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COMPLEX INTERACTION OF STARCHES WITH
CERTAIN DRUG PHARMACEUTICALS

DISSERTATION

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

By

Mohamed Wafik Abdel-Latif Goudah, B. Pharm., M. Sc.

* * * * *

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Approved by

Adviser
College of Pharmacy

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VITA

January 20, 1938 . . . Born - Alexandria, Egypt

1957 B. Pharm., University of Alexandria

1958 Diploma in Hospital Pharmacy,
University of Alexandria

1959 Diploma in Industrial Pharmacy,
University of Alexandria

1959-1960 Demonstrator, College of Pharmacy,
University of Alexandria, Alexandria
Egypt

1961 M. Sc. Pharmacy, The Ohio State University,
Columbus, Ohio

PUBLICATIONS

"Effect of Zinc Oxide Catalyzed Peroxide on Sterols of
Almond, Cottonseed, and Olive Oils". J. Pharm. Sciences 52,
799, 1963.

FIELDS OF STUDY

Major Field: Pharmacy

Studies in Pharmacy Technology. Professor
Earl P. Guth

Studies in Physical Pharmacy. Professor David Guttman

Studies in Pharmaceutical Chemistry. Professors
Lloyd E. Harris and Frank W. Bope

Studies in Physical Chemistry. Professor
Quentin Van Winkle

Studies in Organic Chemistry. Professors Harold Shechter,
Melvin S. Newman and Michael P. Cava

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INTRODUCTION

Starch, one of the most widely distributed naturally occurring organic compounds, is used in many pharmaceutical preparations. Corn starch is official in the United States Pharmacopeia XVI (1). The British Pharmacopeia recognizes both corn and potato starch (2). Starch is used extensively as an absorbent in "weeping" types of dermatitis. It is applied to the skin as dusting powders, pastes and ointments for this purpose (3). Starch paste 10% W/W is often a tablet binder when rapid disintegration is expected. Starch is also used as a disintegrator and as a diluent in tablets. A 3% W/V starch sol is used as an enema, either as a vehicle or for its demulcent action in irritated colons. This same preparation may be used as an antidote in iodine poisoning (4). Starch glycerite U.S.P. XVI is emollient and demulcent. Starch mucilage is used primarily as an aid in suspending insoluble substances in liquids.

In recent years, there have been a great many publications dealing with the complex interactions of polymers with pharmaceuticals. Polyethylene glycols, polystyrene, polyvinylpyrrolidone, carboxymethylcellulose, Pluronics, Tweens, Carbowaxes, and other polymers containing nucleophilic oxygen were found to interact with preservatives as well as with certain drug chemicals (5-22).

Since starch is widely used in pharmacy and since there has been no report on its possible complex interactions with pharmaceutical drugs, this investigation was conducted with the following objectives in mind:

1. To study the complex interaction of starch with certain commonly used preservatives.
2. To determine the effect of molecular structure on the degree of complex interaction.
3. To try to postulate a possible mechanism for such interactions.
4. To report other information disclosed during the course of the investigation.

STARCH

Starch is one of the most widely distributed substances in the vegetable kingdom. Within the protoplast of many plant cells are inclusions in the form of starch granules. These granules are the principal food reserves of plants and are, hence, most abundant in such storage organs as seeds, which may contain as much as 70% starch, and fruits, tubers, roots, and stem pith which may contain as much as 30%. Starches are unique among the carbohydrates in occurring as discrete granules, whose physical and chemical characteristics vary from one plant source to another (23).

The chemical composition of starch is also dependent on its origin. Although mainly composed of polysaccharides of the glucan type, the different starches are also contaminated with varying amounts of fatty acids, proteins, and phosphorus (24). Cereal starches usually contain 0.5% to 1% fatty acids. In starches, such as potato, arrowroot, tapioca, and sago, phosphorus is bound as esterified phosphate at carbon atom 6 of the various D glucose units.

Starch manufacture is an important industry, and a number of continuous and batch extraction processes have been developed commercially to separate starch from grains, roots, tubers, and

pithy stems. These processes usually involve a series of washing, grinding, and pulping treatments.

Unmodified starch granules are insoluble in cold water. When a suspension of starch in water is heated, water is at first slowly and reversibly taken up and limited swelling occurs. Then, at a definite temperature, which is distinctive for different types of starch, the granules undergo irreversibly a sudden swelling and at the same time lose their birefringence. Since the granules swell to several hundred times their original volume, the viscosity of the suspension increases greatly. Starch pastes are complicated colloidal systems, in which there are present not only highly swollen granules but free starch molecules, the empty granule sacs, and aggregates leached from the swollen granules. Because of the differences in the size and form of the granules, starch from different sources may vary greatly in the type of paste which is produced even when the starches are of almost identical chemical composition.

The most common starches may be fractioned essentially by physical or physico-chemical procedures into components which differ in respect to both chemical properties and physical behavior. Accordingly, it has become evident that compared with many other carbohydrates, starch is relatively heterogeneous. Therefore, the structure can best be discussed in relation to the chemical structure of the components, or fractions, which make up the starch granules, and composition is given as the relative proportion of these components which differ in structure (25).

The view is now generally accepted that the majority of starches contain molecules which can be classified according to one of two quite different structural patterns. One type is a linear polymer, and the polymeric bonds are substantially 1-4

α -glucosidic linkages. The other contains a very large proportion of these linkages also, but periodically throughout the structure, linearity is interrupted by an anomalous linkage, such as, for example, a 1-6 glucosidic linkage which would serve to connect another chain of glucose units to the primary chain, thus creating a branched structure.

There is a considerable range of sizes among the molecules of each structural type of starch, even in any one variety of starch. Furthermore, among the non-linear molecules there is some variation in the frequency with which linearity is interrupted and in the length of the terminal branches. Nevertheless, in order to simplify the subject, the custom has prevailed for classifying starch polymer fractions according to whether they are essentially linear or non-linear, and to call the first, amylose, and the second amylopectin. It should be borne in mind that this classification may be somewhat arbitrary and superficial, since there may very well be molecules which contain so few anomalous linkages that they exhibit the properties of linear polymers and there may be linear polymers in starch so gigantic in size that their linear structure is obscured in many tests, owing to the non-linear configurations that are assumed under certain conditions.

Separation of amylose and amylopectin can be made by adding to a starch dispersion certain agents such as butanol, nitropropane, nitrobenzene, and thymol, which form complexes with amylose and cause it to precipitate in semicrystalline form. The amylose complex is collected by centrifugation, and the amylose is regenerated by adding hot water or ethanol. Amylopectin is then isolated from the mother liquor by precipitation with alcohol or by freeze-drying (26-28).

Amylose: Most starches contain 15-25% amylose, (the remainder being amylopectin) but there are exceptions, notably those of the waxy or glutinous varieties of maize, rice, and barley, which are almost pure amylopectin. The starches from certain varieties of pea and lily contain up to 75% amylose. Amylose is responsible for the blue color given by starch. Amylose combines with iodine to form a deep-blue complex. The intensity of the amylose-iodine complex can be measured in a spectrophotometer, or titrimetric measurements can be made of the amount of iodine taken up in forming the amylose-iodine complex (23).

Amylose molecules, because of their linear nature, can coalesce and, hence, retrograde. Retrogradation is a term applied to the reassociation which takes place between molecules in solution. As retrogradation takes place, starch solutions become increasingly cloudy, increasingly resistant to enzyme action, lower in viscosity and, finally, undergo precipitation. When amylose is methylated and hydrolysed, the yield of tetra-O-methyl-D-glucose corresponds with a chain length of 300-400 units (29-30). Determination of the molecular weight of amylose

by physical methods, such as ultracentrifugation, light scattering, osmotic pressure, and viscosity measurements, is more reliable, although difficulties arise since amylose readily retrogrades from solution. Recent studies on amylose have indicated a chain length of 3800 units (31).

