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Review

Alginate graft copolymers and alginate–co-excipient physical mixture in oral drug delivery

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Abstract

Objectives Use of alginate graft copolymers in oral drug delivery reduces dosage form manufacture complexity with reference to mixing or coating processes. It is deemed to give constant or approximately steady weight ratio of alginate to covalently attached co-excipient in copolymers, thereby leading to controllable matrix processing and drug release. This review describes various grafting approaches and their outcome on oral drug release behaviour of alginate graft copolymeric matrices. It examines drug release modulation mechanism of alginate graft copolymers against that of co-excipients in non-grafted formulations.

Key findings Drug release from alginate matrices can be modulated through using either co-excipients or graft copolymers via changing their swelling, erosion, hydrophobicity/hydrophilicity, porosity and/or drug adsorption capacity. However, it is not known if the drug delivery performance of formulations prepared using alginate graft copolymers is superior to those incorporating graft-equivalent co-excipient physically in a dosage form without grafting but at the corresponding graft weight, owing to limited studies being available.

Conclusions The value of alginate graft copolymers as the potential alternative to alginate–co-excipient physical mixture in oral drug delivery cannot be entirely defined by past and present research. Such an issue is complicated by the lack of green chemistry graft copolymer synthesis approach, high grafting process cost, complications and hazards, and the formed graft copolymers having unknown toxicity. Future research will need to address these matters to achieve a widespread commercialization and industrial application of alginate graft copolymers in oral drug delivery

Keywords alginate; co-excipient; graft copolymer; oral drug delivery

Introduction

Alginate is a water-soluble polysaccharide.[1–3] It is a high-molecular-mass hyaluronic acid-like binary copolymer.[4,5] Alginate is commonly isolated from brown algae such as *Laminaria hyperborea*, *Ascophyllum nodosum* and *Macrocystis pyrifera*.[1] It is also available from red seaweed (Corallinaceae) and is found in some bacteria, such as *Azotobacter vinelandii*, and several *Pseudomonas* species.[1,2,4,6] In recent years, there has been an increasing interest in grafting alginate with functional moieties to provide a wider source of biopolymers of this origin.

The alginate chain is made of homopolymeric regions of β-D-mannuronic acid (M) blocks and α-L-guluronic acid (G) blocks, interdispersed with regions of alternating structure of α-L-guluronic and β-D-mannuronic acid blocks (Figure 1).[1–4] The M blocks take the form of extended ribbons, while the G blocks are buckled in shape.[1] Alignment of two G blocks side by side gives rise to diamond configuration cavities that have an ideal dimension for co-operative binding of cations.[1,3] Different algae, parts and sources of algae, and time of harvest produce biopolymers with different monomer composition and block arrangement.[3,4,7] Seaweed alginate exists as mixed salts of various cations found in seawater such as Mg^{2+}, Sr^{2+}, Ba^{2+} and Na^{+}.[1] It has an M/G ratio of 0.45–3.33. Bacterial alginate can have a acetylated moiety in its chemical build-up[2] and can have an extreme composition, containing up to 100% mannuronate residues.[4]

The pKa values of M and G residues of alginate are 3.38 and 3.65, respectively.[3,5] By virtue of carboxyl groups on the constituent uronic acid residues, the pKa value of alginic acid ranges between 3.4 and 4.4, depending on type of alginate and the salt present in mixture.[5] The composition, block sequence and molecular weight of alginate chains dictate

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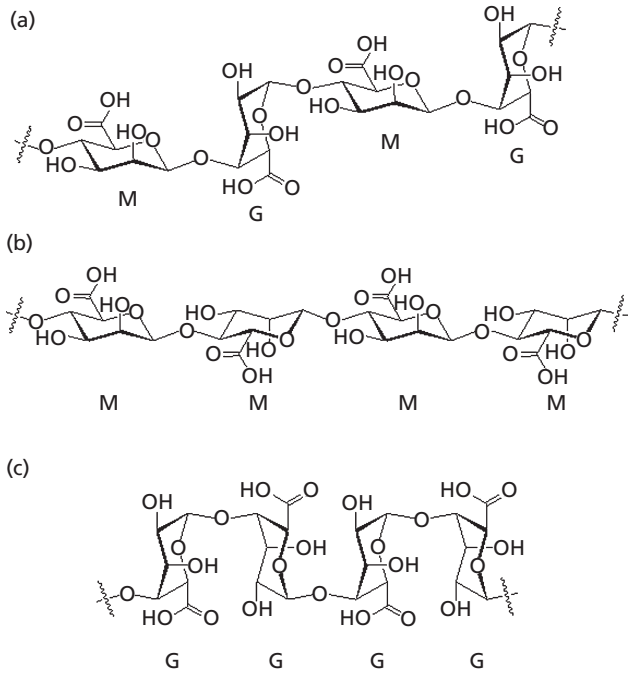


Figure 1 Chemical structures of alginates characterized by block sequences of (a) MG, (b) MM and (c) GG. M, β-D-mannuronic acid; G, α-L-guluronic acid.

the physicochemical properties of the polymer such as viscosity,[3] binding affinity for cations,[1,3,4] gelation,[3,7,8] aqueous solubility,[3] mechanical strength,[4] swelling capacity[4] and bioadhesiveness.[1,4]

Physicochemical and Biological Properties

Alginate is practically soluble in water.[3] It precipitates as alginic acid in low pH or gastric medium.[1,3] In a higher-pH medium or intestinal fluid, the alginic acid is converted to a soluble viscous layer.[1] The viscosity of alginate increases with its molecular weight and is a function of polymer conformation.[3,8] Alginate can form gel under extremely mild conditions without using toxic reactant.[1] It gels upon reacting with divalent cations, such as Ca^{2+},[1] or by forming acid at a pH below the pKa value of uronic acid residues.[4] The latter provides a comparable mechanical property to ionically crosslinked alginate. Alginate is chemically stable at pH values between 5 and 10. It undergoes decarboxylation only under highly acidic conditions.[5]

Na^{+} and Mg^{2+} do not induce gelation with sodium alginate.[1] Sodium alginate form gels with crosslinking solution of Ca^{2+}, Sr^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+} and Ba^{2+}.[1,9,10] The gel is formed via the exchange of Na^{+} from guluronate with divalent cations, and stacking of guluronate moieties to form an egg-box structure.[1,4] Ba^{2+} forms a stronger crosslinked alginate gel than Ca^{2+}.[4,7] However, the gel is not suitable for pharmaceutical application owing to its toxicity.[7] Alginate particles gelled using Al^{3+} are not as spherical as a Ca^{2+}-crosslinked matrix.[11] The trivalent Al^{3+} induces a higher number of aggregation points between two alginate chains than the divalent cations. A high polymer binding propensity distorts the spherical attribute of gelling particles.

The polymannuronate blocks and alternating blocks are almost non-selective in their binding with cations.[4] The mannuronate residue is reported to have a lower affinity for Ca^{2+} than the guluronate group.[12] The gelation of alginate with Ca^{2+} involves mainly the formation of crosslinkages at junctions of GG–GG, MG–GG and MG–MG.[7] The gel strength is markedly enhanced when the average length of the G block increases from 5 to 15 monomers of alginate with a high α-L-guluronic acid content (>70%).[4] The flexibility of the crosslinked alginate gel increases in the order of MG block > MM block > GG block.[8] The alternating block sequence dominates the elasticity attribute of alginate with the ribbon-like M block being less rigid than the buckled G sequence.

