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Research paper

Photosensitive controlled release with polyethylene glycol–anthracene modified alginate

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ABSTRACT

Covalent modification of alginate with polyethylene glycol–conjugated anthracene molecules has the potential to both stabilize the alginate and act as a photosensitive crosslinker. Release studies with Coomassie Blue show lengthy release times from the alginate photogels that extend past 70 days with, for example, 17% versus 27% release at 1750 h (73 days) for photogels with and without 365-nm UV light treatment for 30 min at 10 mW/cm^2 in the initial release period. Photocrosslinking of the photogels after loading effectively "locks" in drug compounds to control their release. Effective crosslinking densities and controls of polyethylene glycol-crosslinked alginate and physically crosslinked calcium alginate gels suggest strong interactions between Coomassie Blue and both alginate and anthracene. Photogels containing anthracene-capped star-polyethylene glycol show increased photosensitivity with modified release profiles. Ultimately, the covalent modification of alginate with photoactive crosslinkers has the potential to produce a long-term, photosensitive, controlled release system.

1. Introduction

Alginate is a non-toxic, inert and hydrophilic polymer conducive to the storage and delivery of active proteins, drugs, and cells [1,2]. Composed of linear copolymers of β-D-mannuronic and α-L-guluronic acid, alginate is generated by renewable resources such as kelp, algae, and bacteria [1,3]. While commonly used physically crosslinked with divalent ions such as calcium, these gels can have weak mechanical properties and uncontrolled degradation kinetics since ions and chelators in solution can lead to their rapid dissolution [4]. Research has focused on the modification of alginate to produce stable, long-term delivery devices [2,4].

The reinforcement of calcium alginate microspheres with oppositely charged materials such as chitosan [5], poly-L-lysine [6,7], and dipalmitoyl phosphatidylcholine liposomes [8] are but a few examples of how researchers have tried to reinforce alginate to control drug delivery from microcapsules and gels. However, covalent modifications along the backbone of the polymer can stabilize the gel matrix and control pore size to specifically control drug delivery [9]. There are several examples of covalent crosslinking of alginate to improve its mechanical and drug delivery properties using crosslinkers such as albumin [10], polyethylene glycol (PEG) [11,12], lysine [12], adipic dihydrazide [12], glutaraldehyde [13], or peptides that cause cellular crosslinking [14]. Hydrophobic modifications in particular have shown great promise in stabilizing the matrix and increasing the release times of various proteins and drugs [9,15–18]. These materials have increased release times versus calcium alginate controls and their swelling/crosslinking properties can be altered with changes in grafting density and changes in the size of the crosslinking molecules [12].

Covalent modification of alginate with stimuli-sensitive molecules cannot only further improve its stability but also introduce other responsive properties to fabricate smart gels. Alginate has already shown natural pH-sensitive properties in past studies with lower release times in lower pH buffers [19,20] making it well suited for oral delivery, where gastric and intestinal fluids have varied pHs. However, pH does not typically vary significantly *in vivo* so the use of other stimuli may be more relevant. Recent examples show the ability for the grafting of or synthesis of interpenetrating networks of alginate with poly(N-isopropyl acrylamide) to produce thermosensitive drug delivery systems [21,22].

Since the research of our group is mainly focused on ophthalmic drug delivery, light can be considered as a potentially valuable drug release stimulus. To produce stimuli-responsive hydrogels that respond to UV light and lasers, we have covalently modified

Abbreviations: PEG, polyethylene glycol; Photogel, PEG–anthracene grafted alginate; Control PEG hydrogel, PEG–diamine crosslinked alginate; Boc-PEG-amine, *O*-(2-aminoethyl)-*O*′-[2-(Boc-amino)ethyl]decaethylene glycol; PEG–diamine, *O*,*O*′-bis(2-aminoethyl)octadecaethylene glycol; EDC, 1-ethyl-(dimethylaminopropyl) carbodiimide hydrochloride; NHS, N-hydroxysuccinimide; MES, 4-morpholinoethanesulfonic acid; PBS, phosphate-buffered saline; TBS, TRIS-buffered saline; UV, ultraviolet.

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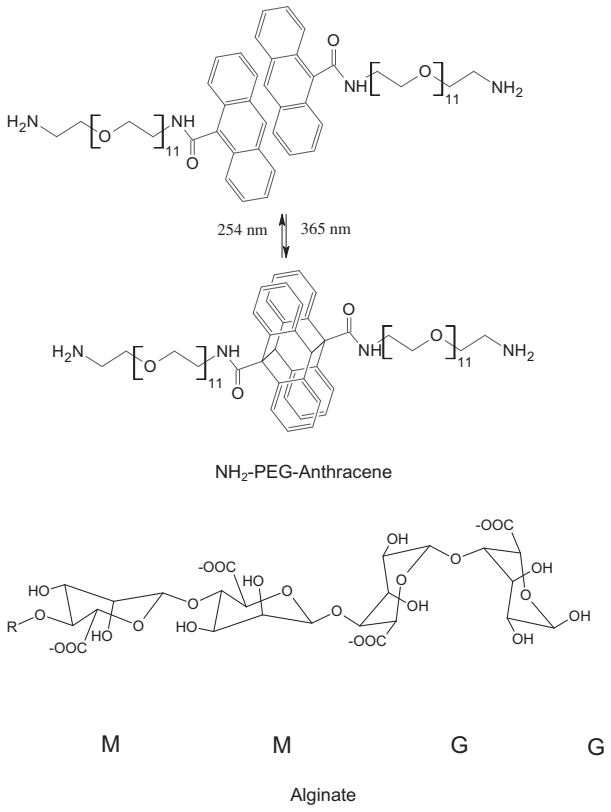

Fig. 1. PEG–anthracene photoreversible dimerization and alginate. PEG–anthracene can dimerize and de-dimerize with 365 nm and 254 nm light. Alginate contains β-D-mannuronic acid (M) and α-L-guluronic acid (G). PEG–anthracene can be grafted onto the carboxyl group of alginate to crosslink it via dimerization.

hydrogels with a PEG–anthracene based graftable photocrosslinker [23] (see Fig. 1) that has shown promise for the introduction of photosensitive properties to hydrogel polymers including alginate while demonstrating cytocompatibility [23]. Anthracene photodimerizes with other anthracene molecules upon exposure to 365 nm light and de-dimerizes with 254/248-nm light treatment in a reversible reaction that causes crosslinking and potentially de-crosslinking when grafted along the backbone of polymers. Alginate covalently linked to PEG–anthracene crosslinkers has been previously demonstrated to generate gels that respond to light treatment.

