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Article

Production, Characterization and Parametric Optimization of Dual Modified Cross Linked-Acetylated Potato Starch as Disintegrant for Tablet Formation

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Abstract

The objective of this study was to produce, characterize, and optimize modified potato starch derived from locally sourced potatoes, and to evaluate the physicochemical properties of native, cross-linked, acetylated, and dual cross-linked–acetylated potato starches as disintegrants for tablet formulation. Starch modification was performed through cross-linking and acetylation using sodium hexametaphosphate (SHMP) and acetic anhydride (AA) as modifying agents, respectively. Native and modified potato starches were characterized using Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), rapid visco analysis (RVA), and X-ray diffraction (XRD). The key modification parameters investigated included reaction temperature, reaction time, pH, concentration of the modifying agent (AA), and concentration of the NaOH catalyst. Based on preliminary experiments, reaction temperature (40, 60, and 80 °C), modifying agent concentration (10, 20, and 30%), and reaction time (40, 55, and 70 min) were selected as the primary variables. Process optimization for dual crosslinked-acetylated potato starch was carried out using response surface methodology based on a Box-Behnken experimental design, with acetyl content as the response variable. The optimized modification conditions were a reaction temperature of 40.22 °C, a reaction time of 69.85 min, and an acetic anhydride concentration of 21.92% (w/w). Under these optimized conditions, an acetyl content of 1.32 ± 0.077% was obtained. Tablets formulated using the dual crosslinked-acetylated potato starch as a disintegrant exhibited a disintegration time of 29.2 ± 0.29 min, a disintegration efficiency ratio of 500 ± 0.99 N min^{-1}, a crushing strength of 92.35 ± 0.86 N, and friability of 0.63 ± 0.08% (w/w). The modified starch was employed as a disintegrant in tablet formulations containing 10% paracetamol as the active pharmaceutical ingredient, magnesium stearate (10%) as a lubricant, and suitable fillers, using a direct compression method.

Keywords: potato starch; acetylation; crosslinking; experimental design; parametric optimization; tablet

1. Introduction

Starch is a carbohydrate with semicrystalline biopolymer characteristics and is widely distributed in many plant parts, including cereal grains, roots, tubers, seeds, and fruits. The botanical origin of starch significantly influences the properties of starch granules due to variations in granule shape, size, structure, and chemical composition [1]. Numerous starch sources exist worldwide, and starch is commonly isolated from different plant organs such as cereals (e.g., corn and wheat), tubers (e.g., potato), and roots (e.g., cassava, taro, sweet potato, and arrowroot) [2]. In recent years, increasing global demand has driven extensive research into alternative starch sources, including

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ginger, cocoyam, sorghum, plantain, rye, barley, yam, enset, and colocasia [3]. The molecular structure of starch, along with its physical, chemical, biochemical, and functional properties, has been extensively studied over several decades, resulting in a vast body of published literature describing its preparation and analytical characterization [4]. Due to its wide range of applications, starch production techniques have diversified, encompassing both native starches (NSs) and numerous modified starches [5]. Starch granules consist primarily of amylose and amylopectin, which together account for approximately 98-99% of the dry weight of starch [6]. Minor non-starch components such as ash, lipids, proteins, and phosphate groups are also present.

Amylose is essentially a linear polymer in which anhydro glucose units are predominantly linked by α-D-(1→4) glycosidic bonds. Amylose can form inclusion complexes with a variety of linear polar and nonpolar compounds, including long-chain fatty acids, fatty alcohols, monoglycerides, and flavor compounds, by wrapping around the ligand to form a left-handed helical structure. In contrast, amylopectin is a highly branched polymer composed of anhydroglucose units connected by α-(1→4) glycosidic bonds with α-(1→6) branch points, forming polymers of α-(1→4)-linked glucopyranose units with α-(1→6) branches [7]. Starch serves as the primary carbohydrate reserve in the plant kingdom and is mainly deposited in seeds, tubers, or roots, accounting for approximately 60–80% of the dry weight of plant materials [8]. It typically consists of 20–30% amylose and 70–80% amylopectin. For example, starch isolated from the tubers of Ethiopian potato (*Plectranthus edulis*, family Lamiaceae) was reported to contain, on a dry weight basis, 0.14% ash, 0.21% lipid, 0.43% protein, and 99.2% combined amylose and amylopectin [4].

However, unmodified native starches are structurally weak and functionally limited for pharmaceutical applications. Consequently, starch is often chemically or physically modified to enhance its suitability for industrial use [9]. Potato is an advantageous source for starch production due to its wide availability and high starch yield. In the pharmaceutical industry, starch is commonly used as a binder, diluent, and disintegrant. During tablet manufacturing, freshly prepared starch pastes with concentrations ranging from 5 to 20% (w/w) are frequently employed, with approximately 15% (w/w) considered optimal for tablet formulation [10]. Chemical modification alters the functional groups of starch molecules, resulting in significant changes to their physicochemical properties [11]. These modifications involve the introduction of functional groups onto the starch backbone without substantially affecting granule shape or size distribution. As a result, starch behavior, gelatinization characteristics, retrogradation tendency, and paste properties are markedly modified. Such alterations enhance the stability of intermolecular interactions at various sites within the starch structure. The chemical and functional properties of modified starches are influenced by several factors, including starch source, reaction conditions (such as reactant concentration, pH, temperature, reaction time, and catalyst presence), type of substituent, degree of substitution (DS), salt sensitivity, covalent cross-linking, and the distribution of substituents within the starch molecule [12]. Chemical modification of starch primarily involves reactions at the hydroxyl groups of the starch polymer and is generally straightforward. Through such modifications, starches with improved functional properties suitable for food and pharmaceutical applications can be produced. Modified starches exhibit enhanced performance as tablet excipients, particularly in direct compression tablet manufacturing.

A disintegrant is an excipient incorporated into pharmaceutical formulations to promote the breakup of solid dosage forms, such as tablets or granules, into smaller particles [13]. Disintegration is a critical step in drug release and absorption, as it increases the available surface area, facilitating rapid dissolution and subsequent drug absorption to achieve the desired therapeutic effect. Starch is an inexpensive and widely used disintegrant, primarily functioning through particle swelling in the presence of water, which disrupts solid bridges and binding forces within the dosage form. The extent of swelling depends on the starch source and type, reflecting differences in the proportion and molecular arrangement of amylose and amylopectin [14].

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2. Materials and Methods

2.1. Materials

The raw material (potato) used in this study was collected from the Holeta Agricultural Research Institute, located in the West Shewa zone of Oromia Regional State, approximately 39 km west of Addis Ababa, Ethiopia. The collected potato samples were placed in internally lined polypropylene bags to prevent contamination and transported to the Chemical Engineering Research Laboratory at Addis Ababa Science and Technology University for further investigation.

All chemicals used in this study were of analytical reagent grade. The chemicals employed included sodium metabisulfite (98%, India), sodium hydroxide (99%, India), sodium carbonate (99.5%, India), sodium hexametaphosphate (SHMP, 68%, China), distilled water (99.9%, AASTU), acetic anhydride (99%, China), hydrochloric acid (35%, China), orthophosphoric acid (85%, India), diethyl ether (>99.6%), sulfuric acid (98%, Ethiopia), hydrogen peroxide (96%, Germany), ethanol (99%, Germany), and iodine solution (>99.8%, India). These chemicals were used without further purification.

2.2. Methods

2.2.1. Proximate Analysis of Raw Potato

The chemical composition of the raw potato, including amylose content, amylopectin content, ash value, protein content, fat content, and moisture content, was determined.

2.2.2. Modified Starches Production

Production of Cross-linked Potato Starch (CLPS)

Cross-linking of potato starch was carried out according to the method described by Woo and Seib [20], with minor modifications. Native potato starch (50 g, dry basis) was dispersed in 100 mL of distilled water and mixed for 30 min using a mechanical mixer. A sodium carbonate solution (0.8 g in 20 mL distilled water) was prepared separately. Sodium hexametaphosphate (SHMP) solutions at three different concentrations (10, 20, and 30%, w/w) were individually prepared by dissolving the required amount of SHMP in 25 mL of distilled water. The sodium carbonate solution and the appropriate SHMP solution were then added to the native starch suspension.

The pH of the mixture was adjusted to 11 using a sodium hydroxide solution (0.6%, w/w in distilled water), and the suspension was stirred using a magnetic stirrer until a homogeneous slurry was obtained. The starch suspension was maintained at reaction temperatures of 40, 60, or 80 °C under continuous stirring and held for reaction times of 40, 55, or 70 min. After completion of the reaction, the suspension was cooled to room temperature, and the pH was adjusted to 6.5 using 1.5 N hydrochloric acid to terminate the cross-linking reaction.

The resulting cross-linked starch (CLS) slurry was recovered by centrifugation at 3000 rpm in 15 min. The starch precipitate was washed three times with distilled water (3 × 100 mL) to remove residual reagents. The slurry was allowed to stand in open air for 10 min to facilitate decantation, and the washing and decantation steps were repeated twice. The starch cake was then filtered using a Büchner funnel and dried in a hot-air oven at 50 °C for 24 h. The dried material was ground using a mortar and pestle, passed through a 200 μm mesh sieve, and stored in an airtight container until further use. The percentage yield of cross-linked potato starch, defined as the amount of starch obtained after reaction with the cross-linking agent (SHMP), was determined on a dry-weight basis according to the method described by the Association of Official Analytical Chemists (AOAC), with slight modifications [16].

$$Yield\ (\%) = \frac{Weight\ of\ Crosslinked\ starch\ x100}{Weight\ of\ native\ starch} \qquad (0.1)$$

Determination of peak viscosity and degree of cross-linking (DCL)

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Viscosity measurements were performed using an Ostwald capillary viscometer, which operates based on Poiseuille's law. According to this law, under laminar flow conditions, the volumetric flow rate of a liquid is directly proportional to the applied pressure difference and inversely proportional to the viscous resistance. The viscous resistance depends directly on the viscosity of the fluid and the length of the capillary tube and is inversely proportional to the fourth power of the capillary radius [21]. For this reason, the Ostwald viscometer is also referred to as a capillary viscometer. In this method, the viscosity of the liquid was determined by measuring the time required for the fluid to flow through a capillary tube between two fixed marks under gravity. The flow time was recorded using a stopwatch. The degree of cross-linking (DCL) of the modified starches was evaluated from the relative viscosity values, as described in the literature [22].

The degree of cross-linking significantly influences the physicochemical properties of starch, including swelling power, viscosity, solubility, and paste characteristics, which are critical for assessing its suitability as a pharmaceutical excipient. Following viscosity measurement, the starch slurry was allowed to settle, and the supernatant was removed by decantation. The washing and decantation steps were repeated twice until the starch cake was clean. The starch cake was subsequently filtered using a Büchner funnel and dried in a hot-air oven at 50 °C for 48 h. The dried modified starch was then ground and passed through a 200 µm sieve, and the final product was stored in an airtight container for further analysis.

$$DCL\ (\%) = \frac{(A - B)\ x100}{A} \qquad (0.2)$$

where A is the peak viscosity of native starch and B is the peak viscosity of CLPS.

Preparation of Acetylated Potato Starch (APS)

Acetylation of potato starch (K) with acetic anhydride (AA) was carried out following the procedure described by [22]. Native starch (10 g, dry basis) was dispersed in 110 mL of distilled water and stirred at 25 °C for 30 minutes. Acetic anhydride was then added dropwise at 10%, 20%, and 30% levels based on the dry weight of starch. A 0.4 M NaOH solution was prepared and gradually added to the starch suspension to maintain the pH within the range of 8.0–8.4, with continuous stirring using a magnetic stirrer. The reaction vessel was sealed, and the mixtures were heated to 40, 60, or 80 °C and held at these temperatures for 40, 55, or 70 minutes, respectively, under constant stirring. After the reaction, the pH of the starch suspension was adjusted to 4.5 using 0.5 N HCl. The modified starch was washed three times with 300 mL of distilled water and allowed to stand in open air for 10 minutes to facilitate settling of the starch cake. The starch slurry was then filtered, and the yield (%) of the acetylated starch was calculated based on its dry weight.

