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EFFECT OF DIFFERENT MODIFICATIONS ON THE EXCIPIENT PROPERTIES OF POTATO STARCH

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Abstract: Excipients are essential to the pharmaceutical delivery system as they assist in getting the drug to the proper site of action. Excipients directly and indirectly influence direct and indirect influence on the rate and duration of a drug's distribution and absorption. This study aimed to determine how thermal modification, carboxymethylation, and pre-gelatinization of potato starch affected the qualities of adjuvants. During pregelatinization and carboxymethylation, it became clear that amylopectin and the high water-holding capacity of amylose both contributed to the drug's rapid breakdown. SEM micrographs show that heat treatment results in the loss of native granular structure. Group acrylamide attachment to starch microparticle chains is visible by Fourier transform infrared spectroscopy (FT-IR) measurement. When compared to potato starch nanoparticles, grafted polyesters had less amorphous regions, according to X-ray diffraction (XRD), while the thermogravimetric analysis (TGA) showed that the composites have more heat stability. Peak temperature in the DSC is greater for carboxymethyl starch but lower for pregelatinized starch.

Index Terms - Starch microparticle, thermal modification, excipient, carboxymethylation, degree of substitution.

I. INTRODUCTION

Polysaccharides are increasingly larger molecules, often referred to as complex carbohydrates. Polysaccharides are polymeric carbohydrate molecules composed of long chains of small units known as monosaccharides. They are linked together by glycosidic bonds, which give the constituent monosaccharide or oligosaccharide on hydrolysis. Plants may produce large amounts of biomass, which is made up of polysaccharides(such as starch) or non-starch polysaccharides. The uses of polysaccharides has been seen in different domain till now, ranging from pharmaceuticals, food to textiles and paper industries, to mention a few. Numerous monosaccharide units linked together via o-glycosidic bonds to produce polysaccharide. The structure of these may range from linear to very branching. These are present in bacteria, mosses, plant, seaweeds, and fungus, and they provide important roles such energy storage, structural, and defensive mechanisms. Structural polysaccharides, storage polysaccharides, and gums are the three types of polysaccharides found in nature. Excipients are defined as "ingredients excluding from the API (active drug substances) final therapeutic dosage form that have already been consistently analysed for safety, quality and efficacy which has been included as well as in a drug delivery system," according to the International Pharmaceutical Excipients Council (IPEC) glossary of words. Chemically modified natural polysaccharides make up a substantial portion of today's synthetic excipients. In related to regulatory clearance, innovative natural polysaccharides, on the other hand, have several downsides, such as quality variations between geographical locations, seasonal fluctuation, structural complexity, contaminants, and loss of

stability during storage. Natural polysaccharide-based excipients, on the other hand, are a hot topic in medication delivery, with an ever-increasing requirement driving research and innovation for commercial applications.

Starch is a natural polymer that is natural, readily accessible, biocompatible, affordable, and the least expensive. It is commonly used to regulate the release of drugs from tablet. Amylose and amylopectin are the two polymers

that make up starch. Native granules include 98-99 percent amylose and amylopectin by dry weight. The sizes of starch granules range from 1 to 100 metres. Native starch is inadequate in a number of ways, making it unsuitable for industrial usage. To produce starch acceptable for industrial application, physical and chemical modification is required. Thermal modification of starches is chosen over chemical modification to keep up with the growing tendency of employing natural excipients. Blending several native starches allows for desired features to be achieved without the requirement for additional monomers or polymerization pathways.

II. MATERIALS & METHODS Materials

The plant Solanum tuberosum consists of starchy tuber potato. The plant belongs to the Solanaceae family of nightshades. Solanum brevicaule is the main source of Potatoes cultivation dated back some 7,000–10,000 years ago. Dioscorea, Colocasia, Xanthosoma, and other tubers are often farmed in Jharkhand. Excipients are commonly employed in pharmaceutical preparations as disintegration agents, coloring agents, diluents, emulsifiers, flavors, preservatives, and foaming agents. Starch is the most commonly used excipient in the production of tablets and capsules, and it can be employed as a fillers, suspending agent, diluent, and other things. Starches are modified by chemical and thermal modifications to improve their excipients.

