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Preparation and characterization of pullulanase debranched starches and their properties for drug controlled-release

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Debranched starches (DBS) with different degrees of debranching (low, L-DBS; moderate, M-DBS; high, H-DBS) were prepared and investigated. After pullulanase modification, the starch granules turned to be more porous, and many small particles containing short glucan chains generated. DBS starches adopt a single-helical V-type crystalline structure with low crystallinity. L-DBS samples contained fewer (20.70%) and longer (degree of polymerization, DP: 21.92) linear short glucan chains than their counterparts (M-DBS: 40.92%, 20.05 DP; H-DBS: 55.52%, 18.52 DP.). Pullulanase enzymatic hydrolyzate for DBS samples with higher degrees of debranching was apt to retrogradation at 20°C. DBS starches could form stable hydrogel, and the higher G′, G″ values indicated DBS samples of higher degrees of debranching formed a stronger gel network. L-DBS could hold more water, and its digestibility was higher. The *in vitro* test convinced DBS is a good candidate to control drug release for over 12 h. Furthermore, the drug release profiles from both DBS-based and HPMC-based tablets were anomalous transport mechanism. The drug release from these four matrices was controlled by a combination of drug diffusion and matrix erosion. The drug release properties from DBS-based tablets were considerably influenced by the degree of debranching. The *in vitro* drug release profile of M-DBS was similar to that of HPMC (f_2 = 60.75), while L-DBS and H-DBS differed from HPMC (f_2 < 50). Summarily, DBS starch is a good hydrogel candidate, and it can be used as excipients in oral tablets to control drug release.

1 Introduction

Starch is the second most abundant carbohydrate in nature only next to cellulose.[1] It is composed of linear amylose and branched amylopectin. Starch is a cheap, nontoxic, biocompatible and biodegradable material. Starch modification technologies, containing physical, chemical and enzymatical modifications, can endow starch derivatives new functional properties. Now, starch and its derivatives have been widely used in food, paper and textile, agriculture, plastic, consumer items, as well as pharmacy industry. [2-6]

Debranching enzymes, like pullulanase and isoamylase, can be utilized to hydrolyze 1,6-α-D-glucosidic bonds at branching points.[7] These enzymatically modified starches contain many linear, low-molecular-weight, recrystallizable glucan chains. These short glucan chains are available for chain realignment via hydrogen bonding and hydrophobic interactions, leading to chain aggregation and gel network formation.[8]

Most of the previous researches focused on the nutritional characteristics of debranched starches. Researchers confirmed the debranched starch (DBS) is an important source for the production of resistant starch (RS) and slowly digestible starch (SDS).[8-11] Temperature-cycling storage or low-temperature incubation can be used to induce retrogradation and recrystallization, thereby improving RS and SDS yields.[9, 11-13]

Retrograded starch,[14] starch acetate,[15] cross-linked starch,[16-18] carboxymethyl starch,[19-21] and grafted starch[22, 23] have been introduced into pharmacy industry used as excipients in oral tablets to control drug release attributed to their hydrogel properties. These modification can rectify the deficiencies of native starch used as extended-release excipients, like low compactibility, elastic compression and enzymatic degradation by α-amylase.[24] Debranched starches are hydrophilic and can form hydrogel in aqueous medium.[25-28] Thus, based on the previous researches, it is feasible to use debranched starches as hydrophilic excipients to extend drug release.

The fine structure of debranched starches can influence the realignment and retrogradation of short glucan chains. Various degrees of debranching generate differences in structural and hydrogel properties. Thus, debranched starches with different degrees of debranching would perform differently in forming hydrogel holding different properties and potential applications. In this work, we focus on investigating the influence of degree of debranching on the hydrophilic properties and drug release properties.

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Simultaneously, this work aims to explore the applications of debranched starches as excipients with different drug controlled-release properties.

2 Experimental

2.1 Materials

Maize starch was provided by Zhucheng Xingmao Corn Development Co., Ltd. (Amylose content: 27.59%±0.01%, batch no. 79696, Shandong, P. R. China). Pullulanase (Promozyme D2, EC 3.2.1.41, enzyme activity: 600 U/mL.) was obtained from Novozymes (Batch no. ATS20036, Tianjin, P. R. China). Pancreatin (batch no. SLBC2100V) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Propranolol hydrochloride was purchased from Wuhan Xinjialing Biotechnology Co., Ltd. (Batch no. 121002, Hubei, P.R. China). All other chemicals used in this study were of analytical grade.

2.2 Preparation of pullulanase debranched starch (DBS)

Maize starch (10% w/w, based on the dry weight of starch) was suspended in 0.01 M acetic acid buffer (pH 5.5), and boiled in a 250-mL pressure tube (ACE Glass, Vineland, NJ, USA) with constant stirring for 15 min. The tube was then transferred to a hot-air oven for 1 h at 130°C to gelatinize the maize starch. When the starch product was cooled to 55°C, pullulanase was added, and the hydrolysis process was maintained for various periods at 55°C. DBS samples with a low debranching degree (L-DBS) and moderate debranching degree (M-DBS) were hydrolyzed for 0.5 h and 4 h, respectively, using 10 μL/mg enzyme (based on dry basis starch). Samples with a high debranching degree (H-DBS) were prepared by hydrolyzing for 4 h with 30 μL/mg. Thereafter, the hydrolyzate was precipitated by slowly adding the solution to 1 L of anhydrous ethanol with continuous stirring. The mixture was stored at room temperature overnight, and the precipitate was collected after centrifugation at 4500 rpm for 15 min (RJ-LD-IIB, Ruijiang Instruments Co., Ltd., Jiangsu, P.R. China), followed by washing three times with 200 mL anhydrous ethanol. The modified starch was finally vacuum-dried (DZG-6050, Senxin Experimental Instrument Co., Ltd., Shanghai, P. R. China) at 40°C, and DBS was obtained after grinding and passing through a 100 mesh sieve.

