

***In-vitro* release from reverse poloxamine/ $\alpha$ -cyclodextrin matrices. Modelling and comparison of dissolution profiles.**

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**ABSTRACT:** Gels obtained by complexation of octablock star PEO/PPO copolymers (Tetronic 90R4) with  $\alpha$ -CD were evaluated as matrices for drug release. Both molecules are biocompatible so they can be potentially applied to drug delivery systems. Two different types of matrices of Tetronic 90R4 and  $\alpha$ -CD were evaluated: gels and tablets. These gels are capable to gelifying in-situ and show sustained erosion kinetics in aqueous media. Tablets were prepared by freeze drying and comprising the gels. Using these two different matrices the release of two model molecules, L-Tryptophan (Trp), and a protein, bovine serum albumin (BSA), was evaluated. The release profiles of these molecules from gels and tablets prove that they are suitable for sustained delivery. Mathematical models were applied to the release curves from tablets in order to elucidate the drug delivery mechanism. Good correlations were found for the fittings of the release curves to different equations. The results point that the release of Trp from different tablets is always governed by Fickian diffusion while the release of BSA is governed by a combination of diffusion and tablet erosion.

## **Keywords**

Cyclodextrins, self-assembly, supramolecular gels, erosion kinetics, controlled release

## **Introduction**

Hydrogel matrices have been gaining relevance throughout the years as drug delivery systems or even as medical devices.<sup>1, 2</sup> This is due mainly to their hydrophilicity, their biocompatibility and their drug loading and release promising properties. Hydrogels can be loaded with a drug or an active substance and be implanted into a patient enabling a controlled drug release. Another type of hydrogels is *in situ* gelling systems. They

possess new promising properties such as a fast degradation process or the capability of forming the gel where it is needed by physical crosslinking between different molecules. In this process of gelation, a protein, a peptide or a drug can be added to the mixture before the crosslinking takes place, so it will finally get immobilised inside the bulk of the gel.<sup>3-8</sup> This last property is very important because it allows us to inject the mixture inside a tissue using a simple needle without any kind of surgery.<sup>9, 10</sup> Self-assembled hydrogels constitute an important family of these *in situ* gelling systems. These kind of hydrogels are supramolecular systems that are formed spontaneously when their constituents come into contact or else after a certain trigger such as temperature<sup>10</sup>. The interest on these materials has grown in recent years.<sup>3, 5-7, 9-11</sup> Self-assembled hydrogels can be obtained using a large variety of natural or synthetic products, and their properties depend strongly on their composition. A group of molecules that are extensively used for this purpose are the amphiphilic polymers.

Amphiphilic polymers have been widely used in drug delivery systems. They can form micelles or aggregates that increase the solubility of hydrophobic drugs and can be applied as drug delivery systems.<sup>12</sup> One of the most common amphiphilic groups of molecules is polyethylene oxide/polypropylene oxide (PEO/PPO) block copolymers.

BASF produces mainly two different types of PEO/PPO block copolymers, namely Pluronic (or poloxamers) and Tetronic (or poloxamines). Pluronics are linear triblock copolymers with the architecture PEO-PPO-PEO. Tetronics are four arm star-like copolymers with two blocks per arm. Depending on the distribution of the blocks in its arms, the molecule can be a Tetronic (normal) or a Tetronic R (reverse). The former have the PEO blocks in the outer part of the arms, while in the latter the PEO blocks are the inner ones.

PEO/PPO block copolymers can be employed as micellar nanocarriers for drug delivery due to their high solubilisation capacity and biocompatibility. These systems are not just inert carriers but they can act as biological response modifiers because they can induce certain cellular responses, such as inhibition of cell efflux transporters and ability to increase drug transport across membranes, which can be advantageous from the therapeutic point of view.<sup>13-15</sup> As a matter of fact, poloxamines have been successfully employed in the preparation of different therapeutic systems, such as micellar drug carriers<sup>16</sup> and nanoparticles.<sup>17,18</sup> In this sense, nanoparticle surface modification with poloxamines was found to determine the biological fate of certain carrier systems. In addition, these polymers have been applied in the field of injectable implants.<sup>19</sup> For instance, cytocompatible implantable matrices for sustained release of ciprofloxacin have been prepared using poli- $\epsilon$ -caprolactone/poloxamine blends.<sup>20</sup>

Some of these amphiphilic copolymers can gelify by themselves in aqueous solutions under certain conditions.<sup>16-19</sup> The addition of cyclodextrins (CDs) enhances the gelation process for PEO/PPO block copolymers, even to the point of making feasible the formation of gels with poloxamers or poloxamines that do not gelify by themselves.<sup>20,21,22</sup> It has been shown that the addition of  $\alpha$ -cyclodextrin ( $\alpha$ -CD) to PEO-PPO solutions leads to the formation of inclusion complexes between  $\alpha$ -CD and the PEO blocks with a polypseudorotaxane structure and the aggregation between uncomplexed PPO blocks by hydrophobic interactions, yielding gels.<sup>20, 21,9,23</sup>

Diverse types of hydrogels based on cyclodextrins have been prepared previously.<sup>24,25</sup> Nevertheless, those were chemically crosslinked gels, so the *in situ* physical hydrogels formed with CD and PEO/PPO block copolymers are a good alternative. Recently, polypseudorotaxanes made of a normal Tetronic (T908) and  $\alpha$ -CD have been used to prepare syringeable viscoelastic gels. Besides a good compatibility,

these materials combined with simvastatin showed an intrinsic ability to promote osteoblast differentiation.<sup>31</sup>

In the present study, an *in situ* hydrogel prepared using  $\alpha$ -CD and the reverse Tetronic 90R4 (90R4) was evaluated as a drug delivery device. The model molecules released from these gels were an aminoacid, L-Tryptophan (Trp), and a protein, bovine serum albumin (BSA). The 90R4/CD gels have proved to be erodible matrices, so they seem to be promising as drug release devices.<sup>20</sup> The release of Trp and BSA from these 90R4/CD matrices was evaluated using 90R4/CD hydrogels, and also with tablets made from these hydrogels. Different mathematical models were used in order to elucidate the mechanisms that predominate in the release processes from the tablets.

