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GASTRO-RETENTIVE FLOATING SYSTEMS PRODUCED BY IONOTROPIC GELATION FOR ENHANCED ORAL DRUG BIOAVAILABILITY

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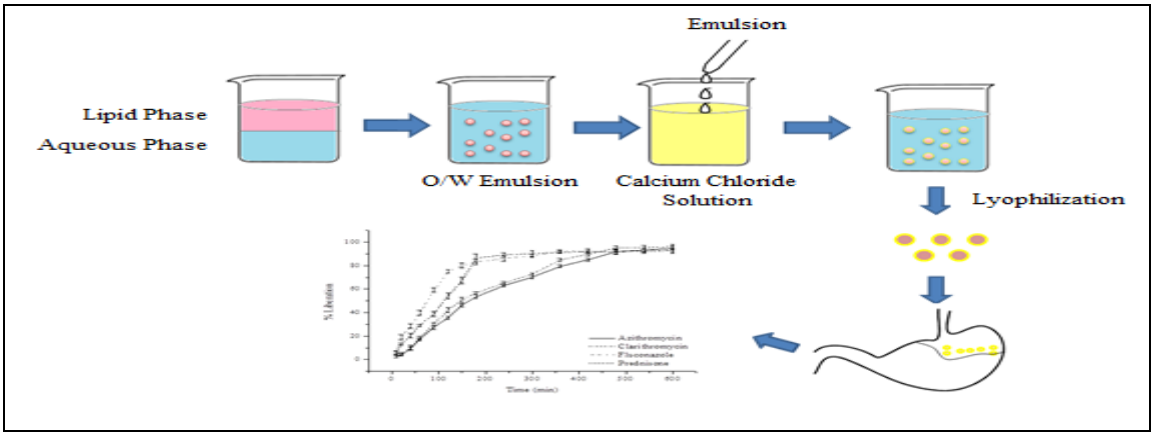
ABSTRACT
The purpose of this study was to develop controlled-release granules for floating gastro-retentive systems. The granules were obtained by ionotropic gelation method between sodium alginate and calcium chloride, from a previously prepared oil-in-water emulsion. Azithromycin, clarithromycin, prednisone, fluconazole and ranitidine were used as model drugs. The physicochemical characterization of the obtained granules was based on the analysis of the loading capacity for the selected drugs, the floating time and lag time, and the sphericity of the beads. Wide Angle X-ray Diffraction (WAXD) and Differential Scanning Calorimetry (DSC) were run for the assessment of the polymorphism of the drugs. A dissolution test was carried out for the evaluation of the release profile of the drugs. Our results demonstrate that the fluctuation of the granules was immediate and the fluctuation time was greater than 24 hours. The loading capacity was influenced by the lipophilicity of the drugs. The low loading capacity of ranitidine was attributed to the hydrophilicity of the drug and to oxidative degradation. DSC and WAXD analyses indicate azithromycin, clarithromycin and prednisone suffered polymorphic changes upon loading by ionotropic gelation. A controlled release profile was observed for azithromycin and clarithromycin, while a release rate reduction was observed for fluconazole and prednisone. From the tested drugs, ionotropic gelation between sodium alginate and calcium chloride, from an oil-in-water emulsion was shown to be an effective strategy for developing floating gastro-retentive systems for azithromycin and clarithromycin.

KEYWORDS: Gastro-retentive floating granules, ionotropic gelation, azithromycin, clarithromycin, prednisone, fluconazole, ranitidine.

Graphical Abstract



INTRODUCTION

The development of new therapeutic systems for increasing the bioavailability of drugs and reduction of side effects is a continuous demand. However, the challenge is maintaining the dosage form in the optimal location for drug absorption in physiological conditions[1]. Generally, the oral route is the most preferable administration route due to higher patients' compliance (since it is more comfortable and painless), low production cost, and can be used for local or systemic drug action[2]. However, the absorption profile of the drugs in the stomach is influenced by the pH[3], solubility, mucosal thickness, and especially by the gastric emptying.

Gastro-retentive systems are being considered a potential approach of prolonged drug release in the gastro-intestinal tract. Gastro-retention can be achieved by the development of mucoadhesive, swellable, of high density or low density (i.e. floating) systems[4-7]. The technology of the floating systems is highly promising because the developed floating granules, which are of low density (less than 1 g/cm³), remain far away from the pylorus and are therefore difficult to be cleaned by the gastric emptying[8]. Literature reports the use of various types of polymers suitable for the production of floating granules, such as chitosan, sodium alginate, calcium pectinate, guar gum, ethyl cellulose and hydroxyl propyl methyl cellulose, acrylic acid derivatives[9].

