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Hydrogels for combination delivery of antineoplastic agents

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Abstract

The systemic delivery of anticancer agents has been widely investigated during the past decade but localized delivery may offer a safer and more effective delivery approach. We have designed and synthesized a novel hydrogel to locally deliver antineoplastic agents, and demonstrate the different types of release that can be achieved from these hydrogels using three model drugs: methotrexate, doxorubicin, and mitoxantrone. Alginate was chemically modified into low molecular weight oligomers and cross-linked with a biodegradable spacer (adipic dihydrazide) to form biodegradable hydrogels. The model antineoplastic agents were loaded into the hydrogel via three different mechanisms. Methotrexate was incorporated within the pores of the hydrogel and was released by diffusion into the surrounding medium. Doxorubicin was covalently attached to the polymer backbone via a hydrolytically labile linker and was released following the chemical hydrolysis of the linker. Mitoxantrone was ionically complexed to the polymer and was released after the dissociation of this complex. These three release mechanisms could potentially be used to deliver a wide selection of antineoplastic agents, based on their chemical structure. This novel delivery system allows for the release of single or combinations of antineoplastic agents, and may find utility in localized antineoplastic agent delivery.

Keywords: Alginate; Controlled release; Biodegradable; Antineoplastic agents; Doxorubicin; Methotrexate; Mitoxantrone

1. Introduction

A broad spectrum of antineoplastic agents has been found to be effective in combating different types of cancer. However, to achieve complete eradication of tumors, antineoplastic agents are administered systemically in high doses, and almost all drugs effective in killing cancer cells cause damage to other healthy tissues and organs. This is due to the non-specific uptake of these agents by healthy organs such as the kidney, liver, bone marrow, and heart. The adverse side effects include severe immune suppression, myelosuppression, nephrotoxicity, and cardiotoxicity [1,2]. During the past decade, many researchers have investigated the sequential and simultaneous delivery of drug combinations to reduce the side effects associated with the systemic delivery of anticancer agents [3–6]. Several drugs have been found to amplify the anticancer activity of other drugs [7–10]. This synergistic effect can potentially lead to reduced doses for each drug administered [11–13]. Hence, the administration of several drugs simultaneously could reduce the side effects caused by high doses of single drugs and could prevent the development of multi-drug resistance (MDR) [14,15].

An alternative approach to systemic delivery of antineoplastic agents is the localized release from a polymer [16]. We have designed and synthesized a novel hydrogel to deliver anticancer agents locally. We have oxidized sodium alginate to form low molecular weight oligomers that are cross-linked with adipic dihydrazide to form hydrogels. We hypothesize that a variety of antineoplastic agents could be locally delivered, alone or in combination from these hydrogels. The kinetics of drug release could potentially be controlled by exploiting three types of drug–hydrogel interaction: no interaction, ionic interaction, and covalent coupling via degradable linkages. We have incorporated three model drugs into

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cross-linked oxidized alginate hydrogels: methotrexate, doxorubicin, and mitoxantrone to test this possibility. All three drugs are potent antineoplastic agents that have been extensively utilized in cancer chemotherapy. Methotrexate is a folic acid antimetabolite inhibitor of dihydrofolate reductase that has been widely used in the treatment of neoplastic diseases [17]. Doxorubicin is an antineoplastic agent from the anthracycline antibiotic family that has been most commonly used to treat solid tumors [18,19]. Mitoxantrone, an anthracenedione, is an intercalating agent that is effective in treating various tumors [18]. Methotrexate, an anionic drug, is used in this study as it is expected to rapidly diffuse out into the surrounding medium. We have used doxorubicin as a model drug for chemical-controlled release. Doxorubicin is expected to be released following the hydrolysis of the degradable bond linking it to the gel [20]. In addition, we have used mitoxantrone as a model drug for ionic-controlled release. Mitoxantrone is expected to form ionic complexes with the carboxylate groups on the sugar residues of the polymer backbone.

2. Experimental section

2.1. Materials

Sodium alginate was purchased from Pronova Biomaterials (Drammen, Norway). Sodium periodate, adipic dihydrazide, ethylene glycol, and anhydrous KBr were purchased from the Aldrich Chemical Company (Milwaukee, WI) and used as received. Ethanol (95%), methanol, and concentrated hydrochloric acid were purchased from Fisher Scientific Company (Fair Lawn, NJ) and were used as received. Doxorubicin and mitoxantrone hydrochlorides were purchased from Sigma Chemical Company (St. Louis, MO). Methotrexate was purchased from Fluka Chemical Corporation (Ronkonkoma, NY). Phosphate buffer saline (PBS) and Dulbecco's Modified Eagles Medium (DMEM) were purchased from Life Technologies (Grand Island, NY). Eagle's Minimum Essential Medium (EMEM), fetal bovine serum, and MCF-7 breast epithelial cell lines were purchased from the American Tissue Culture Collection (Manassas, VA). Cell culture plates (96-well) were purchased from Falcon (Lincoln Park, NJ).

