Chapter 4. Study of dye and anticancer drug release from calcium alginate hydrogels

4.1 Synopsis

Calcium alginate has been shown to be very useful in drug delivery due to the unique properties they possess such as high biocompatibility and biodegradability. In particular, the inert environment within the polymer network of alginates allows the entrapment of a wide range of bioactive substances, cells and drug molecules, with minor interactions between them and the biopolymer. However, there is still much information to be learned about the diffusion processes involved in drug delivery from this material. This chapter describes for the first time the diffusion of rose Bengal (RB) (a hydrophilic molecule), Tris(2,2'-bipyridyl) dichlororuthenium (II) hexahydrate (Rubpy) (a hydrophobic molecule) and an anticancer drug, camptothecin (CPT) (a hydrophobic molecule). The research aims to provide a more complete understanding of using calcium alginate as a delivery vehicle, particularly at different pH's.

This chapter describes the loading and the release of two dyes, RB and Rubpy, as well as the drug, CPT from Ca-Alg_2 hydrogels. The release mechanisms of the loaded dyes and drug from a Ca-Alg_2 hydrogel disc at a pH ~ 2.4 and at ~ 7.4 were investigated by applying two diffusion models, namely the Ritger–Peppas (The power law) and the Weibull models [147, 148]. The diffusion coefficients (D) were obtained from the slope of the fitting curves of the Ritger-Peppas model [149, 150].

4.2 Introduction

Diffusion is the process of the movement of matter from one system to another [151]. Diffusion in polymers depends on the concentration and degree of swelling of polymers [151]. The first mathematical treatment of diffusion was established by Fick who developed a law for diffusion in one dimension [151]. Diffusion in polymer depends on the physical properties of the polymer network and the interaction between the polymer and the solvent [151]. In particular, Fickian diffusion is observed in polymer networks when the temperature of study is above the glass transition temperature (T_g) of the polymer [151, 152]. When the polymer is in the rubbery state, the polymer chains have a higher mobility, which allows penetration of the solvent [151, 152]. Non-Fickian (or anomalous) diffusion is observed in glassy polymers when the temperature of study is below the T_g of the polymer [151]. Alfrey et al. [153] classified the various types of diffusion according to the rate of diffusion relative to the polymer relaxation rate:

1- Fickian diffusion (Case I) is characterised by a diffusion rate (R_{diff}), slower than the polymer relaxation rate (R_{relax}) ($R_{diff} < R_{relax}$) [151, 153, 154].
2- Case II diffusion, the diffusion rate is much faster than the polymer relaxation ($R_{diff} > R_{relax}$) [151, 153, 154].
3- Non-Fickian, or Anomalous diffusion, occurs when the diffusion and polymer relaxation rates are the same order in magnitude ($R_{diff} \sim R_{relax}$) [151, 153, 154].

Alginate has two T_g's, at 31 °C and 113.56 °C due to the guluronic and mannuronic acid content of alginate, respectively [155]. In the work presented here the diffusion experiments are carried out at 25 °C, which is below both the T_g's, of alginate and so the diffusion is assumed to be non-Fickian diffusion.

In order to determine the type of diffusion in this thesis the experimental data derived from the UV-Vis spectra of the release of RB, Rubpy and fluorescence emission spectra of CPT from Ca-Alg_2 hydrogel discs were fitted to known theoretical models, the Ritger-Peppas model and the Weibull model [147, 148], explained in more detail in the following sections.

4.2.1 Diffusion models

4.2.1.1 Ritger - Peppas model

Ritger and Peppas introduced a simple exponential relation (Equation 4.1) to describe the general solute release behaviour from swellable polymeric systems [147, 149], such as alginate hydrogels.

$$\frac{M_t}{M_\infty} = kt^n$$

Equation 4.1

Taking the logarithm of Equation 4.1 results in Equation 4.2.

$$\text{Log}\frac{M_t}{M_\infty} = \log k + n \log t$$

Equation 4.2

Where M_t and M_∞ are the amounts of drug released at time t and infinite time respectively, k is a constant related to the structural and geometric characteristic of the device, and n is the release exponent characteristic of the drug release mechanism [147, 149]. By plotting log M_t/M_∞ against log t, the release exponent, n, can be determined from the slope of a linear regression of Equation 4.2, and the k from the intercept [156].

Equation 4.2 can adequately describe the release of drugs or other solutes from spheres, cylinders and thin films, regardless of the release mechanism [147, 149]. The values of n depends on the shape of the release systems, for thin films, when n = 0.5, diffusion-controlled drug release (Fickian diffusion) is inferred and when n = 1.0, swelling-controlled drug release is inferred [147]. When the n values are between 0.5 and 1.0 then both diffusion-controlled drug release and swelling-controlled drug release (non-Fickian or anomalous transport) is expected. At n values greater than 1.0

super Case II transport is expected [147]. Therefore, once data has been fitted the *n* values can be used to predict the type of diffusive transport from materials, in the case of this thesis from hydrogel discs.

4.2.1.2 Weibull model

The Weibull model is an alternative empirical equation for the description of the release profiles (Equation 4.3) [148].

$$\frac{M_t}{M_\infty} = 1 - \exp\left[-\left(\frac{t}{a}\right)^b\right]$$

Equation 4.3

Equation 4.3 can be arranged to give Equation 4.4 [157, 158].

$$\mathrm{Log}\left[-\ln\left(1 - \frac{\mathrm{M_t}}{\mathrm{M_\infty}}\right)\right] = b\log t - b\log a$$

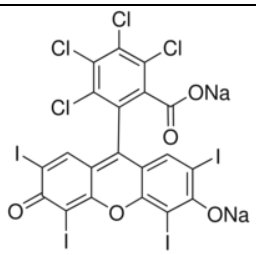
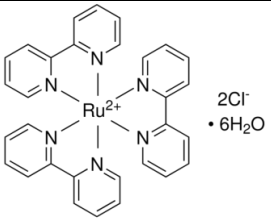
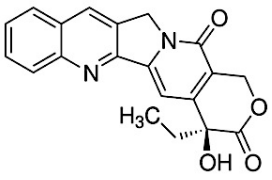
Equation 4.4

Where M_t/M_∞ is the fraction release at time t and infinite time, a is the scale parameter which defines the time scale of the process, b is the shape parameter which characterises the curve shape [157, 158]. By plotting log (-ln (1-M_t/M_∞)) against *log t*, the values of b and a can be determined from the slope and the intercept, respectively of a straight line from Equation 4.4 [157, 158]. There is a link between the values of b and the diffusional mechanism of the release. When the value of $b \leq 0.75$, Fickian diffusion is dominant while when b values are between 0.75 and 1.0, a combined mechanism (Fickian diffusion and Case II transport) occurs [159]. Values of b higher than 1.0, result in the drug release via a complex transport mechanism [159]. Therefore again once data has been fitted the b values can be used to predict the type of diffusive transport from, in the case of this thesis, hydrogel discs.

4.3 Properties of RB, Rubpy and CPT

Table 4.1 shows the chemical formula, molar mass and maximum band position of RB, Rubpy and CPT [84, 160-162]. RB is classified as a hydrophilic dye [161], while both Rubpy [162] and CPT are classified as hydrophobic drugs [93]. The concentration of both RB and Rubpy in the solution can be measured by UV-visible spectroscopy given that RB has one distinct absorption peak in the visible region at 548 nm [160] while Rubpy has one distinct absorption peak in the ultraviolet region at 288 nm [163]. CPT is a fluorescent compound [164] with an fluorescence emission spectrum that is affected by the opening of the lactone ring due to hydrolysis [84] (as discussed in Chapter 1, Section 1.4.1.1).

Table 4-1 Properties of the RB, Rubpy and CPT [84, 160-162].

Compounds	Chemical formula	Molar mass ($g\ mol^{-1}$)	λ_{max} (nm) [84, 160, 162]
Rose Bengal (RB)		1017.64	Absorption λ_{max} = 548
Tris(2,2'bipyridyl) dichlororuthenium (II) hexahydrate (Rubpy)		748.62	Absorption λ_{max} = 288
Camptothecin (CPT)		348.35	Fluorescence λ_{max} = 432 at pH ~ 2.4 λ_{max} = 448 at pH ~ 7.4

At pH < 5 CPT is mainly present as the lactone form while at pH > 5 it is in the carboxylate form [84, 165, 166]. The emission spectrum of CPT is red-shifted by~ 20 nm when it is changes from its lactone form to its carboxylate form [84, 167]. In acidic solution, CPT tends to be in the lactone form with peak emission intensity at 432 nm at an excitation wavelength of 370 nm. Whereas, in alkaline solution, CPT tends to be in the carboxylate form with a peak emission intensity at 448 nm [84, 165, 167].