Dual Crosslinked Acetylated Modified Starch Preparation

The dual modification of potato starch was performed in two steps. First, the starch was crosslinked with sodium hexametaphosphate (SHMP) at varying concentrations, and subsequently, the crosslinked starch was acetylated with different concentrations of acetic anhydride (AA) [23]. For acetylation, 10 g of crosslinked potato starch (dry basis) was dispersed in 110 mL of distilled water and stirred at 25 °C for 30 minutes. Acetic anhydride was added dropwise at 10%, 20%, and 30% based on the dry weight of the crosslinked starch. A 0.4 M NaOH solution was prepared and gradually added to maintain the pH within 8.0-8.4, while stirring continuously with a magnetic stirrer. The reaction vessel was sealed, and the mixtures were heated to 40, 60, or 80 °C and held at these temperatures for 40, 55, or 70 minutes, respectively, under constant stirring. After completion, the pH of the starch suspension was adjusted to 4.5 using 0.5 N HCl. The starch was washed three times with 300 mL of distilled water and left in open air for 10 minutes to allow settling. The slurry was decanted and filtered, repeating the slurring and filtering process twice until the starch cake was clean. The final cake was filtered through a Buchner funnel, oven-dried at 50 °C for 48 hours and then ground to pass through a 200 µm sieve. The dual-modified starch was stored in an airtight container. The yield (%) of the modified starch was calculated on a dry weight basis. Percentage yields of dry

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acetylated potato starch were determined following the method of the Association of Official Analytical Chemists with slight modifications [16].

$$Yield\ (\%) = \frac{Weight\ of\ acetylated\ starch}{Weight\ of\ native\ starch} \quad (0.3)$$

Degree of Substitution (DS) or Degree of Acetylation Determination

The degree of substitution (DS) was determined using the method described by Colossi [46]. Briefly, a 1 g sample of dual crosslinked acetylated starch (DCL-AC) was placed in a 250 mL conical flask, and 50 mL of distilled water was added. The suspension was agitated and heated in a water bath at 100 °C for 30 minutes, then allowed to cool to room temperature. Subsequently, 40 mL of 0.5 N potassium hydroxide solution was added with continuous swirling. The flask was stoppered and left to stand for 72 hours with occasional agitation to ensure complete saponification. After the reaction period, the excess alkali was back titrated with standardized 0.5 N hydrochloric acid using phenolphthalein as an indicator. The same procedure was applied to determine the DS of dual crosslinked acetylated potato starch. The acetyl content of the dual crosslinked acetylated starch, resulting from esterification reactions, was calculated using Eq. (2.4).

$$A = \frac{(V_2 - V_1)xNx43x100}{m\ x1000} \quad (0.4)$$

where, A - acetyl content, V_1- the volume of 0.5 N HCl in mL used for titration of 1 g native starch(ml), V_2- the volume of 0.5 N HCl in mL used for titration of 1 g sample (blank, ml), N- the normality of HCl solution, m -the weight of the sample.

$$Degree\ of\ substitution = \frac{162x\ \%A}{(43x100) - (42x\ \%A)} \quad (0.5)$$

where A acetyl content, 43, molecular weight of the acetyl group, 162 molecular weights of the hydro-glucose unit

2.3. Physicochemical Characterization of Starch

2.3.1. Density

The true density of the starch samples was determined using the fluid displacement method with xylene as the immersion liquid. Approximately 2 g of each starch sample was placed in a 25 mL volumetric flask. The flask was then filled with xylene and weighed, following prior determination of the mass of the empty flask and the flask filled only with xylene. Xylene was added carefully to ensure complete wetting of the sample and to wash down any particles adhering to the flask walls. After standing for 10 minutes, the settled starch was gently stirred using a glass rod to release entrapped air bubbles. The final mass was recorded, and true density was calculated from the measured values.

$$Density\ (\rho) = \frac{W_1\ x\ sg}{(W_1 + W_2 - W_{3)}} \quad (0.6)$$

where, W_1= Weight (g) of a starch sample, W_2= Weight (g) of a volumetric flask filled with xylene, W_3= Weight (g) of volumetric flask plus sample plus xylene left after displaced by the sample, and Sg= Specific gravity of xylene (g/ml) (0.87).

2.3.2. Analysis of Fourier Transform Infrared (FTIR) Spectra

Prior to analysis, starch samples were ground in a mortar to reduce particle size. Approximately 5-10 mg of each sample was equilibrated at 50 °C for 24 h and then finely ground to obtain a uniform particle size distribution. The samples were mixed with potassium bromide (KBr) and pressed into pellets for spectral analysis. Fourier-transform infrared (FTIR) spectra of native starch, crosslinked

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starch, acetylated starch, and dual crosslinked acetylated starch were recorded using a Spectrum 65 FTIR spectrometer (PerkinElmer). Spectra were collected over the wavenumber range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1}, with four scans per sample. The resulting spectra were processed and plotted using Origin Lab software.

2.3.3. pH Determination

The pH of potato starch samples was determined following the method described elsewhere by Nwachukwu [24]. A 1% (w/v) starch dispersion was prepared in distilled water and shaken for 5 minutes to ensure homogeneity. The pH of the resulting suspension was then measured using a calibrated digital pH meter at room temperature.

2.3.4. Determination of Starch Hydration Capacity

The hydration capacity of the starch samples was determined following the method described elsewhere by Jubril [25]. Briefly, 1.0 g of starch was placed in a plastic centrifuge tube, and 10 mL of distilled water was added. The tube was sealed and shaken for 2 minutes, then allowed to stand for 10 minutes before being centrifuged at 1000 rpm for 10 minutes using a bench centrifuge. The supernatant was carefully decanted, and the mass of the hydrated starch sediment was recorded. Hydration capacity was calculated using the equation provided below. All measurements were performed in triplicate. Percent solubility (%S) and swelling power (SP) were determined according to Eqs. (2.7) and (2.8), respectively.

$$Hydration\ capacity = \frac{W_s}{W_d} \qquad (0.7)$$

where W_s and W_d are the weights of the sediment and dry sample, respectively.

2.3.5. Swelling Power and Solubility Determination

The swelling power (SP) and solubility of the starch samples were determined according to a previously reported method [26]. Briefly, 0.5 g of each starch sample was dispersed in 10 mL of distilled water in pre-weighed centrifuge tubes. The starch suspensions were heated at selected temperatures between 20 and 85 °C for 30 minutes, with intermittent shaking every 5 minutes, and then allowed to cool to room temperature. The samples were subsequently centrifuged at 3000 rpm for 15 minutes. The mass of the sediment was recorded and used to calculate swelling power on a dry weight basis. The supernatant obtained at each temperature point was dried in a hot-air oven at 130 °C for 2 hours, cooled in a desiccator, and weighed to determine the amount of dissolved solids. All measurements were carried out in triplicate. Percent solubility (%S) and swelling power (SP) were calculated according to Eqs. (2.8) and (2.9), respectively.

$$Solubility\ (\%) = \frac{W_a}{W_c} \qquad (0.8)$$

$$Swelling\ power\ (\%) = \frac{W_b x 100}{W_c x (100 - S)} \qquad (0.9)$$

where, Wa is the weight (g) of soluble material in the supernatant, Wb is Weight (g) of the precipitate, s is solubility, and Wc is the Weight (g) of a starch sample.

2.3.6. Pasting Properties

Pasting refers to the heat-induced swelling of starch granules in excess water, accompanied by the transition from an ordered to a disordered structure, leaching of molecular components, and ultimately the rupture of starch granules [27]. The pasting properties of native and modified starch samples were determined using a Rapid Visco Analyzer (RVA) according to AACC Method 61-02

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[28], employing Thermocline for Windows (TCW) software. For each measurement, 3.0 g of starch sample was mixed with 25 mL of distilled water in an RVA aluminum canister. The initial (idle) temperature was set at 50 °C, and a 12.5-minute test profile was applied as follows: (i) holding at 50 °C for 1.0 minute, (ii) linear heating to 95 °C over 3.8 minutes, (iii) holding at 95 °C for 2.5 minutes, (iv) linear cooling to 50 °C over 3.8 minutes, and (v) holding at 50 °C for 1.4 minutes. Viscosity values were recorded automatically by the RVA software and expressed in Rapid Visco Units (RVU) or centipoise (cP), as appropriate.

2.3.7. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is a thermoanalytical technique that measures the difference in heat flow between a sample and a reference as a function of temperature, while both are maintained under identical thermal conditions. DSC analysis was performed for native potato starch, crosslinked starch, acetylated starch, and dual crosslinked acetylated potato starch. Samples were placed in sealed aluminum crucibles and analyzed using a SKZ1052B Differential Scanning Calorimeter (Germany) equipped with integrated Synchronous Thermal Analyzer software. The measurements were carried out over a temperature range of 25-200 °C at a heating rate of 10 °C·min^{-1} under a nitrogen atmosphere with a flow rate of 50 cm^3·min^{-1}. The DSC thermograms provided heat flow profiles of native and modified starch samples, which were recorded and displayed by instrument software for subsequent analysis.

2.3.8. X-Ray Diffraction (XRD) Analysis

The crystalline and amorphous structures of native and modified potato starch were revealed using an X-ray diffraction technique. The scattered intensity of an X-ray beam hitting a native and modified starch sample as a function of incident and scattered angle, polarization, and wavelength or energy support these techniques. X-ray diffraction analysis of the samples provides information about the atomic structure of materials and is based on the elastic scattering of X-rays from the electron clouds of the individual atoms within the system. X-ray diffraction (XRD) was used to determine whether the native and modified starch nature was crystalline or amorphous. The analysis was performed by (XRD-7000 X-Ray DIFFRACTOMETER, Japan) using a Cu-Kα (=0.15406 Å) radiation source operating under a voltage of 40 kV and a current of 30 mA. The diffraction angle (2θ) varied from 8º to 80º. The X-ray diffraction patterns were collected in continuous scan mode at a scan rate of 3 ^{0}/min.

2.4. Experimental Design for Starch Modification Process

To investigate the effects of operating conditions on the peak viscosity of crosslinked potato starch and the acetyl content of dual crosslinked acetylated potato starch, a combination of the one-variable-at-a-time (OVAT) approach and response surface methodology (RSM) was employed. Initially, OVAT experiments were conducted to evaluate the individual influence of process variables and to establish suitable experimental ranges. The results obtained from OVAT were subsequently used as inputs for studying the interaction effects of variables using RSM based on a Box-Behnken design (BBD).

Key process parameters influencing starch modification were selected based on an extensive review of the literature. These parameters included solution pH, reaction time, reaction temperature, concentration of the modifying agent, and concentration of the catalyst (NaOH). The optimization of dual crosslinked acetylated potato starch was carried out to identify the optimal combination and interaction of these parameters affecting the selected responses. In this study, potato starch was first crosslinked using sodium hexametaphosphate (SHMP) as the crosslinking agent. The resulting crosslinked starch was subsequently acetylated using acetic anhydride (AA) to obtain dual crosslinked acetylated potato starch. The effects of the selected process variables on the modification outcomes were systematically evaluated using the RSM approach.

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2.4.1. Effect of Individual Process Parameters (OVAT)

The effect of individual process parameters on the acetyl content of dual crosslinked acetylated potato starch was investigated using the one-factor-at a time (OFAT) approach. In this method, the impact of a single variable was assessed while all other factors were held constant. Starch modification experiments were conducted to evaluate the influence of solution pH, concentration of the modifying agent, reaction time, catalyst concentration (NaOH), and reaction temperature on the acetyl content of the dual-modified starch. The acetyl content was calculated according to Equation (2.4).

Effect of temperature on acetyl content

The influence of reaction temperature on the acetyl content of acetylated potato starch was investigated at 20, 40, 60, 80, and 100 °C, while maintaining all other parameters constant: acetic anhydride concentration at 20% (w/w), solution pH at 8, reaction time at 55 minutes, and catalyst (NaOH) concentration at 0.8% (w/w). The acetyl content of each sample was determined according to Equation (3.5). The sample exhibiting the highest acetyl content was considered to represent the optimal condition for the acetylation of potato starch.

Effect of reaction time on acetyl content

The effect of reaction time on the acetyl content of acetylated potato starch was investigated at 25, 40, 55, 70, and 85 minutes, while keeping other parameters constant: solution pH at 8, reaction temperature at 60 °C, acetic anhydride concentration at 20 wt%, and NaOH concentration at 0.8 wt%.

Effect of acetic anhydride (AA) concentration on acetyl content

The influence of acetic anhydride concentration on the acetyl content of acetylated potato starch was investigated at 10, 20, 30, 40, and 50 wt%, while keeping other reaction conditions constant: temperature at 60 °C, solution pH at 8, reaction time at 55 minutes, and NaOH concentration at 0.8 wt%.

The effect of pH on acetyl content

The effect of solution pH on the acetyl content of acetylated potato starch was investigated at pH values of 2, 4, 6, 8, and 10, while maintaining other reaction conditions constant: temperature at 60 °C, reaction time at 55 minutes, acetic anhydride concentration at 20 wt%, and NaOH concentration at 0.8 wt%.

Effect of catalyst (NaOH) on acetyl content

The influence of NaOH concentration on the acetyl content of acetylated potato starch was investigated at 0.6, 0.8, 1.2, 1.4, and 1.8 wt%, while maintaining other reaction conditions constant: temperature at 60 °C, reaction time at 55 minutes, acetic anhydride concentration at 20 wt%, and solution pH at 8.

2.5. Preparation and Evaluation of Tablets

2.5.1. Production of Tablets

Tablets were prepared by direct compression, a process in which the powdered active pharmaceutical ingredient (API) and modified starch excipient are compressed directly into a solid compact without prior granulation. The filler and disintegrant capacities of native, crosslinked (CLPS), acetylated (APS), and dual-modified starch were evaluated following the method described by [29]. For tablet preparation, each modified starch was blended with paracetamol (as the API) in a Turbula mixer (Willy A. Bachofen AG, Turbula 2TF, Basel, Switzerland) for 10 minutes. Subsequently, 0.5% magnesium stearate was added as a lubricant, and the mixture was blended for an additional 5 minutes. Tablets were compressed using a single-punch tablet press (Korsch EKO, Berlin, Germany) equipped with 10 mm flat-faced punches at a fixed compression pressure. Each tablet weighed 500 mg and contained paracetamol at 20%, 30%, or 40% w/w (higher than 40% loading resulted in friable tablets that could not sustain drug release), with the remaining composition consisting of modified starch (5-10%) and magnesium stearate. In this formulation, modified starch served as a disintegrant to facilitate tablet disintegration and drug release.

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Table 2.1. Paracetamol tablet formulation by using CLPS, APS, and dual CLPS-APS as a filler (F1-Formulation).

