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Dissertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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* * * * * * *

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INTRODUCTION

Starch, the chief carbohydrate occurring in a wide variety of plants, has a multitude of applications in many areas of our life; foods, drug therapy, and many industries (1). It is converted to sugars, syrups and dextrins. It is used in textiles, paper and wood products industries. Besides being used as a food for men and animals, starches have been employed in a vast number of situations because of their physical and chemical properties and low cost. In medicine and pharmacy starch had a long history of usefulness. This work will deal with the subject of selected starches, starch fractions and selected drug molecules that are known to complex or are suspected of forming complexes.

Various species of starches have been recognized as official drugs in Pharmacopeias of many countries. In the United States Pharmacopeia XVI, corn starch is officially recognized. Other Pharmacopeias recognize potato starch or wheat starch.

The medicinal use of starches has been entirely empirical. It is described as a protective, an adsorbant or as a diluent in texts on pharmacology, with no explanation of the mode of action. Starch appears to be quite effective in "drying up" dermatitis lesions where
there is a watery exudate; as a consequence starch is found as a component in such pharmaceuticals as dusting powders, pastes, ointments, lotions, emulsions and aerosols. The products may be relatively simple or rather complex in the number and kind of other materials employed. The importance of starch inclusion in these products has not been questioned. There has been some belief that a certain species of starch are superior to another in certain cases.

Starch has been widely used in the manufacture of compressed tablets. In this area it has been employed as an excipient (starch paste) as a diluent, as disintegrator and as a lubricant. The widespread use of starches in the manufacture of compressed tablets is associated primarily with the physical properties of starch and its low cost. Starch is also an important raw material for the manufacture of many other chemicals (2).

The literature of the last twenty-five years contains numerous articles on starch research, especially since T. J. Schoch (3) demonstrated the ability of amylose, one of the fractions of starch, to form water insoluble complex compounds with alcohols, thus enabling the chemist to obtain the pure fraction of amylose.

In view of the fast growing interest in polymeric chemistry, starch and amylose are in particular being investigated much more theoretically with the hope of utilization of these materials in a practical way. Although vast number of researches have been published on
the physical and chemical properties of the various species of starch, very little work has been published regarding the possible involvement of these properties of starch with the stability, availability or absorption of the drug. Furthermore, many therapeutic regimens require that the patient swallow oral preparations just before or just after meals. Since many foods and meals contain starches, there is the possibility of a drug complexation with starches at that time. If such complexes do occur, they could be a factor in absorption of the drug.

Complex interactions of polymers with pharmaceuticals is of special interest to the pharmacist; however, pharmaceutical research has contributed very little in the basic detection and understanding of any such complex interactions that might occur between starch and the wide variety of pharmaceuticals.

Guth and Goudah (4) reported complex interactions of potato and arrowroot starches with a number of organic molecules, most of which are used as preservatives in pharmaceutical preparations. The formation of inclusion complexes with different magnitudes of contributions by hydrogen bonding and dipole-dipole interactions were expected. The tendencies of interaction were estimated. An increase in the size of the drug molecule was not always matched by a decreasing tendency of interaction with starch; that is, the smallest drug molecule tested in their work did not show the greatest degree of complexing with starch.
All the complexing drugs tested were polar organic molecules containing at least one hydrophilic group such as hydroxyl or carboxyl. Although all the molecules had very low solubility in water, it was found in general that the most soluble drug showed the highest tendency of interaction with starch. The degree of interaction of the same drug molecule with arrowroot starch was found to be greater than with potato starch.

A combination of attractive or repulsive forces were expected to be functioning to increase or decrease the affinity of the starch polymers for one drug molecule or another; however, no stochiometric data were available in the study due to the polymolecularity of the starch.

Assuming occlusion complex formation, with the amylose fraction of starch trapping the organic molecule, a very wide possibility of different addition compounds may be assumed to occur since such complexes are mainly a result of the architectural design of the polymer. A chemical affinity contribution to such trapping would broaden the possibility for the existence of more interaction complexes but at the same time would increase the difficulty in understanding the nature of the complexes and the mechanisms involved.

The adsorption affinity of one of the starch fractions, amylose, is manifested in all the reactions of starch paste and sols. It is
responsible for retrogradation, for the blue coloration with iodine, and for the formation of a complex with polar organic molecules which provides the most satisfactory method for fractionation of starch (5).

In this work, we conducted further investigations of starch-pharmaceuticals complexes, to study a variety of drug molecules with different affinities for the starch:

(a) To determine the affinities of various starches for a drug molecule, and the affinity of each fraction of the starch for that molecule; thus detecting the contribution of each of the starch fractions, amylose and amylopectin in the complex.

(b) To try to postulate the nature of these complexes.

(c) To detect any affinity of dry starch granules for organic molecules, and check the existence of adsorption complexes between unruptured starch granules and such molecules, since it was known as early as 1941 that starch adsorbs heavy metal ions, silica, protein, and other polar materials which may be present during growth or biosynthesis of the starch (5).
LITERATURE REVIEW

Physico-chemical Properties of Starch

The most abundant and widely distributed carbohydrate reserves in plants are starches. The principle storage organs for starches are the seeds, stem pith and fruits. The starches occur as discrete granules whose characteristics vary from one plant to another. Different starches vary by size and shape of the granules, the temperatures at which they gelatinize in water, the rate of swelling in various solvents, the degree of isotropism evident in polarized light, and the extent to which they combine with iodine.

Since the granules of one starch of the same plant specie are identical and are characteristic of that specie, it is possible by careful physical examination to differentiate and classify starches as to origin. Detail description of the various starches was given as early as 1913 (6). A review of the early literature on the starch granules was given by C. L. Alsberg (7).

Starch manufacture is an important industry. Starches commercial production is a world-wide industry. Starches are prepared from corn (8), from potatoes (9), from rice and wheat grains (10), and other sources like roots of tapioca and arrowroot.
The shape of starch granules found in different plants varies from round or elliptical to polygonal (11). The optical anisotropism of starch granules in polarized light indicates that the polymers amylose and amylopectin exist in an ordered arrangement. X-ray data provide definite proof that crystalline regions occur.

Baker and Whelan (11) regarded the structure of a starch granule as being built up of alternate layers of the two polysaccharide components of starch, amylose and amylopectin. The thickness of the amylose layer decreased from the center of the granule. Recent studies on the sub-microscopic structure of the starch granules by x-ray small angle scattering (12) did not materially change Whelan's concept. A thorough review of the ultrastructure of starch grains, giving the results obtained with use of electron microscope, x-ray diffraction and molecular weight determination techniques, is found in the recent German literature (13).

The heterogeneity of starch was recognized as early as 1834. Its fractionation into simpler components was first carried out quantitatively by Schoch in 1941 (5). The author showed that slow cooling of a hot aqueous starch solution saturated with n-butanol gives a microcrystalline precipitate of a mixture of unbranched chains which he called "A-fraction" and is known now as amylose.* The second

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fraction, which stays in solution, is a mixture of branched chains called "B-fraction" by Schoch. This fraction is known as amylopectin.**

Although there is good evidence in favor of the heterogeneous nature of starch, very real difficulties exist regarding the exact nature of the association of the two components in the granule. The literature prior to 1941 describing the preparation and properties of the two fractions of starch must be viewed with some skepticism, since the terms then used are now used to designate only relatively pure components.

Presently it is generally accepted that the major component, amylopectin (75-85% in most starches), is a multi-branched polysaccharide of high molecular weight and consists of chains of (1-4)-linked \( \alpha \)-D-glucose residues. The chains normally contain an average of about 20-25 D-glucose residues and are interlinked to form a ramified or bush-like structure by means of \( \alpha \)-D (1-6)-glucosidic linkages.

Amylose, the minor component of starch, is an essentially linear polymer built up from several thousand (1-4)-linked \( \alpha \)-D glucose residues. In certain corn and pea starches amylose content can be as high as 75%.

**Ibid.
There is evidence (14) that some starches contain a small proportion (5-10%) of a third polysaccharide. In potato and rubber seed starches, this material was found to be a short-chain amylopectin with average chain length of 13-16 D-glucose residues. It is not, as might be expected, a polysaccharide that is intermediate in structure between amylose and amylopectin.

Evidence regarding the manner in which D-glucose units are combined in the two starch components has been obtained largely from hydrolysis and methylation experiments, from studies on the kinetics of starch hydrolysis in acid solutions (15), from optical rotation consideration and comparison of the rotations of methylated starches to the rotations of methylated D-glucose (16), and from enzymatic hydrolysis studies. All such studies suggest the presence of α-1-4 glucosidic linkages and support the prevalence of that bond.***

After starch is methylated repeatedly a tri-methyl starch is obtained. Its hydrolysis leads almost entirely to the production of 2, 3, 6-trimethyl-D-glucose, but with some dimethyl-D-glucose and 3.8 - 4.6% of 2, 3, 4, 6-tetramethyl-D-glucose (17). This latter product is obviously derived from the nonreducing chain ends. The yield of this latter product suggests the presence of chains with 26-30 anhydro-glucopyranosidic units per nonreducing end.

***For a review covering the development of our present knowledge in starch chemistry, see C. T. Greenwood, Advances in Carbohydrate Chemistry, 11, 336 (1956).
With the development of better methods for the separation of starch into amylose and amylopectin, the methylation work was repeated on those components. Similar results were found for amylopectin. In amylose, however, the terminal group content, determined by the yield of 2, 3, 4, 6-tetramethyl-D-glucose, was found to be one terminal group to every 300-350 residues (0.32% yield) (18).

Further terminal group determinations have been made with the periodate oxidation procedure (19), in which a measure is made for the amount of formic acid formed in the oxidation. Each non-reducing terminal group forms one mole of formic acid. Each reducing terminal group forms two moles of formic acid, and no formic acid is derived from the glucopyranosidic units within the chains. The degree of polymerization of amylose and amylopectin in many starches was determined by this method.

The amylose content of a number of starches as obtained from such periodate oxidation studies is given by Greenwood (20).

In amylopectin, evidence that branching takes place by means of (1 - 6) linkages was given by methylation and hydrolysis studies (21). Confirmation for chain branching through (1 - 6) linkage came with the actual isolation, from the amylopectin hydrolysis, the disaccharide 6 - (α-D-glucopyranosyl) -β-D-glucose or isomaltose (22). Various types of branched structures have been proposed for amylopectin; the most favored one is that suggested by Meyer (23) (24), which consists
of a branched molecule which has no central chain. All other structures proposed contain different arrangement of the same basic, linear unit chains. According to Peat (25) these chains in amylopectin molecule are termed A, B and C chains where:

A-chains are side chains linked solely through their reducing group to the rest of the molecule. B-chains are those to which A-chains are attached, although they themselves are similarly linked through one reducing group to another chain. C-chains carry the reducing group; thus, there is only one C-chain per amylopectin molecule.

Whatever the type of branched structure in amylopectin, it is possible through a determination of the number of non-reducing terminal groups, after hydrolysis to "limit dextrin" by $\alpha$-amylase, to have an indication of the frequency of chain branching. Such measurements indicate one terminal group exists for every 10-12 D-glucose units (26).

In amylose, methylation studies (18) showed the presence of 0.2-0.4% of non-reducing terminal group corresponding to a length of 200-350 D-glucose units. The linear nature of the molecule was first suggested by Meyer (27). Molecular weight determinations of potato starch amylose by osmotic pressure method were found to agree with the chain length given by the methylation studies. All chemical methods used to answer the question of linearity are questionable. Perhaps the best evidence for assuming that amylose is a long linear
polymer is derived from the simple observation that amylose and its triacetate can cast into strong pliable films which, on elongation, undergo molecular orientation such that the films increase in strength, become birefringent and develop a marked "Fiber" type x-ray pattern (28) (29). Studies on the orientation of the amylose-iodine complex and of amylose alone, as evidenced by streaming birefringence, is a good indication of the existence of amylose as a highly aniso-dimen-
sional molecule (30) (31). The flexibility of amylose fiber is indicated by the production of commercial films of amylose recently prepared by heating a mixture containing solid amylose with glycerol and water (32).

It should be noted here that, in spite of all the differences be-
tween the two starch fractions, these fractions have many points in common. Amylose, as explained above, is a linear chain composed of \( \alpha -D\)-glucopyranose residues linked by \( \alpha -D-(1 - 4) \) glycosidic bonds; on the other hand, each of the branches of the amylopectin molecule has the same structure as amylose but amylopectin contains besides \( \alpha -D-(1 - 6) \)-glucosidic bonds at the points where branching occur. Hence, both polymers contain the same chemical unit of structure in extensive parts of their molecules, and, moreover, all these units have the identical stereochemical configuration. The boat configuration of the glucose residues in amylose was shown by the analysis of the submicro-
scopical structure of starch grain in a recent work by Sterling (33).
Thus one can reasonably say that the main structural features and the highly polymeric nature of the two components of starch are now well established, but the chemical problems connected with the reactions and the behavior of starch and its two components are yet to be elucidated. Chemical methods of investigation supplemented by physical measurements, which became very helpful in polymer sciences in the last fifteen years, are the necessary methods for better understanding of these problems.

From the pharmacist point of view, a complete understanding of the physico-chemical properties of starch, and its behavior in aqueous solutions is very essential for further investigation of the many possible reactions, and the different affinities of this important carbohydrate. An excellent review on the problems concerning starch chemistry with more than one hundred references was recently published in the German scientific literature (34).

