Evaluation of retrograded starch as excipient for controlled release matrix tablets

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High amylose starch (HAS) was retrograded by two different methods. The physicochemical properties of the retrograded materials were evaluated and structural changes were highlighted. Micromeritics properties were demonstrated as suitable for the compression process. Hydrophilic matrices were prepared by dry granulation of the retrograded starch. The in vitro release of diclofenac sodium (DS) in media with different pH values (1.2 and 7.4) was evaluated. The release profiles demonstrated the lowering of drug release rates in acid medium, mainly when pectin was associated to the matrix by physical mixture. In enteric medium, increased rates of drug release were observed, so that t80% occurred at approximately 60 min, while for the tablets obtained with HAS, this time was of approximately 120 min. The matrix obtained with pectin (during retrogradation and by physical mixture) enabled a more effective control over the drug release rates, so that t80% of DS was 150 min and 210 min, respectively.

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1. Introduction

Despite the wide and successful research with alternative routes of drug administration, the oral route remains the preferable choice because of its inherent benefits, such as safety, easy administration, flexibility of formulation, greater patient adherence to the treatment and the possibility of releasing drugs both locally and systematically [1,2]. Among oral controlled release systems, matrix tablets were noteworthy by presenting efficient manufacturing technology and high reproducibility, which reflects in lower costs of production, mainly when the direct compression of the powders is their way of obtainment [3,4].

Swellable hydrophilic matrices are monolithic systems that do not degrade upon contact with the aqueous medium, undergoing hydration, which leads to the formation of a swollen diffusion layer, controlling the rates of release. The polymer swelling, the solute diffusion throughout the matrix, and erosion are also mechanisms involved in the control of drug release [5,6].

Natural polymers play an important role in the design of these systems, since they are materials from renewable and abundant sources, additionally, they are nontoxic and biodegradable. Besides, they can confer or enhance controlled release properties to the systems due to molecular properties of polymers, such as their monomer nature and type/degree of substitution [7–9]. Furthermore, in situ drug release can be reached because of the presence of glycoside bonds in the polymer structure that are hydrolytically cleaved by colonic enzymes [10].

Starch, a natural high molecular weight polysaccharide composed by glucose units, has received great attention as a carrier of matrix tablets, since it can be modified by a number of physical, chemical and enzymatic procedures, such as cross-linking [11], pregelatinization [12], retrogradation [13], complexation [14] and others, considering that native starch does not present suitable properties to reach desired drug release rates [12,13,15].

The retrogradation process of the starch, which occurs by hydrothermal treatments, converts the pregelatinized starch (amorphous) to a more resistant and compacted crystalline form [16,17], known as resistant starch (RS), which can exist in different
2. Materials and methods

2.1. Materials

High amylose corn starch (Hylon VII type – 70% amylose, lot: HA9140) was obtained from National Starch & Chemical (New Jersey, USA), sodium hydroxide (lot: 611648) was supplied by Grupo Química (Rio de Janeiro, Brazil), 37% hydrochloric acid (lot: 29957) was provided by Quimis (Diadema, Brazil), diclofenac sodium was purchased from Henrifarma (São Paulo, Brazil), pectin (type LM-5206CS – DE < 50%, lot: S74431) was provided by CP Kelco (Copenhagen, Denmark), pancreatin (lot: 0903372) was purchased from Vetec (Duque de Caxias, Brazil), 3,5-dinitrosalicylic acid (purity ≥ 98.0%, lot: 125k3664) was provided by Sigma–Aldrich Co. (St. Louis, USA), purified water (Milli Q, Millipore).

2.2. Methods

2.2.1. Retrogradation of high amylose starch (HAS)

Aqueous dispersions of HAS at different concentrations (20 or 40%) were autoclaved at 121 °C (15 min) for starch gelatinization. The gelatinized starch (GS) was retrograded according to two different methods in order to assess the impact of the storage time and temperature in the material properties. In M1, the temperature was kept constant (4 °C) for 8 days (isothermal cycle) and in M2 it was employed alternating cycles of temperature (4 °C and 30 °C; 2 days at each temperature) for 16 days [34]. All samples were dried in a forced air circulation oven–drier at room temperature until constant weight.