2.3 Gel Permeation Chromatography

The molecular weight distribution (MWD) of starch samples was determined by gel permeation chromatography (GPC) using Cai's[29] method, with modifications. Starch samples (30 mg, dry basis) were dissolved in 90% DMSO (3 mL) containing 50 mmol/L $NaNO_3$ and constantly stirred in boiling water for 24 h. Samples were passed through a 0.45-μm organic filter and then injected into a Shimadzu HPLC/GPC instrument (CTO-20A, Shimadzu Corporation, Kyoto, Japan) equipped with a RID-10A refractive index detector, three Phenogel columns, specifically Styragel HR3 (molecular weight, MW: 500–30,000), Styragel HR4 (MW: 5,000–600,000), Styragel HMW7 (MW: 500,000–1×10^8) (Waters, Inc., Torrance, CA, USA), and a differential refractive index detector. The eluent system was composed of 90% (v/v) DMSO containing 0.5 mmol/L $NaNO_3$, and the flow rate was 0.8 mL/min. The column oven temperature was maintained at 80°C. Dextran standards (Sigma-Aldrich, Co., St. Louis, MO, USA) of different MW were used for method calibration. The data were processed using XPS Peak fit software, and after peak fitting, GPC data were used to characterize the MWD of the starch samples.

2.4 Scanning electron microscopy

Starch samples were coated with Au/Pd before examining the micromorphology under a high-resolution field-emission scanning electron microscope (SEM, S4800, Hitachi, Ibaraki, Japan) at an accelerating voltage of 1 kV. Images were obtained at 1200×, 2500×, 5000× and 10000× magnification.

2.5 Particle size analysis

The particle size distribution of DBS samples was determined using a Microtrac S3500 laser particle size analyzer (Microtrac Inc., North Largo, FL, USA) by dry method. The data was analyzed by Microtrac Flex software (version 10.3.3).

2.6 X-ray diffraction analysis

X-ray diffraction (XRD) analysis was conducted using an X-ray diffractometer (D8 Advance, Bruker AXS Co., Karlsruhe, Germany). Starch samples were scanned at 3°/min from 5° to 40° (2θ) at 35 kV and 20 mA with Cu-Kα radiation (λ = 1.5406Å). The data were analyzed using MDI Jade 5.0 software (Materials Data Inc., Livermore, CA, USA).

2.6 Brabender viscosity analysis

Brabender® viscograph (803200 series Brabender® OHG; Duisburg, Germany) was used to investigate the viscosity changes during pasting, pullulanase hydrolysis and retrogradation.

Native starches (45 g, dry basis) were dispersed in HAc-NaAc (0.01 mol/L, pH 5.5). The concentration of starch slurries was 10% (w/w), and the total mass was 450 g. At the pasting stage, the temperature of the slurry rose from 50°C to 95°C at a rate of 1.5°C/min and kept at 95°C for 30 min. Then, the temperature was reduced from 95°C to 55°C at a rate of 1.5°C/min. Pullulanase was added into the starch paste when the temperature reached 55°C. During the pullulanase hydrolysis phase, the temperature was held at 55°C for 0.5 h, 4 h, 4 h for L-DBS, M-DBS, H-DBS whose pullulanase dosages were 450 μL, 450 μL, 1350 μL, respectively. Then, the system was rapidly heated from 55°C to 95°C at a rate of 5°C/min, and the mixture was held at 95°C for 15 min to terminate the reaction. The enzymatic hydrolyzate was cooled to 20°C at a rate of 1.5°C/min and keep at 20°C for 3h aiming to investigate the retrogradation of debranched starch.

2.7 Rheological properties

Rheological properties of DBS samples were measured according to the method mentioned in our previous work.[25, 28] The tests were taken on a TA AR-G2 rotational rheometer (TA Instruments, New Castle, DE, USA) with a 60-mm flat-plate system. The flat gap was 1 mm. Starch slurry (10%, w/w) was prepared for strain sweep, frequency sweep, time sweep. The edge of the flat-plate was covered by silicon oil to prevent the water evaporation during measurements.

Strain sweep was measured at the constant frequency of 1 rad/s over the strain range of 0.1%-100% at 30°C to determine the linear viscoelastic region of the samples. Frequency sweep was conducted at an appropriate strain within the linear viscoelastic region at 30°C over the angular frequency range of 0.1-100 rad/s. Time sweep was measured at 30°C and the frequency of 1 rad/s for 1 h at a strain within the linear viscoelastic region. Storage modulus (G′) and loss modulus (G′′) were used to evaluate the rheological properties of DBS samples.

2.8 Water holding capacity (WHC)

WHC of DBS starches was measured by the modified method of Onofre.[30] DBS starches (40 mg, dry basis, W_0) mixed with 1.5-mL distilled water were added to a 2-mL centrifuge tube (W_1: weight of the tube). Then, the centrifuge tube was incubated in a hot-air oven at 37°C for 1 h. The tube was immediately centrifuged at 15,000 rpm for 10 min (TG16G; Kaida Scientific Instruments Co., Ltd., Hunan, P. R. China). Then, the supernatant was carefully removed, and the tube was accurately weighted again (W_2: weight of the gel and tube). The WHC value was calculated according to Eq. (1).

$$\text{Water Holding Capacity (g/g)} = \frac{W_2 - W_1}{W_0} \quad (1)$$

2.9 Digestibility

The digestibility of DBS samples was measured according to Gurruchaga.[31, 32] The starch samples (150 mg, dry basis) were homogeneously dispersed in 100 mL phosphate buffer solution (simulated intestinal fluid, SIF, pH 6.8). The reaction mixture was poured into a stoppered 250-mL Erlenmeyer flask and 20 glass beads were added. The suspension was first incubated at 37°C for 15 min. Pancreatin was added to the suspension. Pancreatin (0.045 g) was prepared by suspending in 4 mL distilled water, and vigorously and constantly agitating in a 5-mL centrifuge tube using a vortex shaker for 10 min. The pancreatin dispersion was centrifuged at 4000 rpm for 15 min and a 10-μL aliquot of the supernatant was added to the starch suspension. Samples (2 mL) were withdrawn from the starch suspension at various times (0 h, 1 h, 2 h, 6 h, 12 h, and 24 h) and centrifuged (3000 rpm, 5 min). The supernatant (1 mL) was then used to determine the reducing sugar content by 3,5-dinitrosalicylic acid (DNS) according to the method of Miller [33]. An identical sample without pancreatin was used as a control.

