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Effect of the formulation on the in-vitro release of propranolol from gellan beads

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Abstract

Gellan gum beads of propranolol hydrochloride, a hydrophilic model drug, were prepared by solubilising the drug in a dispersion of gellan gum and then dropping the dispersion into calcium chloride solution. The droplets formed gelled beads instantaneously by ionotropic gelation. Major formulation and process variables which might influence the preparation of the beads and the drug release from gellan gum beads were studied. Very high entrapment efficiencies were obtained (92%) after modifying the pH of both the gellan gum dispersion and the calcium chloride solution. The beads could be stored for 3 weeks in a wet or dried state without modification of the drug release. Oven-dried beads released the drug somewhat more slowly than the wet or freeze-dried beads. The drug release from oven-dried beads was slightly affected by the pH of the dissolution medium. Gellan gum could be a useful carrier for the encapsulation of fragile drugs and provides new opportunities in the field of bioencapsulation.

Keywords: Gellan gum; Propranolol hydrochloride; Drug delivery; Beads; Ionotropic gelation

1. Introduction

Gellan gum is a linear anionic polysaccharide produced by the microorganism *Pseudomonas elodea* (Kang et al., 1982; Doner and Douds, 1995). The natural form of the polysaccharide consists of a linear structure of a repeating tetrasaccharide unit of glucose, glucuronic acid and rhamnose (Jansson et al., 1983; O'Neill et al., 1983; Doner and Douds, 1995) in a molar ratio of 2:1:1. Native gellan is partially acylated with acetyl and L-glyceryl groups located on the same glucose residue (Kuo et al., 1986). X-ray diffraction analysis shows that gellan gum exists as a half-staggered, parallel, double helix which is stabilized by hydrogen bonds involving the hydroxymethyl groups of one chain and both

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carboxylate and glyceryl groups of the other (Chandrasekaran et al., 1992). The presence of acetyl or glyceryl groups does not interfere with double helix formation but does alter its ion-binding ability. The commercial gellan gum is the deacylated compound obtained by treatment with alkali (Kang et al., 1982), yielding the gum in its low acyl form in which the acetate groups do not interfere with helix aggregation during gel formation. Gellan forms gels in the presence of mono- and divalent ions, although its affinity for divalent ions is much stronger (Sanderson and Clark, 1983). Milas et al. (1990) showed a mechanism of gelation based on aggregation of the double helix controlled by the thermodynamics of the solution in which the nature of the counter-ion is of prime importance. The apparent viscosity of the gellan gum dispersions can be markedly increased by increases in both pH and cation concentration (Grasdalen and Smidsroed, 1987; Deasy and Quigley, 1991).

Gellan gum is mainly used as a stabilizer or thickening agent and it has a wide variety of applications, particularly in the food industry (Sanderson and Clark, 1983; Anderson et al., 1988), as a bacterial growth media (Shungu et al., 1983; Harris, 1985) and in plant tissue culture (Colegrove, 1983). Its medical and pharmaceutical uses are in the field of sustained release. Due to its characteristic property of temperature-dependent and cation-induced gelation, gellan has been used in the formulation of eye drops which gelify on interaction with the sodium ions naturally present in the eye fluids (Rozier et al., 1989; Greaves et al., 1990; Sanzgiri et al., 1993; Rozier et al., 1997).

Microcapsules containing oil and other core materials have been formed by complex coacervation of gellan gum–gelatin mixtures (Chilvers and Morris, 1987). Deacetylated gellan gum was used to produce a bead formulation containing sulphamethizole by a hot extrusion process into chilled ethylacetate (Quigley and Deasy, 1992). More recently, the ability of gellan gum to form gels in the presence of calcium ions enabled capsules to be prepared by gelation of the polysaccharide around a core containing starch (Alhaique et al., 1995, 1996), or oil (Santucci et al., 1996).