Starch Behavior in Water

The Physical and Chemical Properties of Aqueous Starch Sols

Unmodified starch granules are insoluble in water. In warm water, however, the granules will swell greatly and produce a viscous paste at low concentrations. At a certain temperature, characteristic of the starch type, gelatinization occurs. When a suspension of starch granules in cold water is heated, water is slowly taken up and a
limited swelling occurs; then at a specific temperature (within ± 1°C) the granules undergo a sudden rapid irreversible swelling. The granules swell to several hundred times in original volume, thus losing birefringency. The viscosity of the suspension increases greatly. As the temperature of the suspension is raised the final phase is reached, indicated by diffusion of starch polymers from some granules into the solution and by the complete rupture of others.

A similar swelling may be induced at room temperature by chemical agents like formic acid, chloral hydrate, strong bases and metallic salts.

The swelling process, the appearance and the behavior of the starch granules, and the suggested mechanism for the process have been described by Bear (35). Starch pastes are colloidal systems in which there are highly swollen granules, free molecules of the starch two polymer components, and empty granule sacs. Formation of these sacs taken in conjunction with some other properties of the granules has led some investigators to believe that the granules are surrounded with a distinct membrane. The difference in the properties of various starch pastes is due largely to the degree of swelling which the granules undergo, and the ease with which the rupture of the granules occurs; thus the character of the paste is related to the granular properties of the unswollen starch.
The physical properties of starch suspensions and starch gels may be greatly altered by modifying the starch by oxidation, by pyrolysis, and by hydrolysis with acid or with enzymes. These processes are used in industry to prepare what is known as modified starches or dextrins.

Starch sols prepared by autoclaving are physically unstable and tend to become less soluble on standing, as evidenced by an increase in viscosity and opacity. This phenomenon has been termed as "retrogradation." Amylose in solution is unstable and tends to aggregate and precipitate from the solution. The most outstanding property of amylose is its tendency to crystallize. The difference between amylose and amylopectin, as regards crystallization behavior, is one of degree rather than of kind. Amylose shows a far stronger tendency to crystallize than does amylopectin. Whereas aqueous solutions of amylopectin, containing as much as 20% of the polymer, will remain fluid for several hours at room temperature, while amylose solutions of the same concentration rapidly solidify at 100°C.

The suggestion has been made that amylose in stable form is a helix (36), whereas in retrogradation the molecular chains align to form crystallites, which can then grow to form visible aggregates.
In aqueous solutions of amylose, or starch, the probability of the following equilibrium of amylose configurations with given (20):

"Aggregated helics" ⇐ helical configuration ⇐ linear configuration ↫
aggregated linear chains.

Paschall and Foster (37) provided evidence for "aggregated helics" from light scattering measurements on amylose in solvents of varying pH. The periodate oxidation studies on aggregates of amylose in aqueous solutions favored the existence of amylose in α-helix form in solution (38). The properties of amylose chains, their flexibility and the steric configuration have been the subject of very extensive investigations. In view of the great successes of cellulose chemistry, studies of chain properties of amylose as compared with cellulose are published very frequently (39) (40).

Retrogradation is accompanied by opalescence, increased resistance to enzymatic degradation, and a decrease in viscosity. Different starches retrograde at different rates. The study of three different starches by Whistler (41) showed that the rate of retrogradation was in the order of potato < maize < wheat. The rate depends on the amylose content in the starch, on the size of the amylose molecule and the method by which the starch sol is prepared. Turbidimetric measurements (42) show that retrogradation time is inversely proportional to the chain length until a certain critical value of length is reached,
below which the molecules are too small to crystallize. The low retrogradation tendency of potato starch is related to its large size amylose molecule; the molecules are too large to undergo orientation.

Amylose sols degrade by hydrolysis in the presence of alkali. It was shown that such hydrolysis is an oxidative one (43). Oxygen presence is important in such degradation. Potato amylose was suggested to contain "oxygen sensitive" bonds (44). Aqueous solutions of starch and amylose which had been prepared under oxygen-free conditions were stable in the absence of oxygen, but showed an initial very rapid breakdown when heated in the presence of oxygen. This was followed by a slow oxidative degradation.

Amylopectin is more stable than amylose in solution. Its molecules do not aggregate as easily as those of amylose; thus, amylopectin does not contribute to the retrogradation phenomenon noticed in starch sols. The occurrence of aggregation of amylopectin molecules was shown to be improbable (45).

Slight degradation of the amylopectin fraction in a number of starch sols was shown after the autoclaving of the starch prior to fractionation (46). Although degraded by alkali amylopectin, like amylose, is stable in alkali system in the absence of oxygen.

It was shown that treatment of maize starch granules with 0.1N sulfuric acid results in preferential hydrolysis of amylopectin (47). This was also established by a chromatographic analysis of the
degradation products (48). In general if starch granules are treated with one normal acid or stronger, both components of starch are hydrolyzed by equal proportions. The rates of degradation of amyllose and amylopectin in 7.7N hydrochloric acid at 30°C have been measured by Swanson and Cori (49). From comparison of the rates of degradation of the two polymers, these authors concluded that the 4 - 1 - D bonds found in the linear amyllose and in the short branches of the amylopectin are less stable to acid hydrolysis than the 6 - 1 - D bonds found in amylopectin.

The molecular weight and the degree of polymerization (DP) for amyloses and amylopectins obtained from various starches were subjected to extensive studies. Early measurements were done by chemical methods including the many methods for the determination of chain ends, either the reducing or the non-reducing terminal group, or both, as the case may be, and the methods of measurements of the amount of formic acid produced by oxidation with periodate. These methods give a "number average" molecular weight, and suffer from a disadvantage of determining some present terminal groups which may have been previously produced or lost by degradation.

Physical methods have been used for determining the degree of polymerization and molecular weight of starch components in various solvents. These methods include osmotic pressure measurements (50),
sedimentation measurements (51), light scattering (52), and diffusion measurements (53). Viscosity measurements have been used, but their importance is questioned due to the irregular viscometric behavior of amylopectin.

Physical methods lead to more accurate molecular weights than those obtained by chemical methods. Some of the methods give a "number average" molecular weight (Mn) (osmotic pressure), while some others give a "weight average" molecular weight (Mw) (light scattering). The difficulty in characterizing the molecular weight of starch adequately is caused by the amylopectin fraction. Most methods are undependable if used without further secondary supporting measurements. The validity of light scattering measurements on amylopectin was discussed by Erlander (52). Most physical methods show concentration dependence when used for estimation of high molecular weight amylopectins (51).

The methods for evaluating the molecular weight distribution and the polymolecularity of starch are part of the molecular weight measurements. Estimation of the polymolecularity of a starch sample can be obtained from comparisons of (Mn), (Mw) obtained from two different physical methods. Such estimations have been described frequently in the literature (54).
Starch-Iodine Reaction

The characteristic blue color formed when starch is combined with iodine is well known. Amylose solutions produce with iodine a deep blue complex which is insoluble in water. Amylopectin solutions, on the other hand, produce a red-purple color with iodine. Since the two starch components differ in the colors produced with iodine, spectrophotometric methods have been proposed for the determination of the amylose content of a starch (55).

Solutions of pure amylose in water react with iodine to form "amylose-iodine" complex in which about 200 mg. of iodine are bound per one gram of amylose, roughly one iodine molecule per 7 or 8 D-glucose residues, the amount bound could be determined by a potentiometric titration (56); the method showed that amylose adsorption of iodine to form a complex reaches a level of saturation. Amperometric titration methods have been used for the estimation of iodine absorption of starch (57). X-ray studies (58) (59) showed that in starch-iodine complex the amylose chains assume the configuration of a tight helix 13A in diameter. The affinity of amylose for iodine was shown to depend on its chain length, the shorter chains having the lower affinity (60).

Pure amylopectin does not form a complex with more than minute amounts of iodine. It can adsorb iodine at rather high iodine
concentrations only. The reason for the low affinity of amylopectin for iodine was explained by strong steric limitations of the branched structure, and by the relative short outer branches (20-30 D-glucose units) compared to amylose (500-2000 D-glucose units) (60). It is, however, suggested that at least the outer branches of the amylopectin molecule adsorb iodine, and, as they have essentially the same structure as the amylose molecule, their complexes will possess the same configuration as those of amylose.

The assumed mechanisms of the interaction of iodine with the two starch fractions have been described in several papers. These mechanisms will be reviewed later in the discussion part of this work, for the sake of comparison between "starch-iodine" complexes and those complexes formed with the organic molecules with which this work is concerned.
EXPERIMENTAL

The Determination of Molecular Weights of Starches

Theory

The methods which can be used to determine the molecular weight, size and shape of polysaccharides have been reviewed by Greenwood (61). Special problems exist in the case of starch due to the branched nature of amyllopectin molecule. Problems also arise from the aggregation of the polymer molecules (especially amylose) in aqueous solutions. Studies of the derivatives of the starch components are therefore more convenient.

Light scattering measurements are applied in this work. The subject of light scattering has been treated in an excellent manner by Tanford (62).

In order to develop a satisfactory theory for light scattering from liquids or liquid solutions, it is necessary to treat such dilute solutions as equivalent to a gas in which the solute molecules behave as do the molecules of the gas. The classical theory for light scattering from a gas was developed by Rayleigh and is usually discussed in all the books treating the subject of light scattering (62, 63, 64).

The interest of macromolecular chemist in light scattering arises from the fact that scattering intensity from a single particle
is proportional to the square of the molecular weight. The original work on determining the size and the shape of macromolecules by means of light scattering measurements was done by Debye (65). In his treatment the turbidity $\tau$ is defined as the fractional decrease in the transmitted light intensity or

$$\frac{I}{I_0} = e^{-\tau}$$  \hspace{1cm} (I)

Where $I_0$, is the intensity of the incident beam,

$I$, is the intensity of the transmitted beam,

e, is the base of the natural log,

$\tau$, is the turbidity in cm$^{-1}$, and

$l$, is the length of the scattering medium.

From this equation, turbidity can be called the natural logarithm of the fractional decrease in the transmitted intensity of the light.

From Rayleigh's original "scattering from gases" theory, Debye arrived at the following equation for scattering from solutions

$$\tau = \left(32 \pi^3/3\right) \left(\gamma^2 n^2/N \lambda^4\right) M C$$  \hspace{1cm} (II)

Where $\tau$, is the turbidity as above, in cm$^{-1}$,

$n$, is the refractive index of the solvent,

$N$, is Avogadro's number,

$\lambda$, is the wavelength of light in cm,
\( \gamma \) is the refractive index increment. It can be considered as constant if the dilution is sufficiently great.

\( C \), is the concentration in gm/cm\(^3\), and

\( M \), is the molecular weight of the solute.

All values except \( \gamma \), \( C \), and \( M \) are constant, and can be joined in a new value \( H \); Debye equation thus becomes:

\[
\gamma = H \cdot M \cdot C \tag{III}
\]

or

\[
HC/\gamma = 1/M \tag{IV}
\]

where

\[
H = (32 \pi^{3/3}) \left( \frac{V^2 \cdot n^2}{\lambda^4 N} \right) \tag{IVA}
\]

Thus, the turbidity \( \gamma \), is proportional to the concentration if the molecular weight is the same; or, alternatively, the turbidity is proportional to molecular weight if the concentration is constant.

Equation IV was derived on the assumption that the small isotropic particles are independent scatterers in very dilute solutions. At higher concentrations (non-ideal solutions) interference between the scattered waves in the solution occurs, and turbidity is no longer a linear function of the concentration. The equation was corrected for such concentration interference:

\[
HC/\gamma = 1/M + 2B \cdot C \tag{V}
\]

where \( B \) is a constant.

The final limitation on equation (V) is that it applies on small anisotropic particles, i.e., those which have different optical
properties according to the angle of incidence of light. The relations between scattering and dimensions of the particles become more complicated if the particles are larger than 1/20 of the wavelength of the light. A brief description of such relationships and the treatment of light scattering by large molecules is in order.

When a very small, spherical particle is encountered with a light wave the electrons of the particle are made to vibrate in the incident magnetic field associated with the light wave. The electrons will oscillate in unison with the incident wave, becoming the sources of the scattered light. If the particles are very small compared to the wavelength they can be considered as point sources of radiation and according to Rayleigh the intensity of the scattered light is symmetrically distributed. If the particles are comparable in size with the wavelength, the intensity of the scattering depends on the direction in which the observation is made and the "scattering envelop" is unsymmetrically distributed. *

After Debye presented in 1944-1945 his new theoretical approach of light scattering the method became popular in colloidal chemistry. The method was used for molecular weight determination for synthetic polymers as well as natural ones. In the polysaccharides field it has been applied on starch derivatives, glycogen, and sugar beet arabans.

*Illustrations of classical light scattering envelopes can be seen in references (63) and (66).
It is now possible to apply this method not only to very dilute systems containing very small particles of colloidal solute, but also to comparatively coarse systems; moreover, the method can be used to study the size and shape of spherocolloids and linear colloids as well.

For molecular weight determination of a polymer, the Debye equation (V) is used. \( \gamma \), in the equation, represents the measured turbidity; \( C \), is a known concentration term. \( H \), is a constant which can be determined by calibrations (67). It can also be calculated from the equation given for it (IVa). The only unknown in the equation is \( \gamma \), which is the specific refractive increment. It can be determined by a refractometer at the same wavelength of light used for scattering.