Herein, we report on the properties of PEG–anthracene grafted alginate "photogels". Not only do these gels deliver over lengthy periods beyond 2000 h, but different light treatment times, different model release compounds, and different formulations can be used to alter the release characteristics of the photoresponsive alginate. Furthermore, this technique has the potential for loading of the photogels and locking the drug in place with exposure to UV light.

2. Materials and methods

2.1. Materials

Sodium alginate (61% β-D-mannuronic acid, 39% α-L-guluronic acid, MW = 12–18 kDa, from *Macrocystis pyrifera*), *O*-(2-aminoethyl)-*O*′-[2-(boc-amino)ethyl]decaethylene glycol (Boc-PEG-amine, $n = 11$), *O,O*′-bis(2-aminoethyl)octadecaethylene glycol (PEG–diamine, $n = 20$), and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich (Oakville, ON). Anthracene-9-carboxylic acid was from Alfa Aesar (CA). Coomassie Blue G-250 was purchased from Fluka Chemicals (Switzerland). Anthracene-terminated four-arm poly(ethylene oxide) made with a pentaerythritol core (star-PEG–anthracene) of 9500 Da (polydispersity of 1.15) with over 90% functionality was purchased from Polymer Source (Quebec). Fast Green and other reagents were purchased from Sigma–Aldrich (Oakville, ON) and EM Science (Gibbstown, NJ). NMR spectra were obtained with a Bruker AV 200. The 365-nm lamp source was a 10 mW/cm^2 Curezone II UV lamp from CON-TROL-CURE (Chicago, IL) (400 W, 60 Hz) and the 254-nm source (0.63 mW/cm^2) was an EL Series UVLS-28 UV lamp from UVP (Upland, CA) (8 W).

2.2. PEG–anthracene crosslinker

Amine-terminated PEG–anthracene (MW = 748.9 g/mol) is a water soluble photocrosslinker that can be grafted to different polymer backbones. The preparation of the crosslinker followed a procedure described by Wells et al., by reacting Boc-PEG-amine with anthracene-9-carboxylic acid followed by deprotection in trifluoroacetic acid with triisopropylsilane as a scavenger [23]. Specifically, under dark conditions, 200 mg of Boc-PEG-amine was reacted with 288 mg of anthracene-9-carboxylic acid with 268 mg of EDC in 20 mL of dry dichloromethane (DCM) under nitrogen (respective molar ratio of 1:4.2:4.5). After removing unreacted reagents and side products by extraction with a water/ethyl acetate mixture, the protecting groups were removed in 20 mL of DCM by 4 mL of trifluoroacetic acid with 2 mL of triisopropylsilane as a scavenger followed by purification with filtration or silica columns [23].

2.3. Hydrogel synthesis

2.3.1. Photogels

As depicted in Fig. 1 and as previously described [23], the amine group on the PEG–anthracene was reacted with the carboxyl groups on the alginate. A 6% aqueous solution of alginate was premixed with EDC (394 mg/mL) and N-hydroxysuccinimide (NHS) (118 mg/mL) for 5 min and subsequently added to amine-terminated PEG–anthracene (428 mg/mL). The mixture was then placed between two glass plates using a 1-mm spacer and allowed to react for 16 h at room temperature. All solutions were in 4-morpholinoethanesulfonic acid (MES) buffer at pH 6 (0.1 M MES and 0.5 M NaCl with 1 N NaOH to pH 6) to promote EDC activity [24]. The $COOH_{alginate}$/EDC/NHS was mixed at a molar ratio of 0.8:2:1. Previous work shows that under these conditions, the resulting gels will have 42% grafting of PEG–anthracene onto the alginate polymer backbone [23]. Loose gels resulted due to physical entanglements and pi bond interactions between the aromatic groups on anthracene. These photogels were punched into disks 0.5 cm in diameter, soaked in de-ionized water overnight, and then dried at room temperature for 72 h. Degradation via hydrolysis and de-crosslinking was monitored with gel disks soaking in PBS buffer at 37 °C under shaking. ^{13}C NMR studies were performed on photogels crosslinked in quartz NMR tubes with and without UV light treatments to observe any changes in the carbon bonds. Percent swelling of the gels was of the dry mass divided by the mass after soaking for 24–122 h in PBS or water. Degradation of the gels was monitored by soaking in PBS buffer at 37 °C in a shaking water bath as per the release study procedure outlined below.

2.3.2. Control PEG hydrogels

Hydrogels made from alginate covalently crosslinked with PEG–diamine were used as controls. A bifunctional PEG molecule which can bind to the alginate backbone at both ends was used. The same procedure as with the photogels was used except for either 3 unit or 20 unit amine-terminated PEG molecules (PEG–diamine) were

grafted in a 1:1 amine to carboxyl molar ratio. The resulting control PEG hydrogels were punched into disks 0.5 cm in diameter, soaked in de-ionized water overnight, and then dried at room temperature for 72 h.

2.3.3. High alginate concentration photogels

To synthesize photogels with a higher concentration of alginate, the same synthesis procedure as with the photogels was used except 12% alginate was substituted for the 6% alginate solution. The resulting high concentration alginate photogels were punched into disks 0.5 cm in diameter, soaked in de-ionized water overnight, and then dried at room temperature for 72 h.

2.3.4. Calcium alginate gels and calcium-reinforced photogels

As an additional control, alginate gels crosslinked with calcium were prepared. To make these gels, approximately 0.1 mL of 3% alginate in water was placed between two glass plates with a 1-mm spacer. Calcium chloride (0.1 M) was then injected into the surrounding space, and the solution was allowed to gel for 4 h. At this time, the gels were punched and soaked in 0.1 M calcium chloride for 16 h to ensure complete crosslinking.

Calcium-reinforced photogels were synthesized by soaking water-swollen photogels in 0.1 M calcium chloride for 16 h to allow calcium ions to diffuse and reinforce any unbound guluronate blocks with physical crosslinks.

2.3.5. Star-PEG–anthracene containing photogels

During the synthesis of photogels containing star-PEG–anthracene, the premixed solution of 6% alginate and EDC/NHS was added to a 1:10 M ratio solution of star-PEG–anthracene and PEG–anthracene. More specifically, to prepare these gels, 1 mL of 6% alginate was mixed with 0.354 mL of EDC/NHS solution for 5 min and then added to a 0.443 mL solution containing 60 mg of star-PEG–anthracene and 190 mg of PEG–anthracene. The mixture was then gelled at 4 °C for 72 h. The resulting gels were punched into disks 0.5 cm in diameter, soaked in de-ionized water overnight, and subsequently dried at room temperature for 72 h.