Alginate is reported to be generally non-toxic, biodegradable, non-immunogenic and biocompatible.[1,11,13–15] It induces several biological effects such as anti-cholesterolaemic,[2,16–22] antihypertensive,[23–25] antidiabetic,[2,17,19,26] anti-obesity,[17] antimicrobial,[25,27] anti-cancer,[2,28] anti-hepatotoxicity,[2] wound healing,[29,30] anticoagulation and coagulation[2] activity. The biological effects of alginate are related to its structural assembly and physicochemical attributes. Depolymerized or low-molecular-weight sodium alginate can reduce plasma triglyceride and cholesterol, decrease body weight gain,[16–18] control the development of diabetes[17,19] and hypertension,[23,24] and has antibacterial activity against *Pseudomonas aeruginosa*.[27] The low-molecular-mass potassium alginate produced from *Laminaria japonica*, with an average molecular weight of 1800 Da and a potassium content of 25%, has similarly been shown to decrease blood pressure in hypertensive rats.[25] In the case of haemostatic effect, zinc or calcium alginate and alginate sulfate demonstrate opposing influences. Zinc or calcium alginate potentiate coagulation effects;[2] alginate sulfate prepared from sodium alginate, on the contrary, demonstrates a high anti-coagulation activity.

Oral Drug Delivery

Alginate has a vast range of unique physicochemical and biological characteristics rendering it suitable for many applications. Alginate has been used as emulsion thickener,[1,2] stabilizer,[1] emulsifier,[1,31] carrier polymer for antigen, enzyme, microbe, animal cell and recombinant gene products,[2,4,6–8] bone and cartilage tissue engineering scaffold,[2,14] peripheral nerve regeneration implant,[2] wound dressing,[2,4,12] dental impression material,[4,31,32] anti-heartburn and gastric reflux raft-forming formulation,[4] radioactive and heavy metal absorption agent,[2,33–35] and plasma expander.[36]

Alginate has been used as carrier material in oral drug delivery system design.[1,2,4,37] In oral drug delivery, it has been formulated as microspheres, microcapsules, gel beads, hydrogel, film, nanoparticles and tablets (Table 1).[38–44] The drug release property of an alginate matrix is strongly governed by the composition of the uronic acid sequences.[1,105,106] The mannuronic acid-rich alginate matrix gives lower drug release rates in dissolution medium of pH 1.2 but higher drug release rates at pH 6.8 when compared with guluronic acid-rich

Table 1 Co-excipients and their use in formulation of oral alginate dosage forms

Co-excipient	Dosage form	Formulation approach	Remarks
Chitosan	Microparticles[45–59]	Alginate microparticles are prepared by emulsification/internal gelation or spray drying method. Chitosan is introduced to alginate core in the form of a coat membrane via coacervation process in a continuous stage or two stages, with calcium choride as crosslinker of alginate matrix. Alternatively, the tripolyphosphate crosslinked chitosan forms the core of beads. Alginate is deposited onto the beads and crosslinked using calcium chloride. The coated beads are shredded into microparticles through rotating blade cutting. Multilayers of chitosan can be introduced as coat, together with alginate, onto drug crystals or carboxymethylcellulose-doped calcium carbonate colloidal particles with crosslink by glutaraldehyde and core decomposition by disodium ethylenediamine tetraacetic acid. In the latter, the formed microcapsules contain negatively charged carboxymethylcellulose, either in free state or couples with excess chitosan of the first layer.	Specific targeting purpose of alginate-chitosan microparticles is introduced through covalently attaching wheat germ agglutinin to chitosan of alginate matrix by carbodiimide method. The robustness of microparticles against enzymatic degradation in the upper gastrointestinal tract can be increased through adding pectin which is more resistant to digestion in gastrointestinal tract than alginate and breaks down only in colon by microflora pectinase. Prevention of gastric and intestinal drug dissolution from microparticles can be attained through enteric coating of matrix by hydroxypropylmethylcellulose phthalate.
	Nanoparticles[60–63]	The alginate is pre-gelled with Ca^{2+} followed by its reaction with chitosan. Chitosan may be introduced as a single co-excipient or in combination with other complexation agent such as albumin. Core-shell nanoparticles can be prepared by alternate adsorption of anionic alginate and cationic chitosan onto cationic nanosize phospholipid vesicles by incubation method.	Pluronic and dextran may be needed by nanoparticles to modulate the rate of drug release. The alginate matrix may require chitosan–albumin coating. Albumin from albumin–chitosan coat of alginate matrix serves as an enteric coat to protect protein drugs against degradation by acid and protease.
	Beads[6,64–74]	Chitosan is introduced as chitosan membrane or directly as alginate–chitosan blend in alginate beads crosslinked by calcium chloride via ionotropic gelation method. The alginate–chitosan beads can be further crosslinked through reacting chitosan with sodium sulfate. Alternatively, multilayers of chitosan can be introduced to alginate formulation. The chitosan can be added as both core and coat, and crosslinked by tripolyphosphate ions.	Polyethylene glycol may be added as extra coat onto alginate–chitosan beads.
	Tablets[75–77]	Mixing or polyelectrolyte coacervation of alginate and chitosan followed by compression.	Glycerl monostearate may be needed as additional drug release retardant.
Chitosan derivatives: carboxymethylchitosan-*g*-sodium acrylate, chitosan-*g*-polyethylene glycol, chitosan-*g*-poly(acrylic acid), *N*,*O*-carboxymethylchitosan, *N*-succinyl chitosan and *N*-trimethyl chitosan	Microparticles, nanoparticles, beads, film[13,78–87]	Chitosan derivative may be added directly to alginate before crosslinking by calcium chloride via ionotropic gelation method, or as coat added to alginate or alginate–chitosan derivative formulation. Genipin has been employed as a naturally occurring crosslinking agent. It provides a lower extent of crosslinking than glutaraldehyde to form a semi-interpenetrating polymer network within a hydrogel system.	Vermiculite or attapulgite can be used as additives to further retard drug release by reduced matrix swelling and enhanced drug adsorption.

Table 1 (*Continued*)

Co-excipient	Dosage form	Formulation approach	Remarks
Polyethylene-imine	Beads[88–90]	Polyethyleneimine is deposited onto calcium alginate matrix via polyelectrolyte complexation. Blocking of surface pores of polyethyleneimine-treated calcium alginate matrix can be achieved by further coating of matrix using alginate.	Dense alginate–polyethyleneimine membrane can retard drug release from matrix in both acidic and near neutral media. Further drug release retardation is attained through applying additional alginate coat on the surfaces of alginate-polyethyleneimine matrix.
Poly-*L*-lysine	Microparticles, nanoparticles, beads[48,91,92]	Poly-L-lysine is crosslinked onto calcium alginate matrix. Gelation of alginate by Ca^{2+} is first required to prepare physically well defined particles as the interaction between poly-L-lysine and alginate takes place at mannuronic residues, which leads to loosely packed domains.	Polymers such as pectin are incorporated into calcium alginate matrix to further retard the drug release.
Poly-*L*-histidine	Beads[93]	Incubation of calcium alginate beads in aqueous solution of poly-L-histidine.	–
Layered double hydroxide	Beads[94]	Ionotropic gelation of alginate and layered double hydroxide in the presence of calcium chloride.	–
Hydroxypatite	Beads[95]	Beads of alginate and hydroxypatite are formed via crosslinking of alginate by calcium component of hydroxypatite.	–
Albumin	Hydrogel disk[96]	Alginic acid is crosslinked by human serum albumin via dehydration condensation using *N*-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide where it is first converted into activated ester prior reacting with amino residues in human serum albumin.	–
Gelatin	Microparticles, beads, tablets[97–99]	Mixture of alginate and gelatin is crosslinked by calcium chloride or coacervated through pH modification of the blend. Glutaraldehyde and sodium carboxymethylcellulose may be added as additional crosslinking agent and as particle aggregation inhibitor respectively.	Matrix swelling of calcium alginate matrix ends up in erosion. Gelatin incorporated matrix swells with no complete erosion bringing a greater degree of drug release control.
Acrylate derivatives: Eudragit E-100, Eudragit®E PO and polymethacrylate	Microparticles, nanoparticles, beads, tablets[100–102]	The acrylate derivative may be introduced in alginate core or as coat to alginate matrix through the formation of inter-polyelectrolyte complex.	–
β-cyclodextrin	Complexes[103]	Cationic *β*-cyclodextrin complexes with drug through directly entrapping drug in its core cavity before it is encapsulated by calcium alginate matrix, which undergoes coating with chitosan.	–
Glyceryl palmitostearate	Beads[104]	Ionotropic gelation of alginate carrying drug and lipid by calcium chloride with heat treatment applied on wet beads to melt the lipid and redistribute the drug into molten lipid.	–

