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FREEZE-DRIED NANOCOMPOSITE GEL BEADS FOR ORAL DRUG DELIVERY: IN VITRO SIMULATION OF GASTRO-INTESTINAL DRUG RELEASE

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Abstract
We investigated entrapment efficiency, swelling and drug release from freeze-dried gel beads prepared with Gellan gum and a synthetic clay, Laponite. Polymeric beads loaded with two model molecules having different molecular weights were prepared and subjected to in vitro release studies in simulated gastric and intestinal fluids. The experimental observations confirm that laponite may be an effective additive for fabricating sustained drug delivery systems from gellan gum by means of ionotropic gelation and freeze-drying.

Keywords: *gellan gum/laponite composite, ionotropic gelation, freeze-drying, gastrointestinal drug release; swelling/drug release modelling.*

1. Introduction

Orally administered dosage forms are the most convenient formulations due to the easiness of employment, pre-determined and measured doses and overall non-invasive nature of administration.

The successful oral formulation should deliver the required therapeutic dose to the specific site of action during the treatment period. However, the delivery of a drug by a simple conventional dosage form normally results in the immediate release of the active pharmaceutic ingredient and their use usually requires a high frequency of administration and uncontrolled absorption. These considerations have guided researchers to focus their efforts on improving oral delivery systems with the development of formulations providing more predictable release rates as well as an increased bioavailability. Sustained release formulations are extensively investigated in order to reduce the dosing frequency, thus resulting in increased patience compliance.

Natural gums, such as gellan gum (GG), an anionic, high molecular weight polysaccharide, have gained significant interest in the pharmaceutical field [Osmalek *et al.* 2014]. It consists of tetra-saccharide repeating units: α-l-rhamnose, β-d-glucuronic acid and β-d-glucose in the molar ratio 1:1:2. GG has proven to be a versatile material in the formulation of polymeric hydrogels, including beads systems, due to its temperature sensitivity and ability to gel under mild conditions. In fact, it forms stable hydrogel networks in the presence of cationic cross-linkers [Patil *et al.* 2012, Lopez-Cebral *et al.* 2013], so that ionotropic gelation method can be employed for the synthesis of polymeric networks using divalent cations as cross-linking agents [Patela *et al.* 2017, Benfattoum *et al.* 2018]. The contact of the polymer with cations results in the instantaneous formation of a gel matrix containing uniformly dispersed material throughout the crosslinked gellan gum matrix.

Polymeric beads are widely used for oral sustained release; after beads are ingested, the drug will slowly diffuse out from the polymer matrix, resulting in a prolonged release of the active agent. Nevertheless, some drawbacks, related to the higher porosity of the matrix or poor mechanical resistance of the polymeric network, could lead to a rapid and massive release in acidic dissolution medium [Gupta *et al.* 2000]. Only a few polymers can be used in their pure form for the formulation

of oral sustained release beads and therefore their combination with other biocompatible materials has been investigated in order to overcome these drawbacks. Clay minerals are one of the fillers that can be used, in combination with many biopolymers, to improve their drug delivery properties [Meirelles *et al.* 2017, Li *et al*. 2011, Wu *et al*. 2010, Haraguci *et al.* 2002]. The ultimate goal is to bring together in the same material the best properties of the natural polymer and clay since each component plays a key role in improving the properties of the nanocomposite hydrogels.

In this scenario, clay hydrogel beads have been widely investigated in oral drug delivery applications, showing that mineral clays can be successfully used as functional additives in the development of bead-modified systems [Raut *et al.* 2019].

The most commonly used clay minerals belong to the smectite family. Among the smectite family, laponite $Na_{0.7}[(Si_8Mg_{5.5}Li_{0.3})O_{20}(OH)_4]_{0.7}$ is a synthetic clay composed of a layered structure (30–25 nm diameter, 1 nm thickness) that has been used to synthesize a wide range of nano-composite hydrogels [Yang *et al.* 2011, Li *et al.* 2009, Pacelli *et al.* 2016]. Specifically, laponite (LAPO) nanoparticles can be uniformly dispersed within the polymeric matrix where they self-arrange and act as both filler and cross-linker during gel formation [Haraguchi 2011, Da Silva *et al*. 2018].