Retrograded samples were labeled as M120, M140, M220 and M240 respective to the method of retrogradation (M1 or M2) and concentration of polymers (20 or 40%). In this sense, the M220 sample was those obtained from M2 method and composed by 20% of polymer.

2.2.2. Enzymatic digestion and resistant starch content

In order to eliminate RSI and RSII fractions, a known mass (100 mg) of HAS, M120, M140, M220 and M240 samples were mixed with 2 mL of phosphate buffer (0.1 mol/L; pH 7.1) and kept in water bath (100 °C) by 30 min [35]. After that, cooled samples were incubated in 0.5 mL of a pancreatin enzymatic solution (0.15 g/mL), at 37 °C for different times (20, 60, 120, 150 and 180 min). Ethanol (80%) was added to the samples for stopping the enzymatic activity.

The glucose content provided by starch hydrolysis was quantified by the reaction with 3.5-dinitrosalicylic acid (DNS) [36] based on the standard glucose. Both RDS (rapid digestible starch – digested at 20 min) and SDS (slowly digestible starch – digested between 20 and 120 min) were used to calculate RS content, according to Equation (1) [37]:

\[
RS = \frac{\text{Total Starch} - \text{RDS} - \text{SDS}}{\text{Total Starch}} \times 100
\] (1)

2.2.3. Physicochemical characterization of samples

2.2.3.1. Moisture content. Moisture content of HAS, M120, M140, M220 and M240 samples was assessed gravimetrically on analytical infrared moisture balance (MettlerTM, PL 200/LP 11). A sufficient mass of samples (about 1 g) was uniformly disposed on the metallic pan of the balance and heated by infrared (105 °C) until constant weight was reached. The results were expressed as percentage of water loss in relation to the initial mass [38].

2.2.3.2. X-ray diffraction (XRD) analysis. Crystallinity patterns of the GS, HAS, M120, M140, M220 and M240 samples were evaluated from their diffractograms recorded on a X-ray diffractometer (Siemens® – Model D5000, Germany), using nickel-filtered Cu Kα radiation (λ = 1.5406 Å) (tube operating at 40 kV and 30 mA). The scanning regions were collected from 4 to 70° (2θ) in step size of 0.05° (2θ).
2.2.3.3. Thermogravimetric (TG) and derivative thermogravimetric (DTG) analysis. TG/DTG analysis of the GS, HAS, M120, M140, M220 and M240 samples were performed via a TA Instruments (SDT 600) (New Castle, DE, USA) at a heating rate of 10 °C/min, from 25 to 600 °C, under nitrogen atmosphere (flow rate of 50 mL/min), in open cylindrical alumina pans containing about 5 mg of sample.

2.2.4. Micromeritic properties

2.2.4.1. Granulometric distribution. The particle size distribution analysis was performed using a set of test sieves (0.297; 0.250; 0.210; 0.177; 0.125 mm) attached to a sieve shaker (Haver & Boeker Model EML Digital Plus, Westfalen, Germany) operated by 20 min. The average particle diameter was calculated according Equation (2) [39]:

\[ d_{av} = \frac{\sum (MO - Fr\%) \times 100}{100} \]  

where \( d_{av} \) is the arithmetic average diameter (mm); \( MO \) is the average of passage and retention mesh opening (mm) and \( Fr\% \) is the percentage of mass retained on each sieve.

2.2.4.2. Apparent bulk and tapped densities. The apparent bulk \( d_b \) and tapped \( d_t \) densities were indirectly determined from the apparent and tapped volume measurements, respectively. A known mass (about 10 g) of HAS, M120, M140, M220, M240 samples and the DS + M120, DS + M140, DS + M220 and DS + M240 physical mixtures (PM) were introduced in a 25 mL graduated cylinder glass and the bulk volume \( V_b \) was recorded. After this, to determine the tapped volume \( V_t \), the samples were submitted to cycles of 1250 taps in an automatic volumeter compactor (Volumeter Tapped, model SVM, ERWEKA, Heusenstamm, Germany) until the difference between two consecutive measurements was lesser than 2%. The values of \( d_b \) and \( d_t \) were calculated according to Equations (3) and (4):

\[ d_b = \frac{m}{V_b} \]  

\[ d_t = \frac{m}{V_t} \]  

where \( d_b \) = bulk apparent density (g/mL); \( d_t \) = tapped apparent density; \( m \) = mass (g); \( V_b \) = apparent bulk volume (mL) and \( V_t \) = apparent tapped volume (mL).

