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The Substituent Group Distribution in a Michael Reaction: Carbamoethyl Cellulose

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ERRATA

Figure 7, p. 76, in this thesis is an incorrect figure. The correct figure is shown below:

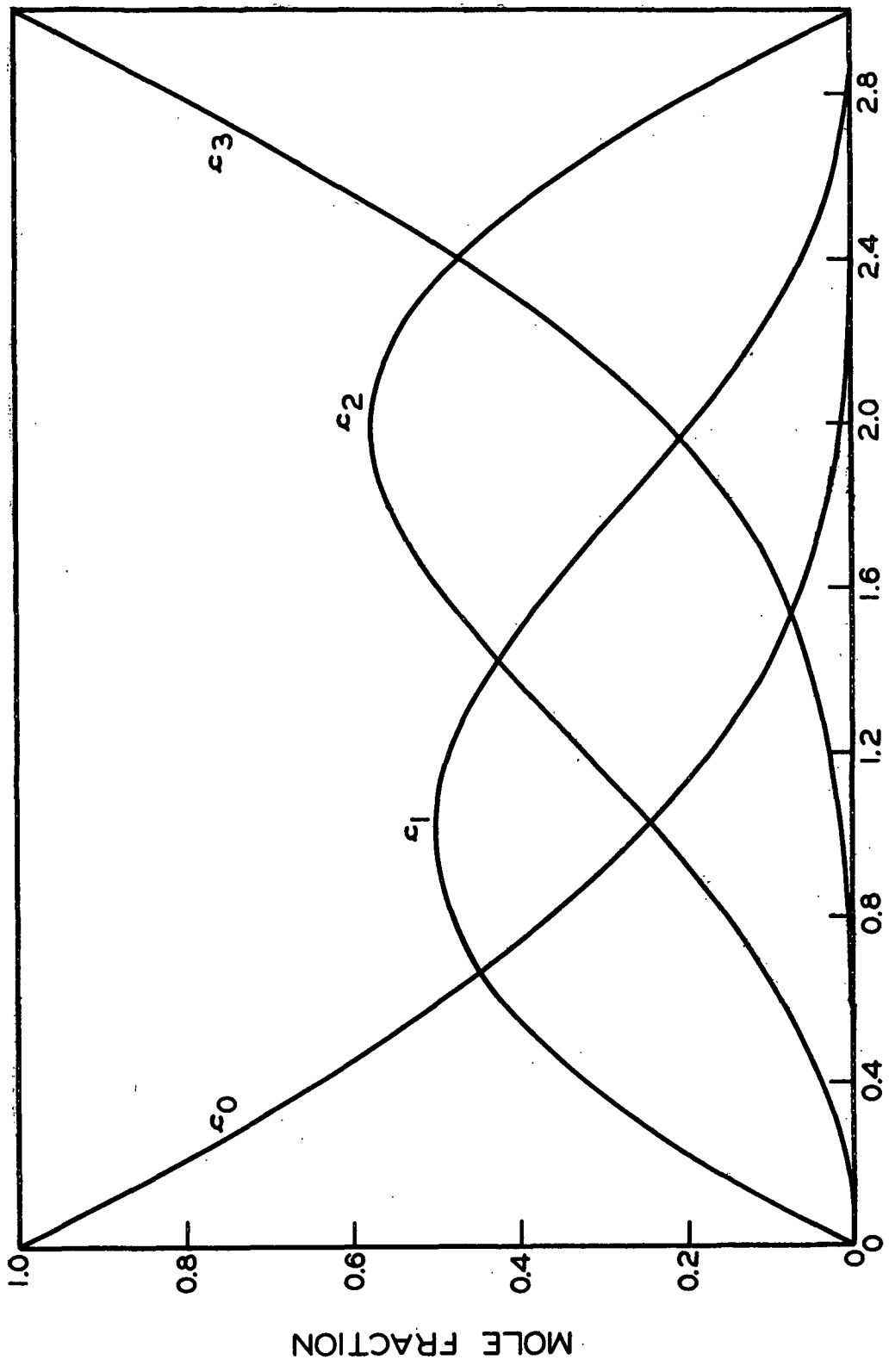

Figure 7. Plot of $c_{\underline{i}}$ values vs. D.S. for $K_{\underline{2}}:K_{\underline{3}}:K_{\underline{6}} = 9:1:19$

THE SUBSTITUENT GROUP DISTRIBUTION IN A MICHAEL REACTION: CARBAMOETHYL CELLULOSE

A thesis submitted by

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SUMMARY

A carbamoethyl cellulose was prepared by the Michael addition reaction of acrylamide with cellulose in the presence of sodium hydroxide. The reaction was allowed to proceed till equilibrium conditions were approached. Nitrogen and carboxyl analyses indicated the derivatives to be 0.48 D.S. carbamoethyl and 0.30 D.S. carboxyethyl, a total of 0.78 D.S.

The distribution of the substituents was studied by the hydrolysis and separation method. The procedure used to hydrolyze the cellulose also completely hydrolyzed the amide groups and gave a mixture of glucose and carboxyethyl glucoses. This mixture was separated by paper chromatography into glucose and the mono-, di-, and trisubstituted glucoses. Identities were assigned to the substituted glucoses on the basis of the molar ratio of their carboxyl content to their aldehyde content and on the basis of chromatographic comparison to carboxyethyl glucoses which were prepared by carbamoethylating suitably blocked glucoses and hydrolyzing the products. The mole fractions were measured by an alkaline hypoiodite oxidation.

The Spurlin equations for the distribution of substituents in an equilibrium reaction were reduced to a cubic equation in the relative equilibrium constant, $\underline{AK}$, in terms of the mole fractions of substituted glucoses, $\underline{c}_j$.

$$c_0(AK)^3 - c_1(AK)^2 + c_2 AK - c_3 = 0$$

This equation was solved for $\underline{AK}$ using the experimentally measured mole fractions to give the relative equilibrium constants, $\underline{K}_2 : \underline{K}_3 : \underline{K}_6 :: 9:1:19$.

INTRODUCTION

SUBSTITUENT GROUP DISTRIBUTIONS

Cellulose is a long-chain, linear polymer of anhydroglucose units linked by β-1, 4-glucosidic bonds. Each of these glucose units has three free hydroxyl groups, one primary and two secondary, which are able to undergo various reactions typical of hydroxyl groups. The substitution reactions of these hydroxyl groups have led to a large number of cellulose derivatives.

One of the important properties of these derivatives is the distribution of the substituent groups among the hydroxyl groups. For any partially substituted cellulose, the substituent groups are distributed among these three hydroxyls in an arrangement which is determined primarily by the relative reactivity of the hydroxyl groups and the nature of the synthesizing reaction. It is also affected to some degree by the cellulose itself and by the particular chemical reagent.

Spurlin (1, 2) presented a mathematical treatment of the statistical distribution of substituents in cellulose derivatives. In it he developed the mathematical relationships between the reaction constants of the substitution reaction and the various measures of substituent group distribution for both rate-controlled distributions and equilibrium-controlled distributions. This work has become the backbone or framework of essentially all of the recent distribution studies. It forms the basis of the treatment of the present work.

In establishing these relationships, Spurlin made certain, definite assumptions. The first assumption is that the distribution of the groups is random and follows the laws of probability. This means that all of the hydroxyl groups must react independently and must be equally free to react; there must be no

biasing effect. There is a major deviation from this condition which is related to the property of accessibility, the fact that some regions of the cellulose structure are not available for reaction with the reagent or are physically restricted in such a way that they react only at reduced rates. Accessibility is related to the physical structure of the cellulose and, hence, will vary with the treatment of the cellulose. It will affect the substituent group distribution by limiting the extent or rate of reaction of a portion of the anhydroglucose units. This deviation can be accommodated by modifying Spurlin's treatment.

The second assumption is that the effect of end groups is negligible. The soundness of this assumption is fairly obvious from a consideration of molecular weights. At a D.P. of 500, either end group represents only two-tenths of one per cent of the glucose units present.

The third assumption is that the ratio of the reaction constants, the rate or equilibrium constants, remains constant. This means that the actual change in rate of reaction which occurs as the D.S. increases and the physical properties change, occurs proportionately at all of the hydroxyls. It also means that there is no change in the reaction constant of a hydroxyl if an adjacent hydroxyl is substituted. Interference has been observed between the 2- and 3-position hydroxyls, but the 6-position hydroxyl and the hydroxyls on adjacent glucose units appear to be too far removed to cause interference. This deviation can also be handled by modifying Spurlin's equations (3).

The fourth assumption is that the reaction may be treated as first order. This requires that all reactant concentrations except those of the hydroxyls remain constant. This is a relatively weak assumption and will hold only for reactions having short reaction times and rather large excesses of reagents.

However, this assumption is more important under rate-controlled conditions than in an equilibrium-controlled situation. Under equilibrium conditions, all of the hydroxyls will be in equilibrium with the same concentrations of reagents. The only concentrations that will be different in the equilibrium equations for the various hydroxyls will be those of the hydroxyls themselves. The concentrations of the other reacting materials can be combined into a common multiplier of the equilibrium constant.

Spurlin defined three kinds of mole fractions; x_i, the mole fraction of glucose units substituted in the i-position; c_j, the mole fraction of glucose units having j number of substituents; and s_i, the mole fraction of glucose units substituted only in the i-position. From these definitions and the laws of probability, he developed the interrelation of these values. Then using the basic laws of chemical kinetics and chemical equilibrium, he developed expressions for these mole fractions in terms of the reaction constants. Appendix VB contains this last development for the case of an equilibrium-controlled carbamoethylation.

Later work, particularly that of Timell (4), confirmed the accuracy and usefulness of these expressions and led to their use in essentially all recent distribution studies.

The distribution of substituent groups in a cellulose derivative can be used to determine the relative reactivity of the various hydroxyl groups to the particular reagent and, to a certain degree, by analogy, the relative reactivities toward similar reagents. It can also be related to some physical properties (5). A great deal of work has been done on the study of the relative reactivities of the hydroxyls with various reagents as indicated in

Sugihara's review (6) and exemplified by Croon's summary article (7), but there is a great deal more work to be done.

TYPES OF SUBSTITUTION REACTIONS

The factor which exercises the most influence on the distribution of substituents in a cellulose derivative is the nature of the chemical reaction. This determines whether the distribution is rate-controlled or equilibrium-controlled and also the relative reactivity of the hydroxyl groups.

In a rate-controlled system the distribution of the substituent groups is determined by the relative rates of reaction of the hydroxyls. The faster a particular hydroxyl reacts with respect to the others, the greater the relative amount of substitution of that hydroxyl at any given D.S. (Degree of Substitution, the average number of substituted groups per anhydroglucose unit). An irreversible reaction always gives a rate-controlled distribution.

In an equilibrium system, the distribution is determined by the relative stability of the substituted group at the various hydroxyl positions. This type of system is possible only with a reversible reaction. However, a reversible reaction can give a rate-controlled distribution if the reaction is interrupted before it can approach equilibrium.

There are several different types of reactions which form cellulose derivatives. Each has its distinctive mechanism and distribution pattern.

ORDINARY NUCLEOPHILIC SUBSTITUTIONS

The most common type of reaction is the simple nucleophilic substitution. The reactions of this type generally consist of the condensation of a cellulosic

hydroxyl group with an alkyl halide or an alkyl ester of an inorganic acid in the presence of alkali. Probably the most important reagents are methyl and ethyl chloride and chloroacetic acid which are used commercially to prepare methyl, ethyl, and carboxymethyl celluloses, respectively. This type of reaction can give rise to a large variety of derivatives, several of which have been subjected to substituent group distribution studies.

The methyl ethers of polysaccharides have received greater study than most derivatives. Generally, in these studies, the 2-position hydroxyl group has been found to be the most reactive. In a fairly typical study of the heterogeneous reaction of methyl chloride with alkali cellulose, the relative rate constants ($k_2:k_3:k_6$) for the 2-, 3-, and 6-position hydroxyl groups were 5, 1, and 2, respectively (8). A very similar ratio of reaction constants ($k_2:k_3:k_6 = 4.5:1:2$) was found for ethyl cellulose prepared under essentially the same conditions (9). Distribution studies of methylated cellulose prepared using dimethyl sulfate have shown that the ratios of the rate constants for this reagent are in the same order as those for the methyl halides and not greatly different numerically ($k_2:k_3:k_6 = 3.5:1:2$) (10).

When carboxymethyl cellulose was studied, a different phenomenon was observed. It was found that substitution on one of the secondary hydroxyls almost completely inhibited substitution on the other hydroxyl. This effect was more clearly evident from hydrolysis and separation studies (3, 11) than from a study using selective reactions (12). Aside from this interference, however, the substituent group distribution is similar to those found with the previous reagents ($k_2:k_3:k_6 = 2:1:2.5$).

A unique reagent which also yields methyl ethers is diazomethane. Accessibility is a very important factor in the reaction of cellulose with this material. Croon (13) studied the reaction of cellulose and diazomethane in moist ether and found the best fit between the calculated and the experimentally measured mole fractions of methylated glucoses was obtained assuming relative rate constants of 1.2, 1.0 and 1.5 for k_2, k_3 and k_6 and an accessibility of only thirty per cent. This example indicates clearly that the dominance of substitution at the 2-position is limited to certain types of reactions.

RING-BREAKING REACTIONS

The reactions of cellulose with compounds containing epoxide rings constitute a different type of reaction. The two most important reagents are ethylene oxide and propylene oxide. The reaction of ethylene oxide in particular has been studied by several workers (14-16). The studies have been complicated by a peculiarity of the reaction. When ethylene oxide reacts with a hydroxyl group it forms another, primary hydroxyl group and this hydroxyl is capable of reacting further with ethylene oxide to form a polyoxyethylene chain at the site of substitution. In spite of these difficulties, it has been possible to show that the primary hydroxyls react more readily than the secondary hydroxyls and that essentially all of the hydroxyls are available to the reagent. Croon modified Spurlin's equations for the relative amounts of substituted glucoses in a rate-controlled reaction and was able to account for substitution on the newly formed hydroxyls. He found the relative rate constants for the 2, 3, 6, and newly formed hydroxyls to be 3, 1, 10, and 20, respectively (14).

The reaction of cellulose with lactones such as β-propiolactone also proceeds with the opening of a ring, but it is of a different character than the

epoxide ring. The reagent can form an ether or an ester linkage and the conditions for forming either exclusively are not known (19).

MICHAEL REACTIONS

The only important type of reaction that has not received significant study is the Michael addition reaction. This reaction is the alkali-catalyzed addition of cellulose hydroxyl groups to activated ethylene groups. The possible reagents include acrylonitrile, acrylamide and substituted acrylamides, acrylic acid and acrylate esters, vinyl sulfones and a variety of other similar compounds. This is the only class of etherification in which the reactions are clearly reversible reactions (18) and in which the substituent group distribution can be equilibrium-controlled rather than rate-controlled.

Cyanoethylation, the reaction of acrylonitrile with a compound containing a reactive hydrogen atom, is an important method in organic synthesis and has been studied with a variety of compounds (18). The cyanoethylation of alcohols is considered to be a typical Michael addition reaction and to proceed by nucleophilic attack of the alkoxide ion on the activated double bond. Recent work by Feit and Zilkha (19) has confirmed the nature and mechanism of the reaction. Most of the studies on cyanoethyl cellulose, though, have reflected the the commercial interest in this material and have dealt with the studies of the useful properties of the derivative or the methods of making it. Practically no work has been done on the distribution of cyanoethyl groups in these derivatives. Moe and co-workers (20, 21) studied a cyanoethylated amylose and a cyanoethyl glucomannan using only periodate oxidation. They were able to conclude that, under the homogeneous reaction conditions, substitution occurred predominantly at the primary hydroxyl, but they could not describe the complete substituent group distribution.

The carbamoethylation of cellulose, the reaction with acrylamide, should take place by the same mechanism as cyanoethylation even though it does not proceed to the same extent. A peculiarity of the reaction is the fact that it gives rise to two kinds of groups, carbamoethyl groups by the addition reaction, and carboxyethyl groups by the hydrolysis of the carbamoethyl groups and the relative amounts of each can be controlled within certain limits by varying reaction conditions (22, 23). The distribution of substituents in these derivatives has not yet been studied.

