Author: J. D. Shiffert

Division: Biochemical Research

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# SCHARDINGER CYCLODEXTRINS

## OBJECT:

To prepare Schardinger Cyclodextrins and investigate their use in selective filtration of cigarettes and commercial applications of their flavorant-inclusion compounds.

## SUMMARY:

The work described was begun, primarily, to investigate the use of cyclodextrins in the selective filtration of cigarettes. During this phase of the work, the commercial potential of cyclodextrin-inclusion compounds as flavorants and other uses was recognized (35-38) and investigated.

Alpha, beta and gamma cyclodextrins were prepared using methods described in the literature. The method of Cramer and Heinglein (10) was found to be superior to the others. When large scale preparation (100 to 150 liters of substrate) of the cyclodextrins was attempted, yields were low due to the lack of proper equipment. It is felt that with proper fermentation equipment, much greater yields of the cyclodextrins could have been obtained. There is presently on hand 215 grams of alpha cyclodextrin, 1457 grams of beta cyclodextrin, and 239 grams of gamma cyclodextrin in this laboratory.

Cigarettes with cyclodextrin treated filter tips were tested for selective filtration properties. There was no significant difference found between the treated and control filter-tip cigarettes in removal of total phenols or fatty acids from the smoke. Analogous results were obtained with cigarettes which had filter tips treated with the cyclodextrin-acetates.

The commercial applications of the beta cyclodextrin flavorant-inclusion compounds were investigated. It was found that in many cases the flavorant-inclusion compounds offer advantages over the regular liquid and powdered flavors used in many commercial products. In the case of cakes, it was found that even the more volatile flavorants (e.g. butter) were retained during baking temperatures, when they were added as cyclodextrin inclusion compounds. The flavorant-inclusion

compounds were also used successfully as flavorants in chewing tobacco. Taste panels preferred the chewing tobacco with the flavorant-inclusion compounds because there appeared to be a slow release effect of the flavor and the over-all flavor was longer lasting. Flavorant-inclusion compounds also offer many advantages for use in smoking tobacco. When volatile flavors are applied to smoking tobacco as inclusion compounds, the flavor is retained in the inclusion compound until the tobacco is smoked, at which time the flavor is released in the smoke. This same advantage holds true for flavorants which react with tobacco (e.g. oil of lime) and flavorants with loud external odors (e.g. vanillin). In panel tests, beta cyclodextrin-flavorant inclusion compounds were found to be unsuitable as flavorants for chewing gum because of an apparent "bitter note" flavor which they imparted to the gum.

Beta cyclodextrin will form an inclusion compound with the "headnote" flavors of fresh ground coffee. When this inclusion compound was added to instant coffee, a panel of coffee drinkers preferred this coffee over the regular instant coffee.

Laundry which was washed with a beta cyclodextrin-fragrance inclusion compound retained a perceptable amount of fragrance even after drying in an automatic dryer and steam ironing. A control sample which had the same amount of fragrance added as a liquid had no perceptable odor.

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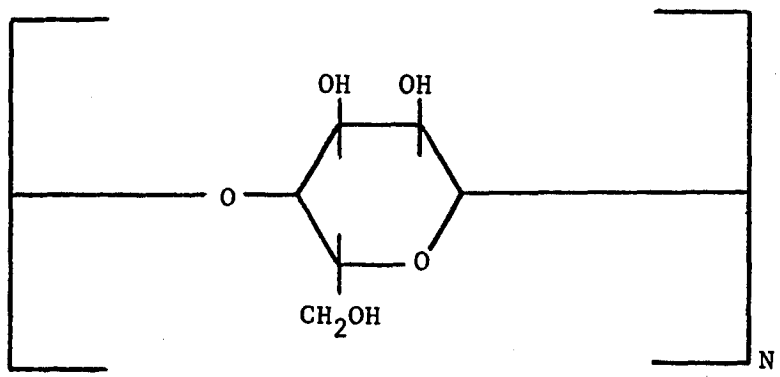
## A. INTRODUCTION

The Schardinger dextrins are a series of homologous oligosaccharides obtained from the breakdown of starch by the action of Bacillus macerans amylase. They are also known (11) as the cyclodextrins, the cycloamyloses, the amylodextrins, and the cycloglucans. The existence of these compounds was first reported by F. Schardinger in 1903 (31, 32).

These compounds are homogeneous cyclic molecules composed of six or more alpha-D-glucopyranose units linked 1-4 as in amylose (13). Their structure is




Where n is 6, 7, or 8, known as the alpha, beta, and gamma cyclodextrins respectively. The existence of higher molecular weight homologues is strongly suspected, but they have not as yet been isolated.

The factor differentiating the Schardinger dextrins is the cyclic configuration of the glucose polymer. This cyclic structure confers upon the molecule several unusual properties:

1. The molecule contains no reducing end group.

2. The compounds are stable to hot aqeous alkali.

3. They are resistant to most enzymes which normally degrade carbohydrates. However, evidence has been obtained (4a) for the existence of two specific cyclodextrinases in Takadiastase.

4. They readily form monomolecular inclusion compounds.

The history of the elucidation of the structure of the Schardinger dextrins presents an interesting picture of the development of various aspects of carbohydrate chemistry. Because of their seemingly improbable cyclic regularity of structure, chemists were slow to accept their cyclic nature and, because of the difficulty in determination of their molecular weights until recent times, slow to realize their number of glucose units per molecule. It is now recognized (6, 39) that the transfer which occurs of alpha 1-4 linked glucose units from amylose and the outer chains of

amylopectin (the two components of starch) to form the cyclodextrins is fascilitated by the natural tendency for chains of alpha 1-4 glucose units to assume a helical configuration.

Because of their cyclic structure and the relatively large open space within each molecule (6Å for alpha, 8Å for beta, and 10-11Å for gamma), they readily form monomolecular clathrate compounds. The cyclodextrins show different degrees of inclusion formation with different sized molecules because of their different internal ring sizes. For example (7), alpha has been shown to form adducts with chlorine, bromine, iodine, hydrocarbons, and benzene derivatives with small substituents. Beta interacts with bromine, iodine and almost any organic molecule. Gamma interacts with iodine and only large organic molecules.

There has been some controversy as to the exact electronic density of the center of the cyclodextrin void. Broser and Lautsch (5) showed that the stability of the inclusion complex in solution increases with basicity of the enclosed molecule. They therefore agreed that the cyclodextrin represents the electrophillic component of the inclusion complex.

Cramer (8) differs with this conclusion. He claims that the hollow space of an occlusion compound is a space of high electron density and behaves in many respects like an electron donor. The hollow spaces of such compounds can be considered as Bronsted and Lewis bases. He states (9) that the Schardinger dextrins form stable inclusion compounds with organic molecules because organic compounds are included in the voids of the cyclodextrin rings where the action upon the electron systems is particularly strong. Because of a state of high electron density in the void, the field energies there are apparently so great that effects appear which are comparable to those in an alkaline medium.

In speaking of the combination of two or more atoms or molecules to form a new chemical entity, we may describe the two distinct phenomena of reaction and interaction. Reaction involves the creation of a new compound by the formation of valence bonds. One or more of the classic types of ionic or covalent bonding is involved. Interaction involves formation of a compound where one or more of the bonds involved cannot be described as being of the classic type. These bonds may arise through hydrogen bonding, dipole-dipole interaction, charge transfer, or other attractive forces. The term "complexation" has come to mean this type of chemical interaction.

Complexation by means of molecular inclusion formation has received little attention by researchers. Schlenk (34) defines inclusion compounds (or occlusion compounds, as they may be called) as addition compounds in which one compound fits into and is surrounded by the crystal lattice of the other compound. These inclusion compounds can be referred to as "no bond" complexes and are characterized by the lack of adhesive forces between the components of the complex. The enclosing compound may be tube shaped, cage shaped or form open layers. The two compounds are present in constant, but not necessarily stoichiometric proportions.

It is not a chemical interaction which causes an inclusion compound to form, although this may be a factor in the net complexation observed. Van der Waals forces may be an important factor here (25), because of the closeness of the components. The steric configurations of the molecules are such that the enclosing, or "host", molecule can spatially enclose the included, or "guest", molecule, leaving uneffected the bonding systems of the components.

Powell (30) notes that the important factor in inclusion formation is geometry, rather than chemistry. Geometrical rather than chemical characteristics of the molecules are limiting factors in the interaction.

The most essential feature (1) of the host is its ability to form a rigid structure containing hollow spaces within which the prospective guest can fit. Pauling point out (29) that often the lattice is formed by molecules linked to one another by hydrogen bonds, host to host.

In view of the interesting properties of the cyclodextrins, it was decided to study their effectiveness in selective filtration of cigarette smoke. During this study, the commercial potential of the cyclodextrin inclusion compounds as flavorants and other uses were recognized (35-38) and investigated.

## B. EXPERIMENTAL

### I. Preparation of Bacillus Macerans Amylase

The Bacillus macerans cultures were prepared by Miss Margaret Long of this laboratory (24).

Amylase was isolated from the Bacillus macerans culture and purified using a modification of the methods of E. Tilden, et al. (40) and R. Kerr (21). The culture was centrifuged and the supernate filtered through a large Seitz filter (E.K size 14), capacity 2 liters. The amylase was precipitated by adding acetone, 33 percent by volume, and storing overnight at -15°C. The mixture was then centrifuged and the precipitate (amylase) dissolved in water. The enzyme (amylase) solution was stored at 5°C. until used for preparation of the cyclodextrin.

