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Final Project Report

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Project title	Starch granule destructurisation and modification under homogeneous aqueous conditions
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Executive summary (maximum 2 sides A4)

The major aim of this study was to establish the feasibility of an aqueous route to chemical modification of starch, and to highlight the way that future starch modification can be best tackled both economically and environmentally. In order to realise the goal, it was first necessary to: (1) fully characterise a range of commercially available native starches; (2) investigate a range of starch solubilisation conditions to ensure that the individual granules are completely destructurised; (3) establish that the component polymers of the destructurised starch granules are not depolymerised to any significant extent. Characterisation was primarily by the techniques of differential scanning calorimetry (DSC), gel permeation chromatography (GPC) and intrinsic viscosity. In the case of GPC, it was necessary to develop a repeatable analytical method to determine the molecular mass distribution of starch types, differing in amylose/amylopectin contents, before and after treatment. After completion of the preliminary solubilisation and characterisation studies, the task of starch modification, according to the aqueous techniques developed, was undertaken.

Four starches were acquired for the study, three of corn (maize) origin and one of wheat. The starches were chosen to offer starches of very high (Hylon VII, 70 %) or high (Hylon V, 50 %) or conventional (wheat starch, about 25 %) amylose content as well as very high (Amioca, 99 %) amylopectin content. This broad selection was used to allow the project to test the applicability of the aqueous modification method to the widest spectrum of starch composition possible.

Work has been carried out to develop a repeatable analytical method to determine the molecular mass distribution of the four starch types, and subsequently their derivatives using GPC and intrinsic viscosity measurements. Measurement of molecular mass can only be made on polymers in solution. Aqueous potassium thiocyanate has been developed as an alternative aqueous solvent to avoid using

DMSO organic solvent for this analytical solubilisation procedure. Potassium thiocyanate disrupts the starch crystallinity and solubilises the component polymers at the same time.

The solubilisation characteristics of four starches at a range of alkali concentrations (0.05 M to 5 M) have been studied. At low alkali concentrations, complete starch gelatinisation did not occur. Furthermore, at low alkali concentration, the corresponding starch concentration was too low to offer an economically viable system at the industrial scale. Conversely, at 5 M alkali concentration then the concentration of starch was too high to result in solution, a prerequisite of homogeneous modification. Results have shown that an alkali concentration in the range 2.5–3 M afforded a 13 to 16 weight % solution of starch that was fully solubilised for all starch samples. Little or no degradation of the starch polymers accompanied this treatment which has been confirmed by GPC analysis. Thus, 2.5 M aqueous sodium hydroxide solution was selected as the optimum solubilisation medium for subsequent starch modification.

A series of starch esters with different side-chain length and low DS-values was prepared and studied. The esters were prepared by acylation of the polymer with the appropriate acid chlorides in aqueous conditions, which represents an economical and facile method for the preparation of esterified starches. The alkali solution acted as the solvent for the derivatives and ensured uniform substitution by enhanced accessibility of the reagent. Successful reaction was limited to acid chlorides containing six to ten carbon chains. Shorter or longer carbon chain acid chlorides did not react under those conditions to form esters, as confirmed by FT-IR spectroscopic analysis.

Modifications to high degrees of substitution (DS-value ~3.0) have not been achieved and it is concluded that the aqueous conditions proposed are unsuited to the preparation of high DS-value starches.

It is concluded that, under optimised aqueous alkali conditions, a range of starches can be modified homogeneously to low levels of substitution (DS-values   0.3) with little or no depolymerisation of the starch chain either during the solubilisation or modification processes. The procedure is equally applicable to high amylose, high amylopectin or conventional (typical amylose: amylopectin ratio of 25:75) starches.

The aqueous route developed represents a viable method for the preparation of homogeneous starch esters from a variety of sources to low degrees of substitution. The method in fact provides a generic solubilisation platform for the broadest possible range of starches. Whilst the range of esters prepared in this study was limited to carbon-chain lengths between six and ten, it should not be presumed to be a limitation of the method for starch. This narrow range was obtained because of the relative solubilities and incompatibilities of the higher and lower carbon chain length acid chlorides from which attempts to prepare esters were made. A range of acid chlorides with tailored solubility profiles is available both from natural and synthetic sources and it is expected that reaction of such acid chlorides under the conditions described here would afford successful modification

Future work should be directed towards exploiting this solubilisation platform and extending the range of modifications for industrial applications for sectors including the paper industry, oil exploration and biodegradable packaging. The absence of organic solvents in the procedure overcomes major exploitation barriers, e.g., economic, environmental, use for food contact applications.

This work forms the basis of two manuscripts for publication in the international journal *Biomacromolecules* describing (1) the solubilisation and (2) the modification of a range of starches under aqueous conditions. Oral presentations of some of the work will be made on 11 July at *SusCompNet* (The Network for Sustainable Composites) at University of Wales, Swansea and 15–19 July at the 6th International Hydrocolloids Conference, Guelph, Canada.

Scientific report (maximum 20 sides A4)

1. INTRODUCTION

1.1 Starch

Starch is a typical homopolysaccharide, it is one of the major polysaccharides used for energy storage and is widely distributed in seeds, roots and tubers as well as in stems, leaves, fruits and even pollen (Perez and Imberty, 1996). The unique chemical and physical characteristics and nutritional quality of starch sets it apart from all other carbohydrates. Starch can also be used in many ways other than as foodstuff, such as in glues, coatings, sizing and flocculating agents, and building materials (Guilbot and Mercier, 1985).

Pure starch is a white, odourless, tasteless, neutral powder existing, in fact, as granules that are insoluble in cold water or organic solvents (Radley, 1953). The shape and size of the granules depend on their origin. Starch granules vary in size from about 2 μm to 150 μm (Kerr, 1950). While it has long been known that the larger granules of any particular type of starch, such as corn, gelatinise more easily than the smaller granules, it has recently been indicated that there may be some correlation between the dispersibility of a type of starch and its average granule size (Whistler and BeMiller, 1997). Starch granules are composed of a mixture of two polymers (Ahmad *et al*., 1999), namely amylose and amylopectin. The amylose content can vary over a broad range, from 0 to about 75 %, but typically 20–25 % (w/w) (Orford *et al*., 1987; Parker and Ring, 1996). The ratio of the two component polysaccharides influences the physical properties of a starch type such as viscosity, solubility, gel formation, gelatinisation temperature (Greets, 1996) and brittleness of formed films.

1.2. Amylose

Amylose is an essentially linear polysaccharide, the D-glucose units of amylose are linked by α-D-(1→4) glycosidic bonds (Scheme 1).

The α-D-(1→4) bond gives rise to a helical conformations. The polysaccharide molecules on slow cooling, gradually align and intermolecular hydrgen bonding occurs. At low amylose concentrations this results in the growth of bundles of molecules, which eventually form visible aggregates and become insoluble particles (Foster, 1965; Langlois and Wagoner, 1967). Rapid cooling of a concentrated amylose solution may cause the formation of a gel by hydrogen bonding of the type shown in Scheme 2. Again, this phenomenon is complicated; potato amylose may not form a gel under the conditions that corn (maize) amylose does (Greenwood, 1970).

1.3. Amylopectin

Amylopectin is usually the major component of the starch granule and is insoluble in cold water due to the hydrogen bonding of polymer chains. On heating, the granules gradually swell and absorb water as hydrogen bonds are broken (Nisperos-Carriedo, 1994). Amylopectin consists of chains of D-glucopyranose residues linked together mainly by α-D-(1→4) linkages, but with 4–5 % of α-D-(1→6) bonds at the branch points (Scheme 3).

It is assumed that the reactivity along the linear amylose chain remains constant, while the highly branched structure of amylopectin results in hindered access to its inner regions and hence lower reactivity. Neutral aqueous solutions of amylopectin are extremely stable (Foster, 1965), and there is little tendency for the molecules to retrograde, although this may occur to a limited extent at low temperatures.