THE PARTICULAR PROBLEM

The particular problem chosen for this thesis was the study of the distribution of substituent groups among the hydroxyls in carbamoethyl cellulose. A derivative prepared by the Michael addition reaction was chosen because this is the only major type of cellulose etherification in which the distribution of the substituent groups has not yet been studied.

To avoid certain experimental difficulties, the carbamoethylation reaction was chosen rather than the commercially more important cyanoethylation even though it does not proceed to the same extent as cyanoethylation and gives two kinds of substituent groups. It was believed that, during hydrolysis, all of the amide groups of carbamoethyl cellulose would be hydrolyzed to carboxyl groups and that some but not all of the nitrile groups of cyanoethyl cellulose would be hydrolyzed. This would give twenty-seven different possible substituted glucoses, including unsubstituted glucose, from cyanoethyl cellulose and only eight from carbamoethyl cellulose. A preliminary experiment substantiated the hypothesis.

The ultimate goal of the thesis was the determination of the distribution of substituent groups in carbamoethyl cellulose, a derivative prepared by the Michael reaction.

METHODS OF APPROACH

There are two basic, chemical approaches to the study of the distribution of substituent groups in cellulose derivatives. The first method is the quantitative reaction of the cellulose with reagents which are specific for particular hydroxyl groups or group combinations. The second method is the complete degradation of the cellulose into known compounds characteristic of the various groups substituted and the subsequent quantitative measurement of these degradation products. Both of these methods have been applied to a variety of cellulose derivatives.

THE METHOD OF SPECIFIC REACTIONS

The specific-reaction method of studying the position of substituent groups in cellulose derivatives consists of treating the cellulose with a reagent that undergoes a reaction characteristic of a particular group and then quantitatively measuring the extent of that reaction. The extent of reaction is a measure of the amount of the specific group present in the cellulose derivative. The greatest difficulty of the method lies in the fact that the reactions are not completely specific but only highly selective under the experimental conditions used. Hence, the results must be carefully interpreted. Nevertheless, when used within their limitations, there are several reactions which do give significant information.

Tritylation is the reaction of cellulose with a triphenylmethyl halide. When first investigated this reaction was thought to take place only at the 6-position hydroxyl which was unsubstituted. However, later work showed that while the reaction is highly preferential for primary hydroxyls, it is not completely specific. Hearon and co-workers (24) studied the distribution of

trityl groups in a 1 D.S. ether. As indicated by tosylation-iodination, only about nine-tenths of the groups were on primary hydroxyls. Hence, a simple measure of trityl content of a cellulose derivative after tritylation will not be a completely dependable measure of the free primary hydroxyl groups in the original cellulose derivative.

Tosylation, reaction with p-toluenesulfonyl chloride, has been used extensively in the study of substituent group distributions. The reagent reacts much more slowly with secondary hydroxyl groups than with primary groups but it does react rapidly enough to prevent the use of this reagent alone. Mahoney and Purves (25) found that treatment of the tosylated cellulose with sodium iodide in acetone replaced the tosyl groups in primary positions with iodine and, hence, the combined reactions could be used to distinguish between free primary and secondary hydroxyl groups in cellulose derivatives. The results must be interpreted with caution; Dyer and Arnold (12) used the method successfully to study carboxymethyl cellulose but Timell (11) reported erratic results in a study of the same derivative. Since tosylation and iodination distinguishes only the free primary hydroxyl groups, it must be combined with other reactions in order to describe the substituent group distribution completely.

Periodate oxidation has been widely used in the study of substituent group distribution. Under carefully controlled conditions, the oxidation selectively oxidizes α-glycols by splitting the carbon-carbon bond (26). Applied to cellulose derivatives, the oxidation measures the number of anhydroglucose units which have free α-glycol groups, that is, which are both unsubstituted in the 2- and 3-positions. In a hydrolyzate of the cellulose derivative, the oxidation measures the amount of 1-2, 2-3, and 3-4 glycol present, which is in turn a measure of the amounts of free 2- and 3-position hydroxyls. This reaction has

been used extensively in combination with tosylation and iodination and has been applied to cyanoethylated amylose and glucomannan (20, 21).

The lead tetraacetate oxidation is, under controlled conditions, selective for cis-glycol groups (27). Since there are no cis-glycol groups in cellulose, the reaction is applied only to hydrolyzates of the cellulose derivative where it measures the 1-2 glycol and hence the amount of free 2-position hydroxyl.

It is obvious that no one group-specific reaction is sufficient to characterize a cellulose derivative. Complete, sound information can be obtained only by the judicious combination of these reactions so that every group and position is measured. A good example of this is the work of Mahoney and Purves already cited (25). They used tosylation and iodination to measure the amount of free 6-position hydroxyl, periodate oxidation to measure the amount of free 2,3-glycol groups and lead tetraacetate oxidation to measure the amount of free 1,2-glycol groups after hydrolysis. Since the 1-position is covered in cellulose, this last value represented a measure of the free 2-position. The amount of free 3-position was then calculated from the total free hydroxyl contents by difference. In this way the specific reaction method is able to give a good indication of the distribution of substituent groups in cellulose derivatives.

THE METHOD OF COMPONENT SEPARATION AND IDENTIFICATION

The component separation method of studying substituent group distributions consists, in practice, of the hydrolysis of the cellulose to the corresponding substituted glucose units. The method has essentially only four basic steps or operations. These are the hydrolysis, the separation of components, the identification of components and the quantitative measurement.

The most common method of hydrolysis is the almost universally used 72% sulfuric acid digestion (28). This method reduces the derivative to the substituted glucoses. Other possible methods are methanolysis in methanolic hydrogen chloride to give the methyl glucosides and acetolysis in strong acetylating mixtures to give substituted glucose acetates. Neither of these methods ordinarily presents any advantage over the sulfuric acid digestion.

The second step is the separation of the components of these substituted glucose mixtures in measurable amounts. This can be accomplished in several ways. In the earlier work, the method usually used was fractional distillation. While this was reasonably satisfactory for the methyl glucoses, which were of prime importance, the method was not applicable to all derivatives. With the development of chromatographic techniques, these methods generally displaced the other methods of separation. At present, paper and column chromatography are almost the only methods used to separate the mixtures of glucose derivatives. Recently, two new methods of separation have been applied to sugar mixtures and may be readily adaptable to cellulose derivative studies. These are the methods of thin-film chromatography and gas chromatography. Thin-film chromatography offers some strong advantages in experimental procedures and technique and a variation in substrates not available from paper chromatographic methods. It has already become an important tool for the separation of sugar mixtures (29). Gas chromatography, also offers some advantages for sugar separations but the techniques are less developed (30). To use this method for substituent distribution studies, it may be desirable or even necessary to go to the less common methods of cellulose depolymerization, methanolysis or acetolysis.

The identification of the components of the hydrolysis mixtures consists either of the comparison of the components to known materials or the actual separation and characterization of these materials.

The quantitative measurement of the substituted glucose units will depend to a certain degree on the method of separation and on the nature of the substituted group. There are several possible methods. The most likely is the colorimetric estimation using a reagent such as o-aminobiphenyl (31) or an oxidative estimation using a quantitative oxidation with alkaline iodine solution (32) or acid dichromate reagent (33). Certain physical methods can be used in quantitative column chromatography. In the case of separations by gas chromatography, quantitative data can be obtained directly by standardizing the instrument.

OTHER METHODS

In general, the purely physical methods have not been useful in the study of substituent group distribution. In most cases the distributions have been studied chemically to help explain the physical phenomena observed. However, O'Connor and co-workers (34, 35) have made a start in the use of infrared spectroscopy. They were able to correlate D.S. and infrared spectra for cyanoethyl cellulose and for cellulose acetate. They also reported spectral details which they believed were related to substituent distribution.

SELECTION OF METHODS FOR THE PRESENT WORK

The method of component separation was chosen rather than the specific-reaction method because this method is potentially capable of giving more information than the specific-reaction method. While the specific-reaction method determines only what fraction of a particular hydroxyl position is substituted, the method of component separation can give the mole fraction of glucose units substituted in each of the eight possible arrangments of substituent groups.

The method of hydrolysis selected was digestion in 72% sulfuric acid followed by dilution and heating to complete the hydrolysis (28). It has been widely used for the hydrolysis of a number of polysaccharides. Croon and co-workers (36), in a study of the effects of various hydrolysis methods on several methylated sugars, found that this method gave relatively little degradation of the methylated sugars and almost no demethylation. Timell (37), in a study of the effect of the method on carboxymethyl cellulose and carboxymethyl glucose, concluded that no carboxymethyl groups were lost during hydrolysis. Hence, the method should give complete hydrolysis of carbamoethyl cellulose with no more than negligible loss of carboxyethyl group.

Paper chromatography was chosen as the method of separation because it has been used successfully in separating a large variety of sugar mixtures including the components of hydrolyzates of a number of cellulose derivatives. The developers and spray reagents were selected empirically.

The method chosen for identification of the separated materials was comparison to known substituted glucoses prepared from glucose derivatives with only certain, specific hydroxyl groups free to react.

Because the separated materials were mixtures and not single compounds, the alkaline hypoiodite oxidation of Willstätter and Schudel (32) was used to measure them quantitatively. This procedure stoichiometrically oxidizes the aldehyde group of the sugar and should be unaffected by substitution on the hydroxyls.

EXPERIMENTAL PROCEDURES

The experimental work was carried out in four phases: the preparation of the derivative, the hydrolysis of the derivative and separation of the components of the hydrolyzate, the identification of the separated materials, and the quantitative estimation of the amounts of materials.

PREPARATION OF THE DERIVATIVES

The first method used to prepare the carbamoethyl cellulose was essentially that used by Frick and co-workers (38) in their work on the modification of cotton fabrics. A typical reaction is found in Appendix IA. This procedure was later modified to use considerably lower temperatures and much longer reaction times (see Appendix IA).

The reference substituted glucoses were prepared by carbamoethylating the blocked glucoses, 1,2:5,6-di-O-isopropylidene-D-glucofuranose and methyl 4,6-O-benzylidene-α-D-glucopyranoside. The methods are described in Appendices IB and IC.

HYDROLYSIS AND SEPARATION

The method of hydrolysis was a slight modification of that of Saeman and co-workers (28). An example is given in Appendix IA.

The separations were made using descending paper chromatography and an acidic developer. (see Appendix III).

IDENTIFICATION OF THE SEPARATED MATERIALS

The methods used to identify the separated materials were chemical analysis and comparison of R_g values and color of chromatographic spots to those of reference compounds on the same chromatograms.

QUANTITATIVE ESTIMATION OF THE AMOUNTS OF MATERIAL

To obtain the mole fraction data needed to calculate relative reaction constants for the carbamoethylation reaction, a step-wise procedure was set up to analyze the carbamoethyl cellulose. The components of the hydrolyzate of carbamoethyl cellulose were separated by paper chromatography and eluted using the procedure of Saeman and co-workers (28). The eluted samples were treated with an anionic resin and titrated conductimetrically with 0.02N sodium hydroxide. The conductimetric procedure made it possible to distinguish between the strong acid introduced into the samples and the carboxyethyl glucoses. Figure 1 shows typical conductance curves. The initial region of zero or negative slope represents the neutralization of the strong acid; the region of smaller positive slope represents the neutralization of the carboxyethyl glucoses and the region of higher slope the final sodium hydroxide addition. The samples were then neutralized and finally oxidized quantitatively by the procedure of Willstätter ans Schudel (32). Details of the procedures are found in Appendix IV.

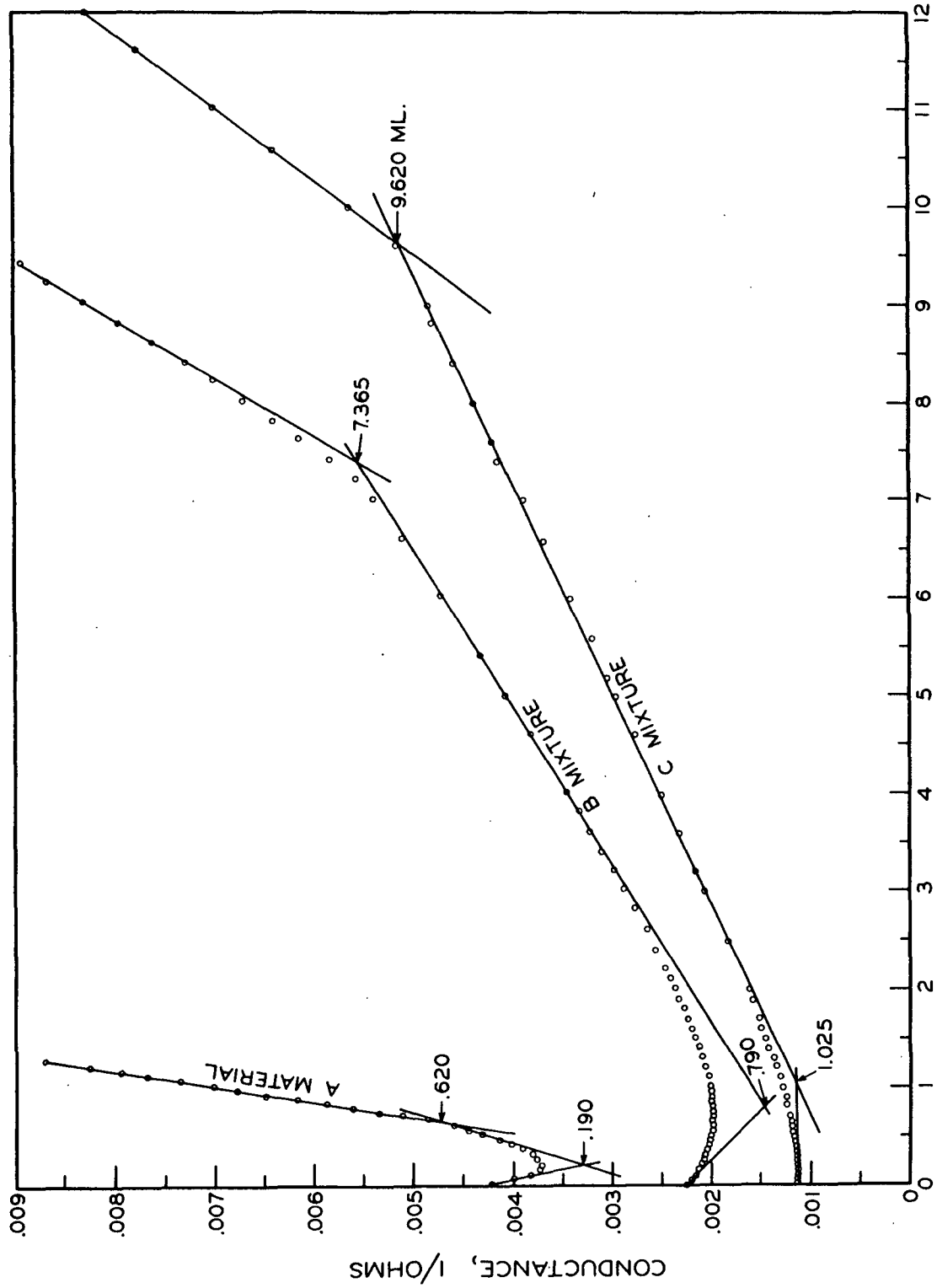

Figure 1. Plot of Conductance vs. Volume NaOH, Conductimetric Titration of Analytical Set VIII

RESULTS AND DISCUSSION

PREPARATION OF THE DERIVATIVES

PREPARATION OF THE CELLULOSE DERIVATIVE

The first step in the investigation was the preparation of a suitable cellulose derivative. The method of Frick and co-workers (38) for carbamoethylating cotton fabrics was tried first. It consisted of absorbing into the cellulose a solution of acrylamide and sodium hydroxide and heating for a relatively short period of time to bring about the reaction. A series of these preparations was made varying the time and temperature of the reaction but the D.S. could not be raised to the desired level, probably because of hydrolysis of the reagent. The highest D.S. obtained using this method was 0.265.

In the course of the above preparations, an attempt was made to increase the reactivity of the cellulose hydroxyls by soaking the cellulose overnight in strong alkali at a low temperature before reacting it with acrylamide. At the same time an actual reaction was run at the same low temperature (4°C.). The same amount of acrylamide was used in both runs but the product obtained from the cold reaction had three times the nitrogen content of the product from the heated reaction (0.18 D.S. vs. 0.049 D.S.).