2.2.4.3. Analysis of flow ability. The angle of repose was determined according to free base cone technique [40]. An accurately mass (20 g) of HAS, M120, M140, M220, M240 samples and the DS + M120, DS + M140, DS + M220 and DS + M240 physical mixtures (PM) was introduced in a stainless steel funnel (14 mm opening) coupled to a vibrating device. The orifice of the funnel was opened and the powder flowed onto a flat surface. The \( \alpha \) angle was calculated by Equation (5):

\[ \tan \alpha = \frac{h}{r} \]  

where \( h \) = cone height (cm) and \( r \) = base radius of cone (cm).

2.2.5. Porosity determination

The porosity of the GS, HAS, M120, M140, M220 and M240 samples was evaluated on ASAP 2010 surface area analyzer (Micrometrics®), according to gas adsorption-desorption technique using \( N_2 \) (\( T = 77.35 \) K) as the adsorptive gas. Specific surface area, pore volume and pore size were calculated from isotherms of nitrogen adsorption/desorption by the Brunauer–Emmet–Teller (BET) method [41] over a relative pressure range of 0.05–0.25 on the adsorption branch.

2.2.6. Liquid uptake ability

The determination of liquid uptake ability of samples was carried out on an Enslin’s device [13,32,42,43]. About 0.05 g of samples were accurately weighed and carefully disposed on the sintered glass filter of funnel. The volume of different media (0.1 N HCl, pH 1.2; 0.1 mol/L phosphate buffer pH 7.4 and 6.8) absorbed by the samples was measured on the graduated pipette of the device at different times for 120 min and expressed as medium absorbed (%) in relation to initial mass of sample. The tests were performed in triplicate. Samples containing DS were also evaluated.

2.2.7. Preparation of tablets

Tablets were produced by dry granulation of excipients, in which retrograded samples and control samples were compressed (single punch machine KORSHI AR 400 –Erweka®, 10 mm punch and die set), milled in a mortar and calibrated on a sieve (0.297 mm mesh opening). The granulated excipients (300 mg) were thoroughly mixed with DS (100 mg) and compressed to producing tablets with a minimal hardness of 30 N. Tablets were labeled according to 2.2.1 section, however M prefix was replaced by the T prefix, resulting in T20, T40, T220 and T240 samples.

For comparative analysis, tablets of retrograded starch/pectin (added RSP suffix) and retrograded starch and pectin physical mixture (added RSP-M suffix) were also evaluated. Retrograded starch/pectin materials were prepared according to procedure previously described by Meneguin et al. (2014).

2.2.8. Physical properties of tablets

Forty tablets were weighed on an analytical balance Owa Labor® and the mean and standard deviation was calculated [44]. Tablets hardness was determined using a durometer Schleuniger Phratmon® – 6D model (\( n = 30 \)). Thickness of 30 tablets was measured using an electronic micrometer (Mitutoyo MDC-M293, Tokyo, Japan) immediately after the compression and after 24 h. Friability was determined on Erweka® friability (TA 20 model) with 20 tablets accurately weighed and submitted to 100 revolutions. After rotations, tablets were carefully cleaned and weighed again. The mass loss during the test was calculated according Equation (6):

\[ \text{Friability(\%)} = \frac{W_i - W_f}{W_f} \times 100 \]  

Where \( W_i \) = initial tablet weight (g) and \( W_f \) = final tablet weight (g).

