%, CAS: 7778-77-0) were acquired from Nuclear, Brazil. Sodium persulfate (SP, 99%, CAS: 7775-27-1) and sodium hydroxide (NaOH, 97%, CAS: 1310-73-2) were supplied from Vetec, Brazil. *N*,*N*-dimethylacrylamide (DMAAm, 99%, CAS: 2680-07-70) was purchased from Aldrich, USA, and cyanocobalamin ($VitB_{12}$, 98,66%, CAS: 68-19-9, $C_{63}H_{88}CoN_{14}O_{14}P$, Figure 1A) and diclofenac potassium (DFK, 99%, CAS: 15307-79-6, $C_{14}H_{10}Cl_2KNO_2$, Figure 1B) were supplied from a local drugstore (Pharma Nostra, Maringá-PR, Brazil). All materials were used as received, unless otherwise indicated.

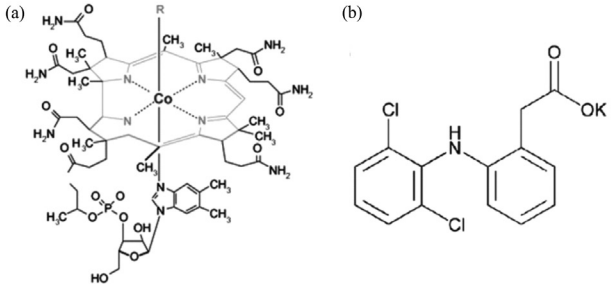

Figure 1. Molecular structures of $VitB_{12}$ (a) and DFK (b).

Chemical modification of Arabic gum

The chemical modification of AG was accomplished based on a previously described procedure.[17] An AG solution was initially prepared by the addition of 75 g of AG to 500 mL of deionized water under constant stirring, for 30 min. After 6 mL of glycidyl methacrylate (GMA, used as modifier) had been added, the pH was adjusted to 3.5 by using HCl aqueous solution, and the temperature was raised to 65 °C. The mixture was stirred for a further 12 h at a constant temperature (65 °C). The as-modified polysaccharide was then precipitated by the addition of 1 L of ethanol to the resultant mixture, separated by filtration and remixed in water. The cycle of dissolution and precipitation was repeated again. The obtained product (AGm) was dried by lyophilization and used for synthesis of hydrogel matrices. In this condition, the reaction occurs more by epoxy ring opening, as previously published.[29]

Preparation of drug-containing hydrogel matrices

The encapsulation of the drug ($VitB_{12}$ and DFK) occurred by loading during hydrogel synthesis, using the following procedure: 4.5 g of AGm were solubilized in a certain volume of water and, subsequently, 5.0 g of co-monomer DMAAm and 110 mg of the initiator sodium persulfate (SP) were added. The volume of the solution was then adjusted to 50 mL using distilled water. After complete homogenization of the pre-gel solution, the drug was added (105.5 mg of $VitB_{12}$, 7.5×10^{-5} mol or 116.5 mg of DFK, 3.9×10^{-4} mol) and stirred until complete solubilization. The drug-containing pre-gel solution was transferred to cylindrical molds and warmed to 75 °C for 30 min.

Hydrogels of AGm-DMAAm with a 60-40 composition (wt.%) loaded with $VitB_{12}$ or DFK were obtained.

Samples preparation for solute release

The samples used for the release assays (cylindrical pastilles) were prepared as follows: just after hydrogel synthesis, cylindrical samples ca. 1 cm^3 in volume were obtained. Samples were dried at room temperature for 48 h and further left in an oven at 40 °C for 12 h; after this period, all samples were weighed. The amount of drug loaded into each weighed sample, C_o, was determined using the equation:

$$C_0 = \frac{M_i M_f}{M_h} \quad (6)$$

where M_i is the mass of the individual sample, M_f is the mass of the solute added during loading (synthesis of the hydrogel), and M_h is the sum of the individual masses of all samples of the drug-loaded xerogel. Considering 100% loading, the amount of loaded drug was 10.9 mg for $VitB_{12}$ and 12 mg for DFK in each 1 g of dried hydrogel.

Release assays

For release assays, different dried hydrogel samples containing a certain amount of solute, C_0, depending on the dry weight, were immersed in a desired volume of buffer solution at pH 6.0 (KH_2PO_4/NaOH) and ionic strength ($\mu = 0.1$ mol L^{-1}) adjusted using KCl. In sequence, the solution was transferred to a glass container under constant stirring at 50 rpm and at a temperature of 25, 35 or 45 °C. For each temperature, the volumes of the buffer solution (external fluid) were 250, 350 or 450 mL. At given time intervals, 3 mL aliquots of the external fluid were collected and the absorbances were measured, at 358 and 271 nm for $VitB_{12}$ and DFK, respectively, using a UV-Vis spectrophotometer (Shimadzu model UV MINI 1240). After each measurement, the sample aliquot was returned to the external fluid in the glass container. The release assays were performed for about 75 h (ca. 4500 min) for $VitB_{12}$ or about 150 h (ca. 9000 min) for DFK, in order to ensure that release equilibrium was attained. The solute concentration in each aliquot was calculated through the use of the corresponding analytical curves, in the concentration range from 0 to 11 mg L^{-1} for $VitB_{12}$ and from 0 to 16 mg mL^{-1} for DFK.

