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Biodegradable intraoperative system for bone infection treatment. I. The drug/polymer interaction

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Abstract

In order to design and evaluate an implantable device of calcium alginate spheres releasing gentamicin sulphate to prevent or treat bone infections, the interaction between the cationic gentamicin and the polyanionic alginate was examined. Such an interaction took place, both in sodium alginate solution—as shown by rheological study, and during the Ca-induced polymer gelation which led to the formation of the calcium alginate spheres. Gentamicin sulphate was found to interact selectively on the mannuronic residues of alginate without competition with calcium ions involved in the polymer gelation. In contrast, calcium ions were found to interact preferentially at the level of the polyguluronic sequences, though polymannuronic sequences can also play a role. This prevented the saturation of the polymannuronic sequences by the drug. Therefore, the alginate having a higher mannuronic acid content, i.e. capable of associating a greater amount of drug, could be considered the more suitable material for the implant design.

Keywords: Alginate; Calcium; Gentamicin sulphate; Implant; Interaction; Viscosity

1. Introduction

Alginates are naturally occurring substances which are extracted from brown seaweeds. They are polysaccharides constituted of homogeneous mannuronic acid segments M-M (M blocks), homogeneous guluronic acid segments G-G (G blocks) and alternating segments M-G (MG blocks) (Rees, 1969).

Alginates have been widely used in the pharmaceutical, food and cosmetic industry. In pharmaceutical technology, the alginates are used as tablet binders, disintegrants, viscosity enhancers and stabilizers of multiple phase systems (McDowell, 1986).

More recently, alginates have been proposed in controlled drug delivery dosage forms [sustained

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release matrices (Stockwell et al., 1986), immobilization matrices for cell (Sun and O'Shea, 1985) and enzymes (Tanaka et al., 1984), microparticles (Sun and O'Shea, 1985; Wan et al., 1992) or nanoparticles (Rajaonarivony et al., 1993) due to their cation-induced gelation. In fact, in the presence of multivalent cations the G-G blocks can associate to form aggregates of the 'egg-box' type (Grant et al., 1973). The interaction of alginates with calcium ions, for which G-G blocks are mainly responsible, has been intensively investigated by circular dichroism, nuclear magnetic resonance and rheological measurements (Penman and Sanderson, 1972; Grant et al., 1973; Stockwell et al., 1986). Also, alginates are described as interacting with cationic compounds such as chlorpheniramine maleate (Stockwell et al., 1986), doxorubicin (Rajaonarivony et al., 1993), propranolol (Segi et al., 1989), polylysin and, more specifically, the interaction with polylysin was reported to take place on mannuronic residues (Bystricky et al., 1990). In addition, aminoglycosidic antibiotic exhibited diminished diffusion coefficients in agar gels containing alginate (Gordon et al., 1988).

Among the numerous reports dealing with alginate controlled delivery systems, few parenteral dosage forms have been investigated (Sun and O'Shea, 1985; Firsov et al., 1987; Kwok et al., 1991; Rajaonarivony et al., 1993), though there are advantageous properties to this material. In fact, alginates are known to be biocompatible and biodegradable (Kronenthal, 1975), they can be sterilized without loss of viscosity (Handbook of Pharmaceutical Excipients, 1986), have hemostatic properties (McDowell, 1986) which are responsible for sustention of the drug release for implants (Firsov et al., 1987) and a low cost.

Therefore, the aim of our work was to design and evaluate an implantable device of calcium alginate spheres releasing gentamicin to prevent or treat bone infections (osteomyelitis). Gentamicin was selected because it is a broad-spectrum antibiotic widely used in the systemic therapy of osteomyelitis. In this first paper, we wish to report a preliminary study on the possibility of interaction between the cationic gentamicin and alginate in calcium alginate spheres and to evaluate the effect of the alginate mannuronic/guluronic ratio (M/G ratio) on the drug association capacity on the polymer. The study involved the evaluation of such an interaction both in sodium alginate solution, by means of rheological investigations, and in calcium alginate spheres, which constitute the implant, by using two types of alginate having a different mannuronic/guluronic ratio, in order to choose the more suitable alginate for the implant design.