2.2.9. In vitro dissolution study

Dissolution tests of tablets were carried out using USP type II dissolution apparatus (paddle) in a Hanson Research (New Hanson SR-8 Plus) dissolution station, according to sink conditions. The system was kept at 37 ± 0.5 °C under 50 rpm rotation speed. Buffer solutions with different pH values were used (900 mL per vessel). At first, test was performed at HCl solution (0.1 N, pH 1.2) containing 1.5% of polysorbate 80 for 120 min, followed by step at phosphate buffer (0.1 mol/L, pH 7.4) for 240 min. Aliquots (5 mL) were collected at different times with replacement of equal volume medium. The amount of DS released from tablets (100 mg) was determined on a UV-VIS spectrophotometer (\( \lambda = 296 \) nm for acid medium and \( \lambda = 276 \) nm for phosphate buffer). The \( t_{80\%} \) (time required to release 80% of drug) was determined. Analysis were performed in triplicate.
2.2.9.1. Analysis of in vitro drug release mechanism. The analysis of mechanisms of drug release process was performed and dissolution data obtained in 2.2.9 section were fitted (SigmaPlot 10.0 software) to mathematical models like Korsmeyer-Peppas and Weibull. Mathematical models were fitted only until 60 and 63.2% of data, respectively.

2.2.10. Statistical analysis
When applied, the results were treated by one-way analysis of variance (ANOVA) to assess the significance of the differences between data. Tukey–Kramer post-test was used to compare the means of different treatment data (GraphPad Prism 5 software). Results with p < 0.05 were considered statistically significant.

3. Results and discussion
3.1. Enzymatic digestion and resistant starch content
The hampered digestibility of RS is due to the packing of amylase double helices, which reduces the access of α-amylase to glycosidic linkages [13,45]. The specific enzymatic digestion of RS in the colon assigns the retrograded samples as a promising material to the development of carriers that aim to target the drug to the colon [46,47].

The results of the resistance against enzymatic digestion of HAS, GS (40% HAS dispersion), M120, M140, M220 and M240 samples are presented in Table 1. It was found that the original RS content present in the HAS was 57.07%, however, the gelatinization process promoted its significant reduction, getting to levels of about 44%, since this process destroys the molecular order of the starch granule, making it more easily digested [48].

The amount of RS in M220 and M240 samples was improved (75.84–76.55%) and greater, in relation to those presented in M120 and M140 samples (72.30–73.85%). This raise in the RS content can be attributed to increased temperature (30 °C) and storage time (16 days) employed in M2, which allowed a higher mobility of polymeric chains, promoting a more extensive structural rearrangement followed by the increasing of crystallinity.

Furthermore, temperatures near to $T_g$ (around 6 °C), favor the nucleation of crystals, while higher temperatures (30–40 °C) contribute for their propagation. So, when retrogradation is followed by the increasing of crystallinity, while higher temperatures (30 °C) and higher times of retrogradation (16 days), which favor the evaporation of water. Moreover, the lower moisture of some materials is frequently associated to a higher structural crystallinity, since less hydroxyl groups will be available for interacting with water molecules [54]. This relation can be confirmed by XRD patterns (3.2.2 section), in which the samples that had lower water content (M2 samples), also showed different crystallinity patterns indicated by the presence of an additional peak at 13° (2θ).

3.2.1. Moisture content
The moisture content can affect a set of physical and mechanical properties, and therefore contributing to decrease the apparent density of particles, affecting the compressibility, porosity, flow ability and the dissolution of tablets [51]. Furthermore, the moisture existent in the drug accelerates its hydrolysis as well as facilitates the reactions to excipients and adjuvants [52].

According to Table 1, the moisture content of samples is within the limits for starch (from 10 to 14%) [53]. The slightly lower water content of M2 samples can be attributed to the alternated temperatures for starch retrogradation (4 °C and 30 °C), and higher time of retrogradation (16 days), which favor the evaporation of water. Moreover, the lower moisture of some materials is frequently associated to a higher structural crystallinity, since less hydroxyl groups will be available for interacting with water molecules [54]. This relation can be confirmed by XRD patterns (3.2.2 section), in which the samples that had lower water content (M2 samples), also showed different crystallinity patterns indicated by the presence of an additional peak at 13° (2θ).