The activity of the Bacillus macerans amylase was determined initially using the method of D. French and co-workers (12) and later using the method of W. Hale and L. Rawlins (17) as modified by W. Blackwell of this laboratory (4).

### II. Preparation of Alpha, Beta and Gamma Cyclodextrins

#### a. Introduction

The original separation method of Schardinger depended on the ease of crystallization of the beta cyclodextrin from water and its low solubility (about 1.5 percent at room temperature); the alpha cyclodextrin was obtained from the mother liquor by treatment with alcohol (33).

A substantial advance in technique was provided by Freudenberg and Jacobi (15), who relied not only on solubility differences of the cyclodextrins themselves, but also on the differences of solubilities and rates of crystallization of the cyclodextrin acetates. Although the method is somwhat tedious, insofar as it required several acetylations and saponifications, it has the distinct merit of producing an alpha dextrin quite free from other dextrin impurities.

In further studies, Freudenberg, Plankenhorn and Knauber (16) modified the previous fractionation scheme, without decreasing its complexity. These authors made use of the observation (14) that bromobenzene does not precipitate alpha cyclodextrin, but the beta and gamma cyclodextrins are readily precipitated. Beta cyclodextrin was separated from gamma cyclodextrin by differential dissolution of the gamma cyclodextrin in warm pyridine.

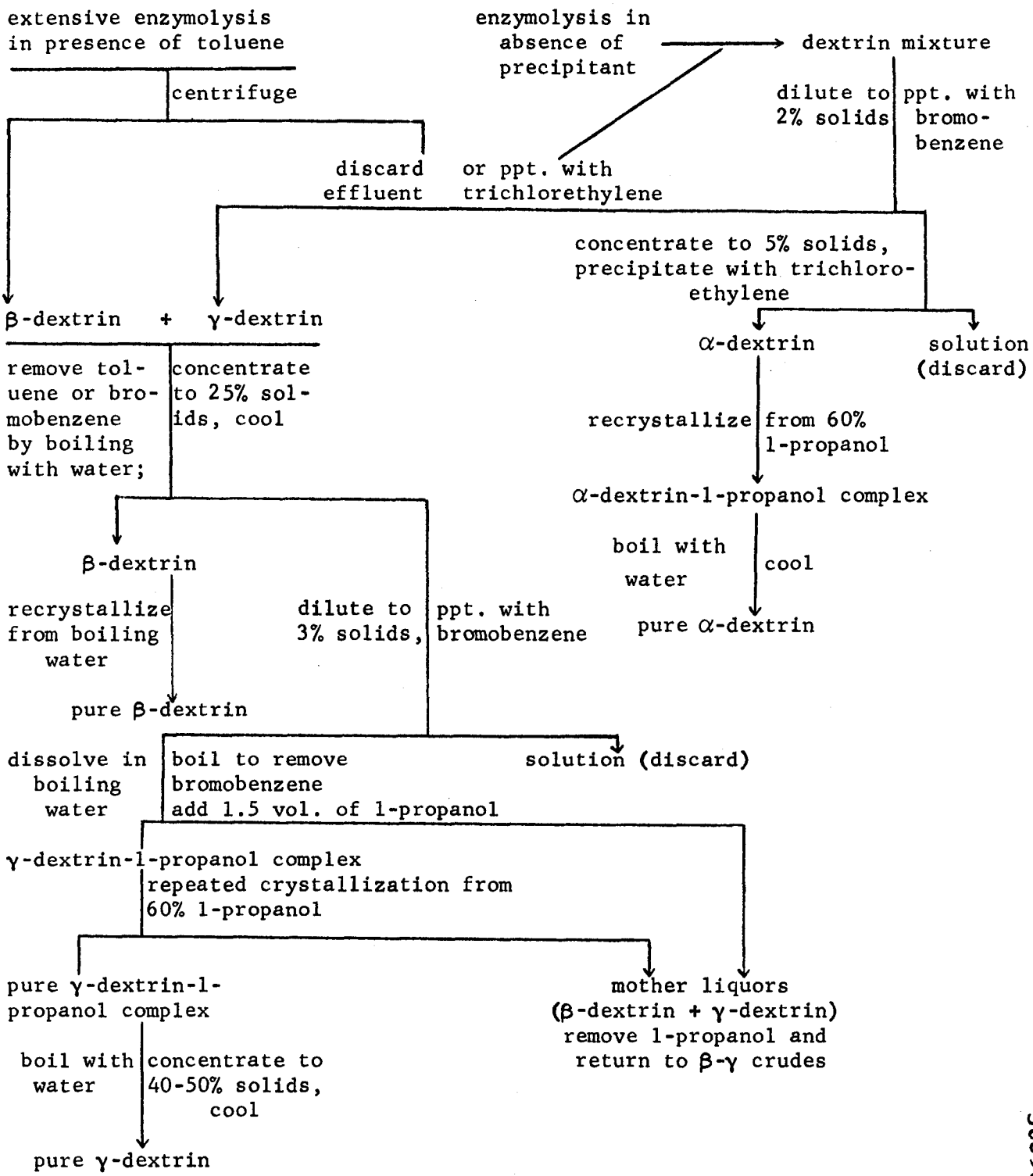
#### b. The French Method

French and co-workers (1949) (12) sought to decrease the complexities of previous fractionation schemes. Fractionation was achieved,

without acetylation of the cyclodextrins, by taking advantage of the low solubility of beta cyclodextrin in water, the differential precipitating action of bromobenzene on beta and gamma cyclodextrin (leaving alpha cyclodextrin in solution), and the low solubility of gamma cyclodextrin in 60 percent n-propanol. These authors also made use of the fact that conditions during the original enzymolysis of the starch can be preadjusted, depending on which cyclodextrin is desired (26). Large yields of beta cyclodextrin are obtained if the enzymolysis is conducted in the presence of a precipitant such as toluene or trichloroethylene. Maximal yields of alpha cyclodextrin are obtained, in the absence of a precipitant, with a relatively short conversion. For preparation of gamma cyclodextrin, the enzymolysis is very extensive, again in the absence of a precipitant (see figure 1).

FIGURE 1

FRACTIONATION SCHEME OF FRENCH, LEVINE, PAZUR AND NORBERG

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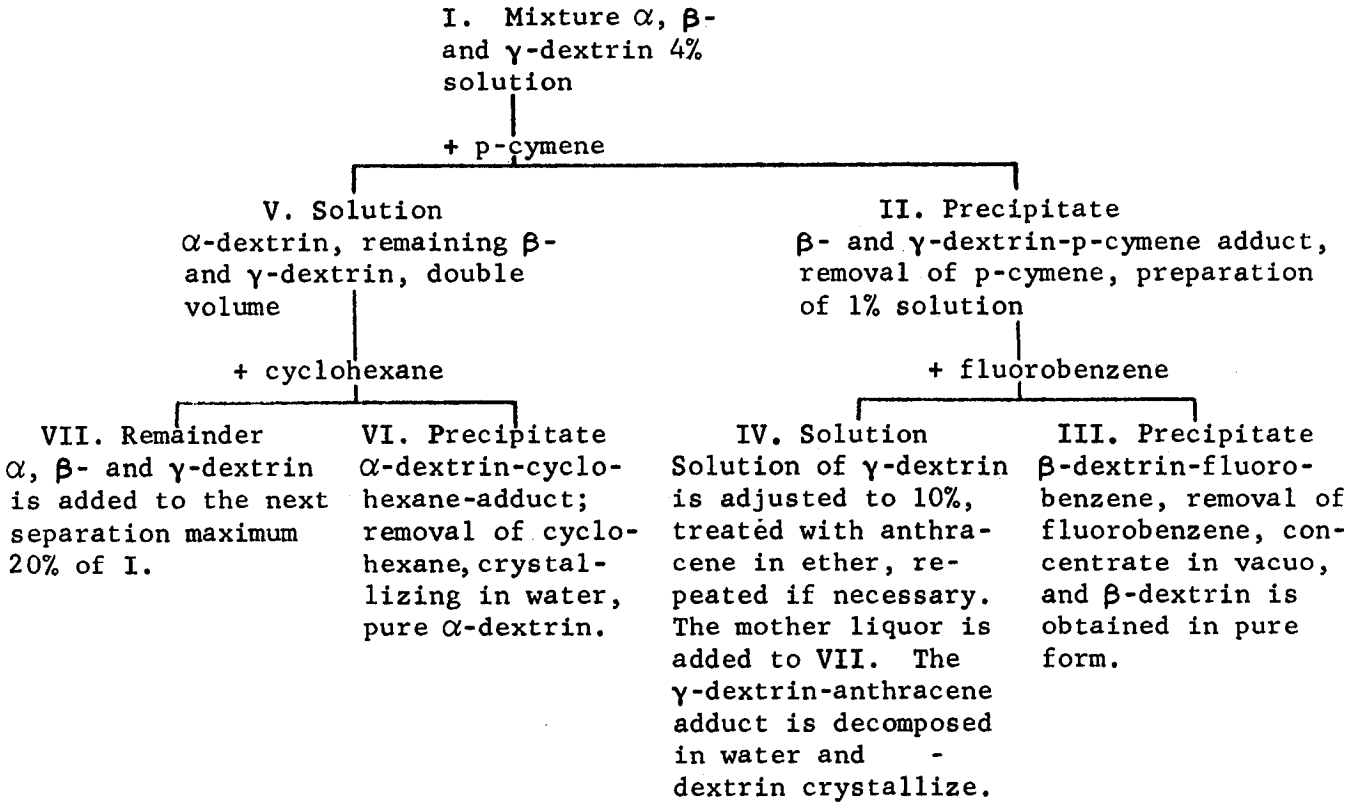
c. The Cramer Method

Cramer and Henglein (10) found that by using suitable cyclodextrin occlusion compounds they were able to work out a procedure for the separation of alpha, beta and gamma cyclodextrin in which specific precipitation agents were employed. The separation procedure also made possible the isolation of gamma cyclodextrin.

