SECTION B

SYNTHESIS AND EVALUATION OF NATURAL POLYMERS
AS A PARTICULATE DRUG DELIVERY SYSTEM
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PART I

• INTRODUCTION
1 INTRODUCTION

1.1 Natural polymers and starch

Vegetable gums were first seen as exudates from injured plants. These viscous fluids dissolved in water and hardened upon drying. They often used as glues and thickening agents. As the time passes, the term ‘vegetable gum’ expanded to include any polysaccharide derived from plant that forms a hydrogel or modified rheological properties of the water (Rutenberg et al, 1984). Natural vegetable gums come from two sources; they are either exudates from or general constituents of plants. The general constituent gums are often structural polymers such as starch, cellulose etc.

While natural combinations of different polysaccharides can produce an almost limited range of properties. Limited availability or expenses in harvesting some gums have created an economic incentive to modify commonly available polymers to produce the desired characteristics. Because of their relative availability starch and cellulose have been the industrial choice for manipulations. Recently they have been modified in a variety of creative ways.

Starch is group of polysaccharides, composed of glucopyranose unit joined together by glucosidic linkages. Industrially starch is broadly divided into two types e.g. Natural and modified. The characteristics of the natural starches are changed by chemical or enzymatic reactions and products of these reactions are termed modified starches.

Starch is a widely distributed material which occurs in roots, seeds and fruits of plants. For commercial use corn is the principle source, though wheat and potatoes are also used. Starch is extracted by grinding with water, filtering, centrifuging and drying a process which yields starch in granular form (John et al., 1991).
Starch is the predominant carbohydrate reserve in many plants. It is found in both photosynthetic and non photosynthetic tissues. Starch found in the chloroplasts of leaves and other photosynthetically competent cells is termed ‘transitory starch’ owing to the diurnal rise and fall of its levels in these tissues. Long term storage of starch is achieved in the amyloplasts, the specialized starch-containing plastid, which is conspicuously evident in non photosynthetic harvestable storage organs such as tubers, roots and seeds. Transitory and reserve starch can be distinguished from one another on the basis of physical characteristics: size, shape and composition. Transitory starch granules tend to be smaller, and reserve granules have species-specific shapes. Transitory starch is composed almost entirely of the branched amylopectin, whereas reserve starch contains significant amounts of linear amylose chains (~11 to 37%, depending on the species) in addition to amylopectin. Because of its greater availability, almost all end-uses of plant starch are of the reserve type. The physical properties of the reserve starch, which in turn are dictated by its structure, are responsible for its specific uses in the food and manufacturing industries. Because starch is the principal constituent of the harvestable organ of many agronomic plants, its synthesis also influences crop yields.

Starch continues to be attractive as a raw material because of its abundant supply, low cost, renewability, biodegradability and ease of chemical modification. Moreover, it is important that starch molecules are multifunctional, i.e. there are three reactive hydroxyl groups per repeating unit, which are, in general, accessible to the typical conversions of primary and secondary alcoholic -OH groups. However, most of the starch derivatives which are available commercially have low degree of substitution from DS 0:01-0:20. Examples are the preparation of starch acetate or succinate, the
reaction with ethylene or propylene oxide to form hydroxyl alkyl starch, with sodium tripolyphosphate to form starch phosphate, and with sodium monochloroacetate to form carboxy methyl starch (Wurzburg, 1981). Many other types of starch derivatives have been prepared, including products with high DS values. DS 0:5; but most of these compounds have not been commercialized. Chemical modification continues to provide a dominant route towards starch utilization in polymer-based materials. In contrast to the recent progress in cellulose chemistry, which is in particular stimulated by the use of non-aqueous cellulose solvents and regioselectively protected or activated derivatives (Heinze et al, 1998), a fully satisfying solution to design high-performance materials based on starch is still missing. Specifically designed starch materials, which can be easily prepared and used for subsequent reactions via different synthesis paths are a challenge of the recently developed polysaccharide chemistry and of great importance for future applications of the renewable and unique polymer starch.

Numbering of starch begins with the reducing end carbon C-1 when –OH group is in the same plane (equilateral), the –OH group is said to be in a β-orientation. If the –OH group is perpendicular (axial) to the hexose ring, the hydroxyl group is said to be in α-orientation. In starch, the polyhexose, the reactivity of the –OH groups at the C-2 positions on the hexose is generally greater than the reactivity at other –OH positions. However the reaction conditions can change the order of reactivity of C-2 (Fydholm, 1967). In a strong caustic solution with high level of methylation, hydroxyl substitution favours a C6 over a C2 or C3 in ratio of 7:6:1 for C6:C2:C3 respectively.

The effect of modification of starch on solutions properly varies with the particular structural change. Cleavage reaction lowers polymer’s size and decreases solution viscosity. The replacement of –OH group can have variety of effects depending upon
the new substituent and polysaccharide, such as amylose chain to chain interaction occurs primarily through hydrogen bonding. These interactions were often facilitated by cations produces junction zone which helps to displace water and control polysaccharide solubility. The addition of a substituent group, may either decrease solubility by enhancing chain to chain interactions or increase solubility by reducing chain to chain interactions.

The addition of substituent groups is controlled by reaction conditions. Depending on the reaction conditions, the reactive sites on a given monomer may be mono substituted, multi substituted or not substituted. Therefore the reaction parameters will dictate the final properties of a polymer. Complex derivatives which create new hydroxyl ends i.e. hydroxyalkyl addition can form new polymer chains on the substituent groups. These branch chains add to the complexity of the end product.

The Latin and English names of starch both represent an epitome of history. The earliest starch was made from wheat by break inhabitant of Island of Chios. The manufacturer was described in his ‘De re rustica’ a means of white powder without use of mill. During the middle ages A.D. 500-1500 the manufacturing becomes an important industry and the starch was used chiefly for stiffing fabrics especially for the use of nobility. Starch began to be made in England, it is from this era of starch that the English name was derived, starch being a variant of stark, meaning stiff. During the 18th century starch was therefore made from other and cheaper sources of starch. In 1765 the manufacture of potato starch started in Germany where it is now made in large quantities. Other sources of starch such as horse chest, nut seeds & corns of *arum maculatum* are available but no appreciable quantity of starch was ever made from them.
About 1800, starch began to be manufactured in the USA from maize and various plants but maize remains the greater source of starch in modern times. Rice forms an important source of starch. The number of Starches are recognized for pharmaceutical use they includes, Maize-Zea mays, Rice-Oryza sativa, Wheat-Tritiaum questium, etc.

1.2 Chemical composition of starch

The term starch actually refers to combination of two polysaccharides amylase and amylopectin. The difference between them is not easily discerned as combination forms may be present. However their simplicity, the composition of starch will be considered to be an amylase tractim with the remainder being amylopectin (Meister, 2000; Nicholsan, 1991).

Starch consists of two types of molecules, amylase (20-30%) and amylopectin (70-80%). Both consist of polymer of D-glucose units. Amylose is linear polymer of glucose connected by $\alpha \ (1\rightarrow4)$ linkage (Figure 1). While amylase is consider to be linear molecule enzymatic study with beta amylose indicates presence of small amount of branching including some long side chain (Takeda et al, 2001; Zobel, 1988). In most cases, amylase composed of about 25% of total starch in plants and has molecular weight that ranges between 100,000 and 210,000 (Johnson, 1979). Depending on the plant species the concentration can be much higher or much lower (Kennedy et al, 1983). Amylopectine is a branched polymer of glucose containing linear strains with $\alpha \ (1\rightarrow4)$ linkage and branches coming off these trainds through $\alpha (1\rightarrow6)$ linkage (Figure 2). The linearsegment of amylopectin are around 20 monomers long shorter than that of amylase (Rutenberg, 1984). However branching raises the size of molecule to a molecular weight of upto 60,00,000. In amylase these are linked with each other at C$_1$ & C$_4$ with ring oxygen atom all on the same side, whereas in amylopectin about one
residue in every twenty or so is also linked at $C_1 \& C_6$ forming branch points. The relative proportions of amylose to amylopectin and branch points both depend on the source of the starch, e.g. amylomaize contain over 50% amylose whereas waxy maize has almost none (Chen et al 1994).

Figure 1: Amylose - Linear polymer of glucose connected by $\alpha$-1, 4-linkage

Figure 2: Amylopectin - Branched polymer of glucose connected
Maize Starch

Maize starch is a white or creamy powder having various direct and indirect applications in industry as binder, stabilizer, yarn sizing, thickening and suspending agent viz. textile paints, detergent, paper, ceramics, pharmaceuticals, e.g. glucose, dextrose monohydrate, dextrose anhydrous, maltodextrin, sorbitol etc. Maize starch finds application in the food processing and Pharmaceutical industry they are also used in bakeries. It is also used for preparing ice cream cones which gives extra strength (Brandon et al, 2006).

Maize starch is widely used in surface sizing and has ability to increase the strength and stiffness of paper (B.P., 2004; European Pharmacopoeia, 2005).

Corn Starch

Corn Starch is white slightly yellow powder with luster. Starch derived from corn. Is dense powdery flour obtained from the endosperm portion of the corn kernel. In British recipes cornstarch is referred as cornflower. Corn starch material is made by pulverizing the ground dried residue of corn grains after the preparatory soaking and removal of the embryo and outer covering. Corn starch is a natural polymer and have formula C₆H₁₁O₅ – (C₆H₁₁O₅)x – C₆ H₁₁ O₆. It is used as caking agent in powdered sugar, as thickening agent in soups and liquids, as a health conscious alternative to talc, as a main ingredient in a biodegradable plastic, as a binder for pudding or similar foods, as a diluents when mixed with water.(International Pharmacopoeia, 2003)

Sago Starch

Sago is a powder starch made from the processed pith found inside the trunks of the sago palm metroxylon sagu. Processed starch is known as sago. Sago forms a major food for the lowland peoples of New Guinea and Moluccas and is a less frequently food
source for some peoples of the Pacific and Indian Oceans. Sago can survive in forest compared to other crops such as cereals (wheat, rice or tapioca) Indonesia is still highest sago producing country others are Thailand and Japan. The probable molecular formula can given as \( C_6H_{11}O_5 - (C_6H_{11}O_5)_x - C_6H_{11}O_6 \).

**Amaranth Starch**

Amaranth is a golden grain of the god. Also, is a natural grass ingredient from seed of the *Amaranthus cruentus*. The genus Amaranthus consists of approximately 60 species only a limited number are of the cultivated types. While most of them considered weedy species. *Amaranthus germplasm* is available in 11 countries. The three principal species considered for grain production include a Hypochondriacus *A.*, *Cruentus A* and *A. Caudatus*. The species grown is vegetable are represented primarily by a tricolor *A. dubius.*, *A Lividus and A. Creuntus*. The weed amaranth comes from *A retroflexus*, is considered one of the world’s worst weeds.

The carbohydrates in amaranth grain consist primarily of starch made up of both glutinous and nonglutinous fraction. The unique aspect of amaranth grain starch is that the size of the starch granules (1 to 31 um) are much smaller than found in other cerebral grains. Due to its unique size and composition it has been suggested that the starch may possess unique granulation and freeze characteristics, which could be of benefit to food industry (Breene, 1991; Becker et al, 1981).

Amaranth grain consist of approximately 5 to 9 % oil which is generally higher than other cereals, lipid fraction 77% and other contains high levels of calcium, iron and sodium.

The swelling power and water absorption capacity was higher for amaranth than for corn starch, however solubility of amaranth starch in comparison lower. The starch
granules of amaranth are very small and modified amaranth starch is proposed as a fat substitute in foods. Its small particle size contributes silky texture, uniform cell structure, excellent moisture retention, good clarity, slightly cohesive properties, viscosity and stability. It forms spherical bodies if allowed to co-dried with other natural gums. It offers non-hazardous replacement of coating and solvent agents in tape and paper applications.

Applications: Baby foods, bakery, breads, cakes, cereals, chips, confectionary, cosmetics, crackers, dairy products, desserts, doughnuts, nutritional bars and nutritional beverages.

1.3 Surface modification of starch

It has been almost a hundred years since commercial production of starch for food and industrial applications was initiated. Two major polymeric components amylose and amylopectin play important roles in the structure, characteristics and properties of the divergent starch sources. Amylose molecules are essentially linear and are comprised predominately of 1, 4-linked D-glucose units with a limited number of 1, 6-branching points (Seib, 1997). Amylose was considered to exist mainly in the amorphous region of starch granules. Diversing significantly from amylose and having an average molecular weight about 100–1000 times that of amylose (Vermeylen et al, 2004), amylopectin molecules are highly branched and are constructed of a large number of short 1,4 linked D-glucose chains, arranged in clusters and linked by 1,6-bonds to longer chains which transverse two or more clusters (Hizukuri et al, 1996, Thompson, 2000; Vermeylen. et al., 2004). The linear arrays of double helices, formed by two neighboring chains in a cluster, alternate with clusters of branch points in the radial direction of the granule. These alternating zones of diversified densities of amylopectin
make the granules both warm and flexible, which might be essential for being an energy reserve in plants (Seib, 1997).

Starch modifications are a means of altering the structure and affecting the hydrogen bonding of amylose and amylopectin in a controllable manner to enhance and extend starch application. When low levels of alterations take place in the molecules, only slight or no change can be observed in the superficial appearance of the granule (Taggart, 2004). Following cross linking, esterification and etherification are the second most important modifications in the starch industry.

1.3.1 Starch modification
Modification or modified starch refers to structural changes in the starch molecule without addition of chemical substituents e.g. an enzymatically depolymerized starch in which the chain length has been shortened. The term modified starch is occasionally used to describe any changes in starch polysaccharide which involves the physical manipulations without addition of chemical constituents and hence it is sometime termed as conversion (Meister, 2000).

1.3.2 Retrogradation
In this process the ordered reassociation of starch molecular strands by hydrogen bonding forms junction zones which can lead to higher viscosity, gelation, and precipitation.

1.3.3 Gelatinization
This consists of irreversible change which occurs in starch granule when heated beyond a specific initial gelatinization temperature. These changes induce a rapid irreversible swelling, crystalline melting and loss of amylase strands from the granule.
1.3.4 Pasting
This is a state of starch chains at temperature above gelatinization where starch loses intermolecular order and its granular nature.