1.4. Analytical characterisation of native starch

Considerable efforts have been made over the last thirty years or so to determine the molecular mass distribution of starches using gel permeation chromatography (GPC). Workers in the field have adopted various strategies and protocols in order to dissolve the starches and perform the GPC experiments. For example, Takeda, et al. performed studies on a variety of starch types (Takeda et al., 1976; 1984; 1992). In studies on maize starch (Takeda et al., 1976) starch was dissolved in perchloric acid and after two hours the starch was clearly dissolved and was then diluted with dilute sodium hydroxide solution. Separation was achieved on a Sepharose 2B[2]

column using water as eluent. No degradation of amylopectin was reported. The gel chromatography traces for amylose and amylopectin varied for normal starch and amylopectin, suggesting differences may be not only quantitative but also qualitative in both components. In further studies by Takeda et al. (1984), various starches were fractionated to separate the amylose and amylopectin components by the method of Lansky et al. (1949), with the minor modifications suggested by Hizuri et al. (1981). The samples were purified before solubilisation for use on the column by recrystallisation 3–6 times from hot, aqueous 1-butanol cooled in an atmosphere of nitrogen. Amylose was re-precipitated by addition of 1-butanol and recrystallisation twice. The amylose was then dissolved (Lansky et al., 1949) and eluted with sodium chloride solution on a Toyopearl column. The molecular mass values obtained for wheat starch were lower than had been previously determined (Arbuckle et al., 1958). The degree of polymerisation of kuzu tapioca and potato amylose were calculated by aqueous dispersion of starch granules. In a further study, Takeda et al. (1992) investigated the structure of amylose subfractions with different molecular sizes by using amylose isolated from defatted (Takeda et al., 1986) maize starch and rice starch. Three subfractions were obtained and each was lyophilised. The weight average molecular mass was determined using TSK-gel G6000PW, G4000PW and G3000PW (Tosoh) columns in series in conjunction with a differential refractometer and a low-angle light scattering photometer. Values were in agreement with previous data.

van Dijk et al. (1976) used GPC to determine the molecular weight distribution of amylose with dimethylsulfoxide (DMSO) as the mobile phase. The number average molecular weight (Mn) was estimated to be 111,000 although the accuracy of the method was questioned since some of the amylose had precipitated out of solution. Biliaderis et al. (1979) determined the molecular mass distributions of a broad range of legume starches. Semi purified starches were prepared from seeds by a wet milling process. The coarse slurry produced was then defibered and the starch was removed and partially purified by centrifugation. Further purification was carried out by passing the starch slurry through a counter-current washing unit. The starches were then dried in a spray drier. Final purification of the starch was achieved by repeated washing with 95 % ethanol and screening. Starch solutions were prepared by dispersion in perchloric acid. Dilute sodium hydroxide solution was added and the volume was made up to 50 ml with distilled water. Aliquots were applied to the column and eluted with water. The molecular mass distribution was determined using a Sepharose 2B column.

Hizukuri and Takagi (1984) determined the molecular mass distribution of amylose obtained from a variety of sources including, potato, sweet potato and tapioca. The samples were characterised by GPC using low-angle laser-light-scattering detection, eluted with sodium phosphate buffer (pH 6.1) containing 0.02 % of sodium azide.

A number of workers have used DMSO as a solvent for starch. Salamis et al. (1984) initially defatted starch samples by stirring in DMSO followed by precipitation by addition of methanol. The samples were then dissolved in DMSO containing methanol and ammonium acetate. From the results it was concluded that the use of DMSO/methanol/ammonium acetate as the eluting solvent avoided absorption of the starch molecules onto the gel and allowed direct dissolution of the polymers.

Mei et al. (1994) determined the molecular mass distribution of several starches including wheat, potato, waxy rice, cassava and sweet potato by GPC coupled to multiangle laser light scattering and refractive index detection. In order to study the fine structure of the amylopectin components, samples were treated with α-amylase. The concentration of the debranched samples was adjusted using sodium phosphate buffer. The solutions were filtered through a syringe filter and injected onto the column. The samples were fractionated using 5 columns connected in series. The column eluent was sodium hydrogen phosphate (0.1 M), sodium dihydrogen phosphate (0.05 M) and sodium azide (0.02 %) at pH 8.6. The elution profiles indicated the polymodal chain-length distributions of the amylopectin components supporting the results reported by Hizukuri (1986). The wheat and waxy rice amylopectins showed at least four distinct peaks with shoulders along the peaks. The potato, cassava and sweet potato profiles displayed broader peaks.

Chen et. al. (1997) also purified starch samples by dissolution in DMSO before undertaking GPC measurements. Separation was achieved using grafted silica columns with potassium hydroxide as eluent and it was claimed that this combination allowed the separation of molecules with a wide range of molecular mass. Elution of the starch with dilute potassium hydroxide solution from the

columns was monitored using light scattering and differential refractometer detectors. Various solvents had been investigated to dissolve the starch. Using aqueous DMSO, it was found that the solubility decreased as the amylopectin content increased. The solubility was found to depend on the DMSO content of the solvent, and also the polysaccharide concentration. Maximum solubility was obtained at the lowest polysaccharide concentration. They concluded that water/DMSO mixtures were not good solvents for starch and that amylopectin has low solubility even in very dilute solutions. Solubility was improved by using 0.1 M potassium hydroxide but it was concluded that complete solubilisation of amylopectin was not achieved and that it is impossible to quantitatively characterise starch samples by aqueous GPC.

Shi et al. (2000) performed a study on the effect of sulfate and citrate salts on the hydroxypropylation of amylose and amylopectin from corn starch and analysed the products by fractionating using a Sepharose CL-2B column. The samples were initially destructured by treatment with DMSO. The starch was precipitated by addition of ethanol. The solution was then centrifuged and washed with ethanol. The starch was re-dissolved in water by heating. It was noted that derivatisation was enhanced to a greater extent in the presence of citrate compared to sulfate.

The use of DMSO to destructure starch prior to dissolving in order to undertake GPC measurements has also been reported by Bello-Perez et. al. (1998). These workers dissolved various starch samples in 95% DMSO. The samples were then precipitated. The precipitates were filtered, washed and dried and the purified samples were prepared for GPC by dissolving in water in a bomb in a microwave oven. Initial experiments had established that pullulan and glycogen could be heated for up to 50 sec without degradation occurring.

Thus, a number of potential routes are available to treat starch in order to determine the molecular mass distribution. Pre-treatment, by dissolution in DMSO, offers a means of destroying the crystallinity of the granules and hence will aid solubility in aqueous solvents. Even so the ‘destructured’ starch still needs to undergo heating in order to disrupt any aggregates and fully dissolve in aqueous media.

1.5. Solubilisation of starch for preparative modification

Native starch properties can be changed in a directed way by physical or chemical modification. This work is concerned with chemical modification. A number of routes to starch modification have been described based on the use of both organic and aqueous solvents.

1.5.1. Organic solvent based methods

1.5.1.1. Preparation of starch esters in organic solvents

Dry, unmodified starch may be heated together with a derivatising agent at temperatures up to 150 °C, to produce low to intermediate DS-value derivatives of starch with its granule structure still intact. With increasing degrees of substitution, the starch granule often swells in the reaction medium, and may become completely soluble. If a non-swelling solvent is used as the reaction medium, substitution does not reach high levels unless the starch is activated first by some technique which destroys the granule structure. Consequently, most high DS-value starch derivatives are in gelatinised form. A number of research workers have used a solvent as swelling agent to prepare high DS-value derivatives, the high DS-value starch derivatives are often thermoplastic. Thiebaud et al. (1997) prepared starch esters (DS-value, 2.7) using fatty acids chlorides in pyridine at 115 °C for 3 h. In the same year, Tanaka (1997) described the use of DMSO as a reaction solvent to prepare starch esters. In 1999, Marcazzan et al. reported that the succinylation of starch was carried out in a solution containing anhydrous pyridine and DMSO.

1.5.1.2. A water/solvent exchange method for starch modification

A novel, hybrid route using both aqueous and organic solvents was developed in our laboratory (Hylnianski, 1997). Starch was gelatinised in hot water to fracture its granules completely by swelling, thus activating the component amylose and amylopectin. The addition of the polar-aprotic solvent, *N,N*-dimethylacetamide (DMA), was made at such a rate as to avoid deactivation of the starch by either precipitation or aggregation. The aqueous solvent was then preferentially removed by distillation and at the same time replaced

by DMA. Eventually, all the water was replaced to give an anhydrous dispersion of gelatinised starch in DMA. An electrolyte was added to the dispersion to disrupt any remaining molecular interactions (e.g., hydrogen bonding) between adjacent intermolecularly-associated chains of either amylose and/or amylopectin and thereby allow the formation of a homogeneous solution.