The main challenge in the development of floating granules is to achieve the required fluctuation time for the complete drug release. A latency period is usually observed between the arrival of the pharmaceutical dosage form to the stomach and the top of the fluctuation (lag time) during which the polymer is hydrated, which might compromise the gastric retention[10]. With the aim to decreasing the periods of latency, the literature suggests the use of low density systems containing oils or air[11,12]. Among the ways of obtaining floating systems, lipid systems reserve possibilities of great biopharmaceutical interest. Simple oil-in-water (o/w) emulsions are obtained by the dispersion of fatty compounds in aqueous medium, in the presence of suitable hydrophilic surfactants. Various drugs (e.g. clarithromycin[13], cefuroxime, metoprolol tartrate[14] zidovudine[12]) have already been processed by this technological approach for the development of floating systems.

In this paper, we report the production and characterization of gastro-retentive floating granules using a technique based on the ionotropic gelation between sodium alginate and calcium chloride. Azithromycin, clarithromycin, prednisone, fluconazole and ranitidine were chosen as model drugs because they have common local action.

MATERIALS AND METHODS

MATERIALS

Azithromycin, clarithromycin, fluconazole, ranitidine HCl and prednisone bought from Henrifarma (São Paulo, Brazil); Polysorbate 80 (Tween®80, Synth, Brazil), sorbitan monostearate (Span®60) and sodium carbonate were purchased from Synth (São Paulo, Brazil); Almond oil, corn oil, olive oil and calcium chloride were kindly provided by Sigma-Aldrich (German, Brazil). Sodium alginate (Protonal® 6650, Brazil), of 150–170 Da, was received from FMC Biopolymer (Philadelphia, USA). Double distilled water was used after filtration in a Millipore system (home supplied).

METHODS

Preparation of gastro-retentive floating systems in o/w emulsions

Gastro-retentive floating systems were prepared by ionotropic gelation between sodium alginate and calcium chloride in o/w emulsions. The inner phase of the o/w emulsion was composed by oil 14 % (almond oil, corn oil or olive oil) and glyceryl monostearate 6 %. The aqueous phase was composed of sodium alginate 1% and surfactant blend (Tween®80 and Span® 80) 1% in purified water. The surfactant blend was adjusted based on the hydrophilic-lipophilic balance (HLB) value of the oil phase of the emulsion (HLB = 14.0). For the production of the emulsion, the aqueous phase was slowly added to the lipid phase, both previously heated at 60 °C. The emulsion was mixed by mechanical stirrer (Tecnal®, TE-039, Brazil) until cooled down to room temperature. For the drug loading, azithromycin (1 %), clarithromycin (1%), fluconazole (1%), ranitidine (2%) or prednisone (0.5%) were incorporated in lipid phase. For the ionotropic gelation, he emulsion was dripped (1 ml/min) through a needle into an aqueous solution of 1 M calcium chloride. The obtained granules were kept in the calcium chloride solution for a further two hours and filtered off. Granules were then washed with water, lyophilized (Thermo Electron Corporation, Modulyod, USA) and stored at ambient temperature for a period of 3months, under dark flask.

Loading capacity

To determine the loading capacity (LC), drug-loaded granules were incubated in ethanol (azithromycin and fluconazole), HCl (0.1 N) (clarithromycin), water (ranitidine) or in methanol (prednisone), for 24 hours under stirring and heated at 30ºC. After that, samples were filtered through membranes (0.22 µm) and diluted, if necessary. Drug content of each sample was determined by UV spectrophotometer (FEMTO, 800XI, Brazil) analysis (azithromycin λ = 215 nm, clarithromycin λ = 760 nm, fluconazole (λ = 261 nm), ranitidine (λ = 313 nm) and prednisone (λ = 244 nm) or VIS (clarithromycin, λ = 760 nm). The tests were performed in triplicate[15].