If the spatial distribution of a chain of glucose units joined 1-4 (α -) is considered, it is found to assume a spiral form as shown in Figure 1 (page 8). This is due to the conformation of the glucosidic bonds. Support for this helical concept comes from x-ray, ultracentrifuge, viscometric, and other studies of the amylose-iodine complex and of certain amylose fractions in solution. Results indicate that the period of each spiral is six glucose units and, in the amylose-iodine complex, it has been shown that the iodine atoms are situated in the core of helically oriented amylose molecules (32-34).

Amylopectin: Amylopectin is the major component of starches. It yields reddish colorations with iodine. Amylopectin is stable in a colloidal sense in that it retrogrades extremely slow. This fraction is stable and functions as a protective colloid for the amylose fraction (35).

Amylopectins, most likely, have a very large and ramified structure with short linear branches as shown in Figure 2 (page 8). This structure is supported by the results of studies employing enzymatic degradation (36-37). Methylation studies have shown that amylopectin has unbranched chains of 20-25 D-glucose units (29-30).

Figure 1. Schematic diagram of the structural configuration of amylose.

Figure 2. Schematic diagram of the structural configuration of amylopectin.

COMPLEXATION

Complex compounds are primarily those molecules in which most of the bonding structure can be described by the classical theories of valency between atoms, but one or more of the bonds is somewhat anomalous and therefore complex (3). Complexes, or coordination compounds, according to the classical definition, result from a donor-acceptor mechanism or Lewis acid-base reaction between two or more different chemical constituents (38).

Martin (38) classifies complexes into the following types:

1. Metal Complexes.
2. Organic Molecular Complexes.
3. Occlusion Compounds.

Metal complexes: This group includes inorganic complexes as hexamine cobalt III chloride $[\text{Co}(\text{NH}_3)_6]^{3+}\text{Cl}_3^-$. The ammonia molecules are known as the ligands and are said to be coordinated to the cobalt ion. Each ligand devotes a pair of electrons to form a coordinate covalent link between itself and the central ion. Another example of this group are the chelates. They are formed by the combination of a metal and a substance containing two or more donor groups. The synthetic chelating agent EDTA is widely used to remove calcium ions from hard water. Chlorophyll and hemoglobin are naturally occurring chelates involved in the life processes of plants and animals respectively.

Organic molecular complexes: An organic molecular complex consists of constituents held together by weak forces of the donor-acceptor type or by hydrogen bonds. These forces are not to be considered as clearly defined bonds but rather as an overall attraction between the constituent molecules.

The type of bonding existing in molecular complexes where hydrogen bonding plays no part is not well understood, but it may be considered for the present as involving an electron donor-acceptor mechanism corresponding to that in metal complexes but ordinarily much weaker.

Many organic complexes are so weak that they cannot be separated from their solutions as definite compounds. The energy of attraction between the constituents is probably less than 5 kcal/mole for most organic complexes. Since the bond distance between the components of the complex is usually greater than 3Å, a covalent link is not involved. Instead, one molecule polarizes the other, resulting in a type of ionic attraction. For example, the polar nitro groups of trinitrobenzene induce a dipole in the readily polarizable benzene molecule and the electrostatic interaction results in complex formation.

A factor of importance in the formation of molecular complexes are the steric requirements. If the approach and close

association of the donor and acceptor molecules are hindered by steric factors, the complex is not likely to form. Hydrogen bonding and other effects play a role in molecular complexation.

Occlusion compounds: This class of addition compound results from the architecture of molecules rather than their chemical affinity. One of the constituents of the complex is trapped in the open lattice or cage-like crystal lattice of the other to yield a stable arrangement. The choleic acids are a group of channel lattice type complexes principally involving desoxycholic acid in combination with paraffins, organic acids, esters, ketones, aromatic compounds and with solvents such as ether, alcohol and dioxane. The crystals of desoxycholic acid are arranged in such a manner as to form a channel into which the complexing molecule can fit.

Some compounds as the clay, montomorillonite, the principal constituents of bentonite, can trap hydrocarbons, alcohols and glycols between the layers of lattice, resulting in the formation of a layer type inclusion complex.

Methods of Analysis

There are many methods for detecting complexation. A limited number of the more important methods related to this study will be discussed:

Solubility method: In this procedure the effect of varying concentrations of a complexing agent on the solubility of the drug in solution is studied. Excess quantities of the drug are placed in well-stoppered containers, together with the complexing

agent, and the bottles are agitated in a constant temperature bath until equilibrium is attained. Aliquot portions of the supernatant liquid are removed and analyzed. If complex action has taken place to any appreciable degree, one observes either an increase or decrease in the solubility, depending on the nature of the complex formed. The phase diagrams obtained from such studies provide, furthermore, a quantitative means of calculating the stoichiometry of the reaction.

Equilibrium dialysis method: The quantitative in vitro study of the interaction of drugs with proteins or other polymers is most conveniently carried out by the dialysis method. The method consists of bringing two solutions, one containing the macromolecular component and the other the drug under study, into equilibrium across a semipermeable membrane. The membrane is so chosen that it permits the low molecular weight drug to pass freely through the barrier and achieve the same activity in both solution phases, yet at the same time, so that it acts as an impermeable barrier toward the polymeric substance. Any increase in the total drug concentration in the polymer side of the barrier can be attributed to complex formation.

Distribution method: The distribution coefficient of the drug between water and another immiscible solvent is determined in the absence of the complexing agent. The distribution coefficient is then redetermined for the drug complex mixture. The difference between the two values is used to detect and determine the constants of complexation. This method is based on the fact

that only the free unbound form of the drug is soluble in the nonaqueous phase.

Pharmaceutical Complexation

One of the early reports on the complex interactions among the components of pharmaceutical preparations was published by Emery and Wright (39) in 1921. They studied the complexing action of caffeine with a number of compounds including sodium benzoate and sodium salicylate. The area of pharmaceutical complexation has been given considerable attention after 1952, when Higuchi and Zuck (40) reported that caffeine has a solubilizing action on benzoic acid. Using the distribution method, they were able to obtain experimental data supporting the existence of the complex in solution, as well as to calculate the equilibrium constant for the reaction. A quantitative study of the caffeine-benzoate interaction was reported by the same authors. In an attempt to gain some insight as to the structural factors favoring complex formation, they studied the interaction of caffeine with aspirin, m-hydroxybenzoic acid, p-hydroxybenzoic acid, salicylic acid, salicylate ion, and butyl paraben. The position of the functional groups were found to influence the stability of the complex. For example, the para form of hydroxybenzoic acid has much greater complexing ability than the ortho form. Esterification of the carboxyl group as well as acetylation of the hydroxyl group of p-hydroxybenzoic and salicylic acid respectively, resulted in a lowered complexing tendency (41).

Further extension studies of these caffeine complexes were reported by Higuchi et al. (42-44). By means of solubility analysis it was shown that caffeine complexes with sulfathiazole, sulfadiazine, p-aminobenzoic acid, benzocaine, phenobarbital, barbital, phthalic acid, and suberic acid. Using the distribution method, procaine and tetracaine were found to interact with caffeine, while valeric acid showed no tendency to interact with the xanthine. Solubilization of riboflavin by complexation with caffeine was reported by Guttman and Athalye (45).