3.2.2. X-ray diffraction measurements (XRD)
HAS is a semi crystalline polymer and it presents different crystalline structures. The X-ray diffractogram of the HAS sample (HYLON VII) (Fig. 1) exhibited pronounced peaks at 17.02° and 23° (2θ), which are characteristic of the B polymorph. The peak at 25° (2θ) can be related to the A polymorphs [55]. The occurrence of peak at 19.8° (2θ) indicates the occurrence of the V polymorph, suggesting a highly ordered crystalline structure of amylose-lipid complexes into starch granules [11,46,56]. XRD of HAS demonstrated that it consists of a heterogenic mix of B and V structures as previously reported by Shamai [57].

The G5 diffractogram evidenced the disappearance of the main characteristic peaks of the HAS, showing a predominantly amorphous pattern as the gelatinization process promotes the disruption of starch granules, leading to disappearing of crystalline regions [58,59].

In the M120 and M140 diffractograms, peaks about 17°, 19.8° and 23° (2θ) were maintained, which are related to the predominance of the B type (~17° and 23° (2θ)), and the V type (19.8° (2θ)) polymorphs while the peak at 25° (2θ) disappeared. In the XRD patterns of M220 and M240 the same peaks observed for M120 and M140 were preserved, but the peak around 13° (2θ) was more intense, which is related to the V crystalline structure [57] and such change is indicative of the resistant starch presence. Cui [27] reported that the V type crystallinity reduces the water uptake of starch granules and make them more resistant to digestive enzymes.

<table>
<thead>
<tr>
<th>Samples</th>
<th>RDS (%)</th>
<th>LDS (%)</th>
<th>RS (%)</th>
<th>Moisture content (%)</th>
<th>Surface area (BET) (m²/g)</th>
<th>Pore volume (cm³/g)</th>
<th>Pore size (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAS</td>
<td>18.48 ± 0.012</td>
<td>24.45 ± 0.025</td>
<td>57.07 ± 0.023</td>
<td>12.23 ± 0.321</td>
<td>--</td>
<td>No measurable</td>
<td>--</td>
</tr>
<tr>
<td>G5</td>
<td>17.13 ± 0.014</td>
<td>38.07 ± 0.014</td>
<td>44.80 ± 0.003</td>
<td>--</td>
<td>--</td>
<td>No measurable</td>
<td>--</td>
</tr>
<tr>
<td>M120</td>
<td>10.58 ± 0.003</td>
<td>17.12 ± 0.018</td>
<td>72.30 ± 0.015</td>
<td>7.30 ± 0.100</td>
<td>0.023</td>
<td>0.000246</td>
<td>28.185</td>
</tr>
<tr>
<td>M140</td>
<td>11.02 ± 0.003</td>
<td>15.12 ± 0.029</td>
<td>73.85 ± 0.026</td>
<td>7.06 ± 0.152</td>
<td>0.206</td>
<td>0.000567</td>
<td>28.218</td>
</tr>
<tr>
<td>M220</td>
<td>10.40 ± 0.002</td>
<td>13.77 ± 0.013</td>
<td>75.84 ± 0.015</td>
<td>7.13 ± 0.057</td>
<td>0.030</td>
<td>0.003504</td>
<td>28.345</td>
</tr>
<tr>
<td>M240</td>
<td>10.56 ± 0.015</td>
<td>12.90 ± 0.007</td>
<td>76.55 ± 0.022</td>
<td>7.00 ± 0.100</td>
<td>1.098</td>
<td>0.000764</td>
<td>28.306</td>
</tr>
</tbody>
</table>
3.2.3. Thermogravimetric (TG) and derivative thermogravimetric (DTG) analysis

The TG/DTG curves of M120, M140, M220 and M240 samples displayed in Fig. 2 show a first degradation stage between 40 and 110 °C, which is related to the moisture evaporation during the heating, according to previous studies with other polysaccharides [33]. The second and main stage of degradation (69–75%) occurred between 250 and 350 °C and it was related to the elimination of polyhydroxyl groups, followed by the depolymerization and decomposition of the starch [60].