The values of the released solute fraction, F_R, for each run were calculated based on the amount of solute released from each aliquot and the total amount of solute loaded in the hydrogel sample. The value of F_{max} equivalent to F_R under equilibrium conditions allows the calculation of the corresponding α value using equation 1. Release kinetic constants, k_R, were computed using linear fitting of equations 7 and 8, derived from equations 4 and 5, respectively, to the experimental data.

$$F_{\max} \ln\left(\frac{F_{\max}}{F_{\max} - F_R}\right) = k_R\, t \quad (7)$$

$$\frac{\alpha}{2} \ln\left(\frac{F_R - 2F_R F_{\max} + F_{\max}}{F_{\max} - F_R}\right) = k_R t \quad (8)$$

In order to better characterize the release systems, half-life time, $t_{1/2}$, for both equations 4 and 5, was calculated, since $t_{1/2}$ corresponds to the time necessary to release 50% of the maximum released drug fraction, that is, $F_R = 0.5F_{max}$. So, equations 9 and 10 are derived from equations 4 and 5, respectively:

$$t_{1/2} = \frac{F_{max}}{k_R} \ln 2 \quad (9)$$

$$t_{1/2} = \frac{\alpha}{2k_R} \ln(3 - 2F_{max}) \quad (10)$$

Results and Discussion

Release of $VitB_{12}$ from AGm-DMAAm hydrogels

Tests in hydrogel without $VitB_{12}$ (blank sample), for evaluating release of any compounds from the blank, showed that no release was detected at λ = 358 nm. Figure 2a-b shows representative plots of the release kinetics, in its linear form (equations 7 and 8), of $VitB_{12}$ from the AGm-DMAAm hydrogel matrix immersed in 250 mL of external fluid. It can be seen that the best fit was obtained using the first-order kinetic law equation (R^2 = 0.99875) rather than the second-order kinetic law equation (R^2 = 0.98848). In the case of similar determination coefficients (R^2) obtained using equations 7 and 8, this suggests, on fundamental grounds, that the release will follow the first-order kinetic law if $t_{1/2}$ is independent of the kinetic law equation (equations 9 and 10); however, this is not the case and, consequently, it can be concluded that the release of $VitB_{12}$ follows first-order kinetics. The fitting procedure shown in Figure 2a-b was based on the experimental determination of F_{max}, which allows for the direct computation of respective α values (Table 1). By keeping these parameters constant for given experimental conditions, the fitting of a straight line equation to the experimental data will allow the direct computation of

rate constant, k_R, which is equal to the slope of that straight line. The procedure was performed for all conditions (three external fluid volumes or three temperatures) as reported and discussed in this paper.

Figure 3 presents the experimental and the predicted profiles for the release of $VitB_{12}$ from AGm-DMAAm hydrogels. The predicted release profiles were obtained by applying equation 4 to the calculated data (F_{max} from equation 1; k_R, from plots of Figure 2).

The experimental data at equilibrium (F_{max}, α and t_{eq}) and the corresponding kinetic data (k_R, α/k_R and $t_{1/2}$) computed by fitting equations 7 and 9 to $VitB_{12}$ release data are shown in Table 1. The goodness of fit was evaluated by the analysis of the obtained determination coefficients values (R^2). As can be seen from the analysis of Figure 3, the partition-diffusion mathematical model, in its first law equation, describes the release of $VitB_{12}$ from AGm-DMAAm matrix very well, for the whole release profile. From the analysis of Figure 3 and the data in Table 1, it is also worth noting the following: in the release of $VitB_{12}$ using 250 mL of external fluid, equilibrium was reached after 2,256, 1,680 and 1,320 min, when the assays were performed at 25, 35 and 45 °C, respectively; i.e., the equilibrium was reached faster as the temperature increased. However, such a trend did not depend on the volume of the external fluid, for a given temperature.

A detailed analysis of the data shown in Table 1 allowed us to verify that release was affected by the temperature. Values of F_{max} increased if the temperature is increased and, consequently, the α and k_R parameters were also affected. This can be simply justified by the dependence of α on F_{max}, according to equation 1. Furthermore, it is worth mentioning that for the three external fluid volumes evaluated, the value of the α/k_R ratio is constant, for a given temperature; i.e., under the investigated conditions, it did