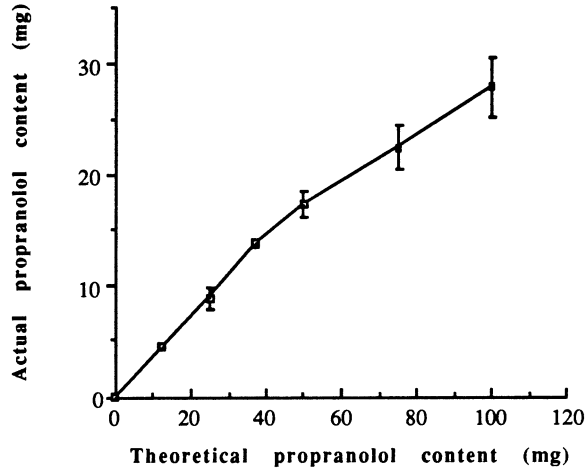

Fig. 1. Effect of propranolol hydrochloride loading on the actual propranolol content in the gellan beads (gellan gum dispersion pH 5.3—pressure 2 bars—$n = 3$).

The objective of this study was to prepare gellan gum beads using a method rather simpler than the ones used so far, i.e. the ionotropic gelation method. Indeed, the interaction between calcium ions and the negatively charged polymer can cause the latter to gelify. Over the last 15 years, a great deal of work has been devoted to this technique. In particular, alginates form beads when dropped into solutions of counter-ions

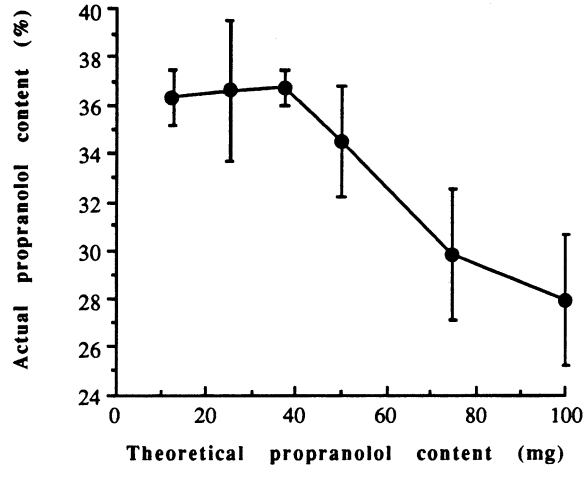

Fig. 2. Effect of propranolol hydrochloride loading on the propranolol content in the gellan beads (gellan gum dispersion pH 5.3—pressure 2 bars—$n = 3$).

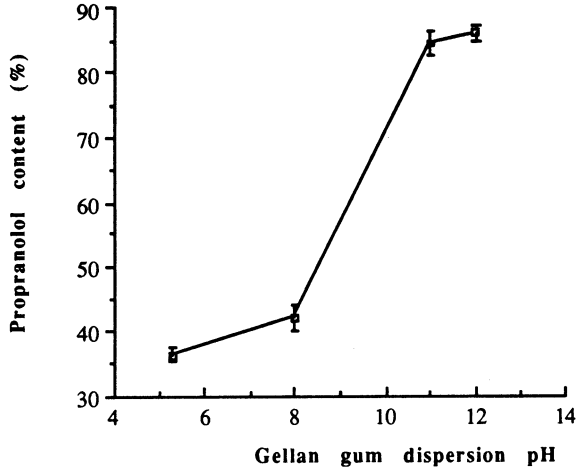

Fig. 3. Effect of the pH of the gellan gum dispersion on propranolol hydrochloride content (%) (Calcium chloride solution pH 6.4—pressure 2 bars—$n = 3$).

(Hwang et al., 1995; Huguet and Dellacherie, 1996; Lim and Wan, 1997). It has the advantage of being carried out under very mild conditions in an aqueous, organic solvent-free environment. In the present study, we developed this method for the preparation of gellan gum beads. Furthermore, we studied the incorporation of a hydrophilic model drug: propranolol hydrochloride. Some formulation and process variables influencing the preparation of the beads and the drug release from gellan gum beads were studied.