4.4 Release profile of RB, Rubpy, and CPT from Ca-Alg_2 hydrogel

The formation of Ca-Alg_2 from an aqueous Na-Alg and $CaCl_2$ solution can occur via two methods, namely the external method (or "diffusion method") and the internal method (as discussed in Chapter 1, Section 1.2.4.1). For loading of molecules into the prepared Ca-Alg_2 then according to literature [124], a dye or drug can be loaded into Ca-Alg_2 using either the *in situ* addition method or the absorption method. For the *in situ* addition method, the dye or drug is first added to the Na-Alg solution then after exposure to an aqueous $CaCl_2$ solution Ca-Alg_2 is formed [124]. However, in the absorption method, the Ca-Alg_2 is first formed and then it is exposed to a dye or drug for 48 h. After this time the Ca-Alg_2 becomes loaded with the dye or drug [124]. Literature shows that the *in situ* addition method has a higher dye and drug loading capacity compared to the absorption method, which requires more drug and time [124].

In this work the Ca-Alg_2 was prepared using the external method, then RB, Rubpy and CPT were loaded into Ca-Alg_2 using the *in situ* addition method. For this, each dye and CPT sample was loaded into the Ca-Alg_2 hydrogels by adding their solution (10 mg mL^{-1} of each dye in water, or 10 mg mL^{-1} of CPT in DMSO) to a Na-Alg solution (2 % wt/wt, 40 mL) prior to cross-linking with aqueous $CaCl_2$ (1 M) (see Chapter 2, Section 2.8.2 for the full synthesis method). A Ca-Alg_2 cylindrical hydrogel was formed using a test tube with 1 cm in diameter and 5 cm in height (see Chapter 2, Section 2.8.2 for the full synthesis method). Once the hydrogel was formed a disc shaped 1 cm in diameter and 0.74 ± 0.07 mm thick (see Chapter 2,

Section 2.8.2 for more details) was cut from the dye, or drug, loaded Ca-Alg_2 cylindrical hydrogel for subsequent release experiments. Every disc had 0.121± 0.010 mg of either RB, Rubpy or CPT, and all the discs contained Na-Alg (10.0 ± 0.5 mg).

Each disc was then immersed in Tris buffer solution (0.02 M, 50 mL) at ambient temperature with the pH modified to pH ~ 2.4 or pH ~ 7.4 by the addition of HCl. After 2, 4, 6, 8, 10, 13, 16, 19, 22, 25, 28, 43 and 103 min the UV-Vis absorption spectra of the Tris buffer (pH ~ 2.4 and ~ 7.4) with RB were recorded at wavelength 550 nm. Figure 4.1 shows only the spectra at pH ~ 7.4 as at pH ~ 2.4 no diffusion (no absorption spectra) was observed. Figure 4.2 and 4.3 shows the UV-Vis spectra recorded for Rubpy at a wavelength of 285 nm, at a pH ~ 2.4 and pH ~ 7.4, respectively.

The fluorescence emission spectra of CPT diffused into Tris buffer solution at pH ~ 2.4 and at pH ~7.4 after 2, 4, 6, 8, 10, 13, 16, 19, 22, 25, 28, 43 and 103 min were recorded at an excitation wavelength of 370 nm and are shown in Figures 4.5 and 4.6, respectively.

The concentrations of RB, Rubpy and CPT in the Tris buffer were then determined from calibration curves, previously recorded from known concentrations of each (see Appendices, Figure 8.2 (a, b and c)).

4.4.1 RB and Rubpy release measured by UV-Vis spectroscopy

Figure 4.1, 4.2 and 4.3 shows the change in the UV-vis spectra of RB at pH ~ 7.4 (550 nm), Rubpy at pH ~ 2.4 (285 nm), Rubpy at pH ~ 7.4 (285 nm), respectively. There is no release was observed for RB at pH ~ 2.4 and the reason for that will be discussed in Section 4.5.1.1. These figures show that the absorbance intensity of the Tris Buffer solution at 550 nm for RB and 285 nm for Rubpy both increase with increasing time. According to Beers law, as the concentration of the dyes increases in the Tris Buffer solution the absorbance increases indicating the release of the dyes from the Ca-Alg_2 hydrogel discs with time [125]. Figure 4.4 shows a plot of concentration versus time for RB at pH ~ 7.4 (Figure 4.4, green circles) and for Rubpy at pH ~ 2.4 (Figure 4.4, red triangles) and pH ~ 7.4 (Figure 4.4, black

squares), indicating an increase in RB and Rubpy concentration with time.

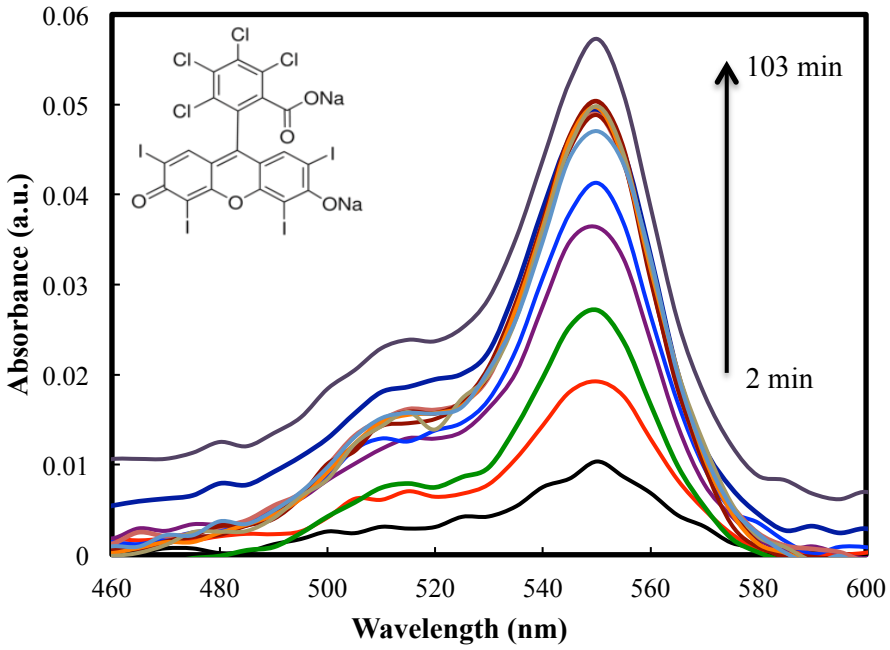


Figure 4-1 UV-Vis spectra of Tris buffer solution at pH ~ 7.4 in the release experiment of RB from Ca-Alg_2 hydrogel discs.

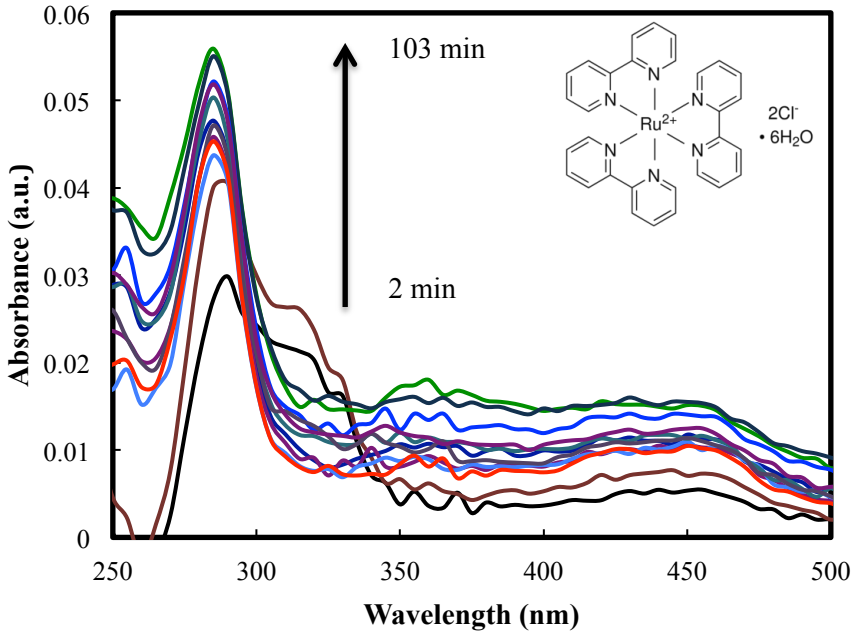


Figure 4-2 UV-Vis spectra of Tris buffer solution at pH ~ 2.4 in the release experiment of Rubpy from Ca-Alg_2 hydrogel discs.