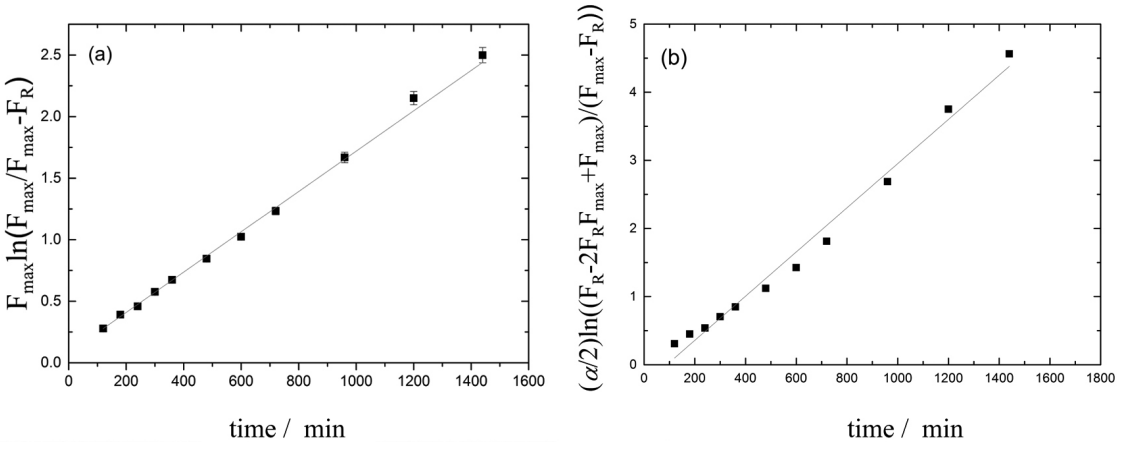

Figure 2. Dependence of the left terms of equations 7 and 8 on the release time for $VitB_{12}$ release from AGm/DMAAm hydrogels immersed in 250 mL of external fluid at 25 °C. Solid lines show the fitting of: (a) equation 7 and (b) equation 8 to the experimental data.

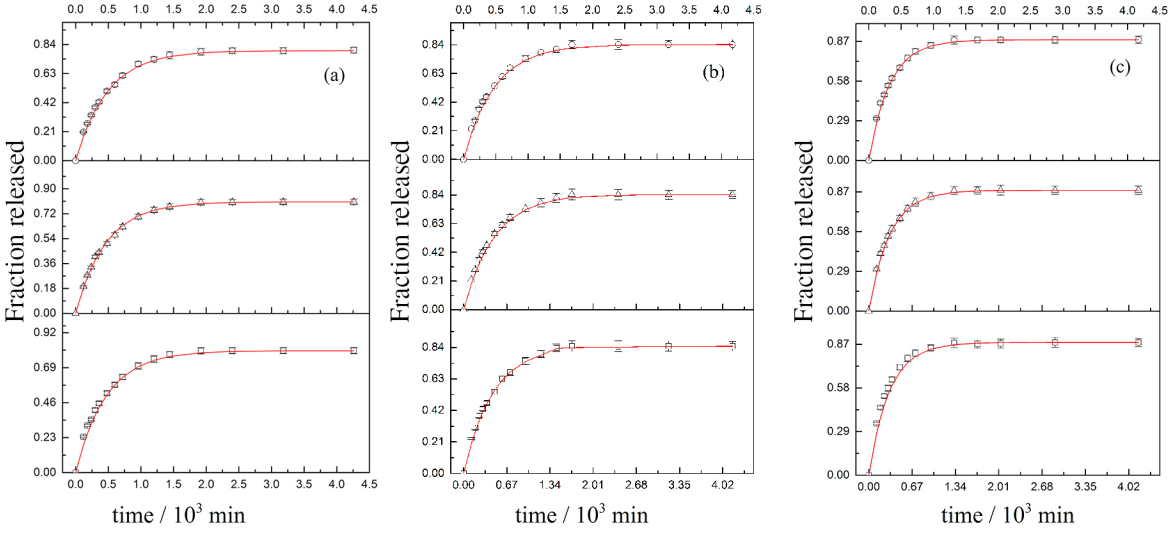

Figure 3. Experimental and predicted profiles for $VitB_{12}$ release from AGm-DMAAm hydrogel immersed in three different volumes (250 (□), 350 (△) and 450 mL (○)) of external fluid at: (a) 25 °C, (b) 35 °C, and (c) 45 °C. Solid lines were estimated using equation 4. The fitting parameters are shown in Table 1.

Table 1. Maximum released fraction (F_{max}), partition activity (α) and kinetic parameters (k_R, t_{eq}, $t_{1/2}$, t_{eq}) and the ratio α/k_R obtained for $VitB_{12}$ releasing from AGm-DMAAc hydrogels at three different volumes of external fluid and three different temperatures