2.2. Oxidation of sodium alginate

A 1 l Erlenmeyer flask was wrapped with aluminum foil and charged with sodium alginate (8.0 g). Double-distilled water (800 ml) was added, and the mixture was stirred until the solid dissolved. An aqueous solution of sodium periodate (0.25 M, 162 ml) was added and the reaction was stirred for 24 h at room temperature. Ethylene glycol (2.3 ml) was then added to the reaction mixture to reduce any unreacted periodate. The reaction was stirred for 0.5 h at ambient temperature, and the solution was filtered and exhaustively dialyzed (Spectra/Pro membrane, MWCO 3500) against double-distilled water (dd. H_2O) for three days. The water was changed at least 3 times a day. The solutions were then concentrated to around 100 ml and freeze dried under reduced pressure to yield a white product (6.9 g, 86%). IR (KBr pellet, cm^{-1}) 3336, 2942, 1730, 1622, 1406, 1321, 1159, 1117, 1026.

2.3. Determination of the degree of oxidation

The degree of oxidation of alginate was determined by measuring the percentage of periodate that was consumed before quenching with ethylene glycol. The consumption of sodium periodate was determined by spectrophotometrically measuring the formation of a complex between unreacted periodate anion and thyodene. Briefly, equal volumes of freshly prepared aqueous solutions of potassium iodide (20% w/v in pH 7.0 sodium phosphate buffer) and thyodene solution (10% w/v in pH 7.0 sodium phosphate buffer) were mixed as an indicator solution. An Erlenmeyer flask (100 ml) was covered with aluminum foil and charged with an aqueous solution of alginate (50 ml, 1.0% w/v) and an aqueous solution of sodium periodate (10.1 ml, 0.25 M). The mixture was stirred at room temperature. At different time intervals, aliquots (0.3 ml) were rapidly removed and diluted to a volume of 100 ml using dd. H_2O. A 0.5 ml aliquot of this solution was immediately mixed with 1.0 ml of the indicator solution in a cuvette. The concentration of the unreacted periodate was measured spectrophotometrically at 486 nm. This number was then subtracted from the original concentration of periodate to yield the amount of periodate that was consumed.

2.4. Size exclusion chromatography (SEC)

SEC analysis was performed on a liquid chromatograph consisting of a SpectraSystem P1000 pump (Thermal Separation Products), a Rheodyne 7010 manual injector, a dual differential viscometer and right-angle laser light scattering (RALLS) detector (Viscotek T 60, $\lambda = 670$ nm) and a laser refractometer detector (Viscotek LR40, $\lambda = 670$ nm), the detectors being connected in parallel. The mobile phase consisted of aqueous sodium nitrate (0.1 M) and was periodically degassed with an on-line degasser. The mobile phase was delivered at ambient temperature with a nominal flow rate of 0.7 ml/min. The separations were carried out on two TSK $GMPW_{XL}$ (TosoHaas, 7.8×300 mm) mix bed columns. Polymers were dissolved in mobile phase solvent at a concentration of 1–3 mg/ml by mechanical stirring for a minimum of 6 h until completely hydrated. A 100 μl injection volume was used for all analyses. The chromatograms were analyzed

using the TriSEC 3.0 GPC software (Viscotek). A differential index of refraction (dn/dc) of 0.154 ml/g was used [21,22].

2.5. Hydrogel formation

Hydrogels were formed at various concentrations of oxidized alginate, adipic dihydrazide and calcium chloride in 24-well plates. The contents of each well were mixed and allowed to gel for 1 h at ambient temperature on a mechanical shaker. The hydrogels were immersed in de-ionized water and incubated at 37°C for 24 h to reach the equilibrium swelling condition. The hydrogels were transferred to 2 ml vials and weighed (wet weight). The gels were then frozen, lyophilized, and the dried samples were weighed (dry weight). The swelling ratio was defined as the ratio of (wet weight − dry weight)/(dry weight). Infrared spectra were recorded as percent transmittance using a Nicolet 5DX FTIR spectrophotometer and a Hewlett Packard 7470A plotter. Samples were pressed as KBr pellets using a hydraulic press (Carver, Inc.). IR (KBr pellet, cm^{-1}) 3554, 3472, 3414, 3236, 1660, 1622, 1406, 1154, 1095, 1036.

2.6. Hydrogel degradation

Aqueous solutions of oxidized alginate (125 μl, 20% w/w) and adipic dihydrazide (125 μl, 0.5 M) containing calcium chloride (80 mM) were mixed in 15 ml conical tubes (in quadruplicates) and allowed to gel for 5 h. Solutions of Dulbecco's Modified Eagles Medium (DMEM, 10 ml) were added, and the tubes were incubated at 37°C. The medium was replaced with fresh medium on a weekly basis. Four tubes were removed every week, and the medium was decanted. The gels were frozen, lyophilized, and the dry solid was weighed.