**Experimental Determination of Turbidity by Light-Scattering and the Determination of H in the Debye Equation**

Turbidities can be measured by determinations of the intensities of scattering at a 90° angle by means of an absolute photoelectric turbidimeter. Detail description of such apparatus is given by Debye (68), by Zimm (69) and by Brice, Halwer and Speiser (70). The important feature of such turbidimeter is that the photomultiplier tube, serving as a detector, is supported on an arm which may be rotated about the cell containing the scattering solution. The scattered intensity may thus be measured directly at any angle.

An alternative experimental procedure is to measure the diminution of the intensity of the incident beam; i.e., the loss of light
intensity as the beam passes through a unit volume of solution ($I_1$ in equation I is unity). This can be done after making certain that absorbance is negligible at the wavelength used. By analogy with absorption spectroscopy using Equation I (Page 23), turbidity $\gamma$, can be defined as:

$$\gamma = -\ln \frac{I}{I_0} \text{ when } (1 = 1 \text{ cm})$$

(VI)

where $\gamma$, is turbidity in cm$^{-1}$.

$I_0$, is the intensity of incidence.

$I_1$, is the intensity of light after passing through a volume of solution placed in a cell of 1 cm width.

In equation (V) the constant $H$ can be determined.

$$H = \left(\frac{32\pi^3}{3}\right) \left(\frac{n^2 \cdot \gamma^2}{N \cdot \lambda^4}\right) \text{ (See before IVa, Page 24)}$$

The only unknown is $\gamma$, which is defined as:

$$\gamma = n - n_0 / C$$

(VII)

where $n_0$, is the refractive index of the solvent,

$n$, is the refractive index of the solution, and

$C$, is the concentration in gm/cm$^3$.

Therefore, this value can be determined with a differential refractometer. All other values needed for determination of $H$ are known.

$N$, is Avogadro's number; $\lambda$, is the wavelength.
Experimental Procedure for Molecular Weight Determinations of Starch

Potassium hydroxide solution, 0.5 M is prepared from analytical grade reagent. The solution is filtered carefully through fritted glass filters.

Dilute solutions of the starches or their fractions to be tested are made using 0.5 M KOH solution. The concentrations used varied between $1 \times 10^{-3}$ gm/ml and $4 \times 10^{-3}$ gm/ml. Pure amylose solutions are prepared with extra special care. Solutions are achieved after addition of the alkali to the amylose solid, stirring with a glass rod driven by a motor, while nitrogen is bubbled through the solution to avoid any oxidative degradation. The starches and amylpectins produce solutions instantly after the addition of alkali. All solutions are gently strained through glass wool, and measurements of the transmitted light at 430 mu are taken immediately on Beckman D. U. Spectrophotometer, using 1 cm cells. Tables 2-11 (Pages 46-50) show the results of the calculated turbidity $\gamma$, using equation (VI).

A series of solutions are prepared for refractive index measurements. Table 1 shows the results of one such measurement in potato starch.

**For more exact light scattering measurements an instrument like "Brice-Phoenix Universal L.S. Photometer" can be used. The scattered light at different angles can be measured, and Zimm treatment of the data must be followed (69).
The solvent scattering is not considered in this work since the spectrophotometer was calibrated on 100% transmittance for the 0.5 M KOH solution.

The treatment of data to calculate the molecular weight of the sample is shown in the chapter dealing with the results (tables and figures on Pages 45-71). The molecular weight values are obtained the following way:

The values \( \frac{HC}{\tau} \) are plotted against concentration \( C \) (gm/ml). The intercept of the line with the ordinate is \( 1/M \) value; the constant \( B \) (equation V) can be calculated from the slope of the straight line.

**Fractionation of Starch**

**Theory**

The fractionation of starch to its components can be done by many techniques. Since 1941 when Schoch (2) demonstrated that slow cooling of a hot aqueous starch solution saturated with 1-butanol gives micro-crystalline precipitate of a linear starch component (A-fraction), it has become generally recognized that practically any polar organic reagent with a high enough solubility in water, can form crystalline precipitate with amylose (71). Reviews covering the literature on starch fractionation up to 1961 have been the subject of three workers: T. J. Schoch (5), C. T. Greenwood (20), and J. Muetgeert (72). The Schoch
method was called fractionation by complexing agents. Many other methods have been developed among which are: the leaching technique, the fractional precipitation method using salt solutions as fractionating media, and other industrial methods. The recent studies dealing with fractionation of starch concentrated on improvements in the yield of fractions and methods for recovering pure amylose in complexing processes (73).

Patented processes for fractionation are mostly those based on the ability of amylose to form insoluble complexes with polar organic compounds. In all such processes the "yield" of amylose varies with the complexing reagent and is not, therefore, quantitative. Other principles have been used for the basis of patented fractionation processes, one of which is an idea developed by Bus and Muetgeert in 1950, using aqueous salt solution as fractionation media for potato starch (74).

**The Experimental Procedure for the Fractionation of Potato Starch to Obtain Crystalline Amylose**

The method of fractionation based on complexing amylose with \( n \)-butanol was followed in this work. The following two different procedures were tried in order to obtain the amylose fraction of potato starch.

The first procedure was to prepare a starch sol by autoclaving. This was saturated with \( n \)-butanol while hot.
Forty grams of potato starch were weighed in a beaker, smoothed into a slurry with 25-50 ml distilled water, then poured rapidly into a 2-liter beaker containing 850 ml of boiling distilled water. The paste was stirred, then transferred to an autoclave and heated at a pressure of 20 lb/square inch for three hours. The sol thus prepared was made up to one liter volume by adding boiling water. The beaker was then placed in a hood and the contents stirred gently. The sol was saturated with 1-butanol, while stirring continued slowly, in order to get well shaped crystals of an amylose-butanol complex (5). The precipitate was observed to occur at 60°C. The stirring of the mixture was continued until the sol was cooled to room temperature. The precipitate was allowed to settle for two hours. A thin layer of clear supernatant was siphoned off. The partially sedimented solid was then centrifuged at 7000 r.p.m. for 20 minutes in polypropylene buckets. The supernatant was siphoned off again and the complex was stirred in 500 ml of 1-butanol saturated water and re-centrifuged as before.

It was reported (75) that this last wet "amylose-butanol" complex is stable if stored in a stoppered container. It is readily soluble in water at room temperature. Most of the residual butanol can be removed from the solution, if desired, by bubbling oxygen-free nitrogen through the sample for 10 minutes in a beaker heated on a steam bath.
This procedure was first implemented to prepare amylose solutions used in the complexing study. It proved impossible, however, to determine the exact concentration of amylose in the solutions. Therefore, the precipitate of amylose-butanol complex was re-suspended in distilled water, and centrifuged at 10,000 r.p.m. as recommended for the removal of butanol from the complex. The new precipitate was dried in the oven at 110°C and stored in a desiccator. A part of the dry crystalline powder thus obtained was micropulverized before it was used for preparing amylose aqueous solutions for the complexing study.

The alternative procedure used in this work was to autoclave the starch sol after saturation with 1-butanol.

Forty grams of starch were gelatinized in boiling mixture of one liter of water and 200 ml of 1-butanol. The pH was adjusted to 6.5 with potassium phosphate buffer. The resulting paste was heated for two hours in the autoclave at 20 lb/square inch pressure, then cooled while being stirred slowly. The complex precipitate was recovered by centrifugation.

This procedure gave a better yield of amylose and was satisfactorily applied on amounts four times greater than described above.

The amylopectin fraction in both procedures stays in solution. The solutions were disregarded, since the fractionation procedures were not efficient to obtain pure amylopectin (free from amylose).
It is obvious that any fractionation product should be adequately characterized. The most accurate method for ascertaining the purity of starch components is that used for determining amylose/amylopectin ratio in starch. It is a potentiometric determination for the amount of iodine bound by the starch fraction (56). Colorimetric methods have been suggested (76) (55). These methods can be used for comparative measurements, but are not absolute.

The precipitation of amylose in this work was not quantitative. Amounts of the linear polymer were obtained by the fractionation of potato starch. The purity of the amylose obtained was not important, since molecular weight values, determined by light scattering were the basis for the comparisons of the starches and the starch fractions. Industrially prepared potato starch amylose was also used in the study after similar molecular weight determinations.

Complexation

Starch Sols

Preparation of starch sols. — The required amount of starch or starch fraction to make 500 ml of the sol was accurately weighed into 125 ml beaker, smoothed into a slorrry with distilled water, then poured all at once into a liter beaker containing 400 ml of boiling distilled water. The gelatinized paste was stirred for five minutes with a motor drive stirrer, then transferred to an autoclave and heated at the
pressure of 20 lb/square inch for three hours. The resulting sol was then stirred until cooled to room temperature. The sol was then transferred to a 500 ml volumetric flask, and distilled water added to obtain 500 ml of the sol. The viscosity of the sol was decreased markedly after autoclaving (4). All sols were discarded after 24 hours of preparation.

The amylose fraction obtained from potato starch was used to prepare similar sols which were autoclaved as above. The sols retrogradate very rapidly after cooling. Another series of sols were prepared from the amylose; after micropulverization of the crystalline fraction both sets of amylose were used in the complexing study.

The amyllopectin, obtained from commercial sources, produced clear viscous solutions which did not show notable retrogradation in a 24 hour period.

Determinaton of complex formation equilibration. — The solubility method of Higuchi and Lach (77) was used to study complex formation.

Methods for determining solubilities under a wide range of experimental conditions have been reviewed by Zimmerman (78). In this study the variations in solubility of the drugs with the composition and the concentration of the starch aqueous sol, are measured at a constant temperature.
An amount of the material to be tested for complexing equivalent to at least three times its normal solubility in water was weighed into 125 ml bottles containing 60 ml of the starch sols of varying concentration. The bottles were stoppered and then agitated in a constant temperature water bath at 30°C ± 0.3°C for 24 hours or 48 hours according to the system. The times needed for equilibrium were evaluated after determinations of the total amount of the drug entering solution with time. Experimentation on the various molecules used showed that equilibration time is more than 15 hours in all cases, and not more than 48 hours as is the case with sorbic acid.

**Analysis.** — After equilibrium was attained, the agitation was stopped and the bottles left in the water bath for one hour. Aliquots of 2-3 ml were removed from each bottle using 10 ml hypodermic syringes kept at 30-32°C temperature and equipped with B-D 15 stainless needles. The aliquots removed were taken from the liquid above the partially sedimented solid residue. A "Swinny" filter adapter, equipped with S & S analytical filter discs, was placed on the tip of the syringe, and 0.5 - 1 ml of the solution was filtered into a tube placed in the 30°C water bath. The filtered solutions were left for 20 minutes in the bath. Aliquots of these were removed, using pipettes kept at 30°C temperature, and appropriately diluted for spectrophotometric analysis. Dilution factors varied from 1:100 to 1:1000 depending on the solubility and the molar absorbancy of the drug tested.
All drugs were assayed spectrophotometrically on a "Beckman" Model DU Spectrophotometer using 1 cm quartz cells. The wavelengths of maximum absorption were determined on the graphs of absorbance vs. wavelength obtained on a "Cary - 15" recording spectrophotometer. Each drug showed the same wavelength of maximum absorbance in water and in the presence of the starch solution. Beer's law plots were made to calculate molar absorbancies at the wavelengths of maximum absorbance (Table 13, Page 56). The absorbance of 50 gm/L starch sol was negligible after dilutions of 1:500 or more at lesser dilutions; readings were made using the proper dilution of the starch sol (without any drug) as a blank in the reference cell.

Results of the interactions of various starch sols at different concentrations with benzoic acid, p-hydroxybenzoic acid, sorbic acid are shown in Tables 14, 15, and 16 (Pages 57, 59 and 61) in the results section.

Potato starch interactions with o-nitrobenzoic acid, m-nitrobenzoic acid, benzoic acid, p-aminobenzoic acid, m-nitroaniline, o-nitrophenol and p-nitrophenol are shown in Tables 17 and 18 (Pages 63 and 64). The necessary phase diagrams are plotted in the results chapter. (See Figures 8, 9 and 11, Pages 65, 66 and 70).

**Dry Starch**

Dry potato starch was the starch used to determine the affinity of unruptured starch granules for some of the organic molecules used
in the study, and to detect the adsorption of fatty acids and fatty alcohols on the dry starch.

When working with fatty acids potato starch was used without previous defatting, since this starch has been shown not to contain any fatty acids or what is known as "fat by hydrolysis" (79). This was proved to be true in the potato starch used in this work as described later when a sample of potato starch is hydrolyzed for the determination of any fat in the starch.

**Determination of "drug-dry starch" adsorption complexes.**

A saturated solution of the drug tested (benzoic acid, sorbic acid, nitrophenols, etc.) was prepared at room temperature; an amount of the substance equivalent to three times its normal solubility was weighed into a 250 ml beaker. One hundred and fifty milliliters of water was added, and the solution was stirred at room temperature for 30 minutes. One hundred milliliters of the solution were filtered into a 250 ml bottle. An amount of 5-25 gm of dry potato starch was added to the bottle. The bottles were stoppered and agitated in a water bath at 30°C ± 0.3 for 24 hours; then the samples were filtered to collect the starch. The filtrates were tested to assure excess amounts of drug in solution, since it is necessary not to take up all the solubilized drug by a large amount of starch. The starch on
the filtered paper was then washed three times with 25 ml of water at 25°C temperature. The washed starch was transferred to clean, one liter beakers to hydrolyze the starch.

