Molecular Weight-Dependent Release Profiles In Acid-Labile poly(o-(α-alkyl)vinylbenzyaldehyde) Microcapsules

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Abstract

The introduction and development of stimuli responsive polymers have garnered considerable application within the field of drug delivery. These polymers are advantageous due to their capability for controlled release of their payload. Release is regulated mainly through polymer degradation or swelling, however additional tuning can be attained by manipulating the payload itself. Release rates of varying molecular weight FITC-Dex (3, 10, 500, 2000 kDa) were studied within an acid-labile poly(o-(\(\alpha\)-alkyl)vinylbenzaldehyde) microcapsule. These rates were modeled according to the Korsmeyer-Peppas equation to define the \(n\) and \(k\)-values. It was found that the \(n\)-values for 3, 10, 500, 2000 kDa dextran conjugates were 5.8e-3, 0.31, 0.71, and 0.85 respectively. Molecular weights above 500 kDa were sufficient to convert the release kinetics from first order to zero order release. This presents a method to accomplish greater control over release profiles.
Acknowledgements

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Introduction

Since the introduction of the first polymer-based delivery system, a high level of interest has led many to the design of novel polymeric systems involving controlled release. Improvements in polymer science have led to considerable advancements in manipulating the release kinetics of entrapped molecules. Initially, polymers were used mainly as solubilizing or structural agents in the release of drugs or other active agents (Kim et al. 2009). However, these systems relied on polymers solely for distribution and therefore lacked any control during release of the encapsulated material. With the advent of stimuli-responsive polymers, a higher degree of specificity in regards to both targeting and duration of release is allowed. Stimuli-responsive polymers undergo either a conformation/ integrity change or degradation in response to shifts in physical properties such as temperature, pH, and ion concentration among others (Bajpai et al. 2008). More specifically, polymeric systems that are responsive to pH have been developed considerably. The sensitivity to pH has led to its prominence for use in drug delivery systems where release of encapsulated material can be localized to specific compartments in the human body (Gao et al. 2007). Furthermore, pH responsive polymers have also found potential use industrial applications where environmental or applied pH changes can activate release.

Upon activation of release, it is important to take into consideration the kinetics associated with it. Controlling release kinetics has chiefly been studied in the context of drug delivery (Zalfen et al. 2008). For the drug to be the most effective, its concentrations must be within the range of a therapeutic window. Concentrations above this window would lead to adverse effects while concentrations below become ineffective. Currently, the most common form of drug release is a “burst” release which follows first-order kinetics. Here, drug concentrations only remain within the therapeutic window for a short duration as there is a concentration spike in the surrounding medium followed by rapid excretion. Therefore drug release should be controlled in a manner in which the drug
concentration is maintained within the upper and lower threshold. Maintaining a constant equilibrium between drug release and excretion can be accomplished by zero order release kinetics (Siegel et al. n.d.). Zero order release is defined by a release rate that is independent of its concentration, thereby the resultant net diffusion out of a barrier is constant throughout the process.

In order to achieve zero-order release, factors that affect diffusion are considered. Among them, matrix degradation, solute diffusion, and polymeric swelling are proposed to play a significant role in solute transport from polymeric matrices (Lager & Peppas 1981). A mathematical description which incorporates these aspects are provided in Fick’s law of diffusion. Fickian diffusion describes solute transport in which the polymer relaxation time is greater than the characteristic solvent diffusion time. Solute transport becomes non-Fickian once the ratio of diffusion times become equal or when solvent diffusion time dominates. The purpose of modeling solute transport serves to elucidate a pattern for release kinetics, as well as to simplify an otherwise complicated process. The Korsmeyer-Peppas model is derived from Fick’s laws and was used to model release (Ritger & Peppas 1987). This model is advantageous due to its simplicity and potential application to non-swellable polymeric delivery systems. In this model, $M_t$ and $M_\infty$ represent the amount of payload released at time $t$ and $\infty$ respectively. The $n$ value is the diffusional exponent which is related to the transport mechanism, while the constant $k$ is the kinetic constant which is dependent on both the encapsulated material and system.

