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Synthesis and evaluation of ampicillin-conjugated gum arabic microspheres for sustained release

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Abstract

Ampicillin was conjugated to periodate-oxidized gum arabic (GA), a branched polysaccharide, to form the imino conjugate of the drug and the polysaccharide. The water-soluble conjugate was dispersed by sonication in a mixture of toluene and liquid paraffin in the presence of a non-ionic surfactant as droplet stabilizer and fabricated into microspheres by heat denaturation at 80°C to obtain spheres less than 2 μm in diameter. These microspheres did not undergo dissolution in water on prolonged incubation. In-vitro release of ampicillin into phosphate buffer from the microspheres was slow and sustained with a cumulative release between 10 and 25% of the drug content in 10 days depending on the degree of oxidation of GA and the drug payload. Release into simulated gastric fluid was faster due to faster hydrolysis of the drug–GA bond in the acid medium, but when the medium was changed to intestinal fluid, the release was slowed down. Ampicillin released was functionally active and inhibited the growth of *S. aureus* and *E. coli* in cultures, although not as actively as free ampicillin. The microspheres underwent slow biodegradation on prolonged incubation in aqueous media. These studies show that ampicillin conjugated with oxidized GA and fabricated into microspheres possesses sustained-release characteristics for prolonged periods.

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Introduction

Antibiotics such as ampicillin are commonly used for systemic therapy as well as for gastric or intestinal infections. Among the semi-synthetic penicillins, ampicillin has found widespread use owing to its broad spectrum of activity. After oral administration, it is absorbed only partially and usually 30–50% of the active compound is excreted in urine (Rolinson 1973). Antimicrobial therapy is also frequently accompanied by gastrointestinal complications, including nausea, vomiting, abdominal pain and diarrhoea (Yost & Gotz 1985). From the therapeutic point of view, antibiotics present problems because of their low solubility in lipid membranes. Antibiotics of several classes, including β-lactams, cross the cell membrane very slowly, or not at all, and are unable to reach therapeutic concentrations at the site of infection (Tulkens 1991; Barza 1994; Chanteux et al 2003).

Since the preferred route of drug delivery is the oral route, oral dosage forms capable of extending the duration of drug delivery have received much attention in recent years (Möes 1993). Particulate formulations have proven efficient in achieving better pharmacokinetic profiles and improving the oral bioavailability of several drugs (McClean et al 1998). Particulate oral delivery systems can protect labile macromolecules, such as proteins and polypeptides, from stomach acid and from first-pass metabolism in the gastrointestinal tract. Likewise, particulate formulations can increase the transit times better than larger dosage forms and can increase the local concentration gradient across absorptive cells (Delie & Blanco-Prieto 2005). Polymeric microspheres are ideal particulate vehicles for many controlled delivery applications due to their ability to encapsulate a variety of drugs, good bioavailability, ease of fabrication and sustained drug-release characteristics (Varde & Pack 2004). Local effects in the gastrointestinal tract, from the release of irritant drug molecules, can also be reduced using particulate carriers.

Oral sustained-release antibiotic formulations are reported to be more effective in the treatment of peptic ulcers (Shu & Zhu 2000; Orienti et al 2002), ulcerative colitis, infections of the small intestine (Yang et al 1999; Portero et al 2002; Tozaki et al 2002) and tuberculosis (Rastogi et al 2006). Ampicillin, which has a short biological half-life of 0.74–1.5 h, is an

ideal candidate for a controlled-release formulation. To make the application of ampicillin more effective, sustained- and controlled-release formulations have been investigated. Thus, ampicillin has been encapsulated in microspheres of ethyl cellulose (Goto et al 1985), chitosan (Chandy & Sharma 1993; Giunchedi et al 1998; Anal et al 2006) and chitosan-alginate (Anal & Stevens 2005) to obtain sustained release. Liposomal ampicillin (Baker-Woundenberg et al 1988; Fattal et al 1991) and nanoparticle-bound ampicillin (Youssef et al 1988; Fattal et al 1989; Pinto-Alphandary et al 1994) were shown to be therapeutically superior to free ampicillin against listeriosis and salmonellosis.

Gum arabic (GA) is a natural polysaccharide obtained from the exudates of the acacia tree, with the major component being arabinogalactan (90%), and is extensively used in the food, pharmaceutical and cosmetic industries (Verbeken et al 2003). It is reported to be fermented and metabolized in the caecum and in the colon (Ross et al 1983, 1984). Oral administration of GA has been shown to accelerate the absorption of some solutes, such as sodium and zinc (Wapnir et al 1996, 1997; Ibrahim & Wapnir 2004a, b), as well as certain pharmaceutical agents, such as paracetamol (acetaminophen) (Codipilly & Wapnir 2004), purportedly due to GA modifying the paracellular water and electrolyte transport in the small intestine (Rehman et al 2003).