T / °C		25			35			45	
V / mL	250	350	450	250	350	450	250	350	450
F_{max}	0.80 (± 0.02)	0.81 (± 0.02)	0.79 (± 0.02)	0.85 (± 0.03)	0.84 (± 0.03)	0.84 (± 0.04)	0.88 (± 0.03)	0.88 (± 0.03)	0.88 (± 0.03)
a	4.0 (± 0.1)	4.1 (± 0.1)	3.9 (± 0.1)	5.6 (± 0.3)	5.4 (± 0.3)	5.3 (± 0.3)	7.5 (± 0.3)	7.5 (± 0.4)	7.5 (± 0.3)
k_R / (10^{-3} min^{-1})	1.70 (± 0.08)	1.74 (± 0.07)	1.62 (± 0.07)	1.9 (± 0.1)	1.8 (± 0.1)	1.8 (± 0.1)	2.8 (± 0.2)	2.7 (± 0.2)	2.8 (± 0.1)
R^2	0.998	0.997	0.996	0.997	0.999	0.999	0.998	0.999	0.996
α/k_R / (10^3 min)	2.4 (± 0.1)	2.4 (± 0.1)	2.4 (± 0.1)	3.0 (± 0.2)	3.0 (± 0.2)	3.0 (± 0.3)	2.7 (± 0.2)	2.7 (± 0.2)	2.7 (± 0.2)
t_{eq} / (10^3 min)	2.25	2.25	2.26	1.68	1.68	1.68	1.32	1.33	1.32
$t_{1/2}$ / min	327	321	340	314	330	334	222	223	222

not depend on the volume of the external fluid used in the $VitB_{12}$ release process. However, the dependence of α/k_R on the temperature showed a maximum at 35 °C. Thus, the initial hypothesis that the α/k_R ratio does not depend on the volume of the external fluid was confirmed for the investigated volume and temperature range, and assuming that $VitB_{12}$ release can be treated by the partition-diffusion mathematical model in its first order kinetic law equation.

Release of DFK from AGm-DMAAm hydrogels

For the *in vitro* assays of DFK release from AGm/DMAAm hydrogels, the values of F_R were obtained in a similar way as for $VitB_{12}$, using equation 1. Tests in hydrogel without DFK (blank sample), for evaluating release of any compounds from the blank, showed that no release was detected at λ = 271 nm. In sequence, the computation of k_R values was carried out as described in the previous section for $VitB_{12}$, i.e., by plotting the data according to equations 4 and 5. As an example, the plots obtained for DFK using the release data for 250 mL of external fluid at 25 °C are presented in Figure 4.

In contrast to what was observed for $VitB_{12}$ release, the release of DFK was better described by the second order kinetic equation (equation 5). In fact, the fitting equation 7 to experimental data showed a poor correlation (R^2 = 0.9566) when compared with the fitting obtained using equation 8 (R^2 = 0.9985). Under different experimental conditions, the conclusions about the equation that better fits the experimental release of DFK are similar. Thus, equation 5 was used to obtain the predicted release profiles for DFK. The experimental and predicted profiles for the three different volumes of external fluid, i.e., 250, 350 and 450 mL at 25, 35 and 45 °C are shown in Figure 5. It can be seen that the predicted profiles match very well to the experimental ones, in the whole range of release time.

Table 2 shows the experimental data (F_{max}, α and t_{eq}) collected under equilibrium conditions for the release of DFK and the respective kinetic parameters (k_R, α/k_R and $t_{1/2}$) calculated using the second-order kinetic law equation developed from the partition-diffusion model. From the analysis of Table 2, one can see that the temperature was an important factor in the release of DFK from AGm-DMAAm hydrogels. The maximum release fraction, F_{max}, was not affected by temperature as only a slightly difference of 4% was observed when the temperature increased from 25 °C to 45 °C; this is slightly below the experimental error (ca. 5%). However, such a small effect was significant enough to be reflected in a non-negligible increase in partition activity, α, when the temperature increased from 25 °C to 35 °C,

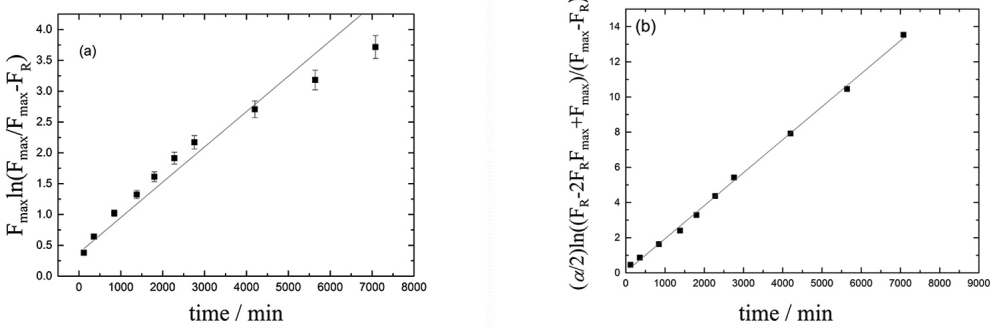

Figure 4. Dependence of the left terms of equations 7 and 8 on the release time of DFK from AGm/DMAAm hydrogels immersed in 250 mL of external fluid at 25 °C. Solid lines show the fitting of: (a) equation 7 and (b) equation 8 to the experimental data.