## EXPERIMENTAL

**Materials.** Tetronic 90R4 ( $M_w$  = 6900 g/mol) was kindly donated by BASF and it was used as received. This poloxamine is a yellow liquid (viscosity 3870 cP at 25°C). The composition of the four blocks in the polymer, determined by <sup>1</sup>H-NMR (Bruker DPX 300 at 298 K), is PO<sub>16</sub>EO<sub>18</sub> per arm.  $\alpha$ -CD was obtained from Wacker Chemie AG Cavamax® W6 Pharma Alfadex) and was used without further purifications. L-Tryptophan (Trp) and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich and were used as received.

**Gel formation.** All complexes were prepared by mixing a determinate amount of the 90R4 poloxamine with an aqueous solution of  $\alpha$ -CD followed by vigorous stirring. Before using them, the resulting mixtures were kept at room temperature for at least one night. Some of the gels were loaded with Trp or BSA in order to perform release

studies. These substances were previously dissolved in the  $\alpha$ -CD solution before mixing it with the poloxamine.

**Tablet preparation.** The resulting gels were freeze dried and the obtained product was compressed using a Specac pellet die. The resulting tablets were weighed and measured prior to their use (see Results and Discussion section). The hardness of the obtained samples was measured using a Caleva Tablet Hardness Tester (see Supporting Information for details). Freeze dried gels loaded with Trp or BSA were used to prepare tablets for release kinetics studies. Besides, additional tablets made from unloaded gels were used for the erosion kinetics analysis. Each BSA tablet contained 200 mg of the protein. On the other hand, the Trp tablets contained 30 mg of the amino-acid for the tablets prepared using 15% (w/w) of Tetronic and 20 mg of the amino acid for the tablets prepared using 25% (w/w).

**Tablet erosion studies.** Seven unloaded tablets were placed into a SOTAX AT 7 Smart USP dissolution testing device at 37 °C in 500 mL of pH 7 phosphate buffer medium; different experiments were performed using stirring speeds of 25 and 100 rpm. During the dissolution process, one tablet was removed from its vessel at different time intervals (see Supporting Information, Figure S1). These partially eroded tablets were placed in an oven at 37 °C until constant weight. Using the initial weight of the tablet ( $W_0$ ) and the weight after been eroded ( $W_1$ ), the percentage of erosion at each time was calculated with the following equation (Eq 1):

$$\% \text{ Erosion} = \frac{W_0 - W_1}{W_0} \times 100 \quad (1)$$

***In vitro* release kinetics.** The release studies were also performed from 500 mL of pH 7 phosphate buffer solutions using a SOTAX AT 7 Smart USP dissolution testing device at 37°C at 25 rpm or 100 rpm stirring speeds. Samples (5 mL) were withdrawn according to a sampling time program of about 10 h, and these sample volumes were replaced with fresh medium. Trp (or BSA) concentrations in each sample were evaluated by UV-vis (Hewlett Packard 8452A) and fluorescence spectrophotometries (Perkin Elmer LS 50 B). Both the poloxamine and the substrate (BSA or Trp) absorb in the same UV region. On the other hand, the fluorescence of Trp (or BSA) is quenched by the poloxamine that is also released as the matrices are eroded. A combination of the results obtained from these two techniques had to be used in order to determine the amount of substrate released as a function of time (see Supporting Information for more details). In addition, it was verified that  $\alpha$ -CD, which is also present in the solution during the erosion process, is not a quencher of Trp. The amount of  $\alpha$ -CD released from the matrix was also evaluated, using size exclusion chromatography (SEC) (Waters 600E with a Waters 2414 Refractive Index detector and an Aquagel OH-30 column). The mobile phase was deionized water and the flow rate was 1 mL/min. The obtained value for the area under the curve for the samples was compared to standard solutions to obtain the concentration of  $\alpha$ -CD. Different release experiments were carried out using gels and tablets. In order to study the release kinetics from the gels, they were formed at the bottom of the dissolution testing device vessels and the dissolution medium was added on top of them at the beginning of the release kinetics. An amount of 20 g of gel was used in each experiment. For the tablets, the standard measuring method was used: the tablets were added into the vessels filled with the buffer media in order to start the release kinetic analyses. The experiments were performed in duplicate for the gels and in quadruplicate for the tablets.

**Analysis of release data.** Different parameters were calculated with the release data obtained from gels and tablets. The mean dissolution time (MDT) can be calculated using the following equation (Eq 2).

$$MDT = \frac{\sum_{j=1}^n \hat{t}_j \cdot \Delta M_j}{\sum_{j=1}^n M_j} \quad (2)$$

where  $j$  is the sample number,  $n$  is the number of dissolution sample times,  $\hat{t}_j$  is the time at the midpoint between  $t_j$  and  $t_{j-1}$  and  $\Delta M_j$  is the additional amount of drug dissolved between  $t_j$  and  $t_{j-1}$ .