This study aims to verify the possibility of using laponite as an additive clay mineral to design new composite gellan gum beads with highly specific characteristics, such as appropriate swelling properties and release kinetics.

Gellan gum (GG) was used to fabricate spherical porous beads suitable as sustained drug delivery systems for oral administration. GG was cross-linked with calcium ions to prepare polymeric beads. Rheological studies and preliminary experiments of beads preparation allowed to identify the GG and the $CaCl_2$ concentrations suitable for obtaining stable and spherical particles before and after freeze-drying. GG beads were formed, through ionotropic gelation technique, with and without the presence of the synthetic clay laponite. The resultant beads were analyzed for dimensions (before and after freeze-drying), morphological aspects and ability to swell in different media miming biological fluids, namely SGF (Simulated Gastric Fluid, HCl 0.1 M) and SIF (Simulated Intestinal Fluid, phosphate buffer, 0.044 M, pH 7.4). The highly porous GG and GG-layered silicate composite beads were loaded with two model drugs having different molecular weight, namely theophylline (MW 180, van der Waals radius 3.7 Å, aqueous solubility 8.3 mg/mL; pKa 8.6) and vitamin B12 (MW 1356, van der Waals radius 21 Å, aqueous solubility 10–33 mg/mL, pKa = 3.28) and subjected to in-vitro release studies in SGF and SIF in order to verify the possible use of freeze-dried beads for the oral administration of drugs.

The experimental observations confirm that laponite may be an effective additive for fabricating sustained drug delivery systems.

2. Material and method

Gellan gum (0.11, 0.165 or 0.22 g) was added to 11 mL of double distilled water and maintained under stirring for 5 h at 80 °C until a homogeneous solution was produced. This solution was cooled and kept at 40 °C. Then, 10 mL of this solution were carefully loaded into a syringe with a 21G needle, ensuring no air bubbles were present, and added to a solution of calcium chloride (50 mL, 0.3% and 0.6% *w/w*) drop wise. The beads were left cross-linking for 10 min (curing time), then filtered and washed four times with 10 mL of deionized water and freeze-dried. The curing time was optimized to have maximum entrapment efficiency of the model molecules used. In fact, while longer curing times increase the degree of crosslinking of the polymer, they also promote the effusion of the loaded molecule out of the beads, thus reducing the final drug loading.

Beads including laponite were produced starting from a solution (11 mL) of GG (1.5% *w/w*) and laponite (1.0% *w/w*) added drop wise to the solution of $CaCl_2$ (0.3% *w/w*), thus following the same procedure adopted for beads without laponite.

The diameter of fresh and freeze-dried beads was measured with a calliper along two orthogonal directions, taking the average of the measurements as the mean diameter of the beads, whereas the ratio between the two measurements was taken as the aspect ratio of the beads.

In order to quantify the swelling degree of the beads, 10 freeze-dried beads were weighed and placed into a tulle net and submerged into 25 mL of simulated gastric fluid (SGF, HCl 0.1 M) or simulated intestinal fluid (SIF, phosphate buffer 0.044 M, pH 7.4), maintained at 37.0 ± 0.5 °C. After

5 min, the beads were removed, lightly blotted on paper to remove the excess liquid and weighed. The beads were then submerged back into the medium and the process was repeated at established time intervals up to 24 h. The experiments were carried out in triplicate with each value representing the mean ± SD. The swelling degree was calculated using the following equation:

$$Swelling\ degree\ (S) = \frac{weight\ of\ swollen\ beads\ (g)\ -\ weight\ of\ dry\ beads\ (g)}{weight\ of\ dry\ beads\ (g)}.$$