2.10 Tablet preparation.

DBS samples (80%, w/w) and the drug model (propranolol hydrochloride, 20%, w/w) were dry-blended in a sealed plastic box, and 250 mg of the mixture was directly compressed at 15 kN using a 10-mm, flat-faced, tooling on a hydraulic press (769YP-15A, Tianjin Keqi High & New Technology Co., Tianjin, P.R. China).

2.11 In vitro release test.

The in vitro drug release properties of DBS-based tablets were measured using an United States Pharmacopeia XXIII dissolution apparatus 2 (paddle apparatus) according to Onofre with minor modifications.[30] The drug dissolution tests were performed in dissolution medium (900 mL) at a paddle rotation speed of 50 rpm at 37°C. The drug release analysis was continuously performed in different dissolution media without replacement. In the first stage, drug release was measured in simulated gastric fluid (SGF, pH 1.2 hydrochloric acid solution with 0.05 mol/L NaCl) for 2 h. Then, the pH of the medium was adjusted to 6.8 by adding an appropriate amount of anhydrous Na_3PO_4. Pretreated pancreatin (0.45 g) was then added to the SIF medium. The pH was increased to 7.4 by adding anhydrous Na_3PO_4 (simulated colon fluid) after 4 h and maintained for up to 24 h. Samples were withdrawn at 0 h, 1 h, 2 h, 6 h, 12 h, and 24 h, and the ultraviolet absorption at 289 nm was evaluated.

3 Results and discussion

3.1 Debranching degree analysis

DBS with various degrees of debranching were prepared under different pullulanase hydrolysis conditions. L-, M-, and H-DBS were used to investigate the influence of debranching degree on the structural and hydrogel properties of DBS starches. The GPC was used to evaluate the debranching degree of DBS samples, and the results are shown in Table 1.

1,6-α-D-glucosidic bonds exist at the branching points of starches, and can be selectively hydrolyzed by pullulanase leading to the formation of linear short glucan chains.[34] Peak 1 represents the larger molecules distribution containing amylopectin, partially debranched amylopectin and large amylose, while Peak 2 (smaller molecules distribution) is composed of smaller amylose and glucan.[12]

The area fractions of Peak 2 were 20.70%, 45.92%, and 55.52% for L-DBS, M-DBS, and H-DBS samples. The MW of Peak 2 indicated that the average chain length (CL) of the

Table 1

Molecular weight and fractions of L-MW and H-MW molecules in DBS.

Starch samples	Mw (Da)	Peak 1*		Peak 2**	
		Peak Area (%)	MW (g/mol)	Peak Area (%)	MW (g/mol)
L-DBS	1555299	79.30	1960429	20.70	3552
M-DBS	176855	54.08	324276	45.92	3248
H-DBS	144518	44.48	321168	55.52	3001

* Peak 1 represents larger molecules (H-MW);

** Peak 2 represents smaller molecules (L-MW).

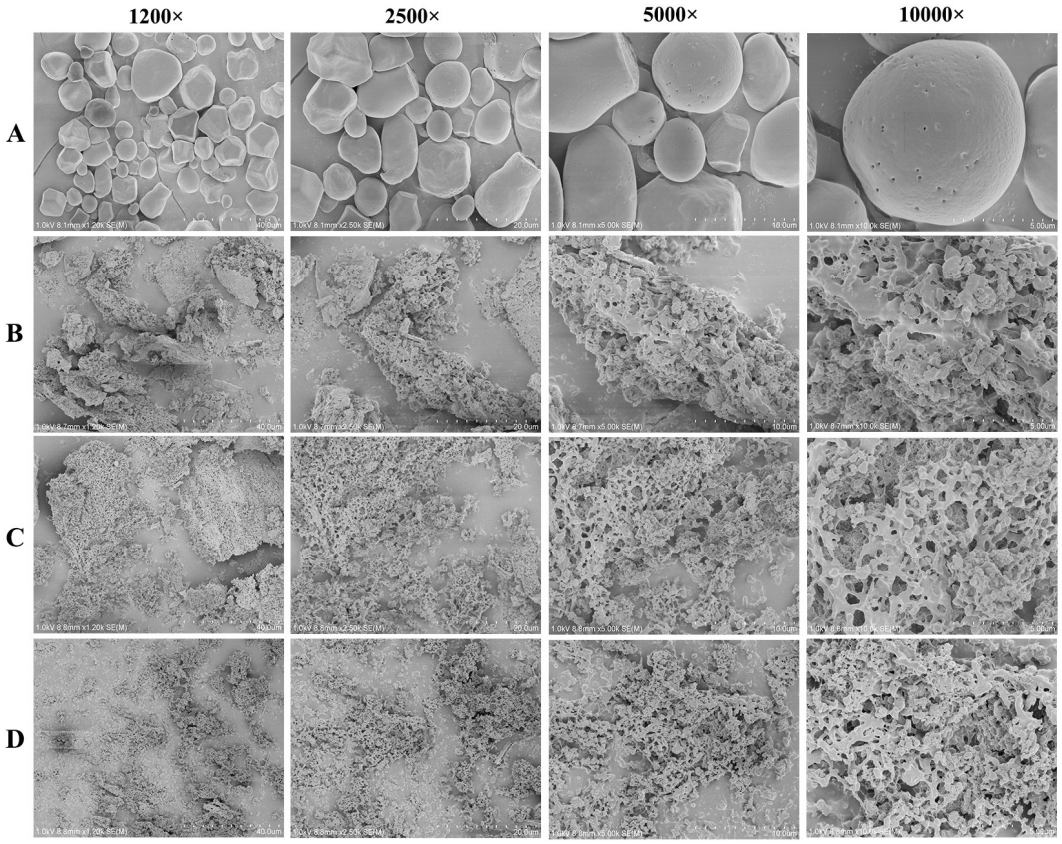


Fig.1 SEM images of starch samples with different magnifications. A: native starch (NS); B: L-DBS; C: M-DBS; D: H-DBS.

