(4) H. C. Haas and N. W. Schuler, *J. Polym. Sci.*, **31**, 237 (1958).
(5) H. L. Marder and C. Schuerch, *J. Polym. Sci.*, **31**, 237 (1958).
(6) G. Hardy in "Encyclopedia of Polymer Science and Technology", Vol. 14, Wiley-Interscience, New York, 1971.
(7) (a) J. Schafer in "Topics in $^{13}C$ NMR", Vol. 1, G. C. Levy, Ed., Wiley-Interscience, New York, 1974, pp 149–208.
(8) (b) F. A. Bovey in "Structural Studies of Macromolecules by Spectroscopic Methods", Wiley-Interscience, New York, 1976, pp 181–200.
(9) G. E. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, 1972, Chapter 7.
(10) L. R. Morris and D. J. Hubbard, *J. Org. Chem.*, **27**, 1451 (1962).
(11) W. R. Sorensen and T. W. Campbell, "Preparative Methods of Polymer Chemistry", Interscience, New York, 1968, p 263.
(12) G. Hardy, K. Nyitrai, F. Cser, and F. Nagy, *Eur. Polym. J.*, **4**, 289 (1968).
(13) F. A. L. Anet, *J. Am. Chem. Soc.*, **84**, 747, (1962).
(14) C. G. Overberger and A. Drucker, *J. Org. Chem.*, **29**, 362, (1964).
(15) P. F. Weit, *Chem. Ber.*, **93**, 116 (1960).
(16) T. Fukuroi, Y. Fujiwara, and S. Fujiwara, *Anal. Chem.*, **40**, 879 (1968).
(17) W. Vielter, E. Breitmaier, G. Jung, T. Keller, and D. Hiss, *Angew. Chem., Int. Ed. Engl.*, **9**, 803 (1970).
(18) Y. Inoue, A. Nishnoka, and R. Chujo, *Makromol. Chem.*, **156**, 207 (1972).
(19) 270-MHz spectra were recorded by Mr. P. Demou, Department of Chemistry, Yale University, New Haven, Conn.
(20) M. K. Akkapeddi and H. K. Reimschuessel, USP 4076680 (Allied Chemical Corp.).

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# Sequential Analysis by Cooperative Reactions on Copolymers. 4. The Structure of Locust Bean Gum and Guaran

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ABSTRACT: Galactomannans like locust bean gum and guaran consist of a backbone of mannose units some of which have galactose side stubs. On reaction of these galactomannans with periodate both galactose and mannose units get oxidized. We show how to correct for the consumption of periodate by the galactose units. After this step the periodate oxidation of the mannan backbone and the methylation analysis of the galactomannan can be described by the theory developed by P. C. Hemmer and the author for reactions on stationary second-order Markovian copolymers. This provides the approximate sequential structure of guaran and locust bean gum.

## I. Introduction

In this paper we apply the theory developed recently[1-4] to describe the periodate oxidation[5,6,8] and the methylation analysis[6,7] of the two important galactomannans locust bean gum and guaran.

For convenience of the reader we give a brief survey of the assumptions made and of the results obtained in our previous work.[1-4]

We consider very long linear polymers consisting of two different monomeric units $i = 1, 2$. The chains are assumed to be stationary second-order Markov chains: $p_{ijl}$ is the conditional probability that a given unit is of type $l$ given that the two preceding ones are of type $i$ and $j$. In a very long copolymer most of the units will conform to stationarity, i.e., end effects will be negligible in the limit of an infinitely long polymer.

Introducing $p_j$ as the probability that an arbitrary unit is of type $j$ and $p_{ij}$ as the conditional probability that a unit is of type $j$ given that it is preceded by an $i$ unit, these probabilities are completely determined in terms of the second-order Markovian probabilities $p_{ijl}$:[2-4]