For the preparation of drug-loaded beads, Gellan gum (0.165g) was dissolved in 9 mL of distilled water using the method described in Section 2.3. Theophylline or vitamin B12 (0.0146 g) were solubilized in 2 mL of water and added to the cooled gellan gum solution, to make a final volume of 11 mL and a concentration of 1.5% *w/w* of GG. The solution was stirred at 100 rpm for 10 min to ensure the drug homogeneously dispersed. The beads were then formed using the method described above. Drug loaded beads including laponite were prepared from a starting solution (9 mL) of gellan gum (0.165 g) and laponite (0.11 g) and then following the same procedure adopted for drug loaded beads without laponite.

In order to determine the quantity of drug loaded into the beads, 15 mg of freeze-dried beads were stirred vigorously in SIF for 1 h, to destroy the beads and extract the drug. The solution was filtered and analysed by HPLC**.** Theophylline was monitored at λ = 280 nm and vitamin B12 at λ = 360 nm. The drug entrapment efficiency was calculated using the following equation:

$$\text{Drug entrapment efficiency (\%)} = \frac{actual\ drug\ content\ of\ beads}{theoretical\ drug\ content\ of\ beads} \times 100.$$

Release studies from drug loaded beads with different formulations were performed separately in SGF (HCl 0.1 M) and in SIF (phosphate buffer, pH 7.4) and sequentially in SGF and SIF to simulate the drug release in the entire gastrointestinal tract. A total of 15 mg of drug loaded beads were added to a known volume V_{ref} of SIF or SGF, warmed to 37 °C in a water bath and stirred continuously at 200 rpm. At defined times, from 1 to 240 min, 1 mL of solution was withdrawn and replaced with 1 mL of fresh solution. Different volumes V_{res} = 50, 75, 100, 150, 175 mL were considered in order to investigate the influence of the release volume V_{res} on release curves.

For gastrointestinal in-vitro release experiments, 15 mg of drug-loaded beads were added to 100 mL of SGF, warmed to 37 °C in a water bath and stirred continuously at 200 rpm. At defined times, from 1 to 120 min, 1 mL of solution was withdrawn and replaced with 1 mL of fresh SGF. After 120 min, the beads were drained to remove excess acid and transferred into 50 mL of SIF. Every 15 min, 1 mL of solution was withdrawn and replaced with the same volume of SIF until 240 min and then again after 24 h. By considering that, after the first 120 min, the beads had released from 60% to 95% of the initially loaded drug, depending on the bead formulation, we chose to carry out the subsequent release in SIF in a smaller release volume (half of that in SGF) to maintain the drug concentration in the release volume high enough to allow the subsequent HPLC analysis. Drug concentrations were determined by HPLC analysis. After 24 h, the beads were collected from the media and destroyed to extract and quantify the drug still embedded into the beads. The release data were reported as drug concentration $C_{res}(t_w^i)$ [mg/mL] at withdrawal times t_w^i [min] and as fraction of drug released up to time t_w^i with respect to the total amount of drug loaded in the beads. The experiments were carried out in triplicate with each value reported representing the mean ± SD.

3. Results and discussion

Based on the rheological properties of GG solutions, different GG beads were prepared dropping GG solution at 1% *w/w* and 1.5% *w/w* into $CaCl_2$ solutions at different concentrations in order to evaluate the effect of the cross-linking agent on the properties of the resulting beads. The crosslinking concentration always exceeded the concentration of the polymer as molar ratios GG:Ca^{2+} of 1:5, 1:7.5, 1:10 and 1:15 mol:mol were investigated. Regular and spherical beads were obtained with GG concentration 1.5% *w/w* for 1:5 and 1:10 GG:Ca^{2+} molar ratios, corresponding to $CaCl_2$ concentrations of 0.3% *w/w* and 0.6% *w/w*, respectively. Concentrations of $CaCl_2$ < 0.3% *w/w* have not produced