Because of the relative success of the reaction run at 4°C., a series of these reactions was run extending the reaction time from sixteen hours to seventy-two hours and increasing the relative amounts of acrylamide used. In this manner, the D.S. of carbamoethyl cellulose was increased to 0.48 D.S. as carbamoethyl and 0.30 D.S. as carboxyethyl, a total of 0.78 D.S. The results obtained from the reaction were fairly definite for any particular set of reaction conditions. In four, essentially replicate preparations the measured

nitrogen content agreed within 0.5% nitrogen, about 15% of the measured value. (see Experiments 267B, 302A, 302B, and 309 in Table II Appendix IA).

The reactions showed an over-all tendency to give higher D.S. with higher acrylamide ratios. Increasing the sodium hydroxide concentration or the reaction temperature decreased the nitrogen content and appeared to increase the carboxyl content of the products. These trends were consistent with those noted by Frick and co-workers (38).

The importance of hydrolysis of the acrylamide as a competing reaction in the carbamoethylation medium was brought out in preparations 284A and 284C. These two reactions differed only in the procedure used in forming the solution of acrylamide and sodium hydroxide prior to absorbing it in the cellulose. In 284A the acrylamide and sodium hydroxide were both weighed into a beaker and then the water was added. All of the acrylamide dissolved rapidly but the sodium hydroxide dissolved more slowly and the solution was heated for about ten minutes to dissolve it. Ammonia was evolved during this heating, indicating that considerable hydrolysis of acrylamide to acrylic acid had taken place. In 284C the sodium hydroxide was first dissolved in the water and then the acrylamide was dissolved with slight heating. Very little ammonia was evolved. This difference in the preparation of the reaction medium was the only difference between 284A and 284C, and yet the 284C product contained more than twice as much nitrogen as product 284A (1.39% vs. 0.59%) and more than twice as much carboxyl as 284A (1.868% vs. 0.739%). The ratios of the carbamoethyl D.S. to carboxyethyl D.S. were almost identical for both products. These results indicate that essentially all of the carboxyethyl groups arise from hydrolysis of the carbamoethyl groups rather than from the Michael reaction of acrylic acid. This is in agreement with the findings of Frick and co-workers (39) for the reactivity of acrylamide and acrylic acid in the Michael reaction with cotton.

The cold reaction method was used to prepare the product which was used through the rest of this study (see Appendix IA). The product was analyzed for nitrogen and carboxyl (for analytical techniques, see Appendix II) and found to be 0.48 D.S. as carbamoethyl and 0.30 D.S. as carboxyethyl, 0.78 D.S. total when calculated using the formula for two substituent groups (see Appendix VA).

The relatively high D.S. (0.5-0.8) carbamoethyl celluloses showed several unusual physical properties. When placed in water, the individual fibers dispersed readily and almost seemed to repel one another. In water they appeared highly swollen and seemed to be almost transparent. Addition of methanol to an aqueous slurry of fibers restored their opacity and greatly increased their tendency to flocculate. When an aqueous slurry was filtered, the filtered pad of fibers was quite translucent and felt quite rubbery. It resembled a homogeneous gel. Yet, when the pad was crushed or torn, the fibers were clearly evident. When the pad was dried in air or soaked in methanol, it became very tough and horny but, if the hard material was soaked in water, it could again be separated into individual fibers. No explanation of these phenomena was attempted.

PREPARATION OF OTHER CARBAMOETHYL DERIVATIVES

In order to aid in the identification of the various components of the carbamoethyl cellulose hydrolyzate, attempts were made to prepare several of the expected substituted glucoses by reacting acrylamide with glucose derivatives having only certain hydroxyl groups available for reaction.

A commercial sample of 1,2:5,6-di-O-isopropylidene-D-glucofuranose was carbamoethylated in a homogeneous reaction. This glucose derivative has only the 3-position hydroxyl group available for reaction and, therefore, should give

only the 3-substituted glucoses, i.e., 3-O-carbamoethyl-D-glucose and 3-O-carboxyethyl-D-glucose.

The reaction of 1,2:5,6-di-O-isopropylidene-D-glucofuranose and acrylamide was carried out in aqueous dioxane using sodium hydroxide as catalyst. After neutralization and concentration the whole mixture was subjected to hydrolysis in 1N sulfuric acid. The resulting product was a mixture of glucose and substituted glucose. When chromatographed in an acidic developer, the product showed three spots indicating the presence of three different materials. The slowest moving of the spots conformed to glucose precisely. The three components were then separated on heavy chromatographic paper. When these separated materials were rechromatographed in acidic developer, they appeared to be pure compounds moving at the same rate as before (R_g's 1.00, 1.34, 1.87). When they were chromatographed in a basic developer, they again appeared to be single compounds but had quite different R_g values (1.00, 0.71, 0.13).

The materials were subjected to a qualitative test for hydrolyzable nitrogen using Nessler reagent, as reported in Feigl (40) (see Appendix IIC). The material which moved fastest in acid medium and slowest in basic medium gave a negative test for nitrogen. The material which moved at the intermediate rate in both developers gave a positive test for nitrogen as did a control sample of ammonium chloride. On the basis of origin, chromatographic behavior in acidic and basic developers and nitrogen content, the material which moved fastest in acidic medium was considered to be 3-O-carboxyethyl-D-glucose and the material which moved at the intermediate speed, 3-O-carbamoethyl-D-glucose.

Methyl 4,6-O-benzylidene-α-D-glucopyranoside was synthesized according to the procedure of Evans and co-workers (41). This material was readily purified

by recrystallization from hot water. The initial attempts to react the material with acrylamide in purely aqueous, alkaline media gave no evidence of any reaction. Reactions using a mixture of dioxane and water also gave no indication of reaction. However, when a tetrahydrofuran-water mixture was used, the reacted mixture, upon complete hydrolysis showed the presence of small amounts of two materials other than glucose. When the reaction in the tetrahydrofuran medium was repeated using only about one-third the amount of solvent, the product, upon complete hydrolysis by the method used to hydrolyze the cellulose derivatives, showed four materials chromatographically (see Appendix IC).

The four materials were believed to be D-glucose, 2-O-carboxyethyl-D-glucose, 3-O-carboxyethyl-D-glucose and 2,3-di-O-carboxyethyl-D-glucose since these are the compounds which would be expected from the preparation and the strong hydrolysis used. The compound which moved slowest in the acidic developer and which appeared to be present in the smallest amount matched glucose. The fastest moving spot was considered to be 2,3-di-O-carboxyethyl-D-glucose, since the higher substitution should have the greater effect on the chromatographic movement of the compound. The second fastest moving material matched the 3-O-carboxyethyl-D-glucose prepared from 1,2:5,6-di-O-isopropylidene-D-glucofuranose and was considered identical with that material. By process of elimination the remaining material was indicated to be 2-O-carboxyethyl-D-glucose.

In this manner, chromatographic samples of 3-O-carbamoethyl-D-glucose, 2-O-carboxyethyl-D-glucose, 3-O-carboxyethyl-D-glucose and 2,3-di-O-carboxyethyl-D-glucose were made available for chromatographic comparisons. All of the products were obtained only as sirups.

HYDROLYSIS AND SEPARATION

CELLULOSE HYDROLYSIS

In order to study the substituent group distribution in the carbamoethyl cellulose it was first necessary to reduce the derivative to monomeric glucose units.

Samples of carbamoethyl cellulose were hydrolyzed according to the 72% sulfuric acid digestion procedure (see Appendix IA). When examined chromatographically, the hydrolyzates were found to be free of cellobiose and of significant amounts of any other sugar material which moved slower than glucose in acidic developers. From this information, it was concluded that the hydrolysis was complete and that all of the materials in the hydrolyzate were present as monomeric glucose units.

The yield of the quantitative hydrolysis of carbamoethyl cellulose, after thorough washing of the filter cake, was 96%. From this yield, it was concluded that the hydrolysis gave very little degradation of the hydrolyzed glucose units and that the mixture in the hydrolyzate was representative of the glucose units in the cellulose derivative.

The hydrolyzate could be chromatographically separated into glucose and three mixtures of materials using an acidic developer. Quantities of each of these materials were separated chromatographically on heavy paper. These materials were then tested for the presence of amide nitrogen using the Nessler reagent test (40) (see Appendix IIC). None of the materials gave a positive test. When the test was run on corresponding molar amounts of ammonium chloride and on 3-O-carbamoethyl-D-glucose, the results were clearly positive.

From this information, it was concluded that none of the components of the hydrolyzate contained hydrolyzable nitrogen and that, therefore, the hydrolysis of amide groups to carboxyl during the cellulose hydrolysis was complete.

The D.S. calculated from the mole fractions of the various components of the hydrolyzate was only very slightly higher than that calculated from the analyses of the cellulose derivative (0.80 vs. 0.78). This fact implies that there is no significant loss of substituent groups during the hydrolysis but rather, if anything, a slight preferential loss of unsubstituted glucose. The amount of this loss necessary to produce the indicated rise in D.S. is less than the error of the method of measurement.

From all of this information, it was concluded that the hydrolysis procedure completely hydrolyzed the carbamoethyl cellulose to a mixture of glucose and carboxyethyl glucoses only, without significant loss of substituent groups or of any individual components of the hydrolysis mixture.

SEPARATION OF SUBSTITUTED GLUCOSES

The hydrolyzates of carbamoethyl cellulose appeared to be satisfactorily separated by paper chromatography. Several different reagents were used to detect the substituted glucoses (see Appendix IIIA). An ether solution of aniline and chloroacetic acid was used on the chromatograms of cellulose hydrolyzates because this reagent gave strong, distinctive colors with the individual components of the separable groups.

A large variety of chromatographic developers was tried in an effort to get a good resolution of the components of the carbamoethyl cellulose hydrolyzates. When an acidic developer was used, the carboxyethyl glucoses were moved ahead of

glucose and fairly well separated, but when a basic developer was used, the acidic components of the hydrolyzate were held back and tended to streak badly.

After comparison of the various results obtained, it was concluded that the best separation was obtained with the solvent system, 9:2:2, ethyl acetate: acetic acid: water. This system separated carbamoethyl cellulose hydrolyzate into glucose and three groups of substituted glucoses (for details of the various separations see Appendix IIIA).

A cellulose powder column was tried in an attempt to separate larger amounts of materials but it showed no advantage over direct separation on Whatman 3 MM paper. (see Appendix IIIB).

The result of these chromatographic investigations was that the carbamoethyl cellulose hydrolyzate could be separated completely into four groups of materials. They were designated, in order of increasing R_g, as glucose, C mixture, B mixture and A. The glucose material chromatographically matched known glucose perfectly. The C mixture consisted of three materials: C_3 which gave a large red-brown spot at R_g 1.60, C_2 which gave a moderate bright orange spot at R_g 1.75, and C_1 which gave a light gray spot at R_g 1.87. These three spots were overlapping. The B mixture consisted of two materials: B_2 which gave an orange spot at R_g 2.33, and B_1 which gave a gray-brown spot at R_g 2.49 adjacent to B_2. The A material appeared to consist of only one, faint spot. Thus, the hydrolyzate of carbamoethyl cellulose contained seven materials which could be separated into four groups. Milligram amounts of each of these groups were separated for further work by heavy paper chromatography.

IDENTIFICATION OF THE SEPARATED GROUPS

Because the separable materials were mixtures rather than single compounds, it was necessary to show what the composition of each group was. It had already been shown that the groups of materials were free from amide and could be considered to consist only of glucose and carboxyethyl glucoses. Among the various groups there were at least six of the seven possible carboxyethyl glucoses. There was good reason to suspect that the groups were separated according to D.S., that is, the unsubstituted, the monosubstituted, the disubstituted and the trisubstituted glucose, but further identification was needed to be sure.

There were three arguments used to characterize the various separated groups of materials. The first was direct analysis, the comparison of the carboxyl and aldehyde content of each group. The second was comparison to compounds of known structure. The third was a comparison of the chromatographic behavior of the series with that of other series.

An initial attempt was made to measure the equivalent weight of the C group of materials. A thoroughly dried sample was titrated potentiometrically with dilute sodium hydroxide but a very indefinite end-point was obtained. This behavior may be attributable to partial lactonization of the carboxyethyl glucoses.

Because a satisfactory method had been worked out for measuring the amount of aldehyde unit in separated groups, it was decided to attempt to measure the carboxyl content and the reducing unit content of a separated material on the same sample. The ratio of these two measures would be a measure of the D.S. of the group separated and, therefore, of its uniformity of substitution level. The

samples were subjected to titration with 0.02N sodium hydroxide, brought to a neutral pH and then subjected to alkaline iodine oxidation (for methods, see Appendix IV). Preliminary work with weighed samples of galacturonic acid and glucuronic acid gave good results; the COOH/CHO for galacturonic acid was 1.02 and for glucuronic acid it was 0.99. The calculated weights of material from duplicate determinations agreed within 3% for the acid titrations and within 2% for the iodine oxidations. The weights were within 4% of the experimental weights for the glucuronic acid titrations and oxidations but were about 12% low for the galacturonic acid in both the titrations and the oxidations. These low values appear to be attributable to the relatively crude sample of galacturonic acid, especially in view of the reproducibility and the agreement of the analyses. The method of consecutive titration and oxidation was considered satisfactory for this study.

Consecutive titrations and oxidations were then run on the groups of materials after they had been separated by quantitative paper chromatography. The separated, eluted samples were each treated with IR-120 ion exchange resin in the free acid form. The samples were titrated conductimetrically. The conductimetric titration was used because any acid introduced during the resin treatment probably would be a strong acid with high conductance. Hence, in the plot of conductance vs. volume of sodium hydroxide added, there would be regions of different slopes for the strong acid and for the carboxyethyl glucoses. A set of four samples was run, treating the eluted samples with resin. Two samples were titrated potentiometrically, and two were titrated conductimetrically. The potentiometric samples gave high carboxyl values and COOH/CHO ratios. The conductimetric samples showed the expected behavior, that is, the plot of conductance vs. volume of sodium hydroxide added showed two regions of different

slope besides the final sodium hydroxide slope. A plot showing the typical regions is found in Fig. 1, p. 19. The total volume of sodium hydroxide consumed agreed well with the amount consumed in the potentiometric samples. Equivalents of acid and COOH/CHO ratios were also calculated for the second increment of the curve, that is, using the volume of sodium hydroxide corresponding only to the range for the gradually rising conductance. These ratios were found to be 0.036, 0.91, 2.06 and 2.8 for glucose, C mixture, B mixture, and A mixture, respectively, and were taken as sufficient evidence that the degrees of substitution of the glucose, C mixture, B mixture, and A mixture were, in fact, zero, one, two, and three, respectively.

The second argument for the identification of the separated materials is a comparison with known materials. The 3-O-carbamoethyl-D-glucose and 3-O-carboxyethyl-D-glucose previously described were compared to the whole carbamoethyl cellulose hydrolyzate chromatographically. The 3-O-carboxyethyl-D-glucose corresponded precisely in both color and position with the weak, light gray C_1 spot, the fastest moving spot in the C mixture. No spot was found to correspond to that of the 3-O-carbamoethyl-D-glucose. The C_1 material, though not characterized, was, therefore, assumed to be 3-O-carboxyethyl-D-glucose.