An amount of the drug molecule was adsorbed by the starch. It was only released from the starch after extensive hydrolysis to a soluble dextrin stage with strong mineral acid. The acid used was hydrochloric acid in normal solution. Hydrolysis with \(\alpha\)-amylase gave comparable results, but the time of hydrolysis needed for the complete release of the adsorbed molecules was better controlled in the acid hydrolysis.

The starch in the one liter beaker obtained from the adsorption experiment was suspended in 500 ml of 1 N hydrochloric acid. The beaker was placed on a steam bath, and the mixture was stirred with a glass rod driven by an electric motor until the starch is homogeneously pasted. The motor was slowed down and the heating was continued for two hours. A blank sample of potato starch was hydrolyzed in a similar manner.

The solutions of the sample and the blank were then cooled to room temperature. Sodium hydroxide solution was added to the point of neutralization (pH = 7, measured on a pH meter in all cases). This was done to all solutions since most of the molecules tested for adsorption were weak acids with low solubility in acidic media.
The neutral solutions were filtered into 1000 ml volumetric flasks. The filter papers were washed several times with distilled water and the washing water added to the volumetric flasks. Each solution was completed to a volume of 1000 ml.

Dilutions were made from the sample and the blank solution, thus prepared, for the measurements of adsorbance on spectrophotometer. The dilutions varied from 1:100 to 1:500 according to the amount of starch used in each sample and the molar absorbancy of the drug adsorbed. The dilute solutions were analyzed spectrophotometrically on a "Beckman DU" at the ultra-violet maximum wavelength of each drug used in the adsorption experiment. The blank solution obtained from the hydrolysis of the starch, diluted in the same manner, was placed in the reference cell of the spectrophotometer.

The time used for hydrolysis of starch was determined after a comparison study of a sample of starch agitated with benzoic acid solution. The starch sample was divided into two equal parts. One part hydrolyzed with N hydrochloric acid for two hours; the second part hydrolyzed with one normal hydrochloric acid for a total of six hours under the same conditions. The rest of the analysis was carried out as before. The amounts of benzoic acid determined in the two parts (hydrolyzed
for different time) were almost identical. Results are shown in Table 21, Page 71.

In a final note, the following control experiment was carried out on potato starch:

After agitation of the starch sample in the drug solution for 24 hours, the starch was filtered and washed as before. The starch was suspended then in 500 ml distilled water, and the suspension was agitated in the water bath of 30°C for 24 hours. The suspension was then filtered and the residue was washed. The filtrate and the washing water was collected and completed to 1000 ml volume. Dilutions were made for spectrophotometric analysis of the drug in question. The results shown in Table 21, Page 71, prove that the release of a minor amount of the adsorbed drug molecule occurred in such experiment.

Determinations of the adsorptions of long chain fatty acids and alcohols on dry potato starch. --- Solutions of the fatty acids or alcohols were prepared in two different solvents, methanol and petroleum ether.*** All solutions had the same concentration**** (15 gm/liter).

***Fat solvents used for defatting starch proved to be of different value. The best were those containing hydrophillic groups (or having some polarity) (80). If fatty acids are going to be introduced to the starch, the choice of solvent might be important.

****It was shown that adsorption of palmitic acid on potato starch follows a typical Freundlich isotherm (81); thus, concentration of the fatty acid in solution is a variable and must be kept constant for our study.
Fifty grams of dry potato starch were placed in a bottle; one hundred milliliters of the fatty acid solution were added; the bottle was tightly stoppered, and agitated in a water bath at 30°C for 24 hours.

All samples thus treated were filtered. The methanol or petroleum ether filtrate was tested for the excess of fatty acid by adding water to precipitate out the fatty acids. The starch collected on the filter paper was washed with 25 ml of petroleum ether three times. (The washing was with petroleum ether in all cases.) The washed starches were transferred into scrupulously clean one liter beakers. The starches were hydrolyzed with 500 ml hydrochloric acid 1 - normal solution in the same procedure described on pages 38 and 39. A blank starch sample was hydrolyzed in the same manner.

The solutions after hydrolysis of two hours were cooled, and placed overnight in a refrigerator to solidify any released fatty acids or fatty alcohols. The cold mixtures were then filtered through "Whatman" fluted filter paper, and the beakers and filters were washed repeatedly until the filtrates were free of hydrochloric acid as shown with methyl orange T.S. An insoluble brown residue on the filter paper which represents decomposition products of the starch was observed. The inside and the lip of the beakers were meticulously scrubbed with small pieces of damp filter paper to pick up adherent fatty acids, and these pieces were placed in the fluted filter of each beaker. Each filter
paper was allowed to dry overnight in the funnel at room temperature. A suitable glass soxhlet extractor was set up and several glass beads were placed in the collection flask. The flask was dried overnight in an oven at 110°C and then weighed. The fluted filter and contents were folded up, placed in an extraction thimble and extracted for three hours with petroleum ether. The flask was removed, the solvent was evaporated on a steam bath in a well ventilated hood, and the flask was dried for one hour in an oven at 110°C. The dry flask was weighed for the determination of the amount of fatty acid or alcohol.

A separate moisture determination on the potato starch done on constant weight basis showed an average 10-11.6% moisture present according to the sample used. Results of fatty acids adsorbed to the starch were calculated on dry starch basis assuming 10% moisture in all cases.

Tables 19 and 20 (Pages 67 and 68) show the results expressed in the amount of fatty acid or fatty alcohol adsorbed by one gram dry potato starch.
MATERIALS

<table>
<thead>
<tr>
<th>Starches</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amioca</td>
<td>Commercial starch claimed to be consisting completely of amylopectin.</td>
</tr>
<tr>
<td>Amylon*</td>
<td>Commercial starch containing 55-60% amylose.</td>
</tr>
<tr>
<td>Amylon VII*</td>
<td>Commercial starch containing 70% amylose.</td>
</tr>
<tr>
<td>Potato Starch</td>
<td>Mallinckrodt chemical.</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>&quot;Argo&quot;</td>
</tr>
<tr>
<td>Arrowroot and Rice Starches</td>
<td>S. B. Penick's &amp; Co.</td>
</tr>
<tr>
<td>α-Amylase</td>
<td>Nutritional Biochem. Corp.</td>
</tr>
<tr>
<td>Fatty Acids</td>
<td>J. T. Baker Co.</td>
</tr>
<tr>
<td>P-Nitrophenol</td>
<td>Eastman Organic Chemicals.</td>
</tr>
<tr>
<td>O-Nitrophenol</td>
<td>Eastman Organic Chemicals.</td>
</tr>
<tr>
<td>M-Nitroaniline</td>
<td></td>
</tr>
<tr>
<td>O-Nitro-benzoic acid</td>
<td></td>
</tr>
<tr>
<td>M-Nitro-benzoic acid</td>
<td></td>
</tr>
</tbody>
</table>

All other chemicals used were commercially available and of reagent grade.

*"National" Starch and Chemical Corp., Chicago.
Molecular Weight Determinations

Debye Equation No. (V),

\[ \frac{HC}{\gamma} = \frac{1}{M} + 2BC \]

The constant \( H \) is determined from equation (IVa), Page 24. The constant \( \gamma \), is the only unknown. A sample determination of the constant \( H \) is given as follows:

\[ n - n_0 = \gamma \cdot C \quad \text{(See equation No. VII, Page 27)} \]

where

\( n_0 \) = the refractive index of the solvent (0.5 M KOH),
\( n \) = the refractive index of the starch sol, and
\( C \) = the concentration of the starch sol in gm/ml.

Table 1 (Page 45) shows the refractive index increment measurements in a series of dilute solutions of potato starch in 0.5 M KOH.

The values \( n - n_0 \) are plotted against \( C \) in Figure 1 (Page 51). The slope calculated by least squares method is, \( \gamma = \frac{dn}{dc} = 0.1141 \).

Calculation of the constant \( H \) in the Debye equation was carried out at the scattering wavelength \( \lambda = 430 \) mp.

\[ H = 3.76 \times 10^{-6} \text{ mole cm}^2/\text{gm}^2 \]
TABLE 1

REFRACTIVE INDEX INCREMENTS IN A SERIES OF POTATO STARCH SOLS AT 430 mu

<table>
<thead>
<tr>
<th>Starch Concentration (gm/ml) x 10^3 in KOH 0.5 M</th>
<th>Refractive Index of the Sol (n)</th>
<th>Refractive Index Increment n - n_o</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.3350</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1.3355</td>
<td>5 x 10^-4</td>
</tr>
<tr>
<td>10</td>
<td>1.3362</td>
<td>12 x 10^-4</td>
</tr>
<tr>
<td>20</td>
<td>1.3372</td>
<td>22 x 10^-4</td>
</tr>
<tr>
<td>40</td>
<td>1.3393</td>
<td>43 x 10^-4</td>
</tr>
<tr>
<td>50</td>
<td>1.3408</td>
<td>58 x 10^-4</td>
</tr>
</tbody>
</table>

Three additional similar determinations of the constant H were carried out on amylopectin, pure amylose, and corn starch. An average value of the four calculated constants was found to be

\[ H = 3.71 \times 10^{-6} \]

This value was used for all subsequent calculations.

Tables 2-11, Pages 46-50, show the turbidimetric measurements of the starch sols used in the study. From these tables HC/\(\gamma\) values are plotted against C (Figures 2, 3 and 4, Pages 52-54). The straight lines obtained show the validity of the method.

The least squares method was used to calculate the intercepts. Table 12, Page 55, shows the calculated intercepts and the corresponding values of "weight average" molecular weights \(M_w\).
TABLE 2

TURBIDIMETRIC MEASUREMENTS OF AMYLOPECTIN
(AMYLOSE FREE)

<table>
<thead>
<tr>
<th>Starch Conc. (gm/ml) x 10^3 in 0.5 M KOH</th>
<th>-log I/I₀</th>
<th>Turbidity, γ' (1/cm) x 10^3</th>
<th>HC/γ' (mole/gm) x 10^10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.070</td>
<td>161.2</td>
<td>230.1</td>
</tr>
<tr>
<td>2</td>
<td>0.100</td>
<td>230.3</td>
<td>322.2</td>
</tr>
<tr>
<td>3</td>
<td>0.120</td>
<td>276.3</td>
<td>402.8</td>
</tr>
<tr>
<td>5</td>
<td>0.140</td>
<td>322.4</td>
<td>575.4</td>
</tr>
</tbody>
</table>

TABLE 3

TURBIDIMETRIC MEASUREMENTS OF "AMILOCA" STARCH

<table>
<thead>
<tr>
<th>Starch Conc. (gm/ml) x 10^3 in 0.5 M KOH</th>
<th>-log I/I₀</th>
<th>Turbidity, γ' (1/cm) x 10^3</th>
<th>HC/γ' (mole/gm) x 10^10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.053</td>
<td>122.0</td>
<td>304.0</td>
</tr>
<tr>
<td>2</td>
<td>0.082</td>
<td>188.9</td>
<td>392.8</td>
</tr>
<tr>
<td>3</td>
<td>0.109</td>
<td>251.0</td>
<td>443.0</td>
</tr>
<tr>
<td>5</td>
<td>0.136</td>
<td>313.2</td>
<td>592.2</td>
</tr>
</tbody>
</table>
### TABLE 4

**TURBIDIMETRIC MEASUREMENTS OF POTATO STARCH**

<table>
<thead>
<tr>
<th>Starch Conc. (gm/ml) x 10³ in 0.5 M KOH</th>
<th>- log I/I₀</th>
<th>Turbidity, γ (1/cm) x 10³</th>
<th>HC/γ (mole/gm) x 10¹⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.054</td>
<td>124.30</td>
<td>298.4</td>
</tr>
<tr>
<td>2</td>
<td>0.0975</td>
<td>224.54</td>
<td>330.4</td>
</tr>
<tr>
<td>3</td>
<td>0.134</td>
<td>308.60</td>
<td>360.6</td>
</tr>
<tr>
<td>5</td>
<td>0.177</td>
<td>407.63</td>
<td>455.0</td>
</tr>
</tbody>
</table>

### TABLE 5

**TURBIDIMETRIC MEASUREMENTS OF ARROWROOT STARCH**

<table>
<thead>
<tr>
<th>Starch Conc. (gm/ml) x 10³ in 0.5 M KOH</th>
<th>- log I/I₀</th>
<th>Turbidity, γ (1/cm) x 10³</th>
<th>HC/γ (mole/gm) x 10¹⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.050</td>
<td>115.1</td>
<td>322.3</td>
</tr>
<tr>
<td>2</td>
<td>0.090</td>
<td>207.2</td>
<td>358.1</td>
</tr>
<tr>
<td>3</td>
<td>0.121</td>
<td>278.6</td>
<td>399.5</td>
</tr>
<tr>
<td>5</td>
<td>0.155</td>
<td>356.9</td>
<td>519.7</td>
</tr>
</tbody>
</table>
TABLE 6
TURBIDIMETRIC MEASUREMENTS OF CORN STARCH