Many starch derivatives were prepared in a manner similar to cellulose modifications. Both heat and alkali are often used to prepare the starch for chemical addition. As with cellulose, the properties of the starch changes with the degree of derivatization. The final characteristics of the starch are controlled by factors like amylase/amylopectin content, the degree of modification, the degree of derivatization and the type of derivatization. (Wurzburg, 1986).

1.3.5 Chain cleavage
Chain cleavage is a process which reduces viscosity and increases aqueous dispersion of most soluble polymers. Cleavage commonly results from oxidation, acid hydrolysis, enzymatic degradation, heat or combination of these factors. Since cleavage reactions are difficult to control the resultant polymer length and by products vary greatly i.e. oxidation of starch with sodium hypochloride, a technique commonly used in the first half of 20\textsuperscript{th} century produces a polymer with greater solubility in water, increase clarity at high concentration and reduces tendency to gel. Unfortunately this oxidation also forms carbonyl groups which creates browning and create instability in mild alkali. Other oxidizing agents i.e. H\textsubscript{2}O\textsubscript{2} and Nitrogen tetra-oxide (N\textsubscript{2}O\textsubscript{4}) have been investigated. H\textsubscript{2}O\textsubscript{2} is used commercially, but its interactions also suffer from a lack of specificity. Nitrogen tetra-oxide oxidizes by a different mechanism creating carbonyl group at the C-6 position (Kerr, 1950). Investigations into controlling carboxyl formation on polysaccharide using N\textsubscript{2}O\textsubscript{4} and creating potential polyuronic acid source have been undertaken. However this process is not yet commercial.
1.3.6 Ether formation

A variety of polysaccharide ethers are produced industrially, e.g. methyl, ethyl, hydroxyethyl, hydroxypropyl and carboxymethyl ethers. Combinations of these with other substituents are often seen. Because of the wide range of the properties produced and low cost, etherification are among the most common industrial modifications. Methylation can be achieved with a simple Williamson synthesis where the hydroxyl group is exposed by the addition of caustic soda (Williamson, 1852).

\[
\text{Polysaccharide}^{-\text{O}^-} + \text{Cl}^- \rightarrow \text{Polysaccharide}^{-\text{O}^-} + \text{Cl}^- 
\]

Hydroxyalkyl substitution represents a different mechanism which utilizes an epoxide ring to create the ether. The polyanion on the epoxide ring permits etherification with substituents such as hydroxyethyl groups.

\[
\text{Polysaccharide}^{-\text{O}^-} + \text{H}^\text{O} \rightarrow \text{Polysaccharide}^{-\text{O}^-} + \text{H}^\text{O}^- 
\]

A final mechanism worth rating is the Michael reaction in which a activated vinyl group is added to the polysaccharide through a O-(2-cyanoethyl) derivative. While other etherification reaction mechanisms have been investigated, they have limited applications.

\[
\text{O}^- + \text{H}_2\text{C}=\text{N} \rightarrow \text{Polysaccharide}^{-\text{O}^-} + \text{NH}_2^- + \text{HO}^- 
\]

Etherification of starch is relatively inexpensive method of derivatization. Hydroxyalkyl starches are stable and maintain their structure during addition,
esterification, oxidation and dextrinization. Hydroxypropyl starches are derivatized starches from waxy maize, corn, potato or tapioca starch, are used for viscosity stabilization for both water and milk based products. They improve freeze-thaw and water holding characteristics of the products.

The addition of charged substituent groups such as sulfonium, phosphonium, tertiary amino and quaternary amino groups creates cationic starches. The most useful are those derived from tertiary and quaternary ammonium addition.

Another technique for producing cationic starches includes, reacting starch with ethylenimine to form 2-aminoethyl ether (Kerr et al, 1952).
Cationic starches are primarily used as wet end additives in the paper industry. The cationic charge promotes binding of the polymer to the cellulose fibres, mineral filters and pigments in paper, creating an ionic adhesive.

As cations have positive charge they are excellent flocculating agent for negatively charged organic material. One group of cationic starches referred to as amphoteric starches. These are cationic starches which have undergone further derivatization with anionic or neutral groups. A common amphoteric addition is the introduction of phosphate ester to the cationic starch. The molar ratio of cationic to anionic group dictates the characteristic of new polymer. For a diethylaminoethyl ether with phosphate monoesters the normal molar ratio of anion to cation is between 0.07 and 0.18 (Caldwell et al, 1949). These derivatized starches have unique characteristics which promote pigment retention as well as dry and wet strength in paper (Carr, 1978)

![Figure 5: Amphoteric starch composed of a tertiary amino cationic starch with phosphate ester](image)

1.3.7 Grafts

A graft refers to the addition of a chemical substituent which forms a polymer at a hydroxyl group of the polysaccharide. e.g. hydroxyalkyl group which contains free hydroxyl groups which promotes chain formation, e.g. the formation of a polymer graft by the addition of hydroxyethyl group.
Generally grafts form a reaction with these polymerizable substituents groups. Agents which have been used to form polymer grafts includes vinyl acetate, acrylamide and methyl methacrylate (Fergason, 1994).

1.3.8 Ester formation
Like other alcohols, polysaccharides can be esterified by variety of agents. Commonly available ester includes acetate, phosphate and sulphate esters. Acetate ester of starch can be formed by reaction of the polysaccharide with acetic acid in presence of catalytic amount of sulfuric acid (Towle et al, 1993). High degree of substitution can be obtained with the addition of small amount of perchloric acid. Starch triacetates (DS=3) have been produced by reaction with acetic acid, sulphuric acid and pyridine at 100ºC for four hrs.

Monophosphate and diphosphate esters of starch are readily formed upon mixing the polysaccharide with sodium tripolyphosphate and dry heating. Diesterification can be induced by the addition of phosphonyl trichloride or phosphorus oxychloride to this process. Sulfate esters can also be formed directly using sulfuric acid. This process can improved by using fuming sulfuric acid and weak organic base such as pyrindine or diphenyl sulfoxide (Jeon et al, 1999; Sagar et al, 1995; Aburto et al, 1999).
1.3.9 Cross linkages

Noncovalent cross link between polymer strands are formed by hydrogen bonds or cation promoted carbonyl bridges. Stronger covalent linkages can be created by the reaction of multifunctional substituents groups on separate polysaccharide strands e.g. crosslinking between phosphate diester groups formed by reagents like trimetaphosphate or phosphoryl trichloride on starch polysaccharide.

Such cross linking on starch increases viscosity. This modified starch is stable under mildly acidic conditions giving it applications in food industry (Towle et al, 1993).
1.3.10 Starch acetate

Starch acetates are the most typical starch ester in the market (Fleche, 1985) and they are used in many food products, such as bakery, frozen, canned foods and white salted noodles (Chen et al, 2004; Schols et al, 2007), to improve texture, stability and appearance. Starch acetates are also used as adhesives, and acid pH-resistant binders in the food industry and as sizing agent in paper manufacture textiles (Fleche, 1985). The reagents used for preparation of starch acetate are normally acetic anhydride or vinyl acetate (Seib, 1997; Traquair, 1990). Previous research (Huang et al, 2008; Schols et al, 2007) on the effect of reagent type on the properties of acetylated granular starches showed that the degree of substitution (DS) is important for the divergently sized starch granule fractions when the starch had been acetylated by the rapidly reacting acetic anhydride. With the slowly reacting vinyl acetate, no divergence in DS of the divergence sized granule fractions was observed. Modifications with vinyl acetate resulted in higher peak viscosity and swelling volume compared to acetic anhydride. However, the divergence in DS values between the two types of acetylation was minor. Thus the substitution pattern was believed to be more important on the properties of acetylated starch. In this study, amylose and amylopectin populations were isolated from two types of acetylated cowpea starch samples and enzymatic digestion in combination with chromatographic and mass spectrometric techniques were used to study the substitution pattern at molecular level to understand the effect of reagent type (acetic anhydride vs. vinyl acetate) (Azronnzan et al, 2004).

Chloroformate derivative of methyl and phenyl salicylate were prepared by reaction with phosgene in presence of triethylamine and these were allowed to react with
soluble starch in pyridine. Dimethyl salicylate, carbonates were isolated as side products.

\[
\begin{align*}
\text{Starch} + (\text{CH}_3\text{CO})_2\text{O} & \rightarrow \text{Acetic anhydride} \\
& \rightarrow \text{Starch acetate} + \text{CH}_3\text{COONa}
\end{align*}
\]

The anti-inflammatory activity of the above polymer was investigated and was compared to that of aspirin starch substituted with methyl salicylate 1. (DS 0.82, 0.82 gm of methyl salicylate per anhydrous glucose unit), starch substituted with phenyl salicylate (DS 0.14) and starch substituted with substituted phenyl salicylate (DS 0.61). All above polymers, especially starch phenyl salicylate exhibited longer duration of activity as compared to aspirin and polymer starch phenyl salicylate was still active after 20 hrs, while aspirin activity ceased much sooner (Khalil, 1995, Wurzburg, 1964).

**1.3.11 Starch succinate**

Derivatization of starch with an ionic substituents group such as succinate, at low degree of substitution converts it into a polyelectrolyte. Starch then acquires typical properties of polyelectrolytes such as increased water solubility and increased solution viscosity. These charged macromolecules are attracted by opposite charge and consequently they find use in paper industry as thickener, flocculants and strengthening agent. Besides pyridine, organic bases such as gama picoline, pyrrole, piperidine have also been reported as catalyst for esterification of starch. Low DS starch succinates could be obtained by refluxing it in pyridine at 115°C in the presence of succinate.
anhydride for varying reaction time without prior gelatinization. (Rutenberg, 1980, Robert, 1967)

\[
\begin{align*}
\text{Starch} & \quad + \quad \text{Succinic anhydride} \\
\text{Starch Succinate} 
\end{align*}
\]

1.3.12 Starch oxidized

Starch has been manipulated by oxidizing for many years by a variety of techniques. Oxidation by sodium hypochloride has been a common treatment for starch in the early 1800’s (Rutenberg et al, 1984). Many paper mills use ammonium persulphate as oxidizing agents to produce high solid, low viscosity, aqueous starch dispersion for use as adhesives in coating (Craig et al, 1968) and sizing (Lauterbach, 1965). A wide variety of techniques that utilize oxidants, such as periodate dichromate and permagnate have been developed for oxidizing starch but rarely used for commercial derivatization. Hydrogen peroxide is used as industrial oxidant for starch, it does not changes the rheological characteristic of starch (Rutenberg et al, 1984).

Hydrochlorite oxidation produces carboxyl and carbonyl substituent groups that replace the hydroxyls of the starch. The ratio of carboxyl to carbonyl group is a function of pH as carbonyl formation is predominant at lower pH. Therefore most industrial oxidation processes are performed at pH 10 to increase carboxyl formation. Oxidation of the glycosidic linkage also occurs, shortening the polymeric chains. This produces short
chain starches that show little retrogradation and can be used to make high solids dispersions with low viscosity. Other benefits of oxidation include the solubilization of nitrogen-containing impurities, decolorization of pigments (Fuller, 1934), and a reduction of free fatty acid contents (Rutenberg et al, 1984). These oxidized starches are referred to as Chlorinated starches, although no chlorine is transferred to the starch (BeMiller, 1993). Reaction works optimally at pH 7, with reaction rate decreasing rapidly as pH increase or decrease (Whistler et al, 1956). The pH is important as the addition of hypochlorite lowers the pH of unbuffered solution. The type and location of the substituent is dependent on several factors. Over a pH range between 2 and 12 with a constant ratio of 0.1 M hypochlorite per monomeric unit, the total number of carbonyl substituent’s increases with decrease in pH while the total number of carbonyl groups remains unchanged. The primary target of oxidation appears to be C2 and/or C3. The presence of carbonyl groups on C2 or C3 eventually depolymerizes the starch by a beta elimination mechanism. Other factors which determine the type and location of substitution includes the ratio of hypochlorite to starch and type of starch utilized (Rutenberg et al, 1984). The whiteness of starch is dependent on the extent of oxidation. The sodium hypochlorite oxidation has some disadvantages. Excessive heat during drying or extended storage will cause the starch to yellow or brown (Prey et al, 1976). The extent of discolorization is proportional to the percentage of aldehyde formed in the product. Oxidized starch is also unstable in alkali. About 80 to 85% of oxidized starch production is used in the paper industry, as a binding and adhesive agent in high solids, pigmented, coating, colors (Lucas et al, 1959; Huber et al, 1966).
Starch and its derivatives are of the utmost importance in different chemical industries. Among these is the textile industry where large quantities of starches are used in textile warp sizing, printing and finishing. It should be noted, however, that the properties of native starches do not often correspond to the properties required for a particular end-use. The main shortcomings of native starches are their very large molecular size, insolubility, instability of the viscous solution under varying temperature and susceptibility to micro-organisms. For this reason, chemical modification of starches has become a must to overcome such problems. Acid treatment, oxidation & etherification, esterification, grafting and preparation of poly (vinyl)-starch composites have been advocated for chemical modification of starches.(Johnson, 1979)

1.4 General characterization starch derivatives

General characterization methods (physiochemical characterization) (Johnson, 1969; Fang et al, 2002).

1.4.1 UV-Vis spectroscopy

UV–Visible spectroscopy can be used to monitor the synthesis of starch (Achar, 1994). Intensity of the absorption band is essentially proportional to the number of chromophoric units and can be a test for the purity of starch derivative. Apart from
synthetic application UV-VIS finds its applications as confirmatory tool for various
drug-starch complexes (Esumi et al, 2005). Also it can be used as a tool to define
morphological information (Caminade et al, 2005).

1.4.2 Infrared spectroscopy
Infra-red spectroscopy is mainly used for the routine analysis of the chemical
transformations occurring at the surface of starch:

1.4.3 NMR spectroscopy
Nuclear Magnetic Resonance is certainly the most widely used in routine analysis for
characterizing starch (Miller et al, 1992), but special techniques have also been used to
probe their size and morphology. Routine NMR analyses are especially useful during
the step by step synthesis of starch, even up to high generations because they afford
information about the chemical transformations undergone by the end groups. In some
cases, selective irradiation or more complex pulse sequences are necessary for a better
assignment of signals. Even three dimensional NMR techniques ($^1$H, $^{13}$C) such as 3D
HMQC-TOCSY and 3D NOESY-HSQC were used for characterizing starch derivative.
$^{15}$N NMR has been rarely used, but it can help in characterizing starch and to detect
their selective protonation.