The dissolution efficiency (DE) of a pharmaceutical dosage form is defined by the percentage of area under the dissolution curve up to a certain time  $t$  expressed as a percentage of the area of the rectangle described by 100% dissolution ( $y_{100}$ ) in the same time (Eq 3).<sup>26</sup>

$$DE = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100 \quad (3)$$

In order to compare different release curves, the difference ( $f_1$ ) and similarity factor ( $f_2$ ) were calculated.<sup>26</sup>

The difference factor ( $f_1$ ) (Eq. 4) calculates the percent (%) difference between the two curves at each time point and is a measurement of the relative error between the two curves: where  $n$  is the number of time points,  $R$  is the dissolution value of the reference  $t$  (prechange) batch at time  $t$ , and  $T$  is the dissolution value of the test (postchange) batch at time  $t$ .



$$f_1 = \left\{ \left[ \sum_{j=1}^n |R_t - T_t| \right] / \left[ \sum_{j=1}^n R_t \right] \right\} \cdot 100 \quad (4)$$

The similarity factor ( $f_2$ ) (Eq. 5) is a logarithmic transformation of the sum-squared error of differences between the test  $T_j$  and reference products  $R_j$  over all time points,  $n$ .

$$f_2 = 50 \cdot \log \left\{ \left[ \left( 1/n \right) \sum_{j=1}^n (R_t - T_t)^2 \right]^{-0.5} \cdot 100 \right\} \quad (5)$$

The data obtained from the *in vitro* release experiments were fitted to different mathematical models of drug release. Several mechanisms are involved in drug release from hydrogel matrix systems: diffusion, swelling, and polymer erosion being the most important ones. Different mathematical models have been proposed to obtain information of the mechanisms, since it is difficult to develop a general expression suitable for every drug device. The most interesting models are the empirical/semi-empirical ones, which provide an accurate fit to the experimental profiles (such as the Peppas equation and the Hopfenberg model), and the mechanistic realistic models, which give some insight into the real phenomena (such as those based on Fick's law).<sup>33</sup> The models used for this study were the Korsmeyer-Peppas equation (Eq. 6), the Higuchi equation (Eq. 7), the Peppas-Sahlin equations (Eq. 8a), the zero-order equation (Eq. 9a), the first-order equation (Eq. 10) and the Hopfenberg equation (Eq. 11).

The Korsmeyer-Peppas model<sup>27</sup> is a simple semiempirical model which exponentially relates drug release with the elapsed time (Eq. 6).

$$\frac{M_t}{M_\infty} = k_{KP} \cdot t^n \quad (6)$$

where  $M_t/M_\infty$  is the drug release fraction at time  $t$ ,  $k_{KP}$  is a constant incorporating the structural and geometric characteristics of the matrix tablets, and  $n$  is the release exponent indicative of the drug release mechanism. The value of  $n$  indicates the mechanism of the release.<sup>27</sup> If the value is around 0.5 (the exact value depends of the geometry) the mechanism is Case I (Fickian) diffusion and a value between 0.5 and 0.89 indicates anomalous (non-Fickian) diffusion. Values of  $n$  equal to 0.89 indicate Case II transport.

$$\frac{M_t}{M_\infty} = k_H \cdot t^{0.5} \quad (7)$$

If the obtained release mechanism is mainly a Fickian diffusion, a dimensionless expression of the Higuchi model was used<sup>26</sup> (Eq. 7).  $M_t/M_\infty$  is the drug release fraction at time  $t$ , and  $k_H$  is the Higuchi constant. In the original Higuchi equation the  $M_t$  (amount of drug released at time  $t$ ) term is found instead of  $M_t/M_\infty$ .

The contribution of Fickian and non-Fickian release can be evaluated by using the Peppas-Sahling model equation<sup>28</sup> (Eq. 8a).

$$\frac{M_t}{M_\infty} = k_D \cdot t^m + k_E \cdot t^{2m} \quad (8a)$$

where  $M_t/M_\infty$  is the drug release fraction at time  $t$ , the first term of the right-hand side is the Fickian contribution ( $k_D$  is the diffusional constant) and the second term is the Case II erosional contribution ( $k_E$  is the erosional constant).  $m$  is the release exponent of purely Fickian diffusion. The value of  $m$  for our tablets was 0.43 as determined from the plot of aspect ratio (diameter/thickness) against diffusional exponent.<sup>29</sup> The  $k_D$  and  $k_E$  values were used to calculate the contribution percentage of diffusion (D) and erosion (E) respectively with Eqs. 8b and 8c.<sup>29</sup>

$$D = \frac{1}{1 + \frac{k_E}{k_D} t^m} \quad (8b)$$

$$\frac{E}{D} = \frac{k_E}{k_D} t^m \quad (8c)$$

The zero-order kinetics equation (Eq 9a) was also used in order to evaluate the type of release mechanism. This model is used for systems where the matrix releases the same amount of drug by unit of time.<sup>26</sup>

$$Q_t = Q_0 + k_{ZO} \cdot t \quad (9a)$$

where  $Q_t$  is the amount of drug dissolved in time  $t$  and  $Q_0$  is the initial amount of drug in the solution (most times,  $Q_0 = 0$ ) and  $K_{ZO}$  is the zero order release constant. We have used a modified zero-order equation (Eq. 9b), where we have replaced the  $Q_t$  term with  $M_t/M_\infty$ .

$$\frac{M_t}{M_\infty} = k_{ZO} \cdot t \quad (9b)$$

The first-order kinetics equation (Eq. 10) is shown below and describes the release of a drug in a way that is proportional to the amount of drug remaining in the interior of the matrix.<sup>26</sup>

$$\ln \frac{Q_t}{Q_0} = k_{FO} \cdot t \quad (10)$$

where  $Q_t$  is the amount of drug dissolved in time  $t$  and  $Q_0$  is the initial amount of drug and  $k_{FO}$  is the first order release constant.