Ingredients of tablet (%)	F1	F2	F3	F4
Paracetamol (%)	52	52	52	32
CLPS (%)	46	-	-	-
APS (%)	-	46	-	-
Dual CLPL-APS (%)	-	-	46	56
Mg Stearate (%)	10	10	10	10
Total (mg)	500	500	500	500

2.5.2. Evaluation of the Formed Tablets

Weight and Thickness

Ten tablets were randomly selected from each batch and weighed individually using an analytical balance. The mean weight and standard deviation were calculated. Tablet thickness was measured using a sliding caliper.

Crushing Strength

The crushing strength of the tablets was determined using a Schleuniger 2E/205 crushing strength tester (Switzerland). Ten tablets were randomly selected from each batch, and the crushing strength of each tablet was measured individually. The mean crushing strength and standard deviation were then calculated.

Tensile Strength

The diametral compression test was performed to evaluate the effect of compression pressure and tablet composition on mechanical resistance. Radial tensile strength was calculated using the measured crushing strength along with the tablet diameter and thickness.

$$\sigma = \frac{2F}{\pi DT} \qquad (0.10)$$

where, σ is the tensile strength, F is the force required to break the tablet, D is the diameter of the tablet, and T is the tablet thickness.

Friability

Friability of the tablets was determined using ten tablets of known weight with a friability tester (ERWEKA, TAR 20, Germany). The tablets were subjected to rotation in the friabilator drum, which induces changes in surface area and diameter due to abrasion and shock [28]. After the test, the tablets were removed, dedusted, and reweighed. Percent friability was calculated as the percentage weight loss relative to the initial tablet weight.

Drug release kinetics

To investigate the mechanism governing drug release, the in vitro release profiles were fitted to several kinetic models. The zero-order model (Equation 2.11) describes systems in which the drug release rate is independent of drug concentration and proceeds at a constant rate. The first-order model (Equation 2.12) applies to systems where the release rate is concentration-dependent, resulting in an exponential decrease over time. According to the Higuchi model (Equation 2.13), drug release from an insoluble matrix is governed by Fickian diffusion and is proportional to the square root of time.

$$Q_t = Q_0 - k_0 t \qquad (2.11)$$

$$lnQ_t = lnQ_0 - k_1 t \qquad (2.12)$$

$$\frac{Q_t}{Q_0} = kH\sqrt{t} \qquad (2.13)$$

$$\sqrt[3]{Q_t} - \sqrt[3]{Q_0} = k_{HC} * t \qquad (2.14)$$

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where k_0, k_1, k_H, and k_{HC} are the release rate constants for the zero-order, first-order, Higuchi, and Hixson-Crowell rate equations, respectively. Q_0 is the initial amount of drug in the tablet, and Qt is the amount of medication released over time t. Dissolution data can be further evaluated with the use of the Korsemeyer- Peppas equation to create a model that will represent a better fit for the formulation (Eq. 2.15)

$$\frac{Q_t}{Q_0} = kt^n \qquad (0.11)$$

where, $\frac{Q_t}{Q_0}$ is the fraction of drug released at time t, k is the kinetic constant, and n is the diffusional exponent. The value of the exponent can be used to characterize the mechanism of drug release.

Disintegration Time

The disintegration time of uncoated tablets was determined according to the United States Pharmacopeia (USP) disintegration test. A disintegration tester (CALEVA, G.B. Caleva Ltd., UK) was used, with 0.1 N HCl maintained at 37 °C as the immersion fluid. Six tablets were tested simultaneously. A tablet was considered fully disintegrated when all fragments passed through the wire mesh of the basket assembly. The average disintegration time and standard deviation for six tablets were calculated. For uncoated tablets, the USP standard disintegration time is less than 45 minutes, while for coated tablets it is less than 1 hour. During testing, the basket-rack assembly was initially at rest with its cylinder in extreme position, and 2.5 L of fluid was placed in the cylindrical jar. The apparatus was adjusted so that the fluid level coincided approximately with the midline of the upper plastic plate. The fluid temperature was maintained at 37 °C throughout the test, after which the basket-rack assembly was removed and disassembled for evaluation.

Disintegration efficiency ratio (DER)

The disintegration efficiency (DER) was used to evaluate the balance between the mechanical strength and disintegrant properties of the tablets. DER was calculated using the following formula:

$$DER = \frac{C_s}{D_T * F_r} \qquad (0.12)$$

where: C_s is the crushing strength, F_r is friability, and D_T is the disintegration time.

3. Results and Discussion

3.1. Proximate Analysis of Raw Potato

The physical and chemical analysis of the isolated potato starch revealed the following composition (w/w, dry basis): moisture 11.82 ± 0.0025%, crude ash 0.25 ± 0.0016%, crude protein 0.17 ± 0.0016%, crude fat 0.3 ± 0.0216%, and total starch 87.46 ± 0.93%. The very low protein and fat contents indicate that the potato starch was highly pure and that residual proteins were effectively removed during isolation. The isolated starch contained 30.52 ± 0.47% amylose and 56.94 ± 0.46% amylopectin.

The yields of the native, crosslinked, acetylated, and dual crosslinked acetylated starches were 25 ± 0.34%, 86.34 ± 0.83%, 87.63 ± 0.83%, and 84.42 ± 0.78%, respectively. For comparison, Gebremariam reported the proximate composition of potato starch as 0.20% ash, 0.20% protein, 0.05% fat, 13.5% moisture, and 29.3% amylose (w/w, dry basis). The present study shows similar results, although slight differences were observed in moisture and fat contents, which may be attributed to variations in the storage duration of raw potatoes and possible starch retrogradation.

3.2. Density of Native Starch and Modified Starch

The true density of native, crosslinked, acetylated, and dual crosslinked acetylated potato starches showed only minor differences. However, higher levels of acetylation, crosslinking, and dual modification (resulting from the combined effects of acetylation and crosslinking) led to measurable

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variations in density. Similar trends have been reported for acetylated starches by Gonzalez and Perez [30]. The observed increase in density may be attributed to the greater compactness of the starch mass induced by crosslinking. An enhancement in absolute density has also been reported for PUSA-44 starch, which exhibited a significantly higher amylose content [31].

Table 3.1. Density of native and modified starch.

Potato Starch (PS)	Density
Native	1.46
Crosslinked	1.48
Acetylated	1.49
Dual crosslinked-acetylated	1.47

3.3. Basic Characteristics of Native and Modified Starch

3.3.1. pH Determination

The pH values of the modified starches varied depending on the type of modification. Acetylated potato starch (APS) exhibited acidic properties with a pH of 4.65, while crosslinked potato starch (CLPS) was near neutral, with a pH of 6.80. The dual crosslinked acetylated starch had an intermediate pH of 5.6. The acidity of APS is due to the use of acetic anhydride as the modifying agent, which introduces acetyl groups and contributes to acidic character. In contrast, CLPS was modified with sodium hexametaphosphate (SHMP), a salt, resulting in a near-neutral product. During dual modification, the near-neutral CLPS was acetylated with acetic anhydride, producing a slightly acidic pH of 5.6. This suggests that some of the acidic components were neutralized during the acetylation of the crosslinked starch, leading to an intermediate pH value in the dual-modified starch.

3.3.2. Analysis of Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of native and modified potato starches revealed characteristic absorption bands corresponding to specific functional groups, which confirmed the chemical modifications. In native potato starch (NPS), the broad absorption band at 3253.2 cm^{-1} was attributed to O-H stretching vibrations of alcohols and phenols. The broad, less intense O-H peaks between 3400 and 3200 cm^{-1} correspond to carboxylic acids, reflecting variations in hydrogen bonding. The peak at 2935.6 cm^{-1} indicated C-H stretching, characteristic of alkanes and sp^3-hybridized carbons. Carbonyl groups (C=O stretching) were observed at 1637.2 cm^{-1}, while the band at 1353 cm^{-1} indicated C-O-C stretching of ethers and esters. The absorption around 995.1 cm^{-1} corresponded to C-O stretching. These peaks provide a reference for determining whether starch has been chemically modified. For crosslinked potato starch (CLPS), the FTIR spectrum showed a -O-H stretching band at 3288 cm^{-1}, while the C–H stretching appeared at 2931.3 cm^{-1}. The band at 1639.2 cm^{-1} corresponded to –C=C stretching, and the absorption at 1149.4 cm^{-1} was assigned to –P=O stretching, indicating successful crosslinking with the SHMP functional group. The C–O stretching band appeared at 991.3 cm^{-1}. In acetylated potato starch (APS), a –O–H stretching band was observed at 3318.9 cm^{-1}, and the C–H stretching at 2931.3 cm^{-1}. A peak at 1153.2 cm^{-1} corresponded to –C=O stretching of the acetyl groups introduced by acetic anhydride. Additional bands at 991 cm^{-1} and 574 cm^{-1} were assigned to –S=O and –C–H stretching, respectively. The FTIR analysis confirmed chemical modifications of starch: in CLPS, the hydroxyl groups of native starch were replaced by phosphorus-containing groups from SHMP, indicating successful crosslinking. In APS, hydroxyl groups were replaced by acetyl groups (–C=O), confirming the acetylation reaction. These spectral changes provide clear evidence that native potato starch was effectively modified through crosslinking and acetylation.

In the dual crosslinked acetylated starch, the crosslinked potato starch (CLPS) was further modified by substitution of hydroxyl groups with acetate groups. The FTIR spectrum displayed a broad absorption band at 3311.2 cm^{-1}, corresponding to –O–H stretching vibrations, reflecting residual hydroxyl groups after acetylation. A weak C–H stretching vibration appeared at 2921.7 cm^{-1},

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indicating the introduction of acetate groups along the starch backbone. The medium-intensity band at 1359.2 cm^{-1} was assigned to $–CH_3$ vibrations from the acetyl groups. The C–4–OH stretching of glucose residues in disaccharide units was observed at 993 cm^{-1}, while the peak at 759.8 cm^{-1} corresponded to cis =C–H out-of-plane bending. These spectral features confirm the stepwise modification of starch, where phosphate-containing groups of the crosslinked starch were replaced by acetyl groups, resulting in the formation of dual crosslinked acetylated starch.

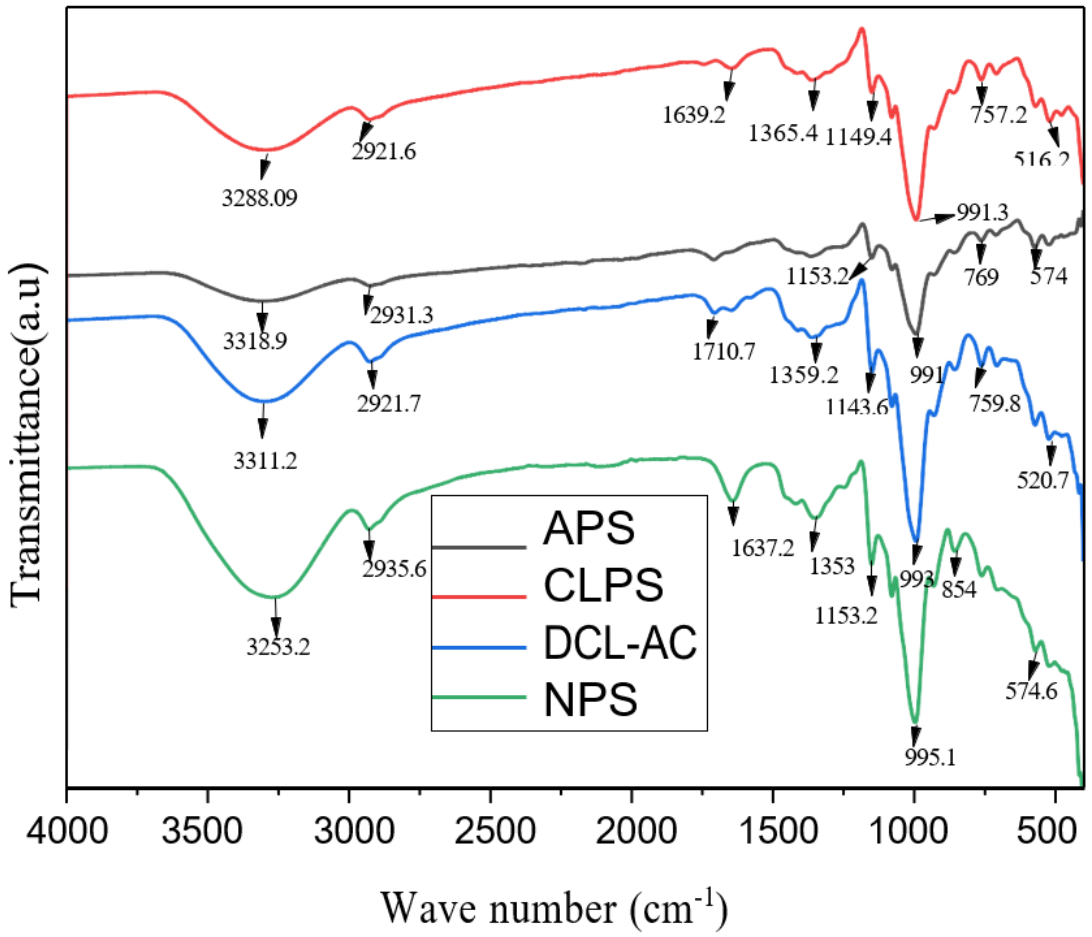

Figure 3.1. FTIR characterization of native (NPS), crosslinked (CLPS), acetylated (APS), and dual crosslinked acetylated (DCL-AC) potato starch.