1.4.4 Differential Scanning Calorimetry (DSC)
The DSC technique is generally used to detect the glass transition temperature (Tg),
which depends on the molecular weight, entanglement and chain-end composition of
polymers. Decomposition of starch in DSC is due to an intermolecular or
intramolecular dehydration reaction (Morita, 1956). Thus, it is conceivable that
substitution of hydroxyl groups of starch into alkyl ester groups resulted in the
improvement of thermal stability of starch. This relationship between DS and thermal
stability was also observed in fatty-acid esters of starch (Shorgren, 1996).
1.4.5 Scanning Electron Microscopy (SEM)

The scanning electron microscopy is a type of electron microscopy that images the sample surface by scanning it with high energy beam of electron in faster scan pattern. The electron interact with the atoms that make up the sample producing signals that contains information about the sample surface topography, composition and other property such as electrical conductivity (Willard, 1986).

The types of signal produced by an SEM includes secondary electrons, back scattered electrons, characteristic X-ray. Secondary electrons can produce very high resolution images of a sample surface. Back scatter electron images can provide information about the distribution of different element in the sample. These X-rays are used to identify the composition and measure the abundance of element in the sample (Skoog, 2001).

1.5 Applications

Starch is a staple in the diet of much of the world’s population, and is also widely used in the western world in the food and beverage industries as a thickener and a sweetener, as well as having some manufacturing applications in the paper and textile industries. The more prevalent use of starch for industrial purposes will only become economically viable when its use as a raw material rivals those derived from petroleum-based products. The use of starch as renewable and biodegradable polymer is becoming increasingly attractive because of the environmental concerns about the industrial wastes generated from petroleum products and the growing awareness of the potential deleterious consequences of greenhouse gas emissions from these activities. For example, foamed starch, which is biodegradable, antistatic, insulating and shock absorbing, is an excellent alternative to polystyrene-based packing material. The ability to produce novel or tailor-made starches would be advantageous in that it would
decrease the quantity of post-harvest modifications currently being used, some of which are environmentally damaging. Starch is composed of two different glucan chains, amylose and amylopectin. These polymers have the same basic structure, but differ in their length and degree of branching, which ultimately affects the physicochemical properties of these polymers. Amylose is an essentially linear polymer of glucosyl residues linked via $\alpha-1, 4$ glucosidic linkages, whereas amylopectin exists as a branched $\alpha-1, 4: \alpha-1, 6$ D-glucan polymer. The physicochemical properties of the $\alpha-1, 4$ glucans are based on the degree of branching and/or polymerization. Therefore it might be of value to produce polymers with features that are intermediate between amylose and amylopectin, or those that are more highly branched or have a higher molecular weight (Muller et al, 1994). This can be achieved by modifying the levels and properties of the starch biosynthetic enzymes, and has been accomplished by the identification of starch biosynthetic mutants and more recently by transgene technology. There is a disadvantage in that many starch mutants also have a significantly lower yield. This can be financially compensated for if the starch produced has novel uses. Alternatively, it would be useful to investigate the basis for the lower yield and to find ways to produce these novel starches at normal or higher levels. The relative amounts of amylose and amylopectin are what give starches their unique physical and chemical properties, which convey specific functionality and could be of biotechnological importance. For example; high amylose starches have numerous industrial applications. These starches are used in fried snack products to create crisp, evenly browned snacks. An added bonus of high amylose starches is that they hamper the penetration of cooking oils, which leads to a decrease in fat intake by the consumer. High amylose starches are widely used as thickeners and are strong gelling agents used
in the production of jellies and owing to their rapid setting properties are used in the production of gum candies (confectionery). Many types of photographic film also have a starch component because of the many features of high amylose starch, including transparency, flexibility, tensile strength and water resistance. High levels of amylose do present the problem of retrogradation, which occurs when gelatinized starch recrystallizes. One way to overcome this is by the introduction of amylopectin, which will also endow starch with new properties depending on the levels of amylopectin present. Starches with high levels of amylopectin are broadly used by the food industry to improve uniformity, stability and texture. Amylopectin also imparts better freeze–thaw stability in frozen foods. The paper industry, exploits the binding and bonding properties of amylopectin to enhance paper strength and printing properties. The binding and bonding properties of starches with high levels of amylopectin make a good addition to adhesives, especially on bottle labels, which are often subject to water and high humidity. As new starches are produced, new applications will be found. Starch quality is also influenced by the presence of lipids, proteins and phosphorous. Granule size and distribution are also qualitative parameters that have an effect on the value and uses of starch (Visser et al, 1993). As all the aforementioned properties are genetically determined, it is possible to manipulate these traits. Therefore, discovery and characterization of the enzymes that affect the quality of starch are of notable worth. The industrial applications of starch are limited because it is used mainly in its unmodified form. The uses of this biological renewable resource could potentially be increased several-fold if it could serve as a raw material to produce modified starches. However, the chemistry of starch is confined because of the limited reactivity of glucose, the building block. The introduction, during starch biosynthesis, of glucose
residues with reactive side-chains or charged groups would increase the number of commercially viable chemical modifications and, in turn, augment the potential uses of starch in applications not feasible today. For example, starch with more charge could create super-absorbent polymers. Reactive groups might decrease the need for some chemical modifications, such as the addition of cross linkers, or could be used to introduce molecules, such as phosphates, which can inhibit retrogradation (Fergason, 1994). If such reactive groups could be introduced, this would be an excellent way to improve the integrity of the starch granule by means of cross linking, which can reinforce hydrogen bonding.

1.5.1 Other applications of starch

1.5.1.1 Gel formulations
Oxidized starch mixed with about 5% sodium alginate has been used in jelly marmalades with no loss of quality (Zubrev et al, 1973). Oxidized starch has been developed as a substitute for gum Arabic, as a possible wall material for spray dried flavours (Bangs et al, 1988; Eden et al, 1989) and for use in gum drops (Voelker et al, 1972; Misra et al, 1990).

1.5.1.2 Film forming agent
Oxidized starch has been reported as a substitute for gum Arabic in the film forming ability. This has been achieved by monitoring the conditions of oxidation of starch in the presence of sodium hypochlorite solution. It has been also used as encapsulating agent (Chattopadhyaya et al, 1997). Many starch derivative were reported as directly compressible material (Timaron S et al, 1992).
1.5.1.3 Water reducing agent

Starch succinate half ester as a water-reducing agent with super retarding performance being used together for massive concrete, rolled concrete and pump concrete to improve the working efficiency of concrete by keeping the fluidity of cement for a long time (Zhang et al, 2008).

1.5.1.4 Pharmaceutical starch

Starch is mainly used in this industry in the medicine preparation, tablet coating, drug formulation etc.

1.5.1.5 Liquid glucose

Starch is a mixture of glucose, maltose and Maltodextrin. In this industry liquid glucose comes in handy and widely used in the manufacture of variety of syrups, antacid preparations and other mixtures. This is because liquid glucose has a unique property of sweetness balanced with providing body and consistency.

1.5.1.6 Dextrose monohydrate

Starch works as a source of energy because unlike cane sugar DMH can be easily assimilated without inversion. This special property of this enables its use as tablet binder and for manufacture of intravenous infections. It is also widely used in antibiotics.

1.5.1.7 Corn steep liquor

Starch plays a vital role in pharmaceutical industry. Whether it is antibiotics like Penicillin G or Aureomycin, or the manufacture of Inositol, corn steep liquor is essential. This is because brown liquid has a vital ingredient, phytin. In some medicines this product acts as a lipotropic agent.
1.5.1.8 Dextrose syrup
Starch is widely used in pharmaceutical industry for the manufacture of antibiotic drugs and penicillin.

1.5.2.9 Food industry
Starch is the principle component of most of the food products as a thickening agent, viz. sauces, pudding and gravies.

1.5.2.10 Textile industry
Starch has most important applications in this industry in the areas of sizing finishing and printing (Frydhlm, 1967).

1.5.2.11 Paper industry
Starch is used in surface sizing and eras ability to improve the strength of paper. Starch succinate is used as a floculant and strengthening agent (Oppermann et al, 1996; Huang et al, 2008).

1.6 Sulphonamides as effective antimicrobials
Bacterial infections remain major causes of morbidity and mortality in hospitals around the world. A new report estimated that *Staphylococcus aureus* (*S. aureus*) infections alone resulted in 9.5 billion dollars in extra hospital charges and nearly 12,000 inpatient deaths per year. Sulfonamides, the development of which is a fascinating and promising area in medicinal chemistry, are widely used in various bacterial infections including enteric and urinary tract, and respiratory tract. The discovery of the antibacterial activity of sulphonamides in the early 1930s marked the beginning of the present era of chemotherapy. Soon after the announcement of the marked antibacterial activity of Prontosil in vivo by Domagk in 1935, it was established that the activity was due to its metabolic product 4-aminobenzensulphonamide (sulphanilamide). When the
potential of the sulphonamides was recognized, research programs were initiated worldwide to prepare analogs and derivatives of sulphanalilide, particularly with a view towards improving its antimicrobial spectrum, therapeutic ratio and pharmacokinetic properties and delaying the emergence of the resistant forms. The antimicrobial activity of sulphonamides (and sulphones) extends to a number of microbial species having a folic acid pathway, which includes many gram positive and gram negative cocci and bacilli, mycobacteria, some large viruses, protozoa, and fungi. However, the clinical use of sulfonamides is limited mostly due to their extremely low solubility in water, rapid elimination in blood, low level of association to plasma proteins and several side effects, which are characterized by fever, skin rash, hepatotoxicity, lymphadenopathy and hematological disorders. In all cases their action is related to PABA antagonism. They are preferred due to the ease of administration and wide spectrum of anti-bacterial activity. The poor solubility of sulfonamides restricts their use in topical and parenteral applications. As poor solubility is generally related to a low bioavailability, this presents a major challenge during drug formulation (Foye, 2002).

1.7 Quinolones as an effective antimicrobials

The synthetic quinoline antibacterial generally include primary substituted 1, 4-Dihydro-4-oxo-3-pyridine carboxylic acid having an additional ring fused at 5,6 position. It was first reported in 1962. Nonquinolone are used for oral and Parenteral treatment of systemic infection. Modification of 6-Fluro and replacement of one ethyl group with cyclic substituent gives number of more effective quinolones. These agent exert their antibacterial activity by inhibition of DNA synthesis. They bind to a subunit of DNA. The quinolones are a family of synthetic broad-spectrum antibiotics. The majority of quinolones in clinical use belong to the subset of fluoroquinolones for
example Ciprofloxacin, Norfloxacin and Gatifloxacin which have a fluorine atom attached the central ring system, typically at the 6-position. The term Quinolone(s) refers to the first generation of the potent and toxic synthetic chemotherapeutic agents derived from Chloroquine used to treat serious, complicated and life threatening bacterial infections. Hans Andersag discovered chloroquine, in 1934 at Bayer I.G. Farbenindustrie A.G. laboratories in Eberfeld, Germany. The first generation of the quinolones begins with the introduction of Nalidixic acid in 1962 for treatment of kidney infections in humans. This drug was discovered by George Lesher and coworkers in a distillate during chloroquine synthesis. Quinolones inhibit the bacterial DNA gyrase or the topoisomerase IV enzyme, thereby inhibiting DNA replication and transcription. Quinolones can enter cells easily via porins and therefore are often used to treat intracellular pathogens such as Legionella pneumophila and Mycoplasma pneumonia. For many gram-negative bacteria DNA gyrase is the target, whereas topoisomerase IV is the target for many gram-positive bacteria. It is believed that Eukaryotic cells do not contain DNA gyrase or topoisomerase IV.

1.8 Antimicrobial susceptibility testing

Antibiotic susceptibility testing is based on either diffusion method or serial dilution method.

1.8.1 Zone of inhibition

This method is preferred over serial dilution method because of the ease with which quantitative results can be obtained. However the method cannot be used when the antibiotic does not diffuse freely due to adsorption or incompatibility with the medium. Dilutions of the antibiotic under test and the standard antibiotic preparation are made in geometric proportions. Plates are prepared by pouring agar, seeding them with test
organism, and allowing setting on a perfectly horizontal surface so that the agar occupies a constant depth throughout the dish. The test organism may be mixed with the agar before pouring or may be applied to the surface of the medium, which is a ready set. Antibiotic solutions are applied in:

1) Cups cut in medium using a sterile cork borer about 10mm in diameter. The cut agar disk is removed by a vacuum device or a splayed-out pen nib.

2) Cylinders of stainless steel glazed porcelain, Pyrex glass or sterilized plastic having an external diameter of about 8 mm and a height of about 10 mm. These are slightly warmed so that they sink to a constant depth when placed on the agar.

3) Filter paper or cellulose disks, which absorb a fixed volume of solution

4) Standard ceramic insulation beads which attracts a definite volume when touched on the surface of the solution. The surface of the agar medium must be dry if this method has to be used.

Plates are left at room temperature to allow diffusion of the antibiotic solution throughout the medium to get ahead of growth of the microorganism. After incubation, inhibition of growth can be observed as a clear zone of inhibition around each container. The diameter of this zone of inhibition is proportional to the log of the concentration of antibiotic (Atlas, 1986).