GA therefore holds promise as a drug delivery vehicle not only to achieve sustained release but also possibly to improve the bioavailability of some pharmaceutical agents. However, literature on the use of this interesting biopolymer is rather scarce (Nishi & Jayakrishnan 2004). We examined oxidized GA as a soluble conjugating agent, as well as an encapsulating agent, for ampicillin. In this communication, we report the preparation and evaluation of GA-conjugated ampicillin as microspheres for sustained release.

Materials and Methods

Materials

GA (from acacia tree) of approximate molecular weight 250 000 (product No. G-9752), borax (sodium tetraborate decahydrate), sodium *m*-periodate and sorbitan sesquioleate were purchased from Sigma (USA). Ampicillin trihydrate IP (Roscillin) was from Ranbaxy Laboratories (India). Dialysis tubing (Spectra/Por, molecular weight cut-off (MWCO) 6000–8000 and 3500) was from Spectrum Laboratories Inc. (CA). Polysaccharide standards ($M_w/M_n < 1.2$) for molecular weight determination were from Polymer Laboratories (Amherst, MA). Liquid paraffin (light, viscosity 18 cP at 30°C) was from S.D. Fine Chemicals (Mumbai, India). Beef infusion, casein hydrolysate and agar were procured from HiMedia Laboratories (Mumbai, India). Starch was procured from Sisco Research Laboratories (Mumbai, India). All other reagents, such as methanol, acetone, toluene, boric acid, disodium hydrogen phosphate, monosodium hydrogen phosphate, sodium chloride, potassium iodide, sodium thiosulphate, etc., were of analytical grade and were procured locally. Doubly distilled water was employed throughout. *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were obtained from American Type Culture Collection (Rockville, MD). The medium used for testing the antibacterial activity was Muller Hinton broth/agar. Medium was prepared using 300 mL of beef infusion, 1.75 g casein hydrolysate, 1.5 g starch, 1 g agar and 1000 mL distilled water. Starch was emulsified in a small quantity of cold water and poured into the beef infusion. Casein hydrolysate and agar were added to this and the volume was made up to 1000 mL with distilled water. The constituents were dissolved by heating gently at 100°C with agitation and the pH was adjusted to 7.4. Simulated gastric fluid (GF) and intestinal fluid (IF) without enzymes were freshly prepared according to the US Pharmacopeia.

Oxidation of GA

GA was oxidized using sodium *m*-periodate as reported earlier (Nishi & Jayakrishnan 2004). Briefly, into 100 mL of a 10% solution of GA (0.058 mol) in distilled water was introduced 6.2 g (0.029 mol) or 2.5 g of periodate (0.0116 mol) to obtain 50 or 20% oxidation and the contents were stirred magnetically at 20°C in the dark for 6 h. The extent of oxidation was determined iodometrically at the end of 6 h. After the reaction, contents were dialysed (MWCO 6000–8000) against water for 48 h with several changes of water until the dialysate was free from periodate. The solution was then frozen and lyophilized to dryness and stored in the desiccator at 4°C until use. Typical yield was 75–80%.

Preparation of ampicillin-conjugated GA microspheres

Oxidized GA (degree of oxidation 50 or 20%) was dissolved in borate buffer (0.1 M, pH 11) to obtain a 10% solution. Into 2 mL of the solution prepared with 50% oxidized GA, 0.1 g or 0.04 g of ampicillin was added in powder form to obtain a theoretical drug payload of 50 and 20 wt%, respectively. With 20% oxidized GA solution, 0.04 g or 0.02 g of ampicillin was added to 2 mL of the solution to obtain a theoretical drug payload of 20 and 10 wt%, respectively. The solutions were stirred magnetically overnight to obtain a yellow-coloured conjugate. Microspheres were prepared from the conjugates by a thermal denaturation process and finally dehydrated using methanol and acetone. Typically, 2 mL of the ampicillin–GA conjugate solution prepared as described above was added to 50 mL of a mixture of liquid paraffin and toluene (1:1) containing 1 g of the oil-soluble surfactant sorbitan sesquioleate in a 100 mL round-bottomed (RB) flask. A reverse emulsion was formed by the dispersion of the aqueous phase in organic phase. The dispersion was cooled on ice and sonicated using the Q-horn of a sonicator (Model 4710; Cole Parmer, IL) at a power setting of 7 (120 W) for 3 min. After sonication, the contents were stirred at 1000 rev min^{-1} with a stainless-steel half-moon paddle stirrer in an oil bath maintained at 80°C using a mechanical stirrer (Model RW-20; IKA Labortechnik, Staufen, Germany) for 2 h. The contents were then centrifuged and washed with 5 mL portions of toluene (5×), methanol (4×) and finally with acetone (2×). The microspheres thus obtained in 75–80% yield were air dried.