3.3.3. Determination of Starch Hydration Capacity

The hydration capacities of native potato starch (NPS), crosslinked potato starch (CLPS), acetylated potato starch (APS), and dual crosslinked acetylated starch (DCL-AC) were 1.68 ± 0.38%, 2.39 ± 1.48%, 2.70 ± 0.54%, and 2.55 ± 1.03%, respectively (Figure). Modification of starch significantly increased its hydration capacity compared to the native starch, which exhibited the lowest value. Among the modified starches, APS demonstrated the highest water-holding capacity, likely due to the introduction of acetyl groups from acetic anhydride, which enhance hydrophilicity. The dual crosslinked acetylated starch (DCL-AC) exhibited intermediate hydration, reflecting a balance between the hydrophilic effect of acetylation and the structural reinforcement provided by crosslinking. These results suggest that chemical modification can be used to tailor the hydration properties of potato starch for specific applications.

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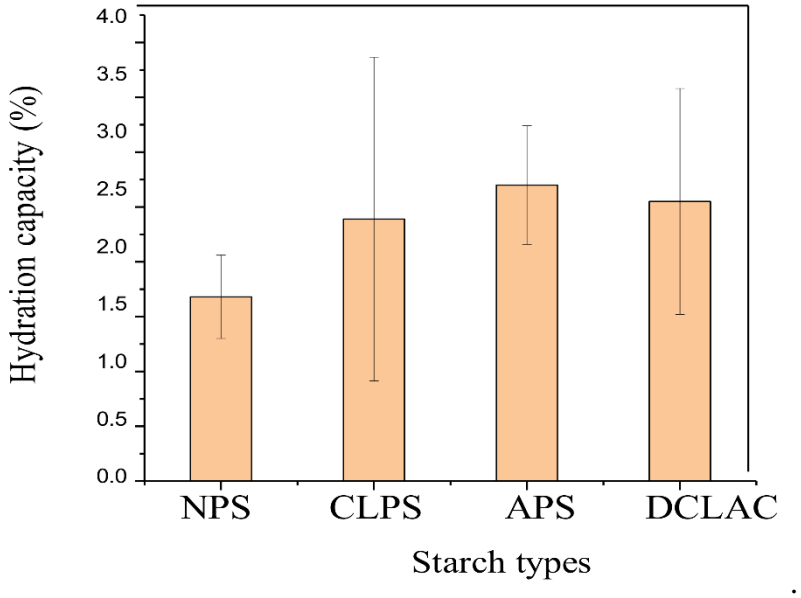

Figure 3.2. Hydration capacity of native and modified starch.

3.3.4. Swelling Power and Solubility Determination

Effect of Temperature

As shown in Figure , temperature had a direct effect on the solubility and swelling power of both native and modified potato starches. An increase in temperature resulted in a corresponding increase in both parameters for all starch samples. Among the modified starches, acetylated potato starch exhibited the greatest increase in solubility and swelling power with rising temperature. This pronounced effect can be attributed to the highly hydrophilic nature of acetylated starch, arising from the introduction of acetyl groups, which weaken intermolecular hydrogen bonding within starch granules and enhance water penetration and granule expansion.

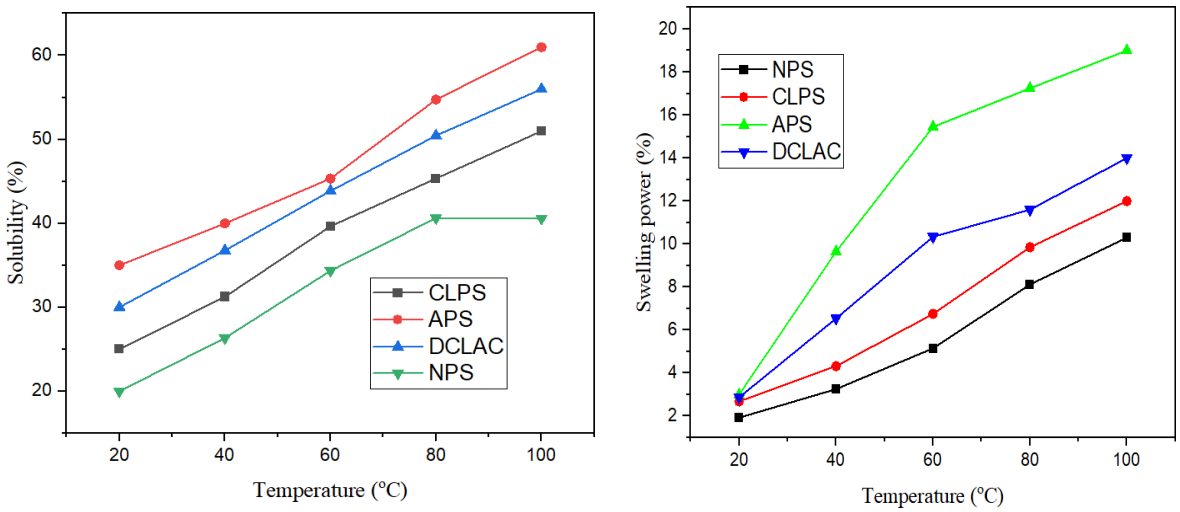

Figure 3.3. Effect of temperature on solubility and swelling power of starch.

Time Effect on Swelling Power and Solubility

As expected, both the swelling power and solubility of the starches increased with increasing time. The solubility of cross-linked, acetylated, and dual cross-linked acetylated potato starch increased markedly from 9.5 to 23%, 15 to 29%, and 11 to 26.5%, respectively. Similarly, the swelling power increased from 2.0 to 3.1 for cross-linked starch, from 1.6 to 3.0 for acetylated starch, and from

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1.7 to 3.0 for dual-modified starch. These results indicate that prolonged interaction time enhances both solubility and swelling behavior in all modified starches. Among them, acetylated potato starch exhibited the highest solubility and swelling power, which can be attributed to the introduction of acetyl groups that increase hydrophilicity and weaken intermolecular hydrogen bonding within the starch granules.

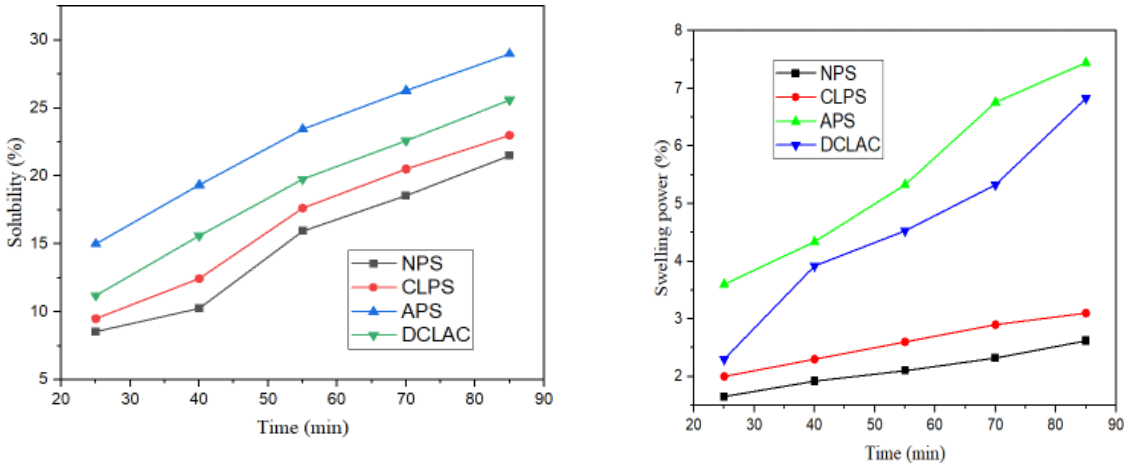

Figure 3.4. Effect of time on solubility and swelling power of starch.

Concentration Effect on Swelling Power and Solubility

The solubility and swelling power of cross-linked, acetylated, and dual cross-linked acetylated potato starch were evaluated at starch concentrations ranging from 10 to 50 wt.%. As expected, both solubility and swelling power increased with increasing starch concentration. The solubility of cross-linked, acetylated, and dual cross-linked acetylated potato starch increased substantially from 40 to 62%, 45 to 61%, and 21 to 60%, respectively. Similarly, the swelling power increased from 0.7 to 1.8 for cross-linked starch, from 4.5 to 20 for acetylated starch, and from 2.3 to 9 for dual-modified starch. These results demonstrate that increasing starch concentration enhances water uptake and granule expansion in all modified starches. Among the samples, acetylated potato starch exhibited the highest solubility and swelling power, which can be attributed to the introduction of acetyl groups that increase hydrophilicity and reduce intermolecular hydrogen bonding, thereby facilitating greater water penetration into the starch matrix.

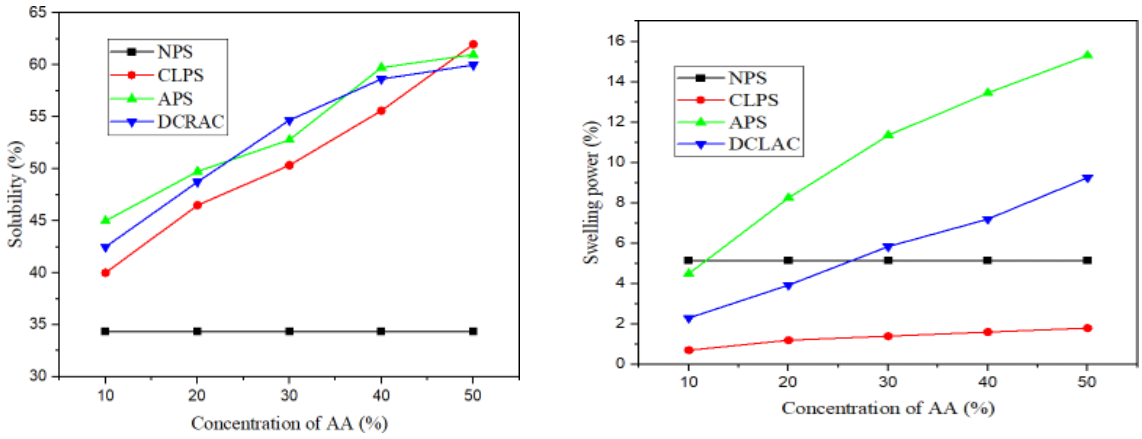

Figure 3.5. Concentration of AA effect on solubility and swelling power of starch.

3.3.5. Pasting Properties

The pasting properties of native potato starch (NPS), cross-linked potato starch (CLPS), acetylated potato starch (APS), and dual cross-linked acetylated potato starch (DCL-APS) were

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analyzed using a Rapid Visco Analyzer (RVA). The RVA viscosity profiles provide insight into the gelatinization behavior, granule stability, and retrogradation tendency of the starches. For native potato starch, gelatinization began at 2.7 min with an onset temperature of 71.9 °C. The peak viscosity reached 11,610 cP at 95.5 °C after approximately 3.33 min, indicating strong swelling and water-binding capacity. The high peak viscosity reflects the high thickening ability of native potato starch, which is associated with extensive granule swelling and amylose leaching. In the case of cross-linked potato starch, gelatinization began slightly later, at 2.9 min and 73.5 °C, indicating increased resistance to thermal swelling due to intermolecular phosphate bridges introduced by sodium hexametaphosphate (SHMP). The peak viscosity, final viscosity, and peak time were 6,189 cP, 9,570 cP, and 6.4 min, respectively. The reduction in peak viscosity compared to native starch suggests restricted granule swelling, while the increased final viscosity indicates enhanced paste stability. Furthermore, increasing SHMP concentration (10, 20, and 30%) resulted in higher final viscosity, confirming stronger cross-link density and improved structural integrity of starch granules. Acetylated potato starch exhibited a gelatinization onset at 2.7 min and 71.9 °C, like native starch, but showed a significantly lower peak viscosity of 1,413 cP with a peak time of 4.67 min. The reduction in viscosity can be attributed to acetyl substitution, which weakens intermolecular hydrogen bonding and reduces granular integrity, thereby facilitating easier disruption during heating and shear.

The dual cross-linked acetylated potato starch showed gelatinization onset at 2.85 min and 72.6 °C, reflecting the combined effects of cross-linking and acetylation. Cross-linking imparted structural rigidity, while acetylation enhanced hydrophilicity. The setback viscosity, which is related to the rate of retrogradation, was highest for starch cross-linked at lower SHMP concentrations. This suggests that limited cross-link density allows greater chain reassociation during cooling. However, after dual modification, the setback value decreased significantly compared to native and solely cross-linked starches. This reduction is attributed to the introduction of acetyl (carboxyl-containing) groups, which hinder amylose–amylose reassociation and suppress retrogradation.

Overall, cross-linking strengthens starch granules, making them more resistant to acidic conditions, high temperature, and mechanical shear, while increasing gelatinization temperature and reducing swelling [33]. These effects arise from reduced mobility of amorphous chains due to intermolecular phosphate bridges within the granules [34]. Dual modification effectively balances structural stability and reduced retrogradation, making DCL-APS particularly suitable for pharmaceutical and controlled-release applications.

Table 3.2. Pasting properties of native, crosslinked, acetylated, and dual crosslinked-acetylated potato starch.