1.8.2 Serial dilution method

Another approach to antimicrobial susceptibility testing is to determine the minimum inhibitory concentration (MIC) using tube dilution procedure. This procedure determines the concentration of an antibiotic that is effective in preventing the growth of the pathogen and gives an indication of the dosage of the antibiotic that should be effective in controlling the infection in the patient. Standardized microbial inoculum is
added to tubes containing serial dilutions of an antibiotic, and the growth of the microorganism is monitored as a change in turbidity. In this way, the break point or MIC of the antibiotic that prevents growth of the microorganism in vitro can be determined. The MIC indicates the minimal concentration of the antibiotic that must be achieved at the site of infection to inhibit the growth of the microorganism being tested. By knowing the MIC and the theoretical levels of the antibiotic that may be achieved in body fluids, such as blood and urine, the physician can select the appropriate antibiotic, the dosage schedule, and the route of administration. MIC’s can even be performed on normally sterile body fluids without isolating and identifying the pathogenic microorganism. For example, blood or cerebrospinal fluid containing an infecting microorganism can be added to tubes containing various dilutions of an antibiotic and a suitable growth medium. An increase in turbidity would indicate the growth of microorganisms and the fact that the antibiotic at that concentration was ineffective in inhibiting microbial growth, whereas a lack of growth would indicate that the pathogenic microorganisms were susceptible to antibiotic at the given concentration.
PART II

• OBJECTIVE
• PLAN OF WORK
2 OBJECTIVE & PLAN OF WORK

2.1 Objective

Starch is an attractive target of extensive research due to its inherent diverse properties. There are different sources of starch that are recognized for pharmaceutical applications which includes maize starch, rice starch, wheat starch and sago starch. Starch consists of two types of molecules, amylose (20-30%) and amylopectin (70-80%). The relative proportions of amylose to amylopectin and branch points both depend on the source of the starch, e.g. amylo maize contain over 50% amylose where as waxy maize has almost none.

Starch modifications are a means of altering the structure and affecting the hydrogen bonding of amylose and amylopectin in a controllable manner to enhance and extend starch application. When low levels of alterations take place in the molecules, only slight or no change can be observed in the superficial appearance of the granule. Following cross linking, esterification and etherification are the second most important modifications in the starch industry (Taggart, 2004). The main shortcoming of making starch is that they are very large molecular size, insolubility, instability of viscous solution under varying temperatures and susceptibility to microorganisms. For this reason, chemical modification of starch has become a must to overcome such problems. Acid treatments, oxidation and etherification, esterification, grafting, etc. have been advocated for chemical modification of starch. Various starch derivatives were reported in the literature, which were used in food industry for various purposes. However their pharmaceutical applications were not reported. Literature survey revealed that no systematic study was reported for synthesis and pharmaceutical applications of...
modified starch obtained from different sources of starch. Hence the objective of the present research work was to synthesize various starch derivatives from different sources of starch viz. maize, sago, amaranth and corn and evaluate starch derivatives for their pharmaceutical application in drug delivery system.
2.2 Plan of work

1. Literature survey.
2. Procurement of raw materials and characterization.
4. Evaluation of synthesized starch derivatives
5. Preparation of starch derivatives complex with Gatifloxacin and Sulphamethaxazole
7. Evaluation of starch derivatives as binding agent.
PART III

• EXPERIMENTAL
3 EXPERIMENTAL

3.1 Drug Profile

3.1.1 Sulphamethoxazole (SMX)

3.1.1.1 IUPAC Name
N-1-(5-methylisoxazol-3-yl)-sulphanilamide

3.1.1.2 Mol. Formula
C₁₀H₁₁N₃O₃S

3.1.1.3 Mol. Wt.
253.28

3.1.1.4 Category
Antibacterial

3.1.1.5 Dose
Initial dose 2 g; subsequent doses, 1 g two or three times daily.

3.1.1.6 Structure

![Chemical structure of SMX](image)

Figure 7: Chemical structure of SMX

3.1.1.7 Description
White or almost white, crystalline powder; almost odourless.

3.1.1.8 Solubility
Freely soluble in acetone, sparingly soluble in ethanol (95%), slightly soluble in chloroform and in ether; practically insoluble in water. It dissolves in dilute solutions of sodium hydroxide.
3.1.1.9 Storage
Store in well closed, light-resistant containers.

3.1.1.10 Standard
Sulphamethoxazole contains not less than 99.0 % and not more than 101.0 % of C_{10}H_{11}N_{3}O_{3}S, calculated with reference to the dried substance.

3.1.1.11 Identification
A: The infra-red absorption spectrum, is in concordant with the reference spectrum of sulphamethoxazole or with the spectrum obtained from sulphamethoxazole RS.
B: Dissolve about 5 mg in 10 ml of 1M hydrochloric acid and dilute 1 ml to 10 ml with water, add 0.2ml of sodium nitrite solution. After 1 or 2 minutes add the solution; to 1ml of 2-naphthol solution an intense orange or red color and, usually, a precipitate of the same color is produced.
C: Melts between 169° and 172°

3.1.1.12 Acidity
Heat 1.25 g of the finely powdered substance with 25 ml of carbon dioxide-free water at 70° for 5 minutes. Cool for about 15 minutes in ice and filter. To 20 ml of the filtrate add 0.1 ml of bromothymol blue solution; not more than 0.3 ml of 0.1M sodium hydroxide is required to change the color of the solution (U.S.P., 2005).

3.1.1.13 Spectrum of activity
Gram negative and gram positive bacteria

Mechanism of action
Sulfonamides are structural analogs and competitive antagonists of para-aminobenzoic acid (PABA). They inhibit normal bacterial utilization of PABA for the synthesis of folic acid, an important metabolite in DNA synthesis. The effects seen are usually bacteriostatic in nature. Folic acid is not synthesized in humans, but is instead a dietary

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requirement. This allows for the selective toxicity to bacterial cells (or any cell dependent on synthesizing folic acid) over human cells. Bacterial resistance to sulphamethoxazole is caused by mutations in the folic acid enzyme that prevents the drug from binding and blocking folic acid synthesis.

3.1.1.14 Bioavailability
Rapidly absorbed following oral administration.
Protein binding 70%, half life 10 hrs.

3.1.1.15 Biotransformation
The metabolism of sulphamethoxazole occurs predominately by N4-acetylation, & glucouronidation.

3.1.2 Gatifloxacin (GTFX)

3.1.2.1 Molecular formula
\( \text{C}_{19}\text{H}_{22}\text{FN}_{3}\text{O}_{4} \)

3.1.2.2 Molecular Weight
375.40

3.1.2.3 Structure

![Chemical structure of GTFX]

3.1.2.4 IUPAC Name
1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid.
3.1.2.5 Description
A white to light yellow crystalline powder.

3.1.2.6 Pharmacokinetics

Absorption
Bioavailability is about 96%. $C_{\text{max}}$ is about 2 to 4.2 µg/ml (dose-proportional). $T_{\text{max}}$ is about 1 h (about 1.5 h after multiple doses), AUC 14.2 to 33 µg/ml after a 200 mg dose and about 33 µg/ml after a 400 mg dose.

Distribution
Protein binding is about 20%. Mean $V_{\text{dss}}$ 1.5 to 2 l/kg.

Metabolism
GTFX undergoes limited biotransformation in humans with less than 1% of the dose excreted in the urine as ethylenediamine and methylethylenediamine metabolites.

Elimination
Mean $t_{1/2}$ ranges from 7 to 14 h. Renal Clearance ranges from 124 to 161 mL/min. less than 1% excreted in urine as ethylenediamine and methylethylenediamine metabolites. Primarily renally excreted as unchanged drug via glomerular filtration and tubular secretion. May also undergo minimal biliary and/or intestinal elimination.

3.1.2.7 Pharmacology
GTFX is a synthetic broad-spectrum 8-methoxyfluoroquinolone antibacterial agent for oral or intravenous administration. It is bactericidal and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to replicate one DNA double helix into two. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian. GTFX is a broad-spectrum antibiotic that is active against both Gram-
positive and Gram-negative bacteria. It should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria.

The bactericidal action of GTFX results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, required for bacterial DNA replication, transcription, repair, and recombination.

3.1.2.8 Spectrum of activity
Gatifloxacin covers a most of aerobic gram-negative, gram-positive and even some anaerobic species responsible for various infections (prostatitis, tuberculosis, pneumonia, bronchitis, and urinary tract, respiratory tract, skin gastrointestinal, bone, soft tissue, abdominal infections, some sexually transmitted diseases and some infections that affect people with AIDS). There is no cross-resistance between Gatifloxacin and the other classes of antibiotics. Cross resistance has been observed between systemic Gatifloxacin and some other fluoroquinolones.

Resistance to Gatifloxacin in vitro develops via multiple-step mutations and occurs at a general frequency of between $1 \times 10^{-7}$ to $10^{-10}$.

3.2 Raw material characterization

3.2.1 Sulphamethoxazole (SMX)

3.2.1.1 Acidity
SMX, 1.25 g was heated with 25 ml of distilled water at 70°C for 5 minutes. After cooling, the solution was filtered. To 20 ml of the filtrate 0.1 ml of bromothymol blue solution was added drop wise till the change in color observed.

3.2.1.2 Melting point
The melting point of a drug was measured by using open capillary tube method.
3.2.1.3 Infra red spectroscopy
SMX was subjected to IR spectroscopy (Thermo IR 200) by KBr pellet technique in the range 400-4000 cm\(^{-1}\).

3.2.1.4 \(\lambda_{\text{max}}\) determination

*Preparation of Phosphate buffer (PBS) pH 7.4:*

A 50 ml of 0.2M potassium dihydrogen phosphate solution was mixed with 39.5 ml of 0.2M sodium hydroxide solution and final volume made up to 200 ml with distilled water.

Various spectrums of SMX were recorded in water & pH 7.4 phosphate buffers using UV-Vis Spectrophotometer (Schimadzu UV 1700 and UV Probe as a data acquisition system) at various concentrations. Wavelength of maximum absorbance (\(\lambda_{\text{max}}\)) was considered as maximum wavelength.

3.2.1.5 Constitution of calibration curve

Weighed accurately 5 mg of SMX and was dissolved in 5 ml of water (stock solution) using methanol as a co-solvent. Suitable volume of stock solutions was diluted to get the concentration in the range of 10, 20, 30, 40, and 50µg/ml. The absorbance of the solution was measured against solvent blank at 258.00 nm and the graph was plotted as absorbance vs. concentration. The same procedure was repeated for pH 7.4 phosphate buffer except concentrations were prepared in the range of 5, 10, 15, 20 and 25 µg/ml and absorbance was measured at 260.20nm.

3.2.1.6 Solubility determination

Saturated solution of SMX in different solvents i.e. in distilled water, pH 7.4 phosphate buffer were prepared and stirred for 24 hrs at room temperature (100 agitations/ min). The solutions were then passed through a Whatmann (No. 1) filter paper. The amount
of the drug dissolved in the solution was determined by measuring absorbance on UV Spectrophotometer at respective wavelengths after suitable dilutions (dilutions for water and pH 7.4 buffer solutions were ~100 times.

3.2.1.7 Differential Scanning Calorimetry
The thermal behavior of drug was studied using Differential Scanning Calorimetry 60 (Shimadzu) with the heating rate of 20°C per min between 100 to 240°C.

3.2.2 Gatifloxacin (GTFX)

3.2.2.1 Melting point
The melting point of a drug was measured by using open capillary tube method.

3.2.2.2 Infra red spectroscopy (IR)
GTFX was subjected to IR spectroscopy by KBr pellet technique in the range 400-4000 cm\(^{-1}\).

3.2.2.3 λ\text{max} determination
Various spectrum of GTFX was recorded in distilled water and pH 7.4 phosphate buffers at various concentrations (i.e. 2 µg to 25 µg). Wavelength of maximum absorbance (λ\text{max}) was considered as maximum wavelength.

3.2.2.4 Constitution of calibration curve of GTFX
Weighed accurately 5 mg of GTFX and dissolved in 5 ml of distilled water (stock solution) using methanol as a co solvent. Suitable volume of stock solution was diluted to get the concentration in the range as 5, 10, 15, 20, 25 and 30µg/ml. The absorbance of the solution was measured against solvent blank at 285.40 nm and the graph was plotted as absorbance vs. conc. The same procedure was repeated for pH 7.4 phosphate buffer except concentrations were prepared in the range of 10, 20, 30, 40, 50 µg/ml and absorbance was measured at 290.50 nm.
3.2.2.5 Solubility determination
Saturated solution of GTFX in different solvents i.e. in distilled water, pH 7.4 phosphate buffer were prepared and stirred for 24 hrs at room temperature (100 agitations/ min). The solutions were then passed through a Whatmann (No.1) filter paper. The amount of the drug dissolved in the solution was determined by measuring absorbance on UV Spectrophotometer at respective wavelengths after suitable dilutions (dilutions for water and pH 7.4 buffer solutions were ~100 times).

3.2.2.6 Differential Scanning Calorimetry
The thermal behavior of drug was studied using Differential Scanning Calorimetry 60 by Shimadzu with the heating rate of 20°C per min between 100 to 240°C.

3.2.3 Starch

3.2.3.1 Identification
A: Suspension of 1g in 50ml of water was heated to boiling for 1 minute.

B: To 10 ml of the mucilage, 0.05 ml of 0.01M iodine solution was added; and change in colour was observed after heating and then cooling the mucilage.
3.2.3.2 pH

Starch slurry, 100 ml was prepared by weighing 20g starch and dispersing in 100ml of water. The slurry was agitated for five minutes moderately and pH was determined immediately to the nearest 0.1 unit potentiometrically.

3.2.3.3 Acidity

10 g of starch was added to 100 ml of ethanol (70%) previously neutralised to phenolphthalein solution, shaked for 1 hour, filtered and titrated 50 ml of the filtrate with 0.1M sodium hydroxide and noted the amount of sodium hydroxide required to change the colour of solution.

3.2.3.4 Fluorescence

The starch was exposed to ultra-violet radiation and fluorescence was observed.

3.2.3.5 Oxidizing substances

5 g starch was added to 10 ml of water and 1 ml of acetic acid and stirred until a homogeneous suspension was obtained. To it 0.5 ml of a freshly prepared saturated solution of potassium iodide was added, mixed and allowed to stand for 5 minutes; and observed for colour change.

3.2.3.6 Loss on drying

0.2 g of starch was heated to 105°C for one hour in an oven and calculated loss on drying.