The evaluated tablets are eroded throughout the drug release process so a model that considers changes in the surface area of the matrix was used: a variation of the Hopfenberg model for tablets (Eq. 11).<sup>30</sup>

$$\frac{M_t}{M_\infty} = 1 - \left[ 1 - \frac{k_{HP} \cdot t}{C_0 \cdot a_0} \right]^2 \left[ 1 - \frac{2 \cdot k_{HP} \cdot t}{C_0 \cdot b_0} \right] \quad (11)$$

Where  $M_t/M_\infty$  is the drug release fraction at time  $t$ ,  $C_0$  is the initial concentration of drug in the tablet,  $a_0$  and  $b_0$  are the initial radius and thickness, respectively, and  $k_{HP}$  is the erosion rate constant.

In order to fit the experimental data to the previous equations, only one portion of the release profile was used, i.e.  $M_t/M_\infty \leq 0.8$ . In the literature, only the portion corresponding to  $M_t/M_\infty \leq 0.6$  is used to study the applicability of the Korsmeyer-Peppas equation.<sup>27, 29, 31</sup> Nevertheless, we have used a wider range in this study because we found an excellent fitting up to a higher value of  $M_t/M_\infty$ .

## RESULTS AND DISCUSSION

**Release from gels.** Gels prepared with Tetronic 90R4 and aqueous  $\alpha$ -CD have been evaluated as release systems. These mixtures exhibit a pseudoplastic behaviour, i.e. the viscosity of the samples decreases when the shear rate is increased, and show an appropriate consistency.<sup>20</sup> Two different types of 90R4/ $\alpha$ -CD gels were evaluated, namely T25a10 and T15a10 (Table 1).

Figure 1 shows the release of BSA and Trp from T25a10 and T15a10 as a function of time at 37°C in a pH 7 aqueous medium. The release process of BSA and Trp from T25a10 is faster than that of T15a10. This is consistent with the gel erosion studies performed by our group in a previous work.<sup>20</sup> Despite the significant differences in the amounts loaded and in the molecular sizes of both substrates, the release processes seem to be equivalent in both cases. In addition, the release of  $\alpha$ -CD from the gels can be used

as an indicator of the erosion kinetics of the matrix, because  $\alpha$ -CD is one of the components of the gel structure.

The comparison between the release of  $\alpha$ -CD and BSA is shown in Figure 2. As can be seen, both profiles, i.e. those of the released molecule (BSA) and the ones related to the gel structure ( $\alpha$ -CD), match each other. This indicates that the release of substances from these gels is dominated by the erosion of the gel matrix.

Table 2 shows the dissolution parameters calculated from the erosion curves for all these gels. Both the  $t_{50\%}$  and the mean dissolution time (MDT) values corresponding to T15a10 gels are always higher than those of T25a10, what indicates a faster release from the latter. The dissolution efficiency (DE) is slightly higher for T25a10 gels; this also indicates that the release is faster for these gels. These quantitative results confirm that the release of BSA can be considered comparable to the release of Trp from the same gel matrix.

Table S1 (Supporting Information) shows the  $f_1/f_2$  comparison factors for the release of BSA, Trp and  $\alpha$ -CD. As can be seen all values of the  $f_1$  factors are lower than 15 and the values of  $f_2$  are higher than 50. Therefore in both types of gel the release curves of BSA, Trp and  $\alpha$ -CD can be considered equivalent.

**Release from tablets.** In order to investigate the mechanism of release from Tetronic 90R4 matrices, a controlled geometry was needed that allows us to apply different mathematical models. Cylindrical tablets were prepared by compressing freeze dried gels. Table 3 shows the size measurements and the composition of the tablets prepared in such a way.

Tablet erosion studies were carried out in order to compare these results with the release of  $\alpha$ -CD from the tablet matrices (Figure 3). The release profile for  $\alpha$ -CD in T15a10 tablets and its erosion profile obtained by weighing the tablets as a function of time (see Experimental Part) are the same (Figure 3a). Nevertheless, the release profile of  $\alpha$ -CD from a BSA loaded T15a10 tablet is slightly faster than the erosion profile of the void tablets (Figure 3b). This can be due to fact that the protein molecules cause some disruption in the structure of the matrix. Small differences between the profiles can also be also seen in the case of the T15a10-100 tablets, so the rotation speed influences the release process of  $\alpha$ -CD (Figure 3c). The erosion process is clearly faster when the stirring speed is 100 rpm instead of 25 rpm. Nevertheless, the differences in these profiles are not very significant and the tablet erosion and the  $\alpha$ -CD release profiles can be considered equivalent in all these cases.