Samples	Peak viscosity	Break	Trough	Final viscosity	Set back	Pasting	Peak
	(cP)	down (cP)	(cP)	(cP)	(cP)	temp. (℃)	time (min)
NPS	11610	10200	1410	3195	1785	71.9	3.33
CLPS	6189	3618	2571	9570	6999	73.5	6.4
APS	1413	460	953	1345	392	71.9	4.67
DCLAC	1440	262	1178	2002	824	72.6	6.73

The pasting profile of dual-modified potato starch is particularly important for the preparation of filmogenic solutions. Dual modification generates a starch structure that, while initially resistant to swelling, becomes susceptible to disruption under prolonged heating and shear. This disruption promotes the formation of linear starch chains that rearrange to produce a viscous and stable paste suitable for film formation. The pasting temperature of crosslinked potato starch was slightly higher than that of the native and acetylated starch. This increase is attributed to the enhanced intermolecular bonding created by crosslinks, which strengthens the starch granules and increases their resistance to thermal swelling, thereby raising. The dual-modified potato starch exhibited slower swelling behavior and a higher onset swelling temperature compared to native potato starch, which swelled rapidly at lower temperatures. This delayed swelling in the dual-modified starch is primarily attributed to crosslinking, which restricts granule expansion by forming intermolecular bridges between starch chains. In addition, acetylation alters the internal structure of the starch

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granules and moderates water penetration, resulting in a slower diffusion of water into the granule interior. Therefore, the combined effects of crosslinking and acetylation reduce granule disintegration during heating. Despite this restricted swelling, the dual-modified starch showed greater molecular rearrangement during the cooling phase than the native starch. This behavior is associated with its higher resistance to shear during heating and the subsequent reorganization of starch chains at elevated temperatures. Structural rearrangement leads to the formation of a more ordered network during cooling, which contributes to paste stability. The gelatinization transition temperature. Native potato starch exhibited the highest peak viscosity among all samples. This behavior is related to its unrestricted granule swelling and extensive amylose leaching during gelatinization. In contrast, the modified starches showed reduced peak viscosity due to structural reinforcement and altered water diffusion, despite their improved water absorption capacity and water solubility index.

3.3.6. Thermal Properties (DSC)

The thermal properties of native, crosslinked, acetylated, and dual crosslinked acetylated potato starch were evaluated using differential scanning calorimetry (DSC), and the thermograms are presented in Figure 3.7. The onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) of native potato starch were 48.5, 78.0, and 108 °C, respectively. Gelatinization of the native starch commenced at the onset temperature, reflecting the disruption of the crystalline regions. The relatively high peak temperature observed for native potato starch is attributed to its strong molecular association and high affinity for water, which requires greater thermal energy to induce complete gelatinization. For crosslinked potato starch, the onset, peak, and conclusion temperatures were 50.0, 80.9, and 110 °C, respectively. Crosslinking with sodium hexametaphosphate (SHMP) did not significantly alter the onset or peak temperatures; however, an increase in the conclusion temperature and gelatinization enthalpy (ΔH) was observed with increasing degree of crosslinking. This behavior suggests enhanced structural rigidity of the starch granules due to the formation of intermolecular phosphate bridges. The influence of crosslinking on the thermal transition properties of starch is known to depend on several factors, including the concentration and nature of the crosslinking agent, reaction conditions, and botanical origin of the starch.

Acetylated potato starch exhibited onset, peak, and conclusion temperatures of 68.0, 78.3, and 104.5 °C, respectively. Acetylation led to a reduction in the gelatinization temperature range, indicating a weakening of the starch granular structure. The introduction of acetyl groups reduced both intragranular and intergranular hydrogen bonding, facilitating water penetration and lowering the thermal energy required for gelatinization. The gelatinization enthalpy (ΔH), which is commonly associated with the degree of crystallinity and molecular order, decreased after acetylation, suggesting disruption of the double-helical structures within the amorphous regions of the starch granules. Moreover, the incorporation of bulky acetyl substituents increased chain mobility and structural flexibility, further contributing to the reduction in gelatinization temperatures. The dual crosslinked acetylated potato starch showed onset, peak, and conclusion temperatures of 48.5, 79.5, and 105 °C, respectively. In this system, crosslinking enhanced the stability of the crystalline regions, making the crystallites more resistant to thermal dissociation, while acetyl substitution weakened hydrogen bonding between starch chains and promoted water absorption. This combined modification resulted in balanced thermal behavior, reflecting the competing effects of structural reinforcement from crosslinking and plasticization from acetylation.

The gelatinization temperature range (T_c-T_o) provides insight into variations in crystallite size, degree of crystalline perfection, and the organization of double-helical starch chains. Lower ΔH values indicate that less energy is required to disrupt the ordered double-helical structures, and ΔH is positively correlated with the relative crystallinity of starch granules. Consequently, starch samples with lower ΔH values generally possess a lower proportion of ordered crystalline regions and reduce crystalline stability. As shown in Figure , native potato starch exhibited the highest peak gelatinization temperature, which can be attributed to its intact crystalline structure and strong

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hydrophilic interactions. Overall, the results of this study are consistent with previous reports indicating that gelatinization temperatures decrease with increasing degrees of chemical substitution, owing to progressive disruption of the starch crystalline architecture.

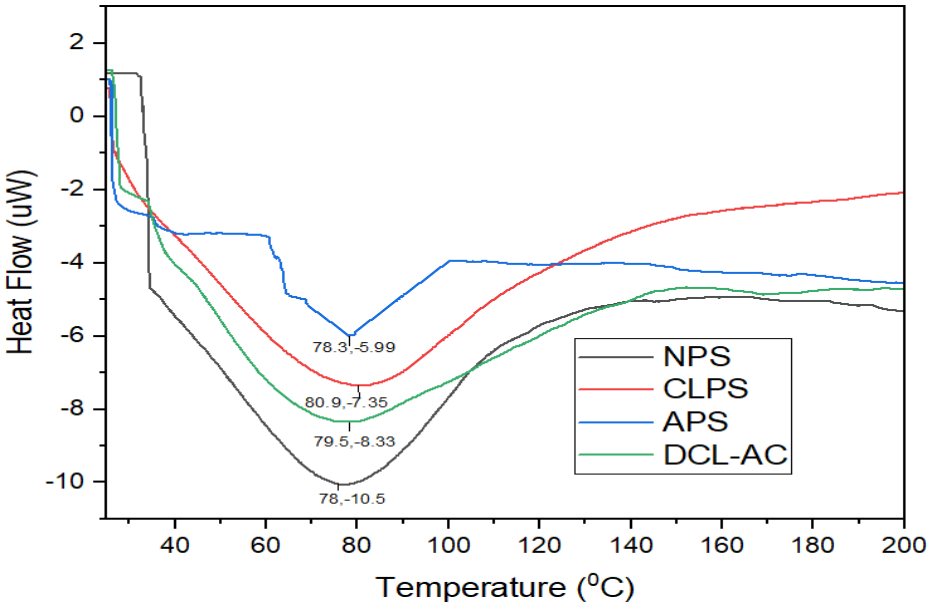

Figure 3.6. DSC analysis of native, crosslinked, acetylated, and dual crosslinked-acetylated potato starch.

The temperature region between the onset temperature (T_o) and the conclusion temperature (T_c), the starch undergoes a phase transition from an ordered crystalline structure to a predominantly amorphous state. At T_o, the crystalline domains of native starch begin to melt, and as the temperature increases toward the peak temperature (T_p), progressive disruption of the double-helical structures occurs. The endothermic transition observed between T_o and T_p corresponds to the energy required to break hydrogen bonds stabilizing the crystalline regions. At T_p, the gelatinization process reaches its maximum extent, indicating the completion of crystalline melting and the formation of an amorphous structure [39]. Beyond T_p, molecular rearrangement and partial reassociation of starch chains may occur during continued heating and subsequent cooling. At the conclusion temperature (T_c), the gelatinization process is completed, and the final molecular organization of the starch system is established. Overall, the dual crosslinked acetylated potato starch effectively compensates for the limitations associated with each individual modification. The dual-modified starch exhibited a broadened gelatinization temperature range and altered gelatinization enthalpy (ΔH_{gel}), resulting from the combined effects of structural reinforcement through crosslinking and hydrogen bond disruption caused by acetyl substitution. This counterbalancing interaction between amylopectin crosslinking and acetyl group substitution produces a thermally stable yet flexible starch network [23]. These observations are consistent with previously reported findings [31]. The increase in peak gelatinization temperature observed for dual crosslinked acetylated potato starch expands the functional application range of native potato starch, particularly in processes requiring enhanced thermal stability.

3.3.7. X-Ray Diffraction (XRD) Analysis of Native and Modified Starch

Starches are commonly classified into A-, B-, and C-types based on the crystalline packing and lamellar organization of amylopectin double helices [40]. Although A- and B-type amylopectin possess similar helical conformations, they differ in their unit cell geometry, with A-type starch exhibiting an orthorhombic unit cell and B-type starch a hexagonal unit cell [41]. Potato starch is known to possess a B-type crystalline structure [40]. The X-ray diffraction (XRD) pattern of native

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potato starch exhibited characteristic B-type reflections, with enhanced crystallinity evidenced by increased peak intensity and broadening at 2θ values of approximately 5.78°, 15.20°, 17.30°, 17.36°, 22.48°, and 26.86°. A prominent diffraction peak was observed at 2θ = 17.36°, accompanied by minor peaks at 34.60°, 63.86°, and 75.94°. These diffraction features are consistent with the B-type crystalline structure commonly reported for potato and other tuber starches [19]. The strong reflection at 2θ = 17.36° and additional peaks above 26.86° within the range of 0° ≤ 2θ ≤ 80° confirm the ordered crystalline arrangement of native potato starch. In such systems, intermolecular association occurs through recrystallization processes, where amylopectin acts as a precipitating framework facilitating amylose aggregation within the amylose–amylopectin-water matrix [42]. XRD analysis further revealed that dual modification through crosslinking and acetylation significantly altered the crystalline organization of the starch granules (

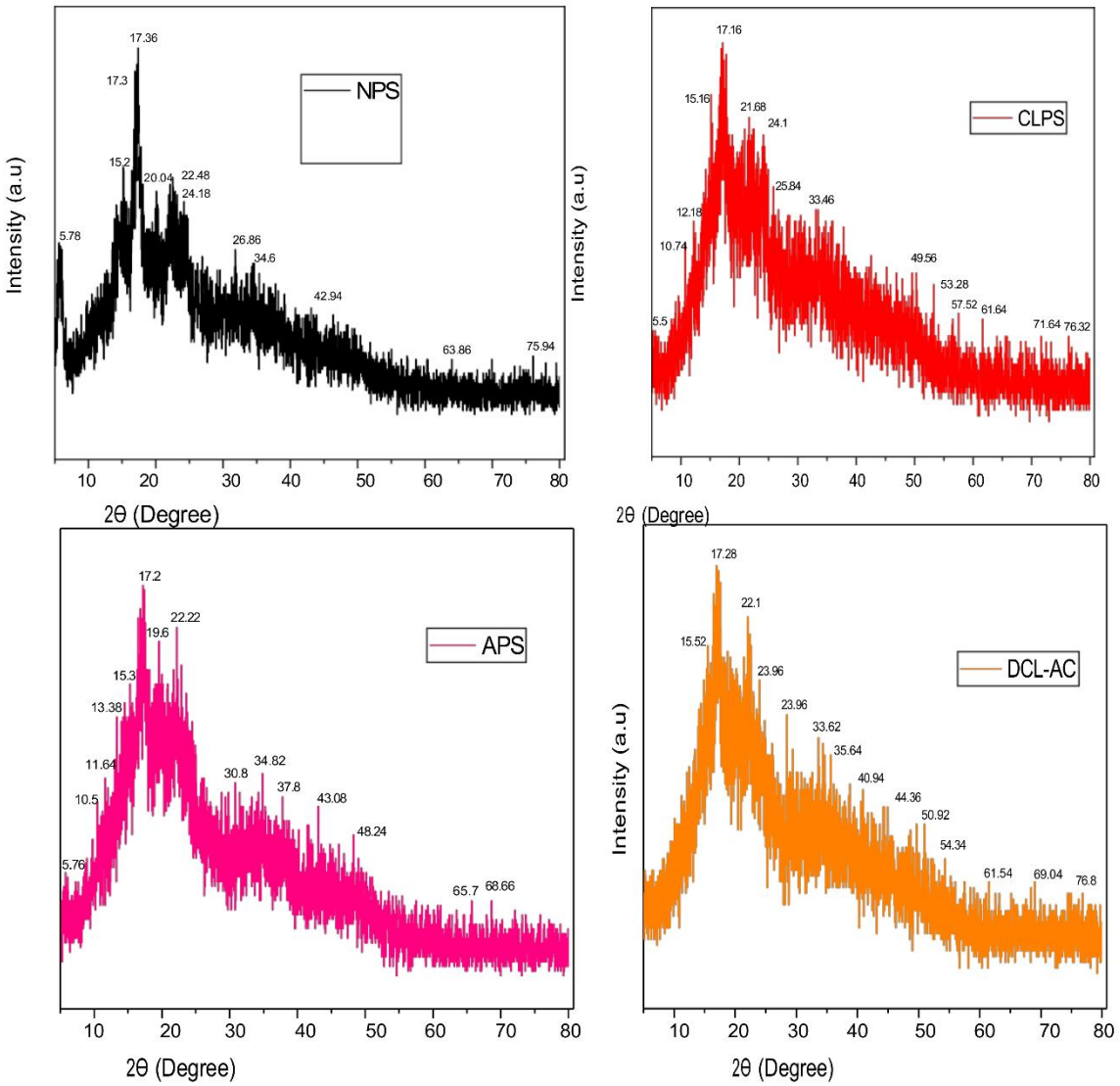

Figure). The rearrangement of amylopectin double helices resulted in a reduction in crystallinity level (CL) from 23.6% in native starch to 18.4% in dual crosslinked acetylated potato starch [39]. The diffraction peaks of dual-modified starch were observed at 2θ values of 15.52°, 27.96°, 33.62°, 50.92°, 69.04°, and 76.80°. Notably, the highest intensity peak occurred at 2θ ≈ 17.28°, closely resembling that of native potato starch, indicating partial retention of the original B-type structure. At lower 2θ values, the dual-modified starch exhibited fewer and less intense diffraction peaks prior to reaching the principal reflection, reflecting a decrease in long-range crystalline order. This reduction in crystalline can be attributed to the combined effects of chemical modification, where multifunctional reagents form ether and ester linkages between hydroxyl groups on starch chains. Crosslinking

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reactions introduce intra- and intermolecular covalent bonds at irregular positions along the polymer backbone, while acetyl substitution disrupts hydrogen bonding within the crystalline lamellae. Together, these modifications stabilize the granules while reducing crystalline perfection and order [39].