Morphology and particle size analysis

The microspheres were dispersed in acetone by sonication and a drop of this suspension was placed on a metal stub, coated with gold and examined by scanning electron microscopy (SEM) (Hitachi, Model S-2400, Japan). The particle size analysis of the microspheres was carried out using SEM pictures of the microspheres. The diameter of about 700 microspheres was measured from the photomicrographs and the distribution was plotted. Morphology of the microspheres after drug release was again examined using SEM.

Analysis of drug content

The conjugate prepared by stirring different weight percentages of ampicillin with oxidized GA was dialysed against water using a dialysis tubing of MWCO 3500 for 48 h with many changes of water, frozen, lyophilized and stored at 4°C. A known weight of the conjugate was dissolved in phosphate buffer and its absorbance was measured at 254 nm. The amount of ampicillin present was determined from a standard curve prepared using known weight of ampicillin dissolved in phosphate buffer.

Infrared (IR) and NMR spectra

IR spectra of the oxidized GA and ampicillin-conjugated GA (lyophilized powders) were recorded in a Fourier transform infrared (FTIR) instrument (Nicolet, Model Impact 410; Madison, WI) using pressed KBr pellets. NMR spectra were recorded in a Bruker 200 MHz spectrometer (Bruker AC-200; Billerica, MA) with TMS as the internal standard and D_2O as the solvent.

Molecular weight measurements

The weight average molar mass (M_w) of GA and oxidized GA was determined by gel permeation chromatography (GPC) using a Waters HPLC system equipped with 510 pump, R401 refractive index detector and 7725 Rheodyne injector. The column used was Ultrahydrogel 1000/500/250. The mobile phase was 0.1 M $NaNO_3$ at a flow rate of 1 mL min^{-1}. A standard curve was prepared with retention time against ln M_W using polysaccharides of known molecular weight, and from this standard curve, M_w of the sample was determined.

Thermal analysis

Thermal stability of the oxidized GA, ampicillin-conjugated GA and ampicillin was analysed by the method of thermogravimetry (Simultaneous DTA-TGA, Model SDT 2960; TA instruments Inc., New Castle, DE). Experiments were carried out in N_2 atmosphere at a heating rate of 10°C min^{-1}. Mass change of the sample was recorded continuously as a function of a combination of temperature with time.

Swelling characteristics

Microspheres, prepared from ampicillin-conjugated GA having a degree of oxidation of 20 and 50% with different ampicillin payloads, were allowed to swell in phosphate buffer. Typically, 100 mg of microspheres was introduced into a glass vial (n = 3) containing 5 mL phosphate buffer and were allowed to swell at 37°C in an incubator for 24 h until they attained equilibrium swelling. The buffer was aspirated using a Pasteur pipette from each vial, the residual buffer was further removed carefully using tissues and the microspheres were weighed in an analytical balance. Degree of swelling (Q) was defined as the reciprocal of the volume fraction of the polymer (υ_2) and was calculated using equation 1.

$$Q = \upsilon_2^{-1} = \{(1/\rho_p)[(Q_m/\rho_s) + (1/\rho_p)]^{-1}\}^{-1} \quad (1)$$

Where ρ_p is the density of microspheres (1.262 g m^{-3}), ρ_s is the density of water (0.9971 g cm^{-3} at 25°C) and Q_m is the swelling ratio, defined as the mass ratio of absorbed water and the dried microspheres (Peppas et al 2000).

In-vitro drug release

In-vitro release was examined into phosphate buffer as well as into GF and IF. A known quantity (50 mg) of the microspheres was suspended in 50 mL phosphate buffer in stoppered Erlenmeyer flasks. The flasks were shaken at 100 times per min in a reciprocating bath-shaker (Model SW-22; Julabo Labortechnik, Seelbach, Germany) thermostatted at 37 ± 1°C. Volumes of 0.5 mL were withdrawn at various times, filtered through a 0.45-μm filter and analysed spectrophotometrically for ampicillin at a wavelength of 254 nm. In the case of release into GF and IF, 50 mg of the microspheres were suspended in 50 mL of the GF and at the end of 2 h, the GF was carefully removed, 50 mL IF was introduced and the release was followed in IF as before.

In-vitro antibacterial activity

Antibacterial activity was studied using two bacterial strains, *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922). Muller Hinton broth (MH broth)/agar, prepared as described before, was sterilized at 121°C for 20 min and poured into sterile tissue culture plates (Nunc, Denmark) to give a uniform depth of 4 mm. Antibacterial activity was checked by disc diffusion method and the disc potency was 10 μg for *E. coli*, with a zone size of 20 mm. Disc potency for *S. aureus* was 10 μg, with a zone size of 40 mm. The minimum inhibitory concentration (MIC) was determined by tube dilution method. Standard strains were used in the log phase of the culture and the cultures contained 10^5 colony-forming units (CFU)/mL (Dizman et al 2005).