2. Materials and methods

2.1. Materials

Gellan gum (Kelcogel®), an anionic polymer in the form of its potassium salt, was a gift from Kelco International (Bagnolet, France). The model drug, propranolol hydrochloride, was obtained from Cooper (Melun, France). Calcium chloride was purchased from Sigma (St Louis, MO). All other products were of analytical grade.

In order to obtain small and reproducible beads, a dispensing system which provided a continuous flow of droplets was used. This consisted of a compressed air spring, a dispensing system (Ultra) made of polypropylene (EFD, Dosage 2000, Bougival, France), a 5-ml disposable barrel/inert polyethylene piston (set EFD, Dosage 2000) and a polypropylene needle.

2.2. Methods

2.2.1. Preparation of the beads

The beads were prepared according to the technique of ionotropic gelation. An appropriate amount of the model drug, propranolol hydrochloride (from 12.5 to 100 mg), was dissolved in 5 ml of deionized water at 55°C. Gellan gum (0.1 g) was added to this stirred solution (300 rpm) at the same temperature until a uniform dispersion was obtained. This homogeneous, bubble-free slurry was added drop-wise, at a pressure of 2 bars, with the disposable syringe into 50 ml of a gently agitated (100 rpm) calcium chloride solution (2% w/v). Immediate formation of small gelled beads was observed. After an appropriate time, the calcium chloride solution was filtered off and the beads were washed with deionized water. Thereafter, the beads were stored either in the wet or dry state. The drying of the gel beads was carried out by either freeze-drying or oven-drying at 37°C for 24 h. All batches were prepared in triplicate. The following preparation variables were investigated in this study: the initial amount of propranolol hydrochloride (12.5, 25, 37.5, 50, 75 or 100 mg), the pH of the calcium chloride solution (6.4, 11 or 12), the pH of the gellan gum dispersion (5.3, 8, 11 or 12) and the stirring time (5 or 7 min).

2.2.2. Characterisation of the beads

2.2.2.1. Drug content and entrapment efficiency. The drug content within the beads was determined indirectly by measuring the concentration of propranolol hydrochloride in the preparation medium and in the washing solutions spectrophotometrically at $\lambda = 289$ nm, using a Perkin-Elmer UV-visible spectrophotometer. The entrapment efficiency (EE) was calculated according to the formula

$$EE = \frac{(\text{amount of added drug} - \text{amount of non encapsulated drug})}{\text{amount of added drug}} \times 100$$

All reported data represent the mean values of three separate experiments.

The solubility of the drug in the calcium chloride solution (2% w/v) was determined by adding an excess of the drug to the medium. Duplicate samples were stirred for 2 days at room temperature. The samples were then filtered through a 0.45-μm filter and the concentration of the drug measured spectrophotometrically after appropriate dilution.

2.2.2.2. Bead size. The diameter of the gel wet beads was measured by optical microscopy (HM-POL, Leitz, Germany). The microscope eyepiece was fitted with a micrometer. The mean diameter was obtained from measurement of 100 beads.

2.2.3. In-vitro drug release

Drug release kinetics were evaluated using 200 ml of dissolution medium (phosphate buffer, pH 6.8 or HCl, pH 2.0) which were placed in a 250-ml beaker, stirred at 300 rpm and maintained at 37 ± 1°C. After addition of the beads, 2-ml samples were taken and replaced with fresh medium at predetermined time intervals. All samples were run in triplicate and assayed spectrophotometrically at $\lambda = 289$ nm either directly or after appropriate dilution. The maximum concentration of propranolol hydrochloride in the release medium was $< 4\%$ of its solubility, so sink conditions were achieved.