2.4. Drug loading

Coomassie Blue and Fast Green were chosen as model drug compounds for release studies since they contain different amounts of aromatic and charged groups and can easily be detected spectrophotometrically at 595 and 630 nm. For loading, dried hydrogel disks were individually soaked in 1 mL of a 0.5 mg/mL solution of Coomassie Blue or Fast Green in phosphate-buffered saline (PBS) for 24 h. The disks were then rinsed twice and soaked for 30 min prior to the release studies in order to remove any residual, loosely associated model compounds. Loading was estimated spectrophotometrically by measuring the concentration of Coomassie Blue or Fast Green in solution before and after loading. The concentration change was assumed to be the amount loaded into the gels.

Calcium alginate gels cannot be loaded using PBS because the phosphate ions in it sequester calcium to help rapidly de-crosslink the gels on the order of hours. Therefore, loading of the calcium alginate gels and the calcium-reinforced photogels was with pre-swollen gels in 0.5 mg/mL of Coomassie Blue in TRIS-buffered saline (TBS) for 24 h followed by the described rinse procedure. TBS was selected for this loading procedure as it will have lower calcium alginate degradation rates than PBS since it has a lower sodium concentration, which remove and replace calcium, and it lacks calcium-binding phosphate ions. Drying was avoided to maintain the properties of the physical gels.

2.5. Effective crosslinking density

In order to correlate the release behavior of the loaded model compounds to the physical characteristics of the gels, experiments were conducted aimed at the calculation of the crosslinking densities of the gels. Dry and swollen or loaded gel masses and polymer/liquid densities were used to calculate the volumetric polymer fraction at maximum swelling ($\upsilon_{2,s}$) and the volumetric polymer fraction in a relaxed state ($\upsilon_{2,r}$) which were used in the Flory–Rehner equation as modified by Bray and Merrill (Eq. (1)) to determine the average molecular weight between crosslinks (Mc) of hydrogels crosslinked in solution [25–27].

$$\frac{1}{Mc} = \frac{2}{Mn} - \frac{(\bar{\upsilon}/V_1)[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1(\upsilon_{2,s})^2]}{\upsilon_{2,r}[(\upsilon_{2,s}/\upsilon_{2,r})^{1/3} - 0.5(\upsilon_{2,s}/\upsilon_{2,r})]} \quad (1)$$

The known variables for calculating Mc are the molar volume of solvent (V_1 = 18 mol/cm^3), the specific volume of dry polymer (υ = 0.60 cm^3/g alginate [28] and 0.89 g/cm^3 for PEG [29]), the Flory polymer–solvent interaction parameter (χ = 0.473 for alginate [30] and PEG [29]), and the average molecular weight of the polymer (Mn = 46,000 g/mol). The effective crosslinking density was then determined by dividing the density of alginate by Mc with and without loading.

2.6. Release studies

The model drugs Coomassie Blue and Fast Green were released from the hydrogel disks into PBS (pH = 7.4) in a shaking water bath at 37 °C, and the buffer was periodically removed and replaced. Releasate concentrations were measured spectrophotometrically at 595 nm for Coomassie Blue and 630 nm for Fast Green. At 1.5 h, some of the hydrogels were irradiated in PBS with 365 nm light for specified treatment times ranging from 10 to 40 min and compared with controls which had no UV treatment. During UV treatments, gels were placed in 1 mL of PBS, covered with a thin layer of parafilm that allowed UV transmission, and placed on cool water to ensure they would stay swollen and not heat up in the UV chamber. Percent release was calculated using the measured loading in the individual gels. To ensure that the absorbance of the Coomassie Blue and Fast Green were not altered by UV exposure, control solutions of the dye molecules with and without crosslinker were irradiated and their absorption profile was noted to remain constant.

2.7. Diffusion exponents and coefficients

Diffusion exponents and coefficients were calculated and compared for the various gel systems before and after UV treatment. In the early stage of release when the release ratio M_t/M_∞ is under 0.6, according to the Ritger and Peppas model [31], release is dependent on $M_t/M_\infty = kt^n$, where M_t and M_∞ are released amounts at time t and at infinite time and n is the diffusion exponent which is indicative of the type of transport. The disks were treated as slabs since their diameter was over four times that of their thickness [32]. The diffusion coefficient (D) can be calculated from Eq. (2) using regression for Fickian release. In this case, l is the width of the slabs (1 mm)

$$\frac{M_t}{M_\infty} = 4\left[\frac{Dt}{\pi l^2}\right]^{1/2} \quad (2)$$

Diffusion coefficients for early release were calculated with release data from 3 to 4 h after UV treatment up to 1700 h for photogels and star-containing photogels or up to 380 h for high alginate concentration photogels. Release from gels was compared by calculating the percent reduction in the diffusion coefficient that occurs with 365-nm UV treatments.

3. Results and discussion

3.1. Synthesis and analysis

Grafting of PEG–anthracene to alginate was successful, resulting in high liquid content hydrogels. Swelling results showed that the gels swelled 96.3 ± 1.3% in water or 97.3 ± 2.1% in PBS ($p = 0.307$). Alginate containing high amounts of mannuronic acid was used, so low viscosity solutions could be maintained with high concentrations of 6% during grafting to allow for sufficient modifications to occur. Carbon NMR showed alterations in the carbon bonds of alginate photogels before and after 365 nm irradiation. Gels showed characteristic anthracene peaks between 125 and 130 ppm. Previous studies show that a peak under 50 ppm is indicative of anthracene dimerization [33]. Following 365-nm UV irradiation for 80 min at 10 mW/cm^2 (48,000 mJ/cm^2), a new peak at 32 ppm was observed corresponding to the carbon at the juncture of the anthracene dimer indicating that dimerization occurred. This peak disappeared after irradiation with 254 nm light for 90 min at 0.6 mW/cm^2 (3240 mW/cm^2) corresponding to partial de-dimerization. Importantly, the appearance of a new peak at 32 ppm with 365 nm light exposure followed by disappearance with 254 nm light proves any observed changes in alginate gel properties from the application of UV light is due to PEG–anthracene dimerization within the gel matrix. ^{13}C NMR de-dimerized alginate photogels (200 MHz, D_2O): δ = 160.5, 130.7, 127.4, 119.3, 69.5, 64.5, 55.4, 52.5, 52.1, 45.5, 42.8, 38.8, 36.6, 35.1, 25.1, 14.6 ppm). ^{13}C NMR dimerized alginate photogels (200 MHz, D_2O): δ = 160.5, 130.6, 128.3, 127.4, 69.5, 64.5, 55.4, 52.4, 52.1, 45.5, 42.8, 36.6, 36.0, 35.1, 32.1, 25.0, 14.6 ppm).