2. Materials and methods

2.1. Materials

The following chemicals were obtained from commercial suppliers and used without further purification. Sodium alginate Manucol DM (M_r about 147 000, extracted from *Laminaria digitata*, containing 62% mannuronic acid and 38% guluronic acid) (high M/G ratio) was donated by Kelco International (Bagnolet Cedex, France). Sodium alginate (M_r about 115 000, extracted from *Laminaria hyperborea*, containing 30% mannuronic acid and 70% guluronic acid) (low M/G ratio), gentamicin sulphate and calcium chloride dihydrate were purchased from Fluka Chemie (Buchs, Switzerland). Tween 20 (polyoxyethylensorbitan monolaurate) was purchased from Atlas Europol (Ternate, Italy), *o*-phthaldialdehyde reagent solution from Sigma Chemical (Milan, Italy). Sodium citrate, sodium phosphate (monobasic), potassium phosphate (monobasic), sodium chloride and all the solvents (analytical grade) were purchased from Carlo Erba (Milan, Italy).

2.2. Methods

2.2.1. Rheological investigations

Dilute water solutions (0.5%, w/v) of both the sodium alginates (low and high M/G ratio) containing gentamicin sulphate differently concentrated (ranged from 0 to 0.43 mM; higher concentrations could not be used because a precipitate was formed) were prepared. After 24 h, the rheological behaviour of the solutions was

determined at 25°C by placing 9 ml of the sample in a coaxial cylinder (radii ratio = 1.02) rheometer (Rotovisco RV 12, Haake, Karlsruhe, Germany) and measuring the shear stress as a function of the shear rate.

2.2.2. Preparation of calcium alginate spheres

Sodium alginate water solutions (5%, w/w) of both the alginates (low and high M/G ratio) were used after being degassed under a vacuum. Calcium alginate spheres were obtained by dropping through a 2 mm orifice 3 ml of sodium alginate water solution, into a medium constituted by 20 ml of n-eptane and 10 ml of gentamicin sulphate (1%, w/v), calcium chloride (5%, w/v), Tween 20 (1%, w/v) water solution. The medium was stirred (1000 rpm) for 10 min at room temperature. The formed spheres were separated from the medium, washed quickly with diethyl ether and vacuum dried for at least 48 h.

The same procedure was followed to prepare spheres without drug.

2.2.3. Preparation of partially-crosslinked spheres

Partially-crosslinked spheres were obtained by the same technique described above but in a medium containing 1% (w/v) calcium chloride, concentration providing spheres with a soft internal zone. The samples were washed with freshly distilled water before drying in order to remove the products not involved in the reaction.

2.2.4. Preparation of gentamicin-alginate adduct

Gentamicin-alginate adducts were obtained as a white precipitate by the same technique described above, but in a medium without calcium chloride. The samples were washed with freshly distilled water and vacuum dried.

2.2.5. Drug content determination

Gentamicin content was determined by placing a weighted amount of all the samples in both water and 3% (w/v) sodium citrate water solution. After 48 h gentamicin concentrations in both the solutions were assayed by spectrophotometrical analysis of the solutions at a wavelength of 332 nm (model Lambda 3B, Perkin-Elmer, Norwalk, CT, USA) after derivatization with *o*-phthaldialdehyde according to Sampath and Robinson (1990). The same amount of the samples without drug produced no changes in the absorbance values ($E < 0.005$). All the data are averaged on three determinations.

2.2.6. Calcium content determination

Calcium content was determined by placing a weighted amount of spheres and partially-crosslinked spheres in both water and 3% (w/v) sodium citrate water solution. After 48 h calcium concentrations in both the solutions were assayed by atomic absorption spectroscopy (model 3030, Perkin-Elmer). All the data are averaged on three determinations.

2.2.7. Drug release analysis

Drug release from the spheres was tested in phosphate buffered saline solution of pH 7.4 at 37°C, without shaking, in order to simulate the implant zone condition. The experiments were carried out under sink conditions. At predetermined time intervals, aliquots of solution were withdrawn and exchanged with new media of the same volume. The amount of gentamicin released was determined by the spectrophotometrical method. The experiments were carried out in triplicate.