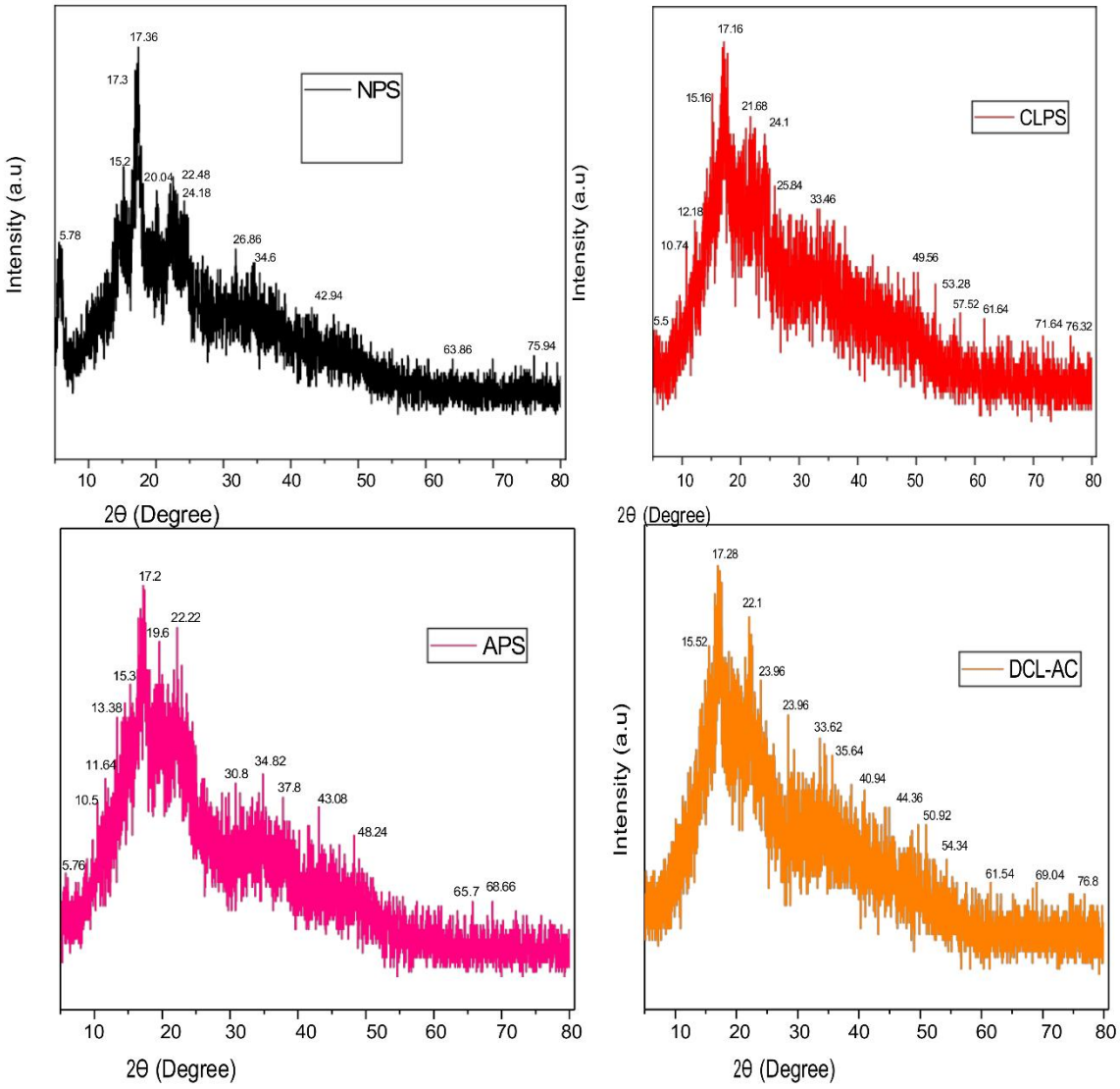

Figure 3.7. XRD analysis of native, crosslinked, acetylated and dual-crosslinked acetylated potato starch.

3.4. Starch Modification Experiment and Effect of Process Parameters

3.4.1. Effect of Reaction Temperature on Acetyl Content

The effect of reaction temperature on the acetyl content of dual crosslinked-acetylated potato starch is presented in Figure . The acetyl content increased from 1.20% to 1.81% as the reaction temperature rose from 20 °C to 60 °C. This increase can be attributed to the enhancement of the esterification reaction rate with increasing temperature, as higher temperatures promote molecular mobility and increase the frequency of effective collisions between reactive sites [23]. Beyond 60 °C, a decline in acetyl content was observed. This decrease is attributed to the onset of starch gelatinization, which limits the accessibility of hydroxyl groups required for esterification. As gelatinization progresses, the structural disruption of starch granules reduces the availability of reactive sites, thereby suppressing further acetyl substitution. These findings are consistent with previous studies, which report that acetylation is commonly employed to lower the gelatinization

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temperature of starch [47]. Once gelatinization begins, the efficiency of acetylation decreases due to reduced chemical accessibility within the starch matrix.

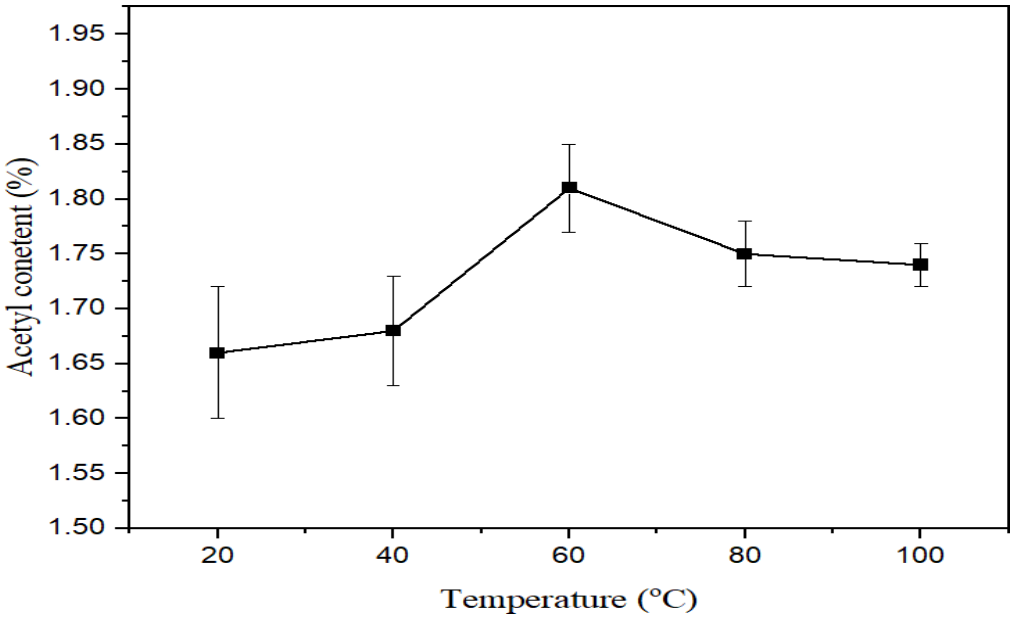

Figure 3.8. Effect of temperature on acetyl content.

3.4.2. Effect of Reaction Time on Acetyl Content

The effect of reaction time on the acetyl content of dual crosslinked-acetylated potato starch is illustrated in Figure . The acetyl content increased from 1.18% to 1.80% as the reaction time was extended from 25 to 55 min, reflecting the progressive substitution of hydroxyl (–OH) groups by acetyl (–CH_3CO–) groups. Beyond 55 min, the acetyl content increased only marginally, indicating that the reaction approached a near-equilibrium state due to saturation of accessible reactive sites. Reaction time plays a critical role in the extent of acetylation, as prolonged exposure allows additional acetyl groups to gradually replace hydroxyl groups within the starch matrix [48]. However, when the reaction time exceeds an optimal value, the rate of substitution decreases significantly, and the acetyl content approaches a constant value. This behavior is attributed to the formation of stable reacted complexes and the limited availability of remaining free hydroxyl groups for further esterification [49].

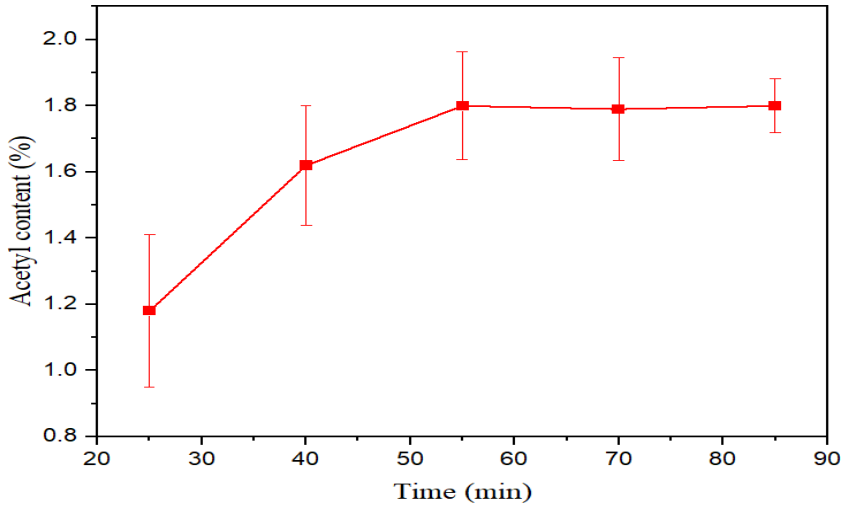

Figure 3.9. Effect of reaction time on acetyl content.

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3.4.3. Effect of Acetic Anhydride on the Acetyl Content Response

The effect of acetic anhydride concentration on the acetyl content of dual crosslinked–acetylated potato starch is shown in Figure . The acetyl content increased from 1.55% to 1.80% as the acetic anhydride concentration was raised from 10 wt.% to 50 wt.%. This positive correlation can be attributed to the greater availability of acetic anhydride molecules around the starch hydroxyl groups at higher concentrations, which facilitates esterification. Since starch hydroxyl groups exhibit limited mobility, the proximity of acetic anhydride molecules is critical for effective substitution [49].

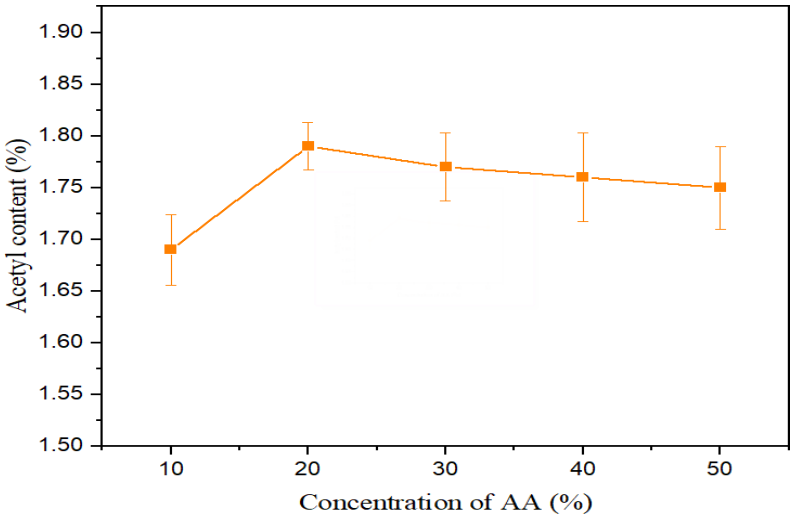

Figure 3.10. Effect of AA concentration on acetyl content.

3.4.4. Effect of pH on Acetyl Content

The effect of pH on the acetyl content of dual crosslinked-acetylated potato starch was found to be negligible compared to other factors such as reaction temperature, reaction time, and concentration of modifying agent. As the pH of the starch solution increased from 2 to 10, the acetyl content showed only minor variations, decreasing slightly from 1.66% to 1.65% and then rising marginally to 1.673% (Figure). This indicates that pH has a minimal influence on acetylation, with changes of less than 0.003% in acetyl content per unit change in pH. The insignificant effect is attributed to insufficient activation of starch hydroxyl groups for nucleophilic attack by acetic anhydride [50]. Based on this negligible impact and supporting literature, pH was not included as a variable in the optimization study.