***In vitro* floating analysis**

The *in vitro* floating capacity of the granules was assessed by determining the time the formulation was kept at the surface of a physiological medium (floating lag time). The granules (100 units) were placed in 25 ml simulated gastric fluid composed by 2% of sodium chloride of hydrochloric acid aqueous solution, pH of about 1.2 without pepsin for 24 hours. At the end of this period the floating particles were collected, filtered in a 0.45 micron cut-off filter paper. The lag time and the percentage of floating granules were recorded for the period of 24 hours. The percentage of floating particles was determined using the Equation 1[12].

$$\text{Floating} = \frac{Nf}{Ni} \times 100 \qquad \textbf{Equation 1}$$

where Ni is the initial number of floating particles and Nf is the final number of floating particles. This study was performed in duplicate

Morphology of granules

The morphology of the granules was analyzed by Scanning Electron Microscope (SEM) (Jeol, JSM 6360, USA) in high vacuum mode. The dried samples were placed in an aluminum base, fixed with glue carbon and covered with gold in order to make them conductive. The photos were taken with 20 kV excitation voltage and magnification up to 1000 times.

Measurement of *in vitro* drug release

The release of drugs was assessed by the USP dissolution test apparatus I, 50 rpm and 37ºC. The granules were carefully weighed to contain 100 µg/mL azithromycin, clarithromycin or fluconazole, or 20 mg/ml prednisone, and it kept in the dissolution vessel containing 900 ml of 0.1 N HCl (pH 1.2) as a release medium. For the assay of prednisone, volume of 500 mL of water was used. The samples were collected from the dissolution medium at predetermined time intervals during a period of 10 hours. To determine the concentration of drug released at each time point, 5 mL of dissolution medium were collected and filtered through a membrane with porosity of 0.45 µm. The concentration of drug was determined by Ultraviolet Spectrophotometry (FEMTO, 800XI, Brazil). The release study of ranitidine was not performed because the beads showed visible signs of degradation.

Wide angle X-ray diffraction (WAXD)

To study the granules of azithromycin, clarithromycin, fluconazole, ranitidine and prednisone WAXD was carried out in an automatic diffractometer X-ray (Shimadzu XRD7000, Japan). WAXD measurements were taken from 5° to 150° in 0.015° steps (1 s per step), voltage/current 40kV/30mA[16].

Differential scanning calorimetry (DSC)

Thermal behavior of granules containing almond oil without drug, granules of azithromycin, clarithromycin, fluconazole, ranitidine and prednisone was assessed by DSC (TA Instruments, MDSC 2910, USA). The samples were heated from 0°C up to 300°C, following cooling down to 10°C, coupled to a cooling mode by nitrogen. A volume of sample containing approximately 10 mg of sample was weighed in an aluminum pan and sealed hermetically, under inert atmosphere (N_2). The analysis was performed at a heating and cooling rate of 5 K/min, using an empty pan as reference[16].

RESULTS AND DISCUSSION

Granules are a versatile carriers for oral administration of drugs. These solid dosage forms can be applied to a variety of drugs to improve the therapeutic activity, prolonged release and decrease collateral effects. Advantageous of granules include their long-term physical, chemical and biological stability, they are easily produced at lab and large scale, the production method is reproducible and it does not release harmful waste for the environment or toxic metabolites[17]. Granules based on emulsified systems produce particle density of less than 1 g/cm^3, which enable them floating in the aqueous medium. The adding of polymers to the aqueous phase of the emulsion and its cross-linking may form a solid matrix.

Alginates are poly-anionic polymers of natural origin, which in contact with divalent cations such as Ca^{2+} or other cationic polymers cause ionotropic gelation, forming inter chain junctions generating particles, which allow the incorporation of drug molecules in a polymer matrix primarily through electrostatic and van der Waals interactions[18].

Azithromycin, clarithromycin, fluconazole, ranitidine and prednisone were chosen due to their better absorption profile in the upper gastrointestinal tract (stomach and proximal duodenum); act on microorganisms (azithromycin, clarithromycin) colonizing the stomach and cause inflammation of the stomach mucosa; have different physicochemical characteristics, such as lipophilicity (Log P), isomerism (prednisone) and oxidation (ranitidine). Three different formulations were developed using 3 distinct oils (almond, corn or olive oil). The best obtained formulation was composed by almond oil, which was chosen for further studies.

Table 1 shows the results obtained in the evaluation of the linearity of azithromycin, clarithromycin, fluconazole, prednisone and ranitidine. The values shown are mean ± standard deviation of three determinations. The coefficients of determination (r^2) and the correlation coefficient (r) show that the studied concentration ranges were directly proportional to the concentration of drug in the sample. For all drugs, the obtained correlation coefficient was greater than 0.99.