3.2.3.7 IR

Maize, Sago, Corn and Amaranth starch were subjected to IR study.
3.3 Synthesis of starch derivative

3.3.1 Starch acetate
Weighed accurately 30 gm of dried maize starch (Dried at 45-50°C for 24 hrs) and mixed with acetic anhydride in ratio of 1:2. Stirred the given mixture for 5 min and then added 50% w/v of aq. sodium hydroxide solution (9 ml). The temperature of reaction mixture was increased to 123°C within 15 min. This temperature was hold for 30, 60, 90, 120, 180 & 240 min. The excess of cold water was added to terminate the reaction. The product obtained was dried at 50°C (Yixiang et al, 2004, Azronnazan et al, 2004). The yield of the reaction was found to be 22 gm. The same procedure was repeated for sago, corn and amaranth starch.

\[
\text{Starch} \quad \text{CH}_2\text{OH} \quad \text{O} \quad \text{OH} \quad \text{CH}_2\text{OH} \quad \text{O} \quad \text{OH} \quad \text{CH}_2\text{OH} \quad \text{O} \quad \text{OCOCH}_3 \quad n \\
\text{Acetic anhydride} \quad \text{NaOH, H}_2\text{O} \quad \rightarrow \\
\text{Starch acetate} \quad \text{CH}_2\text{OH} \quad \text{O} \quad \text{OH} \quad \text{CH}_2\text{OH} \quad \text{O} \quad \text{OCOCH}_3 \quad n
\]

*Figure 9: Reaction for the synthesis of starch acetate derivatives*

3.3.2 Starch succinate
Maize starch was dried at 50°C for 24 hour before reaction. 25 g of dried starch was weighed accurately and transferred to three necked round bottom flask. Accurately weighed 3 gm of succinic anhydride was then transferred to round bottom flask. To the above mixture 25 ml of pyridine was added and the mixture was stirred for 5 min to dissolve succinic anhydride on magnetic stirrer. Reaction temperature was maintained at 115°C for 4-6 hours to complete the reaction. Starch succinate was isolated from
reaction mixture by precipitation with absolute ethanol. The product was washed 3-4 times successively with ethanol to remove traces of pyridine. The resultant product was dried in vacuum dessicator over calcium chloride and finally in oven at 50˚C for 5-6 hours (Bhandari et al, 2002). The yield of reaction was found to be 16 gm. Same procedure was repeated for preparing succinate derivatives of corn, sago and amaranth starch.

![Reaction for the synthesis of starch succinate derivatives](image)

3.3.3 Starch oxidized

Maize starch (1gm) and water (3ml) in the ratio of (1:3) was taken in four necked one litre glass reactor and stirred at 500 rpm; pH was adjusted to 7.5 by 1N sodium hydroxide solution. To it sodium hypochlorite was added dropwise from a dropping funnel up to 90 min at 35 ℃ and pH 7.5 was maintained by adding 1 N Hydrochloric acid. After 2 hour pH was brought down to 7.0 by sodium thiosulphate solution. Product was separated by vacuum filtration and washed until free from chlorine. Product was dried at 50-55˚C for 10-12-hours (Chattopadhyay et al, 1997). The yield
of the reaction was found to be 0.9 gm. Same procedure was repeated for sago, corn and amaranth starch (Whistler et al, 1957).

![Starch Reaction Diagram]

**Figure 11: Reaction for the synthesis of starch oxidized derivative**

### 3.4 Solubility of starch derivatives

Solubility study of starch derivatives was carried out in water and in different organic solvents like benzene, acetone, ethanol and dichloromethane. The previously weighed quantity of starch derivative was dissolved in solvent under study till saturation. Then the saturated solution of starch derivative was ultrasonicated three times with the interval of 10 min each. The solution was then kept overnight and again ultrasonicated for 10 min. The resultant supernatant liquid (5 ml) was separated and evaporated in oven. The residue was dried and weighed, giving the solubility of starch derivatives in the solvent under study. (chen *et al*, 1994)

### 3.5 Characterization of starch derivatives

#### 3.5.1 TLC

Preformulated TLC plates were used with mobile phase composed of Chloroform: Methanol in 4:1 ratio and iodine vapors as visualizing agent.

#### 3.5.2 Physical property

Starch derivatives were evaluated for physical properties such as physical state, viscosity (1.0% w/v dispersion) using Ostwald viscometer and colour.
3.5.3 IR
The prepared starch derivatives were subjected to IR spectroscopy by KBr pellet technique in the range 400-4000 cm\(^{-1}\).

3.5.4 Differential Scanning Calorimetry
Each derivative was subjected to thermal analysis using Differential Scanning Calorimeter TA 60 (Shimadzu) by heating at the rate of 20°C/min in the range of 100 to 240°C.

3.5.5 Scanning Electron Microscopy
Amaranth succinate and Corn succinate were subjected to SEM study.
Scanning electron microscopic (SEM) graphs of dried samples were obtained using Jeol (JSM-6510) SEM (Japan) for morphology analysis. The dried sample was placed in an airtight desiccator, which had silica gel to remove moisture. A small amount of dried samples were dispersed on metal stub containing carbon tape. The samples were made electrically conductive by coating, in a vacuum, with a thin layer of platinum for 60 seconds. Images were obtained at an excitation voltage of 15 kv at different magnifications varying from 100–1500.

3.6 Biocompatibility Study
All synthesized derivatives were subjected to the biocompatibility study to ensure the safety. The study was carried out on fresh animal blood as per following procedure.

Procedure
Biocompatibility study of starch was carried out by haemolytic study of centrifuged blood. Blood was collected in 10% w/v sodium citrate anticoagulant solution; blood was stored carefully at 4-5°C without giving any mechanical jerk. Then it was filled in centrifuge tube and marked its level. On centrifugation at 5000 rpm, the RBC gets
settled down. Centrifuge tubes were then washed with saline water four to five times until the supernatant colour matches with saline colour. 100 µl of RBC suspension was withdrawn and allowed to react with 1% w/v (2 ml) dispersion of starch derivative like starch oxidized, starch succinate, starch acetate. The resultant mixture was transferred to 2 ml small centrifuge tube, again centrifuged at 5000 rpm. 500 µl of supernatant dispersion was withdrawn and its absorbance was measured on UV-VIS spectrophotometer (Schimadzu UV PC 2501), taking the reference of distilled water (100% haemolysis) and saline water (0% haemolysis) as saline water is isotonic with blood. All the readings were observed in the range of 0-100% haemolysis.

3.7 Preparation and characterization of starch derivatives-drug complex

3.7.1 Starch derivative- SMX complex

3.7.1.1 Preparation of SMX dispersion
1% w/v dispersion of Sulphamethoxazole was prepared.

3.7.1.2 Preparation of starch derivative dispersion

Starch acetate
100 mg of maize starch acetate was dispersed in 10 ml of dichloromethane to prepare 1% w/v dispersion. Same procedure was followed for corn, amaranth and sago starch acetate derivatives.

Starch succinate
100 mg of maize starch succinate was dispersed in 10 ml of dichloromethane to prepare 1% w/v dispersion. Same procedure was followed for corn, amaranth and sago starch succinate derivatives.
Starch oxidized

100 mg of maize starch oxidized was dispersed in 10 ml of dichloromethane to prepare 1% w/v dispersion. Same procedure was followed for corn, amaranth and sago starch oxidized derivatives.

3.7.1.3 Preparation of Starch derivative-SMX complex by Co-evaporation method
The above prepared drug dispersion 1% w/v and starch derivative 1% w/v dispersion were mixed in 1:1 ratio; ultrasonicated the said mixture for 10 min 3-4 times and the resultant dispersion was subjected to solvent evaporation by controlled heating at about 45-50 °C, the resulting solid then pulverized and sieved through 60#.

3.7.2 Characterization of starch derivative-SMX complex

3.7.2.1 IR
Starch derivative drug complex were subjected to IR study to confirm complex formation based on positioning and broadening of peaks in comparison to peaks of various starch derivatives.

3.7.2.2 Differential Scanning Calorimetry
Starch derivative drug complex was subjected to the DSC analysis to find out the change in thermal properties of the derivative as compared to complex. DSC analysis was carried out using DSC TA 60 (Shimadzu) in the range of 100 to 300˚C with increase heat rate at 10˚C/min.

3.7.2.3 Scanning Electron Microscopy
Starch derivative drug complex (corn succinate-SMX, amaranth succinate-SMX) were subjected to SEM study.

Scanning electron microscopic (SEM) graphs of dried samples were obtained using Jeol (JSM-6510) SEM (Japan) for morphology analysis. The dried sample was placed
in an airtight desiccator, which had silica gel to remove moisture. A small amount of
dried samples were dispersed on metal stub containing carbon tape. The samples were
made electrically conductive by coating, in a vacuum, with a thin layer of platinum for
60 s. Images were obtained at an excitation voltage of 15 kv at different magnifications
varying from 100–1500.

3.7.3 Starch derivative -GTXF complex preparation

3.7.3.1 Preparation of GTXF dispersion.
1% w/v dispersion of GTXF was prepared in distilled water.

3.7.3.2 Preparation of starch derivative dispersion
Starch acetate
100 mg of maize starch acetate was dispersed in 10 ml of dichloromethane to prepare
1% w/v dispersion. Same procedure was followed for corn, amaranth and sago starch
acetate derivatives.

Starch succinate
100 mg of maize starch succinate was dispersed in 10 ml of dichloromethane to prepare
1% w/v dispersion. Same procedure was followed for corn, amaranth and sago starch
succinate derivatives

Starch oxidized
100 mg of maize starch oxidized was dispersed in 10 ml of dichloromethane to prepare
1% w/v dispersion. Same procedure was followed for corn, amaranth and sago starch
oxidized derivatives.

3.7.3.3 Preparation of starch derivative-GTXF complex by Co-evaporation
method
The above prepared drug dispersion 1% w/v and starch derivative 1% w/v dispersion
were mixed in 1:1 ratio; ultrasonicated the said mixture for 10 min 3-4 times and the
resultant dispersion was subjected to solvent evaporation by controlled heating at about 45-50 °C. the resulting solid then pulverized and sieved through 60#.

3.7.4 Characterization of starch derivative-GTFX complex

3.7.4.1 IR
Starch derivative drug complex were subjected to IR study to confirm complex formation based on positioning and broadening of peaks in comparison to peaks of various starch derivatives.

3.7.4.2 Differential Scanning Calorimetry
Starch derivative drug complex was subjected to the DSC analysis to find out the change in thermal properties of the derivative as compared to complex. DSC analysis was carried out using DSC 60 by Schimadzu in the range of 100 to 300°C with increase heat rate at 20°C/min.

3.7.4.3 SEM
Starch derivative drug complex (Corn succinate-GTFX and amaranth succinate-GTFX) were subjected to SEM study.

Scanning electron microscopic (SEM) graphs of dried samples were obtained using Jeol (JSM-6510) SEM (Japan) for morphology analysis. The dried sample was placed in an airtight desiccator, which had silica gel to remove moisture. A small amount of dried samples were dispersed on metal stub containing carbon tape. The samples were made electrically conductive by coating, in a vacuum, with a thin layer of platinum for 60 s. Images were obtained at an excitation voltage of 15 kv at different magnifications varying from 100–1500.
3.8 Antimicrobial screening of starch derivatives-drug complex

3.8.1 Zone of inhibition (ZOI)
Antimicrobial screening was performed by Disk Diffusion method. Zone of inhibition was measured by using vernier caliper.

3.8.1.1 Disk diffusion method
Disk diffusion refers to the diffusion of an antimicrobial agent of a specified concentration from disks or strips, into the solid culture medium that has been seeded with the selected inoculum isolated in a pure culture. Disk diffusion was based on the determination of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. The diffusion of the antimicrobial agent into the seeded culture media resulted in a gradient of the antimicrobial. When the concentration of the antimicrobial becomes so diluted that it can no longer inhibit the growth of the test. The protocol followed for the ZOI was as follows

Procedure
The antimicrobial susceptibility testing of starch derivative drug complex was carried out by disk diffusion method. Accurately weighed the nutrient medium (Muller Hinton Agar) and dissolve it in a distilled water and heated to aid dissolution. All the accessories were then wrapped in paper and sterilized by autoclave at about 121 °C for 20 min. The freshly grown culture of bacteria (S. aureus,) was suspended in sterile saline solution under the HEPA cabinet. After sterilization the petriplates were then loaded with 20 ml volume of nutrient medium and it was allowed to solidify for some time. The 20 µl bacterial suspension was loaded on each plate and spread on plate surface with help of spreader. The wells were made in the nutrient medium with the
help of sterile cork borer of 10mm size. 100 µl of sample and drug solution was loaded in each well and it was then incubated for 24 hr at about 37°C in BOD incubator. The control of the solvent was also prepared. After 24 hr the zone of inhibition was measured using vernier caliper. Results were then compared for starch derivative drug complex and plane drug.

The experimental setup used for developing ZOI is described below.

<table>
<thead>
<tr>
<th>1. Media</th>
<th>Muller-Hilton agar medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Test organism</td>
<td>S. aureous</td>
</tr>
<tr>
<td>3 Loaded volume of microbial suspension on agar plate</td>
<td>20 µl</td>
</tr>
<tr>
<td>4 Loaded volume</td>
<td>100 µl</td>
</tr>
<tr>
<td>5 Incubation temperature</td>
<td>37°C</td>
</tr>
<tr>
<td>6 Incubation period</td>
<td>24 hrs</td>
</tr>
</tbody>
</table>

### 3.9 Binding study of starch derivatives

Selected starch derivatives like Maize acetate, Corn oxidized, Amaranth succinate and Amaranth oxidized which shows stickiness after heating 1% w/v dispersion were subjected to their binding characteristics. Tablets 300 mg each of plain lactose were prepared using 5% w/v dispersion of the above derivatives as a binding agent and evaluated for their hardness and disintegration time. (Schwartz et al, 1978)

Hardness of the tablet was determined by using Pfizer hardness tester and disintegration study was performed by using Disintegration Rate Test Apparatus. (Mishra et al, 1990, Timarson et al, 1992)
PART IV

- RESULT
- DISCUSSION
4 RESULT & DISCUSSIONS

4.1 Raw material characterization

4.1.1 SMX
Identification of drug

4.1.1.1 Acidity
0.1ml of 0.099 M sodium hydroxide was consumed to change the color of the solution. Thus acidity complies with IP specification as IP Limit is “not more than 0.3 ml of 0.1M sodium hydroxide”

4.1.1.2 Melting point
Melting point of SMX found to be 171-173°C which was in compliance with specification.

4.1.1.3 IR spectroscopy
IR spectral analysis showed presence of all prominent and characteristics peaks, and found identical with reference spectra. IR spectrum is as shown in Figure 11.
From IR study different functional groups observed at characteristic frequencies, summarized are as shown in Table 2.