Similarly, the 2-O-carboxyethyl-D-glucose, 3-O-carboxyethyl-D-glucose and 2,3-di-O-carboxyethyl-D-glucose described above were compared chromatographically with the 3-O-carboxyethyl-D-glucose prepared from 1,2:5,6-di-O-isopropylidene-D-glucose and with the whole hydrolyzate of carbamoethyl cellulose (see Fig. 2). The light gray spots of the 3-O-carboxyethyl-D-glucoses were again found to correspond precisely in both color and position. The slightly slower moving, orange spot of 2-O-carboxyethyl-D-glucose corresponded exactly in color and position to the material C_2. The faster moving, disubstituted glucose spot fell

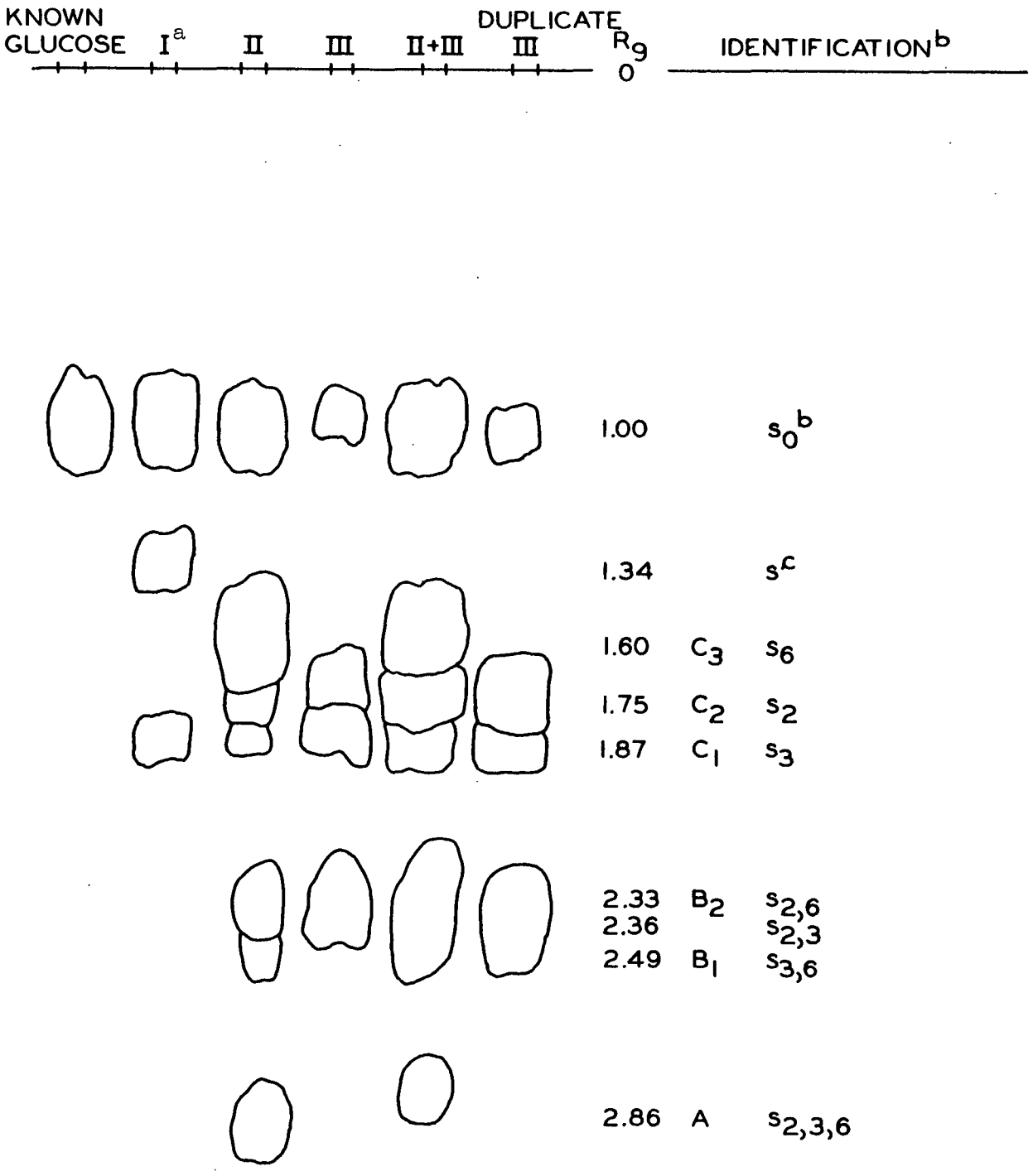

Figure 2. Chromatographic Comparison of Separated Materials

[a] I = Hydrolyzate of carbamoethylated 1,2:5,6-di-O-isopropylidene-D-glucose
II = Hydrolyzate of carbamoethyl cellulose
III = Hydrolyzate of carbamoethylated methyl 4,6-O-benzylidene-α-D-glucopyranoside

[b] S_i = Carboxyethyl glucose substituted only in the i position

[c] 3-O-Carbamoethyl-O-glucose

in almost the same position as the two spots of the B mixture but did not quite match either of them in color or in position. When a mixture of carbamoethyl cellulose hydrolyzate and the products of the carbamoethylation of methyl 4,6-O-benzylidene-α-D-glucopyranoside was chromatographed the C_1 and C_2 spots were increased in intensity, the C_3 and the A spots were essentially unchanged and the spots of the B mixture were rendered less distinct and the B mixture spot as a whole appeared slightly larger.

The third argument is the comparison of the chromatographic behavior of the components of the hydrolyzate with those of similar sets of materials. Whenever a chromatographic developer is used which moves the substituted glucoses faster than glucose itself, the order of the materials separated from a complete mixture has been glucose, 6-O-substituted glucose, 2-O-substituted glucose, 3-O-substituted glucose, 2,6-di-O-substituted glucose, 3,6-di-O-substituted glucose, 2,3-di-O-substituted glucose, and 2,3,6-tri-O-substituted glucose. This order has been found for methyl, ethyl, hydroxyethyl, and carboxymethyl glucoses in a variety of solvents (see Table I). From this general behavior for substituted glucoses, it can be assumed that the carboxyethyl glucoses will fall in the same order for solvent systems which move them faster than glucose. In all of the solvents tried which moved the carboxyethyl glucoses ahead, only the relative separation was affected and not the order of the materials.

From these three arguments, the components of a carbamoethyl cellulose hydrolyzate can be identified. From its very low COOH/CHO ratio and from the agreement of its chromatographic behavior with that of known glucose, the material called glucose in the separation can be safely assumed to be glucose. From the COOH/CHO ratio of the C mixture, its components can be identified as monosubstituted glucoses. By comparison with the carboxyethylated products

TABLE I

SEPARATION OF THE SUBSTITUTED GLUCOSES FROM CELLULOSE DERIVATIVES

Cellulose Derivative	Solvent System	R_g Values of Substituted Glucoses								
		S_0	S_6	S_2	S_3	S_{26}	S_{36}	S_{23}	S_{236}	Reference
Hydroxyethyl cellulose	n-BuOH sat. w/HOH	1.0	1.33	1.89	2.00	2.22	2.33	2.67	3.11	(15)
	n-BuOH sat. w/HOH	1.0	1.30	1.81	1.94	1.93	2.22			(14)
	5:4:1 BuOH:EtOH:HOH	1.0	1.24	1.48	1.58	1.84	1.98	2.05	3.10	(3)
	9:2:2 EtOAc:HOAc:HOH	1.0	1.42	2.36	2.80					(14)
	3:1:1 EtOAc:HOAc:HOH	1.0	1.45	1.70	1.83	2.16	2.25	2.36	3.40	(3)
Ethyl cellulose	3:1:3 EtOAc:HOAc:HOH	1.0	3.43	3.64	3.85					(42)
	Methyl ethyl ketone sat. w/HOH	1.0	5.7	6.3	6.5	23	23	27		(9)
Methyl cellulose	Methyl ethyl ketone sat. w/HOH	1.0	2.85	3.05	3.30	10	11	14		(10)
Carboxymethyl cellulose	12:1:12 UP EtOAc:HOAc:HOH	1.0	1.3	2.7	4.0	4.1	5.1	5.7	7.2	(3)
Carbamoethyl cellulose	9:2:2 EtOAc:HOAc:HOH	1.0	1.7	2.0	2.3	3.0	3.3		4.5	This Work

from the blocked glucoses, C_1 is considered to be 3-O-carboxyethyl-D-glucose and C_2 to be 2-O-carboxyethyl-D-glucose. By elimination and by comparison with the usual order of substituted glucoses upon chromatographic separation, C_3 is considered to be 6-O-carboxyethyl-D-glucose. From the COOH/CHO ratio of the B mixture, its components are identified as disubstituted glucoses. Since the spot for 6-O-carboxyethyl-D-glucose is so much larger than that for the 2- and the 3-substituted glucoses, the amount of disubstituted glucose unsubstituted in the 6-position (i.e., the amount of 2,3-disubstituted glucose) should be quite small. For this reason and because of the usual order of separated glucoses, B_1 and B_2 are tentatively identified as 3,6-di-O-carboxyethyl-D-glucose and 2,6-di-O-carboxyethyl-D-glucose, respectively. No spot for 2,3-di-O-carboxyethyl-D-glucose was found in the chromatogram of the cellulose derivative hydrolyzate although its R_g value would have placed it in the B mixture. From the chromatograms in Fig. 2, particularly that of the mixture of whole hydrolyzate and the hydrolyzed methyl 4,5-O-benzylidene-α-D-glucopyranoside derivative, it is evident that any 2,3-disubstituted glucose is present in very small amounts and in the chromatographic separation is to be found in the B mixture. By COOH/CHO ratios, by ordinary order of substituted glucoses and by the process of elimination the A material is considered to be 2,3,6-tri-O-carboxyethyl-D-glucose. Thus, all of the separated materials are identified with reasonable confidence.

QUANTITATIVE ESTIMATION OF AMOUNTS OF MATERIAL

The final experimental step is the measurement of the amounts of the various components of the hydrolyzate of the derivative. The final analytical procedure consisted of quantitative hydrolysis and neutralization, quantitative separation on heavy chromatographic paper, elution with water, titration with sodium hydroxide and oxidation with alkaline hypoiodate (see Appendix IVB).

The analytical procedures were first tried on weighed amounts of known materials, glucose, glucuronic acid, and galacturonic acid. When washed sheets were used, the amounts recovered and measured were quantitative and the blanks were essentially zero. It was felt that the blank values were less than the experimental error of the method itself and, therefore, no blank corrections were made. (see Appendix IVA).

The procedure was then applied to hydrolyzates of carbamoethyl cellulose. Samples of known weight were chromatographed on heavy paper using 9:2:2 ethyl acetate: acetic acid: water developer. The materials were then cut out and eluted. The samples were titrated with dilute alkali and oxidized with alkaline hypoiodite. The values were then used to calculate the mole fraction of each component and the ratio of carboxyl to aldehyde groups of each material. As described above, the carboxyl-to-aldehyde ratio did not give reasonable results until a conductimetric titration method was used. (see Table VII, Appendix IVC, p. 68). The mole fractions calculated showed fairly good reproducibility. The mole fractions calculated for the various materials were: glucose 0.36, C mixture 0.48, B mixture 0.16, A material 0.006. (see Table VIII, Appendix IVC, p. 69). These values were then used in the Spurlin equations for the calculation of the relative equilibrium constants.

APPLICATION OF SPURLIN'S EQUATIONS

Spurlin's statistical treatment for the calculation of the relative reaction constants was used to calculate the relative equilibrium constants in this study. In order to apply Spurlin's equations, the system must fit the assumptions that were made in developing these equations. (see p. 2-3). The particular system studied here must also meet certain other requisites.

The first of these is that the substituent distribution is, in fact, an equilibrium-controlled distribution. The Michael addition reaction is a very general reaction of compounds having an activated ethylene group with those possessing a labile hydrogen atom (43). Acrylonitrile and acrylamide are typical of the former compounds. It has been well established that the cyanoethylation of alcohols is a reversible reaction proceeding by the nucleophilic attack of the alkoxide ion on the vinyl group (18, 19). By analogy the same mechanism is attributed to the carbamoethylation of alcohols including cellulose, and carbamoethylation is therefore considered to be a reversible reaction.

In order to show that the carbamoethylation had in fact proceeded to such an extent that the distribution would be equilibrium-controlled, a series of carbamoethylations was run. The preparations were begun simultaneously and terminated after various reaction times from eight hours to ninety-six hours. A plot of D.S. vs. reaction time for a reversible reaction with excess reagent would be expected to show an initial rapid rise and a gradual leveling off toward an equilibrium level which would be less than total reaction, that is, less than 3 D.S. In the initial, rapidly rising region, the rate of the forward reaction will be the dominant or controlling influence on the substituent distribution. In the later, nearly level region, the reverse rate becomes important and the true equilibrium situation is approached. Precisely this behavior was found in the D.S. vs. time of reaction plot for the experimental data (see Fig. 3). After seventy-two hours the curve has essentially flattened out into the region of complete equilibrium control. In order to show the importance of the reverse rate in this region, the relative forward and reverse rates were estimated. The initial rate of substitution was estimated from the initial slope of the curve to be 0.20 D.S. units per hour, and from this value and from the rate expression,

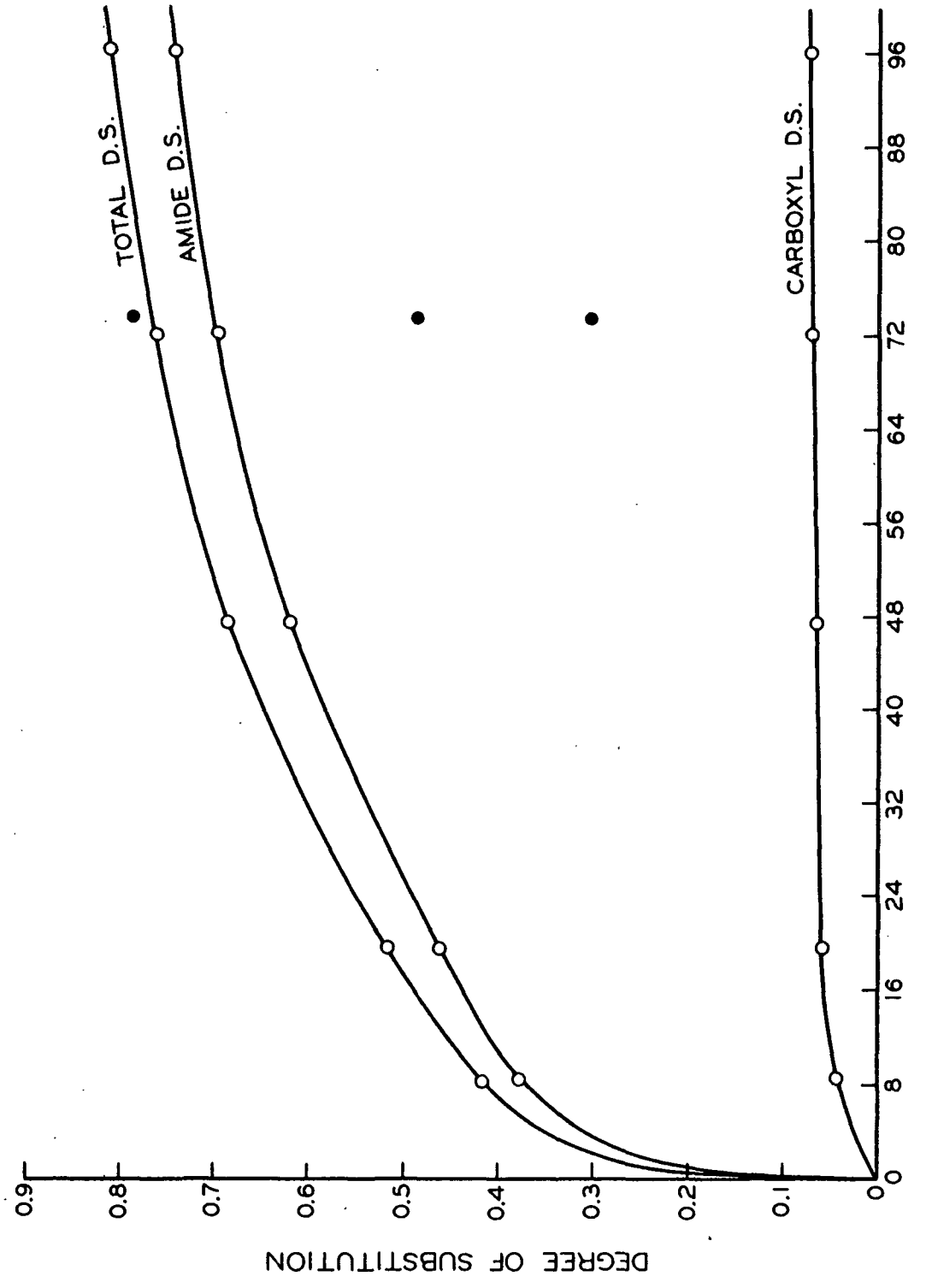

Figure 3. Plot of D.S. vs. Reaction Time for Carbamoethylation at 4°C.