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

**Equation 1. Korsmeyer-Peppas Model for Drug Release**

As mentioned previously, the release kinetics are chiefly dependent on the polymeric system as well as the solute undergoing transport. While there has been an abundance of studies performed on the polymeric design of delivery systems, few have been performed on the manipulating the solute itself. The physical properties of the solute can therefore be tailored to influence the kinetics during
release. Additionally, it is commonly known that larger molecules take longer to diffuse than their smaller counterparts (Soleimani et al. 2012). The release kinetics of the encapsulated material can then be tailored by the addition of a molecular weight (MW) moiety that is conjugated to the active molecule.

In this study, MW-dependent diffusion rates were analyzed through the acid labile polymer poly(α-(α-alkyl)vinylbenzaldehyde) (PMVB) microcapsules. Four different MW conjugates of FITC-Dextran (3, 10, 500, 2000 kDa) were encapsulated within the PMVB microcapsule and subsequently subjected to a low pH environment. The ensuing outward diffusion was monitored via fluorescence microscopy. Capsules with the PMVB polymer comprising the shell was have previously been studied for their pH-dependent degradation rate as well as the mechanism of release. Interestingly, it was found that a multitude of 50 nm cracks were formed in the shell wall during acid exposure unlike the more common mechanism involving bulk or surface erosion (Grolman et al. 2015). This not only suggests a mechanism in which the FITC-Dex is able to diffuse out of the capsule, but potentially introduces a new system for interpreting release profiles. Therefore by modeling the resultant data per each dye conjugate, the release kinetics and general mechanism of diffusion can be inferred. The goal of this project was to determine the range of MWs necessary for a conjugate to have a significant effect on diffusion, as well as the ability of the conjugate to shift release kinetics to a zero-order process.
Materials and Methods

Synthesis of poly(o-(α-alkyl)vinylbenzaldehyde) (PMVB) Polymer

All chemicals used in the synthesis were purchased from Sigma-Aldrich. PMVB monomer was synthesized in a two-step method starting from commercially available 2-bromoacetophenone. In the first step, a two neck round-bottom flask was flame-dried and flushed with nitrogen to remove water. Subsequently, 17.94g of methyl-triphenyl phosphonium bromide (Ph₃PCHBr) 7.05g of potassium tert-butoxide (tBuOK) were mixed together in the flask. To the mixture, 100mL of anhydrous THF was added and the solution was stirred at room-temperature for 30 minutes. After stirring, 5g of 2-bromoacetophenone was added to the flask and stirred at room temperature overnight. The reaction was then quenched with 150 mL NH₄Cl solution and the THF phase was extracted. The organic phase was washed three times with 40mL ethyl acetate and subsequently dried with MgSO₄. The organic phase was gravity filtered and rotovapped. The resulting product was re-dissolved in a small amount of ethyl acetate and run through a column to purify the compound, o-benzylstyrene.

In the second step of the synthesis, a two neck round-bottom flask was flame-dried, capped and placed under nitrogen. Afterward, 40 mL of anhydrous THF was transferred into the flask via a syringe. The o-benzylstyrene from the first synthesis was transferred in the same manner. The flask was then lowered into a dry ice bath and 7.22 mL of n-butyllithium was added via cannula transfer. The solution was allowed to stir in the dry ice bath for 30 minutes. After stirring, 1.4 mL of DMF was added dropwise to the flask and stirred for an additional 20 minutes. The reaction was then quenched with 100 mL of NH₄Cl. The organic layer was extracted twice with 50 mL of diethyl ether and then dried with MgSO₄. The phase was gravity filtered and rotovapped. The resulting mixture was purified via column chromatography using a 1:20 solution of ethyl acetate:hexane. The product was subjected to additional purification using a Kugelrohr short path distillation at 50 °C.
Polymerization was performed in a glovebox. A Schlenck flask was vacuum sealed prior to working in the glovebox. In the glovebox, 0.5 mL of the monomer from the second step and 5 mL of dry DCM were added to the Schlenck flask. The flask was taken out of the glovebox and placed in an acetone dry ice bath under nitrogen. To the flask, 35 μL of boron trifluoride etherate was added and the reaction was stirred for one hour. After stirring, the reaction was quenched with 35 m of quinolone and cooled to room temperature. The polymer was crashed out in 400 mL of methanol and vacuum filtered. The collected precipitate was subsequently dried overnight on vacuum.