alginate matrix.[107–110] Mannuronic acid-rich alginate matrix is deemed to hydrate faster under acidic conditions and has diffusion barrier built up more rapidly, thus giving rise to a slower drug release. Guluronic acid-rich alginate matrix tends to exhibit crack formation and lamination under acidic conditions, which result in burst drug release. At near neutral pH, the guluronic acid-rich alginate matrix forms a more rigid gel upon hydration than the mannuronic acid-rich alginate matrix. It is less susceptible to erosion and provides a more effective barrier to retard drug release.[108,111–113] Typically, low-molecular-weight alginates release encapsulated drugs at a faster rate as a result of lower levels of physical entanglement between polymer chains in the matrix to retain the embedded drugs and faster matrix erosion.[1] The polyanionic alginate is a good mucoadhesive agent.[1] It has the highest mucoadhesive strength with reference to polymers such as polystyrene, chitosan, carboxymethylcellulose and poly(lactic acid).[1] The G block of alginate is able to transiently modify the mucin network structure and manipulate its attachment onto the mucosal surfaces for oral drug delivery.[4]

Alginase is the only enzyme known to degrade alginate.[9] However, it is a bacterial enzyme that is not synthesised by humans or animals. In-vivo degradation of alginate gel occurs mainly due to the sensitivity of the gel towards calcium-chelating compounds, such as phosphate, citrate and lactate ions, or pH and salt content changes of the surrounding medium.[9,114] In gastric fluid, the alginate can be hydrated and converted into an insoluble alginic acid skin.[1] The alginate shrinks at low pH values following reduced electrostatic repulsion between the carboxylate moieties of alginate chains as a result of protonation in the acidic milieu.[1,86,94] This transcribes to a reduction in pore size of matrix thereby rendering a low propensity of drug release.[1] In a higher-pH medium simulating the intestinal fluid, the alginic acid ionizes and converts into a soluble viscous layer.[1] The pores of an alginate gel are large to small molecule drugs or even large proteins with a molecular weight amounting to 3×10^5 Da.[4] Rapid dissolution of alginate matrix in intestinal simulating fluid may induce burst drug release.[1]

Similar degradation events take place with calcium alginate matrix in both gastric and intestinal media. In a high-pH regime, the calcium alginate matrix swells, loses its integrity and exhibits quick drug release. The changes in matrix characteristics are attributed to reduced calcium alginate crosslinkages through an ion exchange process between Ca^{2+} of alginate matrix and monovalent ions of buffer solution such as Na^+ and K^+.[86,94] $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} are three anions in pH 6.8 and 7.4 buffer solutions made of KH_2PO_4 and NaOH.[86] HPO_4^{2-} and PO_4^{3-} are the prevalent forms in buffers pH 6.8 and 7.4, respectively. The rise in drug release is also ascribed to loss of calcium alginate crosslinkages through reaction of Ca^{2+} with PO_4^{3-} and are removed by precipitation as $Ca_3(PO_4)_2$.[86,94]

An abrupt drug release, following the transit of dosage form from the gastric to the intestinal milieu, can result in drug degradation under the influence of proteolytic enzymes.[1] The inability of the dosage form to sustain drug release may negate drug bioavailability and its elements of colon-specific delivery. The drug release property of an alginate formulation can be modified by incorporating additives, such as pectin,[48,57] pluronic,[60] poly(ethylene glycol),[67] vermiculite,[85] attapulgite[86] and Eudragit S100,[46] in the form of matrix or coat substances. The processing of an alginate formulation involves mild conditions that are relatively inert and able to retain the biological activity of drug.[1,114] The common processing approaches for an alginate formulation are crosslinking, coacervation and complexation reactions. Chitosan and Ca^{2+} have received widespread use as a coacervating polyelectrolyte and crosslinking agent, respectively, in the formulation of oral alginate matrices (Table 1).[38,42,115] Most oral alginate formulations employ Ca^{2+} to shape and strengthen the matrices. The addition of chitosan to the calcium alginate matrix enhances its controlled drug release property. Crosslinking the chitosan with tripolyphosphate anion or alternatives further increases the controlled drug release attributes of an alginate formulation.[58,65] Table 1 summarizes the use of chitosan, chitosan derivatives and other co-excipients, such as polyethyleneimine, poly-L-lysine, poly-L-histidine, layered double hydroxide, hydroxypatite, albumin, gelatin, cyclodextrin, glyceryl palmitostearate and acrylates, in drug release modulation of oral alginate formulations.

The incorporation of chitosan into an alginate matrix mutually complements their modes of drug release control. Alginate shrinks in a low-pH medium but it dissolves in a higher-pH milieu. On the contrary, chitosan dissolves at a low pH and is insoluble in a higher-pH medium. Polyelectrolyte coacervation of alginate with chitosan through electrostatic interaction between alginate carboxylate and chitosan ammonium moieties reduces the ease of solvation of alginate at the higher pH and chitosan at the lower-pH regimes,[51,69] similar to alginate–Eudragit complexes.[100] It brings about the formation of a physically robust dosage form with reduced erosion,[49,66] disintegration,[70] dissolution or gel porosity[57] and this provides sustained-release attributes in drug delivery. The use of modified chitosan in the formulation of alginate matrix has similarly been reported to retard drug release.[13,82,83] Modified chitosan, such as *N*-trimethyl chitosan chloride, has a good water solubility over a wide pH range.[13] It has a permanent positive charge and is active as drug absorption enhancer in media of varying pH through regulating the activity of mucosal tight junction, in addition to exerting drug release control over the alginate matrix.

Through the use of a co-excipient, the alginate matrix can be subjected to physicochemical reaction at different domains of the polymer chains to provide a stronger structure, which entails a larger extent of drug release retardation. In a calcium alginate complex, the Ca^{2+} interacts with alginate polymer chains at the oligopolyguluronate sequences into an egg-box model and such complexation leads to the formation of compact domains.[92] The addition of poly-L-lysine co-excipient can further strengthen the alginate matrix.[92] The interaction between poly-L-lysine and alginate takes place at the mannuronate residues to form additional less-ordered and closely packed domains. The use of Ca^{2+} and poly-L-lysine translates to drug release modulation of an alginate matrix at both guluronate and mannuronate junctions instead of largely by guluronate residues.