3. Results and discussion

3.1. Preparation and characterisation of the beads

Ionotropic gelation is a very well known technique for the preparation of alginate beads. As for alginates, the ionic interactions between the negatively charged gellan polymer and the positively charged counter-ion Ca^{2+}, were used to prepare gellan beads. In contrast to alginates which are freely soluble in water, the commercially available product, gellan gum, is not soluble in cold water. Indeed, the commercially available product of gellan gum, Kelcogel, is presented as the potassium salt, but other ions such as sodium, calcium and magnesium are also present. The low level of divalent ions present in the starting material was sufficient to hold the molecular chains together and inhibit hydration (Doner and Douds, 1995). Consequently, the gellan gum solutions were heated to 55°C before being dropped into the counter-ion solution, so that the polymer could be properly hydrated. To obtain regular beads, 55°C was determined as the optimal temperature. The pH of the calcium chloride solution and gellan gum slurry was 6.4 and 5.3, respectively.

Before drying the gellan beads without propranolol were round and transparent and the wet beads containing propranolol showed an opaque globular body. Dried beads with or without drug appeared whitish with a rough surface.

Table 1
Effect of the storage conditions on propranolol hydrochloride release from the beads (prepared with gellan gum dispersions at pH 12.0) in phosphate buffer medium, pH 6.8 ($n = 3$, $\pm$ S.D.)

Time	7 min (%)	15 min (%)	30 min (%)	45 min (%)	60 min (%)
Wet beads	68.5 ± 1.0	80.8 ± 0.7	82.0 ± 1.0	81.2 ± 0.9	80.7 ± 0.9
Oven-dried beads	58.5 ± 0.5	77.9 ± 0.6	83.6 ± 0.4	83.2 ± 0.5	83.1 ± 0.8
Lyophilised beads	71.6 ± 0.5	82.3 ± 0.9	81.7 ± 0.8	80.9 ± 0.9	80.0 ± 0.8

Table 2
Effect of the dissolution medium on propranolol hydrochloride release from wet and dried beads prepared with gum gellan dispersions prepared at pH 5.3 and 12 ($n = 3$, $\pm$S.D)

Time	7 min (%)	15 min (%)	30 min (%)	45 min (%)	60 min (%)
Gellan gum pH 5.3					
Wet beads diss. pH = 2	50.4 ± 0.8	53.9 ± 0.4	53.7 ± 0.4	53.2 ± 0.4	52.8 ± 0.2
Wet beads diss. pH = 6.8	43.6 ± 1.1	51.5 ± 0.9	52.3 ± 1.0	52.0 ± 1.0	51.4 ± 0.9
Oven-dried beads diss. pH = 2	52.3 ± 1.4	55.5 ± 0.9	55.4 ± 0.9	55.1 ± 1.1	54.4 ± 1.4
Oven-dried beads diss. pH = 6.8	36.1 ± 0.3	50.6 ± 1.0	54.9 ± 0.9	54.2 ± 0.9	53.2 ± 0.8
Gellan gum pH 12.0					
Wet beads diss. pH = 2	62.9 ± 0.5	81.5 ± 0.6	82.0 ± 0.7	81.0 ± 0.9	80.5 ± 0.9
Wet beads diss. pH = 6.8	68.5 ± 1.0	80.8 ± 0.7	82.0 ± 1.0	81.2 ± 0.9	80.7 ± 0.9
Oven-dried beads diss. pH = 2	60.5 ± 0.9	83.0 ± 0.4	83.3 ± 0.9	82.6 ± 0.8	82.0 ± 0.8
Oven-dried beads diss. pH = 6.8	58.5 ± 0.5	77.9 ± 0.6	83.6 ± 0.4	83.2 ± 0.5	83.1 ± 0.8

3.2. Influence of the drug loading on the incorporation efficiency

The incorporation efficiency was determined as a function of different initial propranolol hydrochloride loading (12.5–100 mg). When the total amount of propranolol was increased from 12.5 to 75 mg, the beads were still round but their diameter increased with the amount of drug. This effect can be explained by an increase in the viscosity of the gellan solution which therefore flowed more slowly out of the syringe. Consequently, the drops and then the beads had a larger diameter. Moreover, the beads with a theoretical charge of 100 mg of drug were rather soft and sticky. In this case, there were equal quantities of gellan gum and drug in the dispersion and it is possible that the cationic drug could partly mask the negatively charged groups of the gellan gum, which were no longer available to the Ca^{2+} counter-ions.