The interaction between caffeine and the acidic drugs was attributed to a dipole-dipole force or by hydrogen bonding between the polarized carbonyl groups of caffeine and the hydrogen atom of the acid. A secondary interaction probably occurs between the nonpolar parts of the molecules. The complexation of the esters with caffeine is explained on the basis of a dipole-dipole interaction between the nucleophilic carboxyl oxygen of the ester and the electrophilic nitrogen of caffeine (38).

Methylated xanthines were also studied for their complexing tendencies. Theophylline and theobromine were found to interact with p-aminobenzoic acid, salicylic acid, acetylsalicylic acid, and p-hydroxybenzoic acid. Results obtained showed that the complexing interactions of the two methylated xanthines, are qualitatively very similar to the same interactions of caffeine (46). 1-Ethyltheobromine, a synthetic caffeine homolog, has been shown to complex with benzocaine. This interaction is less than the corresponding caffeine-benzocaine interaction (47).

Ethyl, propyl, and butyl derivatives of theobromine, and theophylline interacted with p-hydroxybenzoic acid. The results showed that the compounds investigated behave similarly in their interaction with the acid despite their differences in structure. The solubilities of the complexes formed, however, appear to be markedly dependent on the structure of the parent xanthines (47).

The interaction of drugs with polymers has been the subject of many investigations. Polyvinylpyrrolidone, a polymeric substance which has seen considerable use as a blood extender, was shown to complex with some pharmaceuticals such as sulfathiazole, sodium salicylate, chloramphenicol, mandelic acid, p-aminobenzoic acid, benzoic acid, salicylic acid, p-hydroxybenzoic acid, m-hydroxybenzoic acid, and phenobarbital. No evidence of complex formation was detected between polyvinylpyrrolidone and procaine hydrochloride, benzyl penicillin, caffeine, theophylline, cortisone, aminopyrine and citric acid.

Polyethylene glycols, "Carbowaxes,"¹ polyoxyethylene sorbitan fatty acid esters, "Tweens,"² sorbitan fatty acid esters, "Spans,"² polyoxyethylene acids, "Myrjs,"² and methyl cellulose are macromolecules that fall into the general category of polyethers. These compounds have found extensive and varied applications in pharmaceutical and cosmetic formulation (48). A substantial amount of work has appeared in the literature concerning the fundamental nature of interactions between polyethers and

¹Union Carbide Corp., New York, N.Y.

²Atlas Powder Co., Wilmington, Del.

other pharmaceutical species. Higuchi and Lach (6) made a study of the complexing behavior of high molecular weight polyethers with a number of pharmaceutical compounds. By means of solubility procedures it has been found that although pentobarbital and barbitol have little or no tendency to complex with polyethylene glycols, phenobarbital forms stable molecular compounds with these macromolecular substances. It was also shown that phenolic compounds are bound by the polyethers. Experimental data on the various acids investigated showed that these compounds are only weakly bound. Polyethylene glycol was also shown to form an insoluble complex with iodine (7). Soluble complexes of methyl and propyl paraben with polyethylene glycols were reported. This complexation interfered with the preservative action of the parabens (15-16). The complex interaction of phenols, barbiturates, carboxylic and amino compounds with polyethers is explained on the basis of hydrogen bonding and dipole-dipole interaction. The acidic hydrogen of these compounds is capable of coordinating with basic oxygen of the polyethers. Furthermore, the reaction is favored by the hydrophobic, lipophilic portions of the interacting molecules.

Similar studies were conducted on the various polyoxyethylene sorbitan fatty acid esters, "Tweens."¹ They all showed various degrees of interaction with benzoic acid, hydroxybenzoic acids, and esters of p-hydroxybenzoic acid. It was found that the preservative activity of those esters is primarily a function

¹Atlas Powder Co., Wilmington, Del.

of the concentration of unbound preservatives. Using the data for the complex interactions, it was possible to predict the required preservative concentration (16-18). Other preservatives such as cetylpyridinium chloride and benzalkonium chloride showed a high degree of association and accompanying inhibition of germicidal activity (20). The polyoxyethylene acids "Myrj"¹ surfactants were also shown to interact with benzoic acid derivatives. Other examples of complex interaction between various drug pharmaceuticals was cited in the literature (50-61).

The importance of pharmaceutical complexation cannot be over emphasized. Solubilization of caffeine and other xanthines has been achieved by the formation of soluble complexes (41). Many incompatibilities, which result in wholly unacceptable products, are the result of complex interactions. The softening of polyethylene glycols "Carbowaxes"² based suppositories containing aspirin, the flocculation of suspensions containing polyethylene glycols upon the addition of phenol, the loss of the antimicrobial activity of phenolic preservatives when incorporated with polyethers in pharmaceuticals and cosmetics, are due to such interactions (48).

The phenomenon of molecular association, responsible for the existence of these complexes often determines the pharmacological efficacy of drugs and even the biochemical mechanism by which they operate. The phenomenon is responsible for the

¹Atlas Powder Co., Wilmington, Del.

²Union Carbide Corp., New York, N.Y.

pharmacologically retardant action of drugs and is consequently utilized in the production of sustained release dosage forms (5,59).

Complexation has an effect on the stability of drugs. The oxidative degradation of epinephrine and bilirubin is inhibited by complexation with plasma proteins (38). The hydrolysis of procaine, benzocaine, and tetracaine was retarded by complexation with caffeine (44, 45, 62). It was found that the rate of hydrolysis is a function only of the free uncomplexed drug in solution. The rate of the base catalyzed degradation of riboflavin was decreased by the presence of caffeine (63).

MATERIALS

Starches

Potato Starch¹

St. Vincent Arrowroot Starch²

The physical characteristics of these starches are listed in Table 1 below.

TABLE 1
PHYSICAL CHARACTERISTICS OF STARCHES

	Potato Starch	Arrowroot Starch
Color	White	White
Form	Fine Powder	Fine Powder
Moisture	13%	9.4%
pH	6.4	6.05

Drug Chemicals

Benzoic Acid, A.R., m.p. 122-122.5° C.

Salicylic Acid, A.R., m.p. 157-159° C.

¹Mallinckrodt Chemical Works, New York.

²S. B. Penick & Company, New York.

Para-Hydroxybenzoic Acid, purified, m.p. 213-215° C.

Meta-Hydroxybenzoic Acid, purified, m.p. 198-200° C.

Methyl p-Hydroxybenzoate, purified, m.p. 125-126° C.

Ethyl p-Hydroxybenzoate, purified, m.p. 115-116° C.

Propyl p-Hydroxybenzoate, purified, m.p. 94-95° C.

P-Aminobenzoic Acid, purified, m.p. 185-186° C.

Ethyl p-Aminobenzoate, N.F., m.p. 88-90° C.

Caffeine, U.S.P., m.p. 234-235° C.

All other materials used were of the reagent grade.

EXPERIMENTAL

Preparation of starch sol: Starch, 16.2-24.3 Gm., was accurately weighed into a beaker, smoothed into a slurry with distilled water, then poured slowly with stirring into a flask of hot water at 90-95° C., stirred for five minutes, then transferred to an autoclave and heated at a pressure of 19-21 lb/inch² for three hours. The resulting sol was then stirred until cooled to room temperature. The sol was then transferred to a volumetric flask and distilled water added to volume of 500 ml. Autoclaving insured complete dispersion of the starch granules and markedly decreased the viscosity. All starch sols were discarded after 24 hours of preparation.