2.2.8. Element analysis

The energy dispersive X-ray analysis (EDS, EDAX 9900, Edax International, Prairie View, IL, USA) coupled with SEM (XL-40, Philips, Eindhoven, The Netherlands) was used to detect the sulfur atoms of the gentamicin sulphate molecules associated on the polymer. Thin slabs of the samples were washed with freshly distilled water, vacuum dried and carbonated (model CED 010, Balzers Union, Liechtenstein). The ratio of the characteristic elemental intensity to the total background (p/b) for S (2.31 keV) was obtained with an accelerating voltage 25 kV; detection limit about 0.3%. Because of the low concentration of the element in the washed spheres, only the data concerning the gentamicin-alginate adducts are reported.

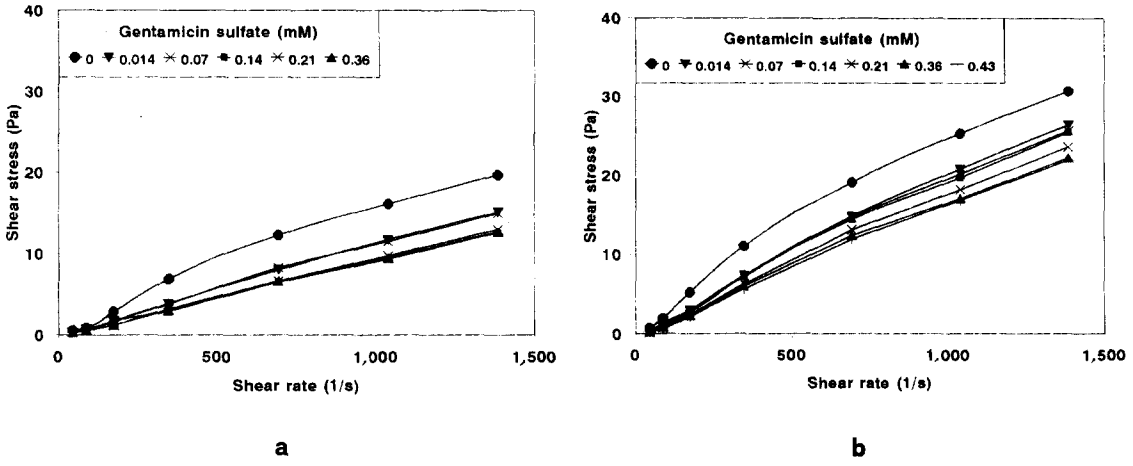

Fig. 1. Flow behaviour of 0.5% w/v alginate solutions containing different gentamicin sulphate concentrations. (a) Low M/G ratio alginate; (b) high M/G ratio alginate.

3. Results and discussion

3.1. Evaluation of drug/polymer interaction by rheological study

Unlike uncharged polymers, dilute solutions of polyelectrolytes show an 'electroviscous effect' which leads to increased viscosities (Fuoss, 1948). This results from a spatial expansion of the hydrodynamic volume of the molecules due to electrostatic repulsions between charged segments subsequent to the dilution of the counter ion layer surrounding the molecules. A 0.5% w/v solution of sodium alginate is considered a dilute polyelectrolyte solution having an expanded configuration (Stockwell et al., 1986). In such conditions, the addition of gentamicin having an opposite charge decreases the intramolecular repulsion leading to a tighter configuration and, consequently, to a reduced viscosity.

Therefore, in order to determine the possible interaction between the cationic gentamicin and the polyanionic alginate leading to a reduced viscosity, rheological measurements were carried out on 0.5% w/v solutions of both the sodium alginate (low and high M/G ratio) alone and in the presence of increasing amounts of gentamicin sulphate such as to produce true solutions, i.e. without precipitate (less than 0.4 and 0.5 mM for low and high M/G ratio alginate, respectively). The flow behaviours of these solutions are depicted in Fig. 1. All the curves showed a pseudo-plastic behaviour without hysteresis loops indicating that there was no change in the structure of the molecules under shear stress. The presence of gentamicin led to a reduced viscosity of both (low and high M/G ratio) the alginate solutions revealing that an electrostatic interaction between drug and alginate had occurred.