In the separation procedure (Figure 2) beta and gamma cyclodextrin are first precipitated with p-cymene and them separated with fluorobenzene. Gamma cyclodextrin is obtained in pure form by precipitating it as an anthracene derivative from the mother liquor. Alpha cyclodextrin is isolated with cyclohexane. The use of specific precipitating agents in this method eliminates the concentration of large liquid volumes. The compounds precipitate in very pure form.

FIGURE 2

FRACTIONATION SCHEME OF CRAMER AND HENGLEIN

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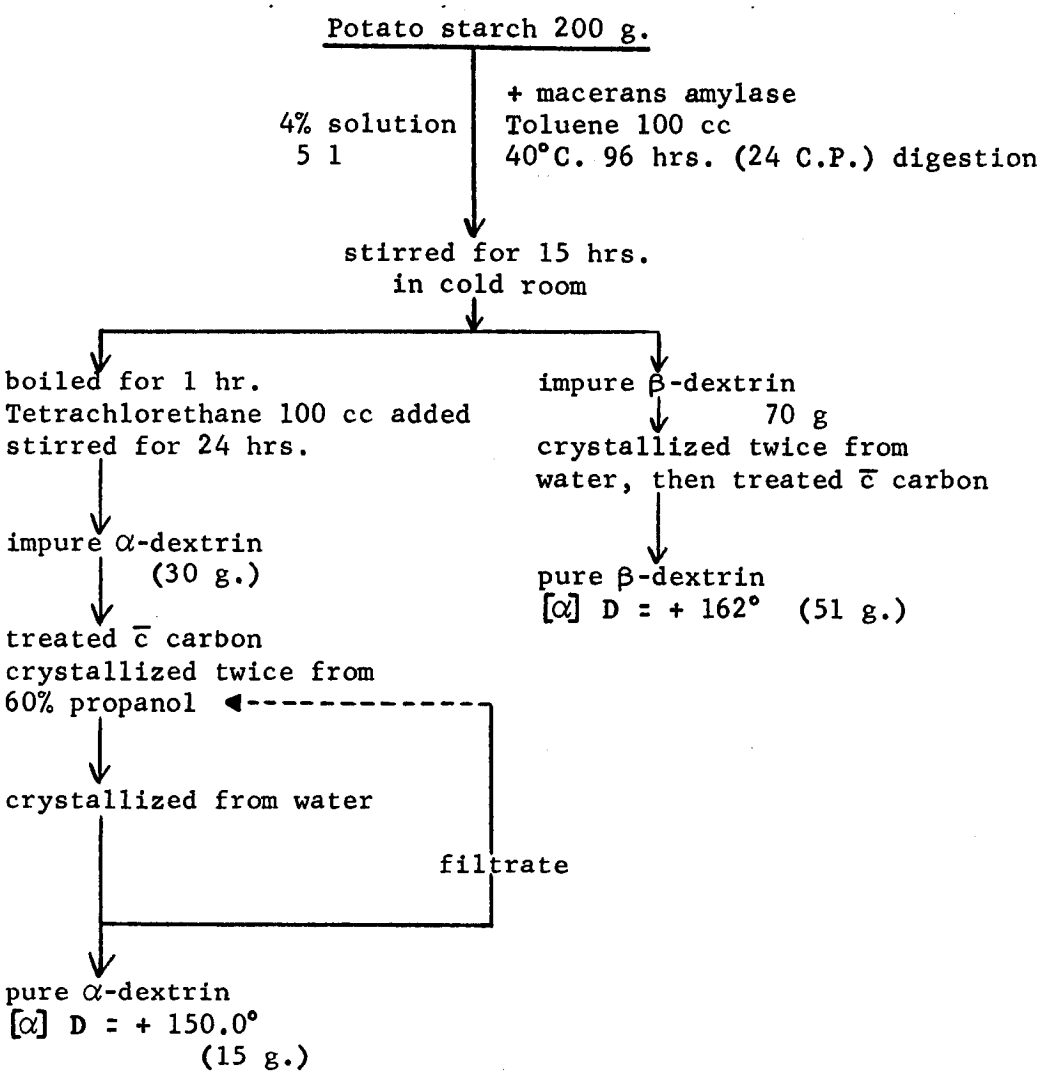
d._ The Kono Method

Kono and Nikuni (22) found that stirring the digestion mixture at a reduced temperature (40°F.) resulted in an increased yield of beta cyclodextrin. Otherwise, this method very closely resembles that of D. French et al. (12). In addition, this method also offers selective preparation of predominately alpha cyclodextrin or of beta cyclodextrin. The fractionation scheme of this method is presented in Figure 3.

FIGURE 3

FRACTIONATION SCHEME OF KONO AND NIKUNI



e._ Effect_of Enzyme_Concentration_on the Yield of_Beta Cyclodextrin

Six different concentrations of Bacillus macerans amylase were added to uniform solutions of soluble starch to determine which concentration of enzyme would give the greatest yield of beta cyclodextrin. Six aqueous solutions of soluble starch were prepared (100 gm./2 liters). The enzyme was added to each flask in different concentration (see Table I). The total volume of each flask was adjusted to 3 liters with distilled water. The flasks were incubated at 40°C. for 11 days for static digestion. Beta cyclodextrin was isolated from the digest according to the method of Cramer (10). The results of this study are presented in Table I.

TABLE I

EFFECT OF THE CONCENTRATION OF B. MACERANS AMYLASE ON THE YIELD OF BETA CYCLODEXTRIN

| Sample No. | Concentration of Amylase (Hale & Rawlins Units) | Yield of Beta Cyclodextrin (gms.) |
|---|---|---|
| 1 | 250 | 3.2 |
| 2 | 200 | 0.3 |
| 3 | 150 | 7.2 |
| 4 | 100 | 6.3 |
| 5 | 50 | 7.1 |
| 6 | 10 | 5.3 |

The data in Table I indicate the optimum concentration of B. macerans amylase for production of beta cyclodextrin is between 50 and 150 Hale & Rawlins units of the enzyme per 100 gms. of soluble starch. However, a reasonably high yield of beta cyclodextrin was obtained even at the low enzyme concentration of 10 Hale & Rawlins units per 100 gms. of soluble starch. The low yield of beta cyclodextrin obtained at an enzyme concentration of 200 Hale & Rawlins units per 100 gms. of soluble starch may have been caused by a contaminant.

f._ Photomicrographs_of the Cyclodextrins

Photomicrographs were made of the alpha, beta and gamma cyclodextrins which were prepared in this laboratory. The crystal structures of the three types of cyclodextrins prepared were found to duplicate those described by French and co-workers (12).

These photomicrographs are presented in Figures 4, 5 and 6.