Method of detecting complexation: The solubility method was employed in determining the extent and the nature of possible complexing reactions between the compounds studied and the starch sol. Excess quantities of the drug were accurately weighed into a 125 ml. bottle together with varying amounts of starch sol, then water was added to a final volume of 60 ml. The bottles were stoppered with a No. 2 gum rubber stopper, and then agitated in a constant temperature water bath at 30° C. $\pm$ 0.5° C., for 3-4 days. After equilibrium was attained the excess solid was allowed to settle while the bottles remained in the water bath. A 1 ml. aliquot of the clear supernatant liquid was then withdrawn

and appropriately diluted for spectrophotometric analysis. A piece of cotton and a Whatman analytical grade filter paper fitted to the delivery end of the pipette served as a suitable filtering device. Dilution factors varied from 1:100 to 1:2000 depending on both the solubility and the molar absorbancy of the drug tested.

To avoid any error that might be due to the viscosity of the sols, each pipette was calibrated to deliver all its contents, by washing the pipette several times with distilled water, and calculations were made accordingly.

Method of assay: All drugs used were assayed spectrophotometrically. A Beckman Model D.U. spectrophotometer was used. The wavelengths of maximum absorption, together with the molar absorbancies, are shown in Table 2. All drugs used obeyed Beer's Law and the molar absorbancies were calculated using the least squares method (64). The absorbancy of the starch sol was negligible at dilutions $\gg$ 1:500. At lesser dilutions the absorption of the starch sol was corrected for by determining the optical density of a series of dilutions of the starch sol at the wavelength of determination of the drug. The specific extinction coefficient of the sols obtained was used to calculate the optical density of the starch sol. This value was subtracted from the optical density obtained for the drug-starch sol mixture.

Results for the interaction of the potato starch and the above mentioned drugs are shown in Tables 3-12 (pages 25-29) and the corresponding phase diagrams are plotted in Figures 3-6 (pages 30-33).

Results for the interaction of arrowroot starch are shown in Tables 13-22 (pages 34-38) and the corresponding phase diagrams are plotted in Figures 7-10 (pages 39-42).

Starch concentrations are expressed in number of equivalents per litre.

$$\text{The number of equivalents} = \frac{\text{weight of starch used}}{\text{molecular weight of the starch repeating unit.}}$$

The molecular weight of the repeating unit in the starch molecule ($\text{C}_6\text{H}_{10}\text{O}_5$), is equal to 162.

Calculations were made on basis of dry weight of starch used.

TABLE 2
SPECTROPHOTOMETRIC CHARACTERISTICS
OF DRUGS TESTED

Drug	$\lambda_{\text{max.}}$, μ	Molar absorbancy $\times 10^{-4}$
Benzoic Acid	272.5	.0873
Salicylic Acid	298	.3530
p-Hydroxybenzoic Acid	251	1.2100
m-Hydroxybenzoic Acid	290	.2040
Methyl p-Hydroxybenzoate	256	1.4800
Ethyl p-Hydroxybenzoate	256	1.5000
Propyl p-Hydroxybenzoate	255	1.5500
p-Aminobenzoic Acid	278	1.2900
Ethyl p-Aminobenzoate	283	1.6700
Caffeine	272	1.0100

TABLE 3
INTERACTION OF POTATO STARCH
WITH BENZOIC ACID IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	3.41
2.17	3.49
4.35	3.60
8.70	3.74
10.88	3.80
13.05	3.87
17.40	4.00
21.75	4.14

TABLE 4
INTERACTION OF POTATO STARCH WITH
SALICYLIC ACID IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	1.79
3.04	1.76
4.56	1.76
6.08	1.76
7.60	1.71
9.13	1.69
10.65	1.66
12.17	1.59
13.69	1.58

TABLE 5

INTERACTION OF POTATO STARCH WITH
p-HYDROXYBENZOIC ACID IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	5.29
4.35	5.52
6.53	5.59
8.70	5.76
10.88	6.00
13.05	6.02
17.40	6.17
19.58	6.29
21.75	6.55

TABLE 6

INTERACTION OF POTATO STARCH WITH
m-HYDROXYBENZOIC ACID IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	8.98
4.35	9.13
6.53	9.32
8.70	9.25
10.88	9.57
15.23	9.74
17.40	9.99
21.75	10.10

TABLE 7

INTERACTION OF POTATO STARCH WITH
METHYL-p-HYDROXYBENZOATE IN
AQUEOUS SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Ester Conc. M/L x 10 ³
0.00	17.4
4.17	17.9
6.53	18.2
8.70	18.5
10.86	19.1
13.05	20.0
17.40	20.8
19.58	21.4
21.75	21.7

TABLE 8

INTERACTION OF POTATO STARCH WITH
ETHYL p-HYDROXYBENZOATE IN
AQUEOUS SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Ester Conc. M/L x 10 ³
0.00	6.39
4.35	6.36
6.53	6.66
8.70	7.19
10.88	7.49
13.05	7.79
15.23	7.96
17.40	8.42
19.58	8.49
21.75	8.72

TABLE 9

INTERACTION OF POTATO STARCH WITH PROPYL
p-HYDROXYBENZOATE IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Ester Conc. M/L x 10 ³
0.00	2.56
4.35	2.54
6.53	2.60
8.70	2.88
10.88	2.95
13.05	3.04
15.23	3.19
17.40	3.21
19.58	3.57
21.75	3.60

TABLE 10

INTERACTION OF POTATO STARCH WITH
p-AMINOBENZOIC ACID IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	4.52
4.35	4.72
6.53	4.82
8.70	4.92
10.88	5.00
13.05	5.10
15.23	5.20
19.58	5.33
21.75	5.45

TABLE 11

INTERACTION OF POTATO STARCH WITH ETHYL
p-AMINOBENZOATE IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Ester Conc. M/L x 10 ³
0.00	7.25
4.35	7.73
6.53	8.00
8.70	8.21
10.88	8.42
13.05	8.63
15.23	8.96
17.40	9.05
19.58	9.35
21.75	9.68

TABLE 12

INTERACTION OF POTATO STARCH
WITH CAFFEINE IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Caffeine Conc. M/L x 10
0.00	1.31
4.35	1.31
6.53	1.31
8.70	1.34
10.88	1.30
13.05	1.27
15.23	1.29
17.40	1.31
19.58	1.28
21.75	1.28

Figure 3. Phase diagram showing the effect of various concentrations of Potato Starch on the apparent solubility of several organic acids in water at 30°C.

Figure 4. Solubility behavior of Salicylic acid in presence of Potato Starch in water at 30° C.

Figure 5. Phase diagram showing the effect of various concentrations of Potato Starch on the apparent solubility of several organic esters in water at 30°C.

Figure 6. Solubility behavior of Caffeine in presence of Potato Starch in water at 30° C.

TABLE 13

INTERACTION OF ARROWROOT STARCH WITH
BENZOIC ACID IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	3.45
3.01	3.52
4.52	3.59
6.03	3.64
9.04	3.71
10.55	3.80
12.05	3.86
13.56	3.90

TABLE 14

INTERACTION OF ARROWROOT STARCH WITH
SALICYLIC ACID IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	1.79
3.01	1.77
4.52	1.78
6.03	1.82
7.53	1.72
9.34	1.70
10.55	1.69
12.05	1.66
13.08	1.63
14.95	1.57

TABLE 15

INTERACTION OF ARROWROOT STARCH WITH
p-HYDROXYBENZOIC ACID IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	4.96
4.52	5.16
6.03	5.13
7.53	5.33
9.04	5.49
10.55	5.56
12.05	5.42
13.56	5.64
15.07	5.77

TABLE 16

INTERACTION OF ARROWROOT STARCH WITH
m-HYDROXYBENZOIC ACID IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	8.81
3.74	9.10
6.03	8.96
7.53	9.18
9.04	9.45
10.55	9.40
12.05	9.50
13.56	9.59