Methods

I. Pregelatinization of native starches from potato

Native potato starch was used to make a 10% w/v starch suspension. The starch suspensions were cooked at 90°C for 15, 20, and 25 minutes, stirring constantly. The thick mixtures were then heat oven drying for 48 hours before being mashed in a mixer and sieved number 20 to produce finely powdered pregelatinized starches. The products were packed in polythene pouches for further use .

II. Carboxymethylation of native potato starch

An electric blender was used to carry out the carboxymethylation of potato starch. In a mixer, potato starch and solid sodium hydroxide were combined and blended to make a homogenous powder combination. A tiny amount of absolute ethanol (2 wt% depending on the starch) was added to the mix. For 1 hour, the mixture was mixed. The Powder SMCA was then added to the mixture in a two different concentration 0.75 and 1.4 and the reaction was carried out for the specified period at ambient or increased temperature. The solution was rinsed with 85 percent aqueous ethanol solution and filtered after the reaction was completed to eliminate the salts produced during the reaction. The washing and filtering were performed numerous times until the silver nitrate test revealed no chloride in the filtrate. The cake was cleaned in 85% ethanol before being dried in a 313 K oven for 48 hours.

III. Determination of the Degree of Substitution(DS)

One of most acceptable approach for determining the degree of substitution (DS) was back titration . Approximately 0.50 g of CMS was dissolved in 20 mL of 0.2 Nach Solution, proceeded by distilled water. The samples were then transferred to a 100 mL volumetric flask and distilled water was added to get the volume up to 100 mL. To dilute the solution, 25 mL was collected and placed in an Erlenmeyer flask with 50–100 mL of distilled water. Excessive NaOH was determined by titration against a phenolphthalein endpoint using a standardized 0.05 M HCl solution. To acquire the value for blank, native starches were also processed in the same way.

The below formula was used to determine DS: DS=160*%carbonyl/4500-58*%carbonyl

The empirical formula of an anhydroglucose unit is 162 g mol1; the total gain in the mass of an anhydroglucose unit (AGU) with each carboxymethyl group replaced is 58 g mol-1.

The calculation for calculating reaction efficiency (RE) was used.

RE (%)= DS/DSt*100

DST is the theoretical degree of substitution (DS), nAGU the number of moles of AGU, and nNaOH number of moles of sodium hydroxide.

IV. Amylose Content

Srichuwong's approach was used to identify the amylose content of native and modified starches (Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005). 100 mg of starch was cooked for 10 minutes with 1 ml of 95 percent ethanol and 9 ml of 1 N sodium hydroxide. Then it was chilled and distilled water was added to get the volume up to 100 ml. 5 ml of the solution was removed, 1 ml of 1N acetic acid and 2 ml of iodine solution were added, and the volume was then increased to 100 ml with distilled water. After 20 minutes, the starch solution's absorbance was taken spectrophotometrically at wavelength 620 nm against the control solution.

% amylose = $3.06* \times \text{Absorbance} \times 20$

*3.06 is conversion factor

V. Water holding capacity

Water holding capacity was used to assess the water retention capacity of native and modified starch samples with some alterations In a pre-weighed centrifuge tube, 100 mg starch was placed, and 5 ml distilled water was added. For one hour, the suspension was shook. After that, it was centrifuged for 10 minutes at 3000 rpm. The liquid from the centrifugation was eliminated, and the wet starch residue was collected.

$$WHC(\%) = Wws/Ws*100$$

Here, WWS = weight of wet starch (g) and WS = initial weight of starch (g) on a dry basis.

VI. Swelling and solubility power

In a temperature-controlled water bath, starch suspensions (1 percent w/v) were produced in distilled water and heated for 30 minutes at varied temperatures of 30°C, 50°C, 70°C, and 90°C. After that, the samples were dried to ambient temperature and centrifuged for 15 minutes at 3000 rpm. The excess liquid obtained was carefully decanted onto a pre-weighed Petri plate, and the sediment was weighed (Gomand et al., 2010). The residue was collected after drying the supernatant liquid overnight at 110°C. The solubilized component of starch in water is represented by the weight of this residue. The equations for calculating swelling and solubility power were used (Jing et al., 2012).