1.5.2. Aqueous based methods

The above methods demonstrate the successful use of organic solvents to disrupt starch granules to afford chemically modified materials, but they are flawed on both economic and environmental grounds. The processing costs are high and the use of toxic solvents is at odds with the production of environmentally benign polymers.

In order to achieve successful aqueous chemical modification of starch, it was necessary to firstly develop a successful aqueous solubilisation technique. Much existing work is reported in the literature.

1.5.2.1. Prior art

Aqueous chemical modification of starch to afford ethers of starch was reported by Gomberg and Buchler (1921) who treated different starches with benzyl chloride in the presence of sodium hydroxide. It was observed that the colloidal and paste properties of these derivatives might warrant their use as industrial products. In 1934 and 1935, Maksorov and Andrianov repeated the work with a number of starches reporting that the properties of the modified material varied with the source of starch.

Chowdhury (1924) treated starch with chloroacetic acid in the presence of sodium hydroxide and obtained a derivative which contained 2 hydroxy acid residues per glucose unit. This product was then methylated with methyl sulfate to give a mixed ether-ester. In the same year, Gault prepared and described the diesters of starch (Gault and Ehrmann, 1924).

Acetyl derivatives were prepared by treating alkali-starch with acetic acid vapours, or by pre-treatment of the starch with ammonia gas, formaldehyde, pyridine, or steam and subsequent treatment with acetic acid vapours, according to a patent issued in 1927 (Farbenindustrie, 1927).

Hughes et al. (1932) reported the ready preparation of acetyl derivatives by pre-treatment of starch with boiling water and subsequent drying with ethanol, followed by addition of acetic anhydride and a trace of sulfuric acid.

A benzoyl-lauroyl ester of starch was prepared by mixing starch with sodium hydroxide solution, refluxing the mixture and adding dropwise a mixture of benzoyl and lauroyl chlorides (Hagedorn et al., 1935).

Ziese (1934, 1935) treated starch with ethylene oxide in alkaline solution and obtained the corresponding hydroxy derivatives of starch.

The ethyl ether of starch has been prepared from ethyl sulfate in the presence of alkali. The resulting product is soluble in organic solvents but insoluble in water (Haworth et al., 1937).

The patent literature discloses the propyl ether of starch (Young, 1921), which has been synthesised by heating together corn starch, water, a solution of NaOH, and *n*-propyl chloride (Degering, 1934; Degering and Rankin, 1945). The reaction mixture was subjected to steam distillation to remove the lower alcohols and lower ethers (Degering and Rankin, 1945).

Starch hydroxy derivatives have been prepared by the reaction of starch with alkene oxides, particularly ethylene oxide, in the presence of aqueous alkali (Farbenindustrie, 1929; Lolkema, 1944).

The monoallyl ethers of both corn and potato starches were prepared by treatment of the respective starches with allyl bromide in the presence of aqueous alkali (Tomecko and Adams, 1923).

Hamilton and Yanovsky (1946) reported a method for preparing the higher alkyl ethers of starch. Thus, starch ethers (DS-value 0.5–2.8) have been prepared from diethyl sulfate, ethyl chloride and iodide, propyl bromide, butyl chloride and iodide, amyl chloride and bromide, and hexyl, heptyl and dodecyl iodides.

MacGregor (1951) reported that the alkali-catalysed reactions of starch with acrylonitrile proceeded rapidly to form 2-cyanoethyl ethers. The reactions were conducted in an aqueous alkaline starch paste from which cyanoethyl-starches precipitated in the DS-value range 1.5–2.5.

Husemana and Kafka (1960) reported the carboxymethylation of high-amylose corn starch in aqueous alkali; under these conditions, the secondary hydroxyl groups reacted preferentially.

Rutenberg et al. (1961) prepared low DS-value *O*-ethylamylose by treating a solution of amylose in 5 % sodium hydroxide solution with ethyl bromide at room temperature.

Hess and Lung (1937, 1938) showed that a single treatment of native starch with methyl bromide gave a DS-value of 2.7–2.8 with little degradation if a 40 % solution of sodium hydroxide was used as solvent and oxygen was excluded from the system.

Amylose was cyanoethylated in aqueous alkaline solution to give water soluble material in the DS-value range of 0.02–0.15. Retrograded amylose was cyanoethylated in suspension in water in the same manner as granular starch. Products in the DS-value range of 0.19–0.88 were insoluble in cold water, but dissolved in water at 125–200 °C (Fisher and Harper, 1962).

Jantas (1997) reacted an aqueous alkaline amylose solution in a two-phase system containing methyl ethyl ketone (MEK) with acryloyl chloride. Reaction was carried out for some scores of minutes with intensive stirring and then the reaction mixture was left to separate. The upper organic layer (esterification product, toluene, MEK) was separated from the aqueous lower one in which remained the unreacted amylose.

Dumoulin et al. (1998) prepared a high amylose starch cross-linked with epichlorohydrin.

In the same year, Bayazeed et al. (1998) treated maize starch with methyl methacrylate in the presence of sodium hydroxide and water. They reported that the extent of esterification increased as the amount of sodium hydroxide increased.

The following recent reactions although not performed on starch indicate the ability of aqueous solubilisation conditions to effect substitution reactions, particularly by esterification.

Descotes et al. (1999) reported the esterification of sucrose with octanoyl chloride at pH 10 in aqueous solution, using different temperatures and a range of organic bases. The reaction of sucrose with octanoyl chloride in alkaline aqueous medium was also reported by Thevenet et al. (1999).

In summary, the preceding discussion highlights the aqueous modification of intact starch granules to low degrees of substitution. Indeed, almost all the starch derivatives manufactured today maintain the granular form of the parent starch.

2. MATERIALS AND METHODS

2.1. Starches

Four starches were acquired for the study, three of corn (maize) origin and one of wheat. Hylon VII (70 % amylose:30 % amylopectin); Hylon V (50 % amylose:50% amylopectin) and Amioca (99 % amylopectin) were from corn and were supplied by National Starch and Chemical. Abrastarch was a commercial wheat starch, provided by ABR Foods Ltd.

2.2. Reagents

Acetyl, butyryl, hexanoyl, heptanoyl, octanoyl and nonanoyl chlorides were obtained from Aldrich. Decanoyl chloride was obtained from Lancaster Synthesis Ltd. Lauroyl and palmitoyl chlorides were purchased from Acros Organics. Stearoyl chloride was obtained from Fluka. All reagents were used without further purification.

2.3. Catalysts

4-Dimethylaminopyridine (DMAP), triethylamine (TEA), pyridine and *N,N*-diisopropylethylamine (DIEA) were purchased from Aldrich.

2.4. Determination of gelatinisation temperatures

Gelatinisation temperatures were determined by differential scanning calorimetry (DSC) using a Micro-DSCII batch and flow calorimeter manufactured by Setaram (Lyon, France). Approximately 0.1 g of starch was weighed accurately into the sample cell and an accurately known weight of water or 1 M potassium thiocycanate (~1.0 g) added. An exactly equivalent weight of solvent was added to the reference cell. The cells were placed in the calorimeter and heated from ambient temperature to 90 °C at a scan rate of 0.5 °C/min.

2.5. GPC determination of native starches

2.5.1. Pre-treatment of native starches

Prior to chromatographic analysis, samples were pre-treated using the method described by Bello-Perez et al. (1998). Starch (1 g) was dissolved in 95 % DMSO (20 ml) with magnetic stirring for three days at room temperature. The sample was then precipitated with pure ethanol and stored overnight at 4 °C. The precipitate was filtered through a G4 glass filter and washed successively with acetone (100 ml) and diethyl ether (10 ml). The sample was then air-dried for 48 h. The weight of the material recovered was determined and the yield calculated (Table 1).

2.5.2. Dissolution in a microwave bomb

Starch solubilisation was achieved by adding distilled water (20 ml) or 1 M KSCN to starch (0.1 g). The sample was then transferred to the teflon cup of a model 4782 polycarbonate microwave bomb (total volume 45 ml) (Parr Instrument Co., Moline, IL, USA). The bomb was placed in a microwave oven (800 W) and heated on full power for varying times up to 90 sec.