TABLE 17

INTERACTION OF ARROWROOT STARCH WITH
METHYL p-HYDROXYBENZOATE IN
AQUEOUS SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Ester Conc. M/L x 10 ³
0.00	17.7
3.74	18.6
5.60	19.0
7.47	19.5
9.04	19.7
9.34	19.8
10.55	20.2
13.08	20.9
14.95	21.3
16.81	21.7

TABLE 18

INTERACTION OF ARROWROOT STARCH WITH
ETHYL p-HYDROXYBENZOATE IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Ester Conc. M/L x 10 ³
0.00	6.30
3.01	6.57
4.52	6.77
6.03	6.93
7.53	7.10
9.04	7.20
10.55	7.50
12.05	7.93
13.56	8.00
15.07	8.10

TABLE 19

INTERACTION OF ARROWROOT STARCH WITH PROPYL
p-HYDROXYBENZOATE IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Ester Conc. M/L x 10 ³
0.00	2.56
3.01	2.47
4.52	2.57
6.03	2.69
7.53	2.86
9.04	2.84
10.55	2.88
12.05	2.94
13.56	3.15
15.07	3.17

TABLE 20

INTERACTION OF ARROWROOT STARCH WITH
p-AMINOBENZOIC ACID IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	4.52
3.74	4.75
5.60	4.80
7.47	4.90
9.34	5.10
11.21	5.13
13.08	5.22
16.81	5.35
18.68	5.50

TABLE 21

INTERACTION OF ARROWROOT STARCH WITH ETHYL
p-AMINO BENZOATE IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Ester Conc. M/L x 10 ³
0.00	7.13
3.74	7.49
4.52	7.64
7.47	7.97
9.34	8.15
11.21	8.39
13.08	8.60
14.95	8.84
16.81	9.02
18.68	9.27

TABLE 22

INTERACTION OF ARROWROOT STARCH
WITH CAFFEINE IN AQUEOUS
SOLUTION AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Caffeine Conc. M/L x 10
0.00	1.31
3.01	1.31
4.52	1.30
6.03	1.29
7.53	1.30
9.04	1.27
10.55	1.25
12.05	1.26
13.56	1.27
15.07	1.26

Figure 7. Phase diagram showing the effect of various concentrations of Arrowroot Starch on the apparent solubility of several organic acids in water at 30°C.

Figure 8. Solubility behavior of Salicylic acid in presence of Arrowroot Starch in water at 30° C.

Figure 9. Phase diagram showing the effect of various concentrations of Arrowroot Starch on the apparent solubility of several organic esters in water at 30°C.

Figure 10. Solubility behavior of Caffeine in presence of Arrowroot Starch in water at 30° C.

Effect of pH on complexation: To determine the effect of pH on the degree of complexation, the interaction of benzoic acid and potato starch was studied over a range of pH from 1.7 to 4.2. The buffer solutions of Clark and Lubs (1) was used to keep the pH constant throughout the period of interaction. Ten ml. of each buffer was used. The procedure was essentially the same, as the one used for detecting complexation. The pH of the drug-starch mixture, after equilibrium, was measured with a Beckman Model G pH Meter. Benzoic acid was assayed spectrophotometrically. The wavelength of maximum absorption showed a shift towards a shorter wavelength of 271 mu. for pH of 1.7 and 2.6, while at pH of 3.3 and 4.2, the shift was towards the longer wavelength of 273 and 276 respectively. The molar absorbancies were recalibrated using the same concentration of buffer that would be in the final dilution of the starch-drug mixture.

Results of the interaction of potato starch with benzoic acid at various pH increments studied are shown in Tables 23-26 (pages 44-45).

The corresponding phase diagrams are shown in Figures 11-12 (pages 46-47).

Complex interaction at 20° C.: The interactions of potato starch with benzoic acid and p-hydroxybenzoic at 20° C. were measured. The procedure was essentially the same, except for the temperature being kept constant at 20° C. $\pm$ 0.5° C. Results are shown in Tables 27-28 (page 48), and the corresponding phase diagram is plotted in Figure 13 (page 49).

TABLE 23
 INTERACTION OF POTATO STARCH
 WITH BENZOIC ACID AT
 PH 1.7, AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	2.97
6.53	3.23
8.70	3.31
10.88	3.36
13.05	3.41
15.23	3.45
17.40	3.50
19.58	3.59
21.75	3.83

TABLE 24
 INTERACTION OF POTATO STARCH
 WITH BENZOIC ACID AT
 PH 2.6, AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	3.24
4.35	3.41
6.53	3.56
8.70	3.61
10.88	3.75
13.05	3.75
15.23	3.86
17.40	3.85
19.58	3.93

TABLE 25

INTERACTION OF POTATO STARCH
WITH BENZOIC ACID AT
PH 3.3, AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	3.50
4.35	3.60
6.53	3.65
10.88	3.69
13.05	3.79
15.23	4.00
17.40	4.03
19.58	4.16
21.75	4.24

TABLE 26

INTERACTION OF POTATO STARCH
WITH BENZOIC ACID AT
PH 4.2, AT 30° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	6.14
4.35	6.28
8.70	6.46
10.88	6.49
13.05	6.56
15.23	6.63
17.40	6.75
19.58	6.88
21.75	6.96

Figure 11. Solubility behavior of Benzoic acid in presence of Potato Starch in water at 30° C., at various pH increments.

Figure 12. Solubility behavior of Benzoic acid in presence of Potato Starch in water at 30° C., at various pH increments.

TABLE 27

INTERACTION OF POTATO STARCH WITH
BENZOIC ACID IN AQUEOUS
SOLUTION AT 20° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	2.40
4.36	2.54
6.53	2.61
8.71	2.75
10.89	2.82
13.07	2.89
17.42	2.98
19.60	3.06
21.78	3.21

TABLE 28

INTERACTION OF POTATO STARCH WITH
p-HYDROXYBENZOIC ACID IN AQUEOUS
SOLUTION AT 20° C.

Orig. Starch Conc. Equiv./L x 10 ²	Satd. Total Acid Conc. M/L x 10 ²
0.00	3.08
4.36	3.44
6.53	3.49
10.89	3.82
13.07	3.95
15.24	4.00
17.42	4.15
19.60	4.25

Figure 13. Phase diagram showing the effect of various concentrations of Potato Starch on the solubility of certain organic acids in water at 20° C.
 O = Benzoic Acid--X = p-Hydroxybenzoic Acid.

DISCUSSION

Part I. Evidence and Importance of Complexation

In the solubility method of analysis, complex interaction is evidenced by either a decrease or an increase in the solubility of the complex formed, depending on the nature. The results of the interaction of benzoic acid and potato starch (Table 3, page 25, and Figure 3, page 30) showed evidence of the formation of a soluble complex. The straight-line plots obtained show that the increase in solubility of the drug is a function of the concentration of the starch. When plots obtained through solubility data show a plateau region, stoichiometries and formation constants of the complexes can be calculated. Where no plateau region is obtained, stoichiometry cannot be determined because the concentration of the free drug present in solution is an invariant. Such was the case in the interaction of benzoic acid with potato starch. However, linear dependence of the complexation upon starch concentration is indicative of a first order dependency of the interaction upon starch concentration. In cases such as this, stoichiometry with respect to the drug molecule is difficult to calculate since the data were obtained at constant drug activity. Excess of drug being present at all times. The results for p-hydroxybenzoic acid, m-hydroxybenzoic

acid, p-aminobenzoic acid (Tables 5-6, page 26, and Table 10, page 28) as well as the corresponding phase diagrams (Figure 3, page 30) indicate that these acids form soluble complexes with potato starch. Similarly to benzoic acid, these interactions showed first order dependency upon the starch concentration. Results for the interaction of salicylic acid with potato starch (Table 4, page 25) and the related phase diagram (Figure 4, page 31) is indicative of complex formation. The complex formed in this system appears to be extremely insoluble, since no initial increase in the apparent solubility of salicylic acid was observed. The total concentration of salicylic acid in the plateau region of this phase diagram is an invariant independent of the amount of starch since any salicylic acid precipitated in the form of the complex is replaced by further dissolution of the solid free salicylic acid. At point B the system is no longer saturated with respect to the salicylic acid, since all of the excess solid acid has been converted to the complex. Further addition of starch results in the depletion of free salicylic acid in solution.