3.2. Degradation

Since humans have no native enzymes to biodegrade alginate, de-crosslinking and slow hydrolysis are thought to be the primary degradation mechanisms for these gels. Therefore, to estimate degradation kinetics, gel integrity was monitored while soaking the gels in buffer under physiologic conditions. The control PEG hydrogels remained intact for over 800 h and the photogels for over 2000 h in PBS with or without 365-nm UV light treatment. Calcium alginate physical gels, however, degraded in under 24 h. Covalent modification with PEG and PEG–anthracene therefore is an effective method to stabilize alginate for a variety of long-term drug delivery and biological applications.

3.3. Effective crosslinking density and loading

The crosslinking density of the different gels can be characteristic of the size of the crosslinking molecules and the level of crosslinking within the gels. Smaller crosslinkers lead to tighter crosslinks and therefore a higher crosslinking density. When grafted with PEG–diamine crosslinkers, effective crosslinking density will be affected by the length (size) of the chains and when grafted with PEG–anthracene, effective crosslinking density of the photogels will be affected by the formation of linked-PEG chains due to the dimerization of the anthracene groups on the ends of the grafted PEG–anthracene molecules. Ultimately, the reactivity and concentration of the crosslinker will affect crosslinking densities, and future studies will focus on the effect differing grafting densities will have on photocrosslinking. From Fig. 2, it can be seen that the calcium alginate gels have a lower crosslinking density than the PEG hydrogels, which is not surprising since the molecular weight of the calcium is 40.1 g/mol compared with the PEG–diamine which is 897.1 g/mol. The addition of calcium to photogels causes an increase in crosslinking density due to the formation of physical crosslinks of calcium with alginate guluronic acid block chains. The photogels, shown in Fig. 2, have a low crosslinking density since at this point, they have not been exposed to UV light so the anthracene molecules have not dimerized and therefore the PEG–anthracene chains are not connected, i.e., not photocrosslinked. Previous studies by Wells et al. have demonstrated the ability for 365-nm UV light to cause an increase in the effective crosslinking density of alginate photogels [23].

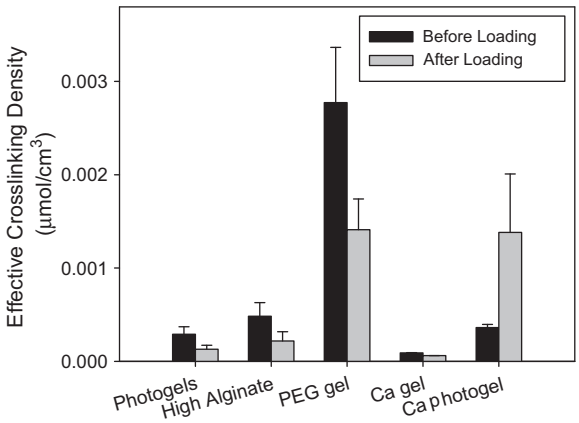

Fig. 2. Effective crosslinking density of gels before and after loading. The effective crosslinking density of the various gels had a trend of decreases after loading with Coomassie Blue (photogels $p = 0.217$, high alginate concentration photogels $p = 0.157$, PEG gel $p = 0.192$, Calcium gel $p = 2.69 \times 10^{-10}$). Calcium-reinforced photogels have an increase in effective crosslinking density because the Coomassie Blue loaded gels also have calcium which causes an overall increase in crosslinking (calcium-reinforced photogel $p = 0.136$). In the Figure, Photogels = alginate photogels, High Alg Photo = high alginate concentration photogels, PEG Gel = control PEG hydrogels, Ca Gel = calcium alginate gels, Ca Photogel = calcium-reinforced photogels.

As illustrated in Table 1, during loading, the photogels uptake the most Coomassie Blue, likely due to their lower crosslinking density relative to the calcium-reinforced photogels and control PEG hydrogels. When loosely crosslinking, soaking provides a very effective technique to promote large amounts of drug diffusion into the gel matrix. UV exposure would then "lock" drugs within the photocrosslinked matrix resulting in slow release. This technique could increase loading amounts and decrease loading times, which are crucial parameters in delivery systems for water-labile drug compounds and proteins [1,34].

The lower loading of alginate crosslinked with short PEG (3 units) versus long PEG (20 units) further demonstrates that tighter, more highly crosslinked gels will absorb less Coomassie Blue due to their smaller mesh size. High concentration alginate photogels were also found to have lower loading of the dye, likely due to the high amount of alginate causing physical entanglements that act as connections to increase the overall crosslink density which will slow diffusion into the gels, decreasing loading. Therefore, not surprisingly, calcium alginate gels load low relative amounts

Table 1
Coomassie Blue loading. Loading of Coomassie Blue into the different types of gels estimated by solution depletion.

Gel type	Loading medium	Average estimated loading (mg/g gel)
Control PEG hydrogels (short)	PBS	1.80 ± 0.69
Control PEG hydrogels (long)	PBS	10.50 ± 1.68
Photogels	PBS	28.50 ± 3.39
High alginate concentration photogels	PBS	9.37 ± 0.47
Star-PEG–anthracene containing photogels	PBS	18.40 ± 2.30
Calcium alginate gels	TBS	3.03 ± 1.65
Calcium-reinforced photogels	TBS	16.83 ± 2.84

of Coomassie Blue and the photogels reinforced with calcium load less than photogels without calcium. Since their loading was into preswollen gels and not into dried gels, there is also a lower driving force for Coomassie Blue uptake. The high overall loading of the relatively small Coomassie Blue into the different gels suggests the possibility that positive interactions between alginate and Coomassie Blue act to increase its absorption. In comparison, the loading of Fast Green into the photogels was on average 1.29 ± 0.11 mg/g gel which suggests there are lower interactions occurring between Fast Green and the photogels. Fast Green was used as a comparison because it has fewer aromatic groups and slightly different charge properties due to less amine groups but is of a similar size as Coomassie Blue.

To investigate the interactions between Coomassie Blue and alginate, effective crosslinking densities before and after loading were calculated and compared, since interactions may act to increase crosslinking to affect future release kinetics. Based on swelling data using the Flory–Rehner equation [25–27] and also illustrated in Fig. 2, the effective crosslinking of the photogels, high alginate concentration photogels, and PEG hydrogels show slight but insignificant decreases after loading of Coomassie Blue. The aromatic groups on Coomassie Blue may interact with the aromatic groups on anthracene. However, the observed decreases in crosslinking density also observed with the control PEG gels suggest that Coomassie Blue likely electrostatically interacts with alginate but not in a crosslinking fashion [35]. It should also be noted that the calcium-reinforced photogels have an increase in crosslinking as shown in Fig. 2 because the gels "before loading" have no calcium reinforcement, whereas the addition of calcium chloride in the Coomassie Blue loaded gels causes an overall increase in their crosslinking density.