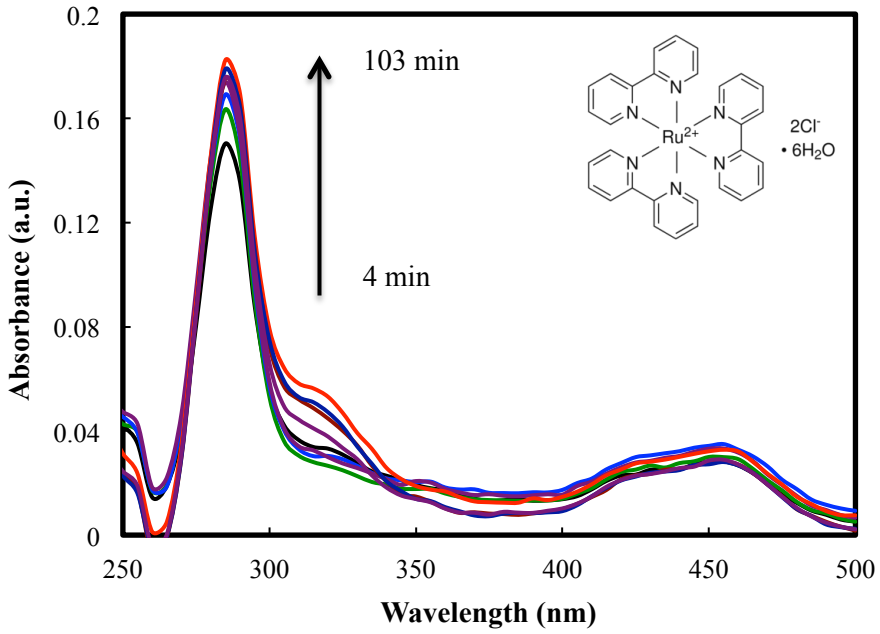


Figure 4-3 UV-Vis spectra of Tris buffer solution at pH ~ 7.4 in the release experiment of Rubpy from Ca-Alg_2 hydrogel discs.

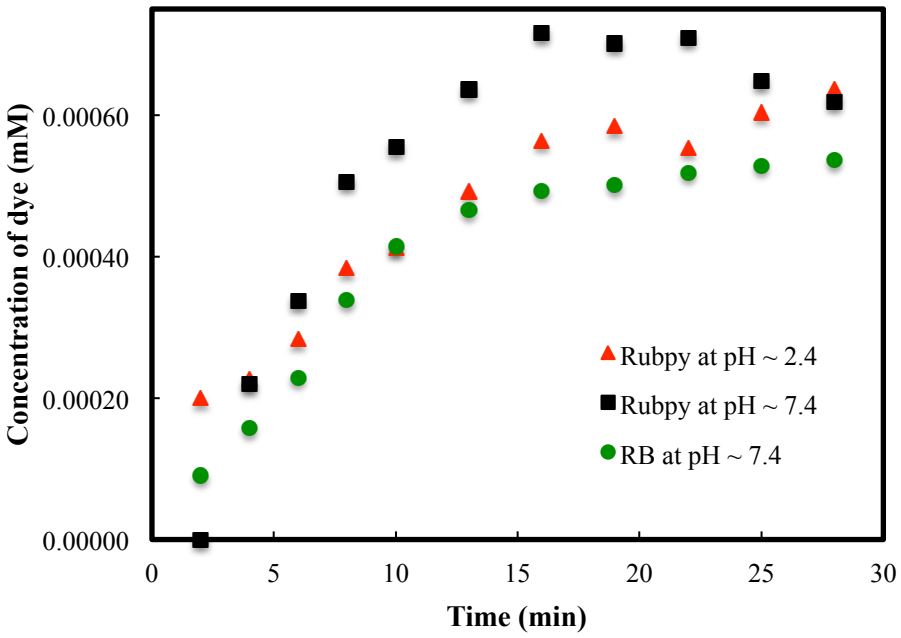


Figure 4-4 Change in concentration of RB and Rubpy in Tris buffer solution at pH ~ 2.4 and pH ~ 7.4 in the release experiment of RB and Rubpy from a Ca-Alg_2 hydrogel disc.

4.4.2 CPT release measured by fluorescence spectroscopy

Figure 4.5 and Figure 4.6 shows the change in the fluorescence emission spectra at 430 nm (lactone form) and 455 nm (carboxylate form) of the CPT at an excitation wavelength of 370 nm for pH ~ 2.4 and pH ~ 7.4, respectively. The downward peaks in the spectra are the instrument changing gratings. Both figures represent an increase in emission intensity with increasing time. In addition, Figure 4.5 shows that the intensity of the lactone emission peak (at 430 nm) is lower than the intensity of the carboxylate emission peak (at 455 nm) in Figure 4.6. The reason for this will be discussed in Section 4.5.1.1. Figure 4.7 shows a plot of concentration versus time, indicating an increase in CPT with time at both pHs, pH ~ 2.4 and pH ~ 7.4.

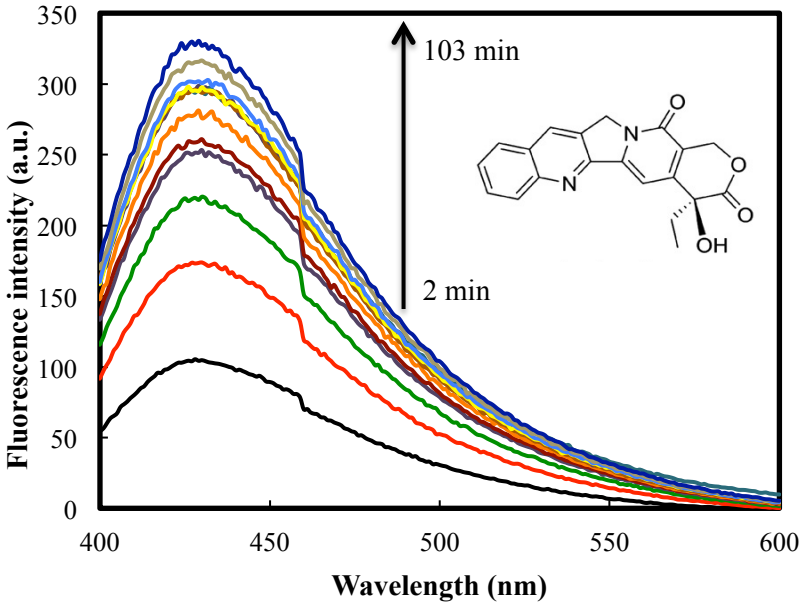


Figure 4-5 Fluorescence emission spectra at an excitation wavelength of 370 nm of the Tris buffer solution at pH ~ 2.4 in the release experiment of CPT (lactone form) from a Ca-Alg_2 hydrogel disc.

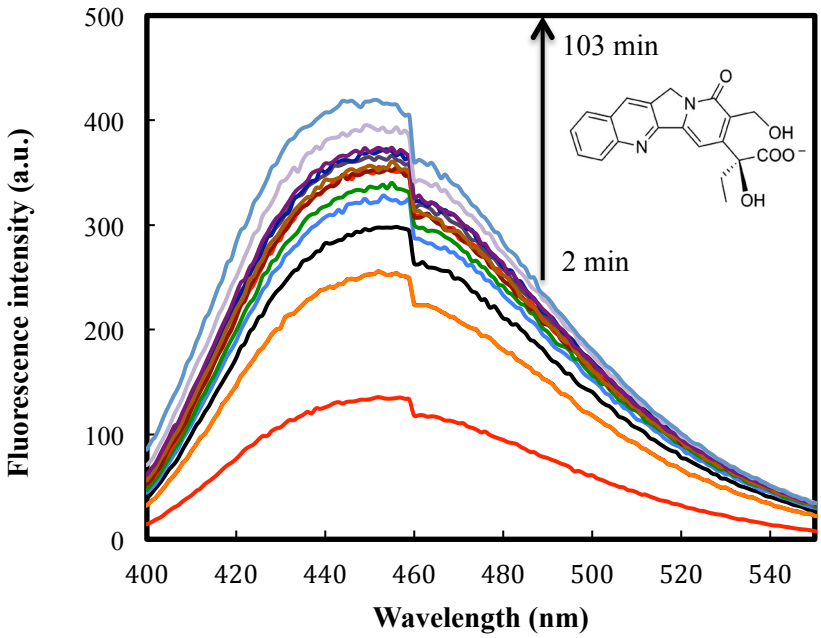


Figure 4-6 Fluorescence emission spectra at an excitation wavelength of 370 nm of the Tris buffer solution at pH ~ 7.4 in the release experiment of CPT (carboxylate form) from a Ca-Alg_2 hydrogel disc.