The stoichiometric ratio of the components of the salicylic acid-starch complex formed in the plateau region can be calculated from the phase diagram. This is possible because, if the above analysis is true, the amount of starch represented by the plateau region is equal to that entering into the complex during this interval. The corresponding amount of salicylic acid being converted to the complex is equal to the difference between

the total amount added to the system and the amount soluble in distilled water at 30° C.

Starch content of complex formed in plateau region

$$= 6.7 \times 10^{-2} \text{ Equiv./L (from diagram, Figure 4, page 31)}$$

Salicylic acid content of complex

= Total acid added to system - salicylic acid in solution at point A

$$= (2.11 - 1.79) \times 10^{-2}$$

$$= 3.2 \times 10^{-3} \text{ M/L}$$

Then the stoichiometric ratio = $\frac{\text{Starch content}}{\text{Salicylic acid content}}$

$$= \frac{6.7 \times 10^{-2}}{3.2 \times 10^{-3}} \approx \frac{21}{1}$$

Examination of the diagram, shows that there is a very slight decrease in the slope of the line B-C. This means that only a relatively small number of salicylic acid molecules reacts with a large number of equivalents of potato starch. This seems to be in accordance with the 21:1 stoichiometric ratio, that is twenty-one equivalents of starch, reacting with one molecule of salicylic acid.

The interactions of methyl p-hydroxybenzoate, ethyl p-hydroxybenzoate, propyl p-hydroxybenzoate, and ethyl p-amino-benzoate with potato starch were shown in Tables 7-9 (pages 27-28) and Table 11 (page 29). The corresponding phase diagrams (Figure 5, page 32) indicate and prove a definite complex interaction between these drug chemicals and the starch. All the complexes formed were of the soluble type.

Caffeine was the only drug tested that showed no evidence of interaction with potato starch (Table 12, page 29 and Figure 6, page 33). There was no increase or decrease in the solubility of caffeine on the addition of the starch sol. The phase diagram was in the form of a plateau without any breaking point, indicating the absence of an insoluble complex. Visual examination of the interaction systems also showed the absence of any insoluble complex.

The interaction of arrowroot starch with the drugs were similar to those of potato starch. Benzoic acid, p-hydroxybenzoic acid, m-hydroxybenzoic acid, p-aminobenzoic acid, methyl p-hydroxybenzoate, ethyl p-aminobenzoate, showed indication of formation of soluble complexes with arrowroot starch (Tables 13 and 15-21, pages 34-38 and Figures 7 and 9, pages 39 and 41).

The interaction of salicylic acid with arrowroot starch resulted in the formation of an insoluble complex (Table 14, page 34 and Figure 8, page 40). The stoichiometric ratio of the components of the salicylic acid--arrowroot starch complex formed in the plateau region can be calculated from the phase diagram.

Starch content of complex formed in plateau region

$$= 6.8 \text{ Equiv./L} \times 10^{-2} \text{ (from diagram, Figure 8, page 40)}$$

Salicylic acid content of complex

$$= \text{Total acid added to system} - \text{salicylic acid in solution at point A}$$

$$= (2.11 - 1.79) \times 10^{-2} = 3.2 \times 10^{-3}$$

$$\text{Then the stoichiometric ratio} = \frac{\text{Starch content}}{\text{Salicylic acid content}}$$

$$= \frac{6.8 \times 10^{-2}}{3.2 \times 10^{-3}} \approx \frac{21}{1}$$

The stoichiometric ratio is again twenty-one equivalents of starch to one molecule of salicylic acid.

Again caffeine did not show any evidence of complex interaction with arrowroot starch (Table 22, page 38 and Figure 10, page 42).

The drug chemicals interacted in the same manner with the two starches tested. This indicates that inherent in the starch structure, is the liability to form complexes with polar drug pharmaceuticals. This is in agreement with Whistler and Hilbert (28) findings that organic compounds which are capable of forming hydrogen bonds form complexes with amylose. Although these workers utilized this phenomenon to affect the fractionation of starch into amylose and amylopectin using special experimental conditions, it is expected that a starch sol, which is a mixture of the two fractions, will show the same tendency of complex formation towards polar drugs. Other macromolecules, which have in their structure the potentiality of hydrogen bonding and dipole-dipole interactions, showed various degrees of interaction with benzoic acid, hydroxybenzoic acids, and esters of p-hydroxybenzoic acid (15-18).

The interaction of starch with drug pharmaceuticals is a factor that should be considered when formulating pharmaceutical preparations. Numerous reports have appeared in recent years concerning the inactivation of various preservatives in the presence of several nonionic surfactants and vegetable gums

(19, 65, 66). Kostenbauder et al. (18) found that the preservative activity of p-hydroxybenzoic acid esters is primarily a function of the concentration of the unbound preservative. Knowing the degree of interaction of the preservative with the macromolecule, would allow prediction of the approximate increase in concentration required to effect adequate preservation. The complex interaction of starches with benzoic acid, and p-hydroxybenzoic acid esters is expected to affect their preservative activity. When the results of complex interaction of these compounds is expressed as the ratio of total (free and complexed) preservative concentration to free (uncomplexed) preservative concentration, as a function of starch concentration, the increased quantity of preservative which might be required can be determined. When the complexation data for benzoic acid, and p-hydroxybenzoic acid ester with potato starch (Tables 3, 7, 8 and 9, pages 25, 27 and 28) are treated in the above mentioned way, it is found that the concentration of benzoic acid employed as a preservative for a 3% W/V starch sol, for example, should be approximately 20% more than the concentration which would be employed in the absence of starch sol. The concentration for methyl, ethyl, and propyl parabens needed for effective preservation should be increased by a factor of 22%, 30%, and 30% respectively.

From the complexation data of arrowroot starch with benzoic acid, and p-hydroxybenzoic acid esters (Tables 13, 17, 18 and 19, pages 34, 36 and 27) the concentration of benzoic acid employed as a preservative for a 3% W/V arrowroot starch sol should be

approximately 20% more than the concentration which would be employed in the absence of starch sol. The concentration for methyl, ethyl, and propyl parabens should be increased by 25%, 35%, and 30% respectively. Obviously even higher concentration of the preservative will be needed for more concentrated starch sols.

While this approach is undoubtedly an over simplification of actual conditions, it nevertheless provides some indication of the quantity of preservative that might be required. In practice if preservatives are employed in concentration well in excess of minimum inhibitory concentration, then binding a portion of the preservative might not reduce the effective concentration to a level which would permit microbial growth. The preservation of pharmaceutical preparations undoubtedly depends upon more factors than the interaction between preservatives and macromolecules. However, consideration of such interactions in formulation work should not be overlooked.

Salicylic acid is widely used, and in various vehicles, for local treatment of a variety of dermatoses. On the scalp the use of a washable type of base makes salicylic acid completely available to the skin, with the result that a relatively low concentration may be used. The finding that salicylic acid forms insoluble complexes with starch sols represents an incompatibility which should be taken into consideration when the two are dispensed together. The phenomenon might be investigated for prolonging the action of salicylic acid when it is applied to the skin in external preparations containing starch pastes.