The GS showed 66% of mass loss and the principal degradation event occurred between 200 °C and 300 °C, while the degradation peak of the samples submitted to retrogradation process shifted to highest values, indicating that the process promoted the increase of thermal stability. Besides, this set of results evidences the lower thermal stability of the GS attributed to the granular structure loss due to the heating in water excess [61].

The preparation method and amylose concentration did not influence the thermal behavior, since no significant differences between the TG/DTG curves of M1 and M2 samples were observed.

3.3. Micromeritic properties

3.3.1. Granulometric distribution

It was possible to observe a great similarity among granulometric distribution profiles of M120, M140, M220 and M240 samples (Fig. 3), and the higher size frequency for all samples ranging between 0.210 mm and 0.250 mm (about 26.15%). There was no difference between the values of the mean diameter (about 0.19 mm) (p > 0.05).

3.3.2. Bulk and tapped apparent densities

Bulk density and tapped density of M120, M140, M220 and M240 samples and their physical mixtures (PM) with DS (Table 2) were statistically different (p < 0.05). Retrograded samples containing 20% of polymer showed lower density values than those prepared with 40% of polymer, indicating that the increase of the starch concentration promoted the building of denser particles, which should lead to better flow properties [62]. Furthermore, PM exhibited higher values of both bulk and tapped densities. This density raise can be related to the presence of the drug, which probably led to a higher number of interparticle contacts, making powder more packed and dense [30].

3.3.3. Analysis of flow ability

The values of repose angle of samples (Table 2) were similar (p > 0.05) and ranged between 20° and 30°, indicating their free flowing ability. On the other hand, the high value of repose angle of HAS (39.96°) demonstrated its poor flow. This behavior can be related to the lower moisture percentage of M120, M140, M220 and M240 (7–7.3%) in relation to HAS (12.23%) (Table 1). According to Albero [63], the decrease of flowability can be caused by the increase of the adhesion in existent points of contact between granules as a result of the water surface tension.

3.4. Porosity determination

Porosity represents the total sum of void spaces in solid materials in the form of pores, cracks and cavities of different sizes and shapes and it can influence important physical-chemical properties, such as adsorptive properties, mechanical strength, dissolution and wettability [64].

According to Table 1, porosity values for GS were not measurable. This fact can be associated to the starch heating in an aqueous medium that allows structural changes by the disruption of hydrogen bonds, which stabilize the granule internal crystal structure. Thus, an amorphous mass is formed, and therefore, it is not possible to observe the occurrence of pores, possibly because they are located in inaccessible regions for the adsorptive gas [65].

According to the results, it was observed that the surface area, the volume and size of these pores were affected mainly through the retrograded starch obtaining method, since samples prepared according M2 were more porous than those prepared by M1 (Table 1). This behavior can be related to the fact that the method that employed alternating cycles of temperature (M2) was
responsible for the construction of more crystalline structures, as already evidenced in the X-rays diffraction analysis (3.2.2 section). Samples obtained with higher concentration of polymers (40%) also showed higher values of surface area in relation to the others (20%). In addition, such data are in agreement with the density data (Table 2), in which 20% samples exhibited lower density than 40% samples.

Based on the IUPAC pore size classification, in which microporous materials have pore diameters of less than 2 nm (20 Å), mesoporous materials between 2 nm and 50 nm (20 Å e 500 Å) and
macroporous materials have a pore diameters greater than 50 nm (500 Å) [66], all samples of this study have predominantly mesoporous structures.

3.5. Liquid uptake ability

The drug release rate from swellable matrices is controlled by three sequential events: water absorption, matrix swelling and drug diffusion throughout gel layer, and it can be influenced by ionic strength and/or dissolution medium pH [67,68].

All samples had a pH-responsive behavior (Fig. 4) with lower liquid uptake in acid medium (pH 1.2) than at pH 7.4, which is an inherent behavior of anionic polymers. In acid medium, the hydrophilicity is lower because the carboxyl groups remain in their protonated form, causing a strong entanglement of the polymeric chains, and thus reducing the water entrance and retention [69].
Otherwise, the increase of pH values promotes the ionization of carboxyl groups and the consequent repulsion of chains with network dilation, which favors the water entrance [70].