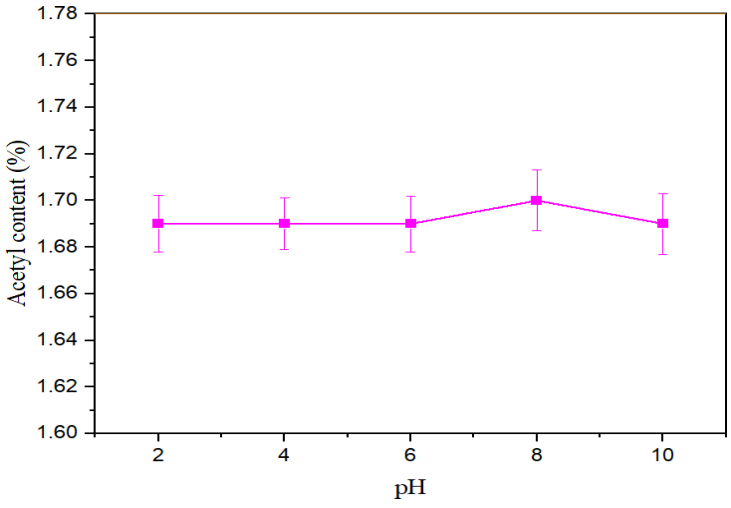

Figure 3.11. Effect of pH on acetyl content.

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3.4.5. Effect of Catalyst (NaOH) Concentration on Acetyl Content

The effects of NaOH concentration on acetyl content of dual crosslinked acetylated potato starch were insignificant as shown in Figure . In dual crosslinked acetylated, the molecules of starch were first held by the crosslinking agents of SHMP, which strengthen the bond in the starch. When the crosslinked potato starch was acetylated with acetic anhydride replacement of the crosslinked phosphate groups by acetyl group, which results in the increment of the amorphous region.

Thus, the negative effect of crosslinking was compensated by acetylation. When the concentration of NaOH increases from 0.6%wt. to 1.4%wt., the acetyl content of the dual crosslinked acetylated potato starch insignificantly increases from 1.7%wt. to 1.72%wt, which indicates a slight effect on the response. As indicated in Figure 3.8, the NaOH concentration was not significantly affecting the response acetyl content and in literature the effect of this parameter was not included in the optimization.

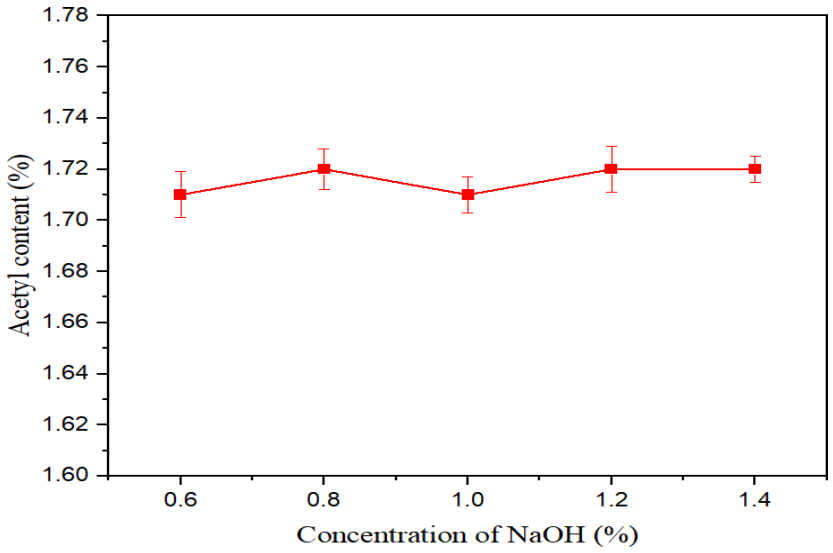

Figure 3.12. Effect of NaOH concentration on acetyl content on the responses.

3.4.6. Estimation of Combined Effects of the Factors

A central composite design (CCD) was employed to evaluate the significant effects of process parameters namely, reaction temperature, reaction time, and concentration of the modifying agent on the removal efficiency of methylene blue. The interactions between two variables at a time were examined while keeping the other parameters constant, and the results were visualized using contour and three-dimensional (3D) response surface plots.

Interaction effect of reaction temperature and time

Figure A, presents the three-dimensional (3D) response surface plot illustrating the interaction between reaction temperature and reaction time at a fixed concentration of the modifying agent (20%). The acetyl content (%) of dual crosslinked acetylated potato starch increased with longer reaction times, while it decreased slightly with increasing reaction temperature. According to the coded model equation, the combined effect of reaction temperature and reaction time on acetyl content was positive, with a coefficient of 0.0275, indicating a very small influence. The interaction showed a statistically significant effect on removal efficiency, with a p-value of 0.0241, although the overall combined effect on acetyl content was nearly negligible.

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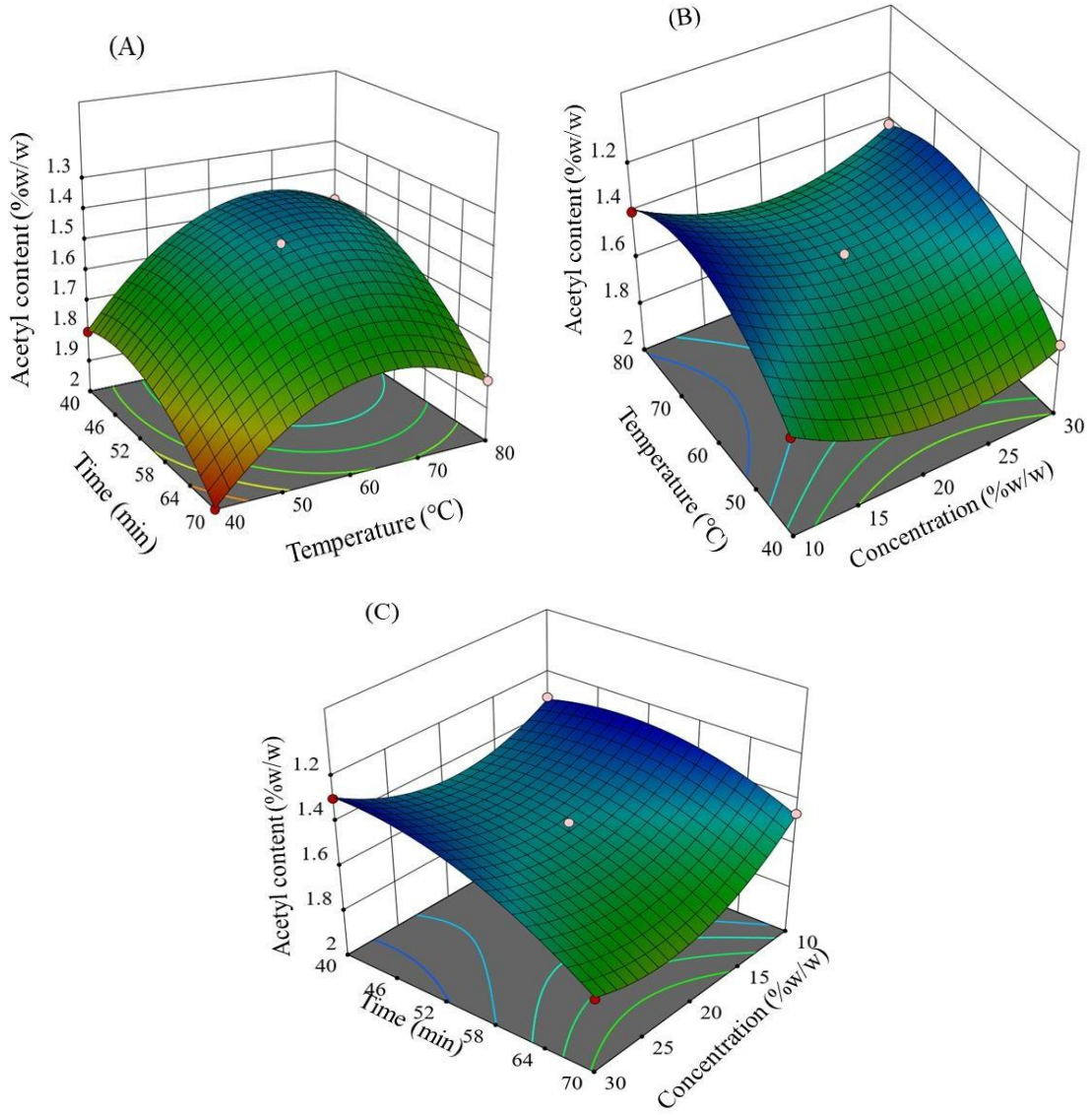

Figure 3.13. Response surface 3D plot of interaction effects, (A) (temperature and time), (B) (temperature and concentration), and (C) (time and concentration).

Interaction effect of temperature and concentration of modifying agent

Figure B, presents the three-dimensional (3D) response surface plot showing the interaction between reaction temperature and the concentration of the modifying agent at a constant reaction time of 55 min. The acetyl content (%) increased with increasing concentration of the modifying agent up to a certain point. However, at higher temperatures, the acetyl content decreased. This behavior indicates that the interactive effect of temperature and modifying agent concentration reaches an optimum region, beyond which the acetyl content declines. The decrease at higher temperatures can be attributed to the inactivation of starch molecules, while excessive concentration of the modifying agent may lead to the formation of starch complexes, reducing the efficiency of acetylation.

Interaction effect of reaction time and concentration of modifying agent

The interaction between reaction time and the concentration of the modifying agent (acetic anhydride, AA) on the acetyl content of dual crosslinked acetylated potato starch is illustrated in Figure C. The acetyl content (%) increases with longer reaction times, as extended durations allow more hydroxyl groups to react with the modifying agent. Similarly, increasing the concentration of acetic anhydride enhances acetylation up to an optimal point. Beyond this point, additional increases in either parameter do not significantly improve acetyl content. These observations are consistent

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with the ANOVA results, which indicate that the interaction between reaction time and modifying agent concentration has an insignificant effect on acetyl content, with a p-value greater than 0.0001.

3.5. Formulation of Tablets

Tablets were prepared by direct compression, containing paracetamol (50% w/w) as the active ingredient, modified starch (10% w/w) as a disintegrant, lubricants (10%), and other excipients. In this study, tablets were formulated using native starch and three types of modified starch: crosslinked potato starch (CLPS) prepared at 60 °C for 55 min with 20% w/w sodium hexametaphosphate, acetylated potato starch (APS) prepared at 60 °C for 55 min with 20% w/w acetic anhydride, and dual crosslinked-acetylated potato starch prepared under optimized conditions (40.2 °C, 69.85 min, 21.924% w/w acetic anhydride). Tablets containing APS required higher amounts of lubricant due to sticking issues during compression, whereas tablets containing CLPS exhibited smoother surfaces and better flow. The dual crosslinked-acetylated potato starch improved the stacking behavior of APS while reducing the viscosity of CLPS, enabling sustained release of the active ingredient. APS exhibited higher moisture content than CLPS and dual-modified starch, making compression more challenging until surface moisture was adequately removed. Regarding density, APS was denser than CLPS and dual crosslinked-acetylated starch, allowing tablets containing APS to form more compact structures due to its lower volume at comparable mass. Both crosslinking and acetylation enhanced particle bonding during tableting, which increased compaction and resulted in higher crushing strength. Furthermore, the degree of substitution of CLPS with acetate functional groups significantly influenced drug release, and the dual-modified starch facilitated controlled disintegration, making it suitable for sustained-release applications.

3.5.1. Basic Characteristics of Tablet

Weight, Tensile Strength, Crushing, Strength, and Thickness

As presented in Table , the weight, tensile strength, and crushing strength of tablets containing dual crosslinked-acetylated potato starch were evaluated to assess the mechanical behavior and performance of the tablets.

Table 3.3. Characteristics of the tablet of dual crosslinked acetylated potato starch.

Run	Weight(g)	Thickness(mm)	Diameter(mm)	Crushing strength(N)	Tensilestrength (10^6)
1	0.445	5	9.8	63	0.82
2	0.454	5	9.83	62	0.8
3	0.465	5	9.7	73	0.93
4	0.445	5	9.8	62	0.72
5	0.4425	5	9.88	70	0.88
6	0.48	5	9.85	62	0.84
7	0.456	5	9.78	70	0.93
8	0.465	5	9.83	65	0.85
9	0.453	5	9.87	63	0.86
10	0.467	5	9.8	68	0.89

3.5.2. Friability of Tablets

Tablets prepared from acetylated potato starch (APS) exhibited lower friability compared to those made from crosslinked potato starch (CLPS). The weight loss due to friability was 0.46% for APS tablets, whereas CLPS tablets showed a higher weight loss of 0.73%. All friability values were below 1%, indicating that the formulated tablets were mechanically stable. In contrast, tablets prepared from native starch (NS) exhibited a friability of 0.9%, approaching the acceptable limit and suggesting that NS may not meet stringent pharmaceutical requirements [51]. The improved compaction observed in crosslinked starches is attributed primarily to crosslinking in amylopectin, which reinforces the crystalline regions and promotes stronger packing within the granules.