2.5.3. Calibration standards

For the calibration, Dextran T500 and Hydroxyethylcellulose (HEC) were used. Dextran T500 was purchased from Pharmacia and had a nominal average molecular mass of 500,000. HEC was a gift from C Kelco Ltd Denmark and had a reported molecular mass of 250,000 and molar substitution of 2.5 respectively. These two polysaccharides were used to establish the optimum temperature and solvent conditions to completely solubilise the starches.

2.5.4. Conditions for measurement of starch

GPC was performed using a linear 10 μm HEMA Bio Column (600 × 8 mm). The output of the column was connected to a Wyatt Dawn (DSP) multiangle laser light scattering (MALLS) instrument (Optilab, Flintshire) in conjunction with the Optilab refractive index (RI) detector. RI provides an accurate concentration profile for the eluting species, and MALLS enables their absolute molecular mass and radius of gyration (R_g) to be determined, thus avoiding the necessity to calibrate the columns.

Starch measurements were recorded under the following conditions: 1 or 2 ml injection loop, 0.5 mg/ml solution (all solutions were filtered using 3.0 μm cellulose acetate filters before injection). 0.1 M KSCN was used as eluent and the flow rate was set at 0.5 ml/min, regulated using a Waters 515 pump. All measurements were made at room temperature. The dextran and HEC standard samples were dissolved in 1 M KSCN, with a 500 μl injection loop, and 2.5 mg/ml solution being used for GPC experiments.

2.6. Treatment of starch with sodium hydroxide

The series of starches was treated with different alkaline concentrations (Table 2) as follows: 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 M NaOH. In all the experiments, 1 mole equivalent of alkali to each starch hydroxyl group was maintained, i.e. 3 mole equivalent alkali to each 1 mole equivalent anhydroglucose unit. The series of experimental procedures is described more fully below.

Experiment series 1

In this experiment, Hylon VII or Abrastarch (1.08 g) was added to 0.05 M NaOH solution (400 ml). The dispersion was stirred magnetically at room temperature, under an atmosphere of N_2 for 16 h. After this time, the acidity of the mixture was adjusted to pH 7.0 with glacial acetic acid. Solid material was isolated by precipitation in 3 volumes of methanol, then collected by filtration. The solid residue was washed with 70 % methanol. This washing procedure was repeated twice to eliminate salt impurities. Residual methanol was removed from the residue by evaporation in air, and the products were dried at 50 °C overnight, then weighed. The dried samples were stored in a desiccator over phosphorus pentoxide for further analysis (Sample Nos 1–2, Table 2).

Experiment series 2

Hylon VII, Hylon V, Abrastarch or Amioca (1.62 g) was added to 0.1 M NaOH solution (300 ml). Further processing was carried out as described for Experiment series 1 (Sample Nos 3–6, Table 2).

Experiment series 3

Hylon VII, Hylon V, Abrastarch or Amioca (2.7 g) was added to 0.5 M NaOH solution (100 ml). Further processing was carried out as described for Experiment series 1 (Sample Nos 7–10, Table 2).

Experiment series 4

Hylon VII, Hylon V, Abrastarch or Amioca (5.4 g) was added to 1.0 M NaOH solution (100 ml). Further processing was carried out as described for Experiment series 1 (Sample Nos 11–14, Table 2).

Experiment series 5

Hylon VII, Hylon V, Abrastarch or Amioca (1.62 g) was added to 1.5 M NaOH solution (20 ml). Further processing was carried out as described for Experiment series 1 (Sample Nos 15–18, Table 2).

Experiment series 6

Hylon VII, Hylon V, Abrastarch or Amioca (5.4 g) was added to 2.0 M NaOH solution (50 ml). Further processing was carried out as described for Experiment series 1 (Sample Nos 19–22, Table 2).

Experiment series 7

Hylon VII, Hylon V, Abrastarch or Amioca (6.75 g) was added to 2.5 M NaOH solution (50 ml). Further processing was carried out as described for Experiment series 1 (Sample Nos 23–26, Table 2).

Experiment series 8

Hylon VII, Hylon V, Abrastarch or Amioca (8.1 g) was added to 3.0 M NaOH solution (50 ml). The resulting paste was stirred mechanically under an atmosphere of N_2 for 16 h. Further processing was carried out as described for Experiment series 1 (Sample Nos 27–30, Table 2).

Experiment series 9

Hylon VII, Hylon V, Abrastarch or Amioca (10.8 g) was added to 4.0 M NaOH solution (50 ml). Further processing was carried out as described for Experiment series 8 above (Sample Nos 31–34, Table 2).

Experiment series 10

In this experiment, 0.54 g, 0.81 g and 1.08 g of Abrastarch were treated with 1.0, 1.5 and 2.0 M NaOH (10 ml), respectively. The resulting dispersions were stirred under an atmosphere of N_2 for 88 h. Further processing was carried out as described for Experiment series 1 (Sample Nos 35–37, Table 2).

Experiment series 11

Hylon VII and Hylon V (1.62 g) were treated with 3.0 M NaOH (10 ml) under an atmosphere of N_2 for 72 h. Further processing was carried out as described for Experiment series 1 (Sample Nos 38–39, Table 2).

Experiment series 12

Hylon V (13.51 g) was added to 5.0 M NaOH (50 ml), The resulting thick paste was stirred mechanically under an atmosphere of N_2 for 40 h. Further processing was carried out as described for Experiment series 1 (Sample No 40, Table 2).

2.7. Synthesis of acylated starches

General method 1—Modification in 2.5 M sodium hydroxide solution

Starch (6.75 g) was added to NaOH solution (50 ml, 2.5 M) at room temperature with mechanical stirring under an atmosphere of N_2, until the starch granules gelatinised fully (30 min). The reaction catalyst (Table 3) was added (or not) and, after 5 min, 0.5 mole equivalents of the required acid chloride was then added to the solution, and the reaction mixture was stirred for 1 h. Upon completion of the reaction, the mixture was neutralised to pH 7 with acetic acid, and the acylated starch was isolated by precipitation in methanol. The product was collected by filtration, re-dissolved in 70 % aqueous solution of methanol, then reprecipitated. This process was repeated twice to eliminate impurities, such as fatty acid and salts. After filtration, residual methanol was removed by evaporation in air, and the starch ester was dried at 50 °C overnight and weighed. The dried samples were kept in a desiccator over phosphorus pentoxide for further analysis.

In the second iteration of this experiment, recovered yields, FT-IR data and calculated DS-values are reported in Table 4.

General method 2—Modification in buffered solutions

(a) Reaction in sodium 4-phenolsulfonate/sodium hydroxide buffer (pH 10)

Starch (Hylon VII, 1.77 g) was stirred in NaOH solution (32.7 ml, 1 M) at room temperature, under a atmosphere of N_2. The mixture was held under these conditions for 30 min, then sodium 4-phenolsulfonate solution (34.7 ml, 1 M) was added to maintain pH 10. The requisite catalyst and acid chloride were subsequently added (Table 5) to the solution and allowed to react for 1h. The reaction mixture was then treated and the reaction product isolated according to Method 1 above.

(b) Reaction in sodium borate/sodium hydroxide buffer

Starch (Hylon VII, 1.73 g) was stirred in NaOH solution (32 ml, 1 M) at room temperature, under an atmosphere of N_2 for 30 min, then sodium borate solution (100 ml, 0.5 M) was added to maintain the acidity at pH 10. The requisite amounts of catalyst and acid chloride were subsequently added (Table 5) to the solution and the reaction was allowed to proceed for 1 h. Subsequent treatment and isolation of the reaction product was performed according to Method 1 above.

(c) Reaction in di-sodium hydrogen orthophosphate/sodium hydroxide buffer

Starch (Hylon VII, 1.45 g) was stirred in NaOH solution (26.9 ml, 1 M) at room temperature, under an atmosphere of N_2 for 30 min, then Na_2HPO_4 solution (50 ml, 0.5 M) was added to adjust the acidity to pH 12. The requisite quantities of catalyst and acid chloride were subsequently added to the solution (Table 5) and reaction was allowed to continue for 1h. Subsequent work-up and isolation of the reaction product was carried out according to Method 1 above.