2.7. In vitro drug release from alginate hydrogels

An aqueous alginate solution was prepared by dissolving sodium alginate (2 g), sodium chloride (0.8 g), and sodium hexametaphosphate (0.4 g) in 96.8 ml of dd. H_2O. A 50 ml conical tube was charged with 5 g of the above solution. A solution of the drug (165 μl, 25 mg/ml in DMSO) was then added and mixed thoroughly. Aqueous calcium sulfate slurry (200 μl, 158 mg/ml) was added, mixed thoroughly, and the mixture was cast between parallel glass plates separated with glass spacers (2 mm in thickness). The gels were allowed to set for 8 h. The glass plates were separated, and the disks were punched out with a 12.7 mm hole puncher (McMaster-Carr). The hydrogel disks were placed in scintillation vials (2 disks in each vial). Aqueous PBS (pH 7.4) was added, and the vials were incubated at 37°C. The medium was replaced periodically, and the amount of drug that was released in the medium was quantified by measuring the absorbance of the drug at 327.5 nm (methotrexate), 480 nm (doxorubicin), or 609 nm (mitoxantrone). UV/VIS spectra were collected on a Perkin Elmer Lambda 12 UV/VIS spectrophotometer.

2.8. In vitro drug release from cross-linked oxidized alginate hydrogels

Sterile tubes were charged with aqueous solutions of adipic dihydrazide (150 μl, 0.5 M), calcium chloride (20 μl, 1.0 M), and DMEM (20 μl). Solutions of methotrexate, doxorubicin hydrochloride, or mitoxantrone (10 μl, 25 mg/ml in DMSO) were then added to the above aqueous solutions, and the mixtures were mixed for 15 min. An aqueous solution of oxidized alginate (300 μl, 10% w/w) was then added, and the contents of the tubes were mixed thoroughly and allowed to gel for 1 h. Aqueous DMEM solutions (5 ml) containing penicillin and streptomycin were added to each tube, and the tubes were incubated at 37°C. The medium was removed periodically and replaced with fresh DMEM, and the released drug was quantified as described previously. A total of 0.25 mg of each drug was always loaded per gel sample. Release data are reported as a percentage of this total incorporated drug.

3. Results

Aqueous solutions of sodium alginate were oxidized in the dark using sodium periodate at room temperature following a modified procedure reported previously [23–25]. The amount of sodium periodate used in these reactions was varied to form alginates with different degrees of oxidation. FTIR analysis of the oxidized product revealed a new peak at 1730 cm^{-1} corresponding for the vibrational symmetric stretching vibration of the aldehyde groups. The degree of oxidation was determined indirectly by measuring the percentage of sodium periodate that was consumed in each reaction. Sodium periodate was almost quantitatively consumed in all conditions except when 100 percentage equivalents was used (Table 1). In this case, only 69% of the sodium periodate was consumed after 24 h. This is consistent with earlier papers reporting that the aldehyde groups of the oxidized uronate react with neighboring alcohol groups to form hemiacetals [23]. The formation of hemiacetals protects the alcohol groups of neighboring uronates from further oxidation.

3.1. Molecular weight distribution of oxidized alginate

The molecular weight distribution of oxidized alginate was analyzed by aqueous gel permeation chromatography. The weight-average molecular weight of the starting alginate was 394 kDa. Alginate oxidized with five

Table 1
The experimental degree of oxidation of cross-linked oxidized alginate hydrogels. Reactions were run at a concentration of 0.8% w/w alginate in the dark at room temperature for 24 h

Periodate equivalents (%)	Periodate consumed (%)	Degree of oxidation (%)
5	98.9 ± 0.2	4.9
10	98.9 ± 0.1	9.9
25	98.8 ± 0.1	24.7
50	98.7 ± 0.1	49.3
100	69 ± 2	69

Table 2
Molecular weight distributions of alginate and oxidized alginates

$NaIO_4$[a] (%)	M_w (kDa)	M_n (kDa)	PDI (M_w/M_n)	IV_w (dl/g)	$F(x)$[b]
0	358	130	2.75	9.40	12.5
10	94	48	1.95	1.73	58
20	69	35	1.99	0.91	61
50	46	27	1.67	0.39	83
100	29	17	1.75	0.40	96

[a] Represents the percentage equivalents of sodium periodate that was initially added to the reaction mixture (based on the uronate groups).
[b] $F(x)$ is the weight fraction of the polymer that have a molecular weight lower than 80 kDa.

equivalents of sodium periodates formed a polymer with a weight-average molecular weight of 198 kDa (Table 2). The weight-average molecular weight then decreased as the percentage equivalents of periodate was increased to reach 29 kDa with 100% equivalents of sodium periodate. As a result, the intrinsic viscosity of the polymers decreased as periodate concentration was increased. Only 12.5% weight fraction of the original unmodified alginate has a molecular weight below 80 kDa. This number increased to reach a value of 96% for alginate that was oxidized with 100 equivalents of sodium periodate (Table 2).