<table>
<thead>
<tr>
<th>Starch Conc. (gm/ml) x 10^3 in 0.5 M KOH</th>
<th>- log I/I_o</th>
<th>Turbidity, T* (1/cm) x 10^3</th>
<th>HC/γ (mole/gm) x 10^10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.038</td>
<td>87.5</td>
<td>424.0</td>
</tr>
<tr>
<td>2</td>
<td>0.068</td>
<td>156.6</td>
<td>473.8</td>
</tr>
<tr>
<td>3</td>
<td>0.092</td>
<td>211.8</td>
<td>525.4</td>
</tr>
<tr>
<td>5</td>
<td>0.134</td>
<td>308.6</td>
<td>601.1</td>
</tr>
</tbody>
</table>

TABLE 7
TURBIDIMETRIC MEASUREMENTS OF RICE STARCH

<table>
<thead>
<tr>
<th>Starch Conc. (gm/ml) x 10^3 in 0.5 M KOH</th>
<th>- log I/I_o</th>
<th>Turbidity, T* (1/cm) x 10^3</th>
<th>HC/γ (mole/gm) x 10^10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.022</td>
<td>50.7</td>
<td>731.7</td>
</tr>
<tr>
<td>2</td>
<td>0.043</td>
<td>99.0</td>
<td>749.5</td>
</tr>
<tr>
<td>3</td>
<td>0.063</td>
<td>145.0</td>
<td>767.5</td>
</tr>
<tr>
<td>5</td>
<td>0.099</td>
<td>255.7</td>
<td>813.8</td>
</tr>
</tbody>
</table>
### TABLE 8

**TURBIDIMETRIC MEASUREMENTS OF "AMYLON" STARCH**

<table>
<thead>
<tr>
<th>Starch Conc. (gm/ml) x 10^3 in 0.5 M KOH</th>
<th>- log I/I₀</th>
<th>Turbidity, ( \gamma ) (1/cm) x 10^3</th>
<th>HC/( \gamma ) (mole/gm) x 10^{10}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.014</td>
<td>32.3</td>
<td>1148.6</td>
</tr>
<tr>
<td>2</td>
<td>0.025</td>
<td>59.9</td>
<td>1238.7</td>
</tr>
<tr>
<td>3</td>
<td>0.038</td>
<td>87.5</td>
<td>1272.0</td>
</tr>
<tr>
<td>5</td>
<td>0.057</td>
<td>131.2</td>
<td>1413.2</td>
</tr>
</tbody>
</table>

### TABLE 9

**TURBIDIMETRIC MEASUREMENTS OF "AMYLON VII" STARCH**

<table>
<thead>
<tr>
<th>Starch Conc. (gm/ml) x 10^3 in 0.5 M KOH</th>
<th>- log I/I₀</th>
<th>Turbidity, ( \gamma ) (1/cm) x 10^3</th>
<th>HC/( \gamma ) (mole/gm) x 10^{10}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0105</td>
<td>24.18</td>
<td>1534.3</td>
</tr>
<tr>
<td>2</td>
<td>0.020</td>
<td>46.10</td>
<td>1609.5</td>
</tr>
<tr>
<td>3</td>
<td>0.028</td>
<td>64.50</td>
<td>1725.6</td>
</tr>
<tr>
<td>5</td>
<td>0.042</td>
<td>96.73</td>
<td>1917.7</td>
</tr>
</tbody>
</table>
### TABLE 10

**TURBIDIMETRIC MEASUREMENTS OF EXPERIMENTALLY FRACTIONATED AMYLOSE FROM POTATO STARCH**

<table>
<thead>
<tr>
<th>Starch Conc. (gm/ml) x 10^3 in 0.5 M KOH</th>
<th>- log I/I₀</th>
<th>Turbidity, γ (1/cm) x 10^3</th>
<th>HC/γ (mole/gm) x 10^10</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.0175</td>
<td>40.30</td>
<td>1841.2</td>
</tr>
<tr>
<td>3</td>
<td>0.025</td>
<td>57.57</td>
<td>1933.3</td>
</tr>
<tr>
<td>4</td>
<td>0.031</td>
<td>71.40</td>
<td>2078.4</td>
</tr>
<tr>
<td>5</td>
<td>0.037</td>
<td>85.20</td>
<td>2177.2</td>
</tr>
</tbody>
</table>

### TABLE 11

**TURBIDIMETRIC MEASUREMENTS OF THE COMMERCIAL AMYLOSE**

<table>
<thead>
<tr>
<th>Starch Conc. (gm/ml) x 10^3 in 0.5 M KOH</th>
<th>- log I/I₀</th>
<th>Turbidity, γ (1/cm) x 10^3</th>
<th>HC/γ (mole/gm) x 10^10</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.019</td>
<td>43.7</td>
<td>1698</td>
</tr>
<tr>
<td>3</td>
<td>0.028</td>
<td>64.4</td>
<td>1728</td>
</tr>
<tr>
<td>4</td>
<td>0.037</td>
<td>35.2</td>
<td>1741</td>
</tr>
<tr>
<td>5</td>
<td>0.045</td>
<td>103.6</td>
<td>1790.5</td>
</tr>
</tbody>
</table>
Figure 1. Refractive Index Increments of Potato Starch Solutions in 0.5 M KOH.
Figure 2. Debye Equation Plots of Turbidimetric Data of Various Starches.
Figure 3. Debye Equation Plots of Turbidimetric Data of Rice Starch and "Amylon" Starch.
Figure 4. Debye Equation Plots of Turbidimetric Data of "Amylon VII" Starch, Potato Amylose and Commercial Amylose.
TABLE 12

"WEIGHT AVERAGE" MOLECULAR WEIGHTS (Mₘ) AS CALCULATED FROM TURBIDIMETRIC DATA BY LEAST SQUARES METHOD

<table>
<thead>
<tr>
<th>Starch</th>
<th>Ordinate Intercept (1/Mₘ) x 10^{10}</th>
<th>Molecular Weight Mₘ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylopectin</td>
<td>148.82</td>
<td>6.719 x 10^{7}</td>
</tr>
<tr>
<td>Amioca</td>
<td>233.21</td>
<td>4.288 x 10^{7}</td>
</tr>
<tr>
<td>Potato</td>
<td>252.57</td>
<td>3.959 x 10^{7}</td>
</tr>
<tr>
<td>Arrowroot</td>
<td>262.68</td>
<td>3.807 x 10^{7}</td>
</tr>
<tr>
<td>Corn</td>
<td>380.74</td>
<td>2.626 x 10^{7}</td>
</tr>
<tr>
<td>Rice</td>
<td>708.95</td>
<td>1.411 x 10^{7}</td>
</tr>
<tr>
<td>Amylon</td>
<td>1087.48</td>
<td>9.195 x 10^{6}</td>
</tr>
<tr>
<td>Amylon VII</td>
<td>1430.25</td>
<td>6.991 x 10^{6}</td>
</tr>
<tr>
<td>Amylose (Fractioned)</td>
<td>1620.42</td>
<td>6.171 x 10^{6}</td>
</tr>
<tr>
<td>Amylose (Commercial)</td>
<td>1665.32</td>
<td>6.004 x 10^{6}</td>
</tr>
</tbody>
</table>

Complexation

The spectrophotometric characteristics of drugs tested are shown in Table 13.

Interactions of benzoic acid with various starches in aqueous solutions at 30°C are shown in Table 14, Page 57.
Interactions of p-hydroxybenzoic acid with the starches in aqueous solutions at 30°C are shown in Table 15, page 59.

Interactions of sorbic acid with the starches in aqueous solution at 30°C are shown in Table 16, Page 61.

Table 17 (Page 63) shows the interactions of potato starch with a number of organic molecules in aqueous solutions at 30°C.

### TABLE 13

**SPECTROPHOTOMETRIC CHARACTERISTICS OF DRUGS TESTED**

<table>
<thead>
<tr>
<th>Drug</th>
<th>$\lambda_{\text{max}}$, μ</th>
<th>Molar Absorbancy x 10^{-4}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic Acid</td>
<td>272.5</td>
<td>0.0873</td>
</tr>
<tr>
<td>p-Hydroxybenzoic Acid</td>
<td>251</td>
<td>1.2100</td>
</tr>
<tr>
<td>Methyl p-hydroxybenzoate</td>
<td>256</td>
<td>1.4800</td>
</tr>
<tr>
<td>p-Amino benzoic Acid</td>
<td>278</td>
<td>1.2900</td>
</tr>
<tr>
<td>m-Nitro benzoic Acid</td>
<td>265</td>
<td>0.5465</td>
</tr>
<tr>
<td>p-Nitro phenol</td>
<td>310</td>
<td>0.8928</td>
</tr>
<tr>
<td>o-Nitro phenol</td>
<td>275</td>
<td>0.6015</td>
</tr>
<tr>
<td>Sorbic Acid</td>
<td>259</td>
<td>2.5600</td>
</tr>
</tbody>
</table>
### TABLE 14

**SOLUBILITIES OF BENZOIC ACID IN AQUEOUS SOLUTIONS OF VARIOUS STARCHES AT 30°C**

\[(\text{Mole/L}) \times 10^3\]

<table>
<thead>
<tr>
<th>Starch Concentration Gm/L</th>
<th>Pure Amylose</th>
<th>&quot;Amioca&quot; Starch</th>
<th>Potato Starch</th>
<th>Arrow-root Starch</th>
<th>Corn Starch</th>
<th>Rice Starch</th>
<th>&quot;Amylon&quot; Starch</th>
<th>&quot;Amylon VII&quot; Starch</th>
<th>Amylose Fractioned</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>34.2</td>
<td>34.2</td>
<td>34.2</td>
<td>34.2</td>
<td>34.2</td>
<td>34.2</td>
<td>34.2</td>
<td>34.2</td>
<td>34.2</td>
</tr>
<tr>
<td>10</td>
<td>34.2</td>
<td>39.7</td>
<td>36.5</td>
<td>36.5</td>
<td>37.4</td>
<td>38.0</td>
<td>38.7</td>
<td>40.6</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>34.8</td>
<td>42.5</td>
<td>37.9</td>
<td>39.3</td>
<td>40.3</td>
<td>41.8</td>
<td>42.9</td>
<td>46.5</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>34.8</td>
<td>42.6</td>
<td>40.2</td>
<td>41.6</td>
<td>42.2</td>
<td>43.3</td>
<td>46.2</td>
<td>47.0</td>
<td>46.6</td>
</tr>
<tr>
<td>40</td>
<td>35.0</td>
<td>42.6</td>
<td>41.5</td>
<td>44.7</td>
<td>44.8</td>
<td>46.0</td>
<td>50.2</td>
<td>51.4</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>--</td>
<td>--</td>
<td>41.6</td>
<td>45.0</td>
<td>45.6</td>
<td>--</td>
<td>--</td>
<td>53.3</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>45.0</td>
<td>45.6</td>
<td>47.4</td>
<td>52.6</td>
<td>54.5</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>47.4</td>
<td>52.6</td>
<td>54.5</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>47.4</td>
<td>52.6</td>
<td>54.5</td>
<td></td>
</tr>
</tbody>
</table>

*Commercial amylose and fractioned amylose from potato starch produced almost identical results.*
Figure 5. The Effect of Various Concentrations of Starches on the Apparent Solubility of Benzoic Acid at 30°C.
TABLE 15

SOLUBILITIES OF p-HYDROXYBENZOIC ACID IN AQUEOUS SOLUTIONS OF VARIOUS STARCHES AT 30°C

(Mole/L) x 10³

<table>
<thead>
<tr>
<th>Starch Concentration Gm/L</th>
<th>Pure Amylopectin</th>
<th>&quot;Amioca&quot; Starch</th>
<th>Potato Starch</th>
<th>Arrow-root Starch</th>
<th>Corn Starch</th>
<th>Rice Starch</th>
<th>&quot;Amylon&quot; Starch</th>
<th>&quot;Amylon VII&quot; Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>58.2</td>
<td>58.2</td>
<td>58.2</td>
<td>58.2</td>
<td>58.2</td>
<td>58.2</td>
<td>58.2</td>
<td>58.2</td>
</tr>
<tr>
<td>10</td>
<td>58.2</td>
<td>73.6</td>
<td>63.6</td>
<td>--</td>
<td>64.2</td>
<td>63.7</td>
<td>--</td>
<td>64.8</td>
</tr>
<tr>
<td>20</td>
<td>58.9</td>
<td>78.5</td>
<td>68.6</td>
<td>69.6</td>
<td>70.0</td>
<td>70.0</td>
<td>70.4</td>
<td>70.9</td>
</tr>
<tr>
<td>30</td>
<td>59.2</td>
<td>78.6</td>
<td>74.3</td>
<td>75.0</td>
<td>75.7</td>
<td>76.4</td>
<td>76.4</td>
<td>76.9</td>
</tr>
<tr>
<td>40</td>
<td>--</td>
<td>78.6</td>
<td>80.2</td>
<td>80.4</td>
<td>81.1</td>
<td>80.9</td>
<td>81.8</td>
<td>83.1</td>
</tr>
<tr>
<td>45</td>
<td>--</td>
<td>--</td>
<td>80.2</td>
<td>80.8</td>
<td>--</td>
<td>82.7</td>
<td>84.5</td>
<td>86.3</td>
</tr>
<tr>
<td>50</td>
<td>--</td>
<td>--</td>
<td>80.3</td>
<td>80.9</td>
<td>81.8</td>
<td>82.7</td>
<td>86.2</td>
<td>89.1</td>
</tr>
<tr>
<td>60</td>
<td>--</td>
<td>--</td>
<td>80.3</td>
<td>80.9</td>
<td>81.8</td>
<td>--</td>
<td>--</td>
<td>89.1</td>
</tr>
<tr>
<td>65</td>
<td>--</td>
<td>--</td>
<td>80.3</td>
<td>80.9</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>89.1</td>
</tr>
</tbody>
</table>
Figure 6. The Effect of Various Concentrations of Starches on the Apparent Solubility of p-Hydroxybenzoic Acid, at 30°C.
### TABLE 16