Fig. 1 shows that an increase in the initial drug loading enhanced the incorporation of the drug. Indeed, the amount of propranolol hydrochloride incorporated varied from 4.5 to 28 mg for initial loadings of 12.5–100 mg for 0.1 g of gellan gum. Fig. 2 presents the incorporation efficiencies as a function of the initial drug concentration. Around 36% of propranolol was incorporated when the initial drug loadings were 12.5–37.5 mg. Thereafter, from 50 mg, the incorporation efficiencies decreased with increasing drug concentration. Although these incorporation figures seem low, it has to be kept in mind that the encapsulation process is carried out in an aqueous environment, where the drug is freely soluble. Therefore, this technique could be used for fragile drugs when it is not possible to use organic solvent.

3.3. Increase of the drug incorporation efficiencies

In order to improve the incorporation of propranolol hydrochloride into the beads, three different procedures were investigated. These methods were selected in order to limit drug losses into the external calcium chloride medium during bead formation.

3.3.1. Modification of the pH of the calcium chloride solution

The pH of the calcium chloride aqueous phase, 6.4 in the first experiments, was adjusted with NaOH to minimize drug solubility. Since the pK_a of propranolol hydrochloride is 9.45, its solubility rapidly decreased above this value. We observed that an increase of the pH of the calcium chloride solution from 6.4 to 11 did not modify the incorporation efficiency of the drug (36%). At pH 12, the percentage of encapsulated propranolol was enhanced (52%). After addition of the slightly acidic gellan solution, the pH of the overall operation medium dropped to 9.9 and 11.9 respectively, for initial calcium chloride solutions of pH 11 and 12. The difference in encapsulation efficiency is due to the properties of the drug (soluble or insoluble) at

Table 3
Effect of time storage on propranolol hydrochloride release from wet and oven-dried beads prepared with gellan gum dispersions at pH 12 ($n = 3$, $\pm$S.D.)

Time	7 min	15 min	30 min	45 min	60 min
Time = 0 weeks					
Wet beads	68.5 ± 1.0	80.8 ± 1.0	82.0 ± 1.0	81.2 ± 0.9	80.7 ± 0.9
Oven-dried beads	58.5 ± 0.5	77.9 ± 0.6	83.6 ± 0.4	83.2 ± 0.5	83.1 ± 0.8
Time = 3 weeks					
Wet beads	68.0 ± 0.2	81.3 ± 0.2	82.1 ± 0.3	81.5 ± 0.2	80.7 ± 0.1
Oven-dried beads	53.2 ± 0.6	78.1 ± 0.9	82.2 ± 1.0	81.5 ± 0.9	80.8 ± 0.4

the two pH values (9.9 and 11.9). At pH 9.9 the drug is still soluble and there is no increase in the encapsulation ratio. When the beads were prepared with a calcium chloride solution adjusted at pH 12, we observed a white halo inside the transparent beads, which was a precipitate of the drug.

3.3.2. Modification of the pH of the gellan gum dispersion

The effect of modifying the pH of the gellan gum dispersion before its mixing with the counter-ion solution was also evaluated. Fig. 3 shows that increasing the pH of the gellan gum dispersion to one greater than the pK_a of the drug, led to a better incorporation of the drug, which precipitated inside the beads. Insoluble drug was entrapped in the gel matrix. Indeed, for gellan gum dispersion pH values of 11 and 12, the incorporation efficiencies were 84 and 86%, respectively, and the beads were completely white.