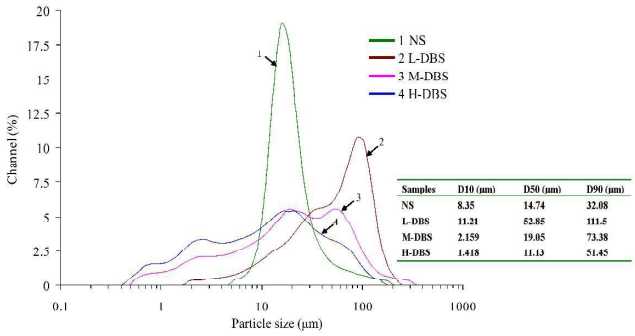


Fig.2 Particle size distribution of NS and DBS starches.

smaller amylose and glucan for L-DBS, M-DBS, and H-DBS were 21.92 DP (MW: 3552 g/mol), 20.05 DP (MW: 3248 g/mol), and 18.52 DP (MW: 3001 g/mol), respectively (Table 1).

The GPC results indicated that DBS with a higher degree of debranching contained more and shorter glucan chains. These lower MW molecules can endow modified starches with novel properties and they are beneficial for holding water, forming hydrogel, as well as retrogradation and RS/SDS formation.[8, 12, 29, 35, 36] Debranched starches with various degrees of debranching would influence the structural and functional properties.

3.2 Morphological properties

SEM images of DBS samples are shown in Fig.1. The starch granule structure was thoroughly destroyed after debranching. Starch fragments and porous structure formed and strengthened with increasing degrees of debranching. The realignment and aggregation of glucan chains contributed to the formation of starch fragments, while chemical desiccation, such as washing with anhydrous ethanol, resulted in the formation of porous structure, generating products with a noticeably increased specific surface area.[37] These new structural properties are beneficial for starches to capture water. Thus, DBS samples would perform better in holding water to form hydrogel.

3.3 Particle size distribution

The particle size of starch is closely related to its physicochemical and functional properties, like pasting properties, rheological properties as well as digestibility.[38-41] Particle size distribution was analyzed using Microtrac S3500 laser particle size analyzer by dry method. The results are shown in Fig.2. Fig.2 shows more small particles (smaller than 10 μm in diameter) exist in DBS samples with higher degrees of debranching.

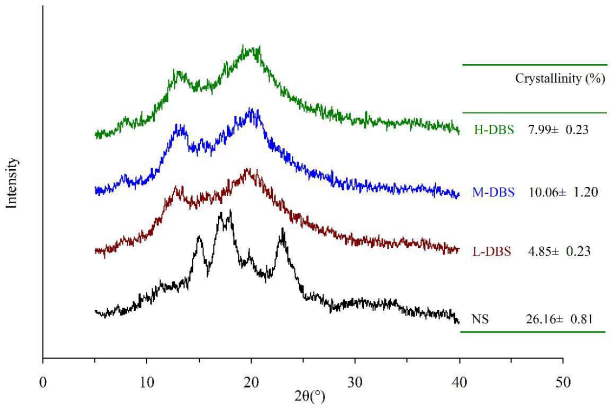


Fig.3 XRD diffraction patterns of NS and DBS starches.

The D50 values (particle size diameter when passing particle percent reaches 50%) are 52.85 μm, 19.05 μm, 11.13 μm for L-, M- and H-DBS samples, respectively. These results compared with the results of GPC (Table 1) and SEM (Fig.1)
confirmed more small particles containing many short glucan chains generated after pullulanase modification. In addition,the generation of particles larger than 100 μm in diameter was mainly caused by granule swelling and caking during the preparation of DBS samples. These small particles containing short glucan chains are beneficial for the hydrophilic properties, which is a prerequisite for holding water to form hydrogel.[42, 43]

3.4 Crystalline characterization

The XRD results are illustrated in Fig.3. Native maize starch had a double-helical A-type crystalline structure, whereas DBS starches adopted a single-helical V-type crystalline structure. The crystallinity of DBS samples was lower than that of native maize starch (26.16%) indicating DBS starches possessed more amorphous structure. In contrast, the crystallinity of L-DBS was the lowest, while that of M-DBS was slightly higher than that of its counterparts.

Although the crystalline structure of native starch is destroyed during gelatinization at high temperatures (130°C), short glucan chains generated by pullulanase hydrolysis can induce retrogradation and recrystallization.[13] The formation of an inclusion complex between the linear short glucan chains and anhydrous alcohol causes striking changes in crystalline structure.[44, 45]

In contrast, the single-helical V-type crystalline structure possesses larger hollow structures beneficial for accommodating small molecules.[46, 47] The increase in amorphous starch also contributes to the formation of gel network [14]. After association, the amorphous and non-ordered starch chains developed into a non-ordered gel network, which is resistant to the erosion of gastrointestinal enzymes.[48-50] The combination of these new crystalline structures plays important roles in hydrogel applications and the delivery of targeting substances.