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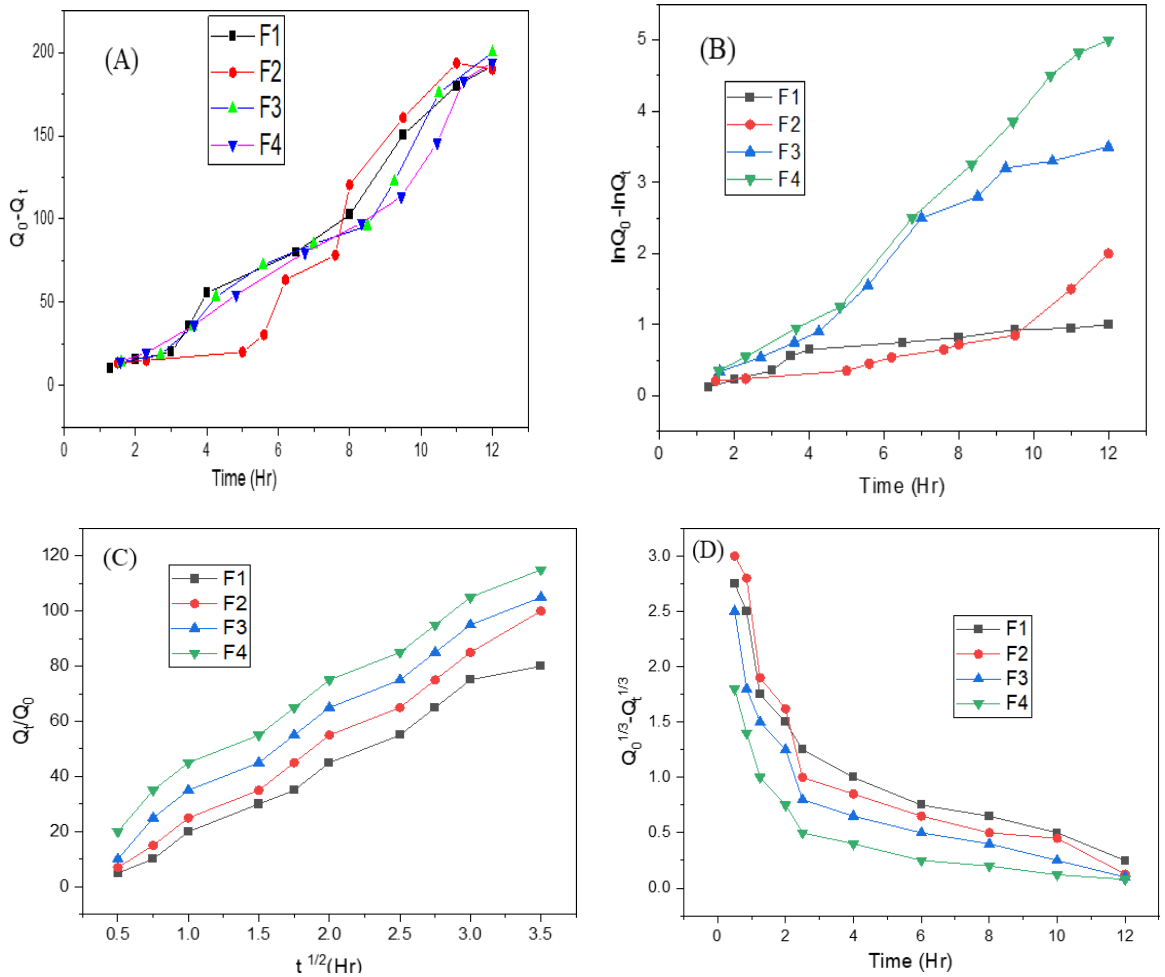
However, crosslinking alone does not fully enhance crushing strength, as the amorphous regions can still disrupt crystalline packing [52]. Tablets formulated with APS demonstrated increased crushing force, consistent with previous reports that acetate moieties act as effective bond-forming substituents. Generally, higher tablet hardness corresponds to lower friability and longer disintegration times [54]. Overall, tablets containing modified starches, particularly APS and dual crosslinked-acetylated starch, exhibit superior mechanical stability compared to native starch tablets.

3.5.3. Tablet Release Kinetics

Quantitative comparison of dissolution profiles can be performed using model-dependent and model-independent approaches. Model-dependent methods rely on fitting the dissolution data to an appropriate mathematical model and evaluating the goodness of fit along with changes in the model parameters. In contrast, model-independent methods allow direct comparison of a test profile with a reference profile without transforming the data into a mathematical expression [55]. In this study, the drug release data were analyzed using various kinetic models to elucidate the mechanisms governing drug release (Figure). The data were fitted to zero-order, first-order, Higuchi, and Hixson-Crowell cube root models to determine the best representation of the release kinetics.



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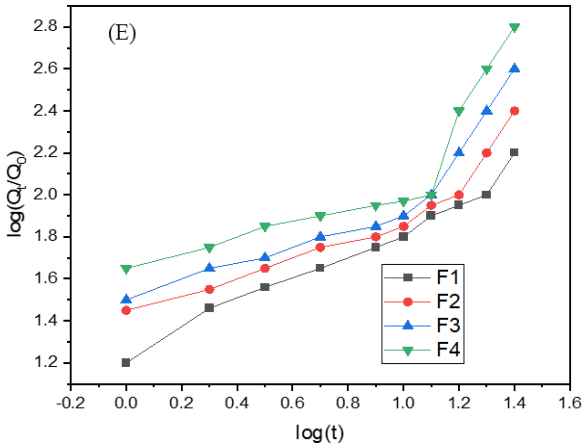

Figure 3.14. The release data from the different formulations fitted to various release kinetic models; zero-order (A), first-order (B), Higuchi (C), Hixson-Crowell (D) and Korsmeyer-Peppas (E) models.

3.5.4. Disintegration Time of Tablets

The disintegration time of tablets prepared with native starch was the shortest among all formulations, which can be attributed to the high number of hydroxyl (-OH) groups present in the starch [56]. These -OH groups enhance water penetration into the tablet matrix, promoting faster disintegration in accordance with the "like dissolves like" principle. In contrast, crosslinked potato starch (CLPS) tablets exhibited longer disintegration times because the crosslinked network restricts water absorption, stabilizing the polymer matrix. The introduction of substituent groups, such as crosslinks or acetyl groups, enhances water-holding capacity and strengthens the matrix, which contributes to increased disintegration times [52]. Acetylated potato starch (APS) tablets displayed enhanced hydrophilicity and water retention, which allowed significant swelling before complete disintegration. This swelling mechanism extended the disintegration time compared to native starch. The dual crosslinked acetylated potato starch (DCL-AC) tablets combined the characteristics of both CLPS and APS, resulting in a balanced disintegration profile. The crosslinking strengthened the granule structure, while acetylation promoted water uptake, leading to disintegration times that reflect a compensation between these opposing effects. Additionally, tablets containing DCL-AC exhibited superior mechanical strength and flow properties during compression. The modification improved the structural integrity and particle flow within the compressive zones, making dual crosslinked acetylated starch highly effective as a disintegrant in tablet formulations [57,58].

3.5.5. Disintegration Efficiency Ratio (DER)

The disintegration efficiency ratio (DER) was used to evaluate the balance between the mechanical strength and disintegrant properties of the tablets. DER was calculated as the ratio of crushing strength to the product of friability and disintegration time. Tablets prepared with native potato starch exhibited the highest DER, which can be attributed to their shortest disintegration time. In contrast, the DER of tablets containing crosslinked potato starch (CLPS) was the lowest, reflecting the increased mechanical strength imparted by crosslinking, which restricted disintegration despite providing greater tablet robustness.

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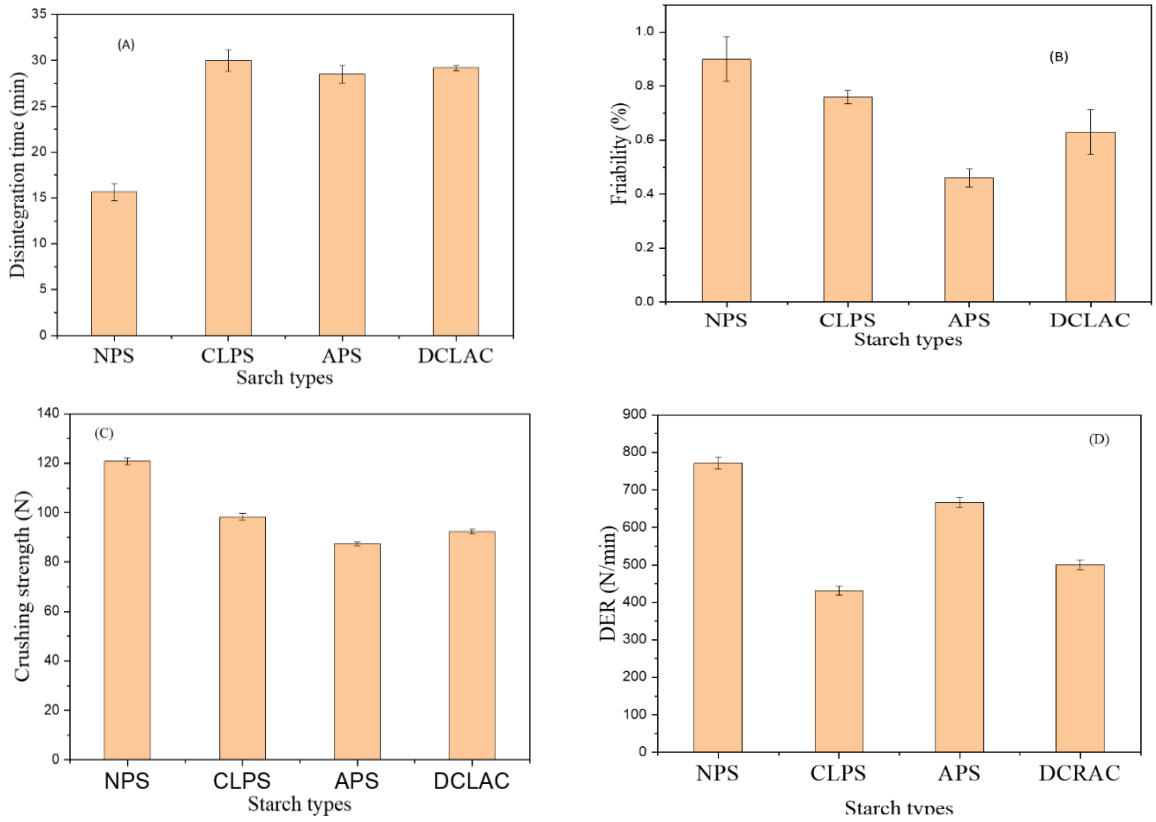

Figure 3.15. Variation of disintegration time (A), friability (B), crushing strength, and disintegration efficiency ratio, DER (D) of native and modified potato starch.

4. Conclusion and Future Perspectives

In this study, potato starch was successfully modified using crosslinking and acetylation in a stepwise dual-modification approach to optimize its properties for tablet excipient applications. Crosslinking enhanced tensile strength, limited swelling, reduced erosion, and improved water uptake and equilibrium swelling. Acetylation of the crosslinked starch further increased swelling, improved paste clarity, and enhanced resistance to retrogradation. By combining these two mechanisms, the negative effects of each individual modification were compensated, resulting in a starch with balanced mechanical strength and disintegration performance. The physicochemical characterization of the native and modified starches revealed their purity and structural features. The native potato starch contained 11.82 ± 0.0025% w/w moisture, 0.25 ± 0.0016% w/w crude ash, 0.3 ± 0.0216% w/w crude protein, 0.17 ± 0.0016% w/w crude fat, and 87.46 ± 0.93% w/w total starch, indicating a highly pure material. X-ray diffraction analysis demonstrated that native, crosslinked, and dual crosslinked-acetylated starches possessed both crystalline (amylopectin-rich regions) and amorphous (amylose-rich regions) structures. FTIR spectroscopy confirmed the presence of functional groups corresponding to crosslinking and acetylation modifications. Thermal analysis via DSC revealed endothermic peaks associated with water evaporation and exothermic peaks corresponding to starch decomposition. Pasting properties determined by Rapid Visco Analyzer (RVA) indicated improved flow and paste-forming behavior of the modified starches compared to native starch.

Preliminary experiments identified the effects of key process parameters reaction temperature, reaction time, and concentration of modifying agent (acetic anhydride) on the acetyl content of dual crosslinked-acetylated potato starch. Parameters such as pH and NaOH concentration were found to have negligible effects and were excluded from optimization. Using response surface methodology based on a Box–Behnken design, the optimal conditions for maximum acetylation (1.32 ± 0.077% w/w)

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were determined to be 40.22 ℃ for reaction temperature, 69.85 min for reaction time, and 21.92% w/w concentration of acetic anhydride. ANOVA confirmed the suitability of the quadratic model for predicting acetyl content, and 3D response surface plots highlighted the interaction effects of the main parameters. The dual-modified starch exhibited improved tablet-forming properties, balancing mechanical strength, swelling, and disintegration, demonstrating its potential as an excipient for sustained-release formulations.

Future Perspectives: Further research could explore the application of this dual-modified starch in various pharmaceutical formulations, including controlled-release tablets and hydrophilic matrices. Additionally, studies on scale-up synthesis, long-term stability, and in vivo drug release performance would be valuable to translate these findings into practical applications. Investigating the incorporation of other functional groups or combination modifications could also expand the versatility of potato starch as a multifunctional excipient.

CRediT authorship contribution statement: Seyoum Misganaw Mengstu: Formal analysis; Investigation; Data curation; Methodology; Writing-original draft; **Sintayehu Mekuria Hailegiorgis:** Conceptualization; Supervision; Writing - review & editing.

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: The authors declare that generative artificial intelligence (ChatGPT) was used solely to improve the clarity, grammar, and English language of the manuscript. The scientific content, data interpretation, and conclusions were entirely developed by the authors, who take full responsibility for the integrity and accuracy of the work.

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