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Synthesis of iron-crosslinked hydroxamated alginic acid and its *in vitro* evaluation as a potential matrix material for oral sustained-release beads

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Hydroxamated alginic acid (HAA) was prepared. The generated semisynthetic polymer was employed in the formation of drug-loaded, iron(III)-crosslinked polymeric beads. Infrared spectroscopy was employed to prove the crosslinking. The degree of hydroxamate substitution was determined by acid-base back titration, and it was found to be ca. 20%. The produced beads were evaluated *in vitro* as orally administered sustained release drug delivery system. Theophylline, paracetamol, and chlorpheniramine maleate were used as model drugs. The generated beads proved to be successful in prolonging drug release, particularly in the case of theophylline. Iron leaching from the generated beads was minimal (<0.5% of the entire complexed iron), and took place during passage through the simulated gastric fluid.

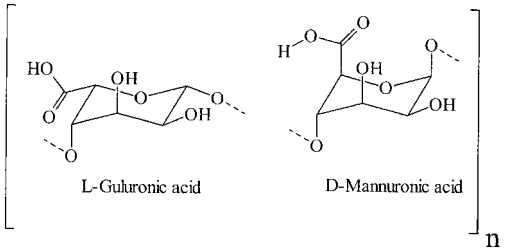
1. Introduction

Sodium alginate (algin) is a purified carbohydrate product isolated from brown seaweads. Algin consists chiefly of the sodium salt of alginic acid, which is a linear copolymer of 1,4-linked mannopyranosyluronic acid and 1,4-linked gulopyranosyluronic acid units. However, D-mannuronic is the major component in the molecule [1, 2].

Sodium alginate has been used as a thickening emulsifying agent [3], tablet binder and disintegrant [4], and recently it was studied together with lactose monohydrate, in the preparation of composite particles suitable as filler for direct tabletting of controlled-release matrix tablets [5]. Algin has also shown useful gel-forming properties when mixed with different polyvalent cations [6, 7]. Calcium alginate has found applications in a number of gelation purposes including the formation of firm gel for the preparation dental impressions [8], and in the preparation of matrices for drug delivery [9]. Alginate is currently being investigated as a carrier material for various controlled release systems [10, 11]. It was recently employed in the preparation of controlled release microspheres or minimatrices for a variety of medicinal agents including metoclopramide and cisapride [12], diclofenac [13], indomethacin [14], propranolol [15], and gentamicin [16]. Furthermore, alginic acid was recently used to encapsulate chitosan bioadhesive microspheres, and vice versa, for intestinal drug delivery [17, 18].

The ratio of mannuronic acid to guluronic acid strongly influences the drug releasing properties of calcium alginate beads [19, 20].

However, calcium alginate matrices were reported to suffer from the following problems. Their dissolution in phosphate buffered saline solution (pH 7.4) occurs completely in a short time period after a certain lag time [7, 21, 22]. Additionally, the matrices were able to extend the release of theophylline and chloramphenicol only when pure water was applied as release medium. While in 0.1 M HCl, simulated gastric fluid (SGF), simulated intestinal fluid (SIF), and 0.1 M NaCl the drug release proceeded much more rapidly. The crosslinking calcium ions were rapidly discharged from the matrices in the presence of acid to yield the protonated alginic acid. This transformation reduced the degree of crosslinking within the matrix, and thus destroyed its ability to provide retarded drug release. In NaCl solutions and SIF, calcium ions were partly exchanged by non-gelling sodium ions or sequestered by the phosphate. This caused swelling and, in the latter case, dissolution of the matrices, thus inducing rapid release of the encapsulated drug. Accordingly, it was concluded that calcium alginate minimatrices do not seem applicable as an oral controlled release system, due to the pronounced sensitivity towards the composition of the release medium and the rapid drug release in media of physiological relevance [22].



These problems prompted our search for a suitable synthetic modification of alginic acid to enhance the stability of the polymer/divalent cation complex, particularly under physiological conditions. Our interest was directed towards iron(III)-hydroxamate complexes.

Hydroxamic acids are known to form particularly stable and relatively pH-independent complexes with ferric ions (i.e., within physiological limits) [23, 24]. Such complexes were used for analytical [25], as well as clinical purposes; for example desferrioxamine B, which is used to treat intoxication resulting from excessive blood iron levels [26].