Microspheres prepared from 50% oxidized GA, having 42 wt% drug payload, were used for antibacterial studies. Microspheres containing 0.1 g of ampicillin were suspended in 10 mL of MH broth to attain an ampicillin concentration of 10000 μg mL^{-1}. From this, dilutions were carried out to attain concentrations in the range 4000–0.1 μg mL^{-1}. To these, bacterial strains were introduced and incubated at 37°C for 18 h with intermittent shaking. With *S. aureus*, turbidity was not seen in the tube containing ampicillin at a concentration of 320 μg mL^{-1} or higher; tubes were turbid with all lower concentrations of ampicillin, indicating the growth of bacteria.

For *E. coli*, turbidity was not seen in the tube containing ampicillin at a concentration of 3200 μg mL^{-1}; bacterial growth occurred at all lower concentrations. These were subcultured on agar plates and observed for growth of bacteria, and the MIC was confirmed from this result.

Statistical analysis

Statistical analysis of all data reported in the studies described was performed by one-way analysis of variance, assuming a confidence level of 95% ($P < 0.05$) for statistical significance. All data were expressed as mean ± standard deviation (s.d.).

Results and Discussion

Oxidized polysaccharides have recently received attention as carriers for drugs containing amino functions that can enter into Schiff's reaction rapidly, with the aldehyde groups on the oxidized polysaccharides resulting in hydrolytically labile imino conjugates. Thus, the polyene antibiotic amphotericin B, which contains an amino group, has been conjugated to oxidized arabiniogalactan via the imine bond to improve the aqueous solubility, reduce the toxicity and enhance the bioavailability of this drug (Falk et al 1999; Kleinman et al 2002). Primaquine, an 8-aminoquinoline that contains a primary as well as a hindered aromatic secondary amino function, has been conjugated to oxidized GA and fabricated into microspheres suitable for phagocytosis and to release the drug for prolonged periods (Nishi & Jayakrishnan 2004). Interaction of both amino groups with the aldehyde functions has been shown to result in an insoluble conjugate that could be fabricated into microspheres and microparticles. Ampicillin has a primary amino group and one would expect the molecule to behave in the same way as amphotericin B, which forms a soluble conjugate on mixing the drug with oxidized GA very rapidly. Indeed, when ampicillin was added to oxidized GA and stirred overnight, the formation of the conjugate was evident by the development of a golden yellow colour and the conjugate remained soluble. Dialysis followed by lyophilization yielded a conjugate in the dry form in 75–80% yield, which could be readily re-dissolved in water.

The formation of the conjugate was confirmed by IR and NMR spectra. The IR spectrum of the conjugate showed a strong absorption at 1400.88 cm^{-1}, which is characteristic of imine stretching vibration. Imines give their characteristic stretching vibrations at 1471–1689 cm^{-1} and these characteristic peaks can be seen at 1607 and 1400.88 cm^{-1}. The peak at 1070 cm^{-1} corresponds to C-O vibration, which is present in GA (Figure 1). Formation of the conjugate was further confirmed by ^{1}H NMR spectrum. NMR spectrum of the conjugate (not shown) showed a peak at 6.85 ppm, characteristic of aromatic protons, which was absent in the spectrum of oxidized GA. This confirmed that ampicillin, which has an aromatic ring, has been covalently conjugated to oxidized GA as shown in Figure 2.

Compounds containing an α-aminoamide moiety are known to condense with aldehydes and ketones, giving 4-imidazolidinones. Ampicillin is known to react with aldehydes and ketones to give the corresponding imidazolidinone

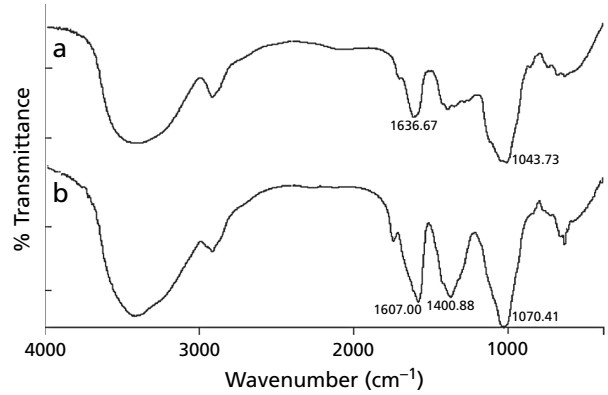

Figure 1 IR spectra of oxidized gum arabic having a degree of oxidation of 50% (a) and ampicillin conjugated to the oxidized gum arabic (b).

adduct (Klixbüll & Bundgaard 1985). The rate of such 4-imidazolidinone adduct formation is depressed with increasing steric hindrance in carbonyl compounds, and formation of this adduct is less likely in the present system where the aldehyde groups residing on the polysaccharide chain would be more sterically hindered, unlike simple aldehydes such as formaldehyde. Further, the ready reversibility of this reaction and its low equilibrium constant with higher aldehydes rather excludes such a possibility even though 4-imidazolidinone adduct in very small amounts might exist in equilibrium with the Schiff base structure.