3.2. Preparation of hydrogels

Hydrogels were subsequently formed by the reaction of adipic dihydrazide and the oxidized alginates. The hydrazide group reacts with the aldehyde groups in oxidized alginate to form hydrazone bonds (Fig. 1). The hydrogels were washed with water and soaked in dd. H_2O for 24 h to release the unreacted adipic dihydrazide. The gels were frozen and lyophilized. FTIR spectroscopic analysis of the discs indicated the disappearance of the peak at 1730 cm^{-1} and the appearance of another peak at 1660 cm^{-1} that corresponds to the stretching vibration of the carbonyl in the hydrazide group.

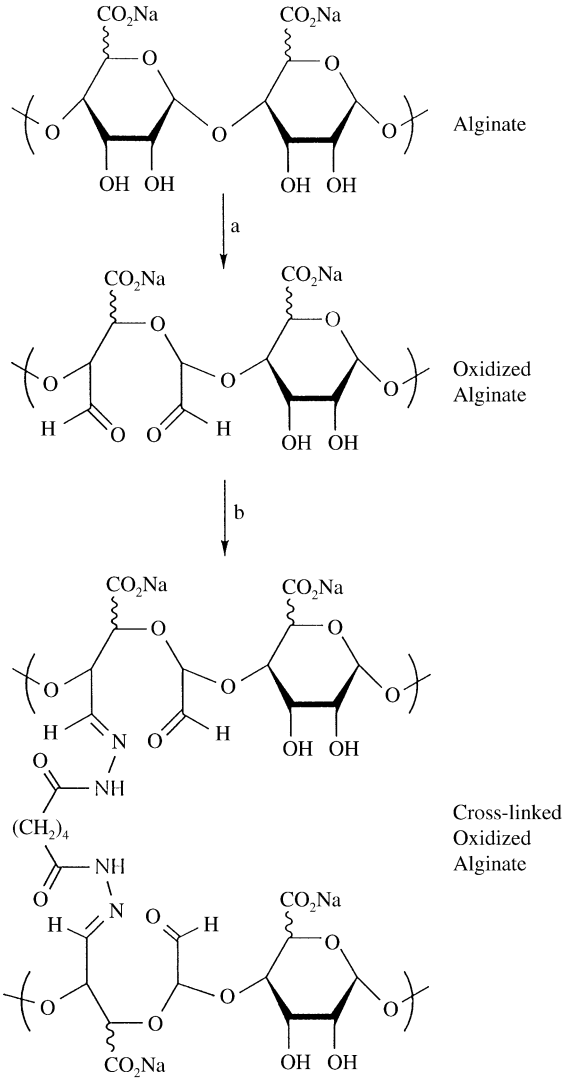

Fig. 1. Synthesis and cross-linking of oxidized alginate: (a) sodium periodate, (b) adipic dihydrazide.

3.3. Swelling behavior

The degree of swelling of cross-linked oxidized alginate hydrogels was analyzed after the hydrogels reached the equilibration swelling in dd. H_2O. The swelling ratio of these hydrogels varied significantly depending on the concentrations of both the ionic and the covalent cross-linkers. The swelling ratio of hydrogels made with oxidized alginate (100 equivalents periodate) and cross-linked at 150 mM adipic dihydrazide was 29.9 ± 1.2 in dd. H_2O (Table 3). The swelling ratio then decreased with increasing concentrations of calcium to reach a minimum of 11.7 ± 0.3 at 40 mM calcium ions. A similar trend was observed when the concentration of covalent cross-links was increased. The swelling ratio was 20.1 ± 1.2 at 40 mM calcium ions at 50 mM adipic dihydrazide, and decreased to 11.7 ± 0.3 at 40 mM as the concentration of adipic

Table 3
Swelling ratio of cross-linked oxidized alginate hydrogels as a function of the concentrations of the ionic and covalent cross-linkers. Hydrogels were formed at 6% w/w oxidized alginate in dd. H_2O

Adipic dihydrazide (mM)	$CaCl_2$ (mM)	Swelling ratio
150	0	29.9 ± 1.2
150	5	29.2 ± 1.5
150	10	29.6 ± 1.5
150	20	17.7 ± 0.9
150	30	14.7 ± 1.9
150	40	11.7 ± 0.3
50	40	20.1 ± 1.2
100	40	13.1 ± 1.1
150	40	11.7 ± 0.3
200	40	11.8 ± 0.2
250	40	12.4 ± 0.2