Consequently, we envisaged the possibility of substituting a fraction of algin's carboxylic acid moieties with hydroxylamine groups, thus replacing the acidic groups with the relatively neutral, iron-complexing hydroxamic acid moieties.

In the present investigation hydroxamated alginic acid (HAA) was successfully prepared as shown in Scheme 1. Moreover, ferric ions were used to crosslink the generated HAA, leading to polymeric matrices that exhibited relatively pH-independent drug releasing profiles.

The ferric ion has been reported only rarely as an ionotropic crosslinking agent in the formation of drug releasing polymeric matrices. Nevertheless, the investigated polymers were carboxymethylchitin and carboxymethylcellulose in those instances [27, 28].

Despite the fact that iron complexing poly(hydroxamic acids) have been reported [23, 24, 29–31], the use of iron-crosslinked hydroxamated polymers in the area of controlled drug delivery is completely new.

The ferric (Fe^{+3}) form of iron is poorly absorbed from the gastrointestinal tract as only 7% of the orally administered iron dose is absorbed. Furthermore, excess iron absorption

is inhibited by a defensive mechanism known as the *mucosal block* [32]. The reported seriously toxic iron dose ranges between 1.0–2.0 g [33]. The total amount of ferric ions released from our newly developed HAA-iron matrix (i.e. under SGF and SIG environments) did not exceed 0.5% of the total complexed iron amount, which indicates the wide margin of safety of the newly developed matrix material.

The generated polymeric beads resisted shape deformation during testing in SGF and SIF, suggesting chemical and physical stability under physiological conditions.

2. Investigations, results and discussion

2.1. HAA synthesis and characterization

The conjugation of alginic acid to hydroxylamine, shown in Scheme 1, was achieved by first activating the carboxylic acid moieties with dicyclohexylcarbodiimide (DCCI), followed by quenching the reaction mixture with hydroxylamine hydrochloride. The activation was carried out under acidic aqueous conditions (pH 4.7) to improve the reactivity of DCCI via imino-nitrogen protonation. The added DCCI was theoretically calculated to activate only 60% of the available carboxylic acid groups. Attempts to increase the activation to more than 60% generated hydroxamated alginic acids with poor solubility in sodium hydroxide media, and thus of limited drug-loading capacity.

The equatorial carboxylic acid groups, within the guluronic acid units, are expected to possess superior reactivity towards DCCI, compared to their axial counterparts in the mannuronic acid moieties. The reason is probably related to the adjacency of the axial carboxylic acid to the hydroxyl group at C3, which is expected to impede the reaction by forming hydrogen bonds with the carboxylic hydroxyl, and by sterically hindering the approach of DCCI molecules. Consequently, hydroxylamine substitution is expected to take place mainly at the guluronic acid units, as shown in Scheme 1.

Hydroxylamine hydrochloride was added in 20-fold excess (compared to DCCI) to ensure the forwardness of the reaction, particularly under the sterically hindering environment of the sugar polymer. Nevertheless, the degree of hydroxylamine substitution, as calculated by back-titration, was found to be *ca.* 20% of the total carboxylic acid moieties in algin, which can be attributed to the polymer-related steric hindrance. The reaction pH was raised to pH 9.0 to ensure the complete liberation of the nucleophilic hydroxylamine from its hydrochloride salt.