VII. Micromeritic properties

Bulk and tapped densities

In a 100 ml measuring cylinder, a weighted amount (Wt) of starch solution was placed, and the volume occupied without tapping was recorded as bulk volume (Vo). The samples was then tapped 100 times, and the volume was recorded as tapped volume (Vf). As a ratio of weight to volume, the bulk density (db) and tapped density (fd) were computed (Kulkarni, Sinha, & Jayaram Kumar,

2013).

Powder flowability

The phrases Hausner's ratio (HR) and Carr's index are used to describe the flow properties of powders (CI). The following equations were used to compute HR and CI (Okunlola, Patel, & Odeku, 2010).

Angle of repose

The fixed funnel method was used to estimate the angle of repose ().A clamp support was used to secure the funnel, and the funnel's mouth was put 2 cm above the horizontal position. The powder was allowed to drain freely down the funnel until the top of the pile of powder, which had been built, reached the funnel tip at a height of (h). The angle of repose was calculated using the formula after determining the radius (r) of the powder heap's base.

 Θ =tan-1 (h/r)

VIII. Scanning electron microscopy

Using a scanning electron microscopy (sem), the microstructure of each starch was examined. Uncoated materials were investigated using a Field emission scanning electron microscope (FESEM). The starch microstructure was captured using a SE detector and a 1 kV accelerating voltage.

IX. Thermogravimetric Analysis

Thermogravimetric analysis was performed by using Thermogravimetric analyzer (DTG-60, Shimadzu, Japan. The sample was subjected to heating (10 oC/min) in the temperature range of 30-600° C under nitrogen environment.

X. Fourier Transform Infra-Red Spectroscopy

Using a Fourier transform infrared spectrophotometer, the FT-IR spectra of native and modified starch samples were determined (FTIR-8400 S, Shimadzu, Japan). Prior to the examination, the starch samples were dried for 2 hours at 120°C. The starches were then combined with potassium bromide (KBr) before being pressed into pellets (Shalviri, Liu, Abdekhodaie, & Wu, 2010). The FT-IR equipment was then used to generate the spectra in the frequency range of 4000-400 cm-1.

XI. Formulation of tablets using native and modified starches

The native and pregelatinized starch and carboxymethylation samples were used to make tablets. Wet granulation was used to make granules using paracetamol as a model medication. 1 g paracetamol, 1.1 g lactose, 0.25 g acacia (10 percent w/w), and 0.125 g starch (5 percent w/w) were mixed with water as the granulating solvent to form 10 tablets. To make granules, the prepared bulk was passed through a 20 mesh. Lubricants such as magnesium and talc were utilized. The following characteristics were investigated: bulk density and tapped density, angle of repose, Hausner's ratio, and compressibility index were all examined.

XII. In vitro dissolution study

A USP Type-II dissolving test device was used to conduct an in-vitro release investigation (Electrolab, Mumbai, India). During the first two hours of the experiment, tablets made with native starches were put separately in 900 mL of hydrochloric acid (pH 1.2) to simulate gastrointestinal pH. Then, for the following 2hours, they were moved to a phosphate buffer solution of pH 6.8 to simulate jejunum pH, and then to phosphate buffer pH 7.4 until the dissolving test was completed. 5 ml of the extract was taken and replace with an equal quantity of new dissolving media at specified intervals of time (10, 20, 30, 40, 50, 60, 70, 80,90,100,110 and 120 minutes). The absorbance at 243 nm was used to quantify the number of paracetamol delivered.

XIII. Physical test of prepared tablets

Granules made from Native, PG15, PG20, PG25, PG15CMC1, PG15CMC2,PG20CMC1,PG20CMC2,PG25CMC1 and PG25CMC2 granules revealed values that were within a wide range (Lachman et al., 1987). The characteristics of the granules imply improved die filling after compressing. The Carr's index for all of the starch granules was less than 20%, indicating a higher flow rate. All of the tablets were between 4 and 8 kg/cm2 in hardness. The greater compressibility of tablets was further demonstrated by elastic recovery.

XIV. X-Ray Diffractometry

The original and modified starch samples were X-Ray diffracted using a Rigaku SmartLab diffractometer. Cu K radiation (1.5406 A) was used, with current and tube voltage of 35 mA and 40 kV, respectively. A finely powdered sample was placed on a standard glass holder with a 20 x 20 x 0.2 mm square indent. The sample was disseminated throughout the indent using a glass slide, and the sample holder was pushed into the sample plate. A continuous scan range of 2-80 was used to detect diffraction patterns, with a step size of 0.040 and a step duration of 31.2 s. The Diffrac plus programme was used to refine the diffraction patterns that were acquired.