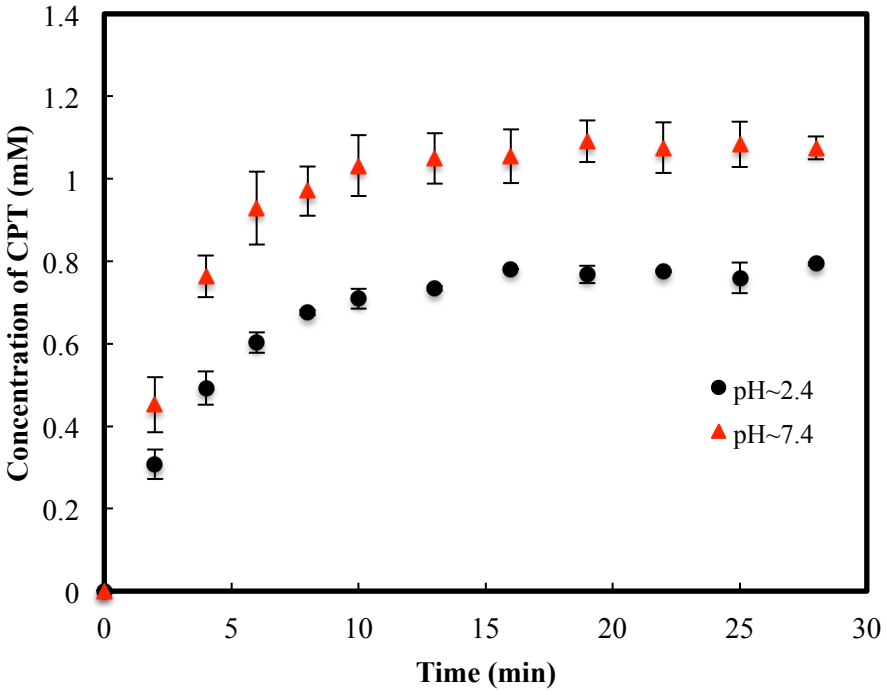


Figure 4-7 Change in concentration of CPT in Tris buffer solution at pH ~ 2.4 and pH ~ 7.4 in the release experiment of CPT from a Ca-Alg_2 hydrogel disc.

4.5 Diffusion kinetics

4.5.1 Fractional amount of RB, Rubpy and CPT release

From the UV-Vis and fluorescence data in Sections 4.4.1 and 4.4.2 the fractional amount (M_t/M_∞) of RB, Rubpy and CPT released was calculated. The fractional amount has been extensively used to describe release behaviour [93].

4.5.1.1 Fractional amount of RB, Rubpy and CPT released at pH ~ 2.4

Figure 4.8 shows the fraction release profile of Rubpy (Figure 4.8, black squares) and CPT (Figure 4.8, red triangles) only; both calculated from the UV-Vis and fluorescence emission data from the Ca-Alg_2 hydrogel discs, respectively. No release of RB was observed from the Ca-Alg_2 hydrogel discs and so this is not represented in the plot.

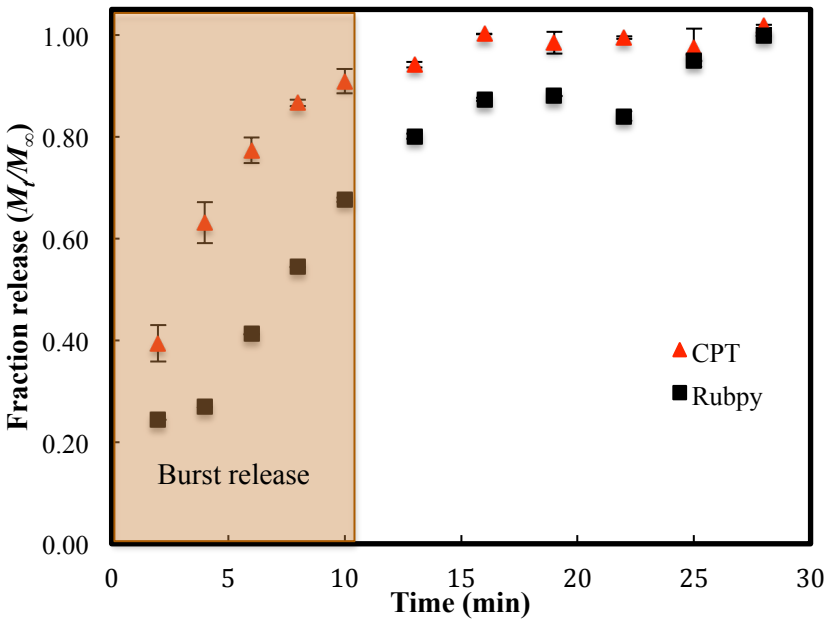


Figure 4-8 Fraction release, M_t/M_∞ of Rubpy (black squares) and CPT (red triangles) from Ca-Alg_2 hydrogel discs at pH ~ 2.4.

The lack of release of RB release at pH ~ 2.4 may be attributed to RB's significantly higher molecular weight (1017.64 g mol^{-1}) compared to Rubpy and CPT (348.35 and 748.62 g mol^{-1}, respectively) (Table 4.1).

In addition one of the most important parameters influencing the release of molecules from Ca-Alg_2 hydrogel discs is the pH of the released medium, which results in either shrinkage or swelling of the polymer. Ca-Alg_2 is an ionic hydrogel containing carboxylic groups, so its behaviour is altered using different pH's for the released medium [168]. In acidic solution, as is the case here, Ca-Alg_2 hydrogel tends to shrink due to the protonation of the mannuronic acid (pKa = 3.38) and guluronic acid (pKa = 3.65) units [97]. The hydrogen bonding that then arises between the carboxylic acid groups then leads to polymer-polymer interactions rather than polymer-water interactions, and thus the electrostatic repulsion among these groups is decreased, causing the Ca-Alg_2 hydrogel to shrink [97, 169].

In order to see the effect of the diffusion media pH on the shrinking or swelling of the alginate hydrogel discs optical analysis was performed on dried hydrogel discs after being exposed to various pH's. As an exemplar CPT loaded Ca-Alg_2 hydrogel discs were used. Figure 4.9 (a-c) shows (a) a pure dried Ca-Alg_2 disc, (b) a dried Ca-Alg_2 disc loaded with CPT after 103 min in pH ~ 2.4 and (c) a dried Ca-Alg_2 disc loaded with CPT after 103 min in pH ~ 7.4 (see Chapter 2, Section 2.10.1.1 for more details). When comparing the diameters of the three discs, it is clearly seen that the diameter of both discs (b) and (c) is different from disc (a). At pH ~ 2.4 the diameter of the dried Ca-Alg_2 hydrogel disc, loaded with CPT after 103 min (Figure 4.9(b)), has significantly decreased compared to the pure dried Ca-Alg_2 disc (Figure 4.9(a)). While at pH ~ 7.4 (Figure 4.9(c)) the disc diameter has significantly increased compared to both the pure discs (Figure 4.9(a)) and the discs at pH ~ 2.4 (Figure 4.9(b)). Furthermore, the pure dried Ca-Alg_2 disc is flexible (Figure 4.9(a)) while the dried Ca-Alg_2 discs immersed in pH ~ 2.4, (Figure 4.9(b)), become stiff and the dried Ca-Alg_2 discs immersed in pH ~ 7.4 (Figure 4.9(c)) become spongy after 103 min.