3.5 Hydrolysis and retrogradation properties of pullulanase enzymatic hydrolyzate

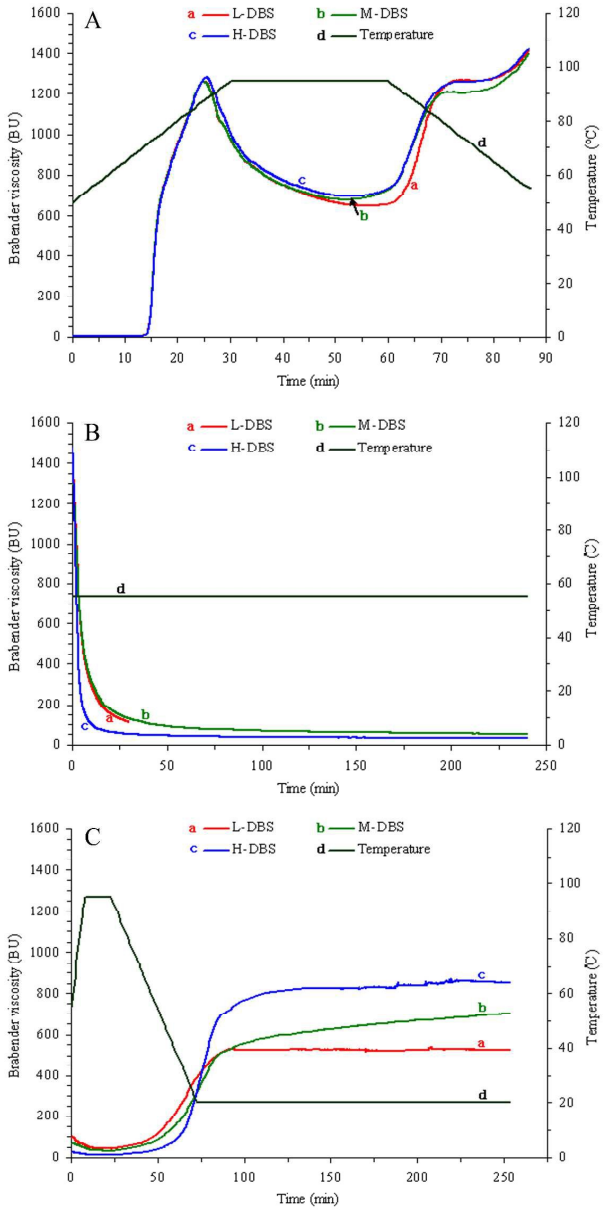


Fig.4 Brabender viscosity curve. (A) Pasting stage; (B) Pullulanase hydrolysis stage; (C) Enzyme inactivation and retrogradation stage.

A 803200 Brabender® viscograph was used to investigate the viscosity changes during pasting, pullulanase hydrolysis and retrogradation aiming to investigate the effect of fine structure of starch molecules on the aging of pullulanase enzymatic hydrolyzate. The results are shown in Fig.4.

Pullulanase was able to reduce the viscosity of starch paste immediately. The pullulanase hydrolysis rate was higher during the preparation of H-DBS compared to that of L- and M- DBS (Fig.4B).The pullulanase enzymatic hydrolyzate performed differently during retrogradation (Fig.4C). The viscosity rapidly rose when the temperature cooled down

below 40°C. The phenomenon was more obvious for the H-DBS sample containing more short glucan chains. After aging

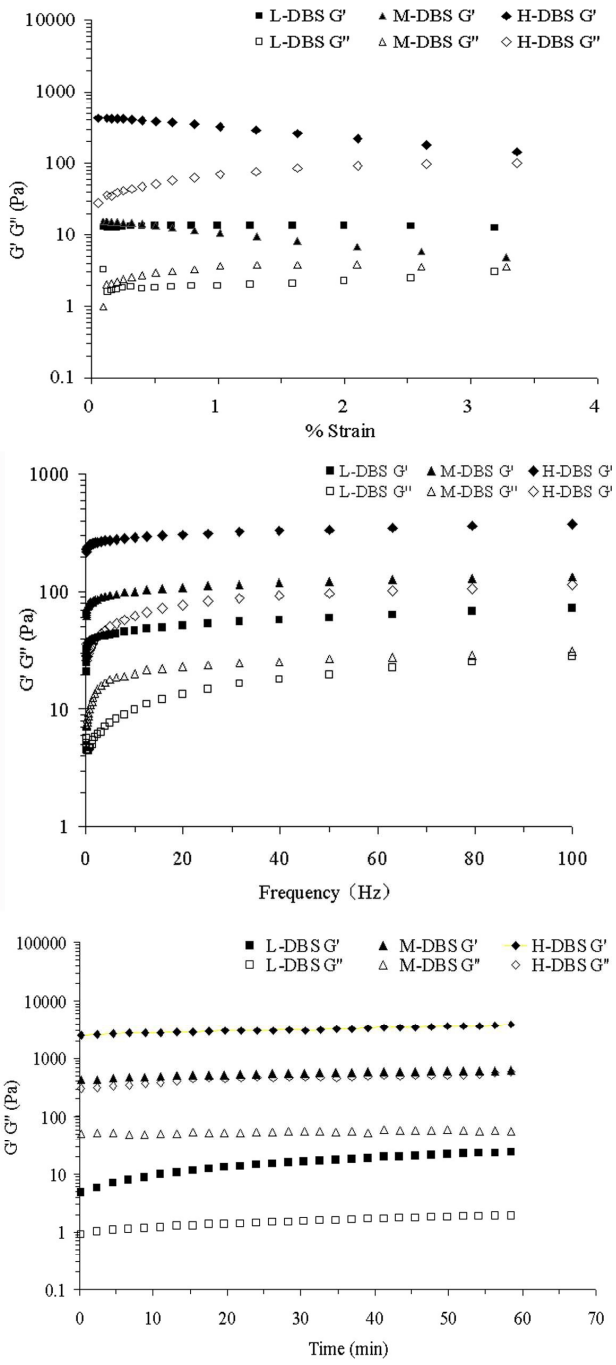


Fig.5 Rheological properties of DBS starches.

at 20°C for 3h, the Brabender® viscosity of H-DBS sample was higher than that of the other two samples. After the enzymatic modification by pullulanase, the chain length,molecular weight as well as the ratios of amylose to amylopectin and crystalline to amorphous structure are tremendously altered. These factors can influence the retrogradation of starch paste.[51] Pullulanase enzymatic hydrolyzate containing more short glucan chains was apt to retrogradation, while L-DBS tended to form more stable system in the retrogradation. Thus, the viscosity increased with the degrees of debranching in the retrogradation stage. These results indicate short glucan chains are beneficial for the realignment and retrogradation of DBS samples.

3.6 Rheological properties

Dynamic rheometer can be used to investigate the viscoelastic or rheological properties of starch samples.[52-54] The rheological properties of DBS samples were investigated aiming to study the hydrogel properties, and the results are illustrated in Fig.5.

The linear viscoelastic region of DBS slurry was identified during strain sweep test. The results indicated that the strain value of 0.5% was within the linear ranges for all the three DBS samples. Thus, the frequency sweep and time sweep were tested with this strain value during hydrogel studies.

DBS samples performed differently during frequency sweep and time sweep. The modulus (G′, G′′) of the DBS samples increased with the increasing frequency. The DBS sample with a higher degree of debranching was of higher G′, G′′ values during both frequency sweep and time sweep. The starches comprising more amylose are of higher G′ and G′′ values.[55] G′ and G′′ values of DBS samples containing more short glucan chains are also higher.