(d) Reaction in sodium hydrogen carbonate/sodium hydroxide buffer

Starch (Hylon VII, 1.73 g) was stirred in NaOH solution (32 ml, 1 M) at room temperature, under an atmosphere of N_2 for 30 min, then $NaHCO_3$ (100 ml, 0.5 M) was added to adjust the acidity to pH 10.5. The required amounts of catalyst and acid chloride were subsequently added (Table 5) to the solution and the reaction was allowed to continue for another 1h. Subsequent treatment and product isolation was carried out as described in Method 1.

General method 3—Modification in a two-phase organic solvent/aqueous system

The following is a modification of a method proposed by Jantas (1997). Starch (Hylon VII, 1.0 g) was added to water (40 ml) at room temperature under an atmosphere of N_2. NaOH solution (20 ml, 3 M) was added and the mixture became clear. Methyl ethyl ketone (MEK) (20 ml) was added, and then the required amounts of acyl chloride (Table 6), MEK (20 ml) and toluene (4 ml) were added dropwise. The reaction was allowed to continue for 1 h with intensive stirring and then the system was left to separate into two distinct phases. The upper organic phase was separated from the aqueous lower one (in which remained any unreacted starch). The organic layer was concentrated under reduced pressure, and the product was dried at room temperature under reduced pressure.

2.8. Characterisation

Elemental analysis was used to establish the DS-values of modified starches. Samples of solid polymer were ground to a powder and dried at 100 °C for 24 h, then analysed on a Carlo Erba EA 1108 CHN S-O instrument to measure the carbon and hydrogen content in the substituted starch samples. The DS-values were calculated from the % carbon contents.

For Fourier transfer infrared (FT-IR) spectroscopic analysis, the modified sample was ground using a Mikro-Dismembrator (2000 rpm for 3 min). The fine powder sample was then mixed with dry potassium bromide (KBr) in a sample to KBr ratio of 1:100, and mixing was performed in a vibratory ball mill capsule for 5 min. The ground mixture was then transferred to a Specadie to produce a 8.5 mm diameter film that was analysed in the beam of the FT-IR spectrometer (Nicolet Magna IR 750, series II).

GPC was used to measure the molecular mass distribution of treated starches. The procedure is described in Section 2.5.4. The measurement conditions are as follows: treated starches were first dissolved in water (0.1 g/20ml) at a microbomb and heating for 60 sec. All measurements were carried out at room temperature using a HEMA Bio Column with mobile phase 0.1 M KSCN and flow rate 0.5 mg/ml.

The solubility of the products was measured at 5 % concentration in different organic solvents with stirring at room temperature.

The intrinsic viscosity of the native and acylated starches was determined in DMSO. Starch (0.25 g) was dissolved in DMSO (5 ml) with heating and stirring and flow times measured in triplicate at 25 °C using a Ubbelohde type 75 viscometer at varying concentration by diluting in situ. The intrinsic viscosities were obtained from the intercept of the plot of reduced viscosity against concentration.

3. RESULTS AND DISCUSSION

3.1. Characterisation of native starches

3.1.1. Gelatinisation temperatures for starches using DSC

Starch granules are insoluble in water but on heating they swell. The surface of the granule develops a translucent envelope, which at a critical stress point ruptures and collapses, releasing amylose molecules into solution. The remaining surface structure degrades to a web-like network. The actual temperature at which the granules burst is very characteristic of the source of the starch and is referred to as the gelatinisation temperature, which can be conveniently followed using differential scanning calorimetry, where an endothermic peak is observed on heating. The DSC heating curves for the four native starch samples dispersed in water are presented in Figures 1 to 4. Abrastarch gave rise to a single endothermic peak and the temperature at the peak maximum T_{max} was 58 °C. The peak is due to starch gelatinisation and the value obtained is typical for wheat starches (53–65 °C). For Amioca starch an endothermic peak was observed with T_{max} at 65 °C and there was a shoulder on the high temperature side. For the high amylose starches, Hylon V and Hylon VII, there was no obvious peak observed on heating to 90 °C, which indicated that gelatinisation probably occurred at higher temperatures.

The DSC heating curves for the starches heated in the presence of 1 M KSCN are given in Figures 5 to 8. For Abrastarch, the peak is shifted to a lower temperature compared to measurement in water (T_{max} ~40 °C), and is of much lower intensity indicating that the enthalpy of the process is significantly less. This supports previous work by Ahmad and Williams (2000) which demonstrated that KSCN was a good solvent for starches. The corresponding peak for Amioca starch (Figure 6) shows a similar trend in that the peak shifts to lower temperatures and is reduced in intensity. No obvious peaks were obtained for the high amylose starches.

The DSC heating curve for a sample of Abrastarch purified in DMSO is given in Figure 9. The fact that the endothermic peak is no longer present indicates that the crystalline structure of the starch has been destroyed by the DMSO treatment.

3.1.2. Determination of molecular mass distribution

During the course of this project various heating protocols adopted by other workers were followed, including heating in glass vials in a microwave oven and in sealed glass tubes in an autoclave. Inconsistent results were obtained and the procedure of Bello-Perez et al. (1998) was adopted in which the samples were heated in a bomb in a microwave oven. The molecular mass distribution values of treated and untreated starches are quoted in Table 7, and the radius of gyration are shown in Table 8.

However, the purification of the starches using DMSO is laborious and inevitably some of the sample is lost. The present study, therefore, has also involved investigating an alternative aqueous solvent so that this pre-treatment stage could be eliminated. The solvent chosen was 1M KSCN. This has previously been shown to be a good solvent for sago starch (Ahmad and Williams, 2000).

3.1.2.1. Optimisation of heating time for starch dissolution

The elution profiles obtained by GPC for 0.5 % dextran and HEC solutions heated in the microwave bomb placed in a microwave for varying periods of time are given in Figures 10 and 11. It can be clearly seen that for both samples the molecular mass is unaffected on heating up to 75 sec, but on heating for 90 sec some depolymerisation became apparent. The results indicate, therefore, that it should be possible to dissolve destructured starch by heating for 75 sec without inducing depolymerisation [see Section 3.1.3].

3.1.2.2. GPC of starches purified by pre-treatment with DMSO

The elution profiles obtained by GPC for the four native starches, which had been purified and destructured by dissolving in DMSO, are given in Figure 12. The samples were dissolved in water by heating in the microwave bomb for 75 sec prior to injection onto the

GPC column. The high amylose starches, namely Hylon V and Hylon VII showed two distinct peaks. The peak at low elution volumes (~10 ml) corresponded to the amylopectin fraction, while the broader peak at higher elution volumes (~14 ml) was due to amylose. The other two starches showed essentially one peak but with a broad shoulder on the low molecular mass side. Similar profiles have been reported by other workers e.g., You and Lim (2000) in their studies on corn starch.

Amylopectin has a very high molecular mass and since it elutes at elution volumes close to the void volume for all the samples it is possible, and perhaps likely, that it is not completely fractionated on the column.

3.1.3. Evaluation of 1 M potassium thiocyanate as a solvent for starch

DMSO-treated starches were dissolved in water by microwaving in the microwave bomb for 75 sec and the non-DMSO treated starches were dispersed in 1 M KSCN and subjected to the same heat treatment. The GPC elution profiles obtained for the four starch smples are presented in Figures 13 to 16. Figures 13 and 14 show the elution profiles obtained for Abrastarch and Amioca starch. It is evident for the samples prepared in the presence of 1 M KSCN that there is a peak present at an elution volume of ~14 ml which is not present in the DMSO-purified samples prepared in water suggesting that some depolymerisation may have occurred. Interestingly, there is no peak at an elution volume of 22 ml as for the samples prepared in water. For the high amylose starches, Hylon V and HylonVII in Figures 15 and 16, a peak appears at an elution volume of ~18 ml again suggesting that some depolymerisation may have occurred.

Confirmation that degradation does occur during heating for 75 sec using 1 M KSCN as solvent was obtained by heating a DMSO purified sample of Abrastarch in both water and 1 M KSCN. The elution profiles are given in Figure 17 and clearly show that there is a peak at ~14 ml for the sample heated in the presence of 1 M KSCN which was not present for the sample heated in water.