Table 2: IR Observations for SMX

<table>
<thead>
<tr>
<th>Frequency observed cm$^{-1}$</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1366</td>
<td>S=O Stretch</td>
</tr>
<tr>
<td>3200 to 3400</td>
<td>N-H Stretch</td>
</tr>
</tbody>
</table>
4.1.4 \( \lambda \) max determination

\( \lambda_{\text{max}} \) of the drug was determined and found to be 258.0 nm with correlation coefficient value of 0.998 in distilled water. Calibration curve was constituted and used for determination of SMX in further experiments. Similarly calibration curve was constituted for SMX in buffer with pH 7.4 at 260.20 nm and used for determination of SMX in further experimentation part.
4.1.1.5 Constitution of calibration curve

Table 3: Calibration curve for SMX in distilled water

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.754</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>1.468</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>2.181</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>2.834</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>3.436</td>
</tr>
</tbody>
</table>
4.1.1.6 Solubility study for SMX

$\lambda_{\text{max}}$ for SMX was observed at 258.0 nm & 260.20 with value of correlation coefficient 0.998 and 0.999 in distilled water and pH 7.4 phosphate buffer respectively. Hence the calibration curve was used for determination of SMX concentration in further experiments. The solubility of SMX was found to be very poor in water and phosphate buffer pH 7.4 (0.4mg/ml ±0.01mg/ml and 0.37 mg/ml ±0.02mg/ml respectively).

4.1.1.7 Differential Scanning Calorimetry

Figure 12: DSC Thermogram of sulphamethoxazole
DSC study reveals that Sulphamethoxazole showed an endothermic peak at 171°C which was in compliance with the standard thermogram. The thermogram is as shown in Figure 13.

4.1.2 Gatifloxacin (GTFX)

4.1.2.1 Melting Point
The melting point of the drug was found to be in the range of 180-182°C and was found to be in compliance with the specification.

4.1.2.2 Infrared Spectroscopy
IR spectral analysis showed presence of all prominent and characteristics peaks, and found identical with reference spectra. IR spectrum is as shown in Figure 14. From IR study different functional groups observed at characteristic frequencies, summarized are as shown in Table 5.

Table 5: IR Observations for GTFX

<table>
<thead>
<tr>
<th>Frequency observed cm(^{-1})</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1632</td>
<td>C=O</td>
</tr>
<tr>
<td>1117</td>
<td>F</td>
</tr>
<tr>
<td>3378</td>
<td>NH</td>
</tr>
</tbody>
</table>

Figure 13: IR spectrum of GTFX
4.1.2.3 \( \lambda_{\text{max}} \) determination

GTFX exhibited absorption maxima i.e. \( \lambda_{\text{max}} \) at 285.4 and 290.50 nm with correlation coefficient value of 0.997 in distilled water and 0.998 pH 7.4 phosphate buffer. Hence the calibration curve was used for determination of GTFX concentration in further experiments.

![Figure 14: UV spectrum of GTFX](image)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc (µg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.619</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>1.247</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>1.86</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>2.498</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>2.96</td>
</tr>
</tbody>
</table>

Table 6: Calibration curve for GTFX in pH 7.4 buffer
Table 7: Calibration curve for GTFX in distilled water.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc (µg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>0.38196</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.80811</td>
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<tr>
<td>3</td>
<td>15</td>
<td>1.11776</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>1.44263</td>
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<td>5</td>
<td>25</td>
<td>1.83272</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>2.21419</td>
</tr>
</tbody>
</table>

4.1.2.5 Solubility study of GTFX

The solubility of GTFX was poor in water and phosphate buffer pH 7.4 (0.56 mg/ml ±0.01mg/ml and 0.51 mg/ml ±0.02mg/ml respectively).

4.1.2.6 Differential Scanning Calorimetry

Thermal analysis of GTFX indicates the sharp endothermic peak at 184.16 with heat of fusion -58.33J/g.
4.1.3 Starch

4.1.3.1 Identification test:
Maize, Sago, Corn and Amaranth starch were subjected to identification test A and B. In test A, a thin cloudy mucilage was observed for Maize, Sago and Corn starch however thick mucilage was observed in case of Amaranth starch. In test B for all the types of starch, dark blue colour was produced which disappears on heating and reappears on cooling. This confirms the identification test of starch.

4.1.3.2 pH Test:
pH of the starch dispersions were measured and found to be 5.6, 5.7, 6.8, 5.6 for Maize, Amaranth, Corn and Sago starch respectively.

4.1.3.3 Acidity:
All the types of starches were subjected for acidity test and amount of 0.1M NaOH required to change the colour of filtrate solution was found to be less than two ml for all the types of starch tested i.e. Maize 1ml, Amaranth 0.4ml, corn 0.6 ml and Sago 0.8ml. Hence all types of starches complies acidity test.

4.1.3.4 Fluorescence test:
No fluorescence was observed when different types of starches exposed to UV light.

4.1.3.5 Oxidizing substances:
Faint brown colour was observed in case of Maize starch whereas no colour was observed for Corn, Sago and Amaranth starch.

4.1.3.6 Loss on drying:
All the types of starches were subjected to LOD study and the moisture content was found to be 7.95%, 8.45%, 9.15% and 6.45% w/w for Maize, Amaranth, Corn and Sago starch respectively.
4.1.3.7 IR study

4.1.3.7.1 Maize starch

Figure 16: IR Spectrum of maize starch

4.1.3.7.2 Corn starch

Figure 17: IR Spectrum of corn starch
4.1.3.7.3 Sago starch

Figure 18: IR Spectrum of sago starch

4.1.3.7.4 Amaranth starch

Figure 19: IR Spectrum of amaranth starch
IR study of all types of starch shows peak of hydroxyl group between 3200-3400 cm\(^{-1}\) and carbon – oxygen peaks between 1080-1160 cm\(^{-1}\) which confirms the presence of hydroxyl group in starch.

From the above studies it can be concluded that the raw material starch (Maize, Sago, Corn and Amaranth) to be used in the present study complies with the different pharmacopoeial and other standards.

### 4.2 Synthesis of starch derivatives

Starch consists of two types of molecules amylose (20-30%) and amylopectin (70-80%). Both consist of polymer of D-glucose units. In amylose these are linked with each other at C\(_1\) & C\(_4\) with ring oxygen atom all on the same side, whereas in amylopectin about one residue in every twenty or so is also linked at C\(_1\) & C\(_6\) forming branch points. The relative proportions of amylose to amylopectin and branch points both depend on the source of the starch, e.g. amylomaize contain over 50% amylose whereas waxy maize has almost none.

Starch is group of polysaccharides, composed of glucopyranose unit joined together by glucosidic linkages. Starch molecules are multifunctional, i.e. there are three reactive hydroxyl groups per repeating unit, which are in general, accessible to the typical conversions of primary and secondary alcoholic -OH groups.

The effect of modification of starch on solutions property varies with the particular structural change. Cleavage reaction lowers polymer’s size and decreases solution viscosity. The replacement of –OH group can have variety of effects depending upon the new substituent and polysaccharide, such as amylose chain to chain interaction occurs primarily through hydrogen bonding. These interactions were often facilitated.
by cations produces junction zone which helps to displace water and control polysaccharide solubility. The addition of a substituent group may either decrease solubility by enhancing chain to chain interactions or increase solubility by reducing chain to chain interactions.

The addition of substituent groups is controlled by reaction conditions. Depending on the reaction conditions, the reactive sites on a given monomer may be mono substituted, multi substituted or not substituted. Therefore the reaction parameters will dictate the final properties of a polymer.

Considering the above parameters starch obtained from different sources like sago starch, maize starch, corn starch and amaranth starch were selected to prepare different derivatives. Starch derivatives viz. acetate, oxidized and succinate were prepared. Acetylated derivatives of maize starch were obtained however acetylated derivatives of corn, sago and amaranth did not formed at the level of acetic anhydride used in the reaction.

Derivatives of different starch were further evaluated for the different properties.

4.3 Solubility of starch derivatives

All the derivatives obtained were solid in nature. All the derivatives were subjected to their interactions with water under various conditions of temperature. The solubility of maize, corn, sago and amaranth starch was found to be 3.6 mg, 1.1 mg, 4.2 mg and 5.1 mg respectively (n=3). The results are shown in Table 8.
Table 8: Effect of water on starch derivatives

<table>
<thead>
<tr>
<th>Starch Derivative</th>
<th>Abbreviation used</th>
<th>Nature</th>
<th>Action of water (RT)</th>
<th>Solubility in water MI at RT</th>
<th>Action of water after 24 hrs (RT)</th>
<th>Action after heating at 90 °C for 10 min</th>
<th>Stickiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize acetylated</td>
<td>MA</td>
<td>Granular</td>
<td>Dispersible, Insoluble</td>
<td>1.3-1.5mg</td>
<td>Swells</td>
<td>Paste formed</td>
<td>Thick mucilage, Sticky</td>
</tr>
<tr>
<td>Maize succinate</td>
<td>MS</td>
<td>Coarse powder</td>
<td>Dispersible, slightly soluble</td>
<td>3.2-3.5mg</td>
<td>Swells</td>
<td>Paste formed</td>
<td>Thin, Nonsticky</td>
</tr>
<tr>
<td>Maize oxidized</td>
<td>MO</td>
<td>Coarse powder</td>
<td>Dispersible, Slightly soluble</td>
<td>3.3-3.6mg</td>
<td>Swells</td>
<td>Paste formed</td>
<td>Thin, Nonsticky</td>
</tr>
<tr>
<td>Corn succinate</td>
<td>CS</td>
<td>Fine powder</td>
<td>Dispersible, Slightly soluble</td>
<td>7.3-9.1mg</td>
<td>Dispersible</td>
<td>Paste formed</td>
<td>Thick mucilage, Sticky</td>
</tr>
<tr>
<td>Corn oxidized</td>
<td>CO</td>
<td>Fine powder</td>
<td>Soluble</td>
<td>47.8-52.4mg</td>
<td>Soluble</td>
<td>Soluble</td>
<td>Thin, Nonsticky</td>
</tr>
<tr>
<td>Sago succinate</td>
<td>SS</td>
<td>Coarse powder</td>
<td>Dispersible, slightly soluble</td>
<td>3.3-3.6mg</td>
<td>Swells</td>
<td>Paste formed</td>
<td>Thin, Nonsticky</td>
</tr>
<tr>
<td>Sago oxidized</td>
<td>SO</td>
<td>Coarse powder</td>
<td>Dispersible, Slightly soluble</td>
<td>6.5-6.9mg</td>
<td>Dispersible</td>
<td>Paste formed</td>
<td>Thin, Nonsticky</td>
</tr>
<tr>
<td>Amaranth succinate</td>
<td>AS</td>
<td>Coarse powder</td>
<td>Dispersible, slightly soluble</td>
<td>6.0-6.4mg</td>
<td>Dispersible</td>
<td>Paste formed</td>
<td>Thick mucilage, Sticky</td>
</tr>
<tr>
<td>Amaranth oxidized</td>
<td>AO</td>
<td>Coarse powder</td>
<td>Slightly Soluble</td>
<td>8.3-8.5mg</td>
<td>Dispersible</td>
<td>Paste formed</td>
<td>Thick mucilage, Sticky</td>
</tr>
</tbody>
</table>

RT – Room temperature

It was observed that maize, corn and sago starch and their derivatives were almost insoluble but dispersible in water at room temperature except corn oxidized and amaranth oxidized, which were soluble in cold water to the extent of four percent and one percent respectively.

However other derivatives were found to have poor solubility but more dispersibility in water at room temperature. Maize acetate showed lowest solubility. On storage for 24 hours at room temperature almost all the derivatives were dispersible except corn oxidized and amaranth oxidized which were dispersible and also soluble. The derivatives dispersible in water at room temperature were further heated at 40-50°C for
about 10 min and subsequently heating up to 90°C and then cooled to room temperature. This results in formation of pasty mass. The pasty mass formed by maize acetylated, corn succinate, amaranth succinate was found to be sticky in nature in comparison to other derivatives. All oxidized derivatives were found to be forming pasty mass with less degree of stickiness.

All derivatives were subjected to their solubility characteristics in different organic solvents like benzene, acetone, ethanol and dichloromethane. The results are expressed in Table 9.

<table>
<thead>
<tr>
<th>Starch Derivative</th>
<th>Benzene</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Dichloromethane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oxidized</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble but Dispersible</td>
</tr>
<tr>
<td>Amaranth oxidized</td>
<td>Insoluble</td>
<td>Slightly Dispersible</td>
<td>Insoluble, Dispersible</td>
<td>Insoluble but Dispersible</td>
</tr>
<tr>
<td>Maize acetate</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble but Dispersible</td>
</tr>
<tr>
<td>Maize oxidized</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble but Dispersible</td>
</tr>
<tr>
<td>Sago succinate</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble but Dispersible</td>
</tr>
<tr>
<td>Amaranth succinate</td>
<td>Insoluble</td>
<td>Slightly Dispersible</td>
<td>Insoluble, Slightly Dispersible</td>
<td>Insoluble but Dispersible</td>
</tr>
<tr>
<td>Maize succinate</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble but Dispersible</td>
</tr>
<tr>
<td>Sago oxidized</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble but Dispersible</td>
</tr>
</tbody>
</table>

The solubility study indicates that all the derivatives were insoluble in organic solvents except dichloromethane in which the derivatives were insoluble but dispersible throughout the phase.
4.4 Characterization of starch derivatives

Starch derivatives were characterized by different tests viz. TLC, IR, DSC and other physical parameters.