3.3.3. Drug loading as a function of stirring time

Due to the hydrophilic nature of the drug and the overall aqueous environment, stirring time may be one parameter strongly influencing the encapsulation. Therefore, in order to decrease the leakage of the incorporated propranolol hydrochloride into the calcium chloride solution during bead preparation, the stirring time was reduced from 7 to 5 min. Consequently, the syringe was emptied much faster. This was achieved by increasing the pressure from 2 to 2.5 bars. However, the encapsulation efficiency was only increased by $\approx 7\%$.

In conclusion, the incorporation of propranolol hydrochloride into the beads can be improved (i) by modification of the pH of both the gellan gum and the calcium chloride solutions before they are mixed; and (ii) by a reduction of the stirring time. When an optimized protocol is employed the incorporation efficiency can be as high as 92%.

3.4. Dissolution studies

3.4.1. Effect of storage conditions

In order to evaluate the influence of the bead storage conditions (in a wet or dry state) on the release of the drug, the beads were stored in sterilized flasks. The drying of the beads was accomplished by either freeze-drying or oven-drying for 24 h at 37°C. The results obtained after release of the propranolol hydrochloride in phosphate buffer medium, pH 6.8, are presented in Table 1. Drug release was fast in all cases and was maximal after 15 min. It has to be noted that $\approx 20\%$ of the drug remains complexed, i.e. non released, within the beads. When the beads were dried in an oven at 37°C, the drug was released somewhat more slowly than from either the wet or the freeze-dried beads. This can be explained by the different swelling kinetics of the oven-dried beads. Furthermore, it has been shown that no degradation of the drug occurred during storage in an aqueous medium, since the percentages of drug released were the same regardless of the storage conditions.

On the other hand, the maximum percentage of propranolol released in medium at pH 6.8 was 80–83%. Similar percentages were obtained in the same medium with gellan capsules using theophylline and benzamide as model drugs (Alhaique et al., 1996). About 20% of the drug was

Table 4
Effect of bead diameter on propranolol hydrochloride release from wet beads in dissolution medium at pH 2.0 ($n = 3$, $\pm$S.D.)

Bead diameter (mm)	Time				
	7 min	15 min	30 min	45 min	60 min
2.09	60.5 ± 0.9	83.0 ± 0.4	83.3 ± 0.9	82.6 ± 0.8	82.0 ± 0.8
2.59	58.8 ± 1.0	81.7 ± 0.5	85.7 ± 0.8	84.9 ± 0.7	84.0 ± 1.0
3.35	41.7 ± 1.9	70.5 ± 0.4	87.1 ± 0.7	86.9 ± 0.8	86.2 ± 0.9

entrapped within the beads and could not be released.

3.4.2. Effect of dissolution medium

In order to evaluate the influence of the dissolution medium on drug release, the results obtained in buffer at pH 6.8 were compared with results from a medium at pH 2.0. The results showed (Table 2) that the wet beads released the drug (maximum 80%) in 15 min in both dissolution media. In contrast, drug release from oven-dried beads was slightly affected by the pH. This small increase in the delivery rate observed after 7 min in the acidic environment cannot be related to the different solubilities of the drug at pH 2.0 and 6.8, because the maximum amount of drug released from the wet beads was the same in the two dissolution media. It could be explained by modifications of the gel structure due to the competition between H^+ and Ca^{2+} ions which could affect the swelling rate of the beads and consequently the penetration of the solvent into the beads (Alhaique et al., 1996). Indeed, reduced ionisation of carboxyl groups results in decreased polymer extension in aqueous medium and a modification of gel structure, which affects the overall release mechanism, may occur (Deasy and Quigley, 1991). This hypothesis was supported by the non-swelling of the beads in medium at pH 2.0. Although the drug was released more quickly in medium at pH 2.0, the total amounts released were the same in the two media and never greater than either 50% for the beads prepared with the gum dispersion at normal pH or 80% when the gum dispersion was alkalinised to pH 12. We calculated that ≈ 2.5 mg of drug was still entrapped within the beads in each case. It is known that gellan gum forms gels with almost all ions, but its affinity for each ion is different. Therefore, a possible explanation is that the propranolol ion had a stronger affinity for the gellan gum than divalent Ca^{2+} ions and therefore formed an association with the gum. The same quantity of propranolol was retained because the same quantity of gum was used. Such an interaction between the polymer and the drug has been reported for propranolol hydrochloride with alginate chains in calcium alginate beads (Lim and Wan, 1997).