Also, with the increase of gentamicin concentration, the viscosity of the alginate with high M/G ratio decreased progressively, whereas it tended to a plateau for the alginate with low M/G ratio, as clearly indicated by the plot of the apparent viscosity, calculated for a shear rate of 1039 s^{-1}, versus gentamicin amount, where 100% is the value of the pure alginate solution (Fig. 2). These results suggest that the effect of the charge neutralization by gentamicin on the alginate with high M/G ratio was not completed, whereas, on the alginate with low M/G ratio, it reached its equilibrium already upon the addition of 0.14 mM gentamicin sulphate. Therefore, a preferential interaction of gentamicin with the mannuronic sequences can be supposed. However, in our opinion, the complexity of the rheological phenomena of alginates involving several factors, such as the different molecular length and the

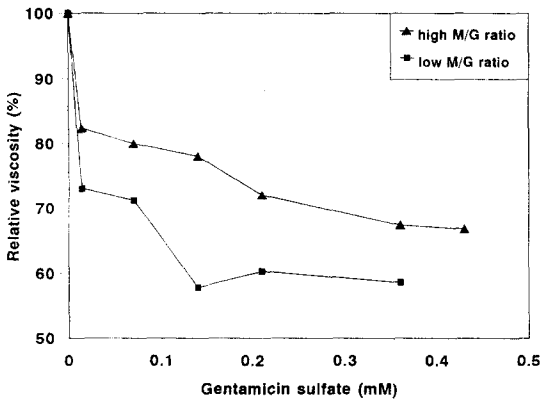

Fig. 2. Relative viscosity values of 0.5% w/v alginate solutions containing different gentamicin sulphate concentrations.

different content in G blocks, rather stiffer than M blocks (McDowell, 1986), makes any relation between the rheological behaviour and the role of M/G ratio on the drug association capacity only an approximation.

3.2. Evaluation of polymer interactions in calcium alginate spheres

Water insoluble calcium alginate spheres containing gentamicin sulphate, sized about 2 mm, resulted from the complexation of the anionic polysaccharide by calcium.

Drug and calcium content determinations carried out in water gave values much smaller than those obtained in sodium citrate solution in which the spheres can dissolve (Table 1). The amounts obtained by water extraction could be considered as unassociated drug fractions, whereas those obtained by dissolving the samples as total drug amounts (drug loading). The differences in these values (1.8 and 4.6% w/w, corresponding to about 44 and 87% of the drug loading, for the low and the high M/G ratio alginate, respectively) were considered as corresponding to associated fractions indicating that the interaction of the drug with the polymer, already observed in sodium alginate water solution by the rheological study reported above, had occurred also in the presence of calcium which acted as polymer crosslinking agent. Furthermore, it could follow that the drug/polymer complex, as well as the known calcium/polymer complex, is stable in water, whereas it was destroyed by media containing drug binding anions. On the other hand, the unassociated fractions of both drug and calcium could indicate that the interactions with the polymer had taken place during the sphere preparation, could be considered istantaneous and had occurred in a medium containing an excess of reactive compounds.

The association capacity of calcium (moles of calcium per gram of polymer) resulted $3.1 \cdot 10^{-3}$ for the polymer with low M/G ratio and $1.7 \cdot 10^{-3}$ for the polymer with high M/G ratio (Table 2), suggesting that the polyguluronic sequences are mainly involved, as it is well known (Grant et al.,

Table 2
Calcium association capacities on the alginate and theoretical values

	Low M/G ratio (mol/g polymer)	High M/G ratio (mol/g polymer)
Theoretical	2.0	1.1
Spheres	3.1 (0.2)	1.7 (0.3)
Unloaded spheres	2.8 (0.3)	2.3 (0.1)

Mean values ± S.D. × 1000.

Table 1
Gentamicin sulphate and calcium contents in calcium alginate spheres

Medium	Alginate M/G ratio	Gentamicin (% w/w)	Calcium (% w/w)
Sodium citrate	Low	4.1 (0.4)	12.6 (0.5)
	High	5.3 (0.3)	10.7 (1.2)
Water	Low	2.3 (0.3)	3.2 (0.1)
	High	0.7 (0.1)	4.7 (0.2)

Mean values ± S.D.