FIGURE 4

ALPHA CYCLODEXTRIN FROM 60% N-PROPANOL,
HEXAGONAL FORM, APPROXIMATELY 700 MAGNIFICATIONS

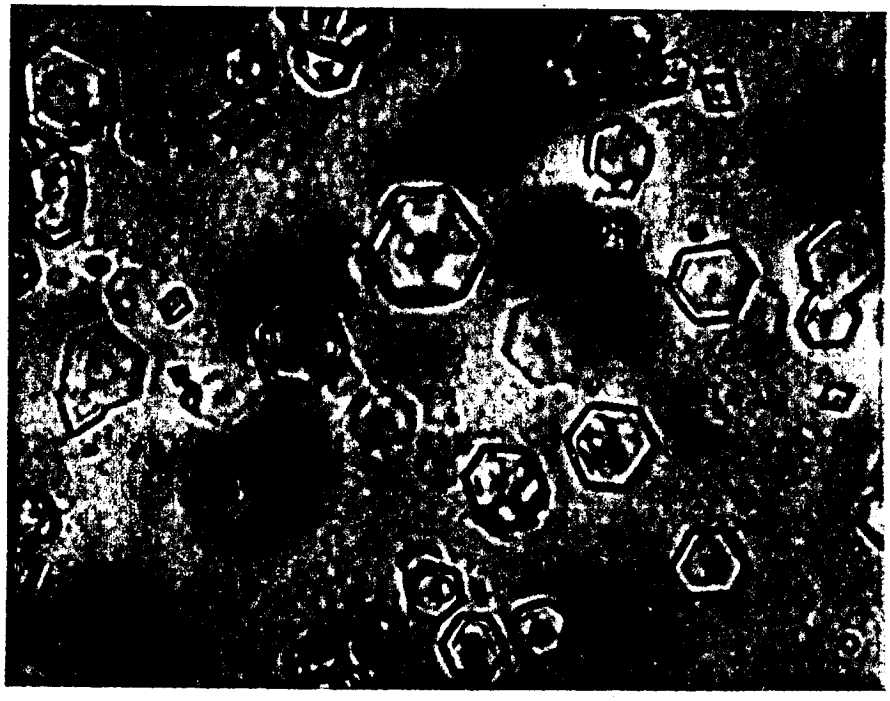


FIGURE 5

BETA CYCLODEXTRIN FROM 60% N-PROPANOL, PARALLELOGRAM CRYSTALS, APPROXIMATELY 700 MAGNIFICATIONS

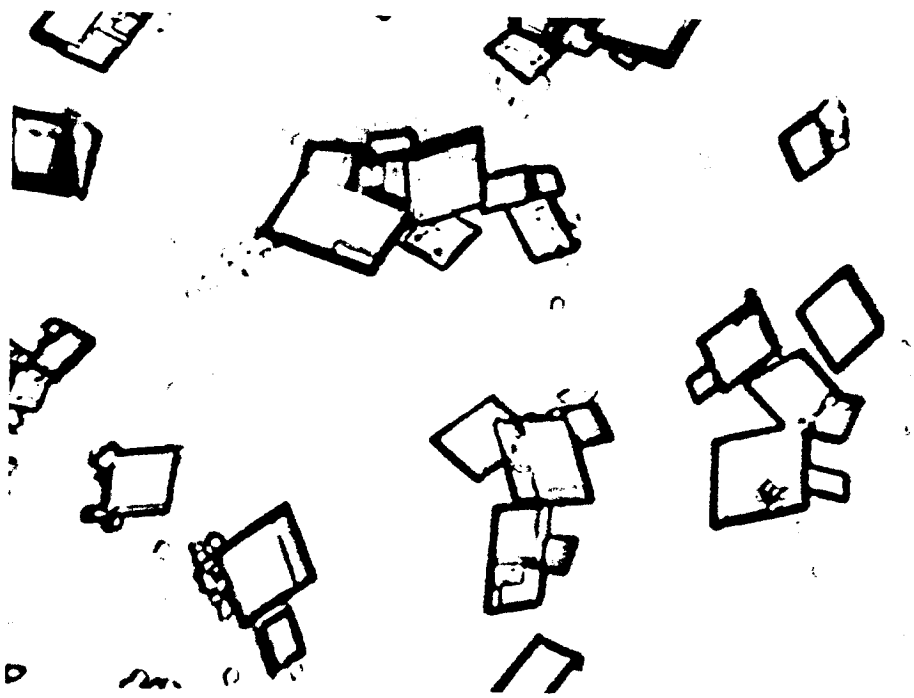
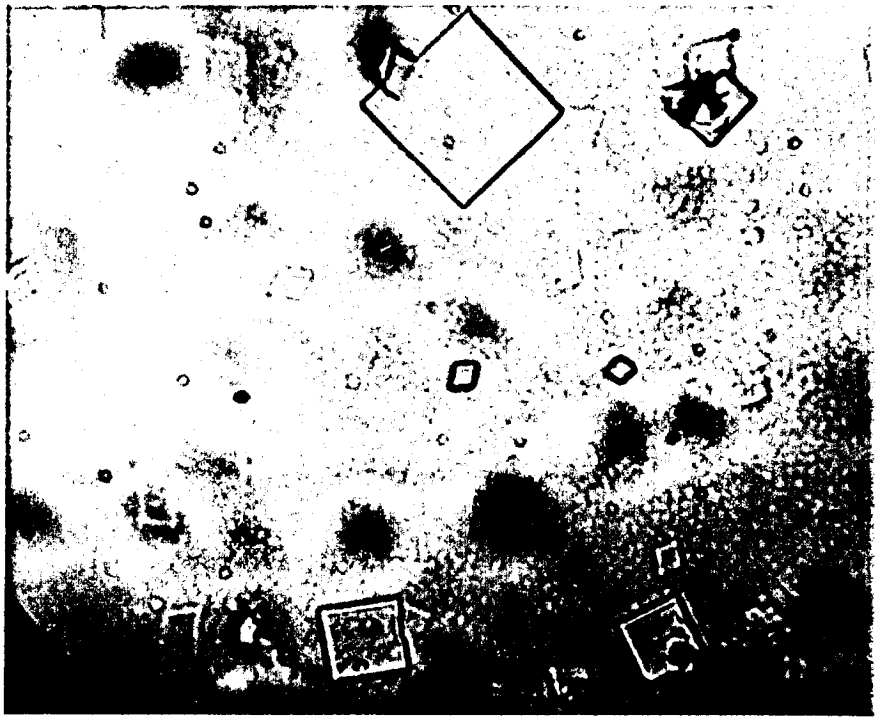


FIGURE 6

GAMMA CYCLODEXTRIN FROM 60% N-PROPANOL,
SQUARE CRYSTALS, APPROXIMATELY 700 MAGNIFICATIONS



g._ Large Batch Preparations

Large batches of beta cyclodextrin were prepared using improvised equipment. All three of the methods (French, Cramer, and Kono) mentioned in Sections B.II.b.,c.,d. of this report were used in these large batch preparations. Soluble starch solution (4-5%) was prepared in 2 liter batches. Usually 150 liters of this starch solution was used as the substrate. This solution was placed in a 55 gallon stainless steel drum and the Bacillus macerans amylase was added; the mixture was then stirred with a large stirring motor. The temperature (40°C.) was controlled by use of copper coils in the drum and circulating water or by locating the entire apparatus in a cubicle (Yerkes Building) at a constant temperature and humidity. After digestion, the mixture was centrifuged. The supernate was concentrated on the "turba-film" evaporator in the pilot plant. The alpha, beta and gamma cyclodextrins were then isolated from this concentrate using one of the methods mentioned above.

The following table illustrates the yields of beta cyclodextrin obtained using the "large batch" methods and laboratory scale methods.

TABLE II

COMPARISON OF THE YIELD OF BETA CYCLODEXTRIN FROM LABORATORY SCALE AND LARGE BATCH PREPARATIONS

| Method | Wt. Soluble Starch (gms.) | Wt. of Beta Cyclodextrin (gms.) | Large Batch % Yield | Ave. Lab. Scale % Yield |
|---|---|---|---|---|
| French | 2800 | 75 | 2.7 | 12.9 |
| Cramer | 5700 | 750 | 13.1 | 29.5 |
| Kono | 5000 | No Yield | 0 | 25.0 |
| Cramer | (Substituted for above) | 244 | 4.9 | 29.5 |
| Cramer | 7500 | 304 | 4.0 | 29.5 |

It is felt that with proper fermentation equipment much greater yields of beta cyclodextrin could have been obtained in the large scale preparations. In the case of the Kono method in the above table, no yield was obtained because the proper experimental conditions could not be maintained. Therefore, the Cramer method was substituted to isolate the beta cyclodextrin which had been formed.

The following amounts of the cyclodextrin are presently on hand in a highly pure form:

alpha cyclodextrin - 215 g.

beta cyclodextrin - 1457 g.

gamma cyclodextrin - 239 g.

## III. Investigation of the Selective Filtration Properties of the Cyclodextrins

### a. Preparation of Cigarettes and Filter Tips

Approximately ten pounds of $C_3$-$C_9$ strips (flue-cured tobacco fraction of the CAMEL blend) were obtained from the Manufacturing Department for making the cigarettes. The strips were cut on an Imhoff cutter set at 32 cuts per inch. The cut tobacco was stored in a chamber at the proper relative humidity to obtain a 12-12.5 percent moisture content in the tobacco. The tobacco was fabricated into cigarettes (68 mm.) on the Hauni machine in this laboratory. The cigarettes were stored in a chamber at the proper relative humidity to maintain a 12-12.5 percent moisture level.

Unplasticized filter plugs (Estron) were obtained from the Manufacturing Department. The "plugs" were wrapped with plastic tape (Scotch Pressure Sensitive Tape-Water Insoluble) and cut into 25.5 mm. sections using a filter plug cutter.

The cyclodextrins were introduced into the filter plug by applying 1.2 ml. of a 2 percent (aqueous) dextrin solution (burette) to the plug and drying it in a forced draft oven for 15 hours at 80°C.

After drying, the "treated" plugs were cut into 17 mm. sections by cutting 4.25 mm. off of each end of the 25.5 mm. plug. These ends were cut from the treated plugs to remove the cyclodextrin which crystallized in these areas during drying. In the case of the controls, 17 mm. sections were cut directly from the original plugs.

The filter plugs (17 mm.) were attached to the $C_3$-$C_9$ cigarettes using plastic tape cut into sections 1/2 inch wide and 55 mm. long.

The final tipped cigarettes which were used for analyses were free of loose ends and soft spots and were selected in a weight range of 1.02-1.06 grams.

### b. Polycyclics

Samples of the cigarettes described in Section III.a. were analyzed for polycyclics in the smoke by the Analytical Division (18). The results of these analyses are presented in Tables III-A and III-B.