**SOLUBILITIES OF SORBIC ACID IN AQUEOUS SOLUTIONS OF VARIOUS STARCHES AT 30°C**

(Mole/L) x 10^3

<table>
<thead>
<tr>
<th>Starch Concentration Gm/L</th>
<th>Pure Amylopectin</th>
<th>&quot;Amioca&quot; Starch</th>
<th>Potato Starch</th>
<th>Arrowroot Starch</th>
<th>Corn Starch</th>
<th>Rice Starch</th>
<th>&quot;Amylon&quot; Starch</th>
<th>&quot;Amylon VII&quot; Starch</th>
<th>Amylose Fractioned</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.7</td>
<td>12.7</td>
<td>12.7</td>
<td>12.7</td>
<td>12.7</td>
<td>12.7</td>
<td>12.7</td>
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<td>12.7</td>
</tr>
<tr>
<td>10</td>
<td>12.7</td>
<td>17.2</td>
<td>--</td>
<td>13.9</td>
<td>14.3</td>
<td>14.7</td>
<td>15.7</td>
<td>16.4</td>
<td>17.4</td>
</tr>
<tr>
<td>20</td>
<td>11.9</td>
<td>19.5</td>
<td>14.6</td>
<td>15.4</td>
<td>15.8</td>
<td>16.6</td>
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<td>22.8</td>
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<td>30</td>
<td>13.1</td>
<td>19.5</td>
<td>16.1</td>
<td>16.6</td>
<td>17.2</td>
<td>18.3</td>
<td>23.5</td>
<td>--</td>
<td>27.1*</td>
</tr>
<tr>
<td>40</td>
<td>13.1</td>
<td>19.5</td>
<td>17.2</td>
<td>18.3</td>
<td>18.7</td>
<td>20.3</td>
<td>27.3</td>
<td>29.3</td>
<td>--</td>
</tr>
<tr>
<td>45</td>
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<td>--</td>
<td>17.6</td>
<td>--</td>
<td>19.5</td>
<td>21.1</td>
<td>28.9</td>
<td>31.3</td>
<td>--</td>
</tr>
<tr>
<td>50</td>
<td>--</td>
<td>--</td>
<td>18.0</td>
<td>19.3</td>
<td>20.0</td>
<td>22.0</td>
<td>30.7</td>
<td>33.4</td>
<td>--</td>
</tr>
<tr>
<td>60</td>
<td>--</td>
<td>--</td>
<td>18.0</td>
<td>19.3</td>
<td>20.1</td>
<td>22.0</td>
<td>31.8</td>
<td>35.0</td>
<td>--</td>
</tr>
<tr>
<td>65</td>
<td>--</td>
<td>--</td>
<td>18.0</td>
<td>19.3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>35.0</td>
<td>--</td>
</tr>
</tbody>
</table>

*Rapid retrogradation in sols of higher concentrations occurred. This made the solubility method for the complex detection insufficient.*
Figure 7. The Effect of Various Concentrations of Starches on the Apparent Solubility of Sorbic Acid at 30°C.
**TABLE 17**

**SOLUBILITIES OF SOME ORGANIC MOLECULES IN AQUEOUS SOLUTIONS OF POTATO STARCH AT 30°C**

(Mole/L) x 10³

<table>
<thead>
<tr>
<th>Starch Concentration Gm/L</th>
<th>Benzoic Acid</th>
<th>Methyl-parahydroxy Benzoate</th>
<th>m-Nitrobenzoic Acid</th>
<th>o-Nitrobenzoic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>34.2</td>
<td>17.5</td>
<td>19.0</td>
<td>40.8</td>
</tr>
<tr>
<td>10</td>
<td>36.5</td>
<td>18.6</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>20</td>
<td>37.9</td>
<td>20.0</td>
<td>25.0</td>
<td>69.5</td>
</tr>
<tr>
<td>30</td>
<td>40.2</td>
<td>21.1</td>
<td>26.6</td>
<td>82.0</td>
</tr>
<tr>
<td>40</td>
<td>41.5</td>
<td>21.7</td>
<td>29.9</td>
<td>97.0</td>
</tr>
<tr>
<td>45</td>
<td>41.6</td>
<td>21.7</td>
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<td>--</td>
</tr>
<tr>
<td>50</td>
<td>--</td>
<td>21.7</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
TABLE 18
SOLUBILITIES OF SOME ORGANIC MOLECULES IN AQUEOUS SOLUTIONS OF POTATO STARCH AT 30°C
(Mole/L) x 10^3

<table>
<thead>
<tr>
<th>Starch Concentration Gm/L</th>
<th>p-Aminobenzoic Acid</th>
<th>m-Nitroaniline</th>
<th>o-Nitrophenol</th>
<th>p-Nitrophenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>45.3</td>
<td>6.5</td>
<td>15.20</td>
<td>113.4</td>
</tr>
<tr>
<td>10</td>
<td>48.0</td>
<td>--</td>
<td>--</td>
<td>136.1</td>
</tr>
<tr>
<td>20</td>
<td>50.5</td>
<td>8.5</td>
<td>18.0</td>
<td>139.0</td>
</tr>
<tr>
<td>30</td>
<td>56.0</td>
<td>9.8</td>
<td>20.0</td>
<td>139.0</td>
</tr>
<tr>
<td>40</td>
<td>56.2</td>
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<td>21.9</td>
<td>--</td>
</tr>
<tr>
<td>45</td>
<td>56.2</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Figure 8. The Effect of Various Concentrations of Potato Starch on the Apparent Solubilities of Several Organic Acids in Water at 30°C.
Figure 9. The Effect of Various Concentrations of Potato Starch on the Apparent Solubilities of Several Organic Acids at 30°C.
TABLE 19
DRY POTATO STARCH ADSORPTION OF FATTY ACIDS AND ALCOHOLS IN PETROLEUM ETHER

<table>
<thead>
<tr>
<th>Fatty Acid or Alcohol</th>
<th>Total Amount of Fatty Acid or Alcohol Adsorbed Per One Gram Dry Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mgm</td>
</tr>
<tr>
<td>Steric Acid</td>
<td>4.30</td>
</tr>
<tr>
<td>Stearyl Alcohol</td>
<td>3.64</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>3.87</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>5.20</td>
</tr>
<tr>
<td>Myristic Acid</td>
<td>6.72</td>
</tr>
<tr>
<td>Lauric Acid</td>
<td>7.62</td>
</tr>
<tr>
<td>Cetyl Alcohol</td>
<td>4.88</td>
</tr>
<tr>
<td>Fatty Acid</td>
<td>Total Amount Adsorbed Per One Gram Dry Starch</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Mgm</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>6.44</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>7.85</td>
</tr>
<tr>
<td>Myristic Acid</td>
<td>9.96</td>
</tr>
<tr>
<td>Lauric Acid</td>
<td>11.10</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>5.86</td>
</tr>
</tbody>
</table>
Figure 10. Dry Starch Adsorption of Several Fatty Acids and Fatty Alcohols from Methanol or Petroleum Ether Solutions.
Figure 11. Solubility Behavior of a Number of Organic Acids in the Presence of Potato Starch.
<table>
<thead>
<tr>
<th>The Organic Molecule</th>
<th>Total Amount Adsorbed Per One Gram Dry Starch* (Mole) x 10^5</th>
<th>Amount Released in Water Per Gram Starch** (Mole) x 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic Acid</td>
<td>6.93</td>
<td>5.9</td>
</tr>
<tr>
<td>Sorbic Acid</td>
<td>4.68</td>
<td>5.1</td>
</tr>
<tr>
<td>o-Nitrophenol</td>
<td>5.83</td>
<td>6.7</td>
</tr>
<tr>
<td>p-Nitrophenol</td>
<td>22.17</td>
<td>23.8</td>
</tr>
</tbody>
</table>

* Determined after hydrolysis of "Dry Starch-Drug" adsorption complex.

** Determined after agitation of the above complex in distilled water. See the Experimental section, Pages 38-40.
DISCUSSION

Evaluation of Results

The Significance and Validity of the Molecular Weights Obtained by Light Scattering

An important feature of the molecular weights obtained by light scattering in general should be noted. Most of the classical physical methods for molecular weight determinations that may be employed for polymers (osmotic pressure for example), depend on the colleagative properties of the solute molecule. Thus, the molecular weight counts the concentration of solute molecules, the larger molecules carrying the same weight as the smaller molecules; hence a "number average" molecular weight (Mn) — total grams of material divided by the total number of molecules — is obtained.

It is shown, on the other hand, that light scattering weighs the various molecules in a heterogeneous system, like starch according to their masses, hence yields a "weight average" molecular weight (Mw) value.

The two values (Mn) and (Mw) are equivalent in homogenous material but differ widely in polymers with heterogeneous nature. The term polymolecular, according to Schulz (82), applies to starch or starch polymer components. The ratio $M_w/M_n$ which always is
greater than one for polymolecular systems, is a measure of poly-
molecularity. For amylose the ratio $M_w/M_n$ might be expected to be
not higher than 1.5 - 2, but for a branched polymer, like amylopectin,
the molecular weight distribution may be extremely broad. It has been
shown that in corn amylopectin the ratio $M_w/M_n$ is of the order of
three hundred.

Using the light scattering measurements thus enables one to
obtain "weight average" molecular weights which count the various
molecules in the sample according to their masses.

The linear amylloses — the commercial and the experimentally
fractioned ones — have the lowest molecular weights.

The amylose fraction, as usually obtained from 1-butanol com-
plex, consists of several subfractions of amylloses of different degrees
of polymerization. Subfraction studies by ultracentrifugation show that
amylose can be separated into seven or eight subfractions of different
molecular sizes (83). As an example, the degree of polymerization of
the subfraction of corn amylose was reported to vary between 300 and
760 glucose units (84).

The values of $(M_w)$ obtained for commercial amylopectin, which
is claimed to be "Amylose-Free" was in the range of reported litera-
ture values of $(M_w)$ obtained by light scattering; these were shown to
be as high as $10^7$. The molecular weights of the remaining starches
were distributed between the two values of amylose and amylopectin.
As the starches are heterogeneous and polymolecular, the values varied from one starch to another according to the content of amylose and amylopectin and according to the sizes of these fractions. In each starch the two polymer fractions, amylose and amylopectin (or even their subfractions) contributed to the value of (Mw) according to their percentage and weight distribution.

The molecular weights then arranged in a decreasing order are: Amylopectin, Amioca Starch, Potato Starch, Arrowroot Starch, Corn Starch, Rice Starch, "Amylon" Starch, "Amylose VII" Starch, Amylose fractioned from potato starch, and commercial Amylose.

Determinations of the amylose content of these starches could have been helpful. An amperometric titration, as described by Larson (87), could give the per cent amylose in the starch. However, the amylose content of a number of the commercial starches used were given by the manufacturers as "Amylon" 55-60%, "Amylon" VII 70%.

Therefore, according to the molecular weights obtained, one can group the starches as follows:

**Group I** — Commercial Amylopectin and "Amioca." Both of these starches have high molecular weight due to the branched nature of the molecule.

**Group II** — Potato, Arrowroot, Corn and Rice. These starches have (Mw) values ranging between $1.4-3.9 \times 10^7$. The differences in molecular weights are explained by: (a) Differences
in amylose/amylopectin ratio; and by (b) differences in the
size of the amylose and the amylopectin from one starch spec-
cie to the other.

Group III — Amylon and Amylon VII. These starches are
reported to be prepared from the same starch source having
different amylose content, "Amylon VII" being richer in
amylose than "Amylon."

Group IV — The fractioned amylose from potato starch
and the commercial amylose from corn starch. Both of these
have relative low molecular weights.

The major sources of error in the light scattering measure-
ments can be summarized:-

1. The constant \( \gamma \) which appears as a squared term in the cal-
culation of \( (H) \) in Debye equation (equations IVa and V, Page 24) is
taken in this study as an average constant after three determinations
of refractive increments on three different starches. This value in-
creases in the starches that are rich in amylopectin.

2. Another source of error is the solvent particle interac-
tions which increases in concentrated solutions. The value of the
virial coefficient \( (B) \) in Debye equation No. V (Page 24) indicated by
the slope of the lines \( HC/\gamma \) vs. \( C \) Debye plots (Figures 2-4, Pages
52-54) show this concentration dependence. The slopes decreased
from amylopectin to amylose showing the lowest virial coefficient in
the case of amylose, i.e. the smallest solvent-polymer interaction is
shown by amylose.