$$p_{11} = p_{211}/(p_{112} + p_{211}) \qquad (1)$$

$$p_{12} = p_{112}/(p_{112} + p_{211}) \qquad (2)$$

$$p_{21} = p_{221}/(p_{122} + p_{221}) \qquad (3)$$

$$p_{22} = p_{122}/(p_{122} + p_{221}) \qquad (4)$$

and

$$p_1 = p_{21}/(p_{12} + p_{21}) \qquad (5)$$

$$p_2 = p_{12}/(p_{12} + p_{21}) \qquad (6)$$

The reaction process is assumed to obey the following rules: The probability that an arbitrarily placed unit of type $j$ reacts in the time interval $dt$ is equal to zero if the unit has already reacted, otherwise equal to $k_j\,dt$ if its two adjacent units are unreacted, equal to $a_l^j k_j\,dt$ if out of its neighbors only the right one has reacted and is of type $l$, equal to $\tilde{a}_i^j/k_j\,dt$ if out of its neighbors only the left one has reacted and is of type $i$, and equal to $c_i{}^j{}_l k_j\,dt$ if it is between two reacted species, from left to right $i,l$.

The extent of reaction $y_j(t)$ of unit type $j = 1, 2$ at time $t$ in an infinitely long polymer chain is given by:[3,4]

$$\begin{aligned} y_j(t) = {} & p_j - p_j \sum_{i=1}^{2}\sum_{l=1}^{2} p_{ji}p_{ijl}e^{-k_jc_i{}^j{}_lt} + \\ & k_jp_j \sum_{i=1}^{2}\sum_{l=1}^{2} p_{ji}p_{ijl}e^{-k_jc_i{}^j{}_lt}\Big\{(1 - a_l^j - \tilde{a}_i^j + \\ & c_i{}^j{}_l)\int_0^t \mathrm{d}s\ e^{k_j(c_i{}^j{}_l-1)s}\pi_{jl}(s)\tilde{\pi}_{ji}(s) + \\ & (a_l^j - c_i{}^j{}_l)\int_0^t \mathrm{d}s\ e^{k_jc_i{}^j{}_ls}\nu_{jl}(s)\tilde{\pi}_{ji}(s) + \\ & (\tilde{a}_i^j - c_i{}^j{}_l)\int_0^t \mathrm{d}s\ e^{k_jc_i{}^j{}_ls}\pi_{jl}(s)\tilde{\nu}_{ji}(s)\Big\} \end{aligned} \qquad (7)$$

The quantities $\nu_{jl}(s)$, $\pi_{jl}(s)$, $\tilde{\nu}_{jl}(s)$, and $\tilde{\pi}_{jl}(s)$ entering eq 7 are probabilities referring to the state of reaction of the first two units in an infinitely long polymer chain[3,4] at time $s = -\ln \zeta$. They are given by

$$\nu_{jl}(\zeta) = \zeta^{k_ja_l^j} \sum_{n,p=1}^{2} \sum_{t,v=1}^{2} \Phi_{jl;np}(\zeta)\Phi^{-1}{}_{np;tv}(1) \qquad (8)$$

$$\pi_{jl}(\zeta) = \sum_{r=1}^{2} p_{jlr}\nu_{lr}(\zeta) \qquad (9)$$

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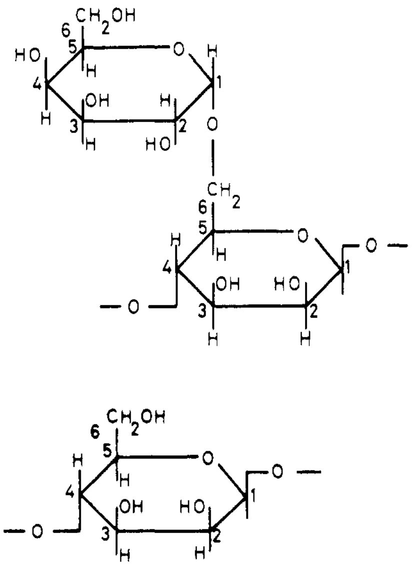


**Figure 1.** (a) Top: Branched mannopyranosyl (unit type 1) residue. (b) Bottom: Unbranched mannopyranosyl residue (unit type 2).

where the 4 × 4 matrix $\Phi_{jl,np}(\zeta)$ is given by a rapidly converging series