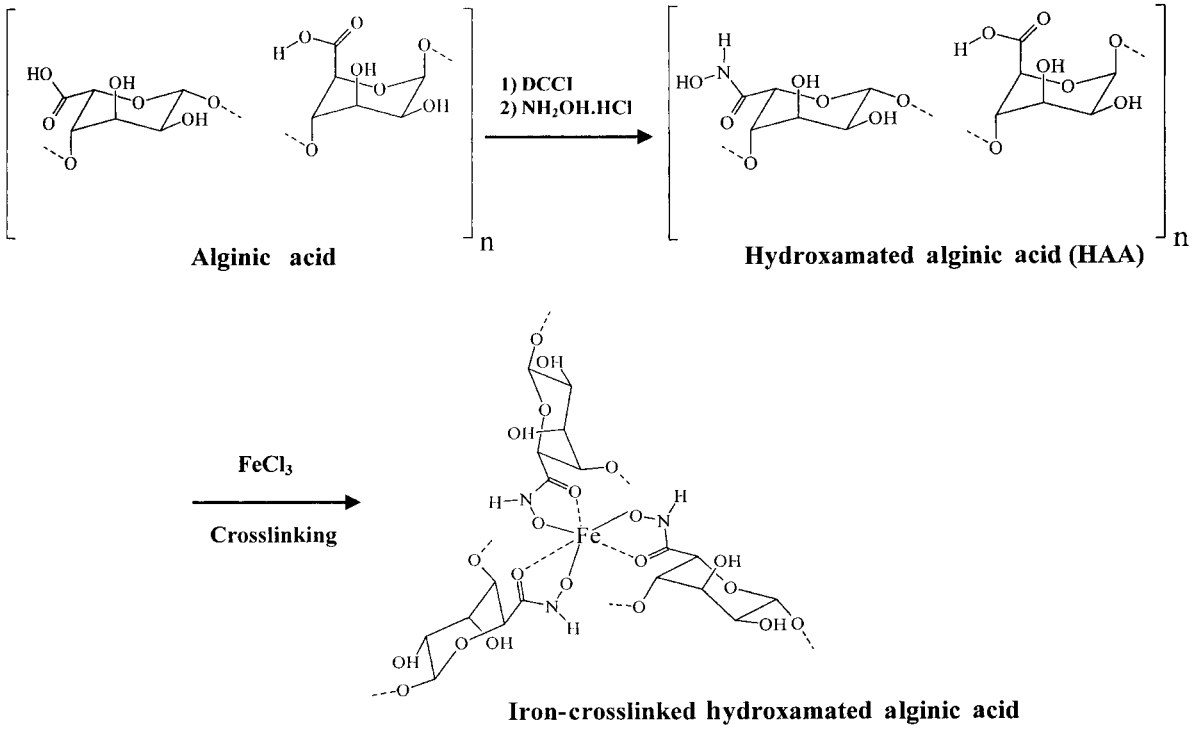
The generated semisynthetic polymer was characterized by IR spectrophotometry and back-titration to determine the degree of hydroxylamine substitution. The IR spectra of HAA, (Fig. 1a), shows the presence of a carbonyl band at 1634 cm^{-1} corresponding to the newly formed amide group. However, a substantial carboxylic carbonyl band, at 1744 cm^{-1} is still present, indicating the presence of a considerable, non-substituted carboxylic acid fraction.

The degree of substitution was determined by acid-base back titration. The analysis is based on the fact that HAA possesses less carboxylic acid groups than the untreated alginic acid. Consequently, the difference in their consumption of sodium hydroxide is unequivocally due to hydroxylamine substitution. Accordingly, the calculated substitution was found to be ca. 20% (number of moles of hydroxamic acid groups within a particular weight of HAA per the total number of moles of carboxylic acid moieties within the same weight of alginic acid).

2.2. Preparation and characterization of Fe^{+3} crosslinked HAA beads

Iron (III)-HAA polymer beads were prepared by dropping a dispersion of the particular drug in HAA/sodium hydroxide solution into ferric chloride solution. The particular