The conjugate was converted into microspheres by heat denaturation at 80°C for 2 h. SEM examination of the microspheres showed that they are predominantly spherical in shape (Figure 3). Particle size analysis of the microspheres showed that their size ranged from 0.4 μm to 1.2 μm, with the median being 0.8 μm. These microspheres were found to be stable in water and did not undergo dissolution even on prolonged incubation for a day or more, suggesting that the GA matrix is crosslinked at the high temperature employed for the fabrication of the microspheres. When oxidized GA alone was dispersed and stirred for 2 h at 80°C, the microspheres obtained slowly underwent dissolution in water. This observation shows that, in the presence of ampicillin, the polysaccharide undergoes some kind of stabilization or crosslinking at the temperature employed for the preparation of microspheres. Similar heat stabilization of microspheres has been reported by others although the exact mechanism by which such stabilization happens is not known (Giunchedi et al 1998). Thermal analysis showed that the free drug is very stable up to 100°C whereas both oxidized GA and ampicillin-conjugated GA showed a weight loss of about 10% up to 100°C (Figure 4). The overall stability of the drug–polymer conjugate was better than the drug and oxidized GA at higher temperatures. With the formation of Schiff's linkage with ampicillin, the aldehyde groups in oxidized GA will become more stabilized, making the conjugate more stable compared with oxidized GA.

Determination of the weight average molar mass (M_w) showed values of 309000 for GA and 481836 and 473805 for oxidized GA having a degree of oxidation of 20 and 50%, respectively, with a polydispersity of 1.4. The increase in the

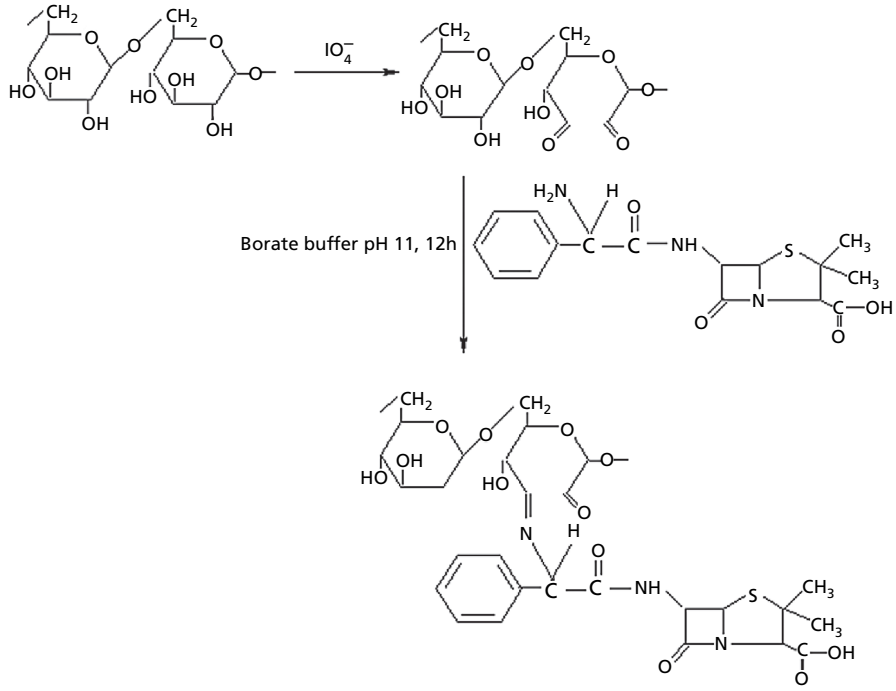

Figure 2 Schematic representation of the conjugation of oxidized GA with ampicillin.

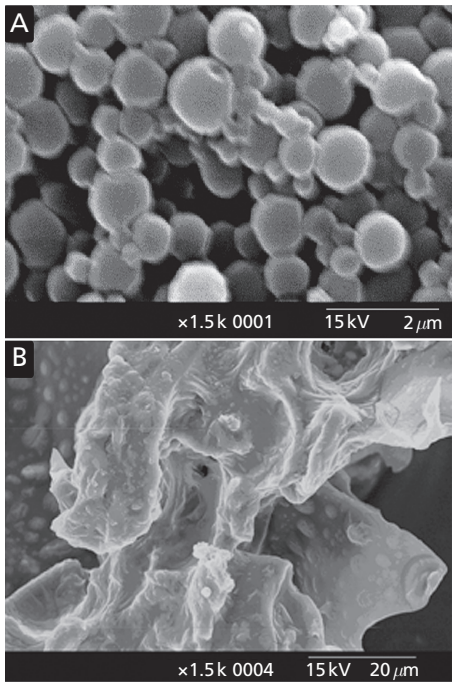

Figure 3 SEM of ampicillin–GA microspheres (A) and microspheres undergoing disintegration after prolonged incubation in phosphate buffer (B).

