Preparation and *in vitro* release of hydrochlorothiazide from gellan beads produced by ionotropic gelation

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The inherent property of gellan gum to gel and form circular beads through a process of ionotropic gelation in the presence of cations was utilized in the formulation of hydrochlorothiazide (HCTZ). The prepared beads exhibited good drug entrapment efficiency, content uniformity, and sustained release potential. The gelatin-HCTZ beads could be utilized in the delivery of HCTZ, by which reduced frequency of the drug administration could be achieved.

**Key words:** Beads, entrapment efficiency, hydrochlorothiazide, ionotropic gelation

**INTRODUCTION**

Gellan gum is a bacterial exopolysaccharide prepared commercially by aerobic submerged fermentation of *Sphingomonas elodea*, in a manner similar to xanthan gum.[1] It forms gels in the presence of mono- and divalent ions.[2] Its ability to form gels makes it suitable as a structuring and gelling agent in foods, as a gel for toothpastes, and as structural additives for personal care products.[3] It has been employed in the recovery of deoxyribonucleic acid (DNA) in gel electrophoresis, in pharmaceutical formulations as a binder,[15] as a sustained release matrix,[5-8] and as a component of ophthalmic preparations.[7] The gum has been used in combination with gelatin to form coacervates.[8] Its ability to form gel in the presence of cations has been utilized in the formulation of drug-gellan beads.[9] The objective of the present study is to prepare hydrochlorothiazide-gellan gum beads by the ionotropic gelation method and to assess its *in vitro* release. Hydrochlorothiazide is a diuretic and is employed as a model drug because it possesses the desirable properties that make it amenable for sustained release formulation.

**MATERIALS AND METHODS**

**Materials**
The following materials were used as procured from their manufacturers, without further purification. Hydrochlorothiazide (Industria chimica profarmaco, S.P.A. Italy), gellan gum (kelcogel, Merck, Sharp and Dome USA), hydrochloric acid, and calcium chloride (sigma chem. Co, USA). All other reagents used were of Analar grade.

**Methods**

**Preparation of beads**

Hydrochlorothiazide-gellan gum beads were prepared by the technique of ionotropic gelation.[10] An appropriate amount of hydrochlorothiazide (50-100 mg) was dissolved in 5 ml of deionized water at 55°C. Gellan gum (0.1 g) was added to it and the mixture was stirred at 55°C until uniform dispersion was obtained. The homogenous bubble-free slurry obtained was added dropwise with a dropper into 40 ml of 2% calcium chloride solution. Circular beads were immediately formed. The beads formed were harvested by filtration, washed with deionized water, and dried in an oven at 37°C for 24 hours. The mixture of calcium chloride solution and the wash deionized water was assayed for hydrochlorothiazide spectrophotometrically at 272 nm in a UV/Vis-2102 PC spectrophotometer.

**Analysis of beads**

Drug content and entrapment efficiency

The drug content of the beads was determined indirectly by measuring the concentration of hydrochlorothiazide in the preparation solution spectrophotometrically.
The entrapment efficiency (EE) was calculated using Equation 1:

\[
EE = \left( \frac{\text{Amount of drug added} - \text{Amount of drug in the preparation medium}}{\text{Amount of drug added}} \right) \times 100\%
\]

Particle size analysis
Mean particle sizes of the beads were estimated using the method of sieves. Weighed quantity (1.0 g) of the beads was placed in a 1.7 mm stainless steel sieve connected to a 1.0 mm stainless steel sieve. A collecting pan was placed at the bottom and the sieves were subjected to shaking in a mechanical shaker for 5 minutes. Beads retained on the 1.7 mm and 1.0 mm sieves were weighed. The average diameter (particle size) of the beads was calculated as the arithmetic mean of the sieve apertures.

Filling of beads into hard gelatin capsule shells
The beads were manually filled into No. 2 hard gelatin capsule shells. Ten capsules from each batch were weighed individually on an electron balance. Their mean and coefficient of variation were calculated.

Content uniformity of filled capsules
Ten capsules picked randomly from each batch were assayed for hydrochlorothiazide content individually. The content of each capsule was emptied into a mortar, pulverized with a pestle, and 10 ml of 0.1 N hydrochloric acid was added. The mixture was stirred and 80 ml of 0.1 N hydrochloric acid was added. The dispersion was stirred with a stirring rod for 5 minutes, filtered into a 100 ml capacity volumetric flask. The volume was made up to 100 ml with 0.1 N hydrochloric acid and the absorbance was read at 272 nm in a spectrophotometer.

Drug release study
A drug release study was carried out in 0.1 N hydrochloric acid (pH 1.2) simulated gastric fluid (SGF, pH 2.2) and simulated intestinal fluid (SIF, pH 7.5). The Erweka dissolution apparatus DF model, fitted with a paddle that was operated at 50 rpm, was used. The capsule was placed in a basket immersed half way into 500 ml dissolution medium. The dissolution temperature was maintained at 37 ± 1°C. Ten milliliters (10 ml) of the sample was withdrawn and replaced with fresh dissolution medium maintained at the same temperature at a predetermined time interval. Withdrawn samples were analyzed for drug content at 272 nm for 0.1 N hydrochloric acid and SIF and at 275 nm for SGF in a spectrophotometer. Data presented here represent the mean of two replicate experiments.