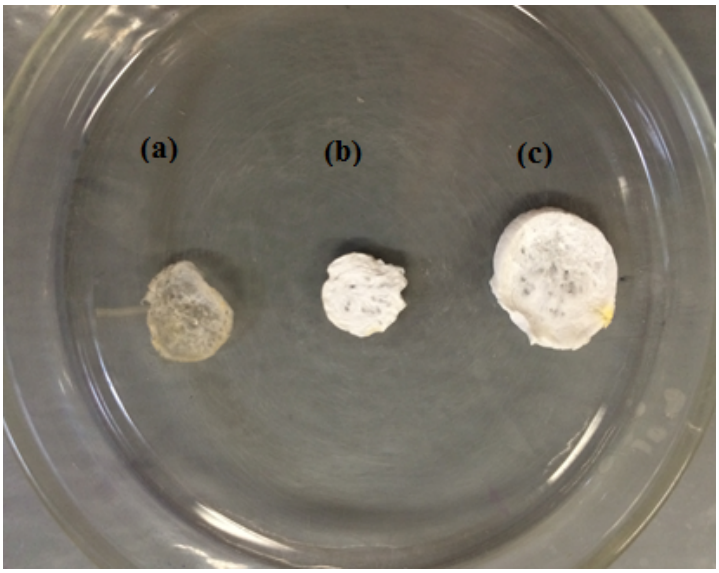


Figure 4-9 Optical images of (a) a dried pure Ca-Alg_2 disc, (b) a dried Ca-Alg_2 disc loaded with CPT after 103 min in pH ~ 2.4 and (c) a dried Ca-Alg_2 disc loaded with CPT after 103 min in pH ~ 7.4

Subsequently, in order to see the effect of the diffusion media pH on the morphology of the alginate hydrogel discs scanning electron microscopy (SEM) analysis was performed on dried hydrogel discs after being exposed to various pH's. As an exemplar CPT loaded Ca-Alg_2 hydrogel discs were used. Figure 4.10(a-c) shows the SEM images of (a) a pure dried Ca-Alg_2 disc, (b) a dried Ca-Alg_2 disc loaded with CPT after 103 min in pH ~ 2.4 and (c) a dried Ca-Alg_2 disc loaded with CPT after 103 min in pH ~ 7.4. The three dry discs for SEM analysis were prepared as described in Chapter 2, Section 2.10.1.1. The pure dried Ca-Alg_2 disc displayed a web like structure with large well-defined pores (Figure 4.10(a)), while after immersion in CPT for 103 min at pH ~ 2.4 the loaded Ca-Alg_2 discs surface became compacted (compressed) and most of the pores collapsed as the Ca-Alg_2 hydrogel shrunk [97] (Figure 4.10(b)). In contrast, after immersion of the Ca-Alg_2 disc in CPT for 103 min at pH ~ 7.4, the surface became sheet-like with an ill-defined porous structure (Figure 4.10(c)).

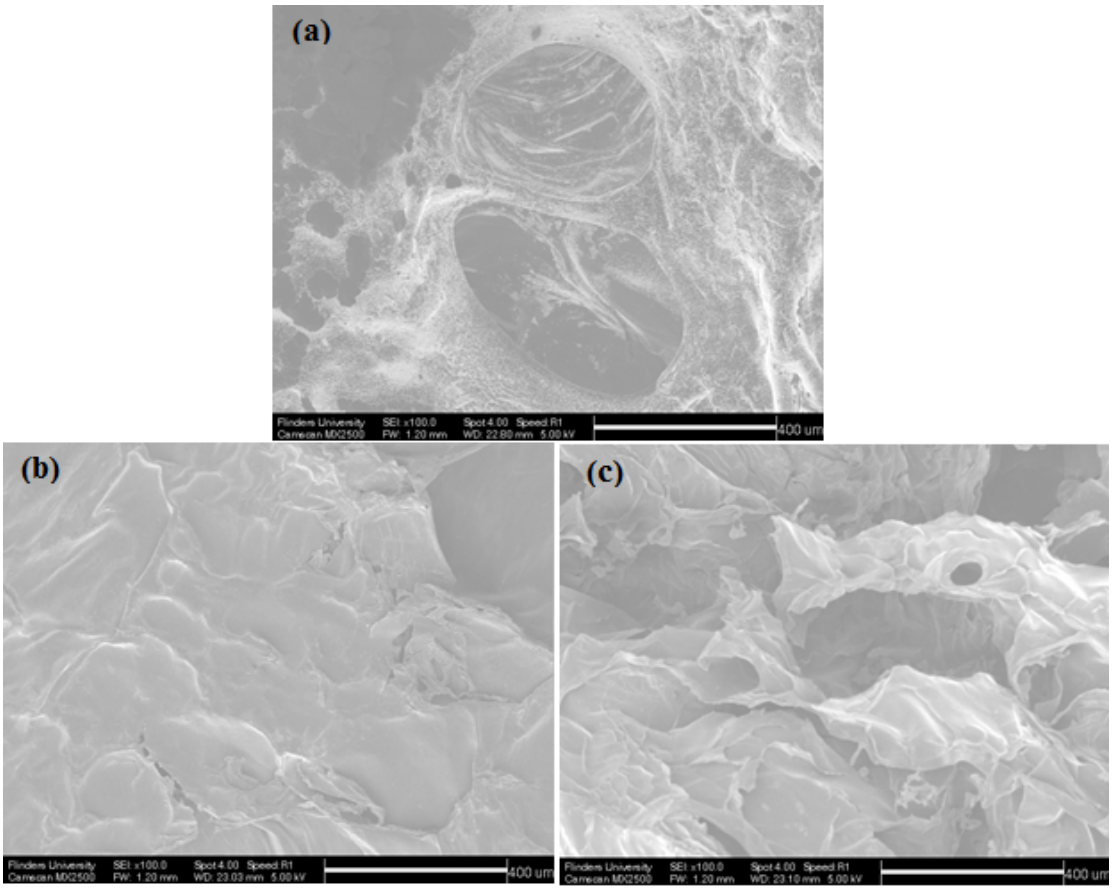


Figure 4-10 SEM images of (a) a dried Ca-Alg_2 disc, (b) a dried Ca-Alg_2 disc loaded with CPT after 103 min in pH ~ 2.4 and (c) a dried Ca-Alg_2 disc loaded with CPT after 103 min in pH ~ 7.4.

From these results, it is therefore proposed that the shrinkage of the hydrogel at pH~2.4, as shown in Figure 4.9(b), along with the higher molecular weight of the RB has led to no diffusion of the RB from the pores of the gel.

In the case of Rubpy and CPT, it can be seen from Figure 4.8 that there are two diffusion stages for the release. The first stage is an initial burst release of Rubpy and CPT (Figure 4.8) from the Ca-Alg_2 hydrogel discs followed by molecular diffusion into the bulk Tris buffer solution. The second diffusion stage (Figure 4.8) is believed to be governed by the behaviour of the Ca-Alg_2 hydrogel in the released medium [170, 171]. This is discussed in more detail later in this Chapter in Section 4.6.

In the first burst release stage the amount of Rubpy (Figure 4.8, black squares) and CPT (Figure 4.8, red triangles) released from Ca-Alg_2 hydrogel disc increased very

rapidly in the first 10 min, after this period it increased gradually until reaching equilibrium after 16 min. The burst release of Rubpy (Figure 4.8, black squares) is slower than the release of CPT (Figure 4.8, red triangles). Given that both compounds are hydrophobic then solubility is not necessarily playing a role in the diffusion. Therefore, the difference in molecular weights may be an influence; Rubpy (748.62 g mol^{-1}) is greater than the molecular weight of CPT (348.35 g mol^{-1}) and so the smaller CPT can escape the pores more easily. As mentioned previously Ca-Alg_2 hydrogels shrink in pH ~ 2.4 release media and so molecular size of the released compound will be crucial.

A further consideration is that the positive charge of Rubpy at pH ~ 2.4 can generate an attractive electrostatic force with the negative charge on the carboxylate groups on the mannuronic acid which are not involved in the egg box structure of the Ca-Alg_2 [97]. This then leads to retardation of the diffusion of the Rubpy from the Ca-Alg_2 hydrogel disc compared with CPT. As described previously CPT is pH-dependent and displays multiple ionisation sites, and therefore multiple p*Ka*'s [172], and exists in two forms, the lactone form and the carboxylate form [84]. The lactone form exists at pH ~ 2.4 whereas the carboxylate form exists at pH ~ 7.4 [84]. Therefore at pH~2.4 CPT has a neutral net charge [167]. In this case it is less likely to be held in the Ca-Alg_2 matrix by electrostatic interactions and therefore more freely able to diffuse out.