**Microfluidic device fabrication:**

Capillaries were supplied by Vitrotube®. A round capillary with inner diameter 0.7mm and outer diameter 0.87mm was pulled using a micropipette puller (P-1000 Sutter Instrument Co., Norfolk, UK) and broken into two identical parts with tapered ends. Another capillary of the same dimension was flame-polished under a Bunsen burner to decrease the diameter of the end. Both the tapered and flame polished capillary were inserted and aligned inside a square capillary with inner diameter of 0.9mm and outer diameter of 1 mm. The capillaries were fixed to a glass slide by using a UV-curable resin. Three wax tips were then glued on the junctions of the capillaries using a two-part epoxy glue (Loctite Epoxy®). The device was left overnight to allow the epoxy to fully cure.

**Formations/ Use of microfluidic device**

The core-shell μCs were formed using the flow-focusing glass capillary device as mentioned before. The oil phase (middle fluid) consisted of the PMVB polymer (10% wt) and rhodamine B (1 mg/mL) dissolved in chloroform. Polyvinyl alcohol (PVA) was included in the inner (5% wt) and outer (10% wt) fluid which comprises the water phases. Additionally, the inner fluid contained 25 mg/ml of the respective MW of FITC-Dextran (3, 10, 500, 2000 kDa). These fluids were loaded into respective syringes and secured onto a syringe pump. A plastic tube then connected the tips of the syringes to the device.
itself. Each set of microcapsules only contained one MW FITC-Dextran molecule as they all have similar fluorescence wavelengths. As the microfluidic device is assembled manually and therefore has small fluctuations in dimensions, the same device was used to encapsulate different conjugates. The device was thoroughly washed in between by running 4 mL of water through it. Images of microcapsule formation were captured on a Phantom high speed camera. Each set of microcapsules only contained one molecular weight conjugate of FITC-Dextran.

**Environmental Scanning Electron Microscopy (ESEM)**

Shell wall thickness was determined primarily through ESEM. After formation, a small amount of microcapsules were separately washed four times with 20 mL distilled water and left to dry under nitrogen. A random selection of these microcapsules were mounted on SEM stubs and subsequently ruptured with a razor blade. The stub was subsequently sputter coated with a thin layer of gold/palladium. Seven capsules from each sample set were measured for size and shell thickness under ESEM. Images were captured under vacuum with a 5 kV electron beam at 10 mm working distance. The thickness of the shell walls were measured through ImageJ software.

**Fluorescence Spectroscopy**

After formation, μCs were collected in a 10% weight PVA solution and left to stir overnight to prevent settling. The μCs were then washed four times with 20 mL distilled water to remove residual PVA. The microcapsules were transferred to a 96 well plate and viewed under a fluorescence microscope. A solution of 12 mM para-toluene sulfonic acid (pTsOH) was added to the wells to trigger PMVB degradation. The average fluorescence intensity per capsule was measured for each time point and averaged. Roughly 150 capsules were monitored during each test condition with varying molecular weights of FITC-Dextran. Controls were also performed in the same manner with a solution of 0.1 M
NaCl solution and 0.1 M phosphate buffer being added to the well plates rather than the pTsOH solution.