## TABLE III-A

(First Determination)

POLYCYCLICS IN THE SMOKE OF CIGARETTES WITH CYCLODEXTRIN-TREATED FILTER TIPS

| Filter | Amount of Cyclodextrin | | Draft Resistence | Fluorathene | Benz [b] Fluorathene | % | 3,4-Benzo-pyrene | % |
|---|---|---|---|---|---|---|---|---|
| Type | Mg. | % | of Filter | γ/150 cig. | γ/150 cig. | Diff. | γ/150 cig. | Diff. |
| Control | -- | -- | 2.12 | 5.75* | 1.45 | -- | 2.23 | -- |
| Alpha | 8.8 | 6.2 | 2.34 | 18.3 | 0.72 | -56.3 | 1.50 | -32.7 |
| Beta | 6.0 | 4.3 | 2.22 | 18.7 | 0.78 | -46.2 | 1.83 | -17.9 |
| Gamma | 12.6 | 8.6 | 2.33 | 13.7 | 0.87 | -40.0 | 2.23 | N.D. |

*This sample was found to have a break in the flask and an undeterminable amount of the sample was lost.

## TABLE III-B

(Second Determination)

POLYCYCLICS IN THE SMOKE OF CIGARETTES WITH CYCLODEXTRIN-TREATED FILTER TIPS

| Filter | Amount of Cyclodextrin | | Puffs | Fluoranthene | % | Benz [b] Fluoranthene | % | 3,4-Benzo-pyrene | % |
|---|---|---|---|---|---|---|---|---|---|
| Type | Mg. | % | Cig. | γ/150 cig. | Diff. | γ/150 cig. | Diff. | γ/150 cig. | Diff |
| Control | -- | -- | 9.21 | 11.95 | -- | 1.01 | -- | 1.31 | -- |
| Alpha | 11.21 | 7.9 | 9.88 | 12.65 | +5.9 | 0.99 | N.D. | 1.41 | +7.6 |
| Beta | 10.77 | 7.7 | 9.98 | 12.65 | +5.9 | 0.83 | -17.8 | 1.14 | -13.0 |
| Gamma | 6.10 | 4.5 | 9.33 | 11.25 | -5.9 | 0.85 | -15.8 | 1.31 | N.D. |

The data in Table III-A suggest a significant removal of 3,4-benzopyrene from the smoke of cigarettes with alpha and beta cyclodextrin-treated filter tips. In addition, there is also a significant removal of benz [b] fluoranthene from the smoke of cigarettes in all three samples treated with cyclodextrins. No definite conclusion could be made in the case of the fluoranthene because of the loss of the control sample.

The data in Table III-B do not substantiate the original conclusions. Instead, it is found there was only slight removal of 3,4-benzopyrene by the beta cyclodextrin, slight removal of benz [b] fluoranthene by the beta and gamma cyclodextrin and also a slight removal of fluoranthene by the gamma cyclodextrin.

Since there were two sets of conflicting data, analysis of another set of these same samples was requested of the Analytical Division. This set of samples has not yet been analyzed. Therefore, no definite conclusion can be made on the removal of polycyclics from the smoke of cigarettes by cyclodextrin-treated filter tips at this time.

c. Phenols

Smoke from the cigarettes described in Section III.a. was analyzed for phenols by the Analytical Division (41). The results of these analyses are presented in Table IV.

## TABLE IV

## PHENOLS IN THE SMOKE OF CIGARETTES WITH CYCLODEXTRIN-TREATED FILTER TIPS

(All the results are expressed as Mg./Cigarette)

| | Phenol | O-Cresol | M-Cresol | Total Cresol | Guiaciol | Xylencl | Total Phenol |
|---|---|---|---|---|---|---|---|
| Control No. 1 | .0621 | .0154 | .0248 | .0397 | .0199 | .0275 | .1492 |
| Control No. 2 | .0627 | .0169 | .0231 | .0396 | .0191 | .0269 | .1483 |
| Control Ave. | .0624±.5% | .0162±4.6% | .0240±3.5% | .0397±0.3% | .0195±2.1% | .0272±1.1% | .1488 |
| Alpha No. 1 | .0646 | .0162 | .0273 | .0429 | .0211 | .0289 | .1575 |
| Alpha No. 2 | .0664 | .0174 | .0261 | .0431 | .0206 | .0286 | .1587 |
| Alpha Ave. | .0655±1.4% | .0168±3.5% | .0267±2.2% | .0430±0.2% | .0209±1.2% | .0288±0.5% | .1581 |
| Beta No. 1 | .0628 | .0166 | .0248 | .0408 | .0198 | .0273 | .1507 |
| Beta No. 2 | .0677 | .0175 | .0275 | .0444 | .0210 | .0296 | .1627 |
| Beta Ave. | .0653±3.5% | .0171±2.6% | .0262±5.3% | .0426±4.2% | .0204±3.0% | .0285±4.0% | .1567 |
| Gamma No. 1 | .0603 | .0155 | .0246 | .0396 | .0189 | .0257 | .1445 |
| Gamma No. 2 | .0597 | .0157 | .0247 | .0399 | .0188 | .0259 | .1443 |
| Gamma Ave. | .0600±.5% | .0156±0.6% | .0247±0.2% | .0398±0.3% | .0189±0.3% | .0258±0.4% | .1444 |

The data in Table IV indicate there is no significant difference between the total phenol content of the smoke from the cigarettes with cyclodextrin-treated filter tips and the control cigarettes.

d. Fatty Acids

Samples of the cigarettes described in Section III.a. were analyzed for fatty acids by Dr. S. A. Bellin (3). The results of these analyses are presented in Table V.

TABLE V

FATTY ACIDS IN THE SMOKE OF CIGARETTES WITH CYCLODEXTRIN-TREATED FILTER TIPS

| Analyses | | Control | Alpha Cyclodextrin | Beta Cyclodextrin | Gamma Cyclodextrin |
|---|---|---|---|---|---|
| Weight of smoke condensates (mg./10 cig.) | | 134 | 144 | 145 | 141 |
| Hexane solubles retained on filters (mg./10 cig.) | | 63 | 65 | 63 | 64 |
| Weight of fatty acids in smoke condenser* (mg./10 cig.) | | 10.3 | 11.6 | 10.6 | 10.4 |
| Unknown acid (As % of Total Fatty Acid) (Mixture) | | 1.4 | 0.4 | -- | -- |
| Unknown acid | " | -- | 0.3 | -- | -- |
| Unknown acid | " | 2.1 | 1.8 | 0.8 | 1.9 |
| Myristate | " | 3.5 | 2.8 | 3.6 | 2.6 |
| Pentadecanoate | " | 2.1 | 2.0 | 2.4 | 2.2 |
| Pentadecanoate | " | 2.5 | 2.0 | 2.3 | 2.0 |
| Palmitate | " | 39.1 | 36.3 | 36.3 | 35.8 |
| Unknown acid | " | 0.9 | 0.5 | -- | 1.0 |
| Stearate | " | 8.5 | 7.7 | 8.6 | 7.9 |
| Oleate | " | 8.9 | 9.6 | 11.0 | 10.2 |
| Linoleate | " | 11.8 | 13.4 | 13.0 | 12.4 |
| Unknown acid | " | 0.1 | 1.4 | -- | 2.6 |
| Linolenate | " | 19.1 | 22.0 | 22.0 | 21.4 |

*Weight of methyl esters of $C_{14}$-$C_{18}$ Acids

There is no significant difference between the control and treated cigarette samples in any of the above analyses (3).

## IV. Investigation of the Selective Filtration Properties of the Cyclodextrin-Acetates

### a. Preparation of the Cyclodextrin Acetates

The cyclodextrin-acetates were prepared by the method of D. French (12). Dry alpha cyclodextrin was added in four equal parts to five parts of boiling acetic anhydride containing one-half part anhydrous sodium acetate. After the final addition, the mixture was refluxed for 30 minutes, allowed to cool to room temperature, and poured with stirring into cracked ice and water. The cyclodextrin-acetate was then suction filtered. Beta and gamma cyclodextrin were acetylated in the same manner. The crude alpha and beta cyclodextrin-acetates were crystallized from 10-15 parts of boiling toluene by cooling to room temperature. Gamma cyclodextrin-acetate was crystallized from 2-3 parts of hot butyl acetate.

### b. Preparation of Cigarettes and Filter Tips

Approximately 1,000 WINSTON cigarettes (untipped) were obtained from the Manufacturing Department. Cigarettes which were free of loose ends and soft spots were selected in a weight range of 0.96-1.02 grams for tipping.

Unplasticized filter plugs were obtained from the Manufacturing Department. The plugs were wrapped with plastic tape (Scotch Pressure Sensitive Tape-Water Insoluble) and cut into 25.5 mm. sections using a filter plug cutter.

The cyclodextrin-acetates were introduced into the filter plugs by applying 1 ml. of a 1 percent (methanol) cyclodextrin-acetate solution (burette) to the plug and drying at room temperature for 4 hours.

After drying, the treated plugs were cut into 17 mm. sections by cutting 4.25 mm. off of each end of the 25.5 mm. plug. These ends were cut from the treated plugs to remove the cyclodextrin-acetate which had crystallized in these areas during drying. In the case of the controls, 17 mm. sections were cut directly from the original plugs. The filter plugs (17 mm.) were attached to the WINSTON cigarettes using plastic tape cut into sections 0.5 inch wide and 55 mm. long. The final tipped cigarettes were submitted to Mr. Wade Williard of Quality Control who selected 20 cigarettes of each cyclodextrin-acetate for submission to Dr. A. Laurene of the Analytical Division for analysis.

c. Smoke Analysis for Phenol

The final tipped cigarettes were submitted to Dr. A. Laurene (23) of the Analytical Division for analysis of phenols. The results are presented in Table VI.