4.4.1 TLC of starch derivatives

Table 10: TLC analysis of starch derivative

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of starch derivative</th>
<th>Abbreviation used</th>
<th>$R_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Maize starch acetylated</td>
<td>MA</td>
<td>0.333</td>
</tr>
<tr>
<td>2</td>
<td>Maize starch succinate</td>
<td>MS</td>
<td>0.389</td>
</tr>
<tr>
<td>3</td>
<td>Corn starch succinate</td>
<td>CS</td>
<td>0.318</td>
</tr>
<tr>
<td>4</td>
<td>Amaranth starch succinate</td>
<td>AS</td>
<td>0.545</td>
</tr>
<tr>
<td>5</td>
<td>Sago starch succinate</td>
<td>SS</td>
<td>0.380</td>
</tr>
<tr>
<td>6</td>
<td>Maize starch oxidized</td>
<td>MO</td>
<td>0.833</td>
</tr>
<tr>
<td>7</td>
<td>Corn starch oxidized</td>
<td>CO</td>
<td>0.388</td>
</tr>
<tr>
<td>8</td>
<td>Amaranth starch oxidized</td>
<td>AO</td>
<td>0.340</td>
</tr>
<tr>
<td>9</td>
<td>Sago starch oxidized</td>
<td>SO</td>
<td>0.803</td>
</tr>
</tbody>
</table>

TLC study was carried out for each derivatives synthesized. $R_f$ values of the different derivatives were compared with the respective source of starch.

4.4.2 Physical properties:

The starch derivatives having solubility more than 8 mg/ml were evaluated for viscosity. 1.0% w/v aqueous dispersions of Amaranth oxidized, Corn oxidized derivatives were prepared and viscosity was determined by ostwald viscometer with respect to water.

Table 11: Viscosity of starch derivatives by Ostwald Viscometer

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Starch derivatives</th>
<th>Density</th>
<th>Viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Corn Oxidized Starch</td>
<td>0.980</td>
<td>1.0952</td>
</tr>
<tr>
<td>2</td>
<td>Amaranth oxidized starch</td>
<td>0.972</td>
<td>0.9069</td>
</tr>
</tbody>
</table>
4.4.3 IR spectroscopy

Starch Acetate

4.4.3.1 Maize starch acetate

Figure 20: IR spectrum of maize starch acetate

Starch Succinate

4.4.3.2 Maize starch Succinate

Figure 21: IR spectrum of maize starch succinate
4.4.3.3 Sago starch succinate

Figure 22: IR spectrum of sago starch succinate

4.4.3.4 Corn starch succinate

Figure 23: IR spectrum of corn starch succinate
4.4.3.5 Amaranth starch succinate

Figure 24: IR spectrum of amaranth starch succinate

Starch Oxidized

4.4.3.6 Maize starch oxidized

Figure 25: IR spectrum of maize starch oxidized
4.4.3.7 Sago starch oxidized

![Figure 26: IR spectrum of sago starch oxidized](image1)

4.4.3.8 Corn starch oxidized

![Figure 27: IR spectrum of corn starch oxidized](image2)
4.4.3.9 Amaranth starch oxidized

![IR spectrum of amaranth starch oxidized](image)

Figure 28: IR spectrum of amaranth starch oxidized

Infra red spectra of synthesized derivatives were recorded to ensure the confirmation of derivative in terms of functional group. IR frequency due to C=O stretch in the region of 1650 to 1800 cm\(^{-1}\) showed that starch acetate, succinate and oxidized derivatives were formed. As starch is a polymeric material, each –OH group may not have replaced and hence the degree of substitution is less for starch derivatives. Hence each spectrum showed the IR frequency peak for –OH stretching in the region of 3200 to 3500 cm\(^{-1}\), C-H aliphatic stretch in the region of 2900 to 3000 cm\(^{-1}\); C-O stretch in the region of 1000 to 1300 cm\(^{-1}\) was also observed.
4.4.4 DSC

Starch acetate

4.4.4.1 Maize starch acetate

![DSC Thermogram of maize starch acetate](image)

Figure 29: DSC Thermogram of maize starch acetate

Starch succinate

4.4.4.2 Maize starch succinate

![DSC Thermogram of maize starch succinate](image)

Figure 30: DSC Thermogram of maize starch succinate
4.4.4.3 Sago starch succinate

![DSC thermogram of sago starch succinate](image1)

Figure 31: DSC thermogram of sago starch succinate

4.4.4.4 Corn starch succinate

![DSC thermogram of corn starch succinate](image2)

Figure 32: DSC thermogram of corn starch succinate
4.4.4.5 Amaranth starch succinate

Starch oxidized

4.4.4.6 Maize starch oxidized
4.4.4.7 Sago starch oxidized

Figure 35: DSC thermogram of sago starch oxidized

4.4.4.8 Corn starch oxidized

Figure 36: DSC thermogram of corn starch oxidized
4.4.4.9 Amaranth starch oxidized

DSC thermograms of starch derivatives were studied and concluded that all the thermogram showed a typical endothermic depression at around 100 °C. This peak is a characteristic of evaporation of water of crystallization from the starch which is basically a carbohydrate. However any carbohydrate does not show any kind of characteristic endothermic or exothermic peak. Broad exothermic peaks were shown by different derivates of starch at different temperature range. Maize starch acetate showed a very small sharp exothermic peak at around 220 °C. Maize starch succinate, corn starch acetate showed a exothermic peak at 150 to 200 °C. Amaranth starch succinate show a broad exothermic peak at 135 to 170 °C. Sago starch succinate showed broad exothermic peak at 180 to 190 °C. Maize starch oxidized showed exothermic peak at around 220 and 300 °C. Starch oxidized showed a deep endothermic peak starting at around 100 °C and extend its peak value at around 140 °C. Amaranth starch oxidized showed a broad exothermic inflection starting at 120 °C and ending at around 190 °C.
Sago starch oxidized showed only initial endothermic depression at around 100 °C (a characteristic of evaporation of water of crystallization). However it does not show any further peaks. At around 170 to 200 °C thermograms showed a small deflections, which cannot be either characterized as exothermic or endothermic.

In all, this concludes that these starch polymers and their derivatives like acetates, succinate or oxidized did not have a characteristic thermal behavior which could be used for its characterization or identification criteria. The exothermic peak at starting and ending at varying temperature indicates that these peaks were due to burning of carbohydrate derivates at varying temperatures.

4.4.5 Scanning Electron Microscopy (SEM)
SEM was performed to observe morphological modifications.

4.4.5.1 Corn succinate (CS)

![SEM image of corn succinate](image)

Figure 38: SEM image of corn succinate
4.4.5.2 Amaranth succinate (AS)

SEM study of the selected derivatives viz Corn succinate and Amaranth succinate showed the surface characteristics behavior of the derivatives. Corn succinate showed elongated granules with rough surface which may get formed due to attachment of small particles towards the surface of the starch derivative granules. Corn succinate exhibits coarse appearance in comparison to Amaranth succinate having smooth surface texture and fine granular appearance.
4.5 Biocompatibility of starch.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Sample ID</th>
<th>% Haemolysis I</th>
<th>% Haemolysis II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>100.0</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Saline</td>
<td>11.03</td>
<td>11.05</td>
</tr>
<tr>
<td>3</td>
<td>MS</td>
<td>23.73</td>
<td>12.98</td>
</tr>
<tr>
<td>4</td>
<td>MA</td>
<td>14.82</td>
<td>16.37</td>
</tr>
<tr>
<td>5</td>
<td>CS</td>
<td>13.54</td>
<td>15.60</td>
</tr>
<tr>
<td>6</td>
<td>AS</td>
<td>86.09</td>
<td>84.73</td>
</tr>
<tr>
<td>7</td>
<td>AO</td>
<td>06.99</td>
<td>05.25</td>
</tr>
<tr>
<td>8</td>
<td>CO</td>
<td>06.07</td>
<td>03.33</td>
</tr>
</tbody>
</table>

MS- Maize starch succinate; MA- Maize starch acetate;
CS- Corn starch succinate; AS- Amaranth starch succinate;
AO- Amaranth starch oxidized; CO- Corn starch oxidized.

Biocompatibility study was carried out to ascertain the safety of synthesized polymers for human beings. Objective of the present study was to calculate the % haemolysis of the polymers with respect to the saline as it is isotonic with the human blood. Amaranth starch succinate was found to be most haemolytic i.e. 86.092% and corn starch oxidized was found to be less haemolytic i.e 6.07% of all repeat trials of the study. Amaranth oxidized and corn oxidized exhibited less than 7% haemolytic activity and hence they can be considered as biocompatible material.

4.6 Preparation of starch derivative – drug complex

Starch derivatives –SMX complexes and starch derivative GTFX complexes were prepared by co evaporation method and characterized for different parameters. All derivatives obtained were solid in nature.
4.7 Characterization of starch derivative -drug complex

4.7.1 IR Spectroscopy

4.7.1.1 MA-SMX

Figure 40: IR spectrum of maize starch acetate-SMX complex

4.7.1.2 MS-SMX

Figure 41: IR spectrum of maize starch succinate-SMX complex
4.7.1.3 SS-SMX

Figure 42: IR spectrum of sago starch succinate-SMX complex

4.7.1.4 CS-SMX

Figure 43: IR spectrum of corn starch succinate-SMX complex
4.7.1.5 AS-SMX

Figure 44: IR spectrum of amaranth starch succinate-SMX complex

4.7.1.6 MO-SMX

Figure 45: IR spectrum of maize starch oxidized-SMX complex
4.7.1.7 SO-SMX

Figure 46: IR spectrum of sago starch oxidized-SMX complex

4.7.1.8 CO-SMX

Figure 47: IR spectrum of corn starch oxidized-SMX complex
4.7.1.9 AO-SMX

Figure 48: IR spectrum of amaranth starch oxidized-SMX complex

4.7.1.10 MA-GTFX

Figure 49: IR spectrum of maize starch acetate-GTFX complex
4.7.1.11 MS-GTFX

Figure 50: IR spectrum of maize starch succinate-GTFX complex

4.7.1.12 SS-GTFX

Figure 51: IR spectrum of sago starch succinate-GTFX complex
4.7.1.13 CS-GTFX

Figure 52: IR spectrum of corn starch succinate-GTFX complex

4.7.1.14 AS-GTFX

Figure 53: IR spectrum of amaranth starch succinate-GTFX complex
4.7.1.15 MO-GTFX

Figure 54: IR spectrum of maize starch oxidized-GTFX complex

4.7.1.16 SO-GTFX

Figure 55: IR spectrum of sago starch oxidized-GTFX complex
4.7.1.17 CO-GTFX

Figure 56: IR spectrum of corn starch oxidized-GTFX complex

4.7.1.18 AO-GTFX

Figure 57: IR spectrum of amaranth starch oxidized-GTFX complex

Infra red spectroscopy is the best technique for the identification of functional groups in the molecule. It is can also be applied to study the effects of different factors like
hydrogen bonding, inductive effect, mesomeric effect, resonance effect, hybridization etc. Complexation of drug with polymers involves weak interactions like Van der Waal interactions and/or intermolecular hydrogen bonding. In the present study various starch derivatives were complexed with SMX and GTFX. IR spectra of the derivatives revealed that in almost all the studies IR frequency was shifted to lower value and peak intensities was also reduced. Increase in bond length which ultimately leads to decrease in bond strength and thereby reduced IR frequency was observed. These all observations lead to the conclusion that complex formation between starch derivatives and drug SMX and GTFX may involve hydrogen bonding and Van der Waal interactions.

4.7.2 Differential Scanning Calorimetry

Starch derivative-SMX complex

4.7.2.1 Maize starch acetate-SMX complex

![DSC thermogram of maize starch acetate-SMX complex](image)

Figure 58: DSC thermogram of maize starch acetate-SMX complex
4.7.2.2 Maize starch succinate-SMX complex

![DSC thermogram of maize starch succinate-SMX complex](image1)

Figure 59: DSC thermogram of maize starch succinate-SMX complex

4.7.2.3 Sago starch succinate-SMX complex

![DSC thermogram sago starch succinate-SMX complex](image2)

Figure 60: DSC thermogram sago starch succinate-SMX complex
4.7.2.4 Corn starch succinate-SMX complex

![DSC thermogram of corn starch succinate-SMX complex](image1.png)

Figure 61: DSC thermogram of corn starch succinate-SMX complex

4.7.2.5 Amaranth starch succinate-SMX complex

![DSC thermogram of amaranth starch succinate-SMX complex](image2.png)

Figure 62: DSC thermogram of amaranth starch succinate-SMX complex
4.7.2.6 Maize starch oxidized-SMX complex

Figure 63: DSC thermogram of maize starch oxidized-SMX complex

4.7.2.7 Sago starch oxidized-SMX complex

Figure 64: DSC thermogram of sago starch oxidized-SMX complex
4.7.2.8 Corn starch oxidized - SMX complex

![DSC thermogram of corn starch oxidized-SMX complex](image1)

Figure 65: DSC thermogram of corn starch oxidized-SMX complex

4.7.2.9 Amaranth starch oxidized-SMX complex

![DSC thermogram of amaranth starch oxidized-SMX complex](image2)

Figure 66: DSC thermogram of amaranth starch oxidized-SMX complex
**Starch derivative-GTFX complex**

4.7.2.10 Maize starch acetate-GTFX complex

4.7.2.11 Maize starch succinate-GTFX complex
4.7.2.12 Sago starch succinate- GTFX complex

![Figure 69: DSC thermogram of sago starch succinate-GTFX complex](image)

4.7.2.13 Corn starch succinate- GTFX complex

![Figure 70: DSC thermogram of corn starch succinate-GTFX complex](image)
4.7.2.14 Amaranth starch succinate- GTFX complex

4.7.2.15 Maize starch oxidized- GTFX complex
4.7.2.16 Sago starch oxidized - GTFX complex

Figure 73: DSC thermogram of sago starch oxidized - GTFX complex

4.7.2.17 Corn starch oxidized - GTFX complex

Figure 74: DSC thermogram of corn starch oxidized - GTFX complex
4.7.2.18 Amaranth starch oxidized- GTFX complex

Differential scanning calorimetry is the best technique to study the thermal behavior of the pharmaceuticals with respect to temperature. In the present study the thermal behavior of plain drug and their complexes with starch derivative were studied in order to confirm the formation of drug polymer complex.

The plain drug and drug starch derivative complexes were subjected to DSC analysis in the range of 100 to 300°C with the heating rate of 20°C/min. The thermal analysis results of drug and drug starch derivative complexes were then compared to draw conclusions. The DSC of SMX & GTFX are shown in Figure 13 and Figure 16 and that of various starch derivative complex in Figure 59 to Figure 76.