**Data modeling**

The fluorescence data was fit according to the Korsmeyer-Peppas model of drug diffusion. The log of the equation was taken to transform it into a linear equation and the values were plotted via OriginLab. A linear regression utilized on the data points with the resulting slope equating to the $n$-value and the $k$-value defined as $10^{y}$ to the power of the y-intercept. Because the net diffusion eventually reaches equilibrium after a certain period of time, only the first 60% of the data attained from fluorescence microscopy was used in the model.
Results and Discussion

PMVB Polymer

Scheme 1. Reaction Diagram of PMVB Synthesis. Step 1 shows the conversion of the starting product 2-bromoacetophene into the o-bromo-(α)-styrene. Step 2 then converts the o-bromo-(α)-styrene into the precursor monomer for polymerization.

Figure 1. Mechanism of PMVB Degradation. Picture of mechanism from (Inci et al. 2013)
Polymerization of PMVB was achieved via a cationic polymerization. Upon exposure to acid, the resultant polymer ($M_n = 46$ kDa, PDI = 2.7) undergoes degradation via protonation of the ether backbone. Subsequent elimination generates an indenyl alcohol, 3-methyl-1H-inden-1-ol. This polymer was previously tested for pH-dependent release properties while keeping the molecular weight of the encapsulated material constant. In these tests, it was determined that PMVB had many desirable traits as a capsule shell including chemical resistance, strong mechanical properties, and formation of a non-porous shell. The microcapsules were formed from the same initial polymer solution as to keep the average molecular weight within the shell constant. The molecular weight of a polymer is crucial in its degradation kinetics, with larger molecular weights generally having slower rates in degradation. Therefore the fact that the microcapsules were derived from the same solution prevents introduction of additional variables that affect the release profiles of FITC-Dex.
Microfluidic Device

Figure 2. Picture of Microfluidic Device. The microfluidic flow-focusing device used to create W/O/W double emulsions

A microfluidic flow focusing device was assembled during the experiment to form water/oil/water (W/O/W) double emulsions comprising the microcapsules. The device itself consists of three nozzles that correspond to the inner (aqueous), middle (oil), and outer (aqueous) phases. The inner fluid contains the encapsulated FITC-Dextran while the middle fluid contains the PMVB to form the capsule shell. The outer phase is the continuous fluid which provides the force necessary to form dispersions resulting in microcapsules.
Flow rates used in microfluidic device

During microcapsule formation, the combination of flow rates (inner-middle-outer: 900-620-6000 in μL/hr) created relatively monodisperse set of capsules. The ratio of middle fluid flow (F2) to inner fluid flow (F1) can be tuned to adjust the shell thickness (D2) with higher ratios corresponding to thicker shell walls (Nabavi et al. 2015). While the outer flow (I3) is inversely proportional to the overall diameter (D1) of the capsule. Because higher D2 corresponds to slow release due to longer degradation times, it was important to maintain an ideal D2 between testing the various MW FITC-Dextran conjugates. This prevents the introduction of any extra variables when measuring the release during acid treatment. D1 was also monitored closely as the mechanical properties of the capsule itself are highly dependent on the diameter. Larger diameter capsules have a propensity to collapse after

Figure 3. Image of Microfluidics during Microcapsule Formation. Monodisperse capsules are able to be formed by keeping the flow rates of the inner, middle, and outer fluid consistent. Individual flows are labeled.
formation while smaller capsules lead to unstable formation. It was found that D2 remained relatively constant so long as F2 was between 650-950 μL/hr. Flow rates beyond this range led to a loss of stability during capsule formation and led to a higher degree of polydispersity within subsequent capsules.
ESEM images of microcapsules depicting overall diameter and shell thickness

Figure 4. ESEM Images of Capsules before Acid Exposure. (A) Non-ruptured capsules (B) Capsules that have been ruptured with a razor blade to measure shell thickness. (C) Magnified view of ruptured shell wall (D) Close up image of shell thickness that was measured through ImageJ software.
Figure 5. Graph of Mean Shell Thickness. Represents the mean shell thickness from seven capsules that were measured from each MW-Dextran set and averaged.

Table 1. Actual Mean Values of Shell Thickness.