Studies were undertaken to ascertain the optimum heating time in 1 M KSCN using amylose and detxtran. It was found that the samples could be heated for 60 sec without depolymerisation but that degradation occurred on heating for 75 sec [data not shown].

3.2. Starch treatment with alkali

Starch contains an abundance of hydroxyl groups. Each anhydro-glucose unit contains two secondary hydroxyl groups and the vast majority also contain primary hydroxyl groups. The hydroxyl groups are able to potentially react with any chemical capable of reacting with alcohols (Nisperos-Carriedo, 1994). In order to overcome the poor reactivity of native starch, a number of techniques have been devised for disrupting the intermolecular association present in the granule. A common method involves heating the starch in water, mechanically disintegrating the greatly swollen granules for improved reactivity. For some reactions, starch is activated by strong bases. Although starch granules can be completely gelatinised in aqueous alkali, the degree of granule swelling depends upon the nature of the starch, the nature of the alkali, the relative amounts of starch, alkali, and water, the temperature, and the presence of absence of neutral salts (Hollo et al., 1959; Hollo et al., 1960). With corn starch, it is possible to heat slurries containing 0.3 mg of sodium hydroxide per gram of starch to 45 °C without loss of granule birefringence. In aqueous alkaline slurries, most of the alkali is absorbed by the starch granules (Leach et al., 1961). The reactivity of the resulting alkali-starch complex is sufficiently enhanced to enable the starch to compete successfully with water in reactions with compounds subject to nucleophilic attack. The increased reactivity due to alkali absorption is a major factor in the manufacture of the starch derivatives of commerce (Roberts, 1965). A further increase in the reactivity of starch in aqueous alkali is obtained by the addition of neutral salts, especially sodium sulfate. These salts shift the starch-alkali absorption equilibrium such that alkali absorption is increased, probably by decreasing the effective water concentration through solvation. An additional effect of the presence of these salts in alkaline slurries is a decrease in the tendency of the starch granules to swell at higher alkali concentrations or to gelatinise as they become increasingly disorganised by the introduction of substituent chemical groups. Again the mechanism probably involves hydration of the salt, so that insufficient free water is available for gelatinisation. In this work, granule disruption and gelatinisation was desired to fully disrupt any molecular interactions and thus render individual starch molecules active for subsequent homogeneous. The commercial route amply evidences the fact that on the intact non-disrupted granule, any chemical modification occurs solely on the granule surface.

In order to prevent the starch oxidation by sodium hydroxide, a range of experiments was carried out under an atmosphere of N_2 at room temperature (Table 2). Samples of starch were stirred in known concentration of sodium hydroxide solution such that the number of moles of sodium hydroxide equalled (more or less) the number of moles of starch hydroxyl groups. Evidence was sought for starch solubilisation by observation of the solutions. After 16 h, the starch solutions were neutralised and the starches reprecipitated by treatment with methanol. When the alkali concentration was low (Experiments 1 and 2, Table 2) using 0.05 M or 0.1 M NaOH, the resulting mixture was opaque, evidencing that low alkali concentration was insufficient to disrupt the granule and amylose/amylopectin intermolecular hydrogen bonds. As the alkali concentration increased from 0.5 to 1.5 M NaOH, all the mixtures obtained for Hylon VII, Hylon V, Abrastarch and Amioca exhibited obvious transparency. The NaOH in solution formed complexes with starch thus resulting in cleavage of adjacent bonds and separation of the starch chains. Upon increasing the sodium hydroxide concentration to 2.0–3.0 M (Table 2) dispersions rapidly formed clear gels, which indicated that more NaOH penetrated into the starch chains, disrupted granular crystallinity, and caused disintegration of H bonds, resulting in fully destructured starch granules. From our observations, the sequence of starch gelatinisation (first to last) was Abrastarch > Amioca > HylonV > HylonVII. In Abrastarch (wheat starch) and Amioca with a high proportion of amylopectin containing branched and short chains, it is proposed that they are easily attacked by sodium hydroxide when compared to the long chain molecules present in amylose. Furthermore, Abrastarch with its larger starch granules undergoes gelatinisation more easily than other starches, with correspondingly smaller granules, as described by Kerr (1950). Scheme 4 illustrates the differing granule sizes. At 5 M alkali concentration, then the concentration of starch was too high to form a homogeneous solution and only a hetergeneous paste was formed. Practical, optimum sodium hydroxide concentration was therefore 2.5 M for a 13 % w/v concentration of starch.

Re-precipitated starches were examined by DSC and light microscopy to evidence full starch destructurisation. The light microscopy experiments revealed at low sodium hydroxide concentration (0.05 M) that the granules remained intact (Scheme 5**a**). Upon increasing concentration to 0.5 M and fully to 1 M complete granule structure was lost (Scheme 5**c**). Thus, the results allowed the conclusion that alkali concentrations in the range 2.5 M to 3 M afforded a 13 to 16 weight % solution of starch that was fully solubilised for all starch samples.

Re-precipitation of the starches by treatment with methanol resulted in less than 100 % recovery. It may be that the lower molecular weight starch molecules dissolved in the alkaline solution, and were not reprecipitated in methanol. The average recovery (%) of sodium hydroxide treated starches is shown in Scheme 6. It can be seen that the high amylose starches were recovered in the highest yield. Abrastarch and Amioca starches were recovered in lower yield. The likely reason is that the branch points [α-(1$\rightarrow$6) glucosidic bonds] of amylopectin are more labile to alkali treatment than are α-(1$\rightarrow$4) glucosidic bonds. Some of these bonds may have been cleaved.

3.3. Molecular mass distribution of starches treated with sodium hydroxide

The main aim of the work has been to establish whether or not treatment of the starches with sodium hydroxide prior to derivatisation leads to depolymerisation. Work has been undertaken to determine the molecular mass distribution of the starch samples before and after dissolving in 2.5 M sodium hydroxide. Molecular mass distributions were obtained for native starch samples prepared by dissolving in the microwave bomb with 1 M KSCN and for samples treated with 2.5 M NaOH and the elution profiles are presented in Figure 18 (a-d). For the Amioca starch, which consists mainly of amylopectin, it is apparent that very little if any depolymerisation occurs since the profiles are virtually superimposible. For the other three starches, whereas the high molecular mass peak, which corresponds to the amylopectin component is uneffected, there is evidence of some depolymerisation of the lower molecular mass peak, which corresponds to the amylose. It should be pointed out, however, that some difference in the profiles might be expected since the recovery of the NaOH treated starches was less than 100 %.

3.4. Modification of solubilised starch

The key parameters believed essential to the promotion of starch modification under optimised solubilisation conditions are reaction time, temperature, pH and reagent concentrations. The feasibility of the modification of the optimally solubilised starches to low degree of substitution (DS < 0.5) was tested using a range of demonstration acylation reactions, namely, acetylation, butyrylation, hexanoylation, heptanoylation, octanoylation, nonanoylation, decanoylation, lauroylation, palmitoylation and stearoylation. Thus, three general acylation methods were investigated based on (1) modification in the optimum solubilisation solvent (2.5 M sodium hydroxide solution); (2) a series of modifications in buffered solution; and (3) modification in a two phase organic solvent/aqueous system. Optimum conditions and reagents for aqueous starch derivatisation reactions were investigated by application of the principles of response surface methodology in conjunction with the technique of combinatorial chemistry. A matrix of experiments was performed to optimise those key parameters deemed to be important in optimising reaction efficiency and, to demonstrate the aqueous modification of starches with a range of acid halides to a maximum degree of substitution. Modified starches were characterised chemically by FT-IR spectroscopic and elemental analyses. All physical characterisations of the modified staches were performed by intrinsic viscosity measurements.

3.4.1. Modification to low degrees of substitution

On the basis of the aqueous solubilisation and characterisation studies of Section 3.2, 2.5 M sodium hydroxide solution was identified as the optimum aqueous solvent for dissolving starch. This solvent system therefore formed the basis of the following derivatisation experiments. However, a number of experiments at high alkali concentration were performed in line with the response surface methodology principle. Additionally, in order to maintain consistent reaction pH a number of buffered aqueous systems were investigated. Finally, in order to increase degrees of substitution obtained, a two phase organic solvent/water based system was investigated.