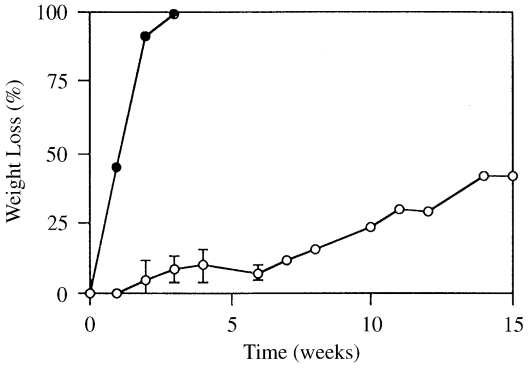

Fig. 2. Percentage weight loss of cross-linked oxidized alginate hydrogels as a function of time. Hydrogels were formed at (●) 100 mM and (○) 150 mM adipic dihydrazide and 40 mM $CaCl_2$. All hydrogels were prepared with 10% w/w oxidized alginates (69% oxidized) in dd. H_2O.

dihydrazide increased to 150 mM (Table 3). The swelling ratio slightly increased as the adipic dihydrazide concentration was further increased. This latter result is consistent with past studies which indicated a decreased cross-link density, and increased content of dangling cross-linkers, above 150 mM adipic dihydrazide [25].

3.4. Hydrogel degradation

Alginate hydrogels degrade in an uncontrolled manner following the release of calcium ions into the surrounding medium. To evaluate whether the degradation of cross-linked oxidized alginates can be controlled, gels were formed with 10% w/w oxidized alginates (oxidized with 100 equivalents of periodate) and cross-linked with adipic dihydrazide and/or calcium. The percentage weight loss of these gels was determined following incubation in medium (Fig. 2). Hydrogels formed at 100 mM adipic dihydrazide degraded after 3 weeks of incubation at a rate of 5% per day. Hydrogels cross-linked at 150 mM adipic dihydrazide and 40 mM calcium chloride degraded at a lower rate of 2.5% per day (Fig. 2). Only 40% of the gel weight dissolved after 15 weeks. Therefore, hydrogels may be formed with this approach that degrade in time frames from 3 weeks to more than 4 months by simply varying the number of covalent and ionic cross-links.

3.5. In vitro drug release

The three model drugs were first separately incorporated in cross-linked oxidized alginate hydrogels. Methotrexate (Fig. 3) was not expected to interact ionically or covalently with the hydrogel. The release profile of methotrexate was not significantly dependent on the concentration of the covalent cross-linker (Fig. 4a), as expected at the high degrees of swelling in these hydrogels. Methotrexate was quantitatively released within 2 days of incubation from hydrogels formed with 50 mM adipic dihydrazide. The overall release time of methotrexate increased to 3 days at 75 mM adipic dihydrazide and 7 days at 150 mM adipic dihydrazide. However, 75% of the loaded drug was released initially at a constant rate of 37.5% per day in all conditions. Drug release from hydrogels formed with concentrations of adipic dihydrazide above 150 mM exhibited similar release kinetics (not shown).

Doxorubicin was covalently incorporated into the hydrogel by reacting it with excess adipic dihydrazide as reported previously for daunomycin [20]. The ketone group on the C13 position of doxorubicin reacts with the hydrazide group to form the doxorubicin–adipoyl hydrazide conjugate (Fig. 3). Upon mixing with oxidized alginate, the free hydrazide group on this conjugate reacts with the pendant aldehyde group on the backbone of oxidized alginate to form a labile hydrazone bond (Fig. 3). The release of doxorubicin from cross-linked oxidized alginate was dependent on the concentration of the covalent cross-linker (Fig. 4b). Doxorubicin was quantitatively released within 2 days from all hydrogels formed at 50 mM adipic dihydrazide. At 75 mM adipic dihydrazide, doxorubicin was released after 3 days. Increasing the concentration of adipic dihydrazide to 100 mM prolonged the total release of doxorubicin to 6 days of incubation, and at a higher concentration of 150 mM adipic dihydrazide, only 20% of doxorubicin was released after 22 days of incubation at 37°C.

Mitoxantrone was expected to form ionic complexes with the hydrogels (Fig. 3), and would thus be released only as the gels degraded. The release of mitoxantrone from cross-linked oxidized alginate hydrogels was influenced by the concentration of adipic dihydrazide (Fig. 4c). The entire loaded drug was released after only 2 days of incubation from hydrogels formed with 50 mM