TABLE VI

PHENOL IN THE SMOKE OF CIGARETTES WITH CYCLODEXTRIN ACETATE-TREATED FILTER TIPS

| Samples | Code | Draft Resistence | Wt. Range Cigarettes | Phenol (mg./cigarette) Vapor Fractometer | Phenol (mg./cigarette) Mass Spectrometer |
|---|---|---|---|---|---|
| alpha-acetate | L-340A | 1.8-2.4 | 1.279-1.313 | 74.5 | 67.7 |
| beta-acetate | L-340C | 1.9-2.1 | 1.275-1.318 | 68.6 | 67.4 |
| gamma-acetate | L-340D | 1.8-2.1 | 1.265-1.301 | 67.3 | 69.1 |
| control | L-340B | 2.7-3.4 | 1.260-1.300 | 63.1 | 58.0 |

There is no removal of phenols from the smoke of cigarettes with cyclodextrin acetate-treated filter tips.

V. Preparation of Cyclodextrin-Flavorant Inclusion Compounds

The method used in the preparation of the cyclodextrin-inclusion compounds was essentially that of H. Schlenk (34). The inclusion component was added to a hot, saturated, aqueous cyclodextrin solution, which was allowed to cool slowly (room temperature) while being shaken for 12-24 hours.

The following list of inclusion compounds were successfully prepared using this technique. The ratio of beta cyclodextrin to the inclusion component was determined by T. G. Harrell (19) of the Analytical Division.

## TABLE VII

## LIST OF BETA CYCLODEXTRIN-FLAVORANT INCLUSION COMPOUNDS PREPARED

1. Imitation prune flavor, No. 50.066/A, Firmenich & Co.
2. Imitation peach flavor, No. 51.976/A, Firmenich & Co.
3. Imitation apple flavor, No. 50.047/A, Firmenich & Co.
4. Imitation grape flavor, No. 59.150/T, Firmenich & Co.
5. Imitation butter flavor, No. 51.561/A, Firmenich & Co.
6. Imitation butter flavor, No. 59.049/A, Firmenich & Co.
7. Imitation lemon juice flavor, No. 51.124/A, Firmenich & Co.
8. Imitation vanilla flavor, No. 59.106/A, Firmenich & Co.
9. Imitation burnt sugar flavor, No. 59.267/AP, Firmenich & Co.
10. Imitation coffee flavor, No. 50.439/AP, Firmenich & Co.
11. Imitation orange juice flavor, No. 59.107/A, Firmenich & Co.
12. Imitation rum flavor, No. 50.860/C, Firmenich & Co.
13. Liquid sunshine, No. 9601, Firmenich & Co.
14. Oil of lemon, No. 1160, Felton Chemical Co.
15. Oil of lime, No. n.o., Felton Chemical Co.
16. Imitation honey base, No. 27, Felton Chemical Co.
17. Imitation fruit flavor, No. 952, Felton Chemical Co.
18. Cinnamic aldehyde, Fritzsche Bros.
19. Oil of peppermint, USP XIV, Fritzsche Bros.
20. Compounded perfume oil, No. 19434, Fritzsche Bros.
21. Vanillin, No. 167, Dow Chemical Co.
22. Diacetyl, No. 1591, Eastman Organic Chemical
23. Ethyl propionate, Source Unknown
24. Imitation McKinley apple, No. N-602, Dragoco, Inc.
25. 50 Spearmint, 50 Carvone, Norda Co.
26. Imitation peach concentrate, F-1975, Givandan Flavors, Inc.
27. Imitation peach flavor, No. F-110, Givandan Flavors, Inc.
28. Imitation apple concentrate, S-3778, International Flavor & Fragrances, Inc.
29. Arome artif. n. alc. lime, No. 13588, Antoine Chiris Bros., Paris
30. Isovaleric acid, Dr. C. Nystrom, this laboratory
31. β-methyl valeric acid, Dr. C. Nystrom, this laboratory
32. α-methyl butyric acid, Dr. C. Nystrom, this laboratory
33. n-butyric acid, Dr. C. Nystrom, this laboratory

A beta cyclodextrin-coffee headnote flavor inclusion compound was also prepared. For this preparation, 100 grams of coffee (A & P, Red Circle, drip grind, freshly ground) was placed in a one-liter Erlenmeyer flask and 500 ml. of water were added. The mixture was slowly heated to boiling and the flask flushed with nitrogen which was passed through a condenser and then bubbled through a solution of beta cyclodextrin. The beta cyclodextrin solution was in a gas-washing bottle fitted with a sintered glass sparger; the solution was held at approximately 30-40°C. with constant stirring on a magnetic stirrer hot plate combination unit. After boiling for 2 hours, a fresh batch of coffee was placed in the Erlenmeyer flask and the process repeated. After two pounds of coffee had been treated in this way, the

beta cyclodextrin solution was allowed to cool to room temperature and stirred for approximately 12 hours. The white precipate of the beta cyclodextrin-coffee headnote flavors was separated by filtering with vacuum and dried.

## VI. Investigation of the Commercial Applications for the Cyclodextrin-Flavorant Inclusion Compounds

### a. Cakes

One of the problems in the Baking Industry today is the use of volatile flavors and their subsequent loss during the baking process. An example of this problem is the butter flavor 51.561/A developed by the Firmenich Company. It was found that a high percentage of this flavor was lost during the baking process (27). With this problem in mind, it was proposed that beta cyclodextrin-inclusion compounds of these flavors might offer a solution. These compounds were prepared and tested in cakes.

Cakes were baked using comparable beta cyclodextrin-flavor inclusion compounds and commercially available powdered flavors as flavorants. For each different flavor tested, three boxes of Duncan Hines, White, Deluxe Cake Mix (1 lb. 2-1/2 oz.) were used and the cakes prepared according to the directions on the package. During the mixing step, the beta cyclodextrin-flavor inclusion compound was added to one mix, the comparable powdered flavor was added to another mix, and the third mix was used per se, as the control.

All of the flavors (both liquid and powdered) used in this experiment were supplied by Firmenich & Co. The preparation of the beta cyclodextrin-flavor inclusion compounds using the Firmenich & Co. liquid flavors is described in Section B.V. of this report. Equivalent amounts of each type of flavor were always added to the cake mixes. Firmenich & Co. recommended that 1 ounce of the powdered flavor be added to 100 pounds of dry cake mix. This recommendation was used as the basis for calculating the equivalent amounts of each flavorant to add to the cake mixes. The ratio of the beta cyclodextrin to the flavor component was determined by T. G. Harrell (19) of the Analytical Division for each of the inclusion compounds used in this experiment. These values were used to calculate equivalent amounts of the beta cyclodextrin-flavor inclusion compounds.

The cakes were panel-tested to determine which cake retained the greatest amount of flavor after baking. The cakes were cooled and approximately 1 inch cubes were cut and presented to the panelists. A paper cup of water was offered for rinsing between samples. The question was asked of each panelist, "Which sample has the strongest _____ flavor?" It was emphasized that we were not interested in preference.

The results of these panel tests are presented in Table VIII.

## TABLE VIII

## COMPARISON OF BETA CYCLODEXTRIN-FLAVOR INCLUSION COMPOUNDS WITH POWDERED FLAVORS IN CAKES

Test No. 1 - Imitation butter flavor, 51.561/A, Firmenich & Co.

| | |
|---|---|
| Total panelists | 47 |
| Number of panelists selecting the beta cyclodextrin complex | 40 |
| Number of panelists selecting the powdered flavor | 5 |
| Number of panelists selecting the control | 2 |

Test No. 2 - Imitation butter flavor, 59.049/A, Firmenich & Co.

| | |
|---|---|
| Total panelists | 50 |
| Number of panelists selecting the beta cyclodextrin complex | 39 |
| Number of panelists selecting the powdered flavor | 8 |
| Number of panelists selecting the control | 3 |

Test No. 3 - Imitation burnt sugar flavor, 59.267/AP, Firmenich & Co.

| | |
|---|---|
| Total panelists | 50 |
| Number of panelists selecting the beta cyclodextrin complex | 38 |
| Number of panelists selecting the powdered flavor | 11 |
| Number of panelists selecting the control | 1 |

Test No. 4 - Imitation orange juice flavor, 59.107/A, Firmenich & Co.

| | |
|---|---|
| Total panelists | 49 |
| Number of panelists selecting the beta cyclodextrin complex | 45 |
| Number of panelists selecting the powdered flavor | 3 |
| Number of panelists selecting the control | 1 |

Test No. 5 - Imitation lemon juice flavor, 51.124/AP, Firmenich & Co.

| | |
|---|---|
| Total panelists | 50 |
| Number of panelists selecting the beta cyclodextrin complex | 20 |
| Number of panelists selecting the powdered flavor | 23 |
| Number of panelists selecting the control | 7 |

The panel overwhelmingly selected the cakes flavored with the beta cyclodextrin-flavor complex over the powdered flavor cakes in four of the five flavors tested. These results indicate that in four of the flavors tested the flavor is retained during baking temperature in the beta cyclodextrin complex. In the case of the lemon juice flavor, it may be rationalized that the powdered form of this flavor is more stable to heat, since the panel could not distinguish between the two types of flavors in the lemon cakes.

b. Chewing Tobacco

Beta cyclodextrin-flavor inclusion compounds were tested as flavorants on chewing tobacco. The flavor inclusion compounds were prepared as described in Section B.V. of this report and were submitted to Mr. E. H. Harwood of this laboratory for panel testing. The following inclusion compounds were prepared with beta cyclodextrin:

1. Imitation McKinley apple, N-602, Dragoco, Inc.
2. Diacetyl, Eastman Organic Chemicals
3. Imitation honey base, Felton Chemical Co.
4. Imitation apple concentrate, S-3778, International Flavors & Fragrances, Inc.
5. Imitation peach concentrate, F-1975, Givandan Flavors, Inc.
6. Imitation peach flavor, F-110, Givandan Flavors, Inc.
7. Imitation prune flavor, 50.066/A, Firmenich & Co.
8. Imitation peach flavor, 51.976/A, Firmenich & Co.
9. Imitation apple flavor, 50.047/A, Firmenich & Co.
10. Imitation grape flavor, 59.150/T, Firmenich & Co.