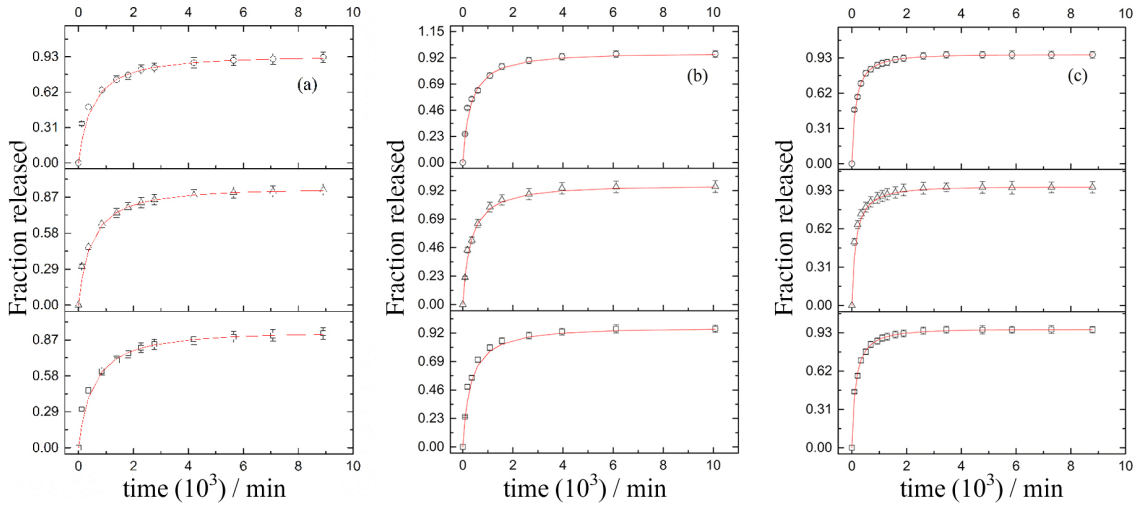

Figure 5. Experimental and predicted profiles for DFK release from AGm-DMAAm hydrogel immersed in three different volumes (250 (□), 350 (△) and 450 mL (○)) of external fluid at: (a) 25 °C, (b) 35 °C, and (c) 45 °C. Solid lines were estimated using equation 5. The fitting parameters are shown in Table 2.

although this remained unchanged by a further increase to 45 °C. Looking to the k_R values, it is possible to observe an increase in the kinetic constant with an increase in temperature from 25 °C to 35 °C, and to 45 °C as well. This behavior can be explained by the fact that, at 35 °C, the system achieved the maximum of F_{max} value for DFK release, i.e., a further increase in temperature did not allow for a sufficient increase in the mobility of the polymer chains to lead to a substantial increase in F_{max}, but this did increase the rate of release. In this way, it can be inferred that after the maximum value of F_{max} is achieved, a further increase in temperature exerts more influence in terms of diffusion (related to solute transport within the pores) than in terms of partition (related to interactions between the solute-hydrogel and solute-fluid).

Evaluation of the parameters α, k_R and α/k_R for the solutes $VitB_{12}$ and DFK

To better understand the effect of temperature on α, k_R and the α/k_R ratio related to the release of $VitB_{12}$ and DFK from AGm-DMAAm hydrogels, plots of α *vs.* T and k_R *vs.* T were made and are shown in Figures 6 and 7.

Important information can be taken from the analysis of Figure 6. Firstly, it became evident that for the investigated temperature range, the temperature exerted more influence

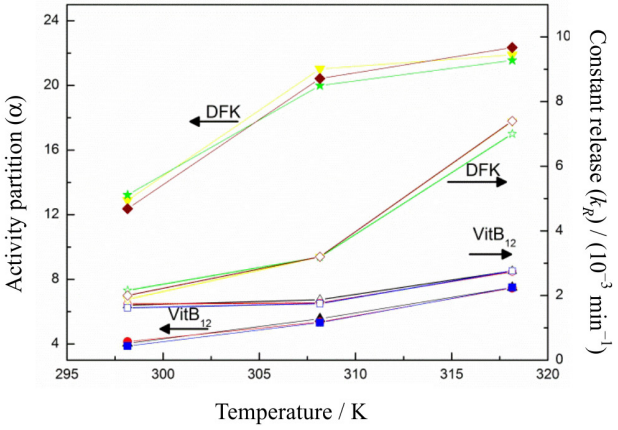

Figure 6. Dependence of α (filled symbols) and k_R (open symbols) on the temperature of release of $VitB_{12}$ and DFK from AGm-DMAAm hydrogels. Error bars were not added for simplicity.

Table 2. Maximum released fraction (F_{max}), partition activity (α) and kinetic parameters (k_R, t_{eq}, $t_{1/2}$, t_{eq}) and the ratio α/k_R obtained for release of DFK from AGm-DMAAc hydrogels at three different volumes of external fluid and three different temperature