3.4.1.1. Modification in 2.5 M sodium hydroxide solution—1st iteration

The results in Section 3.2 have shown that at low alkali concentrations complete gelatinisation of the four starches did not occur. However, at alkali concentrations in the range 2.5 to 3 M, 13 to 16 weight % solutions of starch were obtained that were fully solubilised whilst leaving the polymeric backbone intact. Therefore, 2.5 M sodium hydroxide solution was used for the following reactions.

A series of experiments (Table 3) was carried out to optimise the main parameters towards esterification. In Table 3, reactions M1 to M5 report the treatment of starch with 0.5 equivalents of acetyl chloride under catalytic and non-catalytic reaction conditions. The products of the reactions were analysed by FT-IR spectroscopy. The characteristic ester band (*ca* 1740 cm^{-1}) was not observed in any of FT-IR spectra (M1 to M5), which indicated that acetyl chloride had not reacted with starch hydroxyl (OH) groups. The likely reason was that the short (2) carbon-chain acid chloride was very sensitive to the aqueous basic solution and was readily hydrolysed to its carboxylic acid salt, sodium acetate. Sodium acetate is soluble in methanol and was therefore removed by filtration. The FT-IR spectra only exhibited the unreacted starch absorption bands. In addition, as the gelatinised starch solution was very viscous, in the case where DMAP (solid) was added as a reaction catalyst, it hardly dissolved in the mixture. Thus, DMAP was deemed an unsuitable catalyst under these reaction conditions.

Reactions M6 to M8 with butyryl chloride afforded the same lack of reaction as for acetyl chloride.

A series of hexanoylation reactions of starch was performed, the yields were between 70 to 75 % (Reactions M10 to M13). When using TEA and DIEA as catalysts (Reactions M12 and M13) of the reactions, yields of 74 % and 75 %, respectively were obtained.

Reactions M14, M16 and M17 were performed with heptanoyl chloride. This series of reactions afforded yields of 73 % to 74 %, when either TEA, DIEA or no catalyst was used. However, when pyridine (Reaction M15) was used as catalyst, no reaction product was isolated.

Reactions M18 to M21 comprised a group of octanoylation experiments. In these cases, FT-IR analyses of the products confirmed that starch esters were formed under these reaction conditions. In all cases, the yield of reaction product was over 70 %, with reactions

M18 and M20 affording 75 % yield. When pyridine was used as the reaction catalyst, the yield decreased slightly to 72 % (Reaction M19). When DIEA was used as the reaction catalyst then the yield increased to 84 % (Reaction M21).

A series of nonanoylated starches was prepared (Reactions M23 to M26) using TEA and DIEA as catalysts both in 74 % yield, whereas use of pyridine resulted in a reduction in yield to 68 % (Reaction M24). This result corresponded to reactions M11, M15 and M19. This further confirmed that pyridine was not a suitable catalyst in the reaction.

The lauroylated starch sample obtained by reaction M28 was afforded in the absence of a catalyst, again in 67 % yield. Surprisingly, addition of either pyridine or TEA as catalysts (Reactions M29 and M30) to the above reaction, resulted in no product formation. We have no explanation for these results. Reaction M31 indicated palmitoyl chloride failed to react successfully with starch, but decanoyl chloride did (Reaction M27).

Reactions M32 to M35 were performed with stearoyl chloride under identical reaction conditions to M18-M21 respectively. In these cases, the FT-IR spectra of the isolated products showed the materials after reaction exhibited no characteristic ester carbonyl shifts. It is presumed that the reactivity of the stearoyl chloride reagent was too low to react with activated starch and that the stearoyl chloride was slowly hydrolysed by the aqueous base to its sodium salt. Reaction M31 gave the same results as for stearoyl chloride. In an attempt to reduce the quantity of 'free' water, 4 M NaOH solution was used as the reaction solvent (Reactions M9 and M36). However, in these cases the esters were similarly not obtained and the reactions were presumed to have failed. Reaction M22 was the octanoylation of starch in 4 M NaOH solution which successfully formed the ester, but the yield was relatively low at 69 %.

3.4.1.2. Modification in 2.5 M sodium hydroxide solution—2nd iteration

The first iteration work programme set out to screen a broad range of reaction conditions and indicate where further attention should be directed to optimise esterification. This was achieved by carrying out esterifications using acetyl, butyryl, octanoyl and stearoyl chlorides. In the first round of experiments, reaction with octanoyl chloride appeared to be the most successful approach. It was proposed that the reactivity versus solubility in aqueous conditions was responsible for this phenomenon. In order to test the hypothesis, a second iteration of reactions with acyl chlorides was investigated bearing carbon chains of length ±2 from octanoyl (C_8). Thus, the reactivity of hexanoyl, heptanoyl, nonanoyl and decanoyl chlorides was investigated. Furthermore, all starches would be sequentially studied in the presence of TEA as catalyst. Thus, a matrix of twenty further experiments was performed. The matrix of experiments is tabulated at Table 4 and the outcomes are discussed at Section 3.5.

3.4.1.3. Modification in buffered solutions

A number of literature reports has told that the rates of many chemical reactions are governed by the pH of the solution (Perrin and Dempsey, 1974). Buffer solutions offer advantages for controlling reaction conditions and yields in organic syntheses. In order to investigate whether buffered solutions in the pH range 10 to 12 would sequester hydrochloric acid produced as a by-product of the reaction and thus increase the yield of reaction products, four buffer solutions were selected as solvents in which to attempt starch modification. In this case the buffer acted as two roles, one to disrupt the starch granules, the other to sequester protons, and maintain the reaction mixture at a alkaline pH. Four buffer solutions were prepared, in accordance with method of Perrin and Dempsey (1974).

The first method afforded starch ester which has been confirmed by FT-IR. The second method revealed the modified starch only occurred in sodium 4.phenolsulfonate/sodium hydroxide buffer with a very low yield. The other buffers failed to afford modified product (Table 5). It was concluded that use of such sophisticated buffer systems did not improve product yield, when compared with the basic aqueous alkali procedure. Hence further experiments were not performed.

3.4.1.4. Modification in a two-phase system

The third method was feasible in theory, and has been used to modify starch with acryloyl chloride by Jantas (1997). Unfortunately, in this case, the dried product did not show ester bond in FT-IR spectrum and acylation failed (Table 6). In our hands, it has not been possible to repeat the work of Jantas successfully. In conclusion, this first method was used for the rest of the studies.

3.4.2. Modification to intermediate degrees of substitution

Reactions M37 and M38 (Table 3) were performed with 1.5 equivalents of lauroyl chloride. Reaction M37 resulted in a lauroylated starch ester in 74 % yield. When the reaction temperature was repeated at elevated temperature (40 °C), the reaction (M38) failed and no lauroylated material was obtained. This indicated the high temperature was not good for esterification. Elemental analysis (% C) revealed that no significant increase in DS-value over those obtained for theoretical DS-value of 0.5.

3.4.3. Modification to high degrees of substitution

Reactions M39, M45 to M53 (Table 3) were carried out using 3.0 equivalents of heptanoyl, nonanoyl, decanoyl and lauroyl chlorides with different reaction times and temperatures in an attempt to produce high DS-value esters. All experiments failed. This confirmed that the aqueous conditions did not afforded the acylated starches with high degrees of substitution.

Reactions M40 to M43 were performed using 3.0 equivalents of octanoyl chloride, the reactions were successful but yields were low at 30 % to 39 %. The likely reason was that high concentration reagent caused very thick, non-homogeneous reaction conditions, which lowered the yield and presented ideal substitution. When prolonging the reaction time to 4 h, the yield only increased slightly to 42 % (Reaction M44).

3.5. Extent of modification reactions

Evidence of the reaction of hexanoyl, heptanoyl, octanoyl, nonanoyl and decanoyl chlorides with four types of starches modified in the second iteration in 2.5 M NaOH (Table 4) was obtained by elemental analysis. Increases in the proportions of carbon and hydrogen in the products were taken to indicate introduction of the acyl group. Certain assumptions were required for this to be meaningful. The products were assumed to consist solely of acylated starch. All by-products were removed entirely. Furthermore, the sample under analysis must contain no residual solvents or moisture absorbed from the atmosphere. The DS-value and yield of samples were calculated. The measured and calculated results are shown in Table 4.