M120, M140, M220 and M240 samples had a low water uptake compared to HAS due to changes in the crystallinity as a result of the starch retrogradation by the action of mechanical and thermal energy [71,72]. In fact, the presence of another polymorph, indicated by the appearance of a new peak in the DRX studies (3.2.2 section), may have resulted in different chain arrangements, with lower probability of hydroxyl groups interacting with water molecules [54], lowering the water uptake.

3.6. Physical properties of tablets

The physical parameters values of the tablets' quality (Table 3) showed low weight variation (0.5–0.75%) on them, demonstrating that suitable flow properties of granules allowed the uniform filling of the die and punch set.

All tablets showed hardness greater than 30 N and friability lower than 1.0%, indicating that the tablets have suitable mechanical strength and are resistant to breakage during packing, shipping and handling of the product [44].

After 24 h of obtaining the tablets, there was no significant increase in thickness (Table 3) (p > 0.05), indicating that the material did not undergo significant elastic recovery after compression.

3.7. In vitro drug release profile

There are several factors that can influence the kinetics of the drug release. Among them are drug properties as polymorphic form, crystallinity, particle size, solubility and the amount of drug incorporated into the dosage form. Physical properties of polymers, such as composition, molecular weight, crosslinking density and porosity also are decisive in the control of release rates [73].

When the hydrophilic matrix is in contact with GIT fluids, the surface hydration leads to a liquid uptake and to the formation of a swollen layer, which represents a physical barrier against the drug diffusion and controls the drug release rates [74].

DS, a nonsteroidal benzeneacetic acid derivative, belonging to BSC class II drug, has weak acidic properties (pKa ~4) and pH-dependent solubility, being practically insoluble in hydrochloric acid pH 1.2 (BARTOLOMEI et al., 2006; MERCK INDEX, 2006). DS release profiles from tablets with a different composition are presented in Fig. 5, which demonstrates that drug release rates in acid medium (0.1 N HCl; pH 1.2) were always lower than those shown in phosphate buffer (pH 7.4). This pH-dependent behavior is in agreement with the liquid uptake data, which increased with the change of medium pH of 1.2–7.4. Besides, at acid pH, DS is in the protonated form, and therefore, has lower release rates.

In acid medium (0.1 HCl; pH 1.2), the DS release from T120, T140, T220, and T240 started only after 15 min of test, and after 120 min, 42% of drug was released. Unlike, the HAS tablet started the release after 5 min and reached 49.4% after 120 min.

Although HAS also gelatinizes and possesses the V polymorph, which is characteristic of a highly ordered crystalline structure, it is believed that the higher control of the release rates from retrograded samples is a result of the changes in the crystallinity patterns. Such modifications are caused by the retrogradation process, as the formation of resistant starch in the shape of small crystallites, for example, that can fill the void spaces of the polymeric network.

![Figure 4](image_url)  
**Fig. 4.** Liquid uptake (%) of samples at equilibrium (120 min) in different media.

### Table 3

Physical properties of the tablets.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Thickness T0 (mm)</th>
<th>Thickness T24h (mm)</th>
<th>Average weight (mg)</th>
<th>Hardness (N)</th>
<th>Friability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T120%</td>
<td>3.990 ± 0.162</td>
<td>4.081 ± 0.154</td>
<td>398 ± 0.003</td>
<td>36 ± 0.585</td>
<td>0.8487 ± 0.023</td>
</tr>
<tr>
<td>T140%</td>
<td>4.047 ± 0.100</td>
<td>4.053 ± 0.106</td>
<td>403 ± 0.005</td>
<td>38 ± 0.445</td>
<td>0.8833 ± 0.194</td>
</tr>
<tr>
<td>T220%</td>
<td>3.857 ± 0.150</td>
<td>3.940 ± 0.154</td>
<td>399 ± 0.004</td>
<td>34 ± 0.659</td>
<td>0.8436 ± 0.145</td>
</tr>
<tr>
<td>T240%</td>
<td>4.002 ± 0.051</td>
<td>4.324 ± 0.063</td>
<td>401 ± 0.003</td>
<td>37 ± 0.503</td>
<td>0.8124 ± 15.95</td>
</tr>
</tbody>
</table>
difficulting the entrance of water and subsequent diffusion of drug molecules. Furthermore, these retrograded samples showed inferior liquid uptake in relation to the HAS, so polymeric chains should be in a more entangled state with a more viscous gel layer, hampering the diffusion of drug molecules for the dissolution medium.