T / °C	25			35			45		
V / mL	250	350	450	250	350	450	250	350	450
F_{max}	0.92 ± 0.05	0.93 ± 0.05	0.92 ± 0.05	0.95 ± 0.03	0.95 ± 0.05	0.95 ± 0.03	0.96 ± 0.03	0.96 ± 0.05	0.96 ± 0.03
α	12.1 ± 0.9	13.3 ± 0.9	12.4 ± 0.9	21.0 ± 0.9	20 ± 1	20.4 ± 0.9	22.0 ± 0.8	22 ± 2	22 ± 1
k_R / (10^{-3} min^{-1})	1.9 ± 0.3	2.2 ± 0.2	2.0 ± 0.3	3.2 ± 0.3	3.2 ± 0.3	3.2 ± 0.3	7.39 ± 0.4	7.00 ± 0.6	7.4 ± 0.5
R^2	0.998	0.998	0.997	0.997	0.999	0.995	0.998	0.999	9.990
α/k_R / (10^3 min)	6.4 ± 0.7	6.1 ± 0.6	6.2 ± 0.7	6.6 ± 0.4	6.3 ± 0.6	6.4 ± 0.5	3.0 ± 0.2	3.0 ± 0.3	3.0 ± 0.2
t_{eq} / (10^3 min)	8.91	8.91	8.91	6.10	6.10	6.10	3.36	3.35	3.34
$t_{1/2}$ / min	457	403	431	286	285	285	124	131	124

on α and k_R for DFK release than for $VitB_{12}$. However, the influence of temperature on AGm-DMAAm/DFK system was relatively reduced by changing from 35 °C to 45 °C as compared to the change from 25 °C to 35 °C in the same system. However, the global effect of temperature on the AGm-DMAAm/DFK system was greater than in the AGm-DMAAm/$VitB_{12}$ system. This observation applies to α and k_R parameters. Obviously, this fact will exert an influence on the α/k_R ratio values. Although the maximum released fraction (F_{max}) of DFK, at equilibrium, was around 12.5% higher than that of $VitB_{12}$, it should be emphasized that the α values for the AGm-DMAAm/DFK system were ca. 3 to 5 fold higher than the respective values for the AGm-DMAAm/$VitB_{12}$ system. The k_R values increased, in a similar way, by increasing the temperature from 25 to 45 °C. Another important observation is that the k_R values for both systems were very similar at 25 °C, but became quite distinct as the temperature is increased. The increase in temperature provoked a greater increase in k_R for the AGm-DMAAm/DFK system as compared to the AGm-DMAAm/$VitB_{12}$ system. The k_R for DFK was 2.6 fold higher at 45 °C. The fact that the k_R values for $VitB_{12}$ and DFK were almost the same at 25 °C but different at 35 °C and 45 °C implies that the difference in the molecular weight of the solutes ($VitB_1$ 1,355.3 Da; DFK 296.2 Da) could influence the release process, as it is also dependent on the free volume of hydrogel that expands at higher temperatures. It is worth mentioning that hydrogel expansion due to swelling is concomitant to the drug release process. However, drug-matrix interactions will also influence the release process, as discussed below.

Figure 7 shows the dependence of the α/k_R ratio on the temperature for both systems, in the three different volumes of external fluid. As highlighted and discussed above, the values of the α/k_R ratio practically do not change with the volume of the external fluid, at a given temperature; i.e., whilst for the AGm-DMAAm/$VitB_{12}$ system, the α/k_R ratios were quite similar, for the AGm-DMAAm/DFK system, changes in the ratio values were less than 3%.

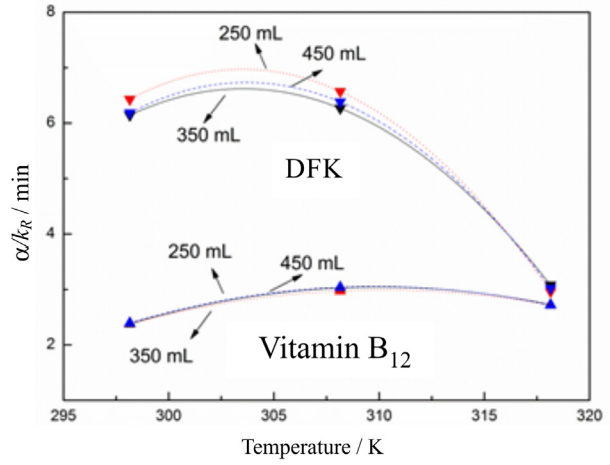

Figure 7. Dependence of α/k_R to the temperature of release of $VitB_{12}$ and DFK from AGm-DMAAm hydrogels in three different volumes of external fluid.

However, for both systems, the dependence of the α/k_R ratio on temperature followed a convex trend with a maximum at 35 °C, and was significant for the AGm-DMAAm/DFK system. Another interesting observation was found as the trends of α/k_R for the two diffusing species were compared: the α/k_R ratio values for $VitB_{12}$ and DFK were almost similar at 45 °C but quite different at 25 °C. This clearly suggests that mass transport is activated by temperature. The different trends presented by the systems for the α/k_R ratio at 35 °C and 45 °C were due to changes in k_R rather than α; only minor changes were found for the latter. It was inferred that possible solute-matrix and solute-external fluid interactions may be the responsible for the distinct behaviors presented by each system. Thus, the activation energy for release, Ea_R, was calculated for each diffusing drug type, at a fixed volume of external fluid.