XV. Differential Scanning Calorimetry

DSC-4000 was used to analyse starch gelatinization (Perkin Elmer, USA). Using a conventional sample pan crimper press, a quantity of 2 mg of starch sample was deposited in an aluminium pan and completely sealed. Using an empty container as a reference, the pan was then put in the calorimeter and heated from 30 to 250°C at a rate of 10°C min1. The starting temperature (), the peak temperature (), and the ending temperature () were all recorded.

III. RESULTS AND DISCUSSION

Proximate analysis of starches

The yield of original starch isolated from solanum tuberosum was originated to be 8.71 %. The content of the amylose of native potato starch (NPS) that were originated to be 17.2 % respectively. The WHC values of NPS were found to be 115.3 % properly. The amylose content and WHC values were originated to be higher for pregelatinized starch samples as compared to the native

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ones. During a hydrothermal treatment such as pregelatinization, macromolecular disorganization and degradation of starch granular structure occurs due to the heat applied, and release of soluble components takes place (Wijanarka, Sudargo, Harmayani, & Marsono, 2017). This could be attributed to the increase in amylose content in the pregelatinized samples. The extended pre-gelatinization time causes amylose (water-insoluble fraction) molecules in the bulk amorphous regions to collaborate with the branched segment of amylopectin (water soluble fraction) in the crystalline region, weakening the starch granules and increasing solubility, contributing to the higher WHC values in pregelatinized starch compared to native starch.

Micromeritic Properties

Based on their micromeritic characteristics, the native starches NPS were determined to have 'good' and 'passable' flow, but the pregelatinized starches had 'outstanding' flow. The improved flow behaviour of pregelatinized starches was due to the reduction in particle size. Physical combinations were discovered to have 'passable flow.' **Table 1** : Micromeritic properties of native and modified starch samples.

Sample	Bulk Density	Tapped Density	Hausner's Ratio	Carr's Index	Angle of Repose
	(dB) g/cc	(dT) g/cc	(HR)	(CI)%	(θ) °
NPS	0.76	0.77	1.16	14.28	35.54
	0.01	0.02	0.05	0.02	0.72
PG15	0.65	0.89	1.18	15.73	45
	0.01	0.01	0.03	0.03	0.51
PG20	0.70	0.91	1.19	16.48	18.43
	0.01	0.02	0.33	0.01	0.03
PG25	0.75	0.91	1.28	21.97	18.45
	0.06	0.06	0.45	0.11	0.21
CMC1	0.89	0.91	1.09	8.79	19.65
	0.48	0.34	0.23	0.24	0.18
CMC2	0.79	0.83	1.07	7.22	17.35
	0.58	0.4	0.5	0.04	0.44
P15CMC1	0.70	0.91	1. <mark>26</mark>	20.87	20.02
	0.44	0.34	0.23	0.24	0.18
P15CMC2	0.68	0.86	1.10	9.30	35.23
	0.01	0.02	0.05	0.02	0.72
P20CMC1	0.76	0.91	1.19	16.48	21.03
	0.04	0.9	0.01	0.23	0.09
P20CMC2	0.61	0.91	1.28	21.97	45
	0.03	0.01	0.03	0.03	0.51
P25CMC1	0.79	0.96	1.04	4.49	19.45
	0.55	0.34	0.23	0.24	0.18
P25CMC2	0.69	0.95	1.20	16.48	16.38
	0.21	0.04	0.83	0.74	0.43