Native, pure and anhydrous starch contains 44.4 % carbon, 6.2 % hydrogen and 49.4 % oxygen by weight. The results in Table 6 show that the carbon content of all samples was higher than 44.4 % indicating an increase in carbon density presumed to result from successful esterification. As can be seen from Table 4, on the basis of % carbon, DS-values obtained in this set of experiments ranged from 0.1 to 0.29. In each series of acyl chlorides, reactions with the four starch types afforded very similar DS-values and yields. The exception was for the octanoylation reaction in which Amioca starch, DS-value 0.16, appeared somewhat lower than expected. All samples had measured DS-values lower than the theoretical (0.5). Product recovery was acceptable.

Extent of reaction was related to the carbon-chain length of acyl chloride reagents: the relationships are illustrated in Figure 19. The esterification only occurred with acyl chlorides of carbon-chain length six to ten. This further evidences that activated starch and 'free' water or hydroxide compete to react with the acyl chloride. In the narrow range C_6-C_{10}, reaction conditions are appropriate for successful reaction with starch. Outside that range, the acyl chlorides are hydrolysed under the reaction conditions and converted to their salts and no starch substitution occurred.

3.6. FT-IR spectra

The FT-IR spectra of the four native starches, HylonVII, Hylon V, Abrastarch and Amioca are shown in Figures 20a, b, c and d. All four spectra have similar profiles. Distinctive peaks in all spectra are described as follows. In the fingerprint region, there are several discernible absorbances at 1156, 1083, 1023 and 937 cm^{-1}, which are associated with native starch and attributed to C–O bond stretching (Goheen and Wool, 1991). The peaks at 1083 and 1023 cm^{-1} are characteristic of the anhydroglucose ring O–C stretch. A characteristic peak occurred at 1640 cm^{-1}, which is presumably a feature of tightly bound water present in the starch (Kacurakova and Wilson, 2001; Kacurakova et al., 1998). A strong absorption band at 1023 cm^{-1}, probably due to the stretching of the C–OH bond, was present in the spectra of the starches consistent with the earlier report by Marcazzan et al. (1999). An extremely broad band due to hydrogen bonded hydroxyl groups (O–H) appeared at 3400 cm^{-1} (Aburto et al., 1997) which was attributed to the complex vibrational stretches associated with free, inter and intra-molecular bound hydroxyl groups which make up the gross structure of starch. The band at 2926 cm^{-1} is characteristic of C–H stretches associated with the ring methine hydrogen atoms.

Evidence of the formation of acylated starches was gained from the FT-IR spectra of the isolated products. The existence of a carbonyl absorption band was assigned to the formation of an ester bond. Figures 21a, b, c, and d show the FT-IR spectra of hexanoylated Hylon VII, Hylon V, Abrastarch and Amioca starches. All of these spectra have similar profiles. In comparison with the spectra of the unmodified starches, the major change is the presence of a carbonyl C=O absorption frequency at 1749 cm^{-1}. The occurrence of a shoulder at 2860 cm^{-1} on the absorbance centred at 2926 cm^{-1} in the spectra was attributed to the methyl and methylene C–H stretching bands associated with the hexanoyl substituents. The strong O–H stretching band at 3400 cm^{-1} in the native starches decreased only slightly in intensity following the esterification reaction, as the relatively low DS-value of hexanoylation meant that a significant quantity of unreacted hydroxyl groups was still present.

FT-IR spectroscopic analysis was similarly applied to the heptanoylated starches (Figure 22), octanoylated starches (Figure 23), nonanoylated starches (Figure 24) and decanoylated starches (Figure 25). In summary, similar spectroscopic profiles to the hexanoylated material were obtained in all cases. These revealed the similar structures of the acylated starches, with all presenting an intense ester carbonyl band at 1749 cm^{-1}. The absence of an absorption at about 1800 cm^{-1} in all spectra indicated that the products were isolated free of the unreacted acyl chloride. Furthermore, the absence of an absorption at 1700 cm^{-1} in all spectra also confirmed that the products were isolated free of any fatty acid which was a likely by-product formed by hydrolysis of the corresponding acyl chloride when reacted under aqueous alkaline conditions and subsquently neutralised.

3.7. Solubility

In general, the introduction of hydrophobic acyl groups into the molecular structure of starch will alter its solubility properties. The solubility of an acylated starch is also dependent upon: (1) the extent of acylation; (2) the nature of the acyl substituent; (3) the type of starch, and (4) the solvent and temperature.

The low DS-value starch esters (hexanoyl to decanoyl starches derived from Hylon VII, Hylon, V, Abrastarch and Amioca, Table 4) obtained have the appearance of a white powder. A 5 % w/v concentration of the starch esters were examined in a range of different organic solvents to investigate their solubility. The solvents used were methanol, acetone, tetrahydrofuran, pyridine, chloroform, dichloromethane, acetonitrile, toluene, xylene, dioxane, *N,N*-dimethylformamide and DMSO. The esters were found to be soluble in warm DMSO, and insoluble in the other solvents at room temperature. However, they were partially soluble in hot pyridine and toluene. The possible reason was due to the lower amount of substituent of acyl groups, and higher amount of remaining hydroxyl groups in the starch esters after esterification. Therefore, the lower DS-value esters offered the poor solubility in organic solvents.

3.8. Intrinsic viscosity

The intrinsic viscosities of the native and acylated Hylon VII, Hylon V Abrastarch starches were determined using DMSO as solvent. The results are reported in Table 9.

Measurements were difficult due to incomplete solubility in the solvent. Within experimental error the intrinsic viscosity of the native starches of the same order as the acylated starches indicating little if any depolymerisation had occurred.

4. CONCLUSIONS

Four starches were acquired for the study, three of corn (maize) origin and one of wheat. The starches were chosen to offer starches of very high (Hylon VII, 70 %) or high (Hylon V, 50 %) or conventional (wheat starch, about 25 %) amylose content as well as very high (Amioca, 99 %) amylopectin content. This broad selection was used to allow the project to test the applicability of the aqueous modification method to the widest spectrum of starch composition possible.

Work has been carried out to develop a repeatable analytical method to determine the molecular mass distribution of the four starch types, and subsequently their derivatives using GPC and intrinsic viscosity measurements. Measurement of molecular mass can only be made on polymers in solution. Aqueous potassium thiocyanate is proposed as an alternative to avoid using DMSO organic solvent for this solubilisation procedure. Potassium thiocyanate disrupt the starch crystallinity and solubilises the component polymers at the same time.

The solubilisation of four starches at a range of alkali concentrations (0.05 M to 5 M) has been achieved. At low alkali concentrations, complete starch gelatinisation did not occur. Furthermore, at low alkali concentration, the corresponding starch concentration was too low to offer an economically viable system at the industrial scale. Conversely, at 5 M alkali concentration then the concentration of starch was too high result in a solution, a necessary prerequisite for homogeneous modification. Results have shown that an alkali concentration in range 2.5–3 M afforded a 13 to 16 weight % solution of starch that was fully solubilised for all starch samples. Little or no degradation of the starch polymers accompanies this treatment which has been confirmed by GPC analysis. Thus, 2.5 M aqueous sodium hydroxide solution was selected as the optimum solubilisation medium for subsequent starch modification.

A series of starch esters with different side-chain length and low DS-values was prepared and studied. The esters were prepared by acylation of the polymer with the appropriate acid chlorides in aqueous conditions, which represents an economical and facile method for the preparation of esterified starches. The alkali solution acted as the solvent for the derivatives and ensured uniform substitution by enhanced accessibility of the reagent. Successful reaction was limited to acid chlorides containing six to ten carbon chains. Shorter or longer carbon chain acid chlorides did not react under those conditions to form esters, as confirmed by FT-IR spectroscopic analysis.

The solubility characteristics of the starches modified with hexanoyl, heptanoyl, octanoyl, nonanoyl and decanoyl substituents to low DS-values remain little changed from those of their native starch counterparts.

It is concluded that, under optimised aqueous alkali conditions, a range of starches can be modified homogeneously to low levels of substitution (DS-values 0.3) with little or no depolymersiation of the starch chain either during the solubilisation or modification processes. The procedure is equally applicable to high amylose, high amylopectin or conventional (typical amylose: amylopectin ratio of 20:80) starches.

Modifications to high degrees of substitution (DS-value ~3.0) have not been achieved.

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