Determination of the activation energy for release, Ea_R

The activation energies for release, Ea_R, of $VitB_{12}$ and DFK from AGm-DMAAm hydrogels, at a given volume of external fluid, were obtained from the slope of ln k_R *vs.* 1/T plot, as shown in Figure 8. The calculation was based on the Arrhenius equation:

$$k_R = Ae^{-Ea_R/RT} \quad (11)$$

For the AGm-DMAAm/$VitB_{12}$ system, the slope was lower compared to that of the AGm-DMAAm/DFK system, regardless of the volume of the external fluid. The values of Ea_R calculated from the slopes of the curves presented in Figure 8 are presented in Table 3. The value of Ea_R for $VitB_{12}$ was ca. 3 times lower that of DFK. The difference was correlated to the presence of more intense interactions in the AGm-DMAAm/DFK system. Considering the chemical structures of both solutes, as shown in Figure 1, the existence of stronger DFK-hydrogel matrix interactions can be addressed. It can be anticipated that the $VitB_{12}$ structure is not strongly affected by the pH (at pH 6.0); however DFK, shows in its structure carboxyl groups that can undergo ionization (pKa close to 4.0).[30] The absence of ionizable groups in $VitB_{12}$ suggests the occurrence of weak interactions with water and also with the 3D matrix. Consequently, $VitB_{12}$ can be entrapped in the matrix and diffuses out basically through polymeric chain relaxation. In the absence of strong interactions between the solute

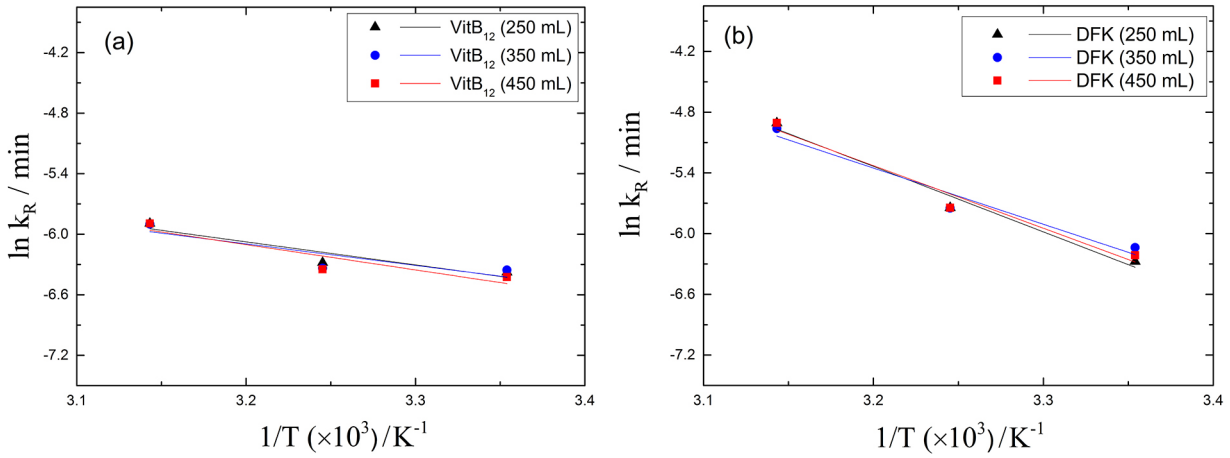

Figure 8. Plot of ln k_R against 1/T for $VitB_{12}$ (a) and DFK (b) release from AGm-DMAAm hydrogels, at three different volumes of external fluid.

Table 3. Values for activation energy, E_{a_R}, for $VitB_{12}$ and DFK releasing from AGm-DMAAm hydrogels at three different volumes of external fluid, obtained by applying the Arrhenius plot

Solute	$VitB_{12}$			DFK		
V / mL	250	350	450	250	350	450
E_{a_R} / (kJ/mol)	19.0 ± 3.2 (R^2 = 0.92)	17.7 ± 3.5 (R^2 = 0.92)	20.8 ± 3.8 (R^2 = 0.91)	53.8 ± 6.0 (R^2 = 0.96)	46.2 ± 6.2 (R^2 = 0.91)	51.4 ± 6.7 (R^2 = 0.94)

and the 3D matrix, the release of the solute occurs mainly through the pores of the hydrogel. In such case, the channels formed by the pores are the preferential pathways for diffusion, and the size of the pores and the solute molecule are important factors in the release process.

As in this study the pH was fixed at 6.0, thus higher than the pKa of the carboxyl groups of DFK close to 4.0.[30] Under these conditions, such groups are negatively charged and should interact with the matrix of the AGm-DMAAm hydrogel that, at this pH, is positively charged at its nitrogen atoms. In this way, the porosity of the matrix, in conjunction with electrostatic and other dipole-dipole interactions like H-bonds, are important factors for the release of DFK from AGm-DMAAm hydrogels. This also explains the fact that the release of DFK could be modeled using a second order kinetic law.