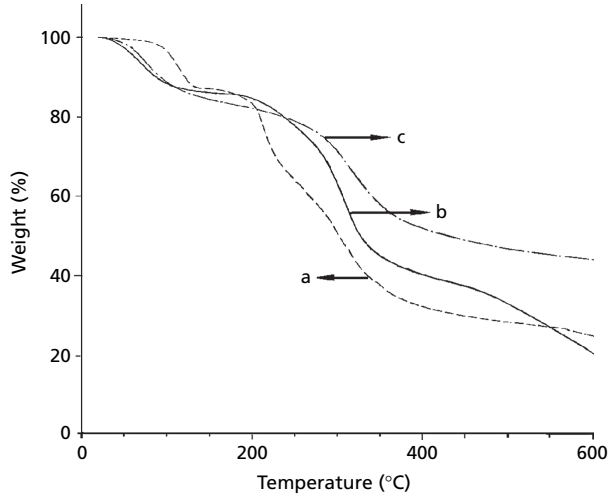

Figure 4 Thermogram of ampicillin (a), oxidized GA (b) and ampicillin conjugated to oxidized GA (c).

molecular weight of oxidized GA compared with GA was due to the inter-chain hemi-acetal bond formation between hydroxyl groups and newly formed aldehyde functionalities. Straight-chain polysaccharides like dextran suffer about 30% decrease in molecular weight after periodate oxidation as they do not present the opportunity for inter-chain hemi-acetal formation (Domb et al 1996). In the case of GA, the vicinal diols available for periodate oxidation are present in side chains and not on the backbone, and therefore there are fewer breaks in resultant polymer chains, and the molecular weight remains stable. Kleinman et al (2002) made similar observations for arabinogalactan, a branched polysaccharide.

Oxidation reduced the solubility of GA. GA is highly soluble in water; about 50% solution of GA can be prepared. Oxidized GA was less soluble than GA. Solubility decreased

with increase in the percentage of oxidation and was limited to 10–15% in water although better solubility was seen in borate buffer due to complexation. GA is reported to have an amphipathic helix structure, with the hydroxyl groups oriented towards the outer surface. As degree of oxidation increased, the number of hydroxyl groups decreased, decreasing solubility. Increase in molecular weight following oxidation also decreases the solubility. The inter-chain hemi-acetal linkages function as crosslinks between different polymer chains, decreasing the solubility of polysaccharide.

The amount of ampicillin conjugated to GA was estimated for different drug payloads and different degrees of oxidation of the polysaccharide before the microspheres were fabricated (Table 1). During the fabrication of microspheres, no ampicillin was found to migrate into the dispersion medium consisting of toluene and liquid paraffin, as examined by UV spectroscopy. Therefore, the amount of ampicillin present in the microsphere matrix is presumed to be the same as that present in the conjugate.

In-vitro release of ampicillin into phosphate buffer was examined from ampicillin-conjugated GA microspheres prepared from GA having a degree of oxidation of 50 and 20% with two different drug contents. Figure 5 shows the release profile from microspheres prepared from 50% oxidized GA. Ampicillin release was slow and sustained from microspheres having high and low drug payloads. As expected, more release was seen from microspheres having a higher payload than from those having a lower payload. No burst release was seen from either preparation as there was no unbound ampicillin. The total amount released in about 10 days was only about 8 and 5% from microspheres having high and low drug contents, respectively. The ratio of aldehyde to amine in the case of 50% oxidized GA with 42 wt% drug payload was about 2.4 whereas with 16 wt% drug payload it was 5.9. The high aldehyde-to-amine ratios therefore should facilitate high drug conjugation that was observed at both these payloads (cf Table 1). When ampicillin was encapsulated in chitosan and methylpyrrolidinone microspheres, both modulated the release in the 30–120 min range and even heat treatment of microspheres did not change the release profile substantially (Giunchedi et al 1998). Chitosan-alginate single layer and multi layer beads encapsulating ampicillin were reported to release 70% of the drug in 4 h and 60% of the drug in 4 h, respectively, into simulated GF (Anal & Stevens 2005). In comparison with systems where the drug is just physically entrapped in swelling-controlled microsphere matrices, the release of drug is highly modulated in systems such as the present one where the drug is chemically conjugated through hydrolytically labile bonds.