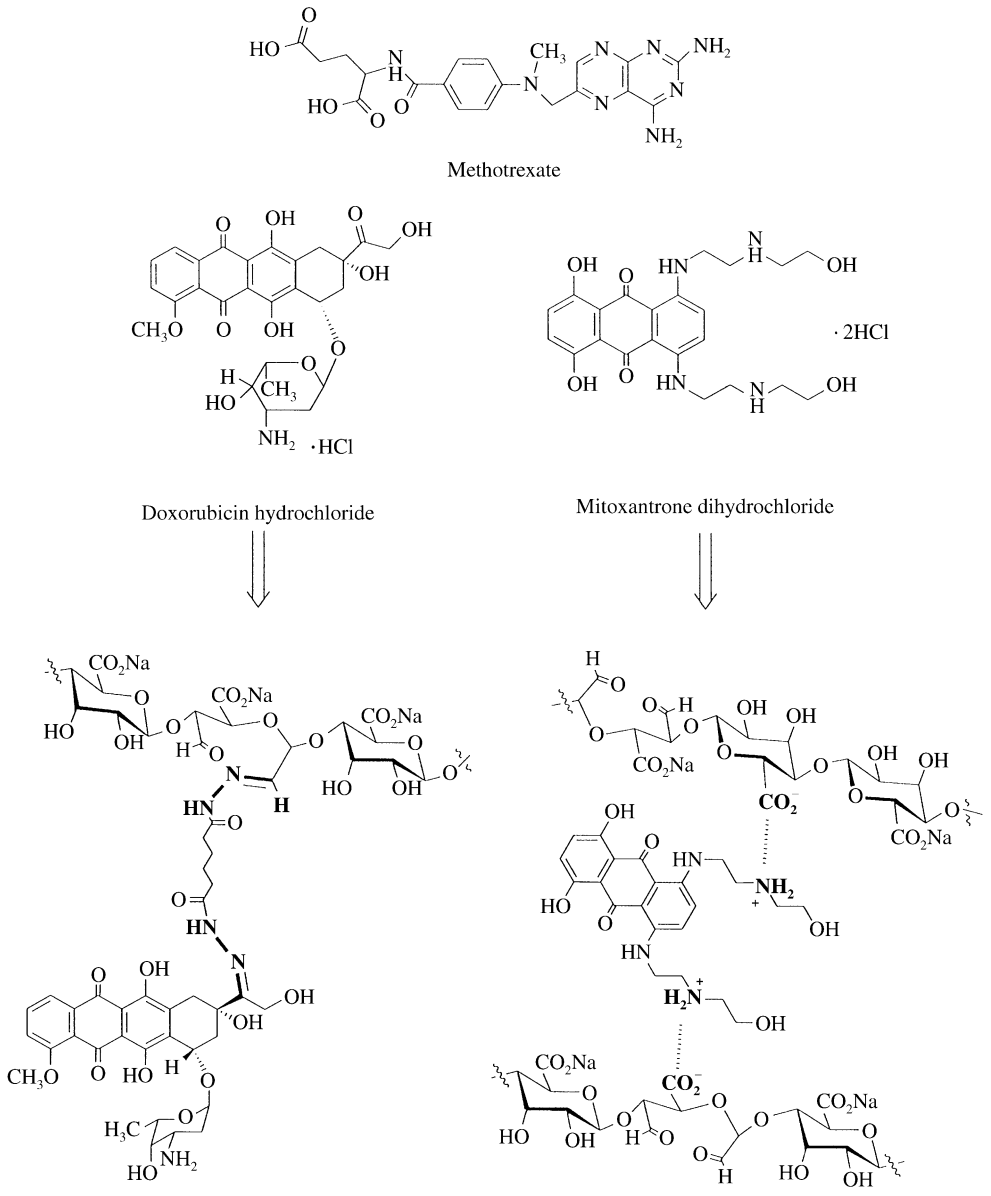

Fig. 3. Proposed mechanism for drug incorporation. Doxorubicin is chemically linked to oxidized alginate with a hydrazone bond. Mitoxantrone forms an ionic complex with the carboxylate groups.

adipic dihydrazide. Hydrogels formed at 75 and 100 mM adipic dihydrazide released the entire drug after 3 and 6 days, respectively. However, hydrogels formed at 150 mM adipic dihydrazide released only 7% of the loaded drug after 21 days of incubation.

Methotrexate, doxorubicin, and mitoxantrone have also been separately incorporated into calcium cross-linked alginate hydrogels, and their release monitored spectrophotometrically as a control. Methotrexate was quantitatively released after 8.5 h of incubation at 37°C as expected. Doxorubicin was released over a 3.5 day time period (Fig. 5). The release of mitoxantrone from alginate hydrogels was negligible over the first week of incubation as expected for an ionically interacting drug.

To test the utility of oxidized alginate hydrogels in delivering combinations of drugs, cross-linked oxidized alginate hydrogels have been loaded with all three drugs simultaneously. Methotrexate was quantitatively released after 9 days of incubation (Fig. 6). Doxorubicin was released slowly at a rate of 1.7% per day for 13 days followed by a rapid release of 19.5% per day during the last 4 days (Fig. 6). Mitoxantrone, on the other hand, was not significantly released during the initial 10 days. However, a rapid release of 24% per day was observed during the last 4 days.