3.4.3. Effect of storage time

In order to evaluate whether the beads could be stored beyond a 24-h period, wet and dried beads prepared with gellan gum dispersion at pH 5.3 and 12.0 were stored for 3 weeks. The beads were dispensed in sterilised flasks and stored in the wet state at ± 2°C and at room temperature when dried. After this storage period, a dissolution study was carried out in dissolution medium at pH 6.8. The results summarised in Table 3 indicate that there was no modification of the release kinetics of propranolol hydrochloride from the beads after 3 weeks of storage.

3.4.4. Effect of the bead diameter

In order to evaluate whether the bead diameter had an influence on the release kinetics of the entrapped drug, beads were prepared with three needle diameters: 0.15, 0.70 and 1.2 mm. The resulting diameters of the beads were 2.09, 2.59 and 3.35 mm, respectively. As indicated in Table 4, the drug was released most quickly from the smallest beads. The maximum amount of drug was still released after 15 min by the small wet beads, but only after 30 min by the largest ones in dissolution medium at pH 2.0. The contact area between the beads and the dissolution medium was larger with

the small beads, which explains the faster release rate of the drug.

4. Conclusion

This study has shown that a hydrophilic drug, propranolol hydrochloride, can be encapsulated in gellan gum beads in an aqueous environment by the ionotropic gelation method. Optimal preparation conditions allowed a very high incorporation efficiency (92%). Storage conditions (wet or dry state) and the time of storage did not modify the release of the drug. With the wet beads, the dissolution medium had no effect on drug release. In contrast, oven-dried beads released the drug slightly faster in medium at pH 2.0. The drug release is too rapid for practical applications, but further research efforts will focus on providing more sustained release. Finally, that ionotropic gelation with gellan gum seems to offer new opportunities in the field of bioencapsulation and could be useful for the encapsulation of fragile drugs.

References

Alhaique, F., Carafa, M., Coviello, T., Murtas, E., Riccieri, F.M., Santucci, E., 1995. Gellan in sustained release formulations: release of theophylline from beads and gel capsules. Proc. Control. Release Soc. 22, 286–287.

Alhaique, F., Santucci, E., Carafa, M., Coviello, T., Murtas, E., Riccieri, F.M., 1996. Gellan in sustained release formulations: preparation of gel capsules and release studies. Biomaterials 17, 1981–1986.

Anderson, D.M.W., Brydon, W.G., Eastwood, M.A., 1988. The dietary effects of gellan gum in humans. Food Addit. Contam. 5, 237–249.

Chandrasekaran, R., Radha, A., Thailambal, V.G., 1992. Roles of potassium ions, acetyl and L-glyceryl groups in native gellan double helix: an X-ray study. Carbohydr. Res. 224, 1–17.

Chilvers, G.R., Morris, V.J., 1987. Coacervation of gelatin–gellan gum mixtures and their use in microencapsulation. Carbohydr. Polym. 7, 111–120.

Colegrove, G.T., 1983. Agricultural applications of microbial polysaccharides. Ind. Eng. Chem. Prod. Res. Dev. 22, 456–460.

Deasy, P.B., Quigley, J., 1991. Rheological evaluation of deacetylated gellan gum (gelrite) for pharmaceutical use. Int. J. Pharm. 73, 117–123.