Table 1 Incorporation efficiency of ampicillin–GA microspheres

Sample	Degree of oxidation of GA (%)	Theoretical drug payload (wt%)	Actual drug payload (wt%)	Incorporation efficiency (%)
50 (50)	50	50	42.3 ± 5.0	84.5 ± 5.0
50 (20)	50	20	16.5 ± 3.8	82.5 ± 3.8
20 (20)	20	20	15.3 ± 3.1	76.5 ± 3.1
20 (10)	20	10	7.3 ± 2.7	73.0 ± 2.7

The release profile seen from 20% oxidized GA having 15 and 7 wt% drug content is shown in Figure 6. Here again, drug release was faster and more extensive from microspheres having a higher drug payload than from microspheres having a lower payload. The amount released in about 10 days was approximately 25 and 15% from microspheres having 15 and 7wt% ampicillin, respectively. Although the aldehyde-to-amine ratios in the case of 15 and 7 wt% drug payload are 2.4 and 4.7, which are comparable with the ratios seen in the case of 50% oxidized GA, the drug release was faster and more extensive from microspheres prepared from 20% oxidized GA. This was intriguing. We therefore examined the swelling behaviour of both microspheres in phosphate buffer since the extent of swelling could make a difference in the release pattern. Microspheres were swollen for 24 h to reach equilibrium and the swelling ratio was

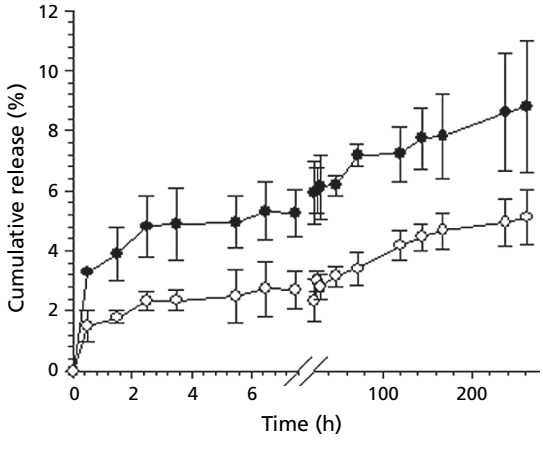

Figure 5 In-vitro release profile of ampicillin from microspheres prepared from 50% oxidized GA into phosphate buffer. 42 wt% drug payload (closed circles), 16 wt% drug payload (open circles). Data are means ± s.d., n = 3.

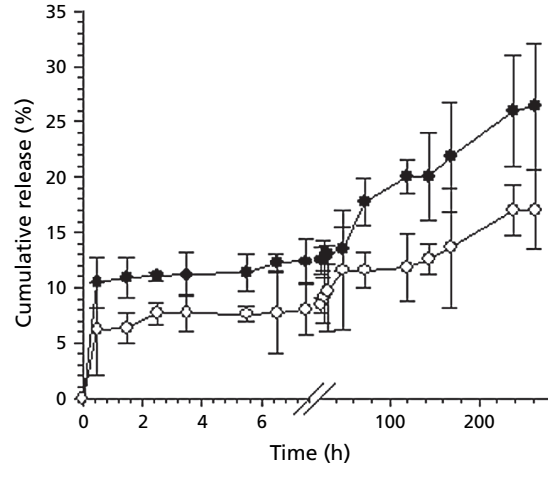

Figure 6 In-vitro release profile of ampicillin from microspheres prepared from 20% oxidized GA into phosphate buffer. 15 wt% drug payload (closed circles), 7 wt% drug payload (open circles). Data are means ± s.d., n = 3.

estimated from the weight of dry and swollen microspheres. The most important parameters that define the structure and properties of swollen polymer networks are the polymer volume fraction in the swollen state and crosslinking density. Highly crosslinked matrix has a tighter structure, and will swell less than the matrix with a lesser crosslinked structure (Peppas et al 2000). The polymer volume fraction in the swollen matrix is a measure of the amount of fluid that the matrix can incorporate into its structure. The swelling ratio (Q_m) was calculated from the ratio of weight of buffer uptake to the weight of dried microspheres. The degree of swelling (Q) is the reciprocal of volume fraction of the polymer (ν_2), which is a measure of interaction between polymer chains.

The swelling parameters of different microsphere preparations are shown in Table 2. Microspheres, prepared from 50% oxidized GA with 42 wt% drug payload and 20% oxidized GA with 15 wt% drug payload have a similar aldehyde-to-amine ratio. The degree of swelling Q for 20% oxidized GA microspheres was 10.79 whereas for 50% oxidized GA it was only 4.95. Oxidized polysaccharides are generally less soluble than their non-oxidized counterparts (Balakrishnan & Jayakrishnan 2005). The larger number of hydroxyl groups left intact in less oxidized GA would contribute to enhanced swelling due to hydrogen bonding compared with the highly oxidized GA wherein more hydroxyls have been oxidized to aldehyde. Therefore, the ability to swell more could be a reason for the increased release that is seen from microspheres prepared from 20% oxidized GA, since it would facilitate the hydrolysis of the drug–GA bond by increased penetration of the solvent and facilitate the release of the drug through wider pores and channels in the microsphere matrix.