Short glucan chains generated from the decomposition of amylopectin tend to reassociate and hold water to form gel network. The content and mean DP values of glucan chains influence the behavior of forming hydrogel. The hydrogel properties were further studies by water holding capacity and digestibility.

3.7 Water holding capacity

The ability of holding water is the prerequisite to form hydrogel for hydrophilic materials.[56, 57] The WHC values of DBS starches are shown in Fig.6.

After debranching modification, more short glucan chains are generated. Short amylose or glucan chains tend to form an insoluble gel network holding less water, while the longer chains can capture more water to form softer hydrogel.[56] Highly branched amylopectin is less prone to

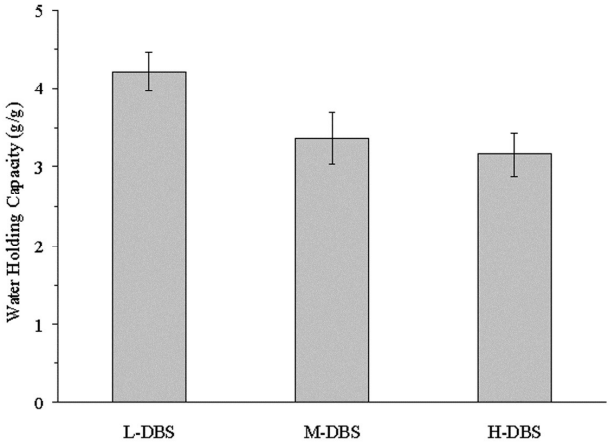
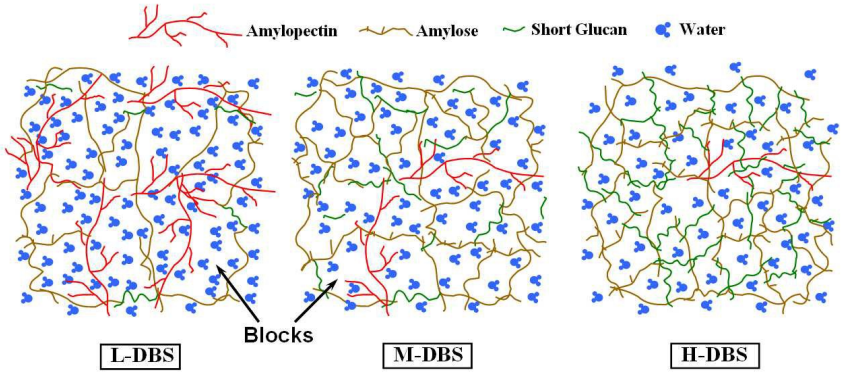


Fig.6 Water holding capacity (WHC) of DBS starches.



Scheme 1 Schematic diagram of gel network formed by DBS starches.

reassociation.[57] Therefore, DBS samples with lower debranching degree possess higher WHC values.

A schematic diagram about the influence of glucan chains on the structural properties of DBS-based hydrogel is illustrated in Scheme 1 based on the results of rheological properties and WHC values.

There are fewer (20.70%) short glucan chains in the L-DBS samples, and the glucan CL (21.92 DP) is longer than that of DBS samples with higher debranching degrees (M-DBS: 40.92%,20.05 DP; H-DBS: 55.52%, 18.52 DP.) (Table 1). In the L-DBS sample, the longer glucan chains tend to form larger blocks via hydrogen bondings and hydrophobic interactions. Shorter amylose and glucans possess better mobility and are most likely to align and aggregate to form smaller blocks. These blocks can hold less water. Thus, the gel network of DBS samples with higher degrees of debranching is more compact and stronger.

3.8 Digestibility

Intestinal α-amylase plays an important role in applying DBS starches as extended-release excipients in oral tablets to control drug release. Starch samples are apt to be hydrolyzed by α-amylase in small intestine. Therefore, it is necessary to investigate the digestibility of DBS samples. The curves in Fig.7 demonstrate that DBS starches can resist the hydrolysis

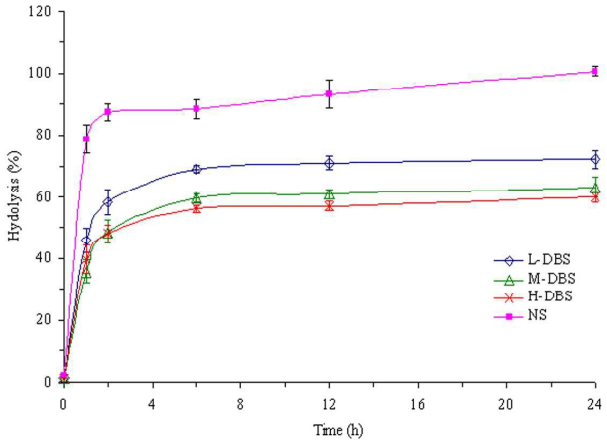


Fig.7 Digestibility of NS and DBS starches.

of intestinal α-amylase compared to native starch.

Furthermore, the hydrolysis values of L-DBS samples are substantially greater than M-DBS and H-DBS. Thus, DBS with a higher degree of debranching can more effectively resisted to the hydrolysis by gastrointestinal enzymes.

Gel network formed by the realignment and aggregation of glucan chains contributes greatly to the resistance to digestive enzymes. This is because the spatial conformation

of the gel network is convinced of obstruction of the erosion and degradation of water and enzymes, thereby leading to low enzymatic hydrolysis of DBS.31 As Fig.6 and Scheme 1 shown, L-DBS can hold more water, and the gel network of L-DBS was softer than that of M-DBS and H-DBS. Higher water holding capacity result in softer hydrogel and higher digestibility; thus, offering greater opportunities for the enzyme to gain access to the gel interior of L-DBS. Therefore, M-DBS and H-DBS were more resistant to digestive enzymes than L-DBS.

3.9 Drug release study.

Based on the above results, it is convinced that DBS samples can hold water to form hydrogel and resist to the degradation of gastrointestinal enzymes. Thus, we explored the feasibility of using DBS starches as excipients to control drug release in oral tablets. Propranolol hydrochloride (PH) was used as drug model. Hydroxypropyl methylcellulose (HPMC), a kind of cellulose candidates, is hydrophilic and performs excellently in forming hydrogel to control drug release. The purpose of this research was to prepare a modified starch which performed similarly to HPMC in extending drug release. Thus, in the *in vitro* tests, HPMC was used as a control. The *in vitro* drug release data were illustrated in Fig.8 and Table 2-3.