3.4. Release studies

3.4.1. Photogels and control PEG hydrogels

Release of Coomassie Blue from alginate photogels into PBS with and without UV treatments is shown in Fig. 3a. After an initial period of instability, where high levels of release were observed, likely due to surface bound dye, irradiation of gels with 30 min of 365 nm light (18,000 mJ/cm^2) results in decreases in the release rate of the model drug Coomassie Blue from the photosensitive gels. Since anthracene photodimerization requires the molecules to absorb light, a minimal exposure is required for adequate dimerization of the grafted PEG–anthracene to cause observable changes in crosslinking and have an effect on release. With an exposure of 6000 mJ/cm^2 obtained for 10 min exposures at 10 mW/cm^2, there is insufficient energy to considerably alter the Coomassie Blue release. This illustrates that increases in 365-nm UV exposure slow the release of Coomassie Blue to produce a tuneable drug delivery material based on UV treatment times/exposures. Since the current alginate photogels are slightly cloudy with a refractive index of 1.348, it is not surprising that higher exposures of 365 nm light are necessary for an effect comparable to our previous studies with hyaluronic acid [23]. Furthermore, highly purified sources of alginate that have less light scattering, and therefore higher photosensitivity, may be more appropriate for future studies.

With 365-nm UV treatment, a slight increase in Coomassie Blue release was observed with the control PEG hydrogels (Fig. 3b). This is thought to have a couple of possible explanations. Despite attempts to store the gels at cool temperatures while under the UV lamp, the slight induced increase in temperature may lead to faster diffusion during this time period. Furthermore, with the absence of anthracene in these systems, the alginate may absorb

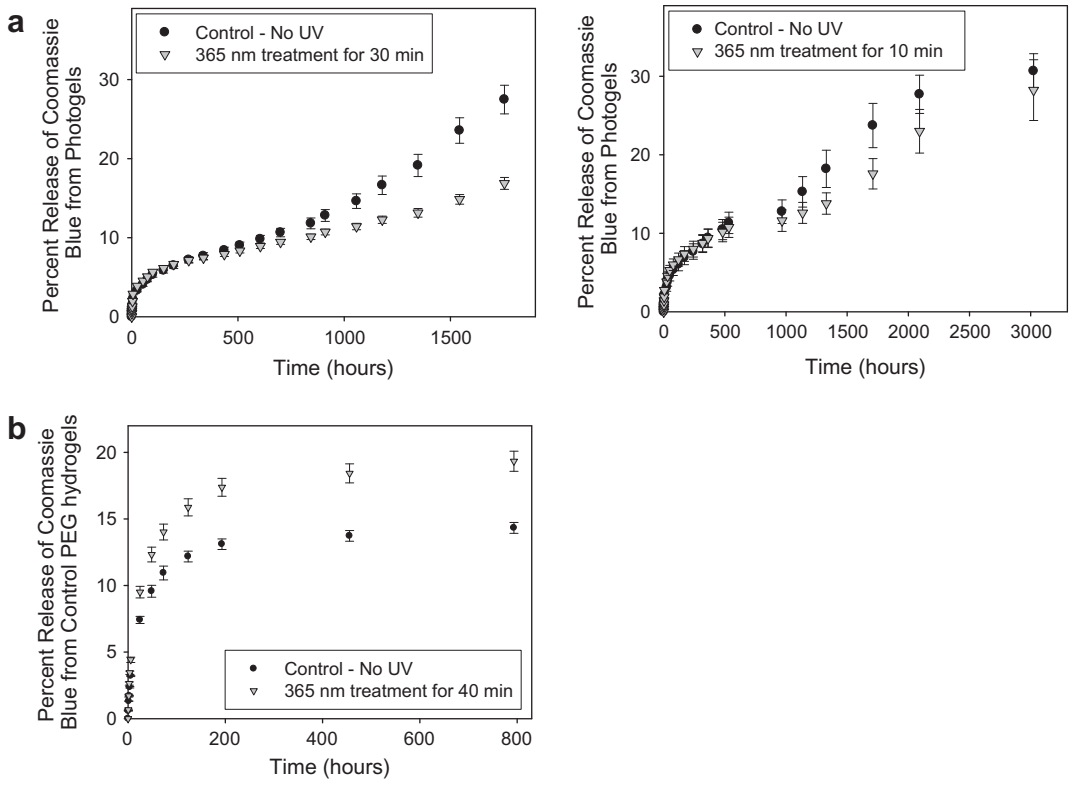

Fig. 3. Coomassie Blue release from photogels and control PEG hydrogels. (a) Coomassie Blue release from photogels into PBS (pH = 7.4) at 37 °C. One-milliliter samples were taken and replenished with fresh PBS periodically. At 1.5 h, gels were irradiated with either no light or 365 nm light (10 mW/cm^2) for either 30 or 10 min. Loading for the 30 min study was on average 25.5 ± 2.1 mg/g gel. Loading for the 10 min study was on average 18.3 ± 1.3 mg/g gel. (b) Coomassie Blue release from control PEG hydrogels into PBS (pH = 7.4) at 37 °C. One-milliliter samples were taken and replenished with fresh PBS periodically. At 1.5 h, gels were irradiated with either no light or 365 nm light (10 mW/cm^2) for 30 min. Loading was on average 10.5 ± 1.7 mg/g gel.

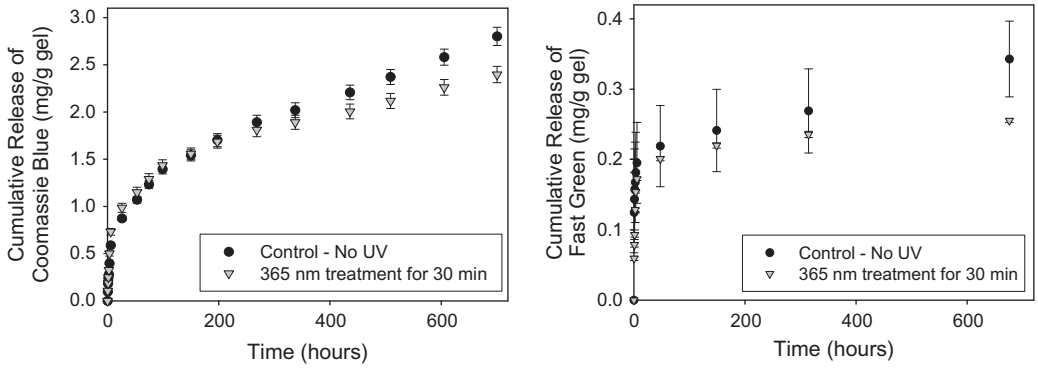

Fig. 4. Fast Green release versus Coomassie Blue release from photogels. Coomassie Blue and Fast Green release from photogels into PBS (pH = 7.4) at 37 °C. One-milliliter samples were taken and replenished with fresh PBS periodically. At 1.5 h, gels were irradiated with either no light or 365 nm light (10 mW/cm^2) for 30 min. Loading for Fast Green was on average 1.29 ± 0.11 mg/g gel. Loading for Coomassie Blue was on average 25.5 ± 2.1 mg/g gel and is the cumulative release representation of the percent release graph shown in Fig. 3a.

the 365-nm UV light, causing slight chain scission along the backbone of the polymer, a slight decrease in crosslinking, and therefore a slight increase in release. The size of the increase is miniscule in comparison with the large decreases in Coomassie Blue release observed with 365-nm UV treatment of photogels verifying the crosslinking effect that PEG–anthracene introduces into the alginate gel matrices.