The  $f_1/f_2$  comparison factors for the release of  $\alpha$ -CD and the erosion curves for the tablets can be seen in Table S2 (Supporting Information). As can be seen all values of the  $f_1$  factors are lower than 15 and the values of  $f_2$  are higher than 50. Therefore the erosion and  $\alpha$ -CD release curves can be considered equivalent. Thus, the release of  $\alpha$ -CD, being a component of the matrices, can be used as an indicator of the tablet erosion. It has to be added that these erosion studies were not carried out for T25a10 tablets because they were too soft and difficult to handle when removed from the bath, in order to be dried and weighed.

Figures 4a and 4b show the release of Trp and  $\alpha$ -CD from T15a10 and T25a10 tablets. It is has to noticed that the differences in the profiles of Trp and  $\alpha$ -CD means that the two release processes do not occur simultaneously as in the case of gels. Another

observation is that a tablet (ca. 1 g) shows a sustained release for a time as long as that of a gel (20 g). The release of Trp from T25a10 seems to be faster than that of T15a10, but it is important to notice that the total amount of Trp loaded in T25a10 is near half the amount of Trp in the T15a10 tablets. Despite having practically the same release profiles, T15a10 tablets are preferred because they use less amount of Tetronic 90R4 and they are more consistent. The dissolution parameters for the release of Trp from T15a10 and T25a10 tablets can be observed in Table 2. The lower values of MDT for the release of  $\alpha$ -CD and Trp from T25a10 indicate that the release process from these tablets is slightly faster than that of T15a10 tablets. Both types of tablets show similar DE values in the release of the substances (ca. 70%). This is consistent with the value of  $f_1$  (lower than 15) and  $f_2$  (higher than 50) for these curves (see Supporting Information, Table S2) indicates that there is no difference between them. In addition  $f_1$  and  $f_2$  values for the release of  $\alpha$ -CD from T25a10 and T15a10 (Table S2) gels points that they can be considered equivalent too.

Figure 4c shows the release of Trp from T15a10-100 (i.e. T15a10 tablets stirred at 100 rpm instead of 25 rpm). As expected, the release of  $\alpha$ -CD and Trp is faster than the release process at 25 rpm (Figure 4b). This is quantitatively confirmed in Table 2, where the dissolution parameters for both substances are displayed. In addition, Table S2 shows  $f_1$  and  $f_2$  factors which suggest that there are differences between the release curves of Trp and  $\alpha$ -CD using 25 and 100 rpm for the study.

The release process for BSA from T15a10 tablets is shown in Figure 4d. The BSA protein is a considerably larger molecule, so its release is slightly slower because it needs more time to leave the matrix. It is important to notice that the amount of BSA

loaded in the T15a10 tablet is much higher than that of Trp (200 vs. 30 mg). As pointed above, the release of  $\alpha$ -CD from BSA loaded tablets is practically equivalent to its release from Trp tablets. In BSA loaded tablets, the release of  $\alpha$ -CD is faster than the release of BSA, suggesting that BSA remains in the tablet with the poloxamine molecules while  $\alpha$ -CD is released. As can be seen in the dissolution parameters comparison (Table 2), the MDT for BSA release (ca. 180 min) is significantly higher than the Trp values, indicative of a slower release. In contrast, for BSA loaded tablets, the release of  $\alpha$ -CD is faster (i.e. lower MDT and  $t_{50\%}$ ) than that of Trp loaded tablets. In contrast the  $f_1$  and  $f_2$  values calculated for the BSA and Trp release indicates that these release curves can be considered as equivalent. The  $f_1$  and  $f_2$  values obtained for the comparison between the  $\alpha$ -CD release from BSA and Trp loaded tablets are not conclusive because  $f_1$  is lower than 15 but the  $f_2$  is lower than 50.

One last type of T15a10 tablets was prepared, using a rotary evaporator to dry out the gels instead of freeze drying them (T15a10-R). The release of Trp from these tablets is slower than the release from the freeze dried ones (Figure 5). This can be due to the more compact structure of the dried gel leading to a slower dissolution process. In fact, the calculated MDT value (Table 2) was the highest of all the studied tablets.

All the release profiles were fitted to different mathematical release models (Table 4). The results for the Korsmeyer-Peppas equation show that the release mechanism of Trp molecules could be diffusion, because of the values found for the  $n$  parameter (ca. 0.5). On the other hand, the  $n$  values obtained for the release of BSA and  $\alpha$ -CD from the different types of gels point to the fact that their release mechanism could be a combination of erosion and diffusion (values between 0.5 and 1). The values of the  $k_{KP}$  are quite similar for all the Trp profiles with the exception of T15a10-100, which is



higher, and T15a10-R, which is lower. The release process is faster for T15a10-100 due to the high stirring speed, and it is slower for T15a10-R due to the different nature of the tablet. The fitting results are good for all the tablets as the higher values of  $R^2$  parameter indicate.

The Higuchi equation was used in order to achieve a better fit for the releases governed by a mechanism of diffusion. For this equation, good  $R^2$  values were obtained for all the Trp releases. The  $\alpha$ -CD release profiles present poorer values for  $R^2$ , clearly showing that the release mechanism is not governed by diffusion. In contrast, the release of BSA shows a better fit to the Higuchi model than those of  $\alpha$ -CD because of its lower value of  $n$  in the Korsmeyer-Peppas equation.

The first and zero-order equations do not show good values of  $R^2$ . The best values correspond to those of zero-order equation for the release of  $\alpha$ -CD. In accordance with the  $n$  values in the Korsmeyer-Peppas for the release of  $\alpha$ -CD (above 0.5), the fittings to the zero-order are better than the Higuchi fittings.