Table 1. Results obtained in evaluation of linearity of azithromycin, clarithromycin, fluconazole, prednisone by UV spectrophotometric method and ranitidine by visible spectrophotometric method.

Drugs	r^2	r	a	b	Equation (y=ax+b)
Azithromycin	0.9984	0.9992	0.0015	0.0123	y=0.0015x + 0.0123
Clarithromycin	0.9988	0.9994	0.0025	0.0082	y=0.0025x + 0.0088
Fluconazole	0.9998	0.9999	0.0024	0.0553	y=0.0024x + 0.0553
Ranitidine	0.9956	0.9978	0.0037	0.0145	y=0.0037x + 0.0145
Prednisone	0.9951	0.9975	0.0433	0.0945	y=0.0433x + 0.0945

*(r^2) coefficient of determination; (r) linear correlation coefficient; (a) slope; (b) point of intersection with the y axis; (y) Absorbance (x) concentration.

Table 2 shows the loading capacity and the percentage of floating granules measured for 24 hours. For the granules of ranitidine, the loading capacity because the low detection of the spectrophotometric method. The variation of the loading capacity can be explained by lipophilic properties of drug. More lipophilic drugs (Log P > 1.0) showed high loading, whereas more hydrophilic drugs (Log P < 1) the loading rate was lower, results that were attributed to the solubility of the drug in the oil phase. The knowledge of the nature and magnitude of pKa and Log P of the loading molecules, namely azithromycin (3.03; 8.74), clarithromycin (3.18; 8.74); fluconazole (0.58; 8.74), Prednisone (2.07; not ionized) and ranitidine (0.79; 8.2 and 2.7), contributes to understanding of the behavior of drugs in binary system type such as oil/water systems[19].

Table 2. Results of loading capacity, floating, solubility, Log P e pKa of AZM, CLT, FLC, PDS and RNT

Drug	Loading capacity (%)	Floating (%) after 24 h	Solubility (g/L)	Log P	pKa
Azithromycin	81.18	100	5.14	3.03	8.74
Clarithromycin	82.16	100	2.17	3.18	8.99
Fluconazole	45.13	X	1.39	0.58	8.74
Prednisone	69.54	100	1.11	2.07	-
Ranitidine	X	100	7.95	0.79	-

(X) there was no loadingor float. (-) no data available

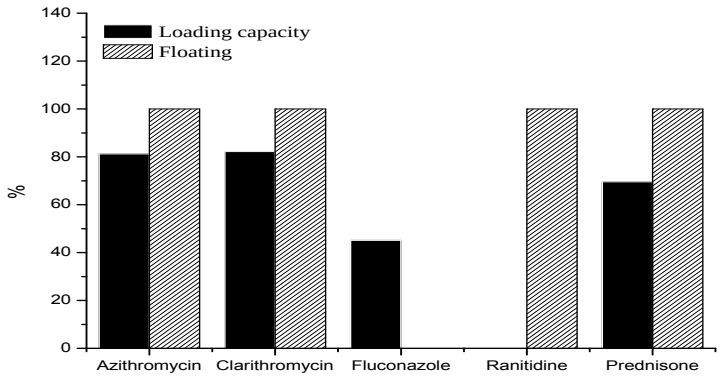


Figure 1. Results of percentage of loading capacity and floating of azithromycin, clarithromycin, fluconazole, prednisone and ranitidine (n=3).

Figure 2 shows the profile of dissolution of granules containing azithromycin, clarithromycin, fluconazole or prednisone. Azithromycin and clarithromycin exhibited similar release profiles corresponding to a sustained release system, with the maximum of release being achieved after 8 hours. Fluconazole and prednisone release peaked at about 3 hours. However, the release of fluconazole was faster than the release of the prednisone. The total released was 94.81%, 96.16%, 92.91% e 92.71% for azithromycin, clarithromycin, fluconazole and prednisone, respectively. Since the lipid matrices are typically passive diffusion systems for loaded molecules, the recorded release was virtually constant as a function of time, suggesting that the release takes place by diffusion of the dissolved drug. These results corroborate

the fact that the release profile of the loaded drug is highly dependent upon its aqueous solubility.