The tendency of starch to form complexes with drug pharmaceuticals proves that starch is not an inert substance, as it is usually regarded. The results of this work indicate that the complexing behavior of starch is a factor to be considered when accounting for the rates of dissolution of drugs from compressed tablets, where starch is one of the ingredients. Carbohydrates, mainly starch, also represent a major part of our food intake. The complexing tendency of starch should not be overlooked when prescribing a drug to be taken immediately before or immediately after meals. Also, the ability of starch to form complexes may be utilized as a mean of stabilization of some drugs.

Part II. Effect of Molecular Structure on the
Degree of Interaction

Several methods have been used to compare relative complexing tendencies using data obtained from solubility studies. Comparison of the per cent increase in solubility has been used as a comparison of relative complexing tendencies with the assumption being made that a higher per cent increase in solubility involves a greater degree of interaction (21, 53). Lach and Cohen (58) in studying the complex interactions of pharmaceuticals with Schardinger dextrans compared the relative complexing tendencies by comparing the slopes of the interaction isotherms. In this study, the method of comparing the slopes is used. The slopes of the interaction isotherms for the drug tested are shown in Table 29 (page 59). These slopes are calculated from the complex interaction data (Tables 3-23, pages 25-29, 34-38 and 44) using the method of least squares (64). Examination of Table 29 (page 59) shows the following order of relative complexing tendencies: m-Hydroxybenzoic > p-Hydroxybenzoic > p-Aminobenzoic acid > Benzoic acid > Methyl p-hydroxybenzoate > Ethyl p-Hydroxybenzoate > Ethyl p-aminobenzoate > Propyl p-Hydroxybenzoate. Both potato and arrowroot interacted with these drugs in the foregoing order. Salicylic acid formed a plateau indicative of insoluble complex, and hence the slope could not be calculated.

TABLE 29
SLOPES OF ISOTHERMS OF INTERACTIONS OF THE
DRUG CHEMICALS WITH THE STARCHES

Drug	Potato Starch Slope $\times 10^2$	Arrowroot Starch Slope $\times 10^2$
m-Hydroxybenzoic Acid	5.55	5.86
p-Hydroxybenzoic Acid	5.48	5.29
p-Aminobenzoic Acid	4.20	5.10
Benzoic Acid	3.31	3.41
Methyl p-hydroxybenzoate	2.15	2.40
Ethyl p-hydroxybenzoate	1.23	1.28
Ethyl p-aminobenzoate	1.08	1.15
Propyl p-hydroxybenzoate	0.536	0.473
O-Hydroxybenzoic Acid	Insoluble Complex	Insoluble Complex
Caffeine	No interaction	No interaction

Caffeine did not interact at all. Where a plateau region is obtained through solubility data, the stoichiometry as well as the formation constant of the complex K_f can usually be determined.

Starch + Salicylic acid = (Starch--Salicylic Acid Complex)

$$K_f = \frac{(\text{Starch-Salicylic Acid Complex})}{(\text{Starch})(\text{Salicylic Acid})}$$

However, since no definite molecular weight of either potato or arrowroot starch is known, the formation constant of the starch-salicylic acid complex could not be calculated. Complex interaction of other macromolecules with the above drugs followed more or less the same order (5,6, and 16). The Schardinger dextrans are a series of homologous oligosaccharides obtained from the breakdown of starch by the action of bacillus macerans amylase. They are homogeneous cyclic molecules composed of six or more alpha-D-glucopyranose units linked 1-4 as in amylose. Lach and Cohen (58) reported the relative complexing tendencies of the above mentioned drugs with B Schardinger dextrin as follows: m-Hydroxybenzoic > p-Hydroxybenzoic > Benzoic = Methyl p-hydroxybenzoate > Ethyl p-hydroxybenzoate > p-Aminobenzoic acid > Ethyl p-aminobenzoate > Propyl p-hydroxybenzoate. Except for p-Aminobenzoic acid, the same order was obtained for the starches. They also found that B Schardinger dextrin formed an insoluble complex with salicylic acid.

The mechanism of interaction of the drug chemicals tested with the starch sols can be elucidated by considering the chemical structure of the starch molecules, as well as the chemical

structure of the drugs. Starch is composed of two fractions, amylose and amylopectin. Amylose is a linear polymer of glucose units, while amylopectin has a branched structure. When the spatial distribution of a chain of glucose units joined 1 (4 $\rightarrow$)-4 is considered, it is found to assume a helical configuration. X-ray studies (34) have shown that in the starch iodine complex, the amylose chains assume the configuration of a tight helix 13 A.^o in diameter, 8 A.^o in period containing six glucose residues per helix turn.

Because of the helical structure of the starch molecules, and the relatively large open space within the molecule, it might be expected that one factor for complexation is the formation of monomolecular inclusion compounds where one molecule of the host encloses one or more molecules of the guest. Another factor that would be expected to play an important part in the complex interaction is hydrogen bonding. The multiplicity of hydroxyl and carboxyl groups on the starch molecule enables it to interact with polar drugs.

Data for the relative complexing tendencies of the drugs tested are in support of this mechanism. If the complex interactions were only due to inclusion formation, the smallest molecule, unsubstituted benzoic acid, would show the greatest degree of inclusion and therefore the greatest slope. Since it does not, other forces are also involved. Meta, and para hydroxybenzoic acids showed the greatest slope, and therefore the greatest degree of interaction. Since these substituted acids are larger than benzoic acid, one would expect them to show less

tendency towards inclusion formation. However, these molecules possess an extra hydroxyl group, causing greater intermolecular hydrogen bonding with the starch molecule. This is definite proof that hydrogen bonding is an important factor in the complexation mechanism. This is further substantiated by the fact that p-aminobenzoic acid has a degree of interaction intermediate between benzoic acid and the hydroxybenzoic acids. P-Aminobenzoic acid has less tendency to form hydrogen bonds than the hydroxyacids probably due to the weak electrophilic nature of amino hydrogen as contrasted to hydroxyl hydrogen.

The importance of inclusion formation as additional factors for the interaction can be appreciated when the degree of interaction of starch with the parabens (Table 29, page 59) are examined. A decrease in slope with increasing molecular weight is observed. This is attributed to spacial filling, as the ester chains become longer and the molecule therefore larger, less inclusion of the ester takes place.

The failure of caffeine to interact with starch sols is probably due to its large stereochemical configuration, which would be difficult to fit in the voids of the helical starch molecule. The stereochemistry of the reactants is an important factor in complex interaction. Higuchi et al. found that caffeine does not interact with polyvinylpyrrolidone (5). The failure of barbital and pentobarbital to complex with polyethylene glycols was attributed to be due in part to stereochemical effect (6).

Salicylic acid forms insoluble complexes with the starches, hence the slope of the isotherm could not be calculated.

Salicylic acid was also found to form insoluble complexes with caffeine and methylated xanthines (41, 46).

Most of the compounds tested showed a greater slope for interaction with arrowroot starch than for interaction with potato starch. This case was reversed for p-hydroxybenzoic acid, and propyl paraben. Due to the heterogeneity and complexity of the starch chemical composition, no satisfactory explanation can be given for this anomaly.