In order to evaluate the relative influence of diffusion and erosion in the release mechanisms, all the profiles were fitted to the Peppas-Sahlin equation. Two constants were obtained,  $k_D$  and  $k_E$ , for the diffusion and for the erosion processes, respectively. The high values of  $R^2$  (ca. 1) show that very good fittings have been obtained in all cases. The values of the diffusional constants were higher for the releases of Trp, while the erosional constants were higher for the release of  $\alpha$ -CD. The release of BSA from T15a10 shows a higher erosion constant than that of Trp from the same tablets but the diffusional constant is higher in the second case, which indicates that the contribution of

erosion is higher in the release mechanism of a big molecule. Using equations 6b and 6c, the percentage contribution of the erosional and diffusional mechanisms can be graphically seen. In all the Trp releases, the main mechanism is diffusion along the whole release process (see Supporting Information, Figure S2). On the other hand, the release of  $\alpha$ -CD in all the tablets is mainly dominated by erosion (Figure S3).

The release of BSA is a special case (Figure 6), because it starts dominated by diffusion but, after 5 hours, the erosion predominates in the process. A possible explanation for this phenomenon is that, during the first part of the release process, the BSA molecules that are located in the external parts of the tablet can diffuse easily. Once these molecules have been released, the BSA molecules in the interior can not diffuse that fast, so the tablet must be eroded in order to deliver the rest. It has to be added that, as occurs with other surfactants <sup>32</sup>, BSA is also partially denatured by these amphiphilic block copolymers. This fact became evident when the quenching of the BSA fluorescence was studied in order to quantify the amount of BSA released. It was found that the poloxamine molecules that come into the solution when the matrix is eroded are responsible for an attenuation in the fluorescence of the protein. This quenching process corresponds to that of a denatured BSA (see Supporting Information, Figure S4) <sup>33</sup>. The reversibility of a possible denaturation process caused by the constituents of the delivery matrix will be obviously an important concern when a protein is the substrate in these devices.

Finally, the last model tested was the Hopfenberg equation. This is an erosion model, so the best values of  $R^2$  are obtained for the release of  $\alpha$ -CD, as it could be expected. Thus, we can conclude that the main mechanism for the release of  $\alpha$ -CD is the erosion. The

release of Trp does not present good values for the  $R^2$  parameter. The main mechanism for the release of Trp is not the erosion of the matrix. Fickian diffusion is more important in this case, as can be seen in the results for the Korsmeyer-Peppas and Higuchi equations. Interestingly, the release of BSA is an intermediate case. On the one hand, it shows a good fit to the Hopfenberg equation (although not as good as that of  $\alpha$ -CD) and, in the other hand, the Peppas-Sahlin equation shows that the release mechanism is a combination of Fickian diffusion and erosion.

## CONCLUSIONS

Two different types of 90R4/ $\alpha$ -CD matrices were evaluated as potentially drug delivery systems, namely gels and tablets. While 90R4/ $\alpha$ -CD gels seem to be good sustained delivery devices, the amount of gel needed could be excessive for some applications. Nevertheless, this could be a promising delivery device for transdermal devices. On the other hand, tablets made by compression of freeze dried 90R4/ $\alpha$ -CD gels release the tested substances in an appropriate fashion using smaller amounts of matrix than the parent gels.

Different drug release mathematical equations were applied to the release profiles of the tablets. After analyzing the results, we can conclude that the release process can be tailored by controlling parameters such as the matrix composition, the stirring speed in the release assay, the nature of the molecule to be released or the gel drying process.

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## SUPPORTING INFORMATION

*Manuscript title:* In-vitro release from reverse poloxamine/ $\alpha$ -cyclodextrin matrices.  
**Modelling and comparison of dissolution profiles.**

*Authors:* Eneko Larrañeta, Cristina Martínez-Ohárriz, Itziar Vélaz, Arantza Zornoza, Rubén Machín, José Ramón Isasi

### Tablet preparation

The tablets can be easily prepared using a Specac evacuable pellet die: the required amount of lyophilized gel (ca. 1 g) is placed using two polyethylene discs as spacers (to avoid sticking) between the plunger and the die stainless steel pellet (13 mm diameter). Handling it with care, a Perkin Elmer hydraulic press can be used to compress the tablets, provided that a minimal pressure is applied to avoid loss of gel by leaking. Nevertheless, a better procedure can be described as follows: a load of ca. 2 kg (i.e. about  $15 \text{ N}\cdot\text{cm}^{-2}$  or 1.5 atm) is applied during five minutes. The obtained tablet is carefully removed from the die and the resulting dimensions and weight are measured. No lubricants were needed. It was observed that an excess load of 3 kg (or a pressure of  $23 \text{ N}\cdot\text{cm}^{-2}$ ) caused a leaking of the gel in the case of T25a10 sample.

The average hardness values of the two types of tablets were  $6.5\pm 0.9 \text{ N}$  for T25a10 and  $10.8\pm 1.8 \text{ N}$  for T15a10.