The carbon NMR study, which proves anthracene dimerization occurs in the photogels, coupled with observed trends in drug release that show decreases upon 365-nm UV light exposure with photogels but not control PEG hydrogels leads to the conclusion that the grafted PEG–anthracene chains act as crosslinkers, which upon dimerization with 365 nm light, can alter the physical properties of alginate gel matrices. Interestingly, the amount of Coomassie Blue release at approximately 800 h is comparable between the control PEG hydrogels (14% at 795 h) and the loose photogels not treated with UV (19% at 845 h). Since the release is similar despite differences in crosslinking densities, interactions between Coomassie Blue and alginate are likely slowing release. There may also be interactions occurring between Coomassie Blue and anthracene since the non-photocrosslinked photogels with no UV exposure also release in a controlled fashion over lengthy periods of time.

To ensure that the observed changes in release were repeatable with other model drugs, Fast Green was also loaded into the alginate photogels and released with and without 365-nm UV treatments. As illustrated in Fig. 4, changes in release after 365-nm light treatment for 30 min appeared by 600 h of release. The lower loading of Fast Green may have reduced the time for occurrence of changes in release to appear. However, importantly, the changes did occur with a different model release compound other than Coomassie Blue. Photoresponsive alginate is a release system that may work on multiple drugs/molecules especially those that contain aromatic groups.

3.4.2. High alginate concentration photogels

Release of Coomassie Blue from photogels containing twice as much alginate but with a 50% lower PEG–anthracene grafting density, shown in Fig. 5, demonstrates that, while there is a trend of decreased release with 365-nm UV treatment, the decrease in release is not to the same degree as the previous photogels that had less alginate but a higher PEG–anthracene grafting density. This demonstrates that the control over the release is in fact due to the presence of anthracene in the gels. In addition, these high alginate concentration photogels released quite quickly in comparison with the other photogels with over 90% delivery at 1200 h

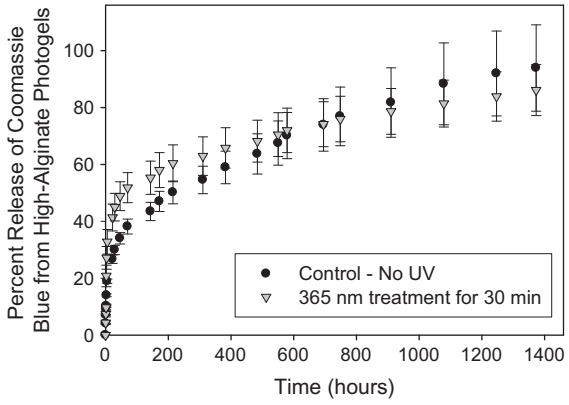

Fig. 5. Coomassie Blue release from high alginate concentration photogels. Coomassie Blue release from high alginate concentration photogels into PBS (pH = 7.4) at 37 °C. One-milliliter samples were taken and replenished with fresh PBS periodically. At 1.5 h, gels were irradiated with either no light or 365 nm light (10 mW/cm^2) for 30 min. Loading was on average 8.80 ± 3.0 mg/g gel.

with or without UV treatment. The increase in alginate in these gels should cause an overall decrease in the diffusion and release of Coomassie Blue. However, the presence of anthracene appears to play a greater role in the release kinetics and when present at a low density it produces gels that release quickly. This demonstrates that a minimum amount of PEG–anthracene grafting is required for either dimerization to occur or for dimerization to significantly affect crosslinking density and drug release.

3.4.3. Calcium alginate gels versus photogels in TBS

To directly compare and observe differences in physically crosslinked alginate gels versus photogels, some photogels were secondarily crosslinked with calcium chloride. Both calcium-crosslinked alginate gels and calcium-reinforced photogels were loaded with Coomassie Blue which was subsequently released into TBS. PBS contains sodium and phosphate ions that quickly degrade calcium alginate gels by the replacement of calcium ions (Ca^{2+}) with two sodium ions (Na^+) to cause de-crosslinking in conjunction with the presence of phosphate which combines with the freed calcium to produce calcium phosphate effectively removing any calcium ions to prevent re-crosslinking [36]. Therefore, TBS was used as a buffer that would slow this degradation and allow adequate observation of the release without the convoluting effects of de-crosslinking [36]. As shown in Fig. 6a, the physically crosslinked calcium alginate gels quickly release the Coomassie

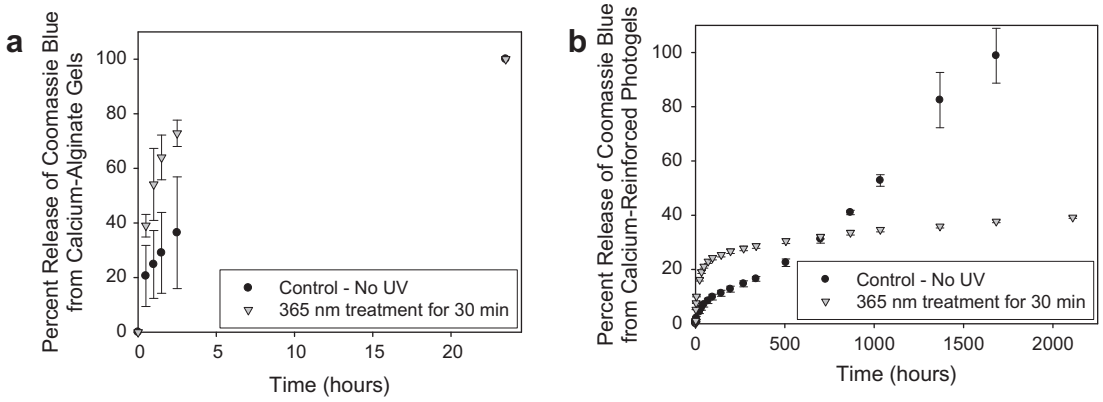

Fig. 6. Coomassie Blue release from calcium alginate gels and calcium-reinforced photogels. (a) Coomassie Blue release from calcium alginate gels into TBS (pH = 7.4) at 37 °C. One-milliliter samples were taken and replenished with fresh TBS periodically. At 1.5 h, gels were irradiated with either no light or 365 nm light (10 mW/cm^2) for 30 min. Loading was on average 3.03 ± 1.65 mg/g gel. (b) Coomassie Blue release from calcium-reinforced photogels into TBS (pH = 7.4) at 37 °C. One-milliliter samples were taken and replenished with fresh TBS periodically. At 1.5 h, gels were irradiated with either no light or 365 nm light (10 mW/cm^2) for 30 min. Loading was on average 16.83 ± 2.84 mg/g gel.