The drug release retardation property of an alginate matrix is promotable by reduced loss of crosslinkages and swelling

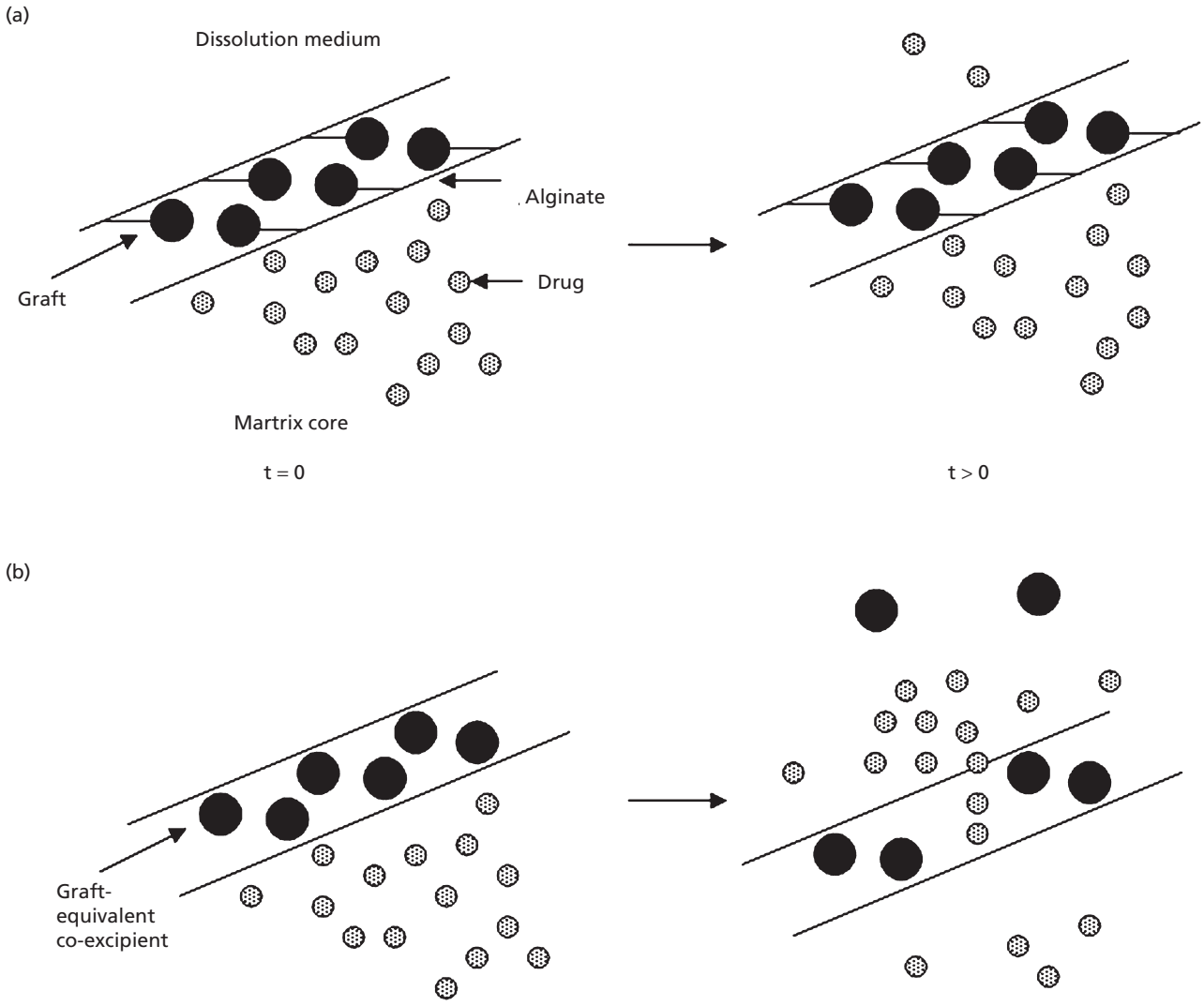


Figure 2 Schematic diagrams of drug release over time from oral formulations prepared from (a) alginate graft copolymer and (b) non-grafted alginate with graft-equivalent co-excipient.

capacity of the polymer bed. A co-excipient, such as positively charged layered double hydroxide, could interact with alginate to reduce its matrix swelling and drug release in an acidic medium. In a high-pH milieu, layered double hydroxide can also complex with PO_4^{3-} from the dissolution medium to prevent the loss of alginate matrix crosslinkages via Ca^{2+} removal in the form of small electrolyte precipitations, thereby sustaining drug release.[94] In alginate matrices containing vermiculite[85] and attapulgite,[86] a low swelling ratio due to alginate–co-excipient interaction affords narrow pores between the polymer chains of the matrix and negates drug release. A fast drug release is further prevented by the drug molecules being adsorbed onto vermiculite or attapulgite.

Alginate Graft Copolymers

Co-excipients can be used to modulate drug release of an alginate formulation. However, it is deemed difficult to keep the mixing ratio of alginate to co-excipient in the course of matrix processing or drug release at a constant or approximately steady level. Demixing and segregation of alginate from co-excipients can occur at any time and to a varied extent. This may result in less controllable or reproducible drug release and therapeutic delivery. The mixture quality of powder has been a common problem faced by industrialists.[116] The quality of polymer powder mixing is dependent on size, density, geometry, moisture content, surface texture and electrostatic charging of the solid particles.[117,118] Optimization of a mixing process often faces constraints as qualification of the characteristics of powder particles requires lengthy and multi-step operations.[116] This is particularly marked when the powder mixing involves a wide variety of excipients, in addition to drug.

Covalent binding of co-excipient to alginate chains produces physically more stable derivatives. It is deemed to give constant or approximately steady weight ratio of alginate to covalently attached co-excipient in copolymers during formulation processing and drug release. Covalent

Table 2 Physicochemical and drug release modulation characteristics of alginate graft copolymers