The thermal analysis results of Sulphamethaxazole showed the sharp endothermic peak at 171.15°C. Heat of fusion and height of the peak was found to be -122.8J/g and -32.5mW respectively. MA-SMX, AS-SMX, CO-SMX showed reduction in endothermic peak value of 5°C or more. MA-SMX, MS-SMX, CS-SMX, A-SMX, AO-
SM & CO-SMX showed peak height of -10.8mW,-15.3mW,-10.6mW,-9.25mW,-14.58mW & -15.mW in comparison to -32.5mW of the pure drug. This fact indicates the formation of starch derivative-SMX complex with the poor interation. The probable lower degree of substitution along with weak bonding of the SMX with starch derivative may contribute to poor interaction.

The thermal analysis results of Gatifloxacin showed the sharp endothermic peak at about 184.16 °C suggesting the melting point of the drug. Heat of fusion and height of the peak was found to be -58.33J/g and -7.63mW respectively. In case of drug-starch derivative complexes, shift in endothermic peak from that of drug by 4 to 6°C was observed. Sago starch succinate GTFX complex showed highest shift of 6°C as shown in Figure 70 suggesting the strong interaction (complex) between drug and starch succinate, whereas, amaranth starch succinate GTFX complex showed lowest shift as shown in Figure 72 suggesting, weak interactions (complex) in drug and starch derivative. In all the complexes heat of fusion as well as peak height was reduced except for sago starch oxidized GTFX complex as shown in Figure 74. DSC analysis result of maize starch acetate GTFX complex showed less interaction i.e. poor complex formation. This might have occurred because of poor solubility of maize acetate in water and most of the polar solvents. The DSC data taken together with less solubility of the complexes in water and common organic solvents, suggest their existence in the solid state as polymeric structure.
4.7.3 Scanning Electron Microscopy:

4.7.3.1 Corn succinate –SMX complex (CS-SMX)

Figure 76: SEM image of corn succinate – SMX complex

4.7.3.2 Amaranth succinate-SMX complex (AS-SMX)

Figure 77: SEM image of amaranth succinate – SMX complex
4.7.3.3 Corn succinate-GTFX complex (CS-GTFX)

Figure 78: SEM image of corn succinate – GTFX complex

4.7.3.4 Amaranth succinate-GTFX complex (AS-GTFX)

Figure 79: SEM image of amaranth succinate – SMX complex

SEM study of the complexes showed surface morphological characteristics of complexes. It indicate formation of very small size granules of CS-SMX complex and
CS-GTFX complex with smooth appearance in comparison to the CS alone which exhibited bigger size granules with rough surface. AS-SMX showed increase in complex granule size whereas AS-GTFX showed smooth surface granules with aggregation. However, SEM study indicates slight change in superficial appearance in the granules and hence low level of attraction in the complex molecules.

4.8 Antimicrobial screening

4.8.1 Starch derivative-SMX complex

4.8.1.1 Zone of inhibition

The diameters of ZOI obtained were 8 mm and minimum of 12 mm for SMX and sago oxidized-SMX & amarnath-oxidized-SMX complex respectively. This clearly indicates the antimicrobial activity was enhanced with starch derivative-SMX complex as compared to SMX alone. The zone of inhibition obtained by the starch derivative alone was undersized as compared to plain SMX or starch derivative – SMX complex. Corn starch succinate-SMX exhibit maximum ZOI of 16 mm. Maize starch acetate-SMX exhibit ZOI of 18mm. This suggests that the improved antibacterial efficiency of the complexes might be due to loose interaction of the SMX with the starch derivative leading to improved hydrophilicity, wettability and/or dissociation of drug from the complex as well as better partitioning of the drug into the microorganisms. The ZOI are shown in Figure 81 to Figure 83.
Figure 80: Zone of inhibition for starch oxidized-SMX complex

Figure 81: Zone of inhibition for starch succinate-SMX complex
4.8.2 GTFX

4.8.2.1 Zone of inhibition

The Diameters of ZOI obtained were 7 mm and minimum of 09 mm for GTFX and sago oxidized-GTFX complex respectively. Amaranth starch succinate-GTFX complex shows maximum ZOI of 18 mm whereas maize starch succinate-GTFX showed 16 mm. Succinate starch-GTFX complex exhibits more ZOI as compared to the starch oxidized –GTFX complexes. Starch acetate –GTFX complexes exhibits ZOI of 16 mm. This clearly indicates the antimicrobial activity was enhanced with starch derivative-GTFX complex as compared to GTFX alone. The zone of inhibition obtained by the starch derivative alone was undersized as compared to plain GTFX or GTFX- starch derivative. This suggests that the improved antibacterial efficiency might be due to complex formation of the GTFX with starch derivative leading to improved hydrophilicity, wettability and /or dissociation of drug from the complex as well as better partitioning of the drug into the microorganisms. The Zone of inhibition are shown in Figure 84 to Figure 86.

![Figure 82: Zone of inhibition for starch acetate-SMX complex](image)
Figure 83: Zone of inhibition for starch oxidized-GTXF complex

Figure 84: Zone of inhibition for starch succinate-GTXF complex
4.9 Binding study of starch derivatives

Table 13: Binding study of selected starch derivative

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Starch Derivative</th>
<th>Hardness Kg/cm²</th>
<th>Disintegration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Maize starch</td>
<td>4.5</td>
<td>2.6</td>
</tr>
<tr>
<td>2</td>
<td>Maize acetylated</td>
<td>8.0</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>Corn oxidized</td>
<td>2.0</td>
<td>1.25</td>
</tr>
<tr>
<td>4</td>
<td>Amaranth succinate</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td>5</td>
<td>Amaranth oxidized</td>
<td>3.5</td>
<td>2.9</td>
</tr>
</tbody>
</table>

The results indicated that in comparison to the maize starch, maize acetylated showed good binding property as exhibited by more hardness for the tablets which were compressed at the same pressure. This may be attributed to the fact that acetylated starch were more dispersible in hot water forming sticky paste which acts as strong binder. However, all the derivatives produced tablets with acceptable hardness except amaranth succinate. The better hardness of the tablet produced from maize acetylated leads to prolonged disintegration time.
PART V

- CONCLUSION
5 CONCLUSION

Starch is an attractive target of extensive research due to its inherent diverse properties. There are different sources of starch that are recognized for pharmaceutical applications which includes maize starch, rice starch, wheat starch and sago starch. Starch consists of two types of molecules amylose (20-30%w/w) and amylopectin (70-80%w/w). The relative proportions of amylose to amylopectin and branch points both depend on the source of the starch, e.g. amylomaize contain over 50% amylose whereas waxy maize has almost none.

Starch modifications are a means of altering the structure and affecting the hydrogen bonding of amylose and amylopectin in a controllable manner to enhance and extend starch application. When low levels of alterations take place in the molecules, only slight or no change can be observed in the superficial appearance of the granule. Following cross linking, esterification and etherification are the second most important modifications in the starch industry (Taggart, 2004). The main shortcoming of making starch derivatives are very large molecular size, insolubility, instability of viscous solution under varying temperatures and susceptibility to microorganisms. For this reason, chemical modification of starch has become a must to overcome such problems. Acid treatments, oxidation and etherification, esterification, grafting, etc. have been advocated for chemical modification of starch. Literature survey indicated that starch obtained from different natural sources and their chemically modified derivatives were not studied in detail. Some of the starch derivatives like Maize and Sago oxidized, Maize and Sago succinate were not reported in the literature. Hence the objective of the present research work was to synthesize, characterize various starch derivatives (oxidized, succinate and acetate) prepared from starch obtained from different natural
sources viz Maize, Sago, Amaranth and Corn and evaluate them for their pharmaceutical application in drug delivery system.

Raw materials like Sago starch, Amaranth starch, Corn starch and Maize starch were characterized by different test such as identification test, pH, acidity, oxidizable substances, fluroscence, and loss on drying. All the starch derivatives complies with the pharmacopoieal test. Similarly the model drug used in this study like Gatifloxacin (GTFX) and Sulphamethaxasol (SMX) were also evaluated and characterized by different tests. These materials were stored as per the recommended conditions and used for further studies.

Succinate derivative of starch were prepared by acetylation of starch obtained from different sources viz Maize, Sago, Corn and Amaranth with succinic anhydride by following the prescribed procedure and reaction conditions. The product obtained was dried. The derivatives (MS, SS, CS, and AS) were further evaluated by TLC technique, physical properties, IR technique and DSC. Selected derivative like corn and amaranth succinate were studied for their surface characterization by SEM technique.

Acetate derivatives of starch obtained from different sources viz Maize, Sago, Corn and Amaranth were prepared by acetylation with acetic anhydride under prescribed reaction conditions and procedure. The product obtained was dried and further evaluated by TLC technique, physical properties, IR technique and DSC. The result of the experiments indicated the formation of only maize starch acetate (MA) derivative. Other acetate derivatives of Sago, Amaranth and Corn starch (SA, AA, CA) were not formed under the prescribed reactions conditions.

Oxidized starch derivatives were synthesized by reacting starch obtained from different sources with sodium hypochloride and sodium hydroxide under standard reaction
conditions. The derivatives were further evaluated by TLC technique, physical properties, IR technique and DSC. MO, SO, AO & CO derivative were prepared

The prepared derivatives were subjected for solubility study/ dispersibility study in a aqueous media and it indicated improved aqueous solubility of almost all the starch derivatives than their respective types of starches. MA, CS, AS, and AO shows formation of sticky/ thick aqueous dispersions/ mucilage. Almost all derivatives were found to be insoluble in organic solvents like benzene, acetone, ethanol and dichloromethane.

TLC study indicates that different derivatives have different Rf values which are less than one.

IR study indicates the formation of starch derivatives which can be confirmed by appearance of strong peaks in the range of 1680-1740cm⁻¹. Starch is a polymeric carbohydrate molecule, starch polymers and their derivatives like acetates, succinate or oxidized did not have a characteristic thermal behavior which could be used for its characterization or identification criteria. Hence DSC thermogram cannot be considered as a tool to characterize the starch derivatives.

Selected starch derivative were subjected to biocompatibility study. The starch derivatives were compared with saline solution as biocompatible and water as producing 100 % heamolytic effect. This study revealed that amaranth succinate exhibited 86% heamolytic activity, and maize succinate 23% activity. MA, CS, AO, and CO derivatives did not exhibit reasonable heamolytic activity and hence may find application in parenteral formulation.

In the present study Sulphamethaxazole (SMX) and Gatifloxacin (GTFX) (antibacterials) were used as a model drugs. They belong to antibacterial category. An
attempt was made to prepare complexes of these two drugs with the different starch derivatives by co evaporation method. Total nine complexes of starch derivatives were prepared with SMX and nine complexes of starch derivatives were prepared with GTFX. The prepared complexes were characterized by IR study, DSC and selected derivatives were studied for their surface characteristics by SEM.

IR study indicates the formation of starch derivative drug complex due to reduction in intensity of corresponding peaks and shifting of peaks to lower frequency. This indicates that the type of complexing forces involved may be hydrogen bonding and weak Vander Wall interaction. DSC study for the derivatives prepared indicated shift in endothermic peak and reduction in enthalpy and peak height. This fact contributes to the confirmation of starch derivative complexes. However DSC study indicated poor complex formation of maize acetate-GTFX complex. Morphological study of succinate derivatives like corn succinate-SMX and corn succinate-GTFX showed appearance of small size granules with comparatively smooth surface as compared to the Corn succinate (CS) granules. Amaranth succinate-SMX and amaranth succinate-GTFX showed increase in granule size. Amaranth succinate-GTFX showed aggregation of granules.

The prepared derivatives, drug starch complexes and the plain drug were evaluated for their antimicrobial activity by performing the zone of inhibition study. The ZOI study indicated that plain SMX had ZOI of 8 mm in comparison to the minimum ZOI of 12 mm for starch derivative SMX complex. Starch derivatives alone did not show any antimicrobial activity. Maximum ZOI was exhibited by maize starch acetate-SMX complex i.e. 18 mm. This study clearly indicates the improved efficiency of complex
for its antimicrobial activity over plain drug and hence can be used as an effective carrier for drug delivery system.

Similarly antimicrobial activity study was performed for plain GTFX, Plain starch derivatives and starch derivatives GTFX complex. ZOI study indicated the ZOI of 7 mm for plain GTFX in comparison to minimum of 9mm for starch derivative-GTFX complex. Maximum ZOI of 18 mm was reported for Amaranth starch succinate-GTFX complex whereas Maize succinate GTFX complex exhibited 16mm. Succinate complexes exhibited more activity than oxidized complexes than acetate derivative-GTFX complexes.

The selected starch derivatives which showed formation of sticky mucilaginous mass after treating them with hot water during earlier studies, like MA, AS, CO and AO were evaluated as a binding agent in tablet formulation in comparison to Maize starch as a binding agent by wet granulation method. The result indicated that MA exhibited strong binding properties by producing tablet having strength of 8 kg/cm² and disintegration time of 24 min in comparison to AO which produces tablet having hardness of 3.5 kg/cm² and disintegration time of 2.9 min. This indicates that MA derivative can be use in retarding the release of the drug from the tablet formulation.

Thus it can be concluded that the prepared derivatives (acetate, succinate and oxidized ) of the starches obtained from the different sources viz. Sago, Amaranth, Corn and Maize which were synthesized, evaluated and characterize for the different properties, exhibited different properties depending on the source of starch. They were further evaluated for their pharmaceutical applications as a drug carrier. Some derivatives like MA, CS, AO and CO were found biocompatible. MA, CS, MS, AS, SS, CO, MO, AO and AS derivatives could be successfully used to prepare complexes with the
antimicrobial drugs like SMX and GTFX with improvement in antimicrobial activity of the drug in complex form, which could lead to decrease in the dose of the drug. This concept can be use in different formulation development. Similarly some derivatives like MA, AS, CO and AO can be use as a binding agent. However these derivatives need to be further evaluated further for their complexation and invivo and other properties.
PART VI

• REFERENCE
6 REFERENCES


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