Scheme 1



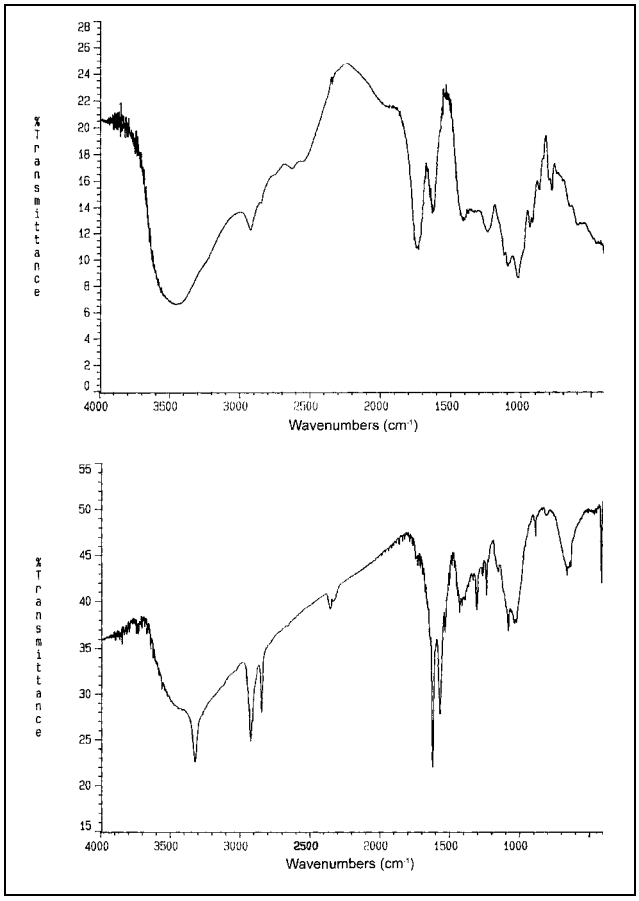


Fig. 1: IR spectra of (a) hydroxamated alginic acid (HAA), (b) iron crosslinked HAA

Table: Amount of theophylline, chlorpheniramine and paracetamol incorporated in 1.0 g iron(III) crosslinked HAA beads

Drug	Theophylline	Chlorpheniramine maleate*	Paracetamol
Total amount loaded	231.5 mg	135.5 mg	223.0 mg

* Crosslinking in etheric ferric chloride solution

drugs and their loaded quantities per 1.0 g polymer are listed in the Table. The amounts of loaded drugs were determined by exhaustive extraction of the finely crushed beads using 0.1 N hydrochloric acid solution (see Experimental, section 3.6).

In the two cases of theophylline and paracetamol, $FeCl_3$ aqueous solutions were employed in the crosslinking procedure. However, ethereal $FeCl_3$ solution was used for the preparation of chlorpheniramine maleate beads. The reason was that when aqueous iron chloride was employed, chlorpheniramine maleate fully migrated from the polymeric spheres (i.e. during curing time). The excessive aqueous solubility of chlorpheniramine maleate is probably the reason for this behavior. The polymeric beads were dried at room temperature over 24–48 h. They underwent a 10-fold reduction in size upon drying.

The generated beads were dark brown in color, spherical in shape, with an average bead-diameter of ca. 1 mm. Further particle size analysis information is to be published in the future.

The complexation of hydroxamic acid moieties with iron(III) is clearly illustrated by IR spectroscopy (Fig. 1a, b). The IR spectrum of iron-crosslinked HAA polymeric matrix shows two principal sharp peaks at 1625 and 1574 cm^{-1}, in contrast, the IR spectrum of the non-crosslinked HAA shows a carboxyl carbonyl band at 1744 cm^{-1} and an amide carbonyl band at 1634 cm^{-1}. Accordingly, one can clearly see that both the carboxyl and amide carbonyls were shifted to lower frequencies upon complexation to iron, i.e. from 1634 to 1574 cm^{-1} for the amide carbonyls and from 1744 to 1625 cm^{-1} for the carboxylic carbonyls. The proposed hydroxamate complexation is shown in Scheme **1**. Carbonyl groups are known to undergo 30 to 60 cm^{-1} lower frequency shifts upon acting as hydrogen acceptors in hydrogen bonding [34]. Similar shifts should be expected upon acting as iron (III) acceptors.

Despite the fact that the IR spectra indicate the involvement of carboxylic acid groups in the complexation with iron (III), it was realized, from experiments carried out on unmodified alginic acid, that hydroxamic acid moieties are essential for the formation of stable hard beads. The generation of useful hard beads was not possible using unmodified alginic acid, both in aqueous or ethereal ferric chloride solutions.

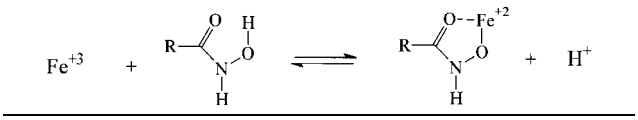
For beads cured in aqueous iron chloride solution, the total amount of iron(III) required to generate 1.0 g of iron-crosslinked dry beads was calculated to be 363.2 mg.

2.3. Dissolution profile of iron(III) from iron(III) crosslinked HAA beads

The release of ferric ion from drug-free beads was studied in SGF (pH 1) followed by SIF (pH 7). The dissolution profile is illustrated in Fig. 2. It is evident that iron (III) release occurred only under acidic conditions. However, the total amount of iron released did not exceed 0.5% of the total chelated iron. The dissolution profile may be explained by the following equilibrium (Scheme 2) [25].