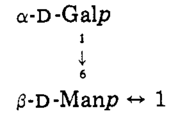
$$\Phi_{jl;np}(\zeta) = \delta_{jn}\delta_{lp} + \delta_{ln}\frac{T_{jlp}}{k_n a_p{}^n - k_j a_l{}^j + k_j}\zeta^{k_n a_p{}^n - k_j a_l{}^j + k_j} + \frac{T_{jln}}{k_n a_p{}^n - k_j a_l{}^j + k_j + k_l}\,\frac{T_{lnp}}{k_n a_p{}^n - k_l a_n{}^l + k_l}\zeta^{k_n a_p{}^n - k_j a_l{}^j + k_j + k_l} + \sum_{m=1}^{2}\frac{T_{jlm}}{k_n a_p{}^n - k_j a_l{}^j + k_j + k_l + k_m}\times \frac{T_{lmn}}{k_n a_p{}^n - k_l a_m{}^l + k_l + k_m}\times \frac{T_{mnp}}{k_n a_p{}^n - k_m a_n{}^m + k_m}\zeta^{k_n a_p{}^n - k_j a_l{}^j + k_j + k_l + k_m} + \sum_{m=1}^{2}\sum_{z=1}^{2}\frac{T_{jlm}}{k_n a_p{}^n - k_j a_l{}^j + k_j + k_l + k_m + k_z}\times \frac{T_{lmz}}{k_n a_p{}^n - k_l a_m{}^l + k_l + k_m + k_z}\times \frac{T_{mzn}}{k_n a_p{}^n - k_m a_z{}^m + k_m + k_z}\times \frac{T_{znp}}{k_n a_p{}^n - k_z a_n{}^z + k_z}\zeta^{k_n a_p{}^n - k_j a_l{}^j + k_j + k_l + k_m + k_z} + \ldots \quad (10)$$

and

$$T_{jlr} = k_j(1 - a_l{}^j)p_{jlr} \quad (11)$$

The probabilities $\tilde{\nu}_{jl}(\zeta)$ and $\tilde{\pi}_{jl}(\zeta)$ are obtained by replacing $a_l{}^j \rightarrow \tilde{a}_l{}^j$ in eq 8, 10, and 11 and by substituting $\nu_{jl}(\zeta) \rightarrow \tilde{\nu}_{jl}(\zeta)$ and $\Phi_{jl;np} \rightarrow \tilde{\Phi}_{jl;np}$ on the rhs of eq 9 and 10.

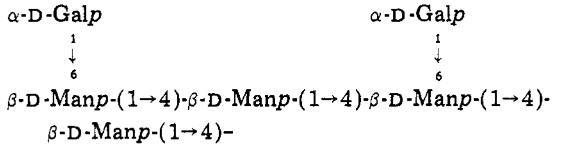
Guaran and locust bean gum belong to the subfamily of galactomannans consisting of a (1→4)-linked backbone of β-D-mannopyranosyl residues some of which (unit type 1) have side stubs linked α-D-(1→6) with α-D-galactopyranosyl groups.[9] The unbranched β-D-mannopyranosyl residues we refer to as unit type 2 (see Figure 1). With the symbols



$$\begin{array}{c}\alpha\text{-D-Gal}p\\ {\scriptstyle 1}\\ \downarrow\\ {\scriptstyle 6}\\ \beta\text{-D-Man}p \leftrightarrow 1\end{array}$$

and

$$\beta\text{-D-Man}p \leftrightarrow 2$$



$$\begin{array}{cccc}\alpha\text{-D-Gal}p & & \alpha\text{-D-Gal}p & \\ {\scriptstyle 1} & & {\scriptstyle 1} & \\ \downarrow & & \downarrow & \\ {\scriptstyle 6} & & {\scriptstyle 6} & \\ \beta\text{-D-Man}p\text{-}(1\rightarrow4)\text{-} & \beta\text{-D-Man}p\text{-}(1\rightarrow4)\text{-} & \beta\text{-D-Man}p\text{-}(1\rightarrow4)\text{-} & \beta\text{-D-Man}p\text{-}(1\rightarrow4)\text{-}\end{array}$$

or in terms of unit type numbers: -1-2-1-2-.

For a moment we forget about the consumption of periodate by the galactose units. Then, the mannan backbone of these galactomannans is a binary copolymer displaying nearest-neighbor effects on oxidation by periodate. Indeed, the oxidation of both branched and unbranched mannose units consists of cleavage of the bond between two vicinal diols to give two aldehyde groups. The aldehydes form immediately six-membered cyclic hemiacetals with hydroxyl groups at neighboring mannose units. As shown by Painter and co-workers[10-12] these hemiacetals are responsible for the protective nearest-neighbor effect.