Graft copolymer	Physicochemical and drug release properties
Alginate-*g*-poly(*N*-isopropylacrylamide) [119,120]	The mechanical strength of alginate increases upon grafting with *N*-isopropylacrylamide. The hydrogel made of graft copolymer expresses a lower swelling capacity due to collapse of *N*-isopropylacrylamide at specific temperature range thereby sustaining drug release.
Alginate-*g*-poly(butyl methacrylate), α-methyl methacrylate-*g*-sodium alginate[15,121]	The microspheres formed from alginate conjugate can prolong drug release when compared to microspheres made of unmodified alginate. The alginate conjugate is more hydrophobic than the parent polymer. An increase in drug encapsulation and controlled drug release can be ascribed to hydrophobic interaction between alginate conjugate and drug.
Oxidised sodium alginate-*g*-poly(2-dimethylamino)ethyl methacrylate[9]	The modified alginate retains its capacity to crosslink with Ca^{2+} to form gel beads. At pH 7.4, the drug release is slower from modified alginate beads than oxidised sodium alginate beads. Sparse long grafted polymer chains are preferable for drug binding. They are flexible and can protrude in solution as tentacles capable of interacting with drug at multiple points. The positively charged grafted chains can also interact with negatively charged COO^- moiety of alginate to prevent bead dissolution and drug release. The tertiary amino group of grafted chains (pKa = 7.0 to 7.3) can protonate at pH 1.8 leading to bead swelling. The C = N bond can be hydrolysed under acidic condition to produce diffusion path of larger pore size. This results in faster drug release from modified alginate beads than oxidised sodium alginate beads in acidic medium. The use of modified alginate in sustained-release drug delivery system design requires additional formulation or processing efforts to circumvent fast drug release in gastric region.
Hydrophobically modified alginate-C_{12}, alginate-C_{18}[10,122–124]	The alginate-C_n conjugate is amphiphilic in nature. The alkyl chains are preferentially bound to mannuronate residues than polyguluronate sequences. The microstructure of Ca^{2+}-amphiphilic alginate hydrogel is more heterogeneous than calcium alginate matrix, despite the alkyl chains not being mainly bonded to the polyguluronate sequence, hampering the fixation of Ca^{2+} on alginate. The Ca^{2+}-amphiphilic alginate hydrogel is characterized by dense hydrophobic domain neighbouring a loose microstructure, which is unable to form egg-box junction in the presence of Ca^{2+}. The gelling of amphiphilic alginate into beads or microparticles requires the use of both calcium chloride and sodium chloride as reactants. The formed hydrogel is stable in the presence of non-gelling cation or calcium sequestering agent. Its mechanical property is reinforced by the combination of calcium bridge and intermolecular hydrophobic interaction. Esterification of alkyl chains onto alginate produces a physical network, which is stabilized by intermolecular hydrophobic interaction between alkyl chains. It affords good protein drug encapsulation and slow drug release. The release of protein drug from matrix is achieved by inducing dissociation of physical hydrophobic network, using surfactant as disrupting agent or esterases such as lipases to hydrolyse ester bond between alkyl chains and alginate backbone. This implies that the conjugate may be used as intestinal-specific drug carrier.
Hydrophobically modified alginate-C_4[125]	Esterification of C_4 alkyl chains onto alginate produces derivative that is capable of encapsulating both hydrophobic and hydrophilic drug molecules, and retaining both gelling and non-toxic attribute of parent alginate. Unlike C_{12}, the attachment of C_4 onto alginate mainly takes place at the guluronic acid moiety. The stiffness of alginate gel decreases in the order of polyguluronic acid, polymannuronic acid and alternating mannuronic and guluronic acid blocks GG > MM > MG. The modulus elasticity of modified alginate gel is higher than native alginate gel as the guluronic acid moiety of the former is lost to esterification and undergoes a lower level of Ca^{2+} crosslinking.
Cholesteryl-*g*-sodium alginate[14]	Sodium alginate is grafted with three cholesteryl groups per hundred hexuronic acid residues. The cholesteryl moiety is used as hydrophobic segment instead of alkyl chains as cholesteryl possesses better biocompatibility, has potential to interact with cholesteryl receptor on cell surface and stronger ability to drive self-assembly of cholesteryl-containing polymer. Its use can lead to the formation of targeted drug delivery system and nanoscale micellar product. The modified alginate can self-aggregate into hydrophobic microdomain surrounded by loose alginate anionic chains and encapsulate hydrophobic compound within the apolar domain. The formed aggregates are more stable for the purpose of drug release modulation and more compact in aqueous sodium chloride solution than parent sodium alginate, following intermolecular hydrophobic interaction between cholesteryl grafts.
Alginate-amide derivative[126]	Covalent attachment of dodecylamine onto alginate chains via amide linkage reduces the progressive loss of associative behaviour encountered by dodecyl ester alginate due to hydrolysis of ester bond. The formed hydrogel is expected to be more stable mechanically over long-term storage and exhibits sustained drug release property.
Propylene glycol alginate[127–130]	The propylene glycol alginate forms a membrane with albumin via transacylation reaction between ester group of propylene glycol alginate and amino group of albumin, initiated by alkaline pH. Peptide drug is released at a slower rate when microparticles are coated with such membrane.

Table 2 (*Continued*)

Graft copolymer	Physicochemical and drug release properties
Sodium alginate-*g*-poly(sodium acrylate) [131,132]	Semi-interpenetrating hydrogel made of sodium alginate-*g*-poly(sodium acrylate) and polyvinylpyrrolidone swells with an increase in medium pH due to a greater ionic repulsion between COO^- moieties. The swelling behaviour of the hydrogel is pulsatile with reversible on-off characteristics in media of high to low pH thereby providing pH-sensitive drug release characteristics.
Alginate-*g*-poly(methacrylamide)[133]	Reversible swelling and deswelling behaviour is demonstrated by hydrogel at pH 2 and 8. At pH 8, the hydrogel swells due to anionic repulsive electrostatic forces between carboxylate moieties. In acidic medium, the swelling capacity of hydrogel decreases following protonation of carboxylate moieties and reduced chain repulsion. The pH-sensitive swelling characteristics of graft copolymer render it suitable for use to control drug release as a function of pH.
Alginate-poly(sodium acrylate-*co*-acrylamide) [134]	Hydrogel exhibits swelling-deswelling pulsatile behaviour at pH 2 and 8. The swelling of hydrogel decreases with an increase in ionic strength of the surrounding medium. The pH and ionic strength sensitivity attributes of hydrogel can be utilised to control drug release.
α-cyclodextrin-alginate conjugate, β-cyclodextrin-alginate conjugate[135,136]	Cyclodextrin is a cyclic oligosaccharide made up of glucopyranose units bonded together via α (1→4) glycoside bonds. The cavity of cyclodextrin with an internal diameter between 6 and 10 Å is relatively hydrophobic. Small apolar drugs are able to be trapped to form inclusion complexes with binding specificity, thereby allowing drug solubilization, stabilization and transport.
	The drug release control of an alginate matrix varies with cyclodextrin graft type. Chemical modification of alginate at carboxylate moiety with β-cyclodextrin may change the characteristics of G blocks, impart polymer chain rigidity and impede interchain junction formation during ionic crosslinking with Ca^{2+}. In the case of α-cyclodextrin-alginate conjugate, its crosslinking capacity with Ca^{2+} is nonetheless less affected by chemical grafting thereby exhibiting a higher degree of drug release retardation than that of β-cyclodextrin.
Poly(acrylamide)-*g*-sodium alginate[31,137–141]	The graft copolymer retains the gelling characteristics of alginate where it can crosslink with divalent or multivalent cations to form beads. Ba^{2+} produces stronger hydrogel beads than Ca^{2+}. Al^{3+}, being trivalent, produces the strongest hydrogel with high crosslink density. The mechanical strength of beads made of graft copolymer can be enhanced using sodium carboxymethylcellulose as co-excipient. At pH 7.4, drug release propensity follows the order of $Al^{3+} < Ba^{2+} < Ca^{2+}$.
	The graft copolymer is easily converted into an ionic form through alkaline hydrolysis of amide group and transformation of $CONH_2$ of poly(acrylamide) to COOH. It provides the copolymer with a pH-sensitive attribute. The hydrogel made of such copolymer exhibits higher swelling capacity and faster drug release in pH 7.4 than 1.2 medium. The drug release propensity is higher in beads containing a higher fraction of alginate grafted with poly(acrylamide) owing to its higher swelling tendency. Poly(acrylamide) is known to have an open porous structure. It swells in water, retains a significant fraction of water in its structure and allows transport of incorporated molecules at a greater ease.
Poly(*N*-vinyl-2-pyrrolidone)-*g*-sodium alginate[142]	Poly(*N*-vinyl-2-pyrrolidone) has been employed as solubilizer of water-insoluble drugs in solid dispersion design. Its grafting into alginate chains may impart similar uses for sodium alginate.
Alginate-*g*-poly(ethylene glycol) [143]	Grafting of poly(ethylene glycol) onto alginate does not negate the gelation process of alginate and increases the pore dimension of the formed gel. It is envisaged to promote drug release.
Lectin-alginate conjugate[144,145]	Lectin is a protein or glycoprotein of non-immunological origin which specifically recognizes sugar molecules and capable of binding to glycosylated membrane components at mammalian mucosa. Examples of non-toxic lectins of plant origin are tomato lectin and wheat germ agglutinin.
	Wheat germ agglutinin binds to *N*-acetylglucosamine and sialic acid residues of mucin exhibiting a molecular weight of 36 kDa. Conjugation of alginate microparticles to wheat germ agglutinin enhances mucoadhesion. Mucoadhesion prolongs residence time of drug at the site of absorption and reduces dosing frequency. In-vivo tests using diabetic rats indicate that the lowest blood glucose level can be attained when the rats are administered orally with insulin-loaded alginate–wheat germ agglutinin microparticles.
Sulfated/thiolated alginate[146–148]	Sulfation of alginate may introduce a polymer with binding affinity for growth factors, chemokines and cell adhesion molecules. The sulfated alginate can be used in the design of a target-specific drug delivery system.
	Conjugating of cysteine to alginate enhances its water uptake, swelling capacity and mucoadhesiveness. The latter expresses through interaction between cysteine-rich mucus and thiolated alginate via disulfide bonds. An increase in mucoadhesiveness translates to prolonged residence of thiolated alginate matrix and drug release. This reduces oral dosing frequency of a drug with a short elimination half-life.
	The transformation of thiolated alginate into the particulate system can be effected by means of crosslinking using zinc chloride or partial formation of disulfide bonds by aqueous alkaline solution. Using zinc chloride as crosslinking agent, the Zn^{2+} complexes with carboxylic acid and amide moieties across the polymeric backbone. The crosslinked matrix is practically insoluble in any solvent and resists drug release in pH 1 medium. Under an acidic ambience, an excessive molecular size of crosslinked polymer at the exterior surfaces of matrix, protonation of carboxylic acid and thiolate anion moiety, and hydrophobic effect of large sulfur atoms within the thiolated alginic acid, are responsible for low drug release. In pH 7, almost all of the drug is released immediately, probably as a result of Zn^{2+} sequestering by phosphate ions of buffer and to a lesser extent, ionization of carboxylic acid and thiol moieties, which induce polymer chain repulsion and matrix disintegration.