stable and spherical beads. GG solution 1.0% *w/w* gave irregular beads even for higher GG:Ca^{2+} molar ratios 1:7 and 1:15. Based on these results, concentrations of GG below 1% *w/w* were not further investigated and the GG concentration of 1.5% *w/w* with $CaCl_2$ concentrations of 0.3% *w/w* and 0.6% *w/w* were adopted because these concentrations did not cause clogging of the syringe needle and produced regular and spherical beads. Further formulations were prepared by adding laponite to GG solution before beads formation. In this case, the beads were formed using the GG solution 1.5% *w/w* with laponite 1% *w/w* and with the lower concentration 0.3% *w/w* of $CaCl_2$, which was chosen by considering that the clay is able to act as cross-linker itself, thus contributing to the polymeric network formation. The beads were recovered by filtration and characterized immediately after preparation in their fresh form and after the freeze-drying process. Specifically, they were observed at the optical microscope and their diameters measured and reported in Table 1.

Table 1. Diameter values of different bead formulations before and after freeze-drying.

Beads Formulation	Beads Diameter (mm ± SD)	Freeze-Dried Beads Diameter (mm ±SD)
GG/Ca 0.3%	2.41 ± 0.06	1.63 ± 0.03
GG/Ca 0.6%	2.44 ± 0.07	1.56 ± 0.09
GG/LAPO/Ca 0.3%	2.79 ± 0.11	2.06 ± 0.08

Different formulations lead to beads with different dimensions: the cross-linker concentration does not influence significantly the particle diameter, whereas the presence of laponite leads to an increase of the particle diameter. For all formulations, the bead population appear homogeneous and with spherical shape (see Figure 1A) characterized by an aspect ratio of about 1.02. The beads containing laponite (Figure 1D) have a smoother and regular surface with respect to the other ones (Figure 1B,C which differ for the $CaCl_2$, concentration), most likely because the clay, acting as filler, increases the particle surface compactness.

Particle density after freeze-drying ρ_b is extremely low and comparable for all formulations. Specifically, $\rho_b = 0.109 \pm 0.02$ g/cm^3 for GG/Ca 0.3% and $\rho_b = 0.0926 \pm 0.02$ g/cm^3 for GG/LAPO/Ca 0.3%.

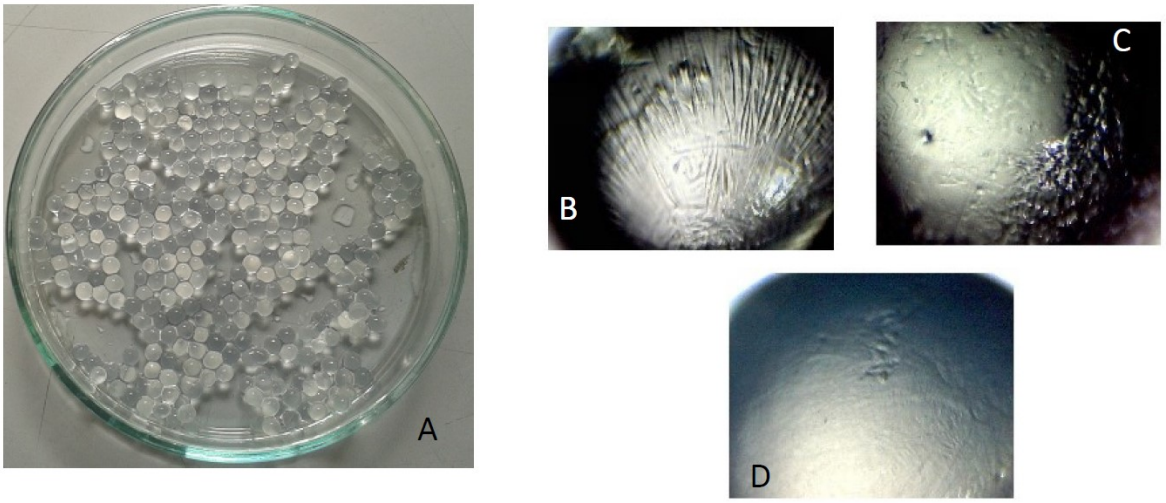

Figure 1. (A) Pictures of beads of GG/LAPO/Ca 0.3%; pictures of beads at the optical microscope; (B) GG/Ca 0.3%; (C) GG/Ca 0.6%; (D) GG/LAPO/Ca 0.3%.