4.5.1.2 Fractional amount of RB, Rubpy and CPT release at pH ~ 7.4

Figure 4.11 shows the fraction release profile of RB (Figure 4.11, green circles), Rubpy (Figure 4.11, black squares) and CPT (Figure 4.11, red triangles) at pH ~ 7.4, calculated from the UV-Vis (RB and Rubpy) and fluorescence (CPT) spectroscopic data.

Figure 4.11 shows that the amount of RB and Rubpy released in the first burst release stage from the Ca-Alg_2 hydrogel discs increased very rapidly in the first 10 min, after this period it increased gradually until reaching equilibrium after 16 min. The release of CPT (Figure 4.11, red triangles) from Ca-Alg_2 hydrogel disc increased very rapidly in the first 6 min. After this period it increased gradually until reaching equilibrium after 16 min.

Figure 4.11 shows that the burst release of RB (Figure 4.11, green circles) is lower than the release of Rubpy (Figure 4.11, black squares) and CPT (Figure 4.11, red triangles) from the Ca-Alg_2 hydrogel discs. It appears that the release of CPT from the Ca-Alg_2 hydrogel discs is higher compared to both the RB and Rubpy. However in the second stage, the release of RB, Rubpy and CPT from the Ca-Alg_2 hydrogel discs appear to be similar, and this behaviour can be interpreted as follows.

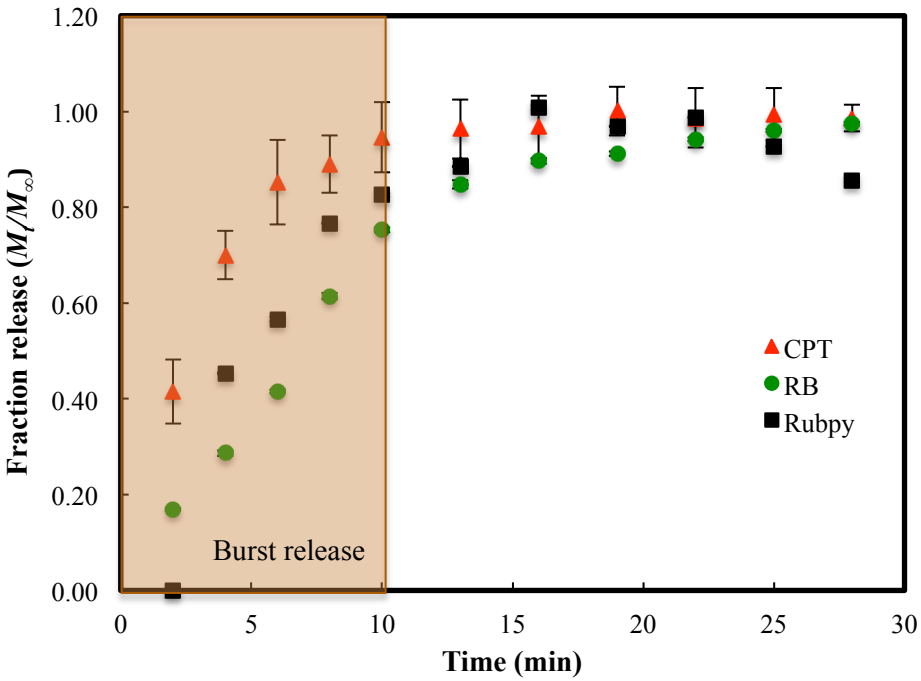


Figure 4-11 Fraction release, M_t/M_∞ of RB (green circles), Rubpy (black squares) and CPT (red triangles) from Ca-Alg_2 discs at pH ~ 7.4.

In alkaline solution Ca-Alg_2 tends to swell, as shown in Figure 4.9(c) [97]. In this case the carboxylic acid groups of Ca-Alg_2 ionise to give $–COO^-$ groups, which facilitate the swelling of the Ca-Alg_2. The osmotic pressure inside the Ca-Alg_2 then increases due to the higher concentration of free H^+ ions and promotes water absorption [97].

The lower initial burst release of RB (Figure 4.11, green circles) compared to Rubpy (Figure 4.11, black squares) and CPT (Figure 4.11, red triangles) may be attributed to the larger size of the RB. At pH ~ 7.4 the alginate is sufficiently swollen to allow the RB molecules to diffuse out unlike the case at pH ~ 2.4 where the alginate has shrunk

[173]. Kikuchi et al. [174] have previously reported that the release of dextran from Ca-Alg_2 hydrogel strongly depends on its molecular weight. Paradee et al. [170] studied the release of tannic acid from Ca-Alg_2 hydrogels and they observed that the slower release of tannic acid was due to its higher molecular weight. The release of the RB from Ca-Alg_2 hydrogel disc may be influenced by the molecular charge of RB in the Tris buffer solution at pH ~ 7.4. As RB carries a negative charge at pH ~ 7.4 it generates a repulsive force with the negative charge on the carboxylate groups on the mannuronic acid which are not involved in the egg box structure of the Ca-Alg_2 [97]. The higher fractional release of CPT (Figure 4.11, red triangles) compared to RB (Figure 4.11, green circles) and Rubpy (Figure 4.11, black squares) from the Ca-Alg_2 hydrogel disc at pH ~ 7.4 may be attributed to CPT's lower molecular weight (348.35 g mol^{-1}) compared to RB and Rubpy (1017.64 and 748.62 g mol^{-1}, respectively). A further consideration is that the p*K*a of the carboxylic group of CPT is about 6.5 thus at pH ~ 7.4 the CPT will carry a negative charge [167]. This gives rise to a repulsive force between the negatively charged carboxylate groups in the Ca-Alg_2 and the negatively charged carboxylate group in the CPT [175]. Even though both the CPT and RB generate a repulsive force, the release of CPT is faster than the release of RB from Ca-Alg_2 hydrogel discs. This can be explained by the smaller size of CPT, which makes the diffusion of CPT through the pore of the Ca-Alg_2 hydrogel discs easier than RB. A similar result has been observed for the release of benzoic acid using Ca-Alg_2 hydrogel [170]. It was found that the higher release of benzoic acid was due to its smaller molecular weight [170].

The slower release of the Rubpy compared to CPT may be attributed to a positive charge on the Rubpy at pH ~ 7.4 and thus a likely attractive electrostatic force with the negative charge of the carboxylate groups in the Ca-Alg_2 hydrogel. However, a repulsive force between the negatively charged carboxylate groups in the Ca-Alg_2 and the negatively charged carboxylate group in the CPT may increase the release of CPT from the Ca-Alg_2 hydrogel disc.

Finally, in the second stage (Figure 4.11), the Ca-Alg_2 hydrogel discs becomes fully swollen with time and with maximum pore size extension and so the release now is at a constant equilibrium rate for RB, Rubpy and CPT.

4.6 Release kinetics

The experimental data in Figures 4.8 and 4.11 were then analysed using two diffusion models. The mechanism for RB, Rubpy and CPT release from Ca-Alg_2 hydrogel discs were described by Ritger-Peppas and Weibull models, according to Equations 4.2 and 4.4. Figures 4.12 (a and b) show the Ritger-Peppas model for RB (green circles), Rubpy (black squares) and CPT (red triangles) released at pH ~ 2.4 and pH~7.4, respectively. The values of the Ritger-Peppas model constants and correlation coefficients are shown in Table 4.2. It can be seen that the coefficient constants, R^2, for the Ritger-Peppas model plots (Figures 4.12) are high, suggesting that the Ritger-Peppas model fits the release data well.