$\frac{d\ D.S.}{dt} = k$ [ROH] [Acrylamide], the forward rate at seventy-two hours was calculated to be 0.142 D.S. per hour. The actual rate, estimated from the slope of the curve, was 0.0022 D.S. per hour. The reverse rate would then be -0.140 D.S. per hour or about ninety-eight per cent of the forward rate. On the basis of this information the reaction was considered to give an essentially equilibrium-controlled distribution of substituent groups.

The requirement that the hydroxyl groups in the cellulose were completely accessible was considered to be satisfied on the basis of two facts. First, the reactions were under strong swelling conditions throughout the reaction time. Croon, using a similar swelling procedure followed by reaction in nonaqueous, nonswelling solvents, found complete accessibility of the cellulose hydroxyls to methyl chloride (8) and sodium chloroacetate (3). Second, limited accessibility in reactions in swelling media is manifest by a reduced rate of reaction in the inaccessible regions. In an equilibrium reaction, all of the hydroxyl groups should reach the same ultimate equilibrium regardless of whether they were substituted by a fast, kinetically controlled reaction or a slow, diffusion-controlled process. For these reasons the carbamoethylation of cellulose was considered to proceed with complete accessibility of all of the cellulose hydroxyl groups.

A further requisite is that essentially all of the substitution comes from the reaction of acrylamide, that is, the reaction of acrylic acid with cellulose is negligible. This was considered to be satisfied on the basis of two pieces of evidence. In the course of their survey of possible reagents for modification of cellulose by the Michael reaction, Frick and co-workers (39) got at least seven times the amount of substitution with acrylamide as with acrylic acid under conditions that were similar except that a higher temperature was used for the

acrylic acid reaction. In the course of this study, two reactions were run which were identical except that in one a fair portion of the acrylamide was hydrolyzed to acrylic acid. In these reactions, 284A and 284C, which were discussed earlier, (see p. 21), the D.S. of the product from the unhydrolyzed reagent, 284C, was three times the D.S. of the hydrolyzed reagent product, 284A. This indicates that the acrylic groups in reaction 284A, though present at the same concentration as in reaction 284C, reacted to a considerably lesser extent. The ratio of carboxyl D.S. to amide D.S. was the same for both products. This indicates that the carboxyethyl groups arose from hydrolysis of the carbamoethyl groups rather than from reaction of acrylic acid. On the basis of this information, the substitution reaction was assumed to be a pure carbamoethylation and the distribution was considered to be controlled by a single, reversible reaction.

Inasmuch as substitution proceeds by a single, reversible reaction, since the reaction has, in fact, proceeded to equilibrium-controlled conditions, and since the hydroxyls of the cellulose may be considered to be completely accessible, Spurlin's equations for the distribution of substituent groups in an equilibrium-controlled reaction can be applied to the system studied.

CALCULATION OF THE REACTION CONSTANTS

To calculate the relative equilibrium constants for the three hydroxyls in cellulose, the Spurlin equations were rearranged and solved for the AK values (see Appendix VB). The AK values are the products of the acrylamide concentration and the equilibrium constant. The result was a cubic equation in AK. Because of the symmetry of the original equations, the three roots of this cubic are the AK values for the three different hydroxyls.

This equation was solved by a trial-and-error method for the various sets of analyses. The resulting AK values were 0.0452, 0.407, and 0.868 or, on a relative basis, 1.0, 9.0, and 19.2. Figure 4 shows a graph of the value of F(AK), the cubic function, against AK, the relative equilibrium constant, for the analysis set VIII.

The graphic representation brings out clearly one of the greatest difficulties of the analytical procedure. The AK values are extremely sensitive functions of the c_j values, the mole fractions of the various levels of substitution. This is brought out further by Fig. 5, which shows the effect of a moderate change in each of the c_j values. This variability reduces the accuracy with which the numerical values of the reaction constants are known but it still allows the assignment of a significant numerical ranking. Thus, the AK values are not known more accurately than ± 10%.

Since the 6-O-carboxyethyl-D-glucose apparently is present in the largest amount of the three monosubstituted glucoses in the cellulose hydrolyzate, the largest of the three AK values was assigned to that position and called AK_6. Similarly, the smallest value was assigned to AK_3 since the 3-O-carboxyethyl-D-glucose is present in the smallest amount. Thus, the relative equilibrium constants for the three hydroxyls are $AK_2:AK_3:AK_6 = 9:1:19$.

The high relative reactivity of the 6-position hydroxyl is considerably different from the general behavior of almost all etherification reactions except hydroxyethylation (14), but it is consistent with the fact that, for cyanoethylation, the extent of reaction is greater for primary alcohols than for secondary alcohols (18). In most reactions, the 2-position hydroxyl is the most reactive; however, these substituent distributions are rate-controlled rather than equilibrium-controlled. Feit and Zilkha (19) found that the rate of cyanoethylation of isopropyl

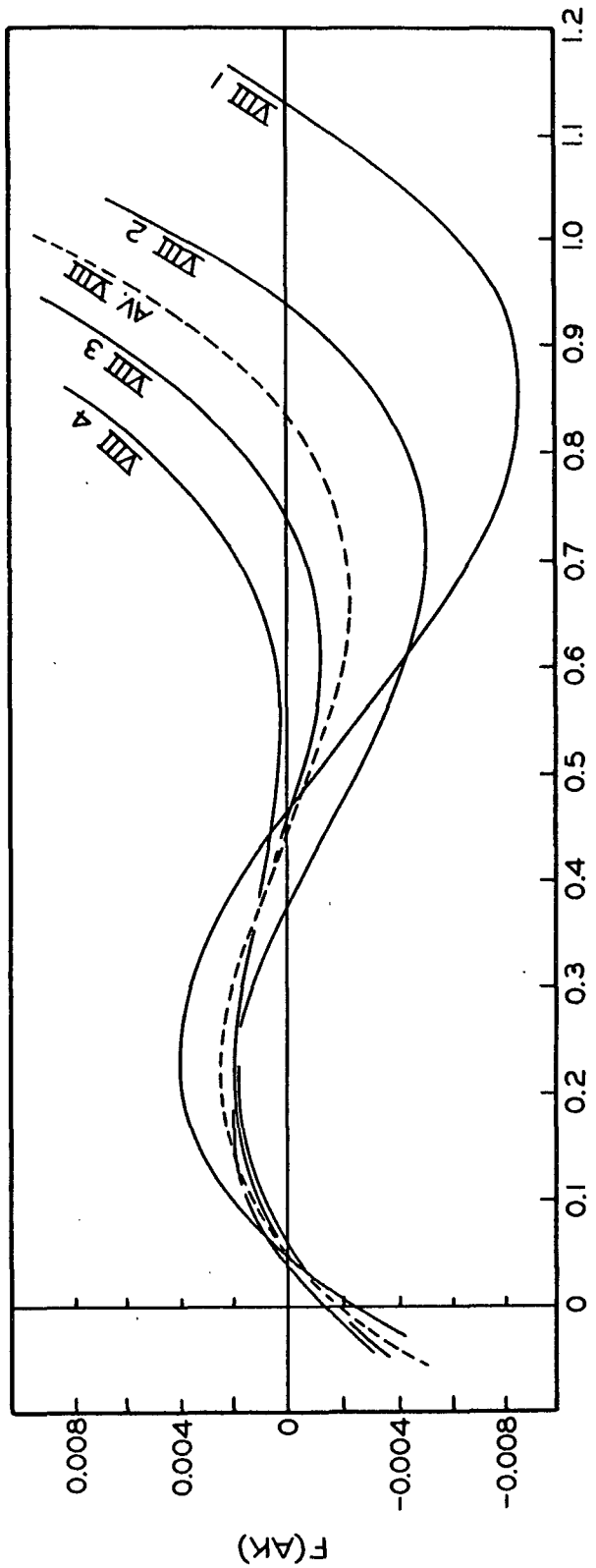

Figure 4. Plot of F(AK), The Cubic Function Developed from Spurlin's Equations, Over a Range of Values for AK, the Relative Equilibrium Constant, Using the Experimentally Measured Mole Fractions

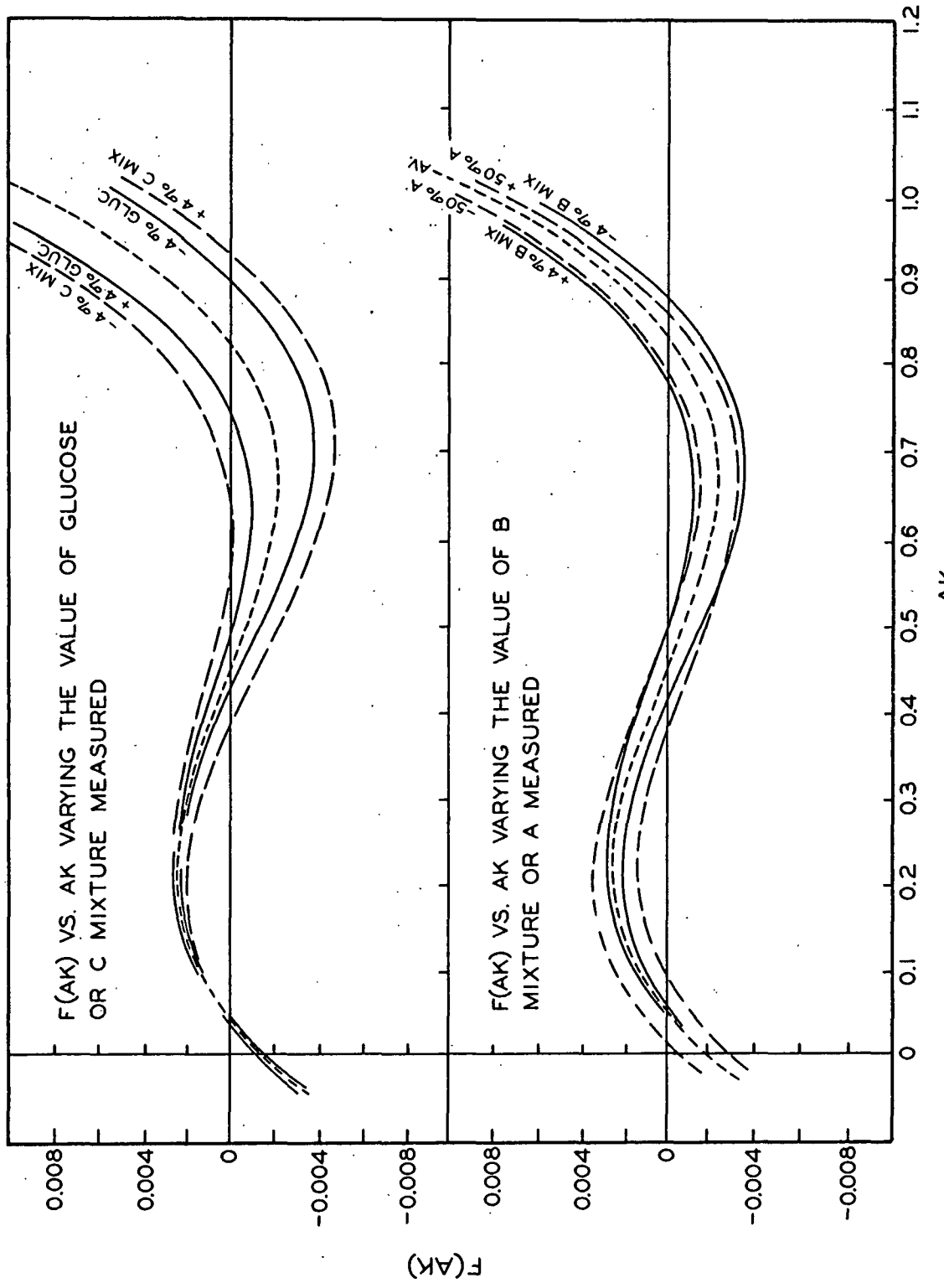

Figure 5. Plot of F(AK) vs. AK Varying the Amounts of Materials Measured

alcohol was much greater than that of n-propyl alcohol or of ethanol. From this it can be conjectured that under rate-controlled conditions, i.e., in the very early stages of reaction, cyanoethylated and carbamoethylated cellulose might show an entirely different substituent distribution in which the 2-position substitution would predominate in a manner similar to those of most cellulose ethers. However, some very recent findings conflict with this suggestion. Garegg and co-workers (44) studied the cyanoethylation of 2-tetrahydropyranyl-α-D-glucosides and found the relative reaction rates for the 2-, 3-, and 6-position hydroxyls to be 3, 1, and 8, respectively.

CONCLUSIONS

From this study it can be concluded that the carbamoethylation of cellulose does proceed by a reversible, Michael reaction, that the distribution of carbamoethyl groups is an equilibrium-controlled distribution in which substitution on the primary hydroxyl group predominates, and that the relative equilibrium constants are $K_2:K_3:K_6 = 9:1:19$.

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APPENDIX I

PREPARATION AND HYDROLYSIS OF DERIVATIVES

The cellulose used in this work was acetate-grade cotton linters, Buckeye type 1A500. The acrylamide was Eastman Kodak EK 5521. The methyl-α-D-glucopyranoside was Eastman Kodak EKP 658. The 1,2:5,6-di-O-isopropylideneglucofuranose was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. All of the other chemicals were ordinary, high-grade laboratory chemicals.

A. CARBAMOETHYL CELLULOSE

The following reaction is typical of the high temperature or oven reactions used in the first stages of this work. It follows the procedure used by Frick and co-workers (38).

Sodium hydroxide, 1.92 g., was dissolved in distilled water, 6.0 ml. Acrylamide, 6.80 g., and hot, distilled water, 4 ml., were added. The solution was then kneaded into the cellulose, 1.0 g. The mixture was placed in an oven at 110°C. for one-hundred minutes. The reaction mixture was then diluted with distilled water, 50 ml., and filtered. The filter pad was washed slowly with 1N sulfuric acid and then with distilled water until the filtrate came through neutral. The product was dried in air.

The product, Pr 284C, analyzed 1.39% nitrogen and 1.87% carboxyl. These values correspond to 0.230 D.S. as amide and 0.075 D.S. as carboxyl, a total D.S. of 0.305.

The following procedure is typical of the low-temperature carbamoethylations. However, it is a larger scale preparation than the rest.

A solution of 100 g. NaOH in 400 ml. distilled water was kneaded into 50.0 g. acetate-grade cotton linters. The mixture was cooled in a refrigerator at 4°C. for thirty minutes. A solution of 340 g. acrylamide in 125 ml. distilled water, dissolved with heating, was added to the cellulose. The mixture was kneaded thoroughly and placed in the refrigerator at 4°C. for seventy-three hours. The mixture was stirred occasionally.

The product was slurried in 1 l. methanol and filtered. The filter pad was reslurried in 1 l. 1N H_2SO_4 and allowed to soak overnight. The product was then filtered and washed with 500 ml. methanol. The product was reslurried in 1 l. methanol, stirred for one hour and filtered. It was then reslurried in 1500 ml. methanol, stirred for one hour and filtered. The product was spread to dry in air.

The product was a fibrous, white material containing 6.68% moisture, 0.72% ash, 3.10% nitrogen and 6.21% carboxyl. These analyses indicate a D.S. as amide of 0.48 and a D.S. as acid of 0.30, a total D.S. of 0.78. For the calculation of D.S. see Appendix VA. The product showed the highly swollen appearance and the other physical properties generally shown by the high D.S. derivatives.

Table II lists the reaction conditions and analyses of the various carbamoethyl celluloses prepared in this study.

The cellulose derivatives were hydrolyzed by the 72% sulfuric acid method essentially as described by Saeman and co-workers (28). The following procedure is typical.