However, a highest delay in the DS release was observed for samples obtained with pectin addition (RSP and RSP-M), as the
release started only after 30 min of test. Moreover, it was verified an important reduction of release rates (~35% from RSP, 30% from RSP-M and 25% from T40RSP-M) after 120 min. This behavior was attributed to the pectin presence, which forms insoluble aggregates in acid medium [75] and allows the building of a swollen layer that controls the release rates [76].

The increase of pH from 1.2 to 7.4 probably caused an expansion of the polymeric network due to the ionization of carboxylate groups, contributing to the drug diffusion and to increase of the release rates [31]. The ionization of DS in increased pH (pka ~4) also contributes to enhance the drug release rates, favoring the dissolution process. So, the DS release from T120, T140, T220 and T240 samples reached 100% in 60 min, against 87% in 120 min from HAS tablets, fact that was attributed to the formation of a more cohesive gelated matrix [62]. Drug release of RSP and RSP-M was significantly sustained to 240 min.

The analysis time for reaching 80% of the drug release \( (t_{80\%}) \) in 0.1 M phosphate buffer (pH 7.4) shows that RSP-M samples allowed control for a longer time \( (t_{80\%} = 210 \text{ min}) \) than RSP \( (t_{80\%} = 150 \text{ min}) \). This behavioral difference was assigned to conditions of retrogradation process for RSP, possibly leading to changes in pectin properties, and therefore, resulting in the viscosity reduction of the gel layer formed during the dissolution process.

### 3.8. Analysis of in vitro drug release mechanisms

Based on the values of adjusted \( r^2 \) (Table 4), it can be noted that in acid medium all samples fitted better with the Weibull model, wherein for samples obtained by M1 and M2 methods and RSP-M samples a complex mechanism of release \( (b = 1.186-1.625) \), involving simultaneously the relaxation of the polymer chains and erosion of the polymer during the release of the drug was observed. However, for HAS tablets in acid and phosphate buffer, the DS release occurred by Fickian diffusion \( (b = 0.2354; b = 0.2770, \text{ respectively}) \), while for the RSP in acid medium the release was reported according to the anomalous Fickian transport \( (b = 0.5095 \text{ to } 0.8531) \), i.e., depending on the processes of diffusion and polymer relaxation.

In phosphate buffer (pH 7.4), M1, M2 and RSP samples fitted better to the Korsmeyer-Peppas. This change of drug release mechanism was considered consistent as these samples also showed pH-dependent swelling and release behavior. A complex mechanism classified as super case II transport \( (n = 1.230-1.981) \) was observed, which implies a high rate of medium permeation through the matrix, enhancing the erosion process of system [77].

For RSP-M, the pH change from 1.2 to 7.4 did not lead to changes in the release mechanism.

### 4. Conclusions

The previously exploited methods for retrogradation of HAS were efficient, as they have provided materials with high RS contents, increased resistance to enzymatic digestion and suitable micromeric properties. However, the structural changes promoted by different retrogradation processes did not significantly affect the release properties. Thus, similar DS release profiles exhibited by both RS from M1 and M2 evidenced that M1 represents a simpler and shorter process that should lead to the reduction of production costs. The RS blending with pectin allows the drug release for a longer time, so that different drug release features can be reached, according to different therapeutic needs, making the applicability of these materials in the development of several controlled drug delivery systems possible.

### Declaration of conflicts of interest

The authors report no declarations of interest.

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