According to Mr. E. H. Harwood (20), two of the above flavor complexes were successfully panel-tested on chewing tobacco. The samples containing the imitation peach flavor, F-110, Givandan Flavors, Inc., inclusion compound was overwhelmingly selected over the control in the panel test. In another panel test, the samples containing the Imitation McKinley apple flavor, N-602, Dragoco, Inc., inclusion compound were also overwhelmingly selected over the control samples. It was also observed that there was a slow release effect of the flavor in the case of the beta cyclodextrin inclusion compound. Many of the panelists remarked that this flavor was "longer lasting". None of the other flavor inclusion compounds were panel tested on a large scale.

c. Smoking Tobacco

Beta cyclodextrin-flavor inclusion compounds became of interest for use as flavors in smoking tobacco from the standpoint of stabilizing volatile flavors. It was also proposed that flavors which reacted with the tobacco itself (e.g. oil of lemon) could also be utilized when applied to tobacco in the form of a cyclodextrin inclusion compound. It was also considered that these inclusion compounds could be used to mask the external odor (if objectionable) of some flavors.

1. BETA CYCLODEXTRIN SMOKING PANEL TEST

Since the cyclodextrins were an unknown entity from the standpoint of smoking quality in cigarettes, a smoking panel test was made on cigarettes which were treated with beta cyclodextrin.

CAMEL blend tobacco (700 g.) was sprayed with an aqueous solution of beta cyclodextrin (7 gm./200 ml.). Another portion of the tobacco (700 g.) was sprayed with 200 ml. of distilled water. The two samples were fabricated into 70 mm. cigarettes. Cigarettes were selected for panel testing which were free of soft spots and loose ends in a weight range of 1.00-1.08 gm. The selected cigarettes were then submitted to Dr. S. A. Bellin for panel testing (2).

Two triangle tests were made, one with the test cigarette odd and the other with the control cigarette odd. In all cases the code numbers, 1, 2, 3, were randomized within the panel.

Dr. Bellin concluded from this test that the 30-member panel was unable to distinguish the cigarettes treated with beta cyclodextrin from the control cigarettes (2).

2. VOLATILE FLAVORANTS

Ethyl propionate was suggested as a good example of a volatile flavorant by Mr. E. H. Harwood. A beta cyclodextrin-ethyl propionate inclusion compound was prepared as described in Section B.V. of this report. The ethyl propionate inclusion compound was applied to CAMEL blend tobacco by sprinkling on the dry powder. Cigarettes were then fabricated using a small hand-operated machine and stored for 30 days at room temperature. When the cigarettes were smoked, the odor and flavor of the ethyl propionate were readily apparent in both the mainstream and side-stream of the smoke. Control cigarettes were prepared in the same way except that a solution of ethyl propionate was sprayed on the tobacco. After 30 days storage, these cigarettes were also smoked and had no detectable flavor of ethyl propionate.

### 3. FLAVORANTS WHICH REACT WITH TOBACCO

It was reported by Mr. E. H. Harwood (20) that when oil of lemon or oil of lime flavors are added to tobacco there is an apparent reaction which after several days completely neutralizes these flavors so that they are not detectable by smell or smoking.

Beta cyclodextrin-inclusion compounds of oil of lemon and oil of lime were prepared as described in Section B.V. of this report. Aqueous slurrys of these compounds were sprayed on CAMEL blend tobacco and, after drying, the tobacco was fabricated into cigarettes (treated) using a hand machine. Control samples were prepared by spraying an equivalent amount of the liquid flavors on equivalent amounts of CAMEL blend tobacco and fabricating cigarettes. Both the "control" cigarettes and "treated" cigarettes were stored in glass jars at room temperature for approximately 30 days. At this time, the cigarettes were smoked and evaluated. It was found that in both bases (lemon and lime flavors) the cigarettes which had been treated with the beta cyclodextrin-inclusion compounds retained these flavors, while neither of the control cigarettes yielded these flavors, upon smoking.

### 4. FLAVORANTS WITH "LOUD" OR OBJECTIONABLE EXTERNAL ODORS

For this study, vanillin was selected as an example of a "loud" flavorant (i.e. one which masks the effect of a group of other flavorants), while isovaleric acid was selected as a flavorant with an objectionable external odor.

The beta cyclodextrin-inclusion compounds of vanillin and isovaleric acid were prepared as described in Section B.V. of this report.

The beta cyclodextrin-vanillin inclusion compounds had no external odor. When a small amount was placed on a burning cigarette, the odor of vanillin was readily apparent in both the mainstream and sidestream of the smoke.

In the case of the beta cyclodextrin-isovaleric acid inclusion compound, the external odor of the isovaleric acid was never completely removed from the inclusion compound. Various techniques including drying under high vacuum and repeated washings with ether did not completely remove the external odor of the isovaleric acid. When the compound was tested on a burning cigarette, there was a substantial release of isovaleric acid in the mainstream and sidestream of the smoke.

d. Laundry Fragrance

The object of this experiment was to determine whether a cyclodextrin-fragrance inclusion compound could withstand the washing,

drying and ironing of the laundry cycle. Mr. C. H. Milton suggested two fragrances for use in this experiment: Liquid sunshine No. 9601, Firmenich & Co. and Compound oil No. 19434, Fritzsche Bros. The beta cyclodextrin-inclusion compounds of these fragrances were prepared as described in Section B.V. of this report. The experiment was conducted at a local launderette (Thruway Shopping Center). Twelve cotton diapers and one bottle of "Downy", concentrated, fabric softener, Procter and Gamble Co. were obtained for the experiment. For the "control", six diapers were washed in a conventional automatic washer through the 30 minute wash cycle. One capful of "Downy" and 0.526 grams of Liquid Sunshine No. 9601 were added at the beginning of the wash cycle. For the treated samples, six diapers were washed exactly as described above except that in addition to one capful of "Downy" 1.35708 grams of beta cyclodextrin Liquid Sunshine No. 9601 inclusion compound were added at the beginning of the wash cycle. Both sets of diapers were dried in separate dryers for ten minutes at 160°F. After drying, the diapers were steam-ironed by Mrs. C. H. Milton.

The diapers were then evaluated for fragrance by Mr. C. H. Milton (28). It was found that the diapers which were washed with the beta cyclodextrin Liquid Sunshine No. 9601 inclusion compound retained a perceptable amount of fragrance even after steam-ironing. The control diapers had no odor at the end of this cycle.

This experiment was repeated twice with the same results.

e. Chewing Gum

Beta cyclodextrin-flavor inclusion compounds were panel-tested in chewing gum. Five flavor inclusion compounds were prepared as described in Section B.V. of this report and are listed below.

1. 50 Spearmint, 50 carvone, Norda, Inc.

2. Oil of peppermint, USP x IV, Fritzsche Bros.

3. Imitation fruit flavor No. 952, Felton Chemical Co.

4. Cinnamic aldehyde, Fritzsche Bros.

5. Imitation licorice flavor, No. 50.691/A, Firmenich & Co.

The method used for making the chewing gum was obtained from Firmenich & Co. and is presented in Appendix I.

Chewing gum samples were prepared using beta cyclodextrin-flavor inclusion compounds and panel-tested with chewing gum made using comparable liquid flavors in equivalent amounts. Some difficulty was encountered in establishing the most acceptable flavor level (i.e. the amount of flavor added to the gum) for panel testing. It became apparent that smaller amounts of the flavor inclusion compounds could

be used since none of the flavor was lost because of the high temperature of the gum when the flavor was added. In the preliminary gum samples, the gum containing the flavor inclusion compound invariably had a stronger flavor than the control gum sample which was prepared using a liquid flavor. Therefore, an acceptable flavor level for the cyclodextrin-flavor chewing gum had to be established by panel testing. For panel testing, the chewing gum was weighed in 3-gram portions and wrapped with aluminum foil. The wrapped gum was then placed in glass tubes fitted with air-tight screw caps. The samples were sent to the panel in this form for testing. The results of panel testing chewing gum are presented in Table IX.