Table 2 (*Continued*)

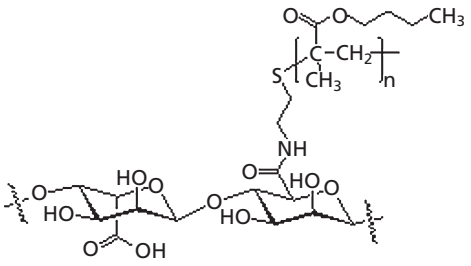
Graft copolymer	Physicochemical and drug release properties
Galactose substituted alginate[149,150]	Coupling of alginate with galactose provides the polymer with a cell-specific binding property. A receptor recognised by an asialoglycoprotein is localized on the hepatocyte cell membrane. The galactose is recognisable by the asiologlycoprotein receptor on hepatocyte surfaces. This phenomenon may be employed to promote galactosylated substance adhesion and targeting at specific organ. The galactosylated alginates with 7 and 19% substitution are able to form beads using Ca^{2+} as crosslinking agent. The galactose moiety linked by an amide bond to the polymer chains interferes with the packing arrangement of polymer chains in gelling process at long G block sequences. It impairs cooperative binding of Ca^{2+} and leads polymer to have a lower affinity for Ca^{2+}. The galactosylated alginate gel swells to a larger extent with a higher level of galactose moiety substitution. This is probably an attribute of reduced Ca^{2+}-alginate crosslinkage. The volume of an ionic gel is governed primarily by positive osmotic pressure, which is counterbalanced at equilibrium by a negative pressure due to network elasticity. The elasticity of a calcium alginate gel is largely driven by the number and strength of crosslinkages formed. Galactosylated alginate can be used to modulate drug release through varying its level of galactose substitution, crosslinking tendency and swelling characteristics.

chemical modification of alginate can introduce several attributes to the parent chains through appropriate selection of grafts. Specific targeting, hydrophilic, hydrophobic, drug encapsulation, mucoadhesive, swelling and bioactivity properties of alginate can be modified by grafting to tailor to the need for sustained drug release. On the basis that reduced segregation or demixing is possible, the grafted alginate is envisaged to be able to express its controlled drug release property in a more consistent manner than unmodified polymer (Figure 2). Covalent chemical modification of alginate represents an approach to reduce the complexity of dosage-form manufacture as shown in Table 1. One example is hydrophobization of alginate, which eliminates the need for the dosage form to undergo sustained-release coating or membrane formation.

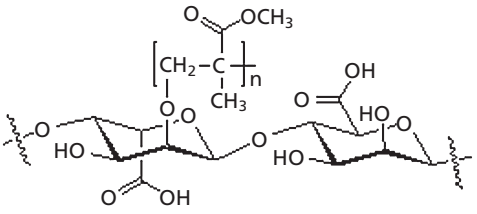
Table 2 summarizes the physicochemical and drug-release properties of various alginate graft copolymers. Alginate has been grafted with poly(*N*-isopropylacrylamide), polyacrylamide, poly(sodium acrylate), poly(sodium acrylate-*co*-acrylamide), poly(butyl methacrylate), poly(methacrylamide), poly(2-dimethylamino)ethyl methacrylate, α-methyl methacrylate, hydrocarbon chain, galactose, lectin, poly(*N*-vinyl-2-pyrrolidone), sulfate, cysteine, α-cyclodextrin, β-cyclodextrin, cholesteryl, poly(ethylene glycol), propylene glycol and dodecylamine. Examples of alginate graft copolymers are shown in Figure 3. Broadly, graft copolymerization of alginate with a hydrophobic or amphiphilic moiety can convert the parent polymer to sustain drug release, whereas attachment of alginate with poly(acrylamide), poly(*N*-vinyl-2-pyrrolidone) or poly(ethylene glycol) reduces its drug release retardation capacity either in a pH-dependent or non-pH-dependent manner. The drug release control property of alginate is modified by grafting through:

1. Increasing the polymer hydrophobicity, which in turn reduces the wetting propensity of dosage form by dissolution medium and drug release (e.g. hydrophobically modified alginate (Figure 3)). Low drug release can also be attributed to hydrophobic interaction between graft copolymer and drug such as protein albumin.
2. Introducing a graft such as poly(acrylamide) of which pH-sensitive drug release characteristics is attained through converting $CONH_2$ of poly(acrylamide) to COOH with a rise in the surrounding pH (e.g. poly(acrylamide)-*g*-alginate (Figure 3)). Ionization of COOH moiety at a high pH induces matrix swelling and drug release.
3. Introducing a cationic graft such as poly(2-dimethylamino)ethyl methacrylate of which the positively charged grafted chains can interact with negatively charged alginate chains or drug molecules to prevent matrix dissolution, drug diffusion and drug release (e.g. oxidised alginate-*g*-poly(2-dimethylamino)ethyl methacrylate (Figure 3)).
4. Coupling alginate with cell-specific ligand such as lectin (Figure 3), which translates drug release at the intended drug absorption site, promotes drug targeting and avoids unnecessary adverse effects incurring on unintended site of drug absorption if any.
5. Increasing the cohesiveness and mucoadhesiveness of alginate via thiolation, which translate to the formation of matrix with a high mechanical strength to survive gastrointestinal transit and a high interaction intensity between mucus and matrix to prolong drug release (Figure 3).
6. Creating cavities in alginate chains for drug encapsulation, via attaching alginate with cyclodextrin carrying a hydrophobic cavity, which allows specific drug binding, solubilization, stabilization and transport (e.g. α-cyclodextrin- and β-cyclodextrin-alginate conjugates (Figure 3)).
7. Transforming the hydrophilic alginate into an amphiphilic polymer thereby promoting aggregation of polymer chains into micelles to encapsulate hydrophilic and hydrophobic drugs (e.g. cholesteryl-*g*-alginate (Figure 3)).