A crucial property of the polymeric beads is the ability to swell in aqueous environments. The results of swelling experiments are reported in Table 2 in terms of the swelling degree at equilibrium S_{eq} (after 24 h).

Table 2. Swelling degree at equilibrium S_{eq} (measured after 24 h) for different bead formulations in Simulated Gastric Fluid (SGF; HCl 0.1 M) and Simulated Intestinal Fluid (SIF; phosphate buffer 0.044 M, pH 7.4).

Beads Formulation	S_{eq} in SGF	S_{eq} in SIF
GG/Ca 0.3%	9.08 ±0.3	45.80±0.9
GG/Ca 0.6%	8.97 ± 0.2	25.44±0.6
GG/LAPO/Ca0.3%	9.14 ± 0.3	20.60±0.3

In general, the swelling degree decreases in both swelling media as the amount of cross-linker is increased. The significant differences observed in swelling degree values in SGF and SIF are related to the nature of GG. The carboxylic groups of GG exist in a protonated form in HCl. This allows the network chains to stay closer to each other, resulting in a smaller swelling degree in acid medium. The beads in SIF exhibit a larger swelling degree as the carboxylic groups are deprotonated, resulting in a repulsion effect between network chains.
The presence of laponite causes a remarkable decrease of the equilibrium value S_{eq} as already observed in [Li *et al.* 2011] dealing with beads made of pH sensitive laponite/alginate/$CaCl_2$ hybrid hydrogel.

Focusing on the entrapment efficiency, Table 3 reports the entrapment efficiency of the two drug molecules into two different bead formulations, GG/Ca 0.3% *w/w* and GG/LAPO/Ca 0.3% *w/w*. It is evident that the entrapment efficiency is influenced by both the bead structure and the steric hindrance of the loaded molecule. Indeed, theophylline, smaller than vitamin B12, is less retained by both bead formulations, whereas the presence of laponite increases the entrapment efficiency of both drug molecules. This will reflect in drug-release data. The fact that the presence of laponite increases the drug entrapment efficiency has been already observed in [Li *et al.* 2011] for methylene blue loaded laponite/alginate beads.

Table 3. Entrapment efficiency of two model drug molecules, theophylline and vitamin

Beads	Drug Molecule	Entrapment Efficiency (%)
GG/Ca 0.3%	Vitamin B12	53.62
GG/LAPO/Ca 0.3%	Vitamin B12	61.26
GG/Ca 0.3%	Theophylline	20.26
GG/LAPO/Ca 0.3%	Theophylline	36.49

The presence of laponite in the GG beads influences significantly the release properties of both drug molecules. Figure 2 shows the integral gastrointestinal release curves of theophylline from beads GG/Ca 0.3% (red squares) without laponite and GG/LAPO/Ca 0.3% with laponite (blue bullets). Continuous lines show the excellent agreement between experimental data and theoretical curves obtained from the swelling/release model developed in [Adrover *et al.* 2019] taking into account the highly porous structure of the beads by including a "diffuse" glassy-rubbery interface [Adrover *et al.* 2015, Adrover *et al.* 2018].

It can be observed that TPH release from beads without laponite is so fast in SGF that 95% of the drug is released in acid medium in the first 120 min. On the contrary, the presence of laponite significantly slows down TPH release in SGF, so that about 70% of drug is released in acid medium, and the remaining 30% is slowly released in the intestinal tract. We conclude that bead formulation including laponite represents a release medium capable of supporting the controlled release of a small non-interacting drug as theophylline.