## TABLE IX

## COMPARISON OF BETA CYCLODEXTRIN-FLAVOR INCLUSION COMPOUNDS WITH LIQUID FLAVORS IN CHEWING GUM

Test No. 1 - Oil of peppermint, USP XIV, Fritzsche Bros.

| | |
|---|---|
| Total panelists | 50 |
| Number of panelists selecting the beta cyclodextrin complex | 4 |
| Number of panelists selecting the control (liquid flavor) | 46 |

Test No. 2 - 50 Spearmint 50 Carvone, Norda, Inc.

| | |
|---|---|
| Total panelists | 50 |
| Number of panelists selecting beta cyclodextrin complex | 14 |
| Number of panelists selecting control (liquid flavor) | 36 |

Test No. 3 - Imitation fruit flavor, No. 952, Felton Chemical Co.

| | |
|---|---|
| Total panelists | 49 |
| Number of panelists selecting beta cyclodextrin complex | 15 |
| Number of panelists selecting control (liquid flavor) | 34 |

Test No. 4 - Cinnamic aldehyde, Fritzsche Bros.

| | |
|---|---|
| Total panelists | 48 |
| Number of panelists selecting beta cyclodextrin complex | 6 |
| Number of panelists selecting control (liquid flavor) | 42 |

In all four panel tests, the panelists overwhelmingly preferred the control (liquid flavor) chewing gum over the chewing gum made with the beta cyclodextrin complex. However, it is felt that the beta cyclodextrin flavor complexes still may have a potential as chewing gum flavors because of the smaller amount of the flavorant which can be used in preparing the chewing gum.

f. Coffee

An apparatus was devised for using a solution of beta cyclodextrin to complex the "headnote" flavors of fresh ground coffee (see Section B.V. of this report). The object of this preparation was to add the "headnote" flavor complex to instant coffee in an attempt to improve the flavor of this product.

For the panel test, 0.050 gm. of Maxwell House Instant Coffee was weighed on a Mettler balance and dissolved in 10 ml. of an aqueous sucrose solution (1 lb./1 qt.) in a paper cup. This solution was termed the "control". The "test" sample was prepared exactly as described above except that 0.050 gm. of the beta cyclodextrin-coffee headnote flavor complex were also added to the solution. Each panelist was presented with two unlabeled cups (1 control and 1 test sample) and asked which liquid had the best coffee flavor. The panel consisted of 30 people who were avowed coffee drinkers. Twenty-one out of thirty panelists preferred the test sample (beta cyclodextrin-coffee complex), while nine out of thirty panelists preferred the control sample of instant coffee.

## VII. Thin Layer Chromatography of the Cyclodextrins

The object of this work was to develop a method for separating alpha, beta and gamma cyclodextrin using thin layer chromatography.

Thin layer glass plates were prepared using the Brinkman apparatus. Kieselguhr G was used as the absorbent. The glass plates were coated with Kieselguhr G and dried for 2 hours at 100°C. and stored in a desiccator. Solutions of the cyclodextrins (0.1 percent) were prepared by dissolving 25 mg. of each in 25 ml. of water. These solutions were spotted (10 ml.) on the plates for chromatography. The plates were irrigated in various solvents, dried and developed by spraying with 0.1N iodine in ethanol.

It was found that the most promising solvents for separation of the cyclodextrins were: methanol, ethanol, n-propanol, n-butanol and water. The best separation was obtained with methanol and ethanol. It is proposed that a combination of these solvents with water in the proper proportions would be the best solvent system for separating the alpha, beta and gamma cyclodextrins.

The cyclodextrin project was suspended shortly after this phase of the work was begun.

## C. DISCUSSION

No appreciable time was spent on the development of better techniques for the preparation of the cyclodextrins. Instead, three published methods were investigated and the method of F. Cramer (10) was found to be superior. When attempting to prepare large scale batches of beta cyclodextrin, the laboratory method was scaled up and the apparatus was improvised. It is felt that with proper fermentation equipment much greater yields of beta cyclodextrin could have been obtained.

During the investigation of the selective filtration properties of the cyclodextrins, some difficulty was encountered in obtaining a uniform distribution of the cyclodextrin throughout the filter plug. Unplasticized Estron filter plugs had to be used, since the aqueous solutions of the cyclodextrins could not be introduced into the plasticized plugs. The filter plugs had to be wrapped with plastic tape (water insoluble) previous to the introduction of the aqueous solution in order to maintain a uniform plug cylinder. Aqueous solutions of the cyclodextrin were introduced into the filter plug, dropwise, using a burette to measure the volume. The filter plug was then dried in a forced draft oven. It was found that during the drying period, the cyclodextrins crystallized in greater amounts at the ends of the filter plugs. It was therefore necessary to cut 4.25 mm. sections off of each end of a 25.5 mm. plug to obtain a 17 mm. filter plug with a uniform distribution of cyclodextrin. The weight of cyclodextrin present in the final filter plug was estimated by the weight difference between treated and untreated plugs of the same exact length. The treated plugs were attached to the cigarettes with plastic tape. Some of the treated plugs had small wrinkles from contact with aqueous solutions and had to be carefully wrapped to avoid air leaks when the cigarettes were smoked on the smoking machine.

Beta cyclodextrin-flavorant inclusion compounds were prepared using a number of commercially available flavors. It is known that some of these flavors contained many individual compounds which comprised the total flavor. When inclusion compounds were prepared with these flavors, it was not established whether all of the compounds comprising the total flavor were included in the final inclusion compound. However, this information could have been obtained using gas chromatographic techniques.

## D. CONCLUSIONS

1. Of the three published methods for the preparations of the cyclodextrins, the method of F. Cramer (10) was found to be superior.

2. The optimum concentration of B. macerans amylase for production of beta cyclodextrin is between 50 and 150 Hale & Rawlins units of enzyme per 100 gms. of substrate.

3. When using "large batch" methods (100-150 liters of substrate) for the preparation of beta cyclodextrin, the yields were 10 to 25 percent lower than with laboratory scale preparations. With proper fermentation equipment, much greater yields of beta cyclodextrins could have been obtained in the large scale preparations.

4. No definite conclusion can be made in regard to the removal of polycyclics from the smoke of cigarettes with cyclodextrin-treated filter tips because two sets of conflicting data were obtained. The priority of another project prevented the analysis of the third set of samples by the Analytical Division.

5. There is no significant difference between the total phenol content of the smoke from cigarettes with cyclodextrin-treated filter tips and the control cigarettes.

6. There was no significant difference in the fatty acid content of the smoke from control cigarettes and cigarettes with cyclodextrin-treated filter tips.

7. There is no removal of phenols from the smoke of cigarettes with cyclodextrin acetate-treated filter tips.

8. Beta cyclodextrin-flavorant inclusion compounds can be used as superior flavorants in cakes. Even the more volatile flavorants are retained during baking temperatures, when present in the cake in the form of cyclodextrin inclusion compounds.

9. Beta cyclodextrin-flavorant inclusion compounds can also be used as superior flavorants in chewing tobacco. There appeared to be a slow release effect of the flavorant in the chewing tobacco; many of the panelists also observed this flavorant was "longer lasting".

10. The presence of 1 percent by weight of beta cyclodextrin on CAMEL blend tobacco has no effect on the smoking quality of the tobacco.

11. When beta cyclodextrin-volatile flavorant inclusion compounds are applied to smoking tobacco, the volatile flavor is retained until the tobacco is smoked, at which time the flavor is released in the smoke.

12. Flavorants which react with tobacco (e.g. oil of lime) can now be utilized as tobacco flavorants when applied as a cyclodextrin inclusion compound.

13. Laundry which was washed with a beta cyclodextrin-fragrance inclusion compound retained a perceptable amount of fragrance even after drying in an automatic dryer and steam ironing.

14. Beta cyclodextrin-flavorant inclusion compounds were found to be unsuitable as flavorants for chewing gum in panel tests because of an apparent "bitter-flavor note" which they imparted into the gum.

15. Beta cyclodextrin will form an inclusion compound with the "headnote" flavors of fresh ground coffee. When this inclusion compound was added to instant coffee, a panel of coffee drinkers preferred the instant coffee with the cyclodextrin flavor additive over regular instant coffee by a ratio of 2:1.

## E. RECOMMENDATIONS

### I. Future Work

In view of the patent situation, no further work is planned on this project.

### II. Patentability

It appears at this time that the patentable material in this report has been pre-empted by other patents.

J. D. Shiffert

/J. D. Shiffert

Distribution:

Mr. Kenneth H. Hoover
Mr. E. H. Harwood
Dr. Murray Senkus
Dr. Eldon D. Nielson
Mr. J. D. Shiffert
Library (2)
Photo for Mr. Cundiff 7-7-67

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Cdh Photo for: Dave Eaker 11-27-84

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## APPENDIX I

### METHOD USED FOR MAKING CHEWING GUM (OBTAINED FROM FIRMENICH & CO.)

| Ingredients | Amount |
|---|---|
| Gum base | 20 grams |
| Corn syrup 45° Be | 37.5 grams |
| Sugar, confectioners | 67.5 grams |
| | 125.0 total |

Procedure

1. Place base and corn syrup in a suitable container. Melt (200-220°F.) and mix for 5 minutes after melting is complete.
2. Remove from heat; add sugar plus liquid flavor. Mix for 3 minutes.
3. Dust with confectioners sugar and roll into surface while warm.

J. D. Shiffert

J. D. Shiffert

February 22, 1963

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## APPENDIX II

### NOTEBOOK PAGE NUMBERS

| | |
|---|---|
| p45051-45100 | October 27, 1961 to April 6, 1962 |
| p103039-46 | May 21, 1962 to June 1, 1962 |
| p109951-110000 | April 26, 1962 to August 21, 1962 |
| p109201-50 | April 11, 1962 to July 23, 1962 |
| p113351-113382 | August 22, 1962 to January 14, 1963 |
| p115151-52 | October 16, 17, 1962 |

J. D. Shiffert

J. D. Shiffert

February 22, 1963