<table>
<thead>
<tr>
<th>Dextran Mw (kDa)</th>
<th>Mean Thickness (nm)</th>
<th>Standard Deviation (nm)</th>
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<tr>
<td>3</td>
<td>2250</td>
<td>98.34</td>
</tr>
<tr>
<td>10</td>
<td>2341</td>
<td>58.07</td>
</tr>
<tr>
<td>500</td>
<td>2259</td>
<td>45.82</td>
</tr>
<tr>
<td>2000</td>
<td>2311</td>
<td>95.31</td>
</tr>
</tbody>
</table>

The shell thickness was measured and visualized through ESEM prior to acid exposure. It was found that the standard deviation between the mean shell thicknesses was relatively low for each set of MW-Dextran capsules- varying by a tenth of a micrometer at max. Since release is also dependent on the thickness of the shell wall, the low deviation minimizes any contribution that it has in influencing diffusion. Therefore keeping the main variables in the experiment dependent on the MW of the dextran conjugates.
Fluorescence Spectroscopy Graph and Fit through Fick’s Laws

**Figure 6. Graph of Fluorescence Measurements.** Monitors the percent release of FITC-Dextran from the capsule after the capsules have been exposed to an acidic medium as well as the power law fit after the Korsmeyer-Peppas model. Graph of raw data provided by J. Grolman.

Upon exposure to acid, the microcapsules began releasing FITC-Dextran immediately. Control experiments were tested in both a solution of NaCl and phosphate buffer. When comparing the effects of the acidic environments with that of the controls, the release profiles of microcapsules subjected to acid was considerably faster. After release, the pH of the supernatants were measured and found to be around 2.47 for the pTsOH solutions. Lower $n$ are indicative of Fickian diffusion while higher values specify more degradation-induced diffusion. As expected, the rate of release was inversely proportional to the molecular weight of the encapsulated drug. Complete release of the 3 kDa FITC-Dextran was achieved in a short period of time whereas the 2000 kDa FITC-Dextran had a much slower rate of release. The 10 kDa depicted a typical “burst” release with a rapid initial outward diffusion followed by slower
release as time increased. The transition from first order to zero order release kinetics can be seen as the MW of the dextran conjugates increases. The rate of release gradually becomes slower and the slope of the percent release becomes almost constant in the 200 kDa dextran conjugate.

N, k values derived from model

![Graph of n, k value dependency on MW](image)

**Figure 8. Graph of n, k value dependency on MW**
Table 2. Table of calculated n, k values

<table>
<thead>
<tr>
<th>Dextran MW (kDa)</th>
<th>n</th>
<th>k</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>5.8e-3</td>
<td>93.32</td>
</tr>
<tr>
<td>10</td>
<td>0.31</td>
<td>52.48</td>
</tr>
<tr>
<td>500</td>
<td>0.71</td>
<td>3.24</td>
</tr>
<tr>
<td>2000</td>
<td>0.85</td>
<td>2.57</td>
</tr>
</tbody>
</table>

To further analyze the release profiles of the microcapsules, the data was fit to a power law derived from Fick’s law of diffusion. Release of the respective FITC-Dextran was modeled after the Korsmeyer-Peppas model for drug diffusion. Generally, $n \leq 0.45$ is indicative of Fickian diffusion while a value $0.45 < n \leq 1$ designates anomalous/ non-Fickian diffusion (Ritger & Peppas 1987). For values of $n > 1$, the model becomes redundant and another model must be chosen to properly characterize release.

As shown in Table 2, the 3 kDa and 10 kDa dextran conjugates demonstrated Fickian release while the release profile of the 500 kDa and 2000 kDa conjugates were degradation induced. There is a sharp decrease in the n which coincides with an increase in k values (Fig 8). Therefore these values are more susceptible to change at lower MW. Since the ratio between the k and n values of the 500 kDa and 2000 kDa conjugates approaches unity, at higher MW they can be fine-tuned in a pseudo-zero order release profile.
Conclusions

In this project, the correlation between molecular weight and diffusion kinetics has been explored for a polymeric release system utilizing the acid labile polymer PMVB. As expected, the molecular weight of the dextran moiety was inversely proportional to the rate of diffusion outside the capsule. A benefit of using the Korsmeyer-Peppas model is that the $n$ value directly states whether diffusion is a zero order or first order kinetic process. As the value $n$ approaches 1, the resultant process becomes more reminiscent of a zero-order rate law- with values between 0.89-1 indicating zero-order processes. It was observed that the $n$-value was directly correlated to the molecular mass of the dextran moiety. Therefore a trend was established as molecules with increasing molecular weight will gradually shift from first order toward zero order diffusion.