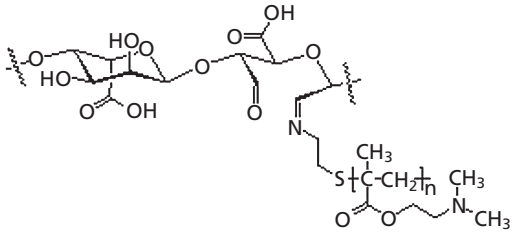
Drug can also be coupled to alginate chains directly or via a linker. The drug release can be delayed by using linker cleaved by specific enzymes in the target site or through protecting the linker against enzymatic attack by means of the steric hindrance effect of alginate. 5-Aminosalicylic acid has been coupled to 6-aminohexanamide-L-phenylalanine and subsequently bound to alginate (Figure 4).[151] The drug is gradually released from the conjugate in the presence of α-chymotrypsin. Chymotrypsin or chymotrypsin-like activity is present in small intestine and colon. The conjugate is expected to undergo hydrolysis and release drug throughout the length of intestine, bypassing the gastric region.



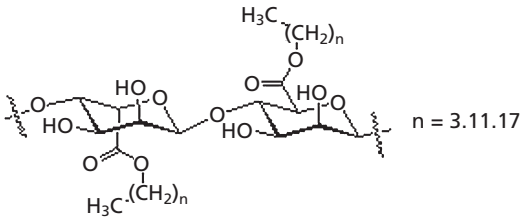
Alginate-*g*-poly(butyl methacrylate)



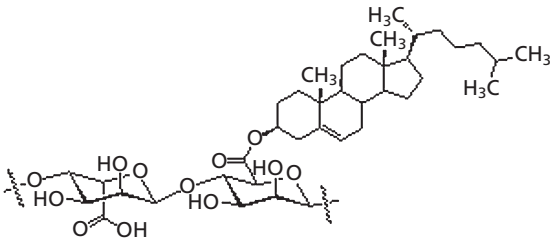
α-methyl methacrylate-*g*-alginate



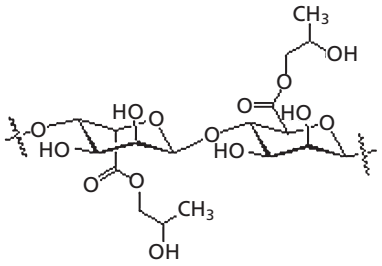
Oxidised alginate-*g*-poly(2-dimethylamino)ethyl methacrylate



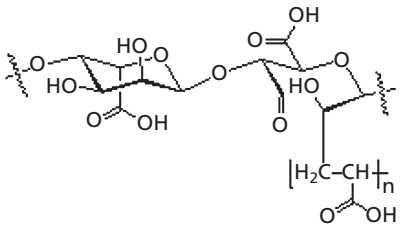
Hydrophobically modified alginate



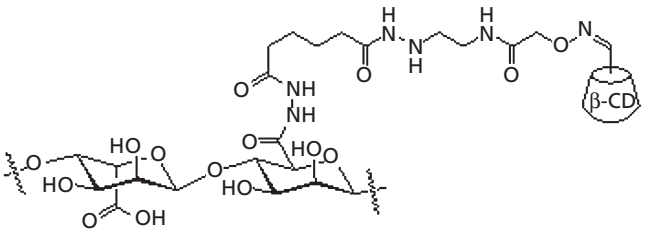
Cholesteryl-*g*-alginate



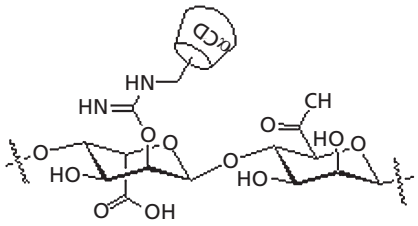
Propylene glycol alginate



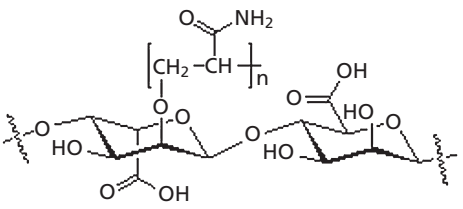
Alginate-*g*-poly(sodium acrylate)



β-cyclodextrin-alginate conjugate

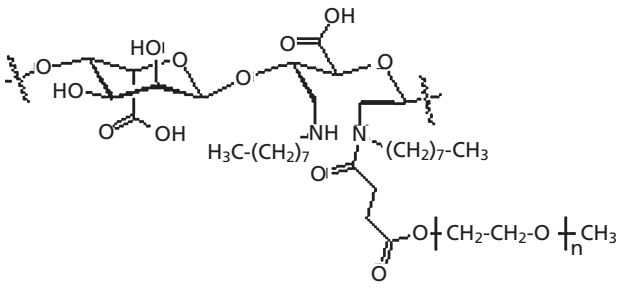


α-cyclodextrin-alginate conjugate

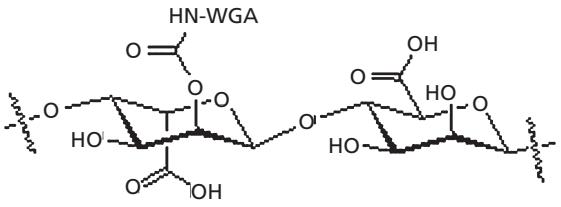


Poly(acrylamide)-*g*-alginate

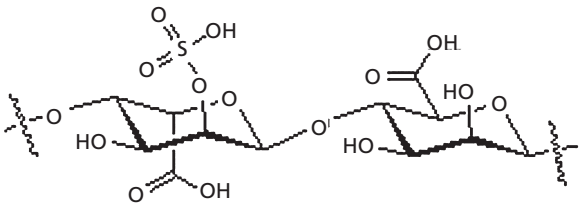
Figure 3 Chemical structures of alginate graft copolymers.



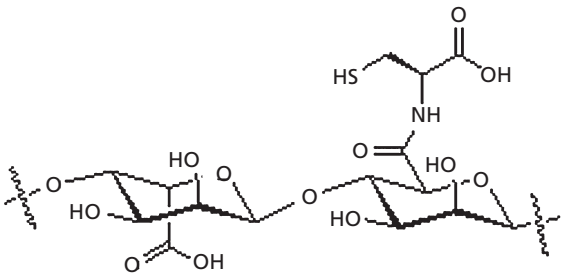
Alginate-*g*-poly(ethylene glycol)



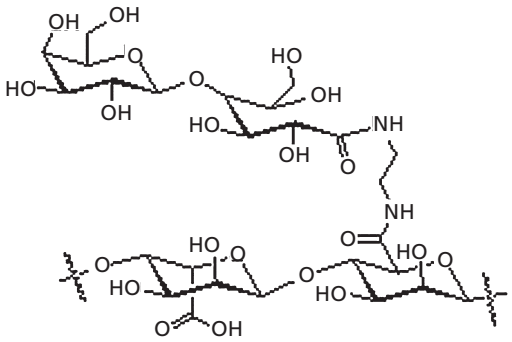
Lectin-alginate conjugate



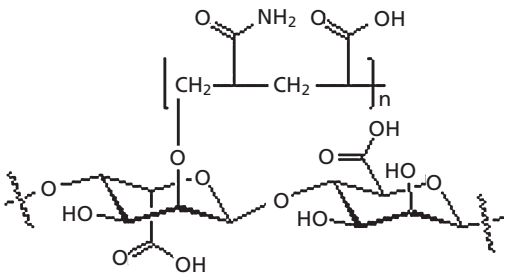
Sulfated alginate



Thiolated alginate



Galactose substituted alginate



Alginate-*g*-poly(methacrylamide)

Figure 3 (*Continued*)

A similar concept of alginate–drug conjugate is adopted in the delivery of daunomycin, an anti-tumour agent (Figure 4).[28] The daunomycin is covalently attached to alginate via acid-sensitive *cis*-aconityl. The drug is released in the acidic milieu of the endosomal and lysosomal compartments of tumour cells or the slightly acidic extracellular fluid of some solid tumours. Complexation of drug with alginate reduces its cytotoxicity, but it does not improve anti-tumour activity as a result of changes in daunomycin release or pharmacokinetic profile.