Evaluation of prepared tablets

Samples	Average	Hardness(kg/cm2)	Disintegration time	Drug
_	weight(mg)			content(%)
Nps	251±0.01	4.01 ± 0.02	0.87 ± 0.01	98.66 ± 0.01
Pg15	252±0.03	5.78 ± 0.01	0.48 ± 0.03	99.55 ± 0.01
Pg20	249.92±0.01	4.2 ± 0.01	0.47 ± 0.01	99.24 ± 0.01
Pg25	248.83±0.01	4.3 ± 0.08	0.58 ± 0.01	99.31 ± 0.08
Cmc1	251±0.01	4.6 ± 0.01	0.32 ± 0.01	98.76 ± 0.01
Cmc2	249.81±0.08	4.1 ± 0.02	0.65 ± 0.08	100.21 ± 0.01
Pg15cmc1	250.06±0.02	4.3 ± 0.01	0.53 ± 0.02	98.25 ± 0.08
Pg15cmc2	249.52±0.02	4.5 ± 0.02	0.50 ± 0.01	100.04 ± 0.01
Pg20cmc1	250.12±0.01	4.2 ± 0.01	0.47 ± 0.01	99.41 ± 0.01
Pg20cmc2	248.92±0.02	5.3 ± 0.02	0.62 ± 0.01	100.32 ± 0.01
-				
Pg25cmc1	250.05±0.02	5.66 ± 0.02	0.23 ± 0.01	99.85 ± 0.01
Pg25cmc2	250.02 ± 0.02	5.89 ± 0.02	0.89 ± 0.01	99.54 ± 0.01

Table 2 : Physical tests of prepared tablets

Table 3 : Degree of substitution(DS)

Samples	Degree of Substitution
CMC1	0.043
CMC2	0.121
P15CMC1	0.189
P15CMC2	0.241
P20CMC1	0.243
P20CMC2	0.260
P25CMC1	0.275

In-vitro dissolution study

The samples P15CMC1 operates as a releasing suppressor when updated in the thermal and chemical modification, according to the in vitro release profile. This might be owing to the physical combinations' high amylose concentration, which prevents solubilization and subsequent release.

The PG15, PG20, and PG25 pregelatinized potato starch samples release quicker than the native NPS. This might be because pregelatinized starches have a higher swelling and solubility than native starches. Release rises with pregelatinization time for the pregelatinized carboxymethylation starch samples P15CMC1, P15CMC2, P20CMC1, P10CMC2, P25CMC1 and P25CMC2, although initial release is slower than native NCS. This might be because a longer pregelatinization period induces a disturbance in the amylose structure, enabling water to soak through and aiding in speedier release.

Due to swelling in pH 1.2 it changes into solubilized form in pH 6.8 as it shows sensitive characteristics of potato starches. In vitro shows that starch can complete the release of drug in stomach and small intestine. It showed that due to high water holding capacity content of amylose contributes and amylopectin contributes super degradation of drug.



Scanning Electron Microscopy

At higher magnification of 5 kv, the SEM analysis showed the complex of the potato starches granules. The natural starch was irregular in shape and uneven in form, with just a little granule having round shaped angles. A small increase in granule volume and the presence of agglomerates were observed after HMT, particularly when greater moisture content (25%) was applied. On the surface of the granules, no fractures or holes formed, and the roughness was only noticeable when compared to the original sample.

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There was some granule aggregation after dual modification, especially in the samples with 19% and 25% moisture content; however, this was quite noticeable than in the single modification sample. The morphological structure of the granule was intact during hydrothermal treatment, comparable to the pregelatinized samples; no substantial alterations were seen as the treatments progressed. The use of ultrasonography enhanced the formation of rugosity in the granules with few exceptions. The level of structural changes in starch granules is determined by various parameters, including US intensity and exposure duration, US power, temperature, and, most importantly, the starch supply. Every starch sources has its own molecular and structural organization, as well as granules of various sizes, all of which might affect the granules' responsiveness to the effects of ultrasound.



FIG1: SEM images of Native , Pregelatinized and CMS Starch

Fourier Transform Infra-Red Spec<mark>trophotometry</mark>

The bending vibration of the carboxyl groups (O–H) cause a band at 3319 cm-1 in native starch, with a diminished band at 2950 cm-1 owing to the C–H stretching vibrations. Stretching vibrations of C–O–C were detected at 1021 cm-1 and 1151 cm1 (Lina M. Rodriquez et al.,2018). The O–H stretching band in the hydroxyl group of starches and the N–H stretching band in the amide group of acrylamides that flap one by one others are ascribed a band at 3334 cm-1 and a shoulder peak at 3150 cm-1. The C–H stretching vibration is allocated to a narrow band at 2950 cm-1. Strong absorption bands exist at 1700 cm-1 and 1600 cm-1, which correspond to C=O and NH amide stretching vibrations, respectively. Because of the C–N stretching, the grafted product revealed an extra peak at 1495 cm-1.