3. One last cause for error is what is known as "interference
effect." As was pointed out previously, the equations used are based
on the assumption that the excited dipoles in the scattering system are
rigorously parallel to the direction of the exciting electromagnetic
vector. Thus, the equations were developed assuming that the scat-
tered components from a molecule are all in phase and are fully
additive. This is true only in the case of molecules which are small
compared to the wavelength of the light employed. If the polymer
molecule has any dimension which exceeds approximately \( \lambda/20 \), in-
ternal interference arises, i.e. in the large molecules the incident
radiation is exciting, oscillating dipoles in many different parts of the
molecule. The component of the scattered radiation from any two
points on the molecule will be out of phase and thus cannot be fully
additive.

Thus, tracing out the complete path length of the light beams
is necessary to determine the extent of the interference coefficient.

The exact light scattering photometers described previously
enable the tracing of the magnitude of the scattered light at different
angles. The Zimm (69) treatment for double extrapolation of light
scattering data must be followed in such a case. It is then clear that the interference effect is greater with amylopectin than with amylose, since amylopectin has a larger molecule than amylose.

It is to be recalled that \((I_0 - I)\), the difference between the intensity of the incident beam and the intensity of the transmitted beam, is considered to represent the scattered light. This does, however, include the amount of light that is absorbed. The scattering in this work was estimated indirectly by measuring the transmitted light; therefore, any absorption of light is a source of error. At the same time the intensity of the incident beam could be decreased by multiple scattering, thus high values of turbidity were obtained.

In general, one can say that the results of molecular weights obtained in this study may be higher than average \((M_w)\) values reported in the literature due to interference effect and concentration dependence. The error committed is highest in amylopectin and the starches rich with amylopectin. Although the validity of such light scattering measurements on amylopectin was questioned, the method was shown more than once to be the most efficient physical method for such large polymer molecules (88). Erlander (52) has shown that the light scattering molecular weight for a given amylopectin solution can be reproduced within the accuracy of the photometer used since the physical aggregation is almost absent in most solvents.
Finally, one can conclude that the molecular weight of a starch, as obtained in this study, is a mean value which counts for all the polymer components of that starch according to their respective masses.

**Evidence of Complexing of Starches with the Drug Pharmaceuticals in Aqueous Solutions**

The solubility method for determining interactions of chemical entities indicates complex formation when the solubility of the pharmaceutical drug is increased or decreased according to the solubility of the complex formed.

The analysis method is U. V. Spectrophotometry which follows the conjugated benzene ring found in most of the molecules tested.

The results are given then as the total amount (Mole/L) of the drug that dissolves in an aqueous solution of the starch. The results obtained with benzoic acid (Table 14 on Page 57 and Figure 5, Page 58) show evidence of the formation of soluble complexes with various starches. The solubilities of benzoic acid in each starch solution increased in a linear relationship with the increase of starch concentration up to a certain limit after which no benzoic acid could be detected in the solution, although excess amounts of the solid benzoic acid were present in the system. At that limit the turbidity of the solutions was notably increased in a very similar manner to the phenomenon noticed in retrogradation as described before. The plateau region thus could
indicate the solubility of the complex formed. In such a case, increasing the starch concentration should cause the disappearance of all the excess of the solid benzoic acid. Further investigation in a higher starch concentration than in the system 50 to 60 gm/L was difficult because of the high viscosities of the solutions.

Examination of Figure 5 on Page 58 shows that the plateau region occurred at approximately the same starch concentration in group II of starches (potato, arrowroot, corn and rice) and at a different starch concentration in Group III ("Amylon" and "Amylon" VII).

Pure amylpectin solution showed no interaction with benzoic acid, while the fractioned amylose* showed a rapid increase in the solubility of benzoic acid with the increase of amylose concentration. The rapid retrogradation of the amylose solutions containing more than 30 gm/L made the investigation by the solubility method inadequate. The amylose precipitated out of solution — probably in a complex form — in lumps of aggregates. When the system was allowed to settle, a thin layer of a supernatant liquid was clearly noticed. Such a system, as will be described later, was successfully centrifuged when drugs other than benzoic acid were used.

*Although it was easier to prepare solutions from the commercial amylose, similar results and behavior were noted in the phase diagram. These results were not shown to avoid repetition.
Looking at the ordinate of the plot in Figure 5 on Page 58, it can be noted that the amount of benzoic acid needed to reach the plateau regions varied from one starch to the other, or in other words, the solubilities of the complexes of benzoic acid with different starches are not similar. Similar results to those obtained with benzoic acid were obtained with p-Hydroxybenzoic acid, as shown in Table 15 on Page 59 and the corresponding phase diagrams (Figure 6, Page 60), and with sorbic acid (Table 16, Page 61 and Figure 7, Page 62).

A summary of the important points that should be made about the results and phase diagrams of benzoic acid, sorbic acid, and p-Hydroxybenzoic acid is as follows:

1. Pure amylopectin solutions showed little or no interaction with the three drugs used.

2. The various starches showed complexation with linear dependence up to a certain limit. The solubilities of the complexes formed with a given drug varied with the type of starch. It can be said, therefore, that when the same solid drug was added to the solutions of the various starches — each being a polymolecular system of its own — the amounts of drug that entered into the solutions before precipitating the macromolecular system, or one of its components, were completely different in one starch from the other.**

**These amounts can be calculated by subtracting the amount of drug in water at 30°C from the total drug in the system at the plateau.
The slopes of the phase diagrams of each drug chemical may be used to compare relative complexing tendencies. However, one look at the graph, Figure 7 on Page 62, shows for example the solubility of sorbic acid in various starch sols at a given starch concentration is dependent on the type of starch sol.

3. Amylose sols show a high uptake of the drug molecule into a solution at a relatively low concentration of amylose. The precipitation of a cake-like product occurred very rapidly in the sol. The precipitate was separated by centrifuging at 5000-7000 r.p.m. when the complexing drug was one of the following: p-aminobenzoic acid, p-nitrophenol, *** myristic acid, lauric acid and cetyl alcohol. The precipitates obtained with these drugs consisted of (amylose-drug) complex in a crystalline form. In the case of p-nitrophenol the precipitate was washed with water several times, then resuspended in water and agitated in the water bath at 30°C. Detection of p-nitrophenol in the water showed very little release of the drug. The hydrolysis of the complex crystals with hydrochloric acid by the method described on Page 38 showed the complete release of p-nitrophenol molecules.

***p-Nitrophenol was the most soluble organic molecule used in the complexing study. When used with other starch solutions the crystalline precipitate was obtained after centrifugation. The system and the precipitate were similar to what was noticed in the fractionation of starch by complexing with n-butanol (Page 30).
4. Finally, one can recognize that the low molecular weight polymer amylose — including the polymolecular subfractions — is the main complexing component with benzoic acid, p-hydroxybenzoic acid, and sorbic acid.

The amylose tendency to crystallize in a complex form with these drugs is far stronger than that of amylopectin.

The three acids, benzoic, p-hydroxybenzoic and sorbic, are polar organic molecules with low solubilities in water. A number of different molecules were used in this complexing study with potato starch and the results (Tables 17 and 18 on Pages 63 and 64, Figures 8, 9 and 11 on Pages 65, 66, and 70) show that the complexing of potato starch with the selected molecules does occur but with wide variance.

It appears at first (Figures 8 and 9 on Pages 65 and 66) that a solid drug tendency to complex with potato starch matches its solubility, i.e., the higher the solubility in water, the higher the tendency for complexing. However, arranging the molecules according to their decreasing order of solubility in water at 30°C and arranging the tendencies of complexing, calculated by the slopes of the lines (Figure 11 on Page 70) in decreasing order (see Table 22, Page 83), shows that the tendencies of complexing do not match the solubilities in water.

Although, for example, the water solubility of p-aminobenzoic is higher than o-nitrobenzoic acid, the latter has a higher tendency to
TABLE 22

SOLUBILITY RELATIVE TO COMPLEXING TENDENCY
OF DRUG MOLECULES

<table>
<thead>
<tr>
<th>Decreasing Order of Solubility in Water at 30°C</th>
<th>Decreasing Order of Tendency to Complex*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: p-nitrophenol</td>
<td>1</td>
</tr>
<tr>
<td>2: p-aminobenzoic acid</td>
<td>3</td>
</tr>
<tr>
<td>3: o-nitrobenzoic acid</td>
<td>5</td>
</tr>
<tr>
<td>4: Benzoic acid</td>
<td>2</td>
</tr>
<tr>
<td>5: m-nitrobenzoic acid</td>
<td>4</td>
</tr>
<tr>
<td>6: Methyl-p-hydroxybenzoate</td>
<td>7</td>
</tr>
<tr>
<td>7: o-nitrophenol</td>
<td>6</td>
</tr>
<tr>
<td>8: m-nitroaniline</td>
<td>8</td>
</tr>
</tbody>
</table>

*See Figure 11, Page 70
complex with potato starch than the former. On the other hand, p-aminobenzoic acid precipitated the complex at low concentrations of starch where no signs of precipitation were shown by o-nitrobenzoic acid. The same can be said of m-nitrobenzoic acid and benzoic acid.

Considering all the results of complexation, the drug molecules with the various starches and starch fractions, and the complexation of potato starch sols with various drug molecules, the interaction phenomenon can be described as follows.

When a polar organic molecule, having a low solubility in water, is added to a starch sol, the amyllose in the sol complexes with the molecule at a tendency related to the concentration of the organic dissolved molecule in water.*** The molecular structure and the polarity of the molecule in particular have a special effect on the tendency for complexing.

The separation of the amyllose complex from the system containing the complexing agent occurs at the saturation concentration of the complex (Cs) (the plateau region in the diagram). This saturation concentration is influenced by the per cent of amyllose in the starch sol.

The most striking influence on the saturation concentration of the complex, however, is that of the solubility of the complexing agent

***Since in this study the solid drug is added to the aqueous sol, the concentration in water is the solubility of the molecule at 30°C.
itself. This is explained in the following example: When phenol was added in a solid form, to a sol of 5 gm potato starch per liter, a rapid precipitation of amylose complex occurred. p-Nitrophenol, on the other hand, precipitated amylose as a complex only in potato starch concentrations higher than 11-12 gm of starch per liter. Finally, o-nitrophenol having a much lower solubility did not precipitate as a complex with amylose in potato starch sols having as high as 40 gm of starch per liter. One can anticipate that the influence of the solubility of the complexing agent will diminish rapidly with increasing the amylose content in the starch. This was proven to be true in this study. This can be recognized by looking at Amylon VII (about 70% amylose) complexing with sorbic acid (the solubility in water is $12.7 \times 10^{-3}$ mole/L) (Figure 7 on Page 62) and looking at Amylon VII complexing with p-hydrobenzoic acid (the solubility in water is $58.2 \times 10^{-3}$ mole/L) (Figure 6 on Page 60). Both complexing agents, although having different solubilities, precipitated the amylose complex at the same concentrations of starch (50-53 gm/L).

In all the examples of complexations demonstrated in this work, the solubility of the "drug-amylose" complex — the saturation concentration (Cs) — is higher than the solubility of the complexing drug. The reverse of the situation must not be overlooked, i.e., some agents although having a low water solubility, might produce complexes with amylose which has a lower solubility.
The results of complexing of starch in aqueous solutions obtained in this work can be related to the aspects available in fractionation of starch by complexing agents. Such comparison will be made in part II of this discussion.

From the phase diagrams of sorbic acid, Figure 7 on Page 62, the values (Cs - Co) are calculated, where Cs is the saturation concentration of sorbic acid in the complex and Co is the concentration of sorbic acid in water at 30°C (12.7 x 10^{-3} mole/L).

When these values of (Cs - Co) were plotted against the reciprocal of the molecular weights of the starches (1/Mw), an approximate linear relationship was noted. Such a relationship simply says that the amount of complexing drug entering solution in a starch sol, before a precipitation of a complex occurs, decreases linearly with increasing the "weight average" molecular weight of the starch.

Adsorption Affinity of Unruptured Starch Granules for Drug Pharmaceuticals

In the last part of this study, potato starch was shown to adsorb fatty acids and fatty alcohols from a methanol medium (Table 20, Page 68) in greater amounts than from a petroleum ether medium (Table 19, Page 67). After potato starch adsorbs the fatty acids or fatty alcohols, the removal of the fatty material was not possible by washing with petroleum ether. The hydrolysis of the starch with hydrochloric acid, however, released these fatty materials. When the amount of fatty
acid or fatty alcohol adsorbed per one gram of potato starch was plotted against the number of carbon of the fatty acids (Figure 10, Page 69), the plot showed approximate linear dependence of the amount adsorbed on the chain length of the fatty acid. The starch granules showed lower affinity for fatty alcohols than for fatty acids of identical chain length. The adsorption affinity also was decreased when the fatty acid contained a double bond (oleic acid). No easy explanation for the higher affinity of starch for the materials in methanol media than its affinity in petroleum ether is possible. It is conceivable that fatty acids can be carried into the granule polymers by an exchange process between the water content of starch and the polar solvent methanol.

It is necessary to note that the concentration of the fatty acid solutions were kept constant. Since it was shown (81) (89) that this adsorption follows a typical Freundlich adsorption isotherm, thus the amount of fatty acid adsorbed is proportional to its concentration in solution.

Similar adsorption affinities of unruptured granules of potato starch for benzoic, sorbic, o-nitrophenol, and p-nitrophenol was shown (Table 21, Page 71).

It is important here to point out that the starch adsorbed the molecules from their aqueous solutions. The concentrations of these solutions were determined by the solubilities of the molecules at room
temperature. The removal of the material adsorbed to the starch granules by agitating the starch in water was not complete. Only 10-12% of the adsorbed amount was released in the water (Table 21, Page 71). Hydrolysis of the starch by hydrochloric acid did release the adsorbed molecule.