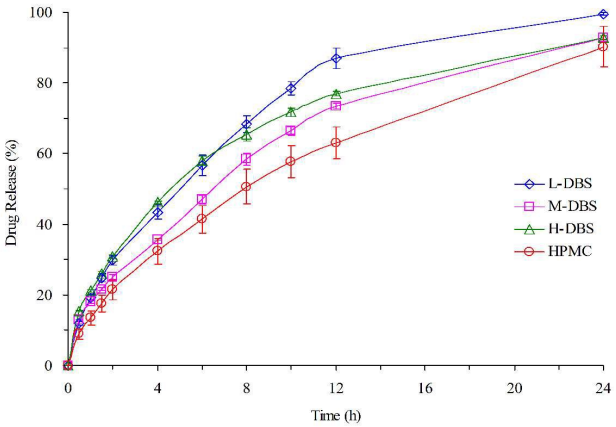


Fig.8 *In vitro* drug release of DBS-based tablets.

Table 2

Similarity factors (f2) of drug release from DBS-based and HPMC based tablets.

Comparison	f_2	Results
L-DBS/HPMC	42.83	Dissimilar
M-DBS/HPMC	60.75	Similar
H-DBS/HPMC	46.65	Dissimilar

The similarity factor (f_2) and Peppas equation were employed to evaluate the *in vitro* drug release properties (Table 2-3). The similarity factor (f_2) was defined by the US Food and Drug Administration (FDA) in order to evaluate the similarity between two different drug release profiles (Eq.(2)).

$$f_2 = 50 \cdot \log\{[1+(\frac{1}{n})\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \cdot 100\} \quad (2)$$

where R_t and T_t are the percentages of drug released at time *t*. An f_2 value between 50 and 100 indicates similarity between two drug release profiles.[58]

The drug release data (ranging from 5% to 60%) were fit to the Peppas[59] (Eq.(3)) to explore the kinetics and mechanism of drug release.

$$\frac{M_t}{M_\infty} = kt^n \quad (3)$$

where M_t/M_∞ is the fraction of drug released at time *t*, and *k* and *n* are the kinetic constant and diffusional exponent, respectively. The values of $n < 0.45$, $0.45 < n < 0.89$, $n > 0.89$ indicate Fickian diffusion, anomalous (non-Fickian) transport, and case-II transport,[60] respectively.

The *in vitro* drug release data in Fig. 8 indicate that both DBS-based and HPMC-based tablets can extend drug release for more than 12 h, and that there is no initial burst effect during drug release. According to f_2 values (Table 2), the *in vitro* drug release profile of M-DBS was similar to that of HPMC (f_2 = 60.75), while L-DBS and H-DBS differed from HPMC ($f_2 < 50$).

Table 3

Mathematical modeling and drug release kinetics of DBS-based and HPMC-based tablets.

Tablets	L-DBS	M-DBS	H-DBS	HPMC
n	0.6147	0.5354	0.5379	0.6266
r^2	0.9982	0.9930	0.9970	0.9997
r^2_{adj}	0.9977	0.9916	0.9963	0.9996
F	2205.36	711.44	1344.61	20223.3

Peppas equation was used to investigate the mechanism and kinetics of the drug release profiles from DBS-based and HPMC-based tablets, with the fitting constants listed in Table 3. All matrices provided *n* values between 0.5354 and 0.6266, revealing that the drug release mechanism from these four matrices was controlled by a combination of drug diffusion and matrix erosion (anomalous transport).[61]

The physical appearance of DBS-based and HPMC-based tablets during *in vitro* dissolution was also studied (Fig.9). The gel layer that formed on the surface of the DBS-based tablets was different. During the in vitro dissolution tests, tablets with a higher degree of debranching tended to form cracks on the surface. Cracks that formed on the surface of M-DBS and H-DBS matrices could decrease the path length for drug diffusion and increase the available area for Fickian release, leading to an increase in drug release from DBS-based tablets at later stages.[62] This property is essential for maintaining constant drug release and increasing the bioavailability of propranolol hydrochloride. The gel network of the L-DBS matrices was soft, and easily degraded by the dissolution

medium. During the *in vitro* test, L-DBS-based tablet was severely degraded, while M-DBS-based and H-DBS-based

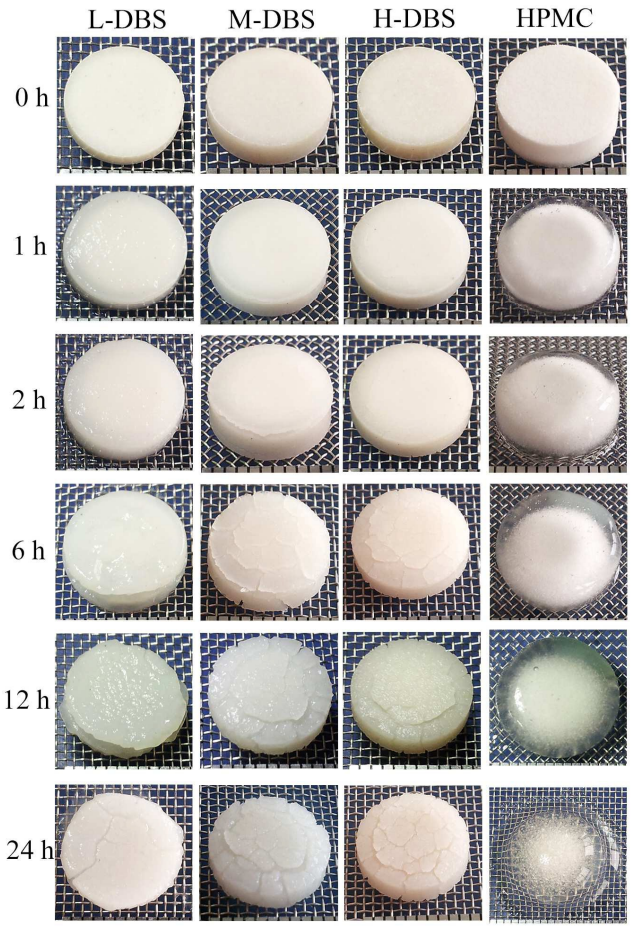


Fig.9. Physical appearance of DBS-based and HPMC-based tablets during in vitro dissolution tests.