Doner, L.W., Douds, D.D., 1995. Purification of commercial gellan to monovalent cation salts results in acute modification of solution and gel-forming properties. Carbohydr. Res. 273, 225–233.

Grasdalen, H., Smidsroed, O., 1987. Gelation of gellan gum. Carbohydr. Polym. 7, 371–393.

Greaves, J.L., Wilson, C.G., Rozier, A. A., Grove, J., Plazonnet, B., 1990. Scintigraphic assessment of an ophthalmic gelling vehicle in man and rabbit. Curr. Eye Res. 9, 415–420.

Harris, J. E., 1985. Gelrite as an agar substitute for the cultivation of mesophilic *Methanobacterium* and *Methanobrevibacter* species. Appl. Environ. Microbiol. 50, 1107–1109.

Huguet, M.L., Dellacherie, E., 1996. Calcium alginate beads coated with chitosan: effect of the structure of encapsulated materials on their release. Process Biochem. 31, 745–751.

Hwang, S.J., Rhee, G.J., Lee, K.M., Oh, K.H., Kim, C.K., 1995. Release characteristics of ibuprofen from excipient-loaded alginate gel beads. Int. J. Pharm. 116, 125–128.

Jansson, P.E., Lindberg, B., Sandford, P.A., 1983. Structural studies of gellan gum, an extracellular polysaccharide elaborated by a *Pseudomonas elodea*. Carbohydr. Res. 124, 135–139.

Kang, K.S., Veeder, G.T., Mirrasoul, P.J., Kaneko, T., Cottrell, I.W., 1982. Agar-like polysaccharide produced by *Pseudomonas* species: production and basic properties. Appl. Environ. Microbiol. 43, 1086–1091.

Kuo, M.S., Mort, A.J., Dell, A., 1986. Identification and location of L-glycerate, an unusual acyl substituent in gellan gum. Carbohydr. Res. 156, 173–187.

Lim, L.Y., Wan, L.S.C., 1997. Propranolol hydrochloride binding in calcium alginate beads. Drug Dev. Ind. Pharm. 23, 973–980.

Milas, M., Shi, X., Rinaudo, M., 1990. On the physicochemical properties of gellan gum. Biopolymers 30, 451–464.

O'Neill, M.A., Selvendran, R.R., Morris, V.J., 1983. Structure of the acidic extracellular gelling polysaccharide produced by *Pseudomonas elodea*. Carbohydr. Res. 124, 123–133.

Quigley, K.J., Deasy, P.B., 1992. Use of deacetylated gellan-gum (Gelrite) for the production of sulphamethizole containing beads. J. Micoencapsulation 9, 1–7.

Rozier, A., Mazuel, C., Grove, J., Plazonnet, B., 1989. Gelrite®: a novel, ion activated, in situ-gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol. Int. J. Pharm. 57, 163–168.

Rozier, A., Mazuel, C., Grove, J., Plazonnet, B., 1997. Functionality testing of gellan gum, a polymeric excipient material for ophthalmic dosage forms. Int. J. Pharm. 153, 191–198.

Sanderson, G.R., Clark, R.C., 1983. Gellan gum. Food Technol. 37, 62–70.

Santucci, E., Alhaique, F., Carafa, M., Coviello, T., Murtas, E., Riccieri, F.M., 1996. Gellan for the formulation of sustained delivery beads. J. Control. Release 42, 157–164.

Sanzgiri, Y.D., Maschi, S., Crescenzi, V., Callegaro, L., Topp, E.M., Stella, V.J., 1993. Gellan-based systems for ophthalmic sustained delivery of methylprednisolone. J. Control. Release 26, 195–201.

Shungu, D., Valiant, M., Tutlane, V., Weinberg, E., Weissberger, B., Koupal, L., Gadebusch, H., Stapley, E., 1983. Gelrite as an agar substitute in bacteriological media. Appl. Environ. Microbiol. 46, 840–845.