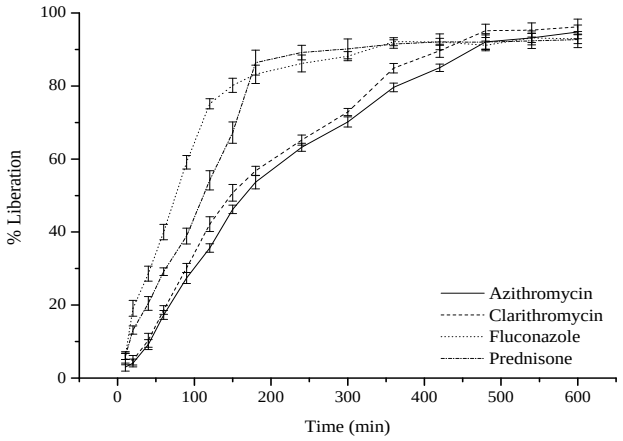
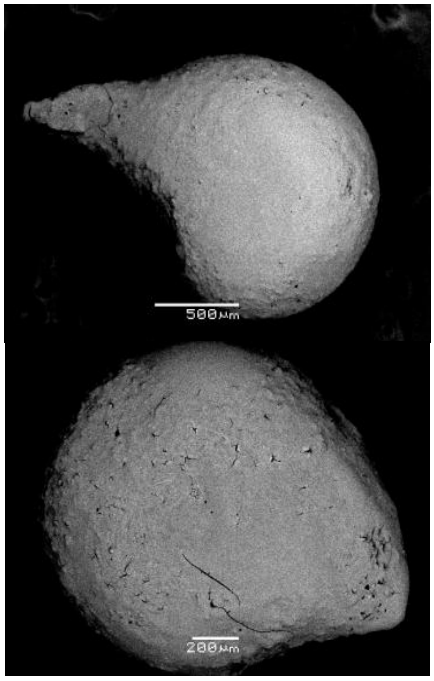


Figure 2. Dissolution profile of clarithromycin, azithromycin, prednisone and fluconazole.

The systems obtained by ionotropic gelation from emulsion coated by sodium alginate were able to control the release of the most lipophilic drugs (i.e., clarithromycin and azithromycin). Taking into account that gastric emptying usually takes about 69-90 minutes in average, the bioavailability of azithromycin, clarithromycin and prednisone may be increased when loaded in floating granules, either by action in the stomach or by absorption in the upper gastrointestinal tract.

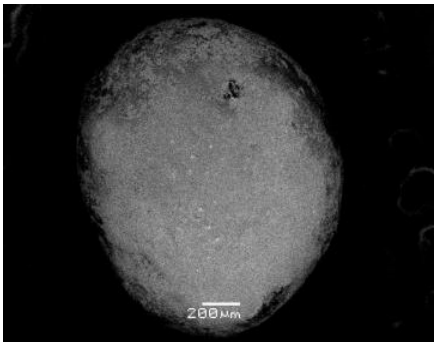
The size, shape, and surface of granules were analyzed by light and scanning electron microscopy (SEM).

The granules size was influenced by the drip rate and distance between the needle tip and the surface of the calcium chloride solution (Figure 3). When the drip rate was greater than 1 mL/min and the distance between the needle tip, and the surface of the calcium chloride solution was greater than 3.0 cm, the granules depicted an elongated shape (Figure 3a). The best conditions of drip rate was 1 mL/min, the distance between the needle tip and the surface of the calcium chloride solution was higher than 3.0 cm drop, the granules showed the form depicted in Figure 3b. When the drip rate was greater than 1 mL/min, and the distance between the needle tip and the surface of the calcium chloride solution was equal to or less than 3.0 cm, the drop presented the rounded form shown in Figure 3c and Figure 4 (a,b,c).