Statistics
The data were expressed as mean ± S.D. The significance of the drug release results was assessed by an analysis of variance (ANOVA). A P value of less than 0.01 was considered significant.

RESULTS AND DISCUSSION

The mean diameter of the beads is shown in Table 1. The EE at drug loads of 50, 75, and 100 mg were 97.72, 98.96, and 99.96%, respectively. The results indicate no significant difference (P > 0.01) in EE, with an increase in drug concentration. The mean diameter of the beads increased with an increase in the drug load [Table 1]. This is accounted for by the increased viscosity of the drug-gellan slurry and the consequent slow flow through the dropper, which resulted in beads with larger diameters.

The release profiles of hydrochlorothiazide in 0.1 N hydrochloric acid, SGF, and SIF are shown in Figures 1-3. There was very slow release of the drug in 0.1 N hydrochloric acid, irrespective of the drug load. Generally, hydrochlorothiazide release was significantly (P < 0.01) faster in SIF than in SGF and 0.1 N HCl, from all the beads. This was consistent with the observed swelling of the beads during the experiment. The beads swelled in SIF and SGF to varying degrees, while no visible swelling was observed in 0.1 N hydrochloric acid. Gellan gum is known to form gel with mono- and divalent ions, to different degrees. It is evident that the presence of cations in SGF (Na+) and in SIF (Na+, K+) altered the solubility of hydrochlorothiazide leading to the enhanced release of the drug in these media. It is possible also that hydrochlorothiazide had a stronger affinity for the gellan gum than the divalent calcium ion, thereby forming association with the gum. Consequently, upon swelling, the solubilized drug inside the beads diffused out into the dissolution medium. Hydrochlorothiazide release from the beads followed a sustained release pattern [Figures 1-3] indicating the usefulness of the bead formulation of hydrochlorothiazide as a means of delivery of the drug in a patient on diuretic medication. For hypertensive patients who are on diuretic medication, in addition to other antihypertensive drugs, the dosing frequency is reduced, patient compliance is assured, and sustained diuretic action is achieved, with resultant reduction in blood pressure. This is an obvious advantage over the conventional immediate release hydrochlorothiazide.

Release mechanisms from the beads
Release data were fitted into the korsmeyer-Peppa’s release model[11] given by Equation 2:

\[
\frac{M_t}{M_\infty} = k^n
\]

Where \( M_t \) is the amount of drug released at time \( t \), \( M_\infty \) is the amount of drug released at infinite time, \( K \) is the kinetic constant incorporating the structural and geometric characteristics of the beads and \( n \) is the diffusional exponent indicative of the release mechanism.

Values of \( n = 0.5 \) represent Fickian diffusion, \( 0.5 < n < 1.0 \).
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The release rate, K, was relatively high in all three media indicating burst drug release from the beads.\[13,14\] From the values of K in Table 2, it is evident that the burst effect was more in the acidic media (0.1 N HCl and SGF) than in the slightly alkaline medium of SIF, irrespective of the amount of drug incorporated. This mechanism may be responsible for the shorter Cmax of the drug in SGF and 0.1 N HCl for all the formulations.

CONCLUSION

Gellan-HCTZ bead system can be employed in the delivery of HCTZ in antihypertensive treatment or in patients requiring diuresis where sustained action of the drug is required.

REFERENCES


Table 2: Release parameters of hydrochlorothiazide from gellan-HCTZ beads

<table>
<thead>
<tr>
<th>Batch</th>
<th>0.1 N HCl</th>
<th>SGF</th>
<th>SIF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>k</td>
<td>n</td>
</tr>
<tr>
<td>I</td>
<td>0.846</td>
<td>2.2623</td>
<td>1.151</td>
</tr>
<tr>
<td>II</td>
<td>0.826</td>
<td>2.623</td>
<td>1.075</td>
</tr>
<tr>
<td>III</td>
<td>0.917</td>
<td>2.621</td>
<td>1.160</td>
</tr>
</tbody>
</table>

n = diffusion exponent; k = release rate constant; Batch-I-50 mg HCTZ; Batch-II-75 mg; Batch-III-100 mg.

\[12\] Results in Table 2 show that, at drug loads of 50, 75, and 100 mg, the release of HCTZ followed non-Fickian diffusion in 0.1N HCl and SIF and supercase II transport in SGF. The release rate, K, was relatively high in all three media indicating burst drug release from the beads.\[13,14\] From the values of K in Table 2, it is evident that the burst effect was more in the acidic media (0.1 N HCl and SGF) than in the slightly alkaline medium of SIF, irrespective of the amount of drug incorporated. This mechanism may be responsible for the shorter Cmax of the drug in SGF and 0.1 N HCl for all the formulations.

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