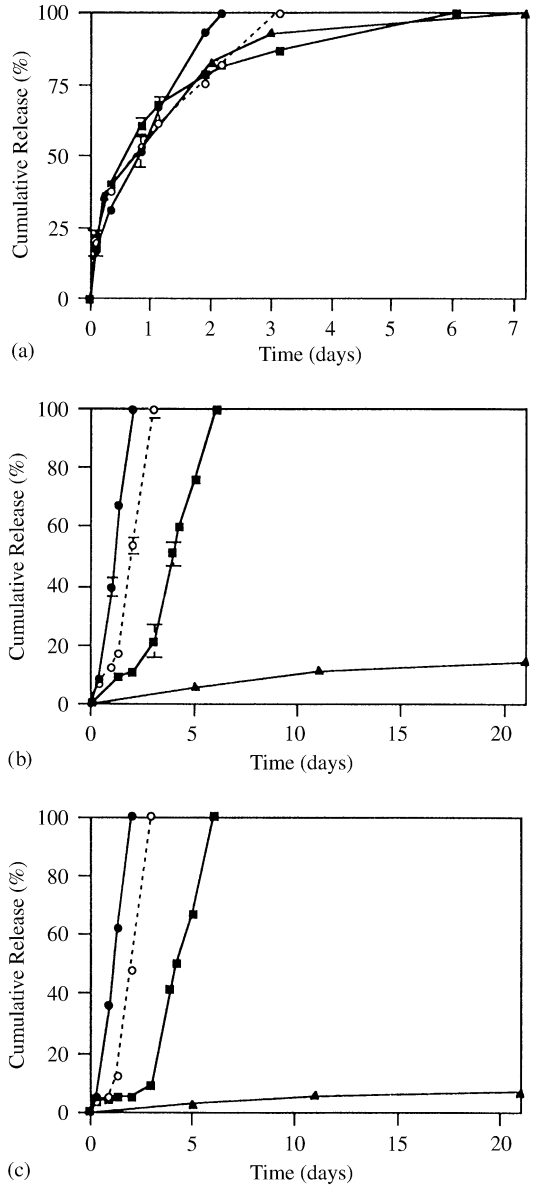

Fig. 4. The cumulative release of (a) methotrexate, (b) doxorubicin, and (c) mitoxantrone from cross-linked oxidized alginate hydrogels. Hydrogels were formed at (●) 50 mM adipic dihydrazide, (○) 75 mM adipic dihydrazide and (■) 100 mM adipic dihydrazide and (▲) 150 mM adipic dihydrazide. All hydrogels were prepared with 6% w/w oxidized alginates (69% oxidized) in dd. H_2O.

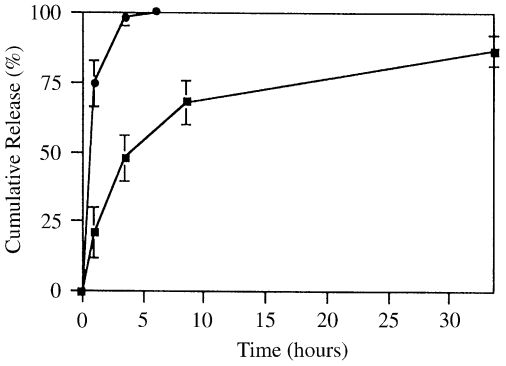

Fig. 5. The cumulative release of (●) methotrexate and (■) doxorubicin from alginate hydrogels. Hydrogels were formed with 2% w/w alginate in dd. H_2O and cross-linked with calcium sulfate.

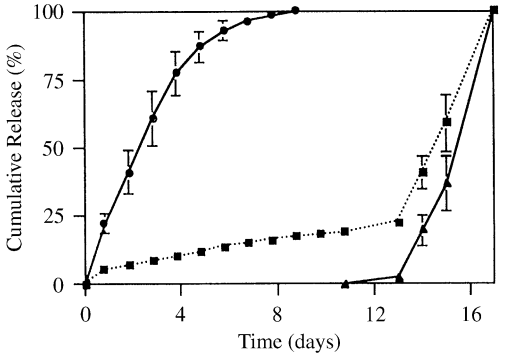

Fig. 6. The release of (●) methotrexate, (■) doxorubicin, and (▲) mitoxantrone from cross-linked oxidized alginate hydrogels loaded with all three drugs. Hydrogels were formed at 100 mM adipic dihydrazide and 6% w/w oxidized alginates (69% oxidized) in dd. H_2O.

4. Discussion

We have synthesized a novel hydrogel derived from alginate for the single or simultaneous delivery of antineoplastic agents. We have previously demonstrated the synthesis and cross-linking of poly(aldehyde guluronate), PAG to form hydrogels [24]. However, we were limited with that polymer by the low molecular weight of PAG (6 kDa), and the synthesis required several labor intensive purification steps. In the present study, we oxidize sodium alginate directly, bypassing the hydrolysis step, and utilized the reactive aldehyde groups to cross-link these polymers and form hydrogels. These polymers could be synthesized in a relatively short period of time and could be formed with a wide range of molecular weights. The weight fraction of the product that had a molecular weight below 80 kDa could also be readily controlled. This is very attractive for biomedical applications of alginate derivatives since polymers with molecular weights lower than 80 kDa are expected to be cleared from the body in a similar manner to low molecular weight alginate [26].