(a)

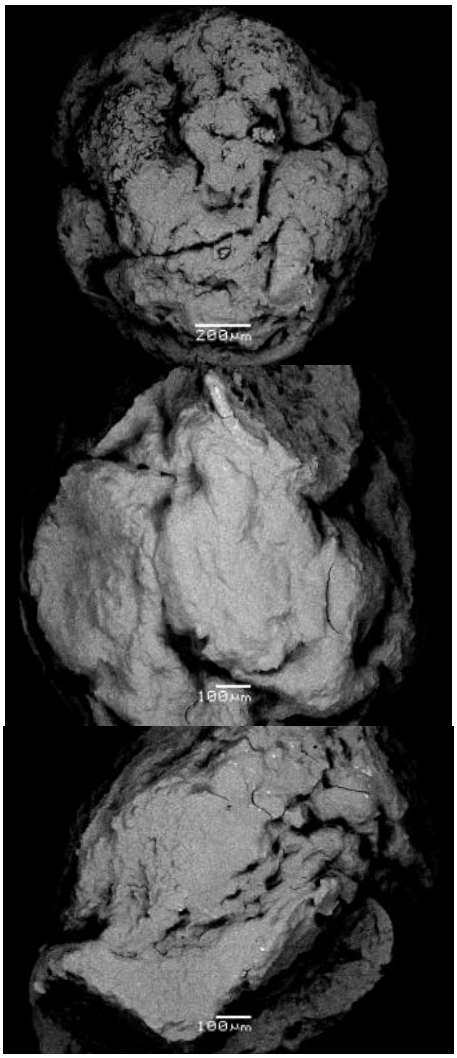
(b)



(c)

Figure 3. Scanning electron micrographs of granules according to the drip rate and the distance between the needle tip and the surface of calcium chloride solution (a) >1 mL/min; >3.0 cm; (b) 1 mL/min, >3.0 cm and (c) >1 mL/min, <3.0 cm.

Other formulations produced with different combinations of oil phase and drug were also analyzed. Figure 4 shows the *beads* containing, respectively, fluconazole/almond oil, clarithromycin/corn oil or clarithromycin/olive oil, after the drying process. These results suggest that both the drug and the choice of the components of the oil phase influence the formation and physical stability of granules. Olive and corn oil loaded granules were more sensitive to high temperatures (60-70°C) used in the drying process, and this might be the first cause of degradation of these oils. This hypothesis is being well-argued because during the manufacturing process, these emulsions reveal a mild odor of decomposing fat, followed by changes in the crystallization of glycerides and loss of the spherical shape of the granules[11,20,21].



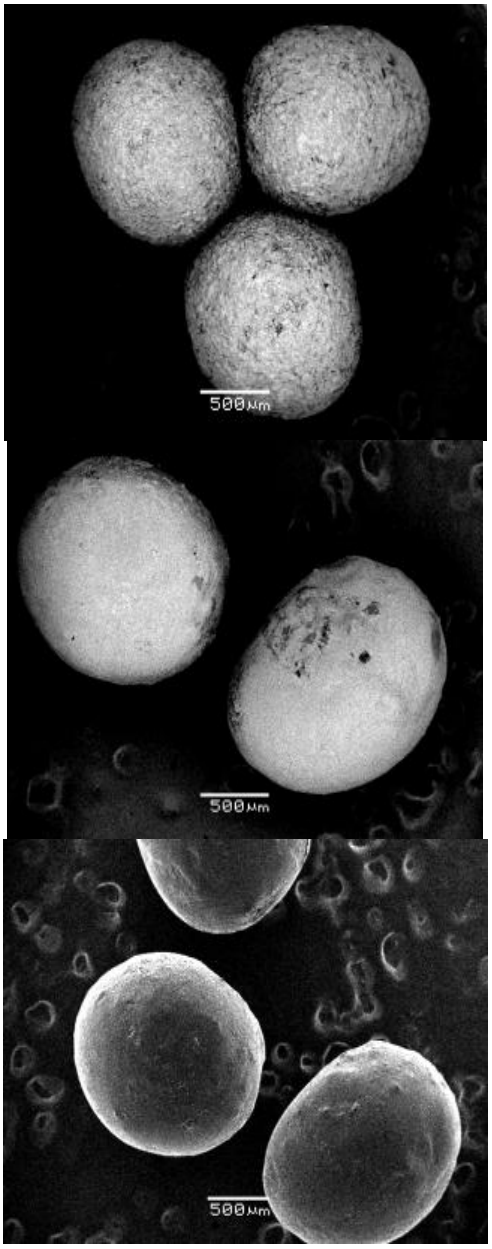
(a)

(b)

(c)

Figure 4. Scanning electron micrographs of the granules prepared with (a) almond oil/fluconazole, (b) corn oil/clarithromycin and (c) olive oil/clarithromycin.