The release into simulated GF and IF from microspheres prepared from 50% oxidized GA having two different ampicillin contents is shown in Figure 7. In comparison with the release profile into phosphate buffer, the release into GF was doubled from microspheres having both high and low drug payloads. This suggests the faster hydrolysis of the drug–GA bond in the acidic medium as expected. When the GF was changed into more neutral IF, the release was not increased substantially at least for 24 h, suggesting the slow hydrolysis of the drug–GA bond in this medium.

Whether ampicillin released is functionally active was checked by microbiological assay of its effectiveness to inhibit the growth of *S. aureus* and *E. coli*. The MIC was 19.2 μg mL^{-1} for *S. aureus* and 192 μg mL^{-1} for *E. coli*. These values were arrived at on the basis of the observation that in-vitro release studies have shown that in 18 h only 6% of the incorporated drug was released from these microspheres. For control ampicillin, MIC values were 3 μg mL^{-1} for *S. aureus* and 100 μg mL^{-1} for *E. coli*. These results show that the ampicillin released is functionally active, although not as active as free ampicillin. The MIC values for most samples of extracted ampicillin have been reported in the range of 12.5–15.5 μg mL^{-1} for *S. aureus* (Anal & Stevens 2005). Oxidized polysaccharides are known to undergo slow biodegradation in aqueous media (Lee et al 2000; Boontheekul et al 2005). It is therefore likely that the released ampicillin is not truly free ampicillin, but ampicillin still bearing small fragments of polysaccharide, which is somewhat less effective than free ampicillin in penetrating the cell walls of bacteria.

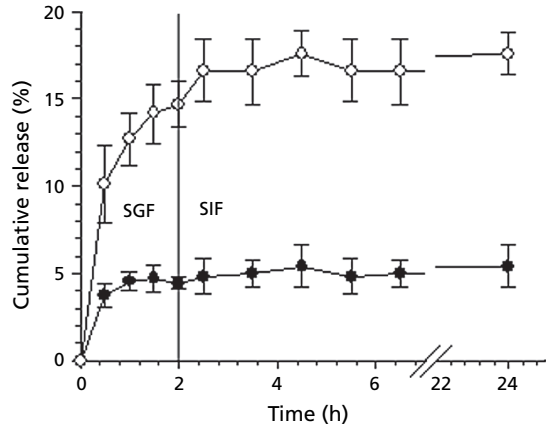

Figure 7 In-vitro release profile of ampicillin from microspheres prepared from 50% oxidized GA into simulated GF (SGF) for 2 h followed by release into simulated IF (SIF). 42 wt% drug payload (open circles), 16 wt% drug payload (closed circles). Data are means ± s.d., n = 3.

Biodegradation of the microspheres was evident when examined by SEM after 10 days in phosphate buffer. Although complete dissolution was not observed, the microspheres had lost their spherical shape on continuous incubation in the medium, suggestive of their slow biodegradation (Figure 3).

Table 2 Swelling parameters of ampicillin–GA microspheres

Degree of oxidation of GA (%)	Drug content (wt%)	Swelling ratio (Q_m)	Polymer volume fraction (ν_2)	Degree of swelling (Q)
50	42.3	3.07 ± 0.58	0.20 ± 0.03	4.95 ± 0.74
20	15.3	7.61 ± 0.12	0.09 ± 0.001	10.79 ± 0.15

Conclusions

In conclusion, ampicillin can be conjugated to oxidized GA rapidly through its primary amino group to form a soluble drug–GA conjugate. Thermal denaturation of the conjugate leads to stabilization or crosslinking of the GA matrix. The exact mechanism by which this happens is not clear at present. The release of ampicillin from microspheres prepared by thermal treatment was examined in phosphate buffer as well as in simulated GF and IF. While the release was slow into phosphate buffer, it was twice as fast into GF due to the rapid hydrolysis of the imine linkages between the drug and GA. Ampicillin released was functionally active, as checked by microbiological assay for its effectiveness to inhibit the growth of *S. aureus* and *E. coli*, although less effective than free ampicillin, presumably due to small fragments of polysaccharide attached to the released ampicillin. Slow biodegradation of microspheres was observed in aqueous media over prolonged incubation. Ampicillin conjugated to oxidized GA and fabricated into microspheres provides a controlled release formulation of the drug with sustained-release properties for prolonged periods, as opposed to encapsulated ampicillin formulations.

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