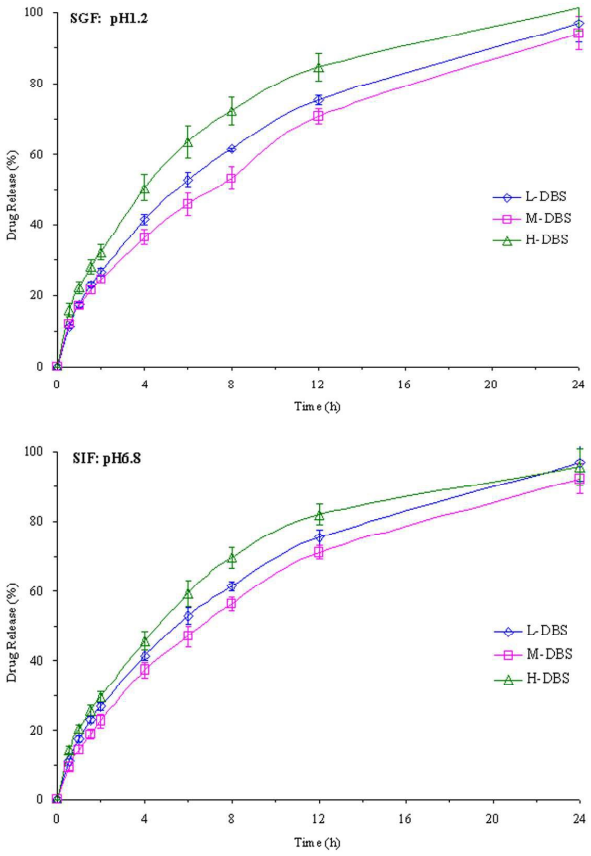


Fig.10. Influence of the pH values of release medium on drug release process from DBS-based tablets.

tablets were slightly eroded. And this result was related to the WHC and digestibility of DBS samples.

The evaluation of pH values of dissolution medium on the drug release properties is an essential part in the investigation of this extended-release systems. Thus, the drug release profiles in pH1.2 (Simulated gastric fluid, SGF) and pH6.8 (Simulated intestinal fluid, SIF) dissolution medium were shown in Fig.10. The results infers that there are no burst effect at the initial stages of the drug release profile, which can lead to a fluctuation of drug plasma concentration and do harm to the treatment of many illnesses, like hypertention. Furthermore, the low pH medium can increase the drug release slightly. However, the drug release profiles from pH1.2 medium and pH6.8 medium judging from the f_2 values (H-DBS: 72.85; M-DBS: 81.61; L-DBS: 77.27).

The above results indicated DBS is a good candidate to control drug release for over 12 h. The drug release properties from DBS-based tablets were considerably influenced by the degree of debranching. Furthermore, by controlling the degree of debranching and the tabletting technology (e.g. compression pressure, drug loading, magnesium stearate, etc.), controlled-release tablets with different drug release properties could be obtained.

4 Conclusions

The above results indicated the structural and hydrogel properties of DBS starches were influenced by the degree of debranching. These differences were attributed to the MWD and CL of DBS. More linear glucan chains and small fragments were released at higher degrees of debranching. The glucan CL of the H-DBS sample was the shortest (18.52 DP, MW: 3001 g/mol), followed by M-DBS (20.05 DP, MW: 3248 g/mol) and L-DBS (21.92 DP, MW: 3552 g/mol). Pullulanase enzymatic hydrolyzate containing more short glucan chains was apt to retrogradation. The glucan CL of L-DBS was longer leading to the formation of larger blocks which were capable to hold more water. And the L-DBS was more easily degraded by the dissolution medium. The *in vitro* drug release data showed that the drug release profiles of M-DBS based and HPMC-based tablets were similar. DBS matrices with a lower degree of debranching were likely degraded and eroded by the dissolution medium. In contrast, more cracks formed on tablets with a higher degree of debranching. The drug release from both DBS-based and HPMC-based tablets were controlled by the combination of drug diffusion and matrix erosion (anomalous transport). And all there four matrices were able to control drug release over 12 h. Thus, DBS starch was a good tablet matrix to extend drug release. Further studies on *in vivo* drug release and the tableting technology are necessary to investigate the drug release properties of DBS-based tablets aiming to prepare DBS-based tablets with different drug release properties.

Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (No. 31571794 and No. 31371787), the Key Program of the National Natural Science Foundation of China (No. 31230057) and the Twelfth Five-Year National Key Technology Research and Development Program of the Ministry of Science and Technology of China (No. 2012BAD37B01).

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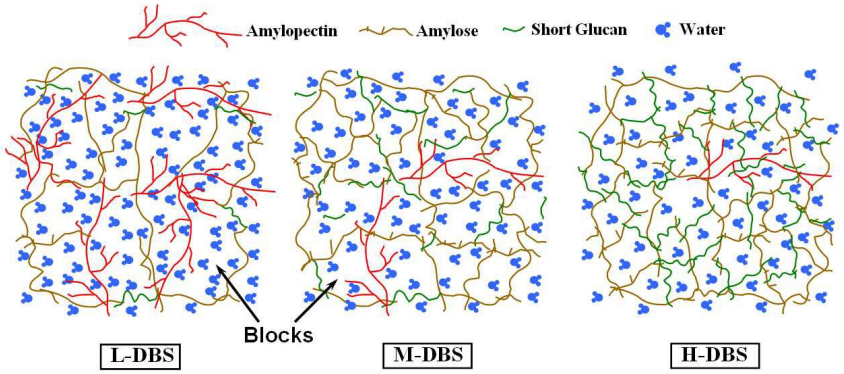

Linear short amylose and glucan tend to align and aggregate to form hydrogel holding less water. The drug release properties of debranched starch based tablets can be controlled by the pullulanase modification conditions.