Figure 5 depicts micrographs of the granules prepared with almond oil and loaded with azithromycin, clarithromycin and prednisone, respectively, showing the spherical and smooth surface of the obtained systems. These combinations produced granules with regular rough surfaces, and have been selected for further studies.



(a)

(b)

(b)

Figure 5. Scanning electron micrographs of the granules prepared with almond oil and loading (a) azithromycin, (b) clarithromycin or (c) prednisone.

WAXD analysis is an important technique to verify the reproducibility of the crystalline state between batches of the same pharmaceutical product. The arrangement of peaks of the structure of a crystal in a powder sample leads to deviation of the peaks of X-rays in a reproducible manner at different angles (θ) relative to the incident beam. The type diffraction is characteristic of a specific crystal structure for a given compound. An amorphous form does not lead to production of a particular type of deviation. Mixtures of different crystalline forms may be analyzed using standardized intensities with specific angles, which are unique for each crystalline form. The analysis of a single crystal X-ray identification and allows accurate description of a crystalline substance. The dimensions of the units and certain angles allow to accurately characterizing the crystal structure, providing differences between the specific crystalline forms of a given compound (LACHMAN et al., 2001; USP, 2007).

Solids can be classified as crystalline, non-crystalline, or a mixture of both forms. In crystalline solids, molecules or atoms are arranged in a three-dimensional matrix, called a grid, within the solid particles. This ordering of

the molecular components not present in non-crystalline solids. Solid non-crystalline, or amorphous solids, when there is no order is repeated in all three dimensions. As can exist in one or two dimensions, resulting in mesomorphs phases (liquid crystals) (USP, 2007).

Figure 6 shows the WAXD profile of free drugs and granules. Figure 5b shows the WAXD profile of granules of azithromycin, clarithromycin and prednisone obtained by ionotropic gelation. The results of Figure 6 show a modification of the solid state of the drug from amorphous to crystalline. Since that is not the result of a process of degradation the solid state modification may have pharmaceutical implications. However, there is no evidence that this change cause pharmacokinetic or pharmacodynamic changes.

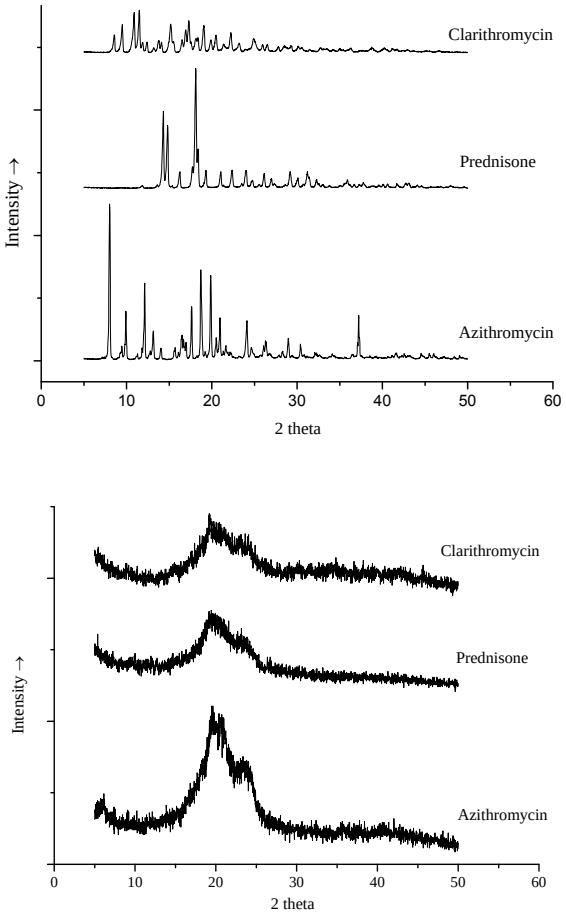


Figure 6. **WAXD diffractogram recorded for (a) free drugs (b) granules**.

Thermodynamic events indicate the identity and purity of the drug. Standards have been established for the boiling temperature of the substance. These transitions occur at specific temperatures, contributing to the identification and characterization of substances. The thermal analysis is the physical chemistry of the materials as a function of temperature, provide information about crystallinity, presence of hydrates, polymorphism, melting temperature, sublimation, glass transitions, degradation, evaporation, solid interactions - solid and purities. The data of thermal analysis are useful also for the characterization of the compatibility and stability of the pure substance or mixture and the packing material. The measurements used more often in thermal analysis, are transition temperature, thermogravimetry and differential calorimetry. The transition temperature occurs when a sample is heated, its heat absorption can be measured by DSC or the resulting temperature difference in an inert reference heated identically, ie, differential thermal analysis[15].