1973). However, these values came over the theoretical ones, calculated for a complete neutralization of only G blocks by calcium ions in a 1:2 calcium/uronic-block molar ratio (Morris et al., 1978). Therefore, it would appear that calcium can also interact with the polymannuronic sequences, as already hypothesized (Grant et al., 1973). Moreover, the presence of gentamicin did not affect significantly the calcium association capacity, as the association values of calcium of the unloaded spheres compared with those of the loaded ones indicated (Table 2).

The association capacity of gentamicin on alginate (moles of gentamicin per gram of polymer) resulted in $0.3 \cdot 10^{-4}$ for the polymer with low M/G ratio and $0.8 \cdot 10^{-4}$ for the polymer with high M/G ratio (Table 3), indicating a preferential association of the drug on the mannuronic residues, as already postulated by means of the rheological study reported above.

Since the M blocks were found to be involved in the interaction with calcium, the possible influence of calcium binding on gentamicin association capacity was assayed by investigating partially crosslinked spheres prepared by using a lower calcium concentration and gentamicin-alginate adducts prepared without calcium, both containing only associated gentamicin. The amounts of gentamicin associated to the polymer increased, according to the M/G ratio, when calcium concentration in the sphere preparation procedure was decreased (partially-crosslinked spheres) reaching the highest values when calcium was not

Table 3
Gentamicin sulphate association capacities on the alginate and theoretical values

	Low M/G ratio (mol/g polymer)	High M/G ratio (mol/g polymer)
Spheres	0.3 (0.02)	0.8 (0.1)
Partially-crosslinked spheres	2.7 (0.2)	3.8 (0.4)
Gentamicin-alginate adduct	4.5 (0.8)	13.0 (0.3)

Mean values ± S.D. × 10 000.

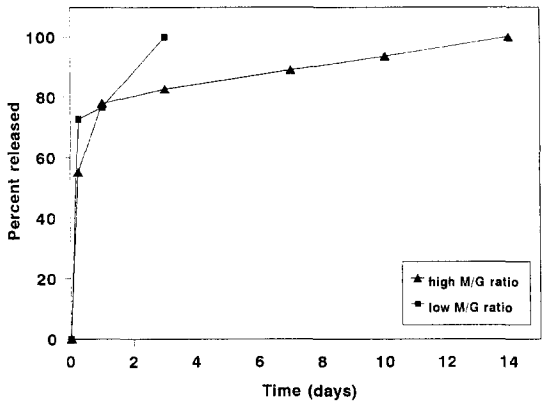

Fig. 3. In vitro drug release profiles of the spheres in phosphate buffered saline solution of pH 7.4.

present (gentamicin-alginate adduct) (Table 3). Besides, gentaimicin would associate in its sulphate form, as the EDS analysis of the adducts, exhibiting a S peak ($p/b = 0.04 \pm 0.01$ and 0.45 ± 0.02 for low and high M/G ratio alginate, respectively), showed.

These results indicated, on one hand, a selectivity of gentamicin for the M blocks, probably owing to the conformational arrangement of the M chains which are more widely spaced that the G chains (Grant et al., 1973). On the other hand, calcium ions were able to compete with gentamicin for the carboxylic acid sites on M blocks, according to the above postulation concerning calcium association, preventing, as a function of their concentration, the saturation of the polymannuronic sequences by the drug.

The drug release profiles for the spheres prepared with either low or high M/G ratio alginate are given in Fig. 3. It can be clearly seen that most of the loaded drug was released within one day, regardless of the M/G ratio. This indicated the disruption of the gentamicin/alginate complex by the ions of the release medium. However, gentamicin was delivered completely within 3 days from the spheres prepared with the low M/G ratio alginate, whereas a sustained drug release of the remaining drug amount was evident for a much longer period for the spheres prepared with the high M/G ratio alginate.

4. Conclusions

The results obtained provided some evidence of the occurrence of an electrostatic interaction between the cationic gentamicin sulphate and the polyanionic alginate. Such an interaction led to significant associated drug fractions within calcium alginate spheres. The extent of drug association was affected by the mannuronic/guluronic ratio of the polysaccharide due to the selective binding of gentamicin at the level of the polymannuronic sequences. Therefore, the alginate having the higher mannuronic acid content, i.e. capable of associating a greater amount of drug and offering a reservoir for a more sustained drug release, is likely to be considered as the more suitable material for the development of a prolonged-action implant.

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