Alginate Graft Copolymer Versus Alginate–Co-Excipient Physical Mixture

Alginate is classified as Generally Regarded as Safe by the FDA.[1] Though biocompatible, alginate has demonstrated varying biological actions as a function of its physicochemical make-up and some can be unorthodox (e.g. anti-coagulation effects of sulfated alginate).[2,4] Graft copolymerization of alginate produces new chemical entities. The use of alginate graft copolymers in the formulation of oral drug delivery systems has raised several concerns, including the issues of pharmacological activity:

1. Complicated processes – preparation of alginate graft copolymers often involves a multi-step approach.[9,143] At each step of synthesis, the type and amount of additive used, process temperature, duration and sequence require optimization to produce derivatives with the intended graft characteristics at a high yield level.
2. Process instability – the graft copolymerization process may be ended with major alterations of alginate chemical structure and conformation thereby negating its controlled

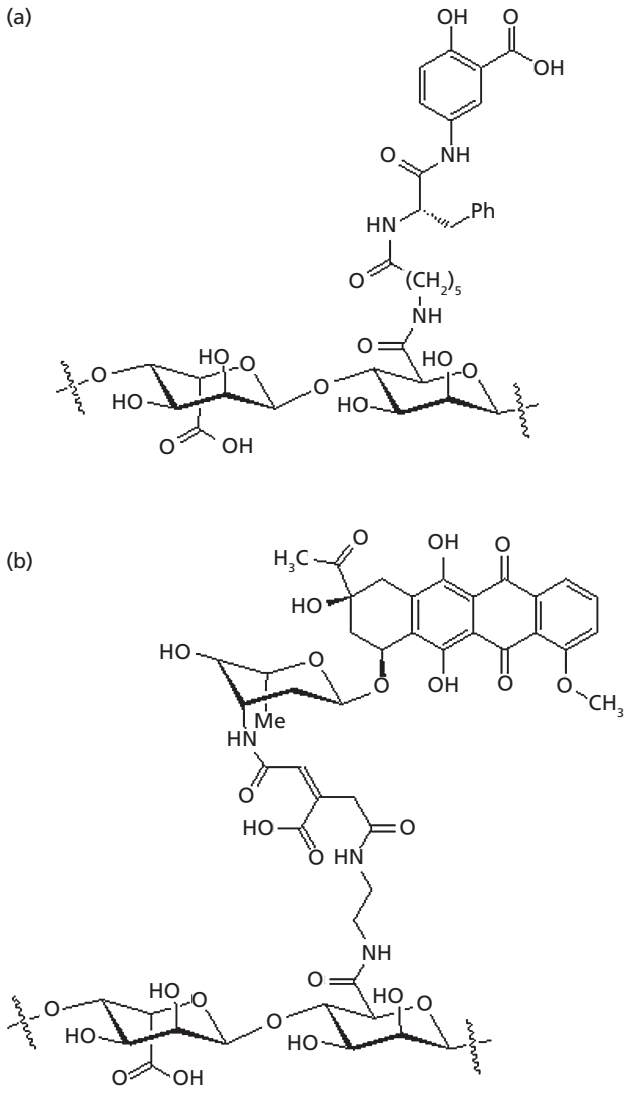


Figure 4 Chemical structures of (a) 5-aminosalicylic acid-alginate and (b) daunomycin-alginate conjugates.

drug release property. One example is grafting of vinyl monomers onto alginate using catalysts, such as ceric (IV) ions, persulfate and hydrogen peroxide, causes alginate ring opening.[15]

3. Product instability – alginate graft copolymer, such as thiolated copolymer, turns brownish rapidly upon exposing to air.[148] This may be due to aerial oxidation. Prevention of product degradation needs additional control measures using airtight container, low storage temperature and/or ascorbic acid reducing agent.
4. Hazardous process – methanol, pyridine, acetone, *p*-toluenesulfonyl chloride, cyanogen bromide, carbodiimide, sodium periodate, chloroform, azobisisobutyronitrile, hexane and sodium cyanoborohydride are some chemicals employed in the graft copolymerization processes as free radical initiator, solvent, linker or washing agent.[9,14,135,136,138,139,142,145–147,149,150] The residual content of these substances poses an additional threat in medication safety. The patient safety issue is aggravated by drugs consumed on a long-term basis.
5. Toxicity of alginate graft copolymers – new chemical entities are introduced with unknown toxicological profiles.[100]
6. High process and product cost – additional derivatization process, critical storage condition and need for safety and toxicity studies incur extra costs. This directly raises the cost of medication incorporating the alginate graft copolymers.

It is apparent that the use of alginate graft copolymers in oral drug formulation is accompanied by a number of drawbacks. When considering its potentials or risks in the commercial sector, it is apparent that very few studies have been conducted to enable direct comparison between the drug delivery performance of oral formulations using alginate graft copolymers with that of formulations that incorporate graft-equivalent co-excipient physically in a dosage form, except partially inferring from some cases of cyclodextrin whereby sustained drug release attributes can be attained by the use of both alginate-cyclodextrin copolymer and alginate-cyclodextrin physical mixture (Tables 1 and 2). In addition, there is no known comparison on drug delivery performance of oral formulation made of alginate graft copolymer with non-grafted alginate dosage form that carries co-excipient at the corresponding graft weight of the former (Tables 1 and 2). Despite these uncertainties, one can, however, regard graft copolymerization of alginate as adding unique features to the processes of drug formulation and delivery that are not attainable by mere physical mixing of alginate and co-excipient into a dosage form. Graft copolymerizing alginate with poly(acrylamide) and lectin augments the pH responsiveness and targeting specificity of alginate in drug release, respectively (Tables 1 and 2). Copolymerization of alginate with the cholesterol moiety and positively charged graft allows the alginate chains to self-assemble into drug carrier without being subject to multi-step processes such as the alternate core adsorption of alginate and co-excipient, or alginate pre-crosslinking followed by coacervation and complexation using co-excipients (Tables 1 and 2).

Synthesis of graft copolymers has been carried out by conventional redox grafting, microwave irradiation, gamma-ray irradiation or using an electron beam method.[31] Of these methods, microwave irradiation demonstrates the best potential for use and fits the concept of green chemistry. In graft copolymerization, free radicals can be generated by microwave without using additional chemicals such as free radical initiators. The interaction between microwave and reagents in a synthesis process is unimpeded by steric hindrance due to the large wavelengths of radiation. Nonetheless, the use of microwave in graft copolymerization of alginate is much less widespread than the redox approach.

Future Perspectives

The drug release profile of oral alginate formulations can be modified using co-excipients. Alternatively, the alginate itself can be converted into a graft copolymer to meet the intended drug release characteristics. In the latter case, the exact usefulness of alginate graft copolymers as an alternative to physical mixture of alginate and co-excipients in drug release

modulation must be first assessed against the physically mixed formulation, before safety and toxicity evaluation, which can be costly and time consuming. In addition, the shortcomings in association with the grafting processes of alginate shall be overcome by designing an appropriate derivatization protocol with the aim of reducing product toxicity, cost and instability as well as process expenditure, complexity and handling risks.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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