A 2.001-g. sample of carbamoethyl cellulose was wet with 10 ml. distilled water. Then 50 ml. 72% sulfuric acid was added and the mixture was allowed to

TABLE II

REACTION CONDITIONS AND ANALYSES FOR CARBAMOETHYL CELLULOSES

Sample No.	Moles of AnGU[a]	Acryl-amide, mole/ mole AnGU	Acryl NaOH, mole/ mole AnGU	H_2O, ml./mole AnGU	Dioxane, ml./mole AnGU	Time, hr.	Temp., °C.	N, %	COOH, %	Ash, %	H_2O, %	D.S.		
												Amide	Acid	Total
7A	0.246	4.07	0.638	506	0	0.15	110							
17A	0.0370	1.80	1.35	2702	0	16.5	45							
23	0.00617	4.58	0.0973	486	0	2.	96	0.466			2.21	0.0554		
25A	0.00617	4.20	0.324	324	0	0.25	125	1.51			2.21	0.190		
25B	0.00617	4.20	4.05	1459	0	0.25	125							
98A	0.0123	1.94	0.827	892	0	0.28	130	0.455			2.40	0.0539		
245A	0.0123	3.88	8.11	1297	0	16.8	4	1.33		5.91	6.90	0.177		
245B	0.0123	3.88	3.79	769	324	0.42	85	0.406			7.84	0.0485		
254A	0.0247	3.88	3.34	696	0	0.42	110	1.87		8.56	6.42	0.265		
267A	0.0123	7.76	8.11	1459	0	71.2	4	2.05	6.41	0.11	7.70	0.297	0.277	0.574
267B	0.0123	15.51	8.11	1762	0	71.8	4	2.96	5.71	0.14	6.30	0.452	0.271	0.723
284A	0.00617	15.5	7.78	1621	0	1.67	110	0.612	0.739		2.96	0.0740	0.0278	0.102
284B	0.00617	15.5	0.0973	973	0	1.67	110	0.861		0.24	3.26	0.103		
284C	0.00617	15.5	7.78	1621	0	1.67	110	1.48	1.868		5.75	0.230	0.0752	0.305
292	0.0123	7.76	5.84	349	14187	3.0	70	0.604	8.57	0.27	6.16	0.0840	0.371	0.455
302A	0.00617	15.5	8.10	2107	0	71.8	4	3.16				0.435		
302B	0.00617	15.5	8.10	2107	0	71.8	4	3.26				0.452		
309	0.309	15.5	8.10	1699	0	73.2	4	3.10	6.21	0.89	4.88	0.483	0.301	0.784
717A	0.0617	7.86	8.10	1530	450	24.	4							
		7.86		90	450	24.	4							
		7.86		90	450	24.	4							
		7.86		90	450	24.	4	2.72	7.92		7.14	0.428	0.388	0.816
935A	0.00617	15.5	8.10	2107	0	8.5	4	2.74	0.959		9.1	0.375	0.0408	0.416
935B	0.00617	15.5	8.10	2107	0	19.5	4	3.24	1.261		8.1	0.460	0.0557	0.516
935C	0.00617	15.5	8.10	2107	0	47.4	4	4.12	1.319		8.5	0.619	0.0617	0.681
935D	0.00617	15.5	8.10	2107	0	72.0	4	4.48	1.414		7.9	0.691	0.0679	0.759
935E	0.00617	15.5	8.10	2107	0	96.0	4	4.70	1.441		8.5	0.737	0.0703	0.807

[a]Moles of anhydroglucose unit.

stand at room temperature for one hour with intermittent stirring. It was then diluted with 500 ml. distilled water and placed on a steam bath for four hours. The hydrolyzate was then neutralized with solid barium carbonate, filtered and concentrated to 25.0 ml. The yield of hydrolyzate was 1.915 g., 94.8% of the theoretical amount.

It was found to be necessary to wet the fibers before adding the 72% sulfuric acid. If this step was omitted, the sulfuric acid swelled the carbamoethyl cellulose into a gel on contact and formed pockets of dry fiber which were surrounded by the gel and were almost impossible to disperse completely. When the fibers were wet, they dissolved smoothly and easily to give a clear colorless solution.

B. CARBAMOETHYLATION OF 1,2:5,6-DI-O-ISOPROPYLIDENE-D-GLUCOSE

The following procedure was used to carbamoethylate 1,2:5,6-di-O-isopropylidene-D-glucose to obtain reference materials substituted in the 3-position.

Acrylamide, 4.40 g., and 1,2:5,6-di-O-isopropylidene-D-glucofuranose, 4.0 g., were dissolved in sodium hydroxide, 7.72 ml. of 0.1N, and dioxane, 15.4 ml. The mixture was allowed to react ten hours at 44°C. The mixture was then treated with IR-120 resin in free acid form, filtered and concentrated to a thin, yellow sirup.

The sirup was diluted with 25 ml. 1N sulfuric acid, allowed to stand at room temperature for three hours and then heated on a steam bath for fifteen minutes. The hydrolyzate was neutralized with barium carbonate, filtered and concentrated to about 15 ml.

When chromatographed in 9:2:2 ethyl acetate: acetic acid: water, the hydrolyzate showed the three expected spots of glucose, the material tentatively

identified as 3-O-carboxyethyl-D-glucose and 3-O-carbamoethyl-D-glucose. Small amounts of each material were separated on heavy paper and when rechromatographed appeared as single materials in both acidic and basic developers.

An attempt was made to prepare the phenylosazone of 3-O-carbamoethyl-D-glucose by warming the material with phenylhydrazine. The result was a dark yellow, apparently crystalline product which, after purfication, had a very light yellow appearance and melted at 200.5 - 201.5°C. with discoloration.

C. CARBAMOETHYLATION OF METHYL 4,6-O-BENZYLIDENE-α-D-GLUCOPYRANOSIDE

In order to prepare 2-O-, 3-O-, and 2,3-di-O-carboxyethyl-D-glucose, a sample of methyl 4,6-O-benzylidene-α-D-glucopyranoside was prepared according to the procedure of Evans and co-workers (41) and carbamoethylated in the following manner. Methyl 4,6-O-benzylidene-α-D-glucopyranoside, 0.5 g., and acrylamide, 1.08 g., were dissolved in tetrahydrofuran, 1.5 ml., and sodium hydroxide, 0.4 ml. 1.0N NaOH. The mixture was allowed to react forty-eight hours at 30°C. The mixture was then neutralized with IR-120, filtered and concentrated. The concentrated product was subjected to the 72% sulfuric acid digestion procedure. The neutralized, concentrated hydrolyzate, upon chromatographing in 9:2:2 ethyl acetate: acetic acid: water, showed four materials tentatively identified as D-glucose, 2-O-carboxyethyl-D-glucose, 3-O-carboxyethyl-D-glucose and 2,3-di-O-carboxyethyl-D-glucose.

APPENDIX II

ANALYSES

A. QUANTITATIVE NITROGEN ANALYSES

The nitrogen analyses were run by the Hengar Kjeldahl method using the procedure of the Analytical Group of The Institute of Paper Chemistry. The procedure was essentially identical with that of Henwood and Garey (45) except that the ammonia was distilled into 4% boric acid solution and then titrated with 0.05N hydrochloric acid using a mixed indicator of bromocresol green and methyl red. The calculated values for duplicate runs agreed within about 0.03% N. This procedure was used for all of the nitrogen analyses made in this study.

B. QUANTITATIVE CARBOXYL ANALYSES

The carboxyl analyses were run using the calcium acetate method as described by Davidson and Nevell (46) except that the cellulose was soaked in the calcium acetate solution for thirty minutes. The results duplicated within about two per cent of the value measured.

The silver nitrophenolate method of Davidson and Nevell (47) was used by the analytical section of the Institute in their analyses.

C. QUALITATIVE TEST FOR NITROGEN

The qualitative test for nitrogen was taken from Feigl's Spot Tests in Organic Analysis 1960 (40). The test was applied without modification to the 3-O-substituted glucoses, to the materials separated from a carbamoethyl cellulose hydrolyzate, and to an equivalent sample of ammonium chloride. In all cases the results of the test were unambiguous.

APPENDIX III

CHROMATOGRAPHY

A. PAPER CHROMATOGRAPHY

Paper chromatography was used almost exclusively as the separation technique of this work. In all cases, descending chromatography on sheets 24 in. long was used. Three different chromatographic papers were used in this study. Whatman No. 1 paper, a good, general grade of paper, proved quite satisfactory for the qualitative work. Schleicher and Schull paper, grade 598, a much faster sheet, was tried but it gave less satisfactory separations. For the preparative separations and the quantitative work, Whatman No. 3 MM was used. This paper is similar to Whatman No. 1 but is a thicker sheet and has a considerably greater capacity. It gave essentially the same separations as Whatman No. 1 but with larger amounts of materials. It was used in the preparative separations and in the quantitative chromatography.

In the course of this study, several different reagents were used to locate the chromatographed materials. A p-anisidine hydrochloride spray (48) was used to locate the reducing sugars at first, but it was replaced with an aniline and chloroacetic acid dip (49) when this latter reagent was shown to give distinguishing colors with the components of the carbamoethyl cellulose hydrolyzates. These colors were very distinctive when they first appeared, but, on further heating of the chromatogram, the colors gradually blended to a dull brown.

Two other reagents were used for the preparations of reference materials. A permanganate-periodate spray (50) was used to detect nonreducing sugar derivatives. A ferric hydroxamate spray (51) was tried in an effort to detect amides. However, no indication was obtained from amide-containing preparations or from known amides.

In an effort to find a chromatographic developer which would separate the materials obtained upon hydrolysis of carbamoethyl cellulose, a large number of solvent systems were tried. These could be grouped into three general classes: acidic developers, basic developers and neutral developers. The acidic developers were the most successful in separating the various carboxyethyl glucoses. These developers moved the substituted glucoses ahead of glucose and separated them into four groups of materials. The solvent ethyl acetate: acetic acid: water in the ratio, 9:2:2, gave the best separations obtained and was used in all of the subsequent work.

Basic developers using ethyl acetate, butyl acetate and butanol systems were tried. All gave essentially the same, poor separation in which the carboxyethyl glucoses were held back and highly streaked. The glucose spot, however, was usually well defined even though the other materials streaked through it.

One exception to this behavior was observed. The hydrolyzate of carbamoethylated 1,2:5,6-di-O-isopropylidene-D-glucofuranose, presumably a mixture of glucose, 3-O-carbamoethyl-D-glucose and 3-O-carboxyethyl-D-glucose, gave equally distinct spots in both acidic and basic developers. All other materials, the whole carbamoethyl cellulose hydrolyzates, the separated groups of materials, and the hydrolyzate of carbamoethylated methyl 4,6-O-benzylidene-α-D-glucopyranoside, gave highly streaked or smeared chromatograms with basic developers. No satisfactory explanation for this behavior has been found. It may be the result of mutual interference of the salts of the acids in the mixtures.

B. COLUMN CHROMATOGRAPHY

In order to separate relatively large amounts of material, a chromatographic column was used. A cellulose column and 9:2:2 developer were used in an attempt to get the same good separation obtained on paper.

A tapered glass column, 4.0 cm. in diameter at the base, 6.0 cm. in diameter at the top, and 25.5 cm. in depth was packed dry with 90 g. of thoroughly washed Whatman standard grade cellulose powder in 10-g. increments. The depth of the packing was 18.5 cm. The column was set up on a fraction cutter actuated by an interval timer.

The carbamoethyl cellulose hydrolyzate, 1.0 g. of material dissolved in 17 g. of water, was absorbed in 10 g. washed cellulose powder. Adding 17 ml. acetic acid and 85 ml. ethyl acetate brought the solvent ratio to 9:2:2. The cellulose powder was then in a pourable slurry.

The column was "pre-wet" by adding 150 ml. of pure 9:2:2 developer to the top of the column and allowing it to soak into the column. When the solvent was almost completely absorbed, the cellulose slurry containing the sample was poured on top of the column. A disk of filter paper was placed on top of the cellulose column to prevent disturbance of the bed during addition of solvent. The column was developed with 2 l. of 9:2:2 developer. The column was allowed to flow under gravity without restraint or constriction. Approximately 25-ml. fractions were taken.

Every eighth fraction was concentrated and monitored chromatographically. Where any two fractions had the same composition, these and all of the intervening fractions were combined. Where there was a difference in composition, the fraction in the middle of the interval, the fourth fraction, was monitored. The combination and remonitoring was continued until all fractions were combined into groups by composition.

The final fractions were all mixtures of the various groups of materials. Figure 6 shows a paper chromatogram of the various grouped fractions. In order to obtain pure materials, it was necessary to refractionate the materials obtained from the column on Whatman No. 3 MM paper. However, chromatographically pure materials were also obtained when the whole carbamoethyl cellulose hydrolyzate was separated directly on 3 MM paper. The column separation were therefore abandoned.

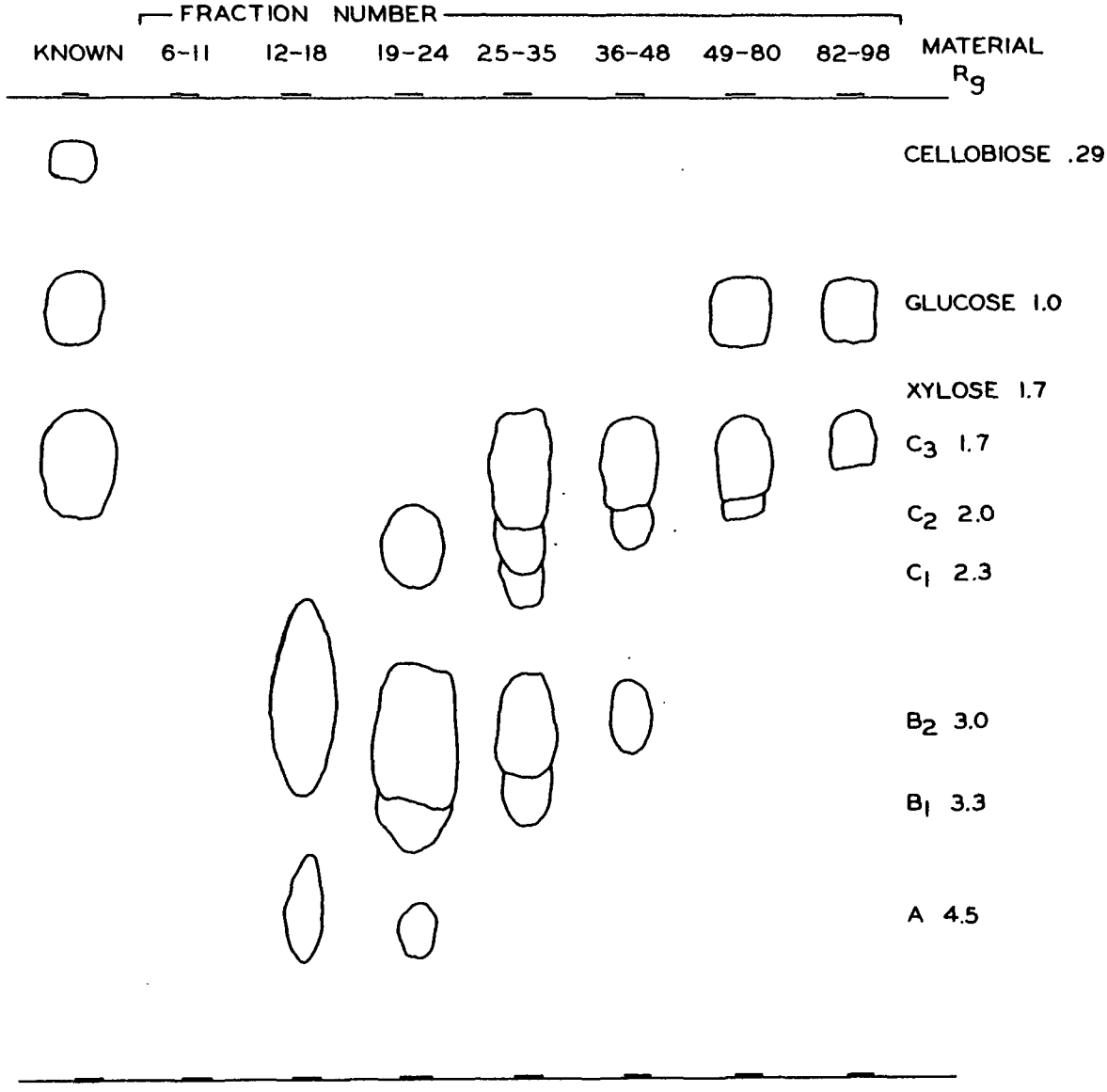

Figure 6. Chromatograms of Fraction Groups from Cellulose Column

APPENDIX IV

QUANTITATIVE SEPARATION AND MEASUREMENTS

A. INDIVIDUAL ANALYTICAL METHODS

In order to obtain data of reasonable confidence, it was necessary to show that each step of the ultimate analytical procedure was a quantitative operation. The first step, the total hydrolysis, was taken to be quantitative because of the essentially one hundred per cent yield of solids in the hydrolyzate.