**Figure S1.** Photograph of T15a10 eroded tablets at different times.

### Determination of released amounts of Trp or BSA

In order to quantify the release amounts of both active ingredients (either Trp or BSA) the procedure consisted on combining their fluorescence signals, quenched by Tetronic 90R4, with the UV-vis signal, which is the addition of both, the active ingredient and the Tetronic contributions. Three calibration curves are needed:

$$\begin{aligned} \text{(a) Fluorescence:} \quad I_f &= K_f^{(Active)} \cdot [Active] \\ \text{(b) UV-vis:} \quad A^{(Active)} &= K_{UV}^{(Active)} \cdot [Active] \\ A^{(90R4)} &= K_{UV}^{(90R4)} \cdot [90R4] \end{aligned}$$

The samples collected by the dissolution testing device were evaluated both by **UV-vis at 280 nm** and by **fluorescence emission at 341 nm** (excitation at 280 nm). With these data, the following equations are obtained:

$$\begin{aligned} \text{UV-vis:} \quad A_{\text{sample}} &= K_{UV}^{(Active)} \cdot [Active] + K_{UV}^{(90R4)} \cdot [90R4] \\ \text{Fluorescence:} \quad I_f/I_Q &= K_Q \cdot [90R4] \quad \rightarrow \quad I_Q = I_f/(K_Q \cdot [90R4]) \end{aligned}$$

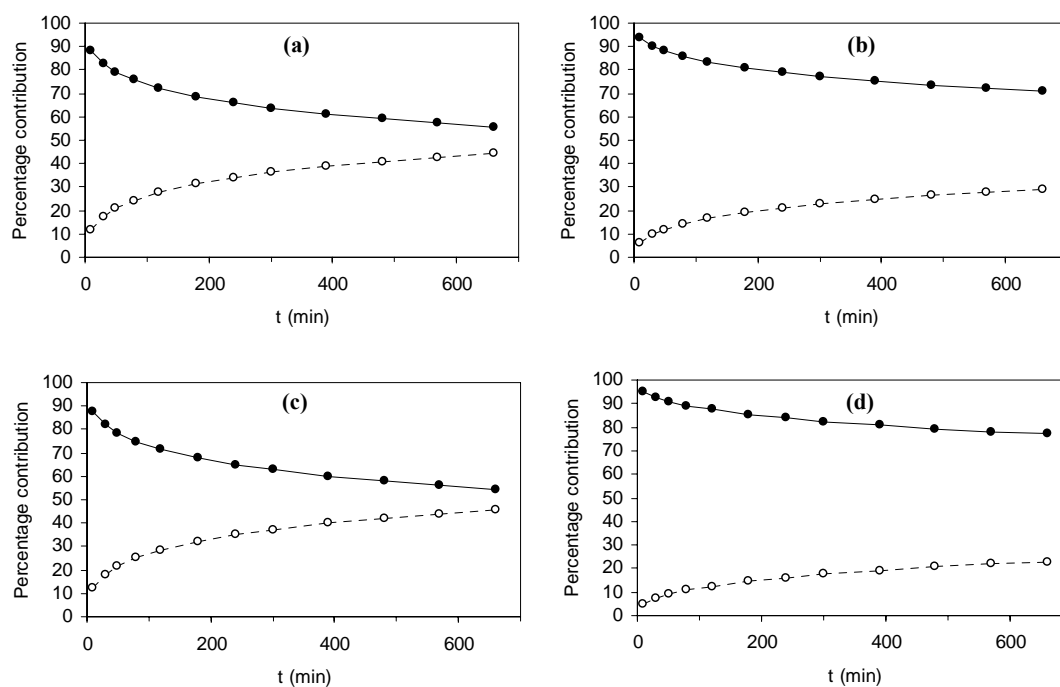
where  $K_Q$  is the quenching Stern-Volmer constant at room temperature and  $I_Q$  is the actual intensity measured for each sample and  $I_f$  would be that in the absence of the quencher, i.e. the one we have in the calibration curve.

Using the constants from the UV-vis calibration curves ( $K_{UV}^{(Active)}$  and  $K_{UV}^{(90R4)}$ ) and, in the case of the fluorescence signal expression, substituting  $I_f$  from its calibration, we obtain two equations with two unknowns ( $[Active]$  and  $[90R4]$ ):

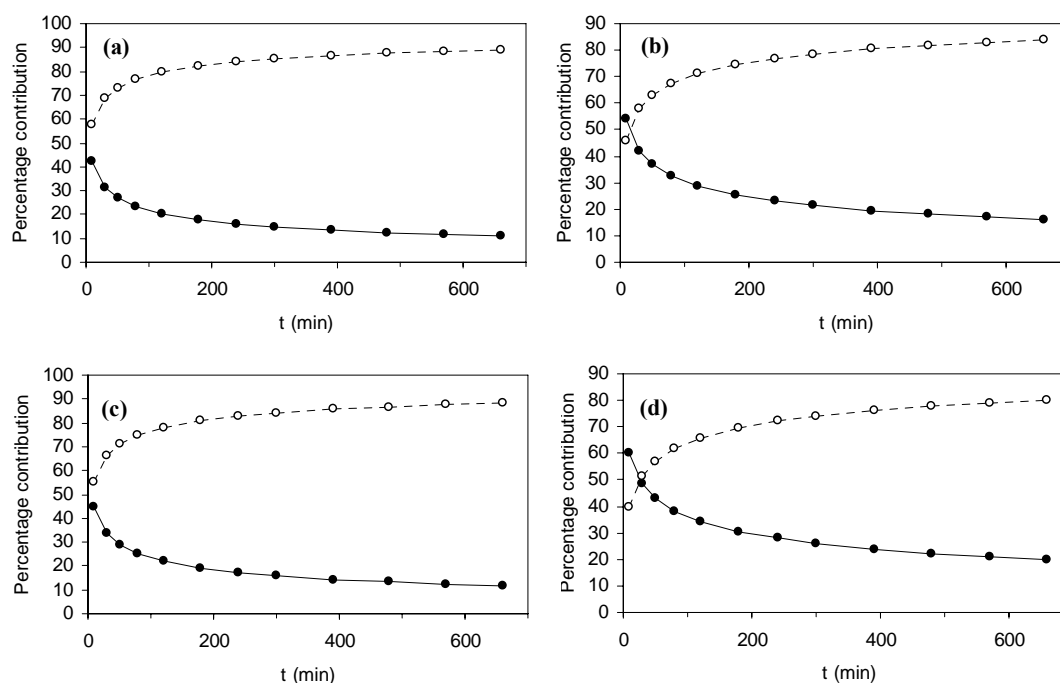
$$\begin{aligned} \text{UV-vis:} \quad A_{\text{sample}} &= K_{UV}^{(Active)} \cdot [Active] + K_{UV}^{(90R4)} \cdot [90R4] \\ \text{Fluorescence:} \quad I_Q &= (K_f^{(Active)} \cdot [Active]) / (K_Q \cdot [90R4]) \end{aligned}$$