Blue consistent with literature [37] and degrade with no observable changes in release with UV treatment. However, the release of Coomassie Blue from the calcium-reinforced photogels, depicted in Fig. 6b, show large decreases in release following 365-nm UV treatment. The addition of calcium to these photogels temporarily increased the crosslinking density of the photogels (Fig. 2). Therefore, during UV treatment at 1.5 h, the anthracene molecules along the backbone of the alginate were likely closer together, thereby increasing the likelihood of dimerization. Hence, there are more significant decreases in percent release following UV treatment. Any calcium introduced into the calcium-reinforced photogels releasing Coomassie Blue in Fig. 6b is likely to have migrated from the photogels after first 24 h. Overall, this demonstrates that increased stability is provided to the alginate gels with covalent grafting of PEG–anthracene and shows that calcium can be used to reinforce alginate photogels in a formulation to increase their photosensitivity.

3.4.4. Star-PEG–anthracene containing photogels

Star-PEG–anthracene was successfully incorporated into the photogels, introducing additional photodimerizing groups into the system in order to increase its overall photosensitivity. As

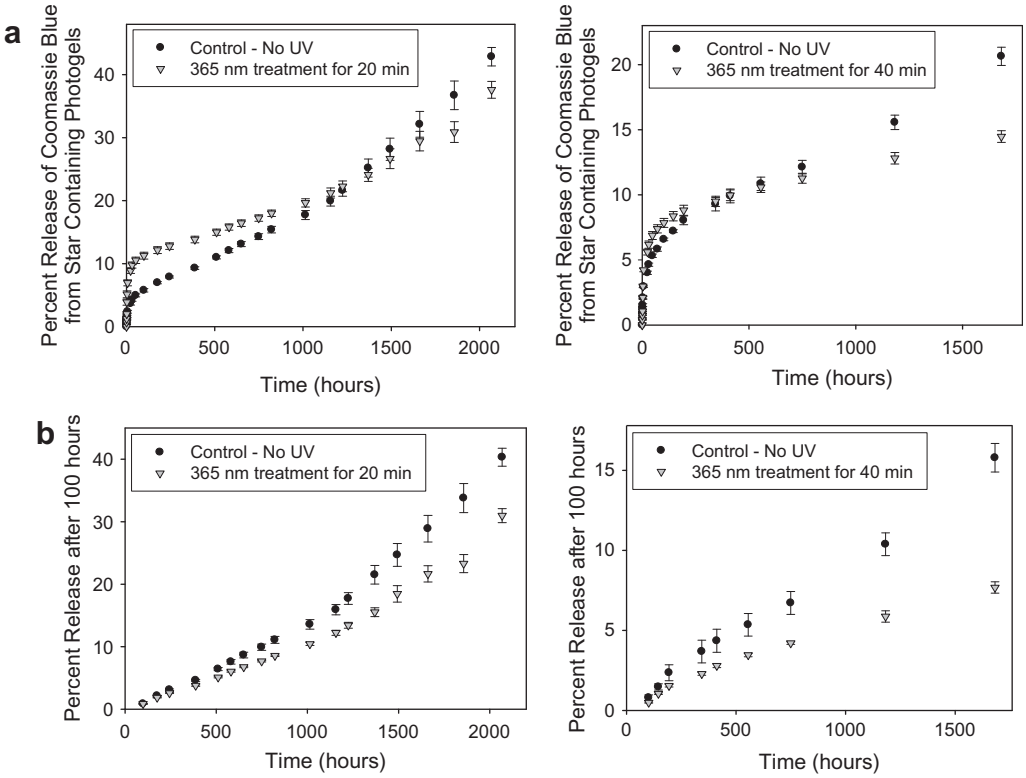

Fig. 7. Coomassie Blue release from star-PEG–anthracene containing photogels. (a) Coomassie Blue release from star-PEG–anthracene containing photogels into PBS (pH = 7.4) at 37 °C. One-milliliter samples were taken and replenished with fresh PBS periodically. At 1.5 h, gels were irradiated with either no light or 365 nm light (10 mW/cm^2) for 20 or 40 min. Loading for the 20 min study was on average 20.71 ± 1.22 mg/g gel. Loading for the 40 min study was on average 16.10 ± 1.27 mg/g gel. (b) The same release studies as in Fig. 7a but with the release calculations starting at 100 h.

Table 2
Changes in the diffusion coefficients with and without UV treatments. The diffusion coefficients and exponents of the different gels with and without UV treatments. The percent decreases in diffusion of 365-nm UV-treated gels versus control with no UV treatments are described as a direct comparison between the different systems. All gels are releasing Coomassie Blue.

Gel type	UV treatment groups	Diffusion coefficient ($cm^2/s \times 10^{-11}$)	Diffusion exponent	Percent decrease with UV (%)
Photogels	No UV	1.23 ± 0.004	0.44 ± 0.02	54
	10 min	0.57 ± 0.003	0.30 ± 0.01	
Photogels	No UV	1.57 ± 0.011	0.42 ± 0.03	67
	30 min	0.51 ± 0.001	0.30 ± 0.02	
High alginate concentration photogels	No UV	31.49 ± 0.121	0.26 ± 0.01	29
	30 min	22.37 ± 0.267	0.16 ± 0.01	
Calcium-reinforced photogels	No UV	12.0 ± 0.093	0.57 ± 0.03	79
	30 min	2.47 ± 0.031	0.21 ± 0.01	
Control PEG hydrogels	No UV	1.57 ± 0.049	0.44 ± 0.04	−44
	40 min	2.81 ± 0.076	0.44 ± 0.04	
Calcium alginate hydrogels	No UV	2186.6 ± 1.47	0.35 ± 0.03	−59
	30 min	5429.1 ± 145.7	0.39 ± 0.04	

shown in Fig. 7a, a decrease in release was observed with 365-nm light treatments of 20 or 40 min after a period of release at approximately 1160 h (approximately 48 days) or 340 h (approximately 14 days), respectively. Therefore, longer treatment times resulted in more rapid changes in release. By removing the initial burst, shown in Fig. 7b, the changes in the release can be more readily observed. This burst is thought to be due to contraction of the star-PEG–anthracene within the photogel system causing changes in pore-structure. Overall, the photosensitivity increased but different types of release curves are noted with the presence of the additional photosensitive molecules.