A justification for comparing the slopes of the isotherms as a truer picture of relative complexing tendency, is the fact that according to this method methyl paraben shows a higher degree of interaction than propyl paraben. If the per cent increase in solubility were to be used, this result would be reversed. It is possible that propyl paraben would interact more than methyl paraben, with macromolecules which have hydrophilic, hydrophobic moieties, the reaction being favored by more interaction of the propyl group with the hydrophobic portion of the macromolecule. However, in case of hydrophilic polymers like starch, methyl paraben would be favored for complex interaction over propyl paraben. It is believed that comparison of the slope is the better method for comparing interaction of hydrophilic polymers with drug pharmaceuticals. However, it must be realized that this comparison of slope is an approximation, and is used solely because of the lack of exact stoichiometric data.

The net interaction observed in this study is believed to be a combination of attractive forces and inclusion formation. No distinction as to the relative contribution of the two forces

can be obtained from the data presented. In general the smallest and most soluble drugs showed the highest slope, while those drugs which are least soluble in water showed the greatest per cent increase in solubility as a function of quantity of starch present.

Part III. Effect of pH on Complexation

The effect of pH on the degree of interaction of benzoic acid with potato starch was measured. The relative degree of interaction is again measured by comparing the slopes of the isotherms. The complex interaction was studied at pH 1.7, 2.6, 3.3, and 4.2. The study was confined to the acidic range of the pH. At higher pH benzoic acid would be converted into benzoate ion, which is very soluble in water. This will necessitate the use of large amounts of the acid, and hence a great error of dilution when it is assayed. The slopes of the isotherms of the interaction of benzoic acid at the various pH studied (Figures 11-12, pages 46-47) are shown in Table 30 below.

TABLE 30

SLOPES OF ISOTHERMS OF INTERACTIONS OF BENZOIC
ACID WITH POTATO STARCH AT VARIOUS pH,
IN AQUEOUS SOLUTION AT 30° C.

pH	Slope x 10 ²
1.7	3.39
2.6	3.50
3.3	3.50
4.2	3.72

Benzoic acid was chosen as representing the effect of pH on the interaction of the organic acids studied since the esters would hydrolyze in acidic or in an alkaline pH.

Examination of the data shown in Table 30, page 65, shows that there is a slight increase in the degree of interaction with increase in pH. Benzoic acid is a weak acid that has an acidity or ionization constant pKa of 4.2 at 25° C. (38).

The ionization of benzoic acid is represented as follows:

$$K_a = \frac{(\text{B}) (\text{H}_3\text{O}^+)}{(\text{HB})}$$

pKa is defined as the reciprocal of the logarithm of Ka. At a pH of 1.7 benzoic acid would be mainly in the form of undissociated molecules. As the pH is increased dissociation increases. At pH 4.2 benzoic acid is 50% dissociated. According to the results of Table 30, page 65, it seems that the benzoate ion has a slightly greater tendency to interact than the undissociated molecule. The mechanism of interaction of the drug chemicals with the starch sol was elucidated as due to a combination of inclusion formation and attractive forces such as dipole-dipole interaction, hydrogen bonding, and other forces. It is doubtful that the benzoate anion would be favored over the undissociated acid in inclusion formation, because the latter depends on the stereochemical configuration rather than the charge on the molecule. It is possible, however, that due to the hydrophilic nature of the starch molecule that the anion is more quantitatively bound to the molecule resulting in an overall increase in the degree of interaction when the pH is increased. Another factor that would account for the apparent slight increase in the degree of interaction, is the formation of a soluble complex

between benzoic acid and the benzoate anion. As the pH is increased, the anion formed would solubilize more of the acid as an acid-anion complex. This view is supported by the finding of Bolton and Valinoti (57), and Kolthof and Bosch (67). These workers reported that the solubility of benzoic acid is increased in the presence of sodium benzoate. They attributed the phenomenon to formation of acid-anion complex. Other workers (68-69) have reported the existence of complexes between various acids and their anions. This extra factor of solubilizing the acid as acid-anion complex at higher pH, would account for the slight increase in the degree of interaction as the pH is increased.

Part IV. Complex Interaction at 20° C.

The interaction of benzoic acid and p-hydroxybenzoic acid with potato starch was studied at 20° C. The relative degree of interaction is compared by comparing the slopes of the interaction isotherms (Figure 13, page 49). Table 31 below shows the slopes of interaction of the two acids with potato starch at 20° C., and 30° C.

TABLE 31
SLOPES OF ISOTHERMS OF INTERACTION OF BENZOIC
ACID, AND p-HYDROXYBENZOIC ACID
WITH POTATO STARCH

Acid	Temperature	Slope x 10 ²
Benzoic Acid	20° C.	3.54
Benzoic Acid	30° C.	3.31
p-Hydroxybenzoic Acid	20° C.	5.88
p-Hydroxybenzoic Acid	30° C.	5.48

The data represented in Table 31, indicate that the degree of interaction of p-hydroxybenzoic acid, and benzoic acid is greater at 20° C., than at 30° C. This was rather expected in view of the forces that play a part in the complex interaction. Hydrogen bonding as well as all types of dipole-dipole interaction requires orientation of the interacting species. Since heat increases the speed with which molecules move, it follows that the subsequent disorientation results in a lesser degree of

interaction. It can be interpreted from the results shown in Table 31, page 68, that a decrease in temperature results in a net increase in the magnitudes of the forces responsible for complexation, and hence a greater complexing tendency. Again in this study, p-hydroxybenzoic acid was found to have a higher relative complexing tendency than benzoic acid. The results of Higuchi and Zuck (41) agree with these conclusions. These workers found that the formation constants, K_f , of the complexes formed between caffeine and several organic acids always increase with a decrease in temperature. Higher formation constants are due to a greater degree of interaction.

SUMMARY AND CONCLUSIONS

Potato starch and arrowroot starch were selected to study the tendency of starches to form complexes with certain drug pharmaceuticals.

To reduce the viscosity of the starch sols, these were autoclaved at 19-21 lb/inch² for three hours.

Benzoic acid, o-hydroxybenzoic acid, p-hydroxybenzoic acid, m-hydroxybenzoic acid, p-aminobenzoic acid, methyl p-hydroxybenzoate, ethyl p-hydroxybenzoate, propyl p-hydroxybenzoate, ethyl p-aminobenzoate, and caffeine were selected to show and evaluate the complexing tendency of the starches.

The above drugs were selected partly because of pharmaceutical use as preservatives and partly because of molecular structure.

The solubility method of analysis was employed for detection of complexation.

All drugs used were assayed spectrophotometrically, at the wavelength of maximum absorption.

1. Except for caffeine, all drugs tested showed evidence of complex interaction with both potato and arrowroot starch.

2. Salicylic acid formed an insoluble complex with both starches.

3. The relative complexing tendencies of the drug chemicals were measured by comparing the slopes of the interaction isotherms.

4. The relative complexing tendencies were in the following decreasing order: m-Hydroxybenzoic acid - p-Hydroxybenzoic acid - p-Aminobenzoic acid - Benzoic acid - Methyl p-hydroxybenzoate - Ethyl p-hydroxybenzoate - Ethyl p-aminobenzoate - Propyl p-hydroxybenzoate.

5. The same order of interaction was obtained for potato and arrowroot starch.

6. The mechanism of interaction of the drug chemicals with the starch sols is believed to be a combination of attractive forces and inclusion formation.

7. The effect of pH on the degree of interaction of benzoic acid with potato starch was studied. A slight increase in the degree of interaction with increase of pH was observed. The pH range studied was 1.7 to 4.2.

8. The increase in the degree of interaction at higher pH seemed to be due to an extra interaction between the anion present in solution with the acid, resulting in the formation of a soluble acid-anion complex.

9. The interaction of benzoic acid, and p-hydroxybenzoic acid with potato starch at 20° C. was studied. The degree of interaction was found to increase as the temperature decreased. This was due to a net increase in the magnitude of forces responsible for complexation with decrease in temperature.

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