Even better results in slowing down the drug release in the gastric media are obtained for a larger drug molecule as vitamin B12. Figure 3 shows the integral gastrointestinal release curves of vitamin B12 from beads GG/Ca 0.3% (red squares) without laponite and GG/LAPO/Ca 0.3% with laponite (blue bullets). In the presence of laponite, only 60% of the loaded vitamin B12 is released in the gastric tract (first 120 min), and also the remaining 40% is slowly released in the intestinal tract, as

the complete release requires about 280 min in SIF medium. Therefore, the bead formulation including laponite is suitable for sustained release of a medium/large interacting drug molecule.

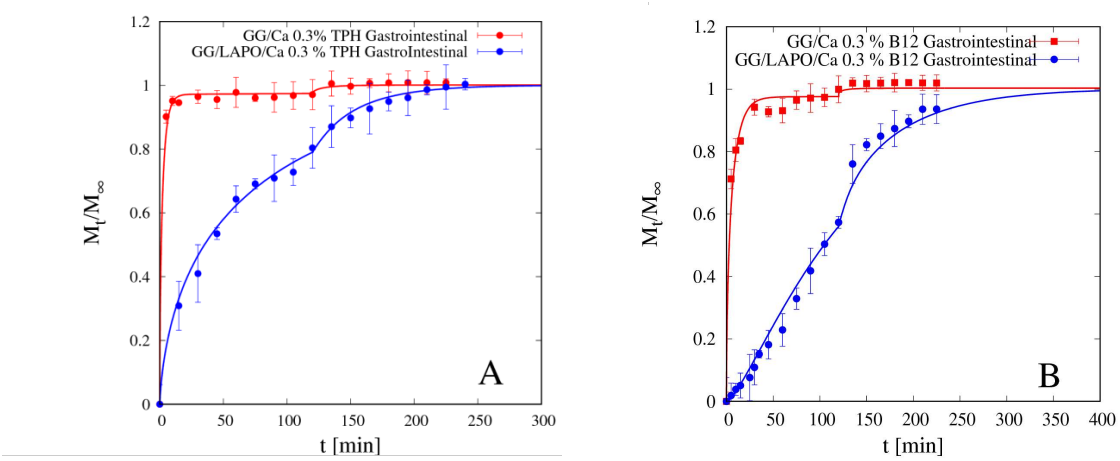

Figure 3. Gastrointestinal integral release curve for theophylline (A) and vitamin B12 (B) from beads GG/Ca 0.3% with (blue points) and without laponite (red points).

4. Conclusions

Gellan gum was employed together with calcium chloride, selected as cross-linker, in order to prepare freeze-dried beads using the ionotropic gelation method. Laponite was also included in the formulations. Stable and spherical beads (aspect ratio ≈ 1.02) were obtained from GG solutions (GG 0.15 % *w/w*) and GG/laponite solutions (GG 0.15% *w/w*, laponite 0.1% *w/w*) with $CaCl_2$ 0.3% *w/w*.

Gellan gum beads including laponite have shown a smoother and regular surface and a larger diameter, namely $d_0 \approx 2.8$ mm and $d_0 \approx 2.1$ mm before and after freeze-drying, respectively. The ability to swell in different media mimicking biological fluids, namely SGF and SIF, was investigated. The bead swelling degree at equilibrium was lower in SGF than in SIF and further reduced in the presence of laponite.

Two model drugs, theophylline and vitamin B12, having different molecular weight and steric hindrance, were loaded into different bead formulations. The presence of laponite in the bead formulation increased the drug entrapment efficiency for both model drugs. Sustained release of both model drugs was obtained from beads including laponite, as a small fraction of the incorporated drugs was released in the gastric medium. This suggests that laponite may be an effective additive in the development of GG beads for sustained release of drugs.

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