X-Ray Diffraction

It depicts the X-RD patterns of native and modified starch. A, B, and C crystallinities are the most common crystallinities found in starch. A-type crystals have a prominent diffraction peak between 12° and 28° 2 Θ , as well as an unresolved doublet between 16° and 19° 2 Θ . The strongest diffraction peak in B-type crystals is about 17° 2 Θ with some small peaks around 15° , 22° , and 24° 2 Θ . The C-type crystallinity is a hybrid of the A and B crystallinities.

The XRD peaks of native potato starch were B-type, while those of pregelatinized starch were A-type. C-type crystallinities were found in the pregelatinized cmc starch samples p15cmc1, p20cmc1, and p25cmc1. B-type crystallinities were observed in the physical mixing of starches p15cmc2, P20cmc2, and P25cmc2. When starch is warmed in the presence of moisture, crystalline packing is disrupted and crystalline rearrangement occurs.



Native starch crystallinity was gradually reduced, resulting in amorphous forms.



Thermogravimetric analysis

Thermogravimetric is the current equivalent of gravimetric, and it deals with mass increases in dynamic or isothermal environments (Beckett & Stenlake,2007). Three forms of deterioration are shown on the TGA curve. The 1st mass loss was 8.04 % and happened between 27 and 230 °C owing to the evaporation of bound water. Weight reduction continued in the second range (230-400 ° C). The degradation of starch can be linked to the 2 nd mass loss. The weight reduction percentage was discovered to be 69.67 %. The oxidation of organic matter causes the 3rd mass loss, which occurs among 322 and 690 ° C. The weight reduction percentage was discovered to be 17.67%

At 700 $^{\circ}$ C, the percent weight loss for all the carboxymethylated derivatives was less than the native polysaccharide and this data represents that carboxymethylation increased the thermal stability of the samples. This might be due to the presence of sodium ions which altered the decomposition pattern





The temperatures at which native and modified starch samples gelatinize are displayed. Because to the reduction of crystallinity after hydration, the peak temperature (Tp) of native starches decreases. Starches are exhibited in their natural state, as well as after heat and chemical alteration. The temperature of pregelatinization reduces the gelatinization temperature of starches. In a same sequence as their x-ray crystallinity, the To value, as well as the peak (Tp) and final (Tc) transition temperatures, declined.

Native is stable due to onset high temperature. Due to heat pregelatinized , bond get weak reduction in onset, peak and conclusion temperature.

Due to chemical, Hydroxyl bond get inserted into the starch. Due to strong bond formed, it get increase than pregelatinized starch.

Due to pregelatinized carboxymethylation, peak temperature is higher than pregelatinized starch but lower than carboxymethyl starch.



IV. CONCLUSION

Native starches of solanum tuberosum are modified by the process of pregelatinization. During hydrothermal treatment starch granules get degraded and release of soluble components takes place. The extended pregelatinization time cause amylose molecules in the bulk amorphous region to collaborate in the crystalline region, weakening the starch granules and increasing solubility compared to native starches. Micromeritic properties revealed outstanding flow than native starches. The improved flow behaviour of pregelatinized starches due to reduction in particle size. Native starches of solanum tuberosum are modified by the process of carboxymethylation. Degree of substitution in all the derivatives are found to be increased with an increase in the concentration of monochloroacetic acid.DS of all carboxymethyl derivatives are found in the range of 0.043- 0.121.Carboxymethylation altered the morphology of starch granules. The result indicates the carboxymethylation lead to improvement in the physical properties of starch. In-vitro released data revealed that with an increase in the DS, the percentage of starches get decreased. Pregelatinized starches are modified by the process of carboxymethylation. Degree of substitution in all the derivatives are found to be increased with an increase in the concentration of monochloroacetic acid.DS of all carboxymethyl derivatives are found in the range of 0.189-0.275. Carboxymethylation altered the morphology of starch granules. In DSC, due to pregelatinized carboxymethylation peak temperature is higher than pregelatinized but lower than carboxymethyl. TGA data revealed that carboxymethylation mended the stability of the samples and increased the amorphous region of the starch. The result indicates the carboxymethylation lead to improvement in the physical properties of starch. In-vitro released data revealed that with an increase in the DS, the percentage of starches get increased.

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