When using the Ritger-Peppas model, only 60 % of the first data should be used ($M_t/M_\infty < 0.6$) [176]. This has its limitation in this work as this gives either 2 or 3 data points. However, we have still gone ahead and fitted the data to the Ritger-Peppas model [176]. The n values (Table 4.2) for CPT and Rubpy diffusion at pH ~ 2.4 are 0.6211 and 1.022, respectively. This therefore implies that the release mechanisms of CPT and Rubpy are anomalous transport and case II transport mechanisms, respectively (Refer to Section 4.2.1.1). The Ritger-Peppas model describes swelling controlled devices but the Ca-Alg_2 hydrogel discs in pH ~ 2.4 tend to shrink as described previously [150]. So, in the case of CPT release from Ca-Alg_2 hydrogel disc, the diffusion-controlled release is dominant, while in the case of Rubpy release, the case II mechanism must be the major mechanism of release [150]. Thus, the actual drug release mechanism includes two phenomena: shrinkage of the Ca-Alg_2 hydrogel disc that expels water out of the disc, and the diffusion of the drug molecules out of the Ca-Alg_2 hydrogel disc as the acidic aqueous medium hydrates the disc [150]. A similar result has been reported by Pasparakis and Bouropoulos [150]. It was found that the release mechanism of verapamil (a model of drug) from Ca-Alg_2 hydrogel beads at pH ~ 1.2 was via case II transport.

It was observed that the values of n (Table 4.2) range from 0.6667 to 0.9048 for RB, Rubpy and CPT release at pH ~ 7.4, which signifies anomalous transport meaning the drug is released by the combined mechanisms of diffusion-controlled drug release and swelling-controlled drug release for the Ca-Alg_2 hydrogel (Refer to Section

4.2.1.1). This observation confirmed that the swelling of Ca-Alg_2 hydrogel at pH~7.4 plays an important role in the diffusion process.

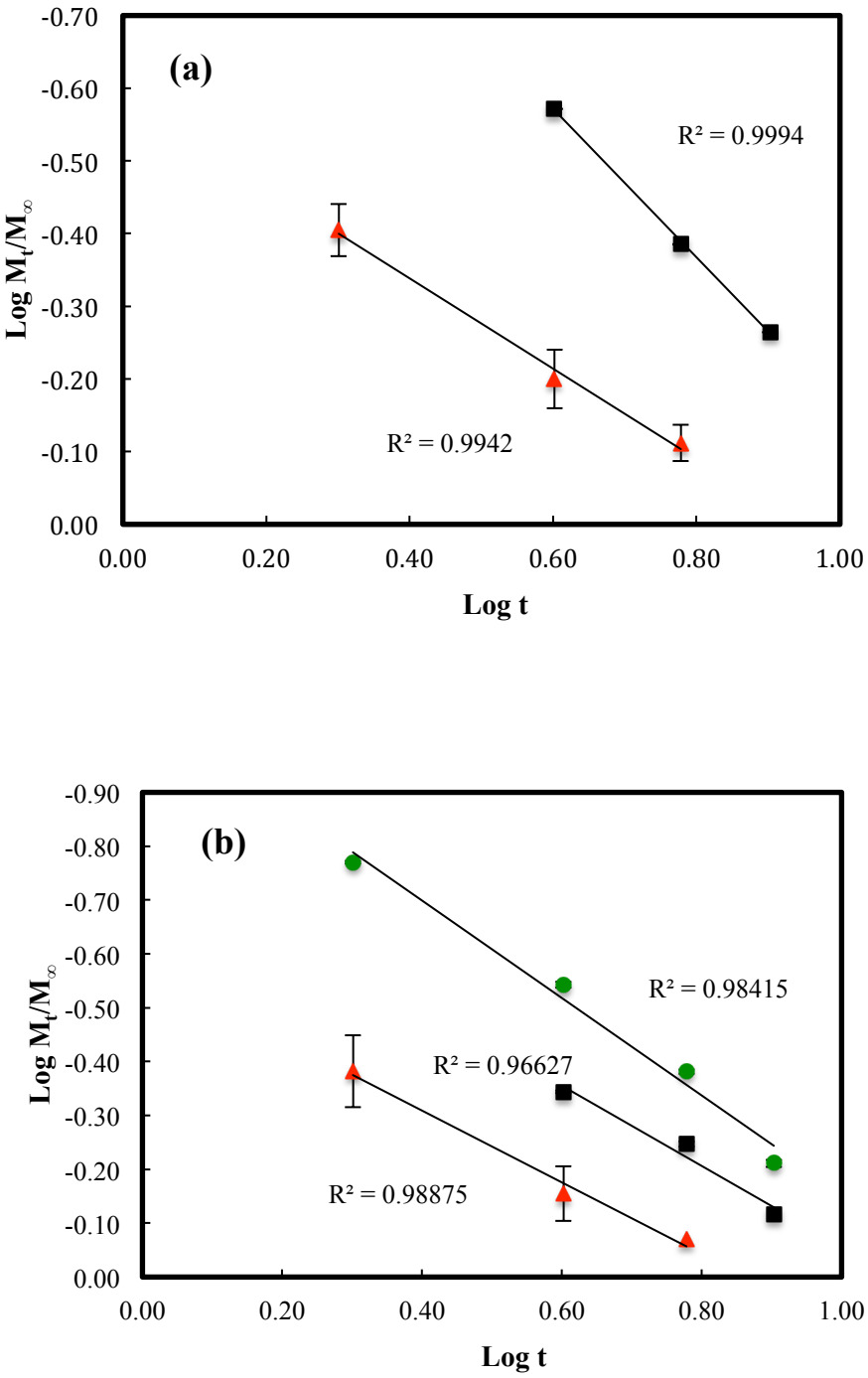


Figure 4-12 Ritger-Peppas model of RB (green circles), Rubpy (black squares) and CPT (red triangles), (a) at pH ~ 2.4 and (b) at pH ~ 7.4.

Table 4-2 Ritger-Peppas and Weibull model parameters with correlation coefficients for RB, Rubpy and CPT release from Ca-Alg_2 hydrogel disc at pH ~ 2.4 and pH ~ 7.4.

Drug	**Ritger-Peppas model**				**Weibull model**		
	n	k	$D \times 10^3$ cm^2 s^{-1}	R^2	a	b	R^2
Rubpy (pH ~ 2.4)	1.0220	0.3057	4.690	0.9994	2.6403	1.3855	0.9989
CPT (pH ~ 2.4)	0.6211	0.5561	3.090	0.9942	1.8327	0.9469	0.9960
RB (pH ~ 7.4)	0.9048	0.3461	3.889	0.9841	2.5395	1.4448	0.9873
Rubpy (pH ~ 7.4)	0.7446	0.4484	3.077	0.9662	2.2299	1.1459	0.9734
CPT (pH ~ 7.4)	0.6667	0.5623	3.065	0.9887	1.7196	0.9828	0.9848

Note: RB at pH ~ 2.4 is not shown as no diffusion was observed.

As the Ritger-Peppas model has a short time approximation and its use is limited to the first 60 % of the release, it is necessary and reasonable to fit the data release to another model, which is the Weibull model [159]. The Weibull model adequately fits the entire release kinetics and has been successfully tested against a large set of experimental release data [177]. The Weibull model of RB (green circles), Rubpy (black squares) and CPT (red triangles) released from the Ca-Alg_2 hydrogel discs at pH ~ 2.4 and pH ~ 7.4, are represented in Figure 4.13 (a and b), respectively. It can clearly be seen that the Weibull model fits the experimental data well with coefficient constants, R^2, ranging from 0.9734 to 0.9989. Table 4.2 shows the Weibull model constants and the correlation coefficients. It can be seen that the values of *b* for CPT and Rubpy (Table 4.2) are 0.9469 and 1.3855 at pH ~ 2.4, respectively. The mechanism of the release of CPT and Rubpy molecules from Ca-Alg_2 hydrogel is therefore via Fickian diffusion and complex release transport (Refer to Section 4.2.1.2) as the shrinkage of the Ca-Alg_2 hydrogel disc, as described previously by the Ritger-Peppas model.

The values of *b* (Table 4.2) ranges from 0.9828 to 1.4448 for RB, Rubpy and CPT released at pH ~ 7.4, implying that the dominant mechanism for RB and Rubpy release is complex release transport while the CPT release mechanism is anomalous transport (Refer to Section 4.2.1.2). It is observed that the results of Ritger-Peppas and Weibull models are identical for CPT only in the swollen regime, and this was not observed for RB and Rubpy.