The 3 kDa and 10 kDa dextran conjugates demonstrated Fickian release while the 500 kDa and 2000 kDa conjugates were non-Fickian in release and trended towards induction by polymer degradation. These values are logical when considering the mechanism of capsule degradation. Rather than the more conventional bulk or surface erosion of many microcapsules, the PMVB shell forms slits less than 50 nm wide during acid exposure. In the case of the 3, 10 kDa conjugates, the diameter of the initial slit is large enough for rapid outward diffusion characteristic of burst release. As higher MW conjugates have a correspondingly larger radius of gyration, their diffusion is restricted during the early stages of slit formation. After prolonged exposure, this restriction is gradually lessened as the slit diameter increases and the conjugates have a higher propensity to diffuse out. Thus the 100, 2000 kDa conjugates have non-Fickian release as they rely on degradation of the shell wall in determining release profiles.

An additional explanation for the release profiles may be attributed to the physical properties and hydrodynamics of the conjugates themselves. The rate of diffusion is inversely proportional to the mass of a substance, as well as the specific volume and irregularity of the structure. The latter two
components are important aspects in hydrodynamics. Substances with a larger specific volumes and shapes deviating from a spherical nature will have an increased surface area. The increased exposure to the solvent leads to a higher degree of frictional interaction and decreases the diffusion rate. While it is simple to approximate the dextran moiety as a rough sphere, dextran molecules with molecular weights greater than 5 kDa form extended coils in solution. The propensity to form a secondary structure may not only make the particle less hydrodynamic, but also increase the radius of gyration to further restrict diffusion through the slit. This facet of using dextran should be considered before further application of this system, especially when considering encapsulation of proteins as proteins generally form globules in solution. Therefore it is important to establish that increases in MW will not cause the same rate of spacial increase when comparing different types of molecules.

Currently, the majority of polymeric delivery systems rely on their design and degradation/swelling mechanisms to influence release rates (Allen & Cullis 2004). The ability of the dextran conjugate to manipulate diffusion rates presents another method of tuning the release of active molecules. Therefore an additional degree of control can be achieved for finer release. However, in regards to the conjugation of a molecular weight moiety to a molecule, it is important to consider the liberation of the molecule from the MW moiety after release. The relative sizes of the conjugate to the molecule itself is immense and is prone to decrease the efficacy of the molecule to its target through steric hindrance. In drug delivery applications, the targeting of in vivo environments often has the presence of enzymes that can target specific bonds for release. The method of conjugation can therefore take advantage of these enzymes for liberation by choosing a specific bond or linker. Indeed, this has been the strategy of many prodrugs that utilize a specific sequence of peptides as a linker such as matrix-metalloproteins present in the tumor environment (Maeda et al. 2000). A certain enzyme subsequently recognizes the sequence and proceeds to cleave it- release the free drug. In this case
however, the enzyme in question should also ideally be immobilized to prevent its diffusion into the capsule- thereby eliminating the potential for any premature release.

The implications of controlling release rates also leads to the possibility of stepwise multicomponent release. As mentioned previously, many studies have focused on the polymeric aspect of delivery systems. There is a limitation in only focusing on the polymeric degradation as the release of molecules is solely dependent on the rate of degradation. Therefore, barring any large variations in the molecular weight of the encapsulated particles, the contents will all release at roughly the same time frame. However, conjugation of a molecular weight moiety to specific molecules allows for control of their release kinetics. It is therefore possible to time release in such a way that the effective concentration of one molecule is reached after another.

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