We have separately incorporated three model drugs into cross-linked oxidized alginate hydrogels: methotrexate, doxorubicin, and mitoxantrone to demonstrate that various drug–hydrogel interactions can be exploited to control the kinetics of drug release. Methotrexate was quantitatively released over a 2 days period from hydrogels formed at 50 mM adipic dihydrazide, and the release was extended up to 7 days from hydrogels formed at higher concentration of adipic dihydrazide. This rapid release is expected for a drug with minimal hydrogel

interaction. The higher concentrations of adipic dihydrazide led to a lower swelling ratio, and this likely caused the slower release. These findings are consistent with many past reports of the rapid release of non-coupled or non-interacting drugs from alginate hydrogels [27].

To demonstrate the utility of these hydrogels to deliver drugs via a chemical-controlled release mechanism, we have incorporated doxorubicin by covalent coupling to the alginate backbone. Doxorubicin was released from days to weeks depending on the concentration of adipic dihydrazide used (Fig. 5b). This clearly indicates that doxorubicin is not merely interacting ionically with the hydrogel but is covalently linked to the polymer backbone [20]. Doxorubicin is released following the hydrolysis of the hydrazone bond connecting it to the hydrogel. We can potentially release a variety of drugs that contain an aldehyde, a ketone, or a hydrazide group in a similar manner. A detailed analysis of this mechanism of drug release from these types of polymers has been provided in a previous publication [20].

To confirm that doxorubicin was covalently linked to oxidized alginate hydrogels, we used calcium cross-linked alginate hydrogels as a control. The release of doxorubicin is expected to follow a diffusion-controlled release in a similar manner to methotrexate in these gels, due to the lack of potential for covalent coupling. Doxorubicin was released from these gels over 3.5 days (Fig. 5), which was somewhat longer than expected. This suggests that doxorubicin, a positively charged molecule, may be interacting ionically with the hydrogel, and this interaction is slowing down the release of doxorubicin. However, doxorubicin was released over a longer time from oxidized alginate hydrogels (formed at 150 mM adipic dihydrazide) indicating a chemical attachment between the drug and the polymer backbone [20].

We have also incorporated and released mitoxantrone from oxidized alginate hydrogels to determine if ionic interactions can be used to control drug release. The release of mitoxantrone was coupled with the degradation of the hydrogels, as expected for this mechanism. Mitoxantrone was completely released from 2 to 6 days from hydrogels cross-linked with 50–100 mM adipic dihydrazide (Fig. 4). However, less than 10% of the drug was released after 22 days from hydrogels formed at high concentrations of adipic dihydrazide (150 mM) suggesting that mitoxantrone is forming ionic junctions with the carboxylate groups on the polymer backbone similar to divalent cations. Mitoxantrone was not significantly released from calcium cross-linked alginate hydrogels during the initial 4 weeks of incubation (data not shown). These gels are not expected to degrade in vitro. This finding supports our prediction that mitoxantrone is forming ionic junctions between the carboxylate groups on the polymer backbone (Fig. 3). Ionic interactions between alginate and many drugs are possible, and have been previously described [28].

The simultaneous delivery of a combination of therapeutical active agents has recently been shown to be beneficial in combating cancer and HIV infection. However, it might even be more attractive to deliver each drug with a different release profile. We have incorporated all the three drugs simultaneously into cross-linked oxidized alginate hydrogels and have observed three different release profiles (Fig. 6). Methotrexate was completely released after 9 days of incubation. Both doxorubicin and mitoxantrone were completely released following the degradation of the hydrogel after 17 days of incubation. However, over 20% of doxorubicin was slowly released during the first 13 days whereas mitoxantrone was not significantly released during that time. This demonstrates clearly that we can indeed load a variety of drugs and release them simultaneously over a wide range of time frame. Another approach to achieve delivery of multiple drugs simultaneously or in sequence is to deliver each from a different polymer, and either mix the formulations prior to delivery or introduce each separately. However, the data in this paper demonstrate a single hydrogel may be used to deliver multiple drugs either simultaneously or sequentially. This may simplify multi-drug delivery, and biomaterial development and regulatory approval.

5. Conclusions

We have designed and synthesized novel hydrogels derived from alginate to simultaneously deliver a variety of drugs. We can control the degradation profile of the hydrogel from days to months and the release of model antineoplastic agents over a similarly wide range of time frames. Three different release mechanisms: diffusion-controlled, covalent bond degradation, and ionic dissociation-controlled mechanisms, can be utilized in this system to control the kinetics of drug release. This novel delivery system could be potentially used for the controlled delivery of a variety of anticancer compounds sequentially or simultaneously, and in a localized manner.

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