Correlation of the Results to the Previously Reported Complexes with Starch

The adsorption affinity of starch was given consideration only after recognizing the heterogeneity of starch polymers and the successful fractionation studies of starch.

Most starch complexing studies were done with one purpose in mind, the fractionation of the starch. Amylose and amylopectin were found to have many of the properties of isotactic polymers. They were both found to belong to the class of the crystalline polymers. The precipitation of these crystalline polymers by complexing agents was sought. Now it is well recognized after a series of studies (72) that practically any polar organic reagent (containing at least one hydrophilic group) can complex with the amylose fraction of starch. If the complexing reagent has high enough solubility, the "amylose-reagent" complex will precipitate as a wet cake and will have a microcrystalline structure. Amylopectin was found to have much less affinity to crystallize as a complex with the polar organic materials. After the
"amylose-reagent" complex is obtained, the removal of the complexing agent from the complex to obtain the amylose was a subject for study of a number of papers.****

Supercentrifugation, washing with aqueous solutions of the complexing agents (containing low concentrations of the agent), with centrifuging the complex precipitates in such media proved to remove 90% of the complexing agent.

The main difference in the fractionation by complexing studies is the use of a complexing molecule with a relative high solubility compared to the drug pharmaceuticals used in this study. The complexing agents used in fractionation are those which precipitate amylose complexes, when they are added in small amounts (in concentrations much less than their solubilities). The most used complexing agents were alcohols like n-butanol and n-propanol, although a very wide variety of molecules were tried (72). Whistler and co-workers (71) have suggested that any water-soluble compound possessing either electron donor or acceptor groups capable of hydrogen bond formation is a suitable complexing agent. The authors reported the use of nitrobenzene, pyridine, amylacetate and 2-heptanone as complexing agents for the precipitation of amylose as a complex. Thymol and cyclohexanol were found to be suitable complexing agents for the amylose of potato starch.

**** See the reviews on starch fractionation (5, 20, 72).
The influence of the concentration of the complexing agent on the formation of the complex precipitate was shown to be critical (72).

The adsorption powers of starch and its two component polymers is very clearly demonstrated in iodine adsorption to produce a blue coloration as described in the introduction of this work. As was mentioned before, x-ray data (58) have pointed to a general similarity in the structure of "iodine-amylose" complex and 'butanol-amylose' complex.

It is reasonable to say that the complex formation of starch with the drug complexing molecules takes the same route as the adsorption of iodine by starch. The mechanism of the interaction of iodine with starch and amylose in particular with which it appears to form a definite inclusion complex has been discussed by Rundle (92). It was explained to be an attraction of a dipolar nature. The helical configuration of amylose was suggested to have a large dipole along the axis of the helix due to the summation of reinforcement of the dipoles of the individual D-glucose molecules in the chain. Thus, when an iodine molecule enters the helix, it will be polarized by the permanent dipole of the amylose molecule and be given an induced dipole, and the two will interact through dipolar forces.

A theory was presented (93) for the dipolar attraction between amylose and iodine in the solid state on the basis of the helical
configuration of amylose in which dipolar forces were shown to be sufficient to account for the stabilizing energy of the complex. The x-ray diffraction patterns of starches and the properties of starch components in the solid state were reviewed by Greenwood (61). The diffraction patterns of amylose complexes with iodine and with fatty acids were shown (94) (95) to be similar. In both cases the complexing molecule enters the center of the amylose helix. The helical configuration of amylose involving 6-D glucose units per turn was suggested. The external diameter of the helix was 13°A and the pitch 8°A. A hexagonal unit cell was suggested as being formed by the close-packing of such "cylinders" of amylose.

The interaction of amylopectin with iodine was studied (96) in terms of the concentrations of iodine needed for different amylopectins samples. It was suggested that the branches of (4 - 1) D-glucose take up iodine in a route similar to that in amylose. The uptake of these branches from iodine is proportional to the length of the chains in the units.

In simulating the chain length of the branches of amylopectin Whistler (97) hydrolyzed amylose to a degree of polymerization of 20 to 40, to study the effect of chain length on the ability to form complexes. It was shown that such short chains no longer form complexes with nitrobenzene or 2-heptanone, although it still does with n-butanol and 2-nitropropane.
Potato starch and arrowroot starch were reported (4) to interact with drug chemicals, the evidences of complex interactions being shown by solubility method. The mechanism of interaction of the drug chemicals with the starch sols was deduced to be caused by inclusion complex formation with the $\alpha$-helical form of amylose supplemented by hydrogen bonding caused by the multiplicity of free hydroxyl groups in the starch molecule.

The name inclusion compounds was given by Schlenk (98) (99) to molecular complexes in which hydrogen bonding is negligible. They were classified (100) as:

a. Polymer inclusion compounds including those with channel-like space and those with cage-like spaces.

b. Monomolecular inclusion compounds.

c. Macromolecular inclusion compounds.

The general understanding of an inclusion compound is that it is a combination of complete organic molecules that are united spatially, leaving unaffected the bonding systems of the components. Inclusion is now believed to be a result of the ability of one compound, because of its peculiar stereochemical properties, and possibly its polarity, to enclose a second compound spatially. The forces operating to stabilize the inclusion in general may be said to depend on the type of inclusion complex. Weak Van der Waal's forces are considered to be very important.
Although the mechanism of the starch complexes with the drug molecules is not the subject of this study, the similarity in behavior of the complexes reported in this work with those of amylose complexes with iodine and the molecules used for the fractionation of starch, makes it reasonable to deduce that the complexes are polymer inclusion complexes where the "host" has channel-like spaces due to its linearity and α-helical configuration.

Cyclodextrins (α, β, and γ) which are called Schardinger dextrins, are prepared by partial enzymatic hydrolysis of starch. These dextrins consist of four, five, or six glucose units which are joined by (1 - 4)α glucosidic linkages. The terminal groups are joined to produce a large ring. These cyclodextrins were reported to show monomolecular complexes with numerous drug pharmaceuticals (101, 102, 103). The complexes formed were considered to be inclusion complexes where the molecules are enclosed in the void of the cyclodextrin "host" molecule. Attractive forces were considered to be effective in those complexes.

The Significance of Starch Complexation in Pharmaceuticals

With the description of the behavior and affinity of starch to complex as a background, the scientist should not be surprised to find applications for such complexing phenomenon in his everyday life, or to
find answers for many unexplained aspects in the chemistry of living systems, like the \textit{in vivo} synthesis of chemicals in animals or biosynthesis in plants and other biological phenomena.

A comparison of the fine structure of starch and its components with the highly branched polysaccharides that exist in animals and plants is very important; thus, investigations on the "amylopectin-type" polysaccharide synthesized by certain bacteria (104) (105) and protozoa (106) (107), and on the "glycogen-type" material synthesized by some plants (108), could prove to be practically useful in joining together the polysaccharides in animals and plants. Thus, an understanding of one of these polysaccharides might be helpful in explaining the roles of others and shed light on many aspects of living systems. The possible complexations of polysaccharides similar to starch might prove to be as important as chelation of proteins in living systems.

Glycogen is well known to complex with iodine in a manner similar to that of amylopectin. It is also known to be precipitated by ethyl alcohol. It is possible that such a macromolecule interacts with some solutes, which could be drugs, chemicals, or even hormones. The reversibility of such binding would be explained by the reversible continuous glycolysis and phosphorylysis in the human body.

A drug complexed with a macromolecule like starch, starch fraction or any similar polysaccharide, might differ from the free
drug with respect to solubility, diffusivity, partition coefficient and other properties. More important, a drug pharmacological effectiveness might be altered if the drug is bound in a complex. When the complex dissociates, i.e. by the hydrolysis of the macromolecular polysaccharide at some stage in the body, the drug can then be available for its usual pharmacological effect.

Another important difference of a drug complexed with a macromolecule from a free drug might be demonstrated in its ability to penetrate biological membranes. A drug complexed with starch cannot be expected to penetrate through a biological membrane as easily as a free drug could; thus complexation of a drug with starch or with a polysaccharide might hinder its movement from one compartment in the body to another compartment. In this respect such complexation of a drug might reduce the absorbability of the drug. The availability of a drug for external therapeutic treatment and its effectiveness should be investigated when the drug is included in a starch paste, sol or any base containing polysaccharides in general. The possible complexation of the drug with such macromolecules in aqueous bases should be questioned in terms of the release of the drug from the base, its absorbance and its effectiveness.

In a similar manner with the fundamental understanding of the high affinity of starch for molecules varying from phenols and alcohols to weak carboxylic acids, the pharmaceutical compounding should
investigate the effects of the complexation of the various drugs introduced in very small quantities in a tablet containing starch, especially when starch as a paste or polysaccharide excipients increase the magnitude of the complexing in the wet granulation technique. In general, the possible interactions between starch and many organic molecules found in small concentration dosage forms where starch is used as a pharmaceutical adjunct must be considered carefully.

A drug added to a liquid pharmaceutical preparation as a preservative for example might interact with the starch, if present in the system. The therapeutic drug, like the preservative, is sometimes added in small quantities compared to the large amount of the starch adjunct; such an amount of drug might be so small that it would be virtually impossible to detect a complex even if an insoluble complex precipitated.

If one considers the drug to be benzoic acid, employed in an aqueous preparation containing 3% potato starch in solution, a simple calculation (Figure 5 on Page 58) shows the amount of benzoic acid expected to be bound to the starch sol. A prediction can be made for the approximate increase in concentration of benzoic acid that is needed to obtain the effectiveness of benzoic acid as a preservative in the system, or if a drug, the amount to be released.

Drug complexing with starch can be a useful method of retarding absorption of a very irritant drug that might complex with starch,
and thereby reduce its toxicity. Starch has been used in this sense as an antidote for iodine poisoning since it proved effective in reducing the oral toxicity of iodine.

The starch complexing affinity could also explain the possible binding to bacterial excretion products in certain dermatitis conditions, thus providing the rationale of the wide use of starch as anti-irritant and adsorbent in dusting powders, lotions and pastes.

It is important to appreciate that the understanding of the many applications of a fundamental phenomenon, like starch complexation, can help the pharmaceutical compounder to predict any unfavorable incompatibilities, or to use these complexations to his advantage.

Some interesting future studies are indicated by the results of this study. The complexing with starch can serve as a means of increasing the solubility of a drug compared with a suspension; also, as the precipitation of "amylose-drug" complex is not a technical problem, such highly important questions as the stability of a drug in the complexed form should not remain long unanswered. The nature and behavior of these complexes appear to suggest their possible use in a sustained dosage form, since the release of the drug from the complex is possibly caused by enzymatic hydrolysis of the macromolecule.
SUMMARY AND CONCLUSIONS

A number of starches were selected to study their complexation with certain organic drug pharmaceuticals.

The "weight average" molecular weights of the starches were determined by a light scattering method. Commercially available amylose and amylopectin were used in the complexation study after molecular weight determinations were made.

Aqueous sols of the starches and the starch fractions were prepared by autoclaving at 20 lb/inch² for three hours.

Benzoic acid, p-hydroxybenzoic acid, and sorbic acid were used to detect the complexing behavior of the different starches.

A number of organic drug molecules which differ in their water solubilities and their polarity were selected to evaluate the complexing affinities with potato starch sols.

The solubility method was used for the detection of complexation, and all drugs were assayed spectrophotometrically.

The affinity of the unruptured potato starch granules to adsorb a number of the organic drug molecules from their water solutions were studied. In a similar manner the affinity of the granules to adsorb a number of fatty acids from methanol solutions was determined.
1. The molecular weight of a given starch as obtained in this work is an approximate mean value which averages all the polymeric components of the starch according to their masses.

2. All drugs tested with starch aqueous sols showed complexation evidenced by the increase in solubilities in water as the starch concentration in the sols increased.

3. Amylopectin (amylose-free) showed no evidence of interaction, while amylose sols showed a high uptake of the drug molecules into solution at relatively low amylose concentration in the sol (2-15 gm/L). A cake-like product which showed similarity in the crystalline nature to "amylose-butanol" complex, was separated by centrifuging. The release of drugs from the amylose complex was accomplished by the hydrolysis of the amylose.

4. When the same solid drug was added to the solutions of various starches, the amounts of drug that entered into solution before precipitating the complexes were inversely proportional to the "weight average" molecular weight of the starch.*

5. The solubility of the drug in water influences its tendency to complex with the starch. The higher the solubility, in general, the more the drug is solubilized by a given starch sol. However, the

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*Reaching a saturation concentration of a drug was, as expected, shown to depend on the solubility of the drug in water and on the starch content of the complexing low molecular weight amylose.
effect of some proton donating moieties was noted. Those moieties affect the affinity of interaction in a fashion not parallel to their effect on solubility.

6. Unruptured potato starch granules were shown to adsorb fatty acids and fatty alcohols from methanol solutions. The adsorption affinity was decreased with the increase of the hydrophobic chain of the fatty acid or fatty alcohol.

7. Correlations of the reported complexes with "amylose-alcohols" complexes used for the fractionation of starch and with "amylose-iodine" complexes reported in the literature, made it possible to assume that the drug-starch complexing takes the same route and may be the same mechanism as that of "starch-iodine" and "starch-n-butanol" complexes.
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