Figure 7 shows the results of DSC of the granules produced with almond oil, namely, inert granules, azithromycin, clarithromycin and prednisone. The endothermic peak at 68°C corresponds to the melting point of the lipid mixture of granules; the onset of endothermic peaks at 143.5°C, 229°C and 245.17°C correspond, respectively, to the melting points of azithromycin, clarithromycin and prednisone. Azithromycin granules shows only the endothermic peak related to the melting point of the glycerides (62.18°C) are present, clarithromycin endothermic peak was 63.5°C corresponds to the melting point of the glycerides and the endothermic peak at 209°C to melting point of

clarithromycin. Reducing energy 106.6 J/g (azithromycin) to 48.3 J/g (clarithromycin) in the merger of clarithromycin is the crystallinity reduction. Prednisone granules the endothermic peak was at 62.0°C corresponds to the melting point of the glycerides. The melting points of 93.54°C, 159.0°C and 185.29°C are indicative of changes in the chemical structure of the prednisone. This result shows chemical incompatibilities of the system.

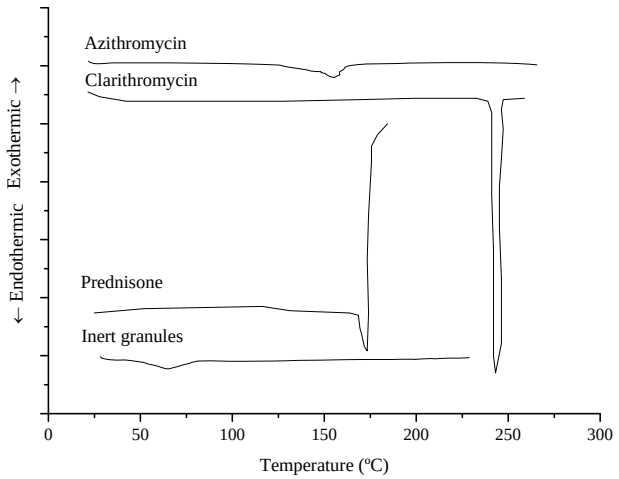


Figure 7. DSC thermograms of granules produced with almond oil, without drug (inert granules) and loaded with azithromycin, clarithromycin and prednisone

The choice of a suitable method of the encapsulation of drugs produced by ionotropic gelation to improve the oral bioavailability of drugs depends on the physicochemical characteristics of the drug to be loaded, the operating conditions and the chosen system. The encapsulation techniques using natural polymers are considered privileged since they use cleaner materials[22,23].

In this study, the chosen method for the encapsulation of azithromycin, clarithromycin, fluconazole, ranitidine and prednisone proved to be selective, simple, reproducible, fast, and able to be transported to the industrial scale. The floating beads were obtained by formation of a prior emulsion, in which the internal phase was solidified by ionotropic gelation to give spherical granules. The solidification of the particles was achieved by reaction *in situ* of this sodium alginate in the emulsion with the calcium chloride in aqueous 1.0 M. The lipophilic characteristics of the system mainly consisting of lipids and calcium alginate, was responsible for the higher encapsulation efficiency of the lipophilic drugs (i.e. azithromycin and clarithromycin).

The floating of the beads occurs because of the lower density of the same in relation to the aqueous medium, and the chosen method was not fully satisfactory for the placement of ranitidine, prednisone and fluconazole.

The Prednisone suffered isomerism, probably caused by the heating temperature of the oil phase of the emulsion or by chemical incompatibility between the components of the formulation. The SEM used to assess morphological characteristics of the granules showed the influence of chemical characteristics of fluconazole in the physical structure of the granules, leading probably the occurrence of metastable forms of lipids.

CONCLUSIONS

This paper reports the development of floating granules by ionotropic gelation technique with sodium alginate/calcium chloride. We have succeeded to obtain gastro-retentive and floating systems for azithromycin and clarithromycin. The morphological characteristics of the granules were influenced by the emulsion dripping speed, the distance formed between the droplet and the surface of the calcium chloride solution. Prednisone was influenced by the preparation process and resulted in the formation of polymorphs and fluconazole alter the chemical structure of lipid granules.

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