The above equilibrium seems to be shifted to the right, as iron(III)-hydroxamate chelates produce analytically optimal colors at pH 1.4 [25]. Still, under the relatively extreme pH of the SGF (pH 1.0), the equilibrium is expected to slightly shift to the left, thus releasing trace quantities of iron(III) into the dissolution medium. On the other hand, the basic conditions of the SIF move the equilibrium to the right, and consequently rendering the ferric

Scheme 2



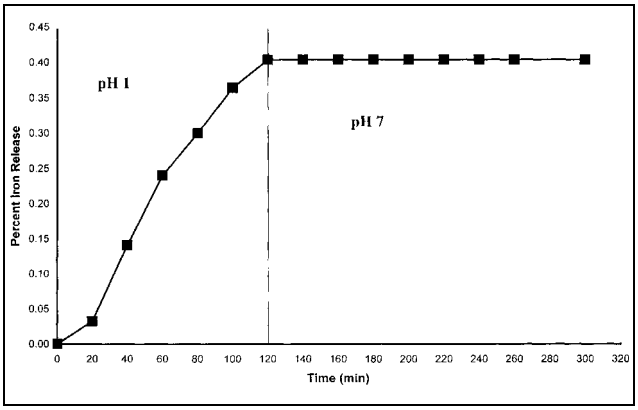


Fig. 2: Iron release profile from iron crosslinked HAA beads

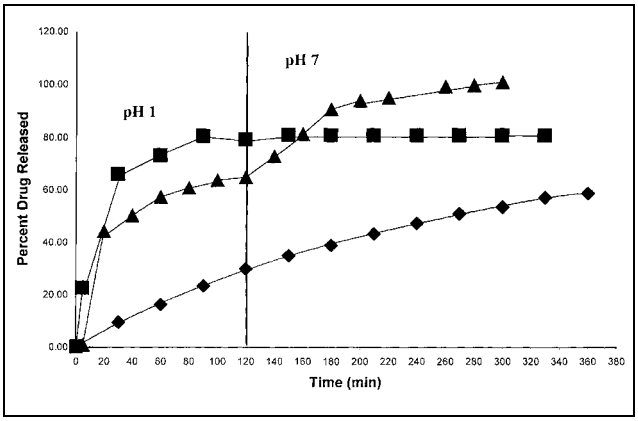


Fig. 3: Release profile of theophylline ◆, chlorpheniramine maleate ■, and paracetamol ▲, from iron crosslinked HAA beads

ion tightly complexed, with the concomitant cessation of iron leaching.

2.4. Drug dissolution profiles from iron(III) crosslinked HAA beads

Figure 3 illustrates the dissolution profiles of theophylline, paracetamol and chlorpheniramine maleate from iron-crosslinked HAA beads. The release profile of theophylline approximates zero order kinetics, i.e. independent of the beads' drug content, that can be compared with the quick theophylline release from calcium alginate matrices reported earlier [22]. Furthermore, the release rate was clearly independent of the pH of the surrounding media. This behavior can be attributed to a hydration-dissolution mechanism: Initially, the outermost layer of the polymeric matrix is hydrated with the concomitant dissolution and diffusion of the dispersed theophylline particles. Afterwards, the gradual hydration of subsequent polymeric layers allows continuous drug release. Around 85% of the total loaded theophylline was released after 24 h.

On the other hand, chlorpheniramine maleate was relatively quickly liberated from the polymeric beads, particularly under the SGF environment. Approximately, 80% of the drug loaded was released after 2 h as shown in Fig. 3. The most probable explanation for this quick release is based on the excellent water solubility of chlorpheniramine, particularly under acidic conditions. Initially, the outermost layer of the polymeric matrix is hydrated, and subsequently, the dispersed fraction of the drug within this particular layer is dissolved. However, the high-water solubility of chlorpheniramine generates a considerable osmotic pressure within the hydrated matrix layer, thus attracting more water from the dissolution medium leading to quicker hydration of the subsequent matrix layers with concomitant drug release.

Paracetamol exhibited a release profile between that of chlorpheniramine and theophylline. The overall shape of paracetamol's release profile is probably related to two factors: (i) the intermediate water solubility of paracetamol compared to theophylline and chlorpheniramine, and (ii) the Fe^{+3}-complexing capacity of paracetamol. It is a well-known fact that phenols form complexes with ferric ion [35]. Phenolic-iron complexes are of reduced stability under acidic conditions which undoubtedly explains the slower release rate of paracetamol in SIF compared to that in SGF.