The aldose determinations were run according to the method of Willstätter and Schudel (32) as described in U.S. Department of Commerce Circular C440 (52). To each sample was added about twice the volume of 0.1N iodine solution theoretically needed to oxidize the sample, from 4 ml. for glucose to 1 ml. for A material. At room temperature and with stirring, a volume of 0.1N sodium hydroxide equal to 1.5 times the volume of iodine solution was added over an interval of two to four minutes. After a total time of fifteen minutes, the samples were acidified with sulfuric acid and the excess iodine was titrated with 0.1N sodium thiosulfate solution using a starch indicator. Several sets of weighed samples were analyzed by this method. The calculated amounts of glucose agreed with the weighed samples within about five per cent. The data from the analyses are found in Table III.

In order to show that the chromatographic separation and elution was quantitative, known amounts of glucose were chromatographed, eluted, and oxidized. It was necessary to wash the sheets twice by developing them with distilled water in order to get good results. When this was done, the amount of glucose calculated was equal to the theoretical amount and the blank was essentially zero (see Table IV). It was found that a great deal of acid from the chromatographic

TABLE III

IODIMETRIC OXIDATION AND TITRATION OF WEIGHED SAMPLES

Analysis	Neutralization Wt.Fract.[a]	Neutralization Eq.Wt.	Theoret. Eq.Wt.	Oxidation Wt.Fract.[a]	Oxidation Eq.Wt.	Theoret. Eq.Wt.	COOH / CHO
527A Glucose	--	--	--	1.027		180.16	
B "	--	--	--	1.030		180.16	
C "	--	--	--	1.275		180.16	
D Blank	--	--	--	(.47 mg.)[b]			
530A Glucose	--	--	--	0.977		180.16	
B "	--	--	--	1.076		180.16	
C "	--	--	--	0.993		180.16	
D Blank	--	--	--	(.107 mg.)[b]			
537B Glucose	--	--	--	1.005	179.2	180.16	
C "	--	--	--	0.958	188.0	180.16	
D Blank	--	--	--	(.694 mg.)[b]			
549A Glucuronic A	0.932	208.4	194.14	1.048	185.2	194.14	0.889
B "	0.922	210.5	194.14	1.038	187.1	194.14	0.889
555A Glucuronic A	0.953	203.7	194.14	0.974	199.4	194.14	0.979
B "	0.978	198.5	194.14	0.974	199.3	194.14	1.001
C Galacturonic A	0.872	227.7	194.14	0.869	223.4	194.14	1.003
D "	0.893	217.3	194.14	0.850	228.3	194.14	1.050

[a] Wt. Fract. = calculated weight/weight sample.

[b] Calculated weight as glucose.

TABLE IV

QUANTITATIVE CHROMATOGRAPHY OF KNOWN GLUCOSE SAMPLES

Sheet	Sample	Wt. Sheet, g.	Glucose, mg.	mg. Glucose / g. sheet	Correction	Glucose Cor., mg.	Glucose Theoret., mg.	Wt. Fract. Uncor.	Wt. Fract. Cor.
611	Glucose 1	1.7329	22.380		-7.88296	14.497	24.000	0.9325	0.6040
Unwashed A	Blank 1	1.6014	10.062	6.283			0.00		
	Blank 2	1.7304	4.873	2.8161			0.00		
611	Glucose 2	1.6848	23.643		+0.56303	24.2060	24.00	0.9851	1.0086
Unwashed B	Blank 3	1.6956	-1.6695	-.9846			0.00		
	Blank 4	1.5692	0.4962	.3162			0.00		
611	Glucose 3	1.8061	23.9592		-0.55883	23.4004	24.00	0.9983	0.9750
1 Washed A	Blank 5	1.7049	0.7670	.4499			0.00		
	Blank 6	1.8697	0.3158	.1689			0.00		
611	Glucose 4(1/2)	2.0911	12.7692		-0.22164	12.4476	12.00	1.0641	1.0456
1 Washed B	Blank 7	1.8653							
	Blank 8	1.9152	0.4060	.2120			0.00		
611	Glucose 5	2.0725	22.7410		+0.38555	23.1265	24.00	0.9475	0.9631
2 Washed A	Blank 9	1.4556	-0.2708	-.1860			0.00		
	Blank 10	1.3222							
611	Glucose 6	2.0362	23.3726		+0.90094	24.2735	24.00	0.9739	1.0114
2 Washed B	Blank 11	1.6975	-1.2183	-.7185			0.00		
	Blank 12	1.5084	-0.2511	-.1664			0.00		
611	Glucose 7	2.0233	23.5983		-0.41124	23.1871	24.00	0.9833	0.9661
3 Washed	Blank 13	1.7338	0.1353	.0780			0.00		
	Blank 14	1.5108	0.4963	.3285			0.00		
637	Glucose A	1.9932	23.869		-0.6317	23.237	24.00	0.9945	0.9682
Untreated	Blank A	1.9877	0.6316	.3178			0.00		
637	Glucose B	1.9932	24.062		-0.3158	23.746	24.00	1.0026	0.9894
Steamed	Blank B	1.6576	0.3158	.1905			0.00		
637	Glucose C	1.6098	16.063		-0.2268	15.836	16.00	1.004	0.9898
EtOac Wash	Blank C	1.6594	0.2268	.1376			0.00		
637	Blank D	0.00	0.04506				0.00		
H_2O Blanks	Blank E	0.00	-0.04518				0.00		
	Blank F	0.00	-0.04518				0.00		

developer remained in the chromatographic sheet after drying. It was necessary to remove this acid before an accurate titration of the separated sample could be made. This was accomplished by steaming the sheet for fifteen minutes after drying and before cutting of the guide strips. Analysis Set 637, Table IV shows that the sugar analysis was unaffected by the steaming procedure.

These methods were then combined into the procedure used to analyze the hydrolyzate of carbamoethyl cellulose.

B. OVER-ALL ANALYTICAL PROCEDURE

The following is the analytical procedure used to measure the amount of each of the groups of materials in order to calculate the mole fraction of each and ultimately to estimate the relative equilibrium constants.

A sample of carbamoethyl cellulose was hydrolyzed quantitatively using the 72% sulfuric acid digestion procedure of Saeman and co-workers (28) (see Appendix IA).

Aliquots of the neutralized, concentrated hydrolyzates were separated on Whatman No. 3 MM paper. The sheets were steamed, cut into sections according to the guide strips and eluted by the method of Saeman and co-workers (28). Sheets 30-cm. wide were used and about 50 mg. of total hydrolyzate solids were spotted per sheet in two sections along with a center and two edge guide strips. The sheets were developed in 9:2:2 ethyl acetate: acetic acid: water for about twelve hours and air dried. The sheets were steamed for fifteen minutes to remove acetic acid and then cut into sections containing the various groups of materials according to the guide strips. These sections were then eluted using pipets of about 1.0-ml. volume. At this point in the final procedures each sample was treated with IR-120 resin in the free acid form.

The separated samples were titrated with 0.02N sodium hydroxide using a microburet. At first, the end point was estimated potentiometrically. In the final procedure, however, it was determined conductimetrically. After the titration, the pH of each sample was carefully adjusted with sulfuric acid to between 6.5 and 7.0.

The aldose content was then measured according to the alkaline hypoiodite method.

C. CALCULATION OF DATA

From the data obtained in these analyses, the following values were calculated for each sample; the milliequivalents of aldehyde, the milliequivalents of acid, the milligrams of material, the carboxyl-to-aldehyde ratio and the mole fraction of the material. The following are the equations used and a typical example using analysis Series VIII C3.

$$\text{meq.CHO} = 1/2[(N\ I_2)(\text{Vol.}I_2)-(N\ \text{Thio})(\text{Vol.Thio})] \quad (1)$$

$$\text{meq.acid} = (N\ \text{NaOH})(\text{Vol.NaOH}) \quad (2)$$

$$\text{mg. CHO} = (\text{Eq.wt.})(\text{meq.CHO}) \quad (3)$$

$$\text{COOH/CHO} = \text{meq.acid/meq.CHO} \quad (4)$$

$$\text{Mole Fract.A} = \text{meq.CHO A/Total meq.CHO} \quad (5)$$

SERIES VIII C3

$$\text{meq.CHO} = 1/2[(0.09616)(8.000)-(0.09784)(3.695)] \quad (6)$$

$$= 0.2039 \text{ meq.CHO}$$

$$\text{meq.acid} = (0.02184)(8.750) \quad (7)$$

$$= 0.1912 \text{ meq.acid}$$

$$\text{mg.mono acid} = (252.2)(0.2039) \quad (8)$$

$$= 51.42 \text{ mg.mono acid}$$

$$COOH/CHO = 0.1912/0.2039 \quad (9)$$

$$= 0.948$$

$$\text{Mole Fract.C3} = 0.2039/0.4359 \quad (10)$$

$$= 0.468$$

Tables V through VIII show the actual values calculated for the various sets of analyses.

TABLE V

IODINE OXIDATION DATA FROM CARBAMOETHYL CELLULOSE ANALYSIS

Hydrolyzate	Set		Glucose[a]	C	B	A	Blank	Wt. Calc. / Wt. Theor.
Hyd 874A	VIII	1	0.1254	0.2054	0.0747	0.00270		
		2	0.1565	0.2117	0.0632	0.00185		
		3	0.1640	0.2039	0.0651	0.00283	0.00420	
		4	0.1863	0.2152	0.0688	0.00311	0.00567	
		Av.	0.1580	0.2090	0.0680	0.00262		
Hyd 701 50.70 mg.	VII	1	0.0674	0.0933	0.0277	0.00215	0.00507	0.903
		2	0.0656	0.0898	0.0232	0.00141		0.807
		3	0.0773	0.0943	0.0307	0.00279	0.00381	0.964
		Av.	0.0701	0.0925	0.0272	0.00212	0.00063	
Hyd 701 50.70 mg.	VI	1	0.0758	0.0941	0.0294	--		
		2	0.0768	0.0937	0.0257	0.00255		0.930
Hyd 701 50.70 mg.	V	1	0.0840	0.1040	0.0363	0.00208		1.062
		2	0.0803	0.1041	0.0353	0.00264		1.050
		3	0.0825	0.1054	0.0458	0.00243	0.00358	1.121
		4	0.1060	0.1062	0.0378	0.00243		1.166
		5	0.0852	0.1049	0.0375	0.00265		1.087
		6	0.0838	0.1045	0.0360	0.00242		1.067
Hyd 701 46.64 mg.	IV	1	0.0787	0.0970	0.0319	0.00357		1.077
		2	0.0804	0.0990	0.0329	0.00386		1.107
		3	0.0816	0.1082	0.0355	0.00135		1.068
		4	0.0679	0.0955	0.0258	0.00708		0.937

[a]Milliequivalents of compounds as aldehydes.

TABLE VI

CARBOXYL TITER DATA FROM CARBAMOETHYL CELLULOSE ANALYSES

Hydrolyzate	Set		Glucose[a]	C	B	A	Blank
Hyd 874A	VIII	1	0.0132	0.1985	0.1636	0.00411	
		2	0.0136	0.2023	0.1520	0.01027	
		Av.	0.0134	0.2004	0.1578	0.00715	
		3	0.0130	0.1912	0.1322	0.00940	
		4	0.0129	0.1879	0.1438	0.00688	
		Av.	0.0130	0.1896	0.1380	0.00814	
Hyd 701	VII	1	0.0007	0.0972	0.0716	0.00964	0.0159
		2	0.0057	0.0962	0.0692	0.00569	0.0072
		3	0.0	0.0749	0.0539	0.00193	0.0009
		Av.	0.0031	0.0967	0.0704	0.00766	
Hyd 701	VI	1	0.0039	0.0837	0.0629	--	
		2	0.0027	0.0805	0.0575	0.00197	
Hyd 701	V	1	0.0	0.0820	0.0578	0.0	
		2	0.0012	0.0843	0.0453	0.00151	
		3	0.0010	0.0801	0.0564	0.00147	0.0005
		4	0.0	0.0801	0.0570	0.00147	
		5	0.0	0.0833	0.0564	0.00151	
		6	0.0013	0.0807	0.0594	0.00147	.0016
Hyd 701	IV	1	0.0015	0.0798	0.0574	0.00351	
		2	0.0008	0.0870	0.0636	0.0122	
		3	0.0006	0.1020	0.0353	0.00201	
		4	0.0044	0.0932	0.0458	0.00374	

[a]Milliequivalents of compounds as carboxylic acids.

TABLE VII

COOH/CHO RATIOS FROM CARBAMOETHYL CELLULOSE ANALYSES

Hydrolyzate	Set	Glucose	C	B	A
Hyd 874A	VIII 1	0.1051	0.967	2.192	1.52
	2	0.1147	0.956	2.404	5.55
	Av.	0.1099	0.962	2.298	3.54
	3	0.0079	0.948	2.030	3.316
	4	0.0693	0.874	2.090	2.210
	Av.	0.0386	0.911	2.060	2.763
Hyd 701	VII 1	0.0097	1.042	2.585	4.463
	2	0.087	1.071	2.987	4.032
	3	0.0	0.794	1.754	0.691
	Av.	0.049	1.056	2.781	4.248
Hyd 701	VI 1	0.0519	0.890	2.137	
	2	0.0356	0.859	2.236	0.773
Hyd 701	V 1	0.0	0.789	1.59	0.0
	2	0.0150	0.810	1.28	0.569
	3	0.0121	0.760	1.23	0.606
	4	0.0	0.754	1.59	0.606
	5	0.0	0.793	1.50	0.570
	6	0.0154	0.773	1.65	0.607
Hyd 701	IV 1	0.0149	0.823	1.802	0.984
	2	0.0100	0.876	1.934	3.158
	3	0.0739	0.942	0.996	1.484
	4	0.0651	0.976	1.776	0.528

TABLE VIII

MOLE FRACTIONS OF THE COMPONENTS OF CARBAMOETHYL CELLULOSE HYDROLYZATES

Hydrolyzate	Set		c_0	c_1	c_2	c_3	D.S. Total[a]
Hyd 874A	VIII	1	0.307	0.503	0.183	0.00661	0.889
		2	0.361	0.489	0.146	0.00427	0.794
		3	0.376	0.468	0.149	0.00649	0.786
		4	0.394	0.454	0.145	0.00656	0.765
		Av.	0.360	0.478	0.156	0.00598	0.808
Hyd 701	VII	1	0.354	0.489	0.145	0.0113	0.815
		2	0.364	0.498	0.129	0.00784	0.783
		3	0.377	0.460	0.150	0.0136	0.800
		Av.	0.359	0.494	0.137	0.00957	0.799
Hyd 701	VI	1	0.380	0.472	0.147	0.	
		2	0.386	0.472	0.129	0.0128	0.769
Hyd 701	V	1	0.371	0.459	0.160	0.0092	0.809
		2	0.363	0.471	0.160	0.0119	0.809
		3	0.350	0.447	0.194	0.0103	0.862
		4	0.420	0.420	0.150	0.0096	0.750
		5	0.370	0.460	0.163	0.0115	0.807
		6	0.370	0.460	0.159	0.0107	0.811
Hyd 701	IV	1	0.373	0.459	0.151	0.0169	0.812
		2	0.372	0.458	0.152	0.018	0.816
		3	0.360	0.477	0.157	0.006	0.809
		4	0.346	0,487	0.131	0.036	0.857

[a]Total D.S. calculated from the mole fractions.

APPENDIX V

CALCULATIONS

A. D.S. CALCULATIONS

In order to calculate the Degree of Substitution of the various products it was necessary to develop an expression for D.S. in terms of the nitrogen and carboxyl content of the derivative. The following expression was developed for the D.S. as amide:

$$\text{D.S. amide} = \frac{(\%N)\ (45.0)\ (162)}{(100)(14.0)(45.0)-(\%COOH)(14.0)(72.1)-(\%N)(45.0)(71.1)} \qquad (11).$$

The corresponding expression for D.S. as acid is

$$\text{D.S. acid} = \frac{(\%COOH)(14.0)(162)}{(100)(14.0)(45.0)-(\%N)(45.0)(71.1)-(\%COOH)(14.0)(72.1)} \qquad (12).$$

Total D.S. was calculated as the sum of D.S. amide and D.S. acid.