These procedure yields excellent results for  $[Active]$ , i.e. either Trp or BSA concentrations. In the case of  $[90R4]$  the results are poorer because its molar absorptivity is considerably smaller, so the errors are higher.

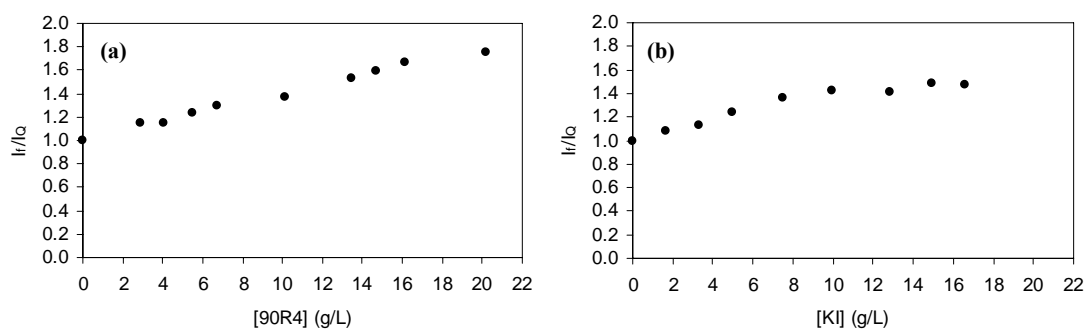
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**Figure S2.** The percentage contribution of the diffusion (●) and the erosion (○) mechanisms for the TRP release from T25a10 (a), T15a10 (b), T15a10-100 (c) and T15a10-R (d).



**Figure S3.** The percentage contribution of the diffusion (●) and the erosion (○) mechanisms for the α-CD release from T25a10 (a), T15a10 (Trp loaded tablet) (b), T15a10-100 (c) and T15a10 (BSA loaded tablet) (d).



**Figure S4.** Stern-Volmer quenching plots for BSA with two different quenchers: Tetronic 90R4 (a) and KI (b). For the native protein, only one of its two tryptophans ( $\text{Trp}_a$  and  $\text{Trp}_b$ ) is accessible to iodide quenching, and a nonlinear Stern-Volmer plot is obtained. In the first plot, a linear fit would indicate that both Trp residues are accessible to the quencher, i.e. the native conformation has been lost.

**Table S1.**  $f_1$  and  $f_2$  comparison factors for BSA, Trp and  $\alpha$ -CD release curves from Tetronic/ $\alpha$ -CD gels.

Curve 1	Curve 2	$f_1$	$f_2$
T25a10 BSA release	T25a10 Trp release	7.5	51.9
T15a10 BSA release	T15a10 Trp release	11.1	53.1
BSA T25a10 $\alpha$ -CD release	T25a10 BSA release	4.9	68.3
BSA T15a10 $\alpha$ -CD release	T15a10 BSA release	6.4	66.5
Trp T25a10 $\alpha$ -CD release	T25a10 BSA release	2.3	83.2
Trp T15a10 $\alpha$ -CD release	T15a10 BSA release	10.0	63.6

**Table S2.**  $f_1$  and  $f_2$  comparison factors for erosion kinetics curves and  $\alpha$ -CD and release curves from Tetronic/ $\alpha$ -CD tablets.

Curve 1	Curve 2	$f_1$	$f_2$
Erosion of T15a10	Trp T15a10 $\alpha$ -CD release	8.5	60.3
Erosion of T15a10	BSA T15a10 $\alpha$ -CD release	12.4	51.9
Erosion of T15a10-100	Trp T15a10-100 $\alpha$ -CD release	14.2	53.1
Erosion of T15a10-R	Trp T15a10-R $\alpha$ -CD release	43.8	24.9
T25a10 Trp release	T15a10 Trp release	6.8	58.8
T15a10 Trp release	T15a10-100 Trp release	17.6	40.2
T15a10 BSA release	T15a10 Trp release	11.6	53.1
Trp T25a10 $\alpha$ -CD release	Trp T15a10 $\alpha$ -CD release	11.4	50.3
Trp T15a10 $\alpha$ -CD release	Trp T15a10-100 $\alpha$ -CD release	27.1	32.6
BSA T15a10 $\alpha$ -CD release	Trp T15a10 $\alpha$ -CD release	14.4	46.2
T15a10 Trp release	T15a10-R Trp release	23.3	36.4