In conclusion, hydroxamated alginic acid (HAA) was successfully prepared. Furthermore, drug-loaded, iron-crosslinked HAA beads were produced. The generated beads resisted deformation under acidic (SGF) and basic (SIF) environments. Moreover, the new matrix material proved to be superior over calcium-crosslinked alginate in the formation of drug-prolonging beads. Iron-crosslinked HAA beads resisted iron(III) leaching in contrast to calcium alginate beads, which were reported to loose their calcium content when exposed to simulated physiological fluids. These results suggest the potential usefulness of iron-crosslinked HAA in orally administered sustained release dosage forms.

3. Experimental

3.1. Chemicals

Reagent grade chemicals were purchased from the following companies: sodium alginate (Hipure, Genzyme-England), dicyclohexylcarbodiimide (DCCI, Fluka-Switzerland), hydroxylamine hydrochloride (M & B Laboratory chemicals-England), ferric chloride anhydrous LR (S.D. Fine-Chem. Ltd. Boisar-India). Chlopheniramine maleate, paracetamol, and theophylline were all received as a kind gift from Hikma Pharmaceuticals (Amman-Jordan). All chemicals were used as obtained from the manufacturers without further purification.

3.2. Synthesis of hydroxamated alginic acid (HAA)

Sodium alginate (5.20 g, equivalent to 0.025 mol carboxylate) was dissolved in H_2O (250 ml), then HCl solution (1.0 N) was added dropwise to the stirred polymer solution until pH 4.0–4.5. Subsequently, DCCI (3.1 g, 0.015 mol) was added to the stirred mixture. After 2 h, hydroxylamine hydrochloride (20.7 g, 0.3 mol) was added and the reaction was further stirred for 1 h. Afterwards, the pH of the reaction was raised to 6.0 using NaOH solution (1.0 N), and the reaction was stirred for 2 h. Thereafter, the pH was raised again to 9.0, and the reaction mixture was stirred for 24 h at room temperature. The reaction was terminated by precipitation with concentrated HCl (10 ml) and acetone (250 ml). The precipitated polymer was filtered, washed thoroughly with ethanol (250 ml) (to remove the resulting DCC urea) followed by acetone (200 ml) and diethyl ether (200 ml), respectively. The polymer was left to dry at room temperature overnight.

3.3. Preparation of the loaded polymeric beads and crosslinking with Fe^{+3}

The prepared HAA (0.5 g) was dissolved in 0.1 N NaOH solution (12 ml). The particular drug (0.5 g) was added to the viscous polymeric solution. Chlorpheniramine maleate was left stirring in the polymeric solution for 2 h. The stirring time with the other drugs was 10 min only. The resulting solution (or suspension) was slowly dropped into a solution of $FeCl_3$ (0.5 g) in H_2O or ether (100 ml). Aqueous $FeCl_3$ solution was used in the formation of theophylline or paracetamol beads, while ethereal $FeCl_3$ solution was used for chlorpheniramine maleate. The beads were left in the $FeCl_3$ solution for 40–60 min, then they were collected and washed twice with H_2O or ether (20 ml). The beads were left to dry at room temperature for 24–48 h.

3.4. Infrared characterization of HAA and iron crosslinked HAA

HAA and iron(III)-crosslinked HAA matrices were characterized using IR spectrophotometry. IR spectra were recorded on a Hitachi 270–50 IR spectrophotometer using KBr disks.

Iron(III)-crosslinked HAA matrix was prepared by adding $FeCl_3$ aqueous solution (0.5 g in 10 ml) to a vigorously stirred HAA (0.5 g) in NaOH solution (0.1 N, 30 ml). The solid brown mass was filtered, washed with H_2O until a colorless filtrate was attained, then washed with acetone and left to dry at room temperature for one week. The dried brown matrix was crushed and a KBr disk was prepared from the resulting fine powder.

3.5. Determination of the degree of substitution

The particular polymer (alginic acid or HAA) (5.0 g) was dispersed in HCl (0.1 N, 500 ml) and the mixture was stirred at room temperature for 1 h. Consequently, the polymeric dispersion was filtered, washed with acetone several times (3×100 ml) and left to dry at room temperature. The dry polymer (2.5 g) was added to a magnetically stirred aqueous NaOH solution (0.5 N, 100 ml) and left for 20 min. The resulting solution was back titrated with HCl (1.0 N) using phenolphthalein as indicator. Unsubstituted alginic acid was used as a blank.