3.5. Diffusion coefficients

Diffusion coefficients are indicative of the rate and the mechanism by which molecules diffuse through the gels, providing a good method of directly comparing gels of different compositions with and without UV treatments. As shown in Table 2, the diffusion coefficients between control PEG hydrogels and loosely crosslinked control photogels (no UV) were similar likely due to aromatic interactions between Coomassie Blue and anthracene slowing release. Despite having a higher concentration of alginate that would formulate a tight network prone to slowing release, the lower amount of anthracene in the high alginate concentration photogels appeared to promote an increase in the diffusion coefficient of Coomassie Blue. Calcium caused lower diffusion coefficients when added to photogels but when solely crosslinking alginate it produces a loose gel prone to fast diffusion and quick de-crosslinking/degradation.

As illustrated in Table 2, the percent reduction increased with additional UV treatment time by 54–67% for 10 and 30 min treatments of photogels indicating increases in UV doses do change the release kinetics. The alginate photogels are tuneable dependent on UV times/exposures. Calcium-reinforced photogels had the highest decrease of 79% with 365-nm UV treatment for 30 min for reasons described earlier, specifically calcium tightening the gels to allow for more efficient dimerization of anthracene with UV treatments. The 29% decrease in diffusion coefficient with 30 min 365-nm UV treatments in high alginate concentration photogels is likely due to the lower overall grafting density compared with regular photogels, demonstrating that grafting may be altered to change photosensitivity.

Control PEG hydrogels have equal diffusion exponents of 0.44. However, there is a slight increase in the diffusion coefficient by 20% with 365-nm UV treatment. The physical calcium alginate gels demonstrated a similar trend over a shorter period of time. There are two possible reasons for this. As previously discussed, it might be due to slight heating in the system or, because there are no anthracene molecules to absorb the light, UV light may be absorbed by the alginate leading to slight alginate break down and a resulting increase in release. Overall, the control gels in comparison with the photogels demonstrate that the slowing of the release of compounds from the photogels is due to dimerization and crosslinking.

The photogels containing star-PEG–anthracene had overall decreases in diffusion coefficients of 48% and 57% with UV treatments of 20 and 40 min, respectively. However, as described in Table 3, after the initial burst, the decrease in the diffusion coefficient is 57% and 72% with 20 min and 40 min UV treatments, respectively, demonstrating the possibility that an increase in the number of anthracene groups due to the presence of the star-PEG–anthracene may increase the overall photosensitivity of the photogels.

Taken together, the results suggest that the photogels, control PEG hydrogels, and variations thereof have the potential to act as effective long-term alginate-based drug delivery systems. There have been several examples in the literature that have shown extended release and degradation with covalently modified alginate. Some hydrophilic modifications include, for example, adipic dihydrazide as a covalent crosslinker to produce alginate gels that

Table 3
Changes in the diffusion coefficients of star-PEG–anthracene containing photogels with and without UV treatments. The diffusion coefficients and exponents of the gels with and without UV treatments. Both the total release time and release after the burst (after 100 h) were investigated to fully explore the changes in release that occur with these gels. All gels are releasing Coomassie Blue.

Star-PEG–anthracene containing photogels	UV treatment groups	Diffusion coefficient ($cm^2/s \times 10^{-11}$)	Diffusion exponent	Percent decrease with UV (%)
Total release time	No UV	2.45 ± 0.014	0.44 ± 0.03	48
	20 min	1.28 ± 0.005	0.23 ± 0.02	
Total release time	No UV	0.92 ± 0.002	0.33 ± 0.01	57
	40 min	0.394 ± 0.002	0.19 ± 0.01	
Release > 100 h	No UV	3.54 ± 0.025	0.60 ± 0.04	53
	20 min	1.65 ± 0.011	0.33 ± 0.03	
Release > 100 h	No UV	0.981 ± 0.005	0.38 ± 0.03	72
	40 min	0.272 ± 0.00001	0.18 ± 0.01	

release 60% of daunomycin (527.5 Da) over 40 days in Dulbecco's modified Eagle's medium [38]. Hydrophobic modifications have shown much promise since hydrophobicity may improve alginate stability and lengthen release times [9,15,16] and degradation times [39]. For example, alginate modification with a vinyl polymer increased release of N,N-diethyl-3-methylbenzamide to over 400 h [40] or modification with long alkyl chains extended release to 5–45% at 122 h [41]. However, while sustained release has been shown in previous studies, the release profiles in Figs. 3, 4, 5, 6b and 7 are considerably lengthier than those in the literature, with some showing controlled release for up to 2000 h (83 days). For example, photogels releasing Coomassie Blue had 17% or 27% release at 1750 h (73 days) for photogels with and without 365-nm UV light treatment for 30 min at 10 mW/cm^2. Since anthracene is quite hydrophobic, the covalent modification of alginate with PEG–anthracene likely lowers the hydrophilicity of the alginate gels, increasing their stability and allowing for controlled release. In addition, it is thought that the Coomassie Blue interacts with alginate and anthracene to both increase loading and release times. While not as dramatic, possibly due to the lower amount of aromatic and amine groups present in Fast Green, increased release durations were also observed with Fast Green demonstrating the potential of these systems. Therefore, not only do the photogels produce a light-sensitive system but they also present an effective modification method to extend the life time of alginate drug delivery materials.

4. Conclusions

PEG–anthracene grafting introduces light-sensitive crosslinking into alginate photogels in a controllable manner dependent on light treatment time/exposure, the model drug compound being released, and the photogel formulation. Covalent modification with both PEG–diamine and PEG–anthracene increases the lifetime of alginate hydrogels even when releasing into high ion containing buffers, such as PBS, which are known to cause high swelling and known to de-crosslink physical alginate gels. Decreases in the release of Coomassie Blue with 365-nm UV treatments were tuneable with larger decreases in release with longer photogel UV treatment times/exposures. Changes in release can occur with a variety of different small molecules; however, Coomassie Blue interacts with alginate in a non-crosslinking fashion resulting in long-term controlled release into high ion containing buffers. The photosensitivity of the photogels to 365-nm UV light can be increased by changing the formulation of the photogels to contain calcium or star-PEG–anthracene and by increasing the amount of grafted PEG–anthracene. Furthermore, the light sensitivity of these materials lends itself to the possibility of a novel loading mechanism that involves absorption of model drug compounds into uncrosslinked photogels followed by photocrosslinking to "lock" the drugs within the gel matrix. Ultimately, the ability to slow the release of small model drugs from alginate photogels presents a long-term, smart delivery material that can be tailored *in vitro* or *in situ* to slow drug release.

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