B. DEVELOPMENT AND MODIFICATION OF SPURLIN'S EQUATIONS FOR THE DISTRIBUTION OF SUBSTITUENT GROUPS IN CELLULOSE DERIVATIVES

Spurlin's equations relating mole fractions and reaction constants for an equilibrium reaction were redeveloped for carbamoethylation using the accepted mechanism of Michael addition.

Consider the reactions for carbamoethylation:

$$ROH+OH^- = RO^-+HOH \qquad (13)$$

$$RO^- + \text{Acrylamide} = RO\text{-}CH_2CHCONH_2^- \qquad (14)$$

$$ROCH_2CHCONH_2^-+HOH = ROH_2CH_2CONH_2+OH^- \qquad (15).$$

From Reaction (13) we get

$$\frac{[RO^-]\ [HOH]}{[ROH]\ [OH^-]} = K_1' \tag{16}$$

$$[RO^-] = \frac{[ROH][OH^-]K_1'}{[HOH]} \tag{17}.$$

From Reaction (14) we get

$$\frac{[ROCH_2CHCONH_2^-]}{[RO^-][Acrylamide]} = K_2' \tag{18}$$

$$[ROCH_2CHCONH_2^-] = [RO^-][Acrylamide]\ K_2' \tag{19}.$$

From Reaction (15) we get

$$\frac{[ROCH_2CH_2CONH_2][OH^-]}{[ROCH_2CHCONH_2^-][HOH]} = K_3' \tag{20}.$$

Substituting Equations (17) and (19) into Equation (20) we get

$$\frac{[ROCH_2CH_2CONH_2][OH^-][HOH]}{[ROH][OH^-]K_1'[Acrylamide]K_2'[HOH]} = K_3' \tag{21}$$

$$\frac{[ROCH_2CH_2CONH_2]}{[ROH]} = [Acrylamide]\ K_1' K_2' K_3' \tag{22}.$$

Considering the reaction at one particular hydroxyl position, $\underline{i}$, and assuming that all of the groups are completely accessible, then, if [CellOH] is the total concentration of glucose units and x_i is the mole fraction of glucose units substituted in the $\underline{i}$ position, we have for the concentration of glucose units substituted in the $\underline{i}$ position

$$[ROCH_2CH_2CONH_2]_i = x_i\ [CellOH] \tag{23}$$

and for the concentration of unsubstituted units

$$[ROH]_i = (1-x_i)\ [CellOH] \tag{24}.$$

Equation (22) then becomes

$$\frac{x_i[\text{CellOH}]}{(1-x_1)[\text{CellOH}]} = [\text{Acrylamide}]\, K_1' K_2' K_3' \tag{25}$$

$$\frac{x_i}{1-x_i} = A K_i \tag{26}.$$

Solving for x_i we get

$$x_i = \frac{A K_i}{1+AK_i} \tag{27}.$$

Because of the interrelation of the various mole fraction expressions, we can then obtain any set of mole fraction values in terms of equilibrium constants, including the following c_j values, the mole fractions of glucose units having j substituents, which are of interest in this study.

$$c_0 = \frac{1}{(1+AK_2)(1+AK_3)(1+AK_6)} \tag{28}$$

$$c_1 = \frac{AK_2+AK_3+AK_6}{(1+AK_2)(1+AK_3)(1+AK_6)} \tag{29}$$

$$c_2 = \frac{AK_2AK_3+AK_2AK_6+AK_3AK_6}{(1+AK_2)(1+AK_3)(1+AK_6)} \tag{30}$$

$$c_3 = \frac{AK_2AK_3AK_6}{(1+AK_2)(1+AK_3(1+AK_6)} \tag{31}.$$

Since the mole fractions are the experimentally measurable values and the equilibrium constants are the values sought, it is desirable to modify the equations. Any set of the equations can be reduced to an explicit set of functions of the mole fractions. For example,

$$AK_2 = \frac{x_2}{1-x_2} \tag{32}$$

$$AK_3 = \frac{x_3}{1-x_3} \tag{33}$$

$$AK_6 = \frac{x_6}{1-x_6} \tag{34}$$

In this work the experimentally measured values were $\underline{c}_j$ values. Therefore, the equations which were solved for the $\underline{AK}$ values were Equations (28), (29), (30), and (31). Equations (29), (30), and (31) can be simplified greatly by dividing each of them by Equation (28). The ratios $\underline{R}$, $\underline{S}$, and $\underline{T}$ can be calculated directly from the experimental data eliminating the intermediate summation of moles of glucose units.

$$R = \frac{\underline{c}_1}{c_0} = \frac{\dfrac{\text{moles monosub}}{\text{Moles Glucose+Moles monosub.+Moles disub.+Moles trisub.}}}{\dfrac{\text{moles glucose}}{\text{Moles Glucose+Moles monosub.+Moles disub.+Moles trisub.}}} \tag{35}$$

$$= \frac{\text{moles monosub.}}{\text{moles glucose}} \tag{36}$$

$$S = \frac{\underline{c}_2}{c_0} = \frac{\text{moles disub.}}{\text{moles glucose}} \tag{37}$$

$$T = \frac{\underline{c}_3}{c_0} = \frac{\text{moles trisub.}}{\text{moles glucose}} \tag{38}$$

There was further consideration from the experimental work. The value of $\underline{c}_3$ was quite small and was measured with only limited accuracy. Therefore, the equation involving $\underline{c}_3$ was not used in the solution for $\underline{AK}$.

Substituting $\underline{x} = \underline{AK}_2$, $\underline{y} = \underline{AK}_3$ and $\underline{z} = \underline{AK}_6$, the three equations solved were:

$$R = x+y+z \tag{39}$$

$$S = xy+xz+yz \tag{40}$$

$$c_0 = \frac{1}{(1+x)(1+y)(1+z)} \tag{41}.$$

By eliminating first $\underline{x}$ and then $\underline{y}$, these three expressions were reduced to the single cubic equation in $\underline{z}$,

$$z^3-Rz^2+Sz+R+S+1-1/c_0 = 0 \tag{42}.$$

This last equation can be solved readily by trial and error. It can be rewritten:

$$c_0z^3-c_1z^2+c_2z+c_0+c_1+c_2-1 = 0 \tag{43}.$$

The equation can also be written:

$$z^3-Rz^2+Sz-T = 0 \tag{44}$$

$$c_0z^3-c_1z^2+c_2z-c_3 = 0 \tag{45}.$$

It should be noted that the original equations, (28, 29, 30, 31), are completely symmetrical; that is, the interchanging of any two of the variables does not change the equation. For this reason, the same equation is obtained whether the original set is solved for $\underline{x}$, $\underline{y}$, or $\underline{z}$. As a result, to fit the physical system, the equation must have three real, positive roots, one of which corresponds to each reaction constant, $\underline{AK}_2$, $\underline{AK}_3$, $\underline{AK}_6$.

The mathematical trick of dividing the equations for c_1 and c_2 by the equation for c_0 greatly simplified the solution of these equations and allowed the

$\underline{AK}$ and relative $\underline{K}$ values to be calculated directly. This is a simpler and easier procedure than estimating the $\underline{AK}$ values, calculating the mole fractions, comparing the values to the experimental values and re-estimating and recalculating until a good fit is obtained. This simplifying trick is especially useful when the mole fractions of the individual components, the $\underline{s}_{\underline{i}}$ values, the mole fractions of glucose units substituted only in the $\underline{i}$ position, can be measured. In this case the calculation is extremely simple because $\underline{s}_{\underline{i}}/\underline{s}_0 = \underline{AK}_{\underline{i}}$. It is not even necessary to measure the total amount of glucose units since $\underline{s}_{\underline{i}}/\underline{s}_0$ = moles of $\underline{i}$-substituted material/moles of glucose. [see Equations (35), (36)]. Under these conditions, the reaction constants can be calculated with the same accuracy as the original analytical data.

C. CALCULATION OF SUBSTITUENT DISTRIBUTION CURVES

The reduced Equation (42) was solved for the $\underline{AK}$ values using the data from the analyses. This gave relative equilibrium constants, $\underline{K}_2$, $\underline{K}_3$ and $\underline{K}_6$ of 9, 1, and 19, respectively. These values were used to calculate the data for the distribution curves found in Fig. 7. For comparison, a similar plot of $\underline{c}_{\underline{j}}$ values against D.S. was made using the relative reaction constants $\underline{K}_2:\underline{K}_3:\underline{K}_6 = 1:1:1$ (see Fig. 8).

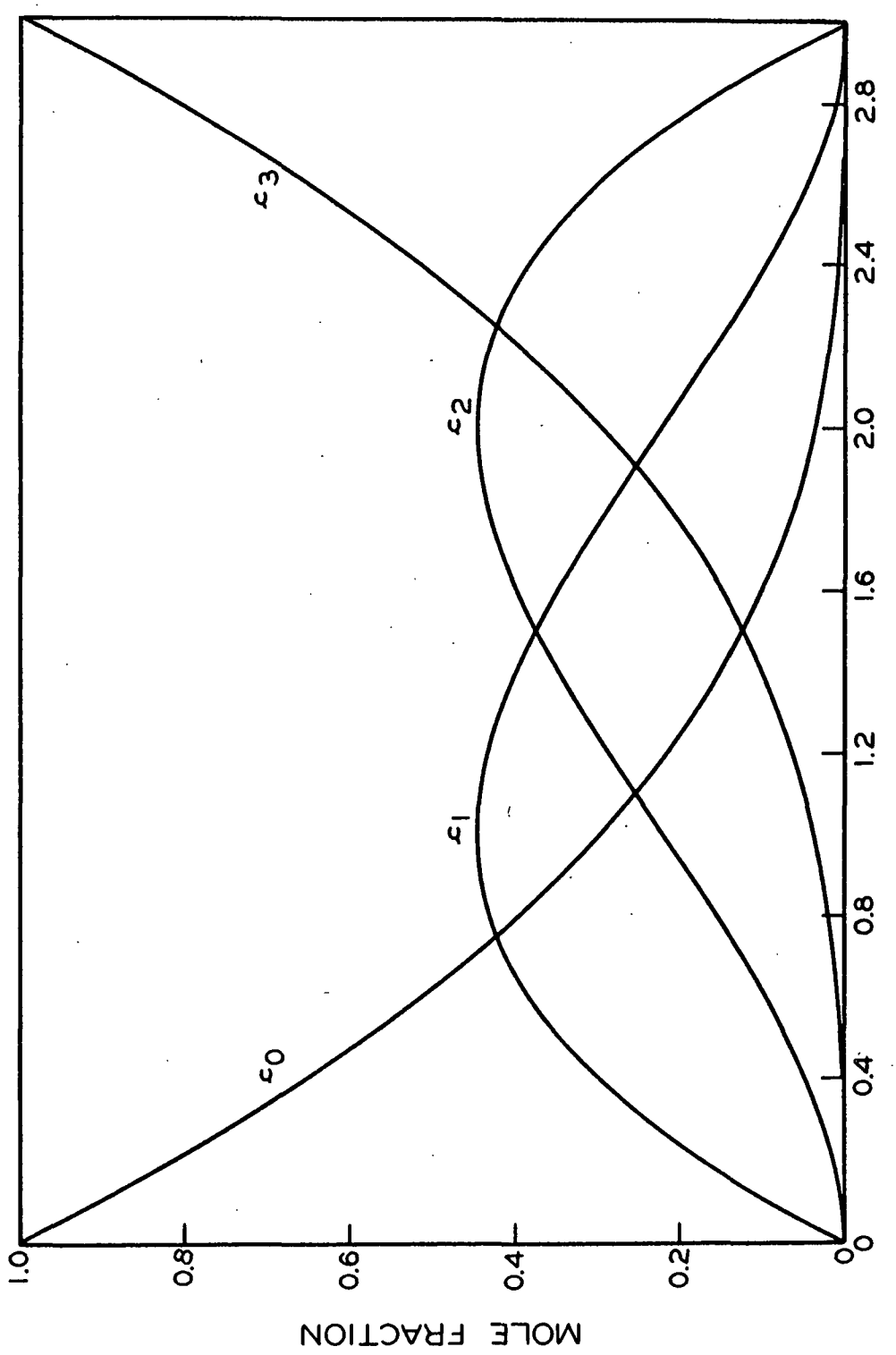

Figure 7. Plot of c_i values vs. D.S. for $K_0:K_1:K_2:K_3 = 9:1:19$

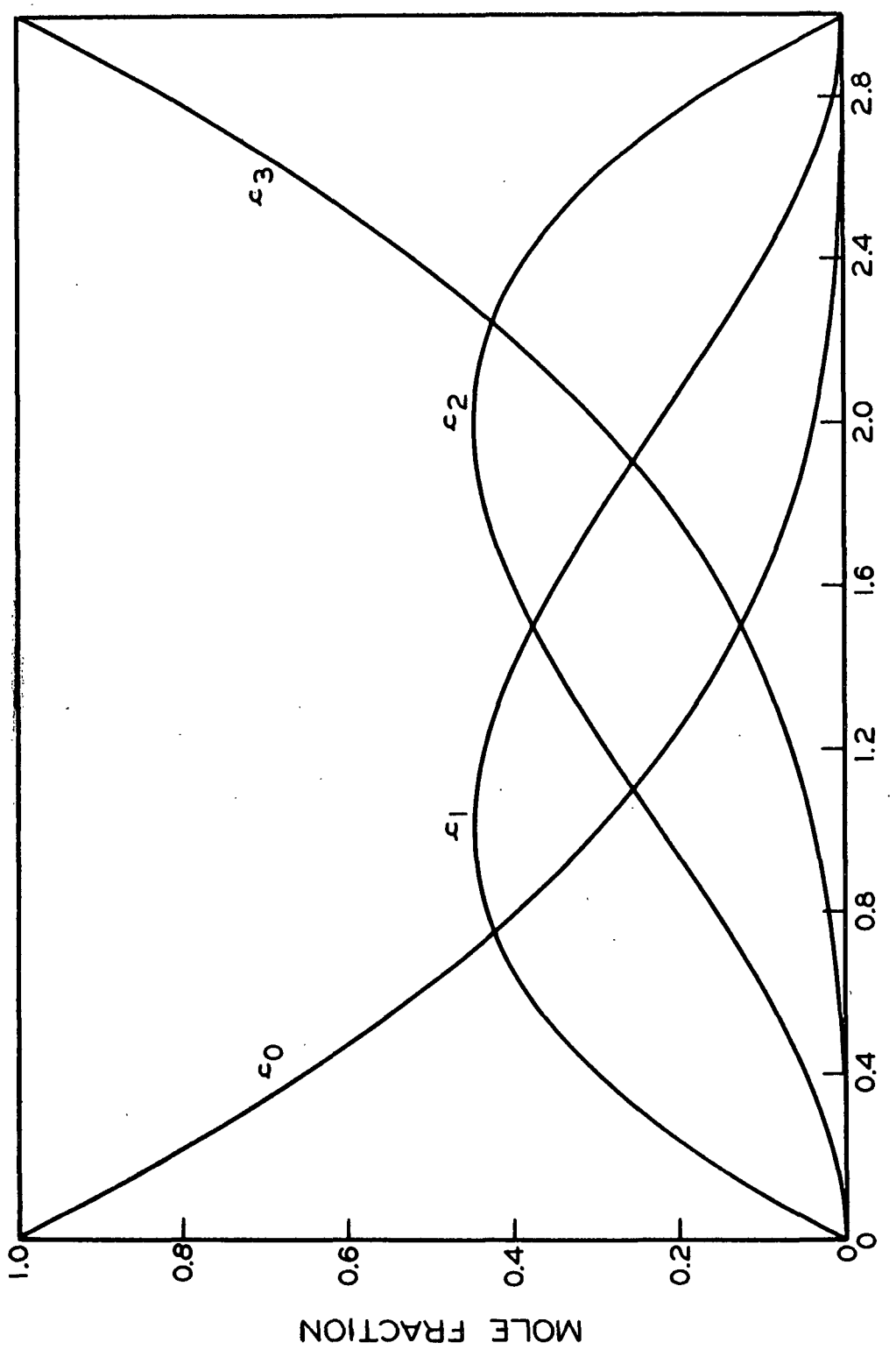

Figure 8. Plot of $\underline{c_j}$ values vs. D.S. for $\underline{K_2}:\underline{K_3}:\underline{K_6}$ = 1:1:1