3.6. Determination of drugs' dissolution profiles

A rotating basket apparatus (Erweka DTD) fitted with a 0.125 mm-stainless steel basket was used. In each case, dried beads (0.3 g) were placed in

the basket. Two buffered dissolution media were used over two subsequent stages: pH 1 for 2 h then pH 7.0 for 3–4 h. The dissolution medium (900 ml) was maintained at 37 °C. The basket was rotated at 100 rounds/min and samples (2 ml) were withdrawn appropriately at predetermined time intervals (every 20 or 30 min) for the analysis of the released drug. The withdrawn volume was immediately replaced with an equivalent volume of the fresh medium maintained at the same temperature. The absorbencies of the undiluted samples were reported at 270 nm for theophylline, 264 nm for chlorpheniramine maleate, and 242 nm for paracetamol using a Cary 1E UV-visible spectrophotometer. Unloaded beads were used as blanks. The concentration of each drug was calculated from an appropriately drawn calibration plot (for each pH value). The concentrations of the released drug were converted into percent-released drug by dividing the released quantities over the total loaded drug (Table).

The total loaded drugs per g polymer were calculated as follows. Drug-containing beads were crushed, and a predetermined weight of the finely crushed beads (0.1–0.2 g) was dispersed in HCl (0.1 N, 200 ml) and stirred for 2 h at room temperature. Subsequently, the polymeric dispersions were filtered, 5 ml of the filtrate was diluted to 25 ml with 0.1 N HCl and the absorbency of the resulting solution was recorded at the corresponding wave lengths. The concentration of each drug was calculated from an appropriately drawn calibration plot. Unloaded iron-crosslinked HAA polymer beads were used as blanks. The total quantities of drugs loaded per g polymer are listed in the Table.

3.7. Determination of the iron(III) dissolution profile

The dissolution apparatus and conditions of the experiment were described as in section 3.6. The following procedures were carried out to determine the released iron(III) in the subsequent buffered media.

Iron determination in samples collected from the SGF (pH 1.0). Samples (2.0 ml) were collected every 20 min. Potassium thiocyanate solution (20% w/v, 5 ml) was added to each sample, followed by dilution to 25 ml with distilled water. The absorbencies were collected at 480 nm.

Iron determination in samples collected from the SIF (pH 7.0): Samples (2.0 ml) were collected every 20 min. KSCN solution (20% w/v, 5 ml) was added to each sample. Subsequently each sample was diluted to 25 ml with HCl (0.1 N). The absorbencies were determined at 480 nm.

Samples collected at zero release time (for each SIG and SGF) were used as blanks. The concentrations of ferric ions were calculated from appropriately drawn calibration plots. Two calibration plots were drawn to cover samples collected from SGF and SIF.

The total complexed iron(III) per g polymer was determined as follows: Unloaded polymeric iron-crosslinked beads were crushed and a predetermined weight (0.1–0.2 g) of the finely crushed beads was dispersed in HCl (20% v/v, 200 ml) and stirred for 2 h at room temperature. Subsequently, the polymeric dispersion was filtered, and 5 ml of the filtrate was treated with KSCN (20% w/v, 5 ml) and the resulting solution was diluted to 25 ml by distilled H_2O. The absorbency of the final solution was determined at 480 nm. Iron-free HAA was employed as a blank.

The preparation of the calibration curve used to measure complexed iron(III) was carried out as follows. Aqueous $FeCl_3$ solution (30 mg in 5 ml) was added to a magnetically stirred solution of HAA (0.5 g) in NaOH solution (0.1 N, 12 ml). The resulting mass was filtered, washed with acetone and left to dry at room temperature for 2 d. Afterwards, the resulting solid matrix was finely crushed, and a predetermined weight of the powder (0.2 g) was suspended for 2 h in a vigorously stirred HCl solution (20% w/v, 200 ml). The resulting dispersion was then filtered, and several sample volumes (2, 4, 6, 8 ml) were collected. KSCN solution (20% w/v, 5 ml) was added to each sample, and the volume was completed to 50 ml with distilled H_2O. The absorbencies at 480 nm were determined to draw the calibration curve.

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