Another aspect that can be considered in this discussion is related to the values of F_{max} obtained for the release of both solutes from the AGm-DMAAm hydrogel. If F_{max} is equal to 1.0, it means that almost 100% of the solute, initially loaded in the matrix, diffused out to the external fluid. Under these conditions, it can be inferred that the solute has strong affinity for the fluid and no affinity for the matrix. In the case of the solute possessing equal affinity for the matrix and the fluid, the value of F_{max} should be 0.5; this is equivalent to α equal to 1.0 (equation 1). For both solutes, values of F_{max} were higher than 0.5 and α > 1.0; this demonstrates that both solutes possess the tendency to diffuse out instead of remaining inside the matrix. However, another issue that should be considered is the smaller size of the DFK molecule compared to $VitB_{12}$, considering that the ratio of $VitB_{12}$/DFK molecular weights is about 5 fold. This would allow easier release of DFK, especially if an increase in temperature leads to weaker DFK-gel interactions. Thus, based on the data in Tables 1 and 2, ca. 92% of the loaded DFK was released (F_{max} = 0.92) at 25 °C; however, only ca. 80% of $VitB_{12}$ was released (F_{max} = 0.80) at this temperature. The amount (mg/g) of loaded solute, in both cases, was almost the same. In this way, at 25 °C, a higher amount of DFK was released as compared to $VitB_{12}$, even though DFK has stronger interactions with the matrix. This is likely be due to the smaller size of DFK compared to $VitB_{12}$.

The effect of matrix-solute interactions can be evaluated, in a semi-quantitative way, by the dependence of the α parameter on temperature. The change from 25 °C to 45 °C led to an increase in α from ca. 4.0 to 7.5 for the release of $VitB_{12}$, while for DFK release, such a change in temperature led to an increase in α from ca. 12.5 to 22.5. As stressed in the above discussion, this was attributed to interactions between DFK and AGm-DMAAm. This was corroborated by the longer time needed to achieve equilibrium for DFK release as compared to $VitB_{12}$ release under the same conditions of temperature and volume of the external fluid, despite the smaller size of DFK. Although the F_{max} for DFK (F_{max} = 0.92) was higher than for $VitB_{12}$

(F_{max} = 0.80), t_{eq} was much higher for DFK (ca. 9,000 min) as compared to $VitB_{12}$ (ca. 2,250 min). This confirms that the mechanisms for release the two solutes are different.

As expected, the values of t_{eq} for both solutes decrease as the temperature increases. However, the reduction was more significant for DFK than for $VitB_{12}$. For $VitB_{12}$, the value of t_{eq} at 45 °C was ca. 58% of the respective value at 25 °C. For DFK, the t_{eq} at 45 °C was only 37% of the value at 25 °C. As DFK/matrix interactions were weakened by an increase in temperature, DFK diffusion out of the matrix was facilitated and became more dependent on polymer chain relaxation (or the pores of the 3D matrix). In this way, an increase in temperature evidently intensified more the DFK release than $VitB_{12}$ one. The same conclusion can be made taking into account the dependence of $t_{1/2}$ on temperature for both solutes.

Broken or weakened matrix-solute interactions can be thermally triggered.[31] In this way, considering the release of a solute from the AGm-DMAAm hydrogel matrix to the external fluid, the value of Ea_R should be higher for DFK than for $VitB_{12}$. The values of Ea_R shown in Table 3 are consistent with this and with the values published in the literature for other systems.[32]

Conclusions

The partition-diffusion mathematical model[17] predicted, with robustness, almost 100% of the profile for $VitB_{12}$ and DFK release from an AGm-DMAAm (60-40, wt%) hydrogel, using a KH_2PO_4/NaOH solution [ionic strength = 0.1 mol L^{-1}, at pH 6.0] as the external fluid. The results show that $VitB_{12}$ release from the AGm-DMAAm hydrogel could be fitted to a first order kinetic model, while DFK release from the same hydrogel matrix was predicted by a second order kinetic model. The temperature was a factor that significantly affected the release process and, as a consequence, a change of 10 °C provoked alterations in the partition activity (α) and release rate constant (k_R). Such a change in temperature had a greater effect on DFK release from the AGm-DMAAm hydrogel than on $VitB_{12}$ release. This was attributed to the existence of stronger interactions between DFK and the hydrogel matrix as compared to $VitB_{12}$ and the matrix.

The partition-diffusion mathematical model allows for calculating several kinetic parameters, such as the rate constant for release (k_R) and the half-life time $t_{1/2}$ for the release process. The activation energy for release, Ea_R, was obtained from the dependence of k_R on the temperature. The values of Ea_R are consistent with those published in the literature for other systems. The initial hypothesis that the α/k_R ratio does not depend on the volume of external fluid was confirmed within the range of investigated volumes. However, the α/k_R ratio changed with the temperature. So, this ratio can be considered as an intensive parameter for a given system at a fixed temperature. The results presented in this work contribute to consolidating the partition-diffusion mathematical model[17] for predicting the release profile. The data collected using this model are sensitive to the presence of solute-matrix interactions, so it can be used to elucidate different behaviors often observed in drug release systems. It is expected that improvements in the partition-diffusion mathematical model will widen its use in different fields, such as pharmacy and agriculture.

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