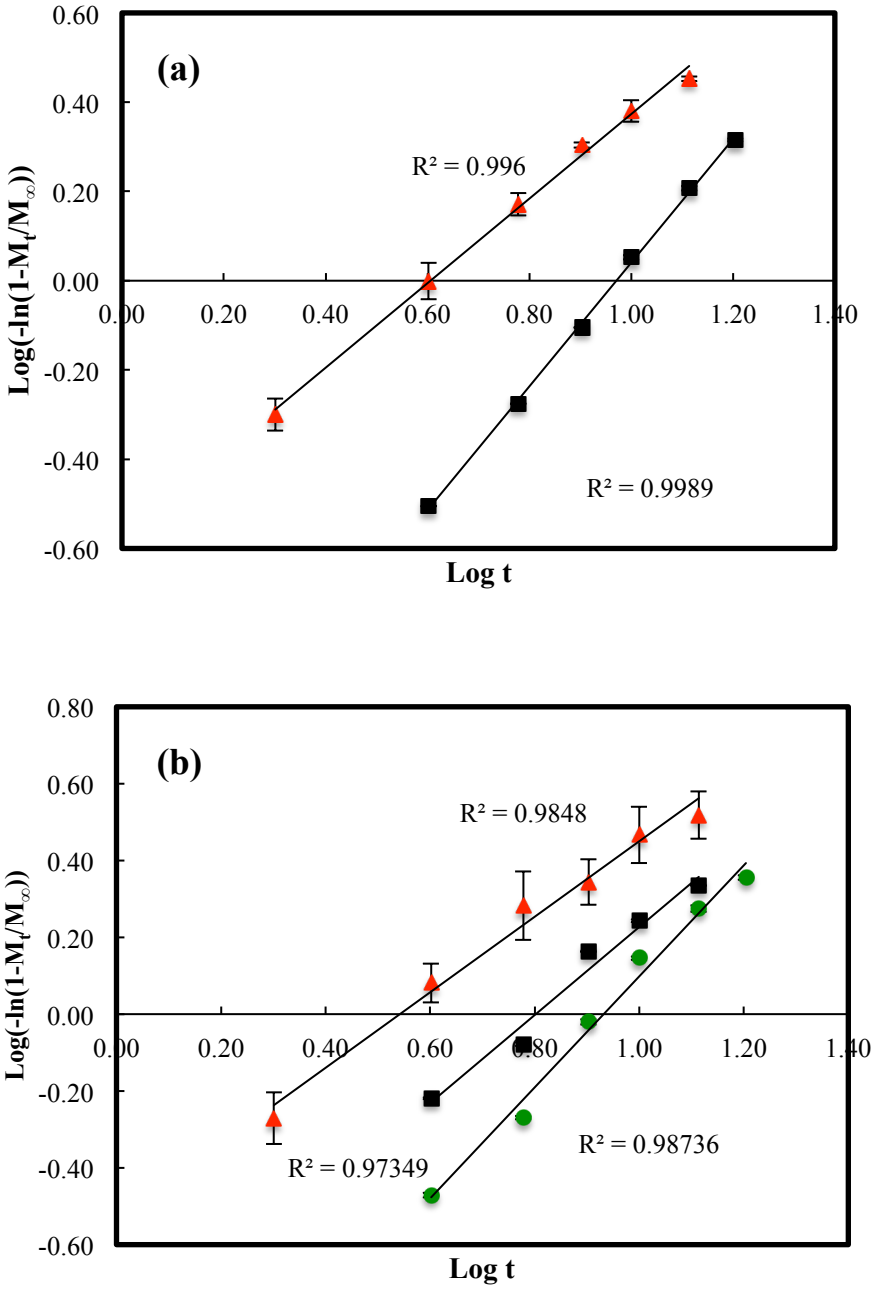


Figure 4-13 Weibull model of RB (green circles), Rubpy (black squares) and CPT (red triangles), (a) at pH ~ 2.4 and (b) at pH ~ 7.4.

By using the values of k and n from the Ritger-Peppas model, the diffusion coefficients were calculated according to the following equation [150, 178].

$$D = \left(\frac{k}{4(\pi l^2)^n}\right)^{\frac{1}{n}}$$

Equation 4.5

Where D is the diffusion coefficient and l is the thickness of the Ca-Alg_2 hydrogel disc. The values of D are presented in Table 4.2. It can be seen that the D of the release of the RB from the Ca-Alg_2 hydrogel discs (3.889×10^3 cm^2 s^{-1}) is higher than the D of the Rubpy and CPT at pH ~ 7.4 (3.077×10^3 and 3.065×10^3 cm^2 s^{-1}, respectively). This may be attributed to a repulsive force between the RB (anionic) and the negative charge of the carboxylate group in the Ca-Alg_2 hydrogel, while the interaction between the Rubpy (cationic) and the negative charge of carboxylate group in Ca-Alg_2 hydrogel decreased the D [170, 179]. Another reason may be attributed to the different wettabilities of the molecules studied, RB is hydrophilic and both Rubpy and CPT are hydrophobic. The diffusion coefficient of the RB is higher than the Rubpy and CPT, due to the rapid water ingress to diffuse RB out as the alginate swells at pH ~ 7.4 [180]. The D values in this study are lower than the literature value for Ca-Alg_2 hydrogel beads at 22.89×10^5 cm^2 s^{-1} for the release verapamil (model of drug) which is a hydrophobic drug with a 491.06 g mol^{-1} molecular weight at pH ~ 1.2 [150]. This was modelled using Peppas model to be Case II transport along with shrinkage at pH ~ 1.2 [150].

4.7 Concluding remarks

This chapter investigated the release of three different molecules, namely rose Bengal (RB), Rubpy, and camptothecin (CPT) from Ca-Alg_2 hydrogel discs. The Ca-Alg_2 hydrogel was prepared by sol-gel transformation in the presence of cross-linking cations (Ca^{2+}) by the *in situ* addition method. The release of the three molecules from the Ca-Alg_2 hydrogel discs was performed in Tris buffer solutions at pH ~ 2.4 and pH~7.4 as a function of time. The release of the molecules was investigated using UV-Vis (for RB and Rubpy) and fluorescence (for CPT) spectrophotometry. The mechanisms of transport of the three molecules from Ca-Alg_2 hydrogel were investigated using two different diffusion models, namely, the Ritger-Peppas and Weibull models. In addition the diffusion coefficients of the three molecules were obtained from the Ritger-Peppas model.

It was observed that the release of the three molecules from the Ca-Alg_2 hydrogel reached equilibrium after 16 min, and the release depended upon two factors, namely the molecular weight and the charge of the dye or drug. It was found that there is no release of RB from Ca-Alg_2 hydrogel at pH ~ 2.4 due to its high molecular weight (1017.64 g mol^{-1}) and the shrinkage of Ca-Alg_2 hydrogel in acidic solution. It was observed that the release of CPT molecules is higher than the release of Rubpy, and this was attributed to the lower molecular weight of CPT (348.35 g mol^{-1}) compared to Rubpy (748.62 g mol^{-1}). It was found that the release of the three molecules from Ca-Alg_2 hydrogel at pH ~ 7.4 was higher than the release at pH ~ 2.4, and this is because of the swelling of Ca-Alg_2 hydrogel in a higher pH solution. It was observed that the positive charge of Rubpy at pH ~ 7.4 delay its release.

A study of the release mechanisms of RB, Rubpy and CPT from the Ca-Alg_2 hydrogels at pH ~ 2.4 revealed that the release occurred via Fickian diffusion and Case II transport. However, at pH ~ 7.4, the release mechanisms of the three molecules from the Ca-Alg_2 hydrogel revealed that an anomalous transport mechanism dominated, implying that the release occurred via diffusion and swelling controlled drug release.

The diffusion coefficients were then calculated by fitting the data to the Ritger-

Peppas model, and it was observed that the diffusion coefficient of RB (3.889×10^3 cm^2 s^{-1}) was higher than the diffusion coefficients of Rubpy and CPT (3.077×10^3 and 3.065×10^3 cm^2 s^{-1}, respectively) at pH ~ 7.4, and this was attributed to the anionic nature and the hydrophilicity of RB compared to that of RB and CPT.

The results of this chapter show the mechanisms of release at various pH's from the Ca-Alg_2 hydrogels but did not appear to provide any sustained or prolonged release of the dye or drug beyond 2 hours. Therefore the following chapters aim to address this by modification of the sodium alginate (Na-Alg) with β–cyclodextrin (β-CD) so that an inclusion complex can be made with CPT in order to control the drug release. The synthesis and characterisation of the β–cyclodextrin grafted alginate is discussed in Chapter 5 while Chapter 6 discusses the investigation of the release of CPT from the inclusion complexes.