Since the oxidation of a mannose unit can be safely assumed to be independent of whether its neighbors are branched and, furthermore, the protecting mechanism of hemiacetal formation[5,10-12] involves hydroxyl groups in the mannose residue of the *protected* unit only, we put

$$a_l{}^j = a_l; \quad \tilde{a}_l{}^j = \tilde{a}_l; \quad c_l{}^j{}_l = c_{il} \quad (12)$$

I.e., we expect the nearest-neighbor effect to depend only on the protecting and not on the protected unit. It is easy to see that the protective effects by the branched mannose and the unbranched mannose units are different. Indeed, the total degree of oxidation

$$y(t) = y_1(t) + y_2(t) \quad (13)$$

of both guaran and locust bean gum approaches a limit less than unity for very large times $t \rightarrow \infty$. For instance, for guaran[5,6,8] $y(\infty) \simeq 0.59$ while for locust bean gum[13] $y(\infty) \simeq 0.86$. This means

$$a_1 = \tilde{a}_1 = c_{1j} = c_{j1} = 0 \quad (14)$$

while $a_2$ and $\tilde{a}_2$ must be larger than zero, since otherwise $y(\infty)$ would be about $(1 - e^{-2})/2 \simeq 0.43$, viz., the limiting extent of reaction of a homopolymer consisting of (identical) totally protecting units. In section III we shall give the chemical mechanism responsible for the total protective effect by unit type 1 and for the partial one by unit type 2.

The fact expressed by eq 14 implies that the limit of the total degree of oxidation $y(\infty)$ will be a function of the remaining parameters $k_1$, $k_2$, $a_2$, and $\tilde{a}_2$ (but not $c_{22}$!) and of three independent conditional probabilities $p_{11}$, $p_{111}$, and $p_{222}$, say.[3,4] Important information about these remaining parameters can be gained by matching to the experimental reaction limit $y_{\text{exptl}}(\infty)$ the theoretical quantity $y(\infty)$ as found via eq 7-10.[32] Previous computations[3,4] show that $y(\infty)$ depends on $a_2$ and $\tilde{a}_2$ mainly through the sum $a_2 + \tilde{a}_2$ and not through $a_2$ and $\tilde{a}_2$ separately. In the following we set

$$a \equiv a_2 = \tilde{a}_2 \equiv \tilde{a} \quad (15)$$

and we call

$$c \equiv c_{22} \quad (16)$$

Although we speak of the total degree of oxidation $y(t)$, eq 13, as experimentally accessible, the quantities that can be directly measured are the total consumption of periodate $P(t)$[5] by the manno- and galactopyranosyl residues, the ratio of unreacted units[6,7] (reaction ratio, for brevity):

$$d(t) = [p_1 - y_1(t)]/[p_2 - y_2(t)] \qquad (17)$$

and the formic acid, $F(t)$, and formaldehyde, $A(t)$, released from the galactopyranosyl residues under periodate oxidation.[5]

Sequential analysis of locust bean gum and guaran means matching the experimental values $P_{\text{exptl}}(t)$ and $d_{\text{exptl}}(t)$ with theoretical values $P(t)$ and $d(t)$ as predicted by our theory. The set of conditional probabilities $p_{11}$, $p_{111}$, $p_{222}$ together with the known global composition $p_1$ that produces agreement of $P(t)$, $d(t)$ with $P_{\text{exptl}}(t)$, $d_{\text{exptl}}(t)$ completely describes the sequential structure. Inherent to our approach is the assumption that the polymers are stationary second-order Markov chains. While it cannot be excluded that these galactomannans are higher order Markov chains the approximation of a second-order Markov chain is forced upon us by the disparate amount of parameters in a, say, third-order Markov chain (seven independent conditional probabilities). Markovian copolymers of order higher than second play virtually no role in the literature. Stationarity of the chain is a convenient assumption but there may be enzymatic copolymerization where owing to depletion of the monomers the product may not be representable as a stationary Markov chain. This point should be kept in mind when comparing the quality of agreement between theory and experiment.

By assuming the feasibility of the sequential analysis we are presupposing that guaran and locust bean gum indeed react according to our model with nearest-neighbor cooperative effects and with neighbor-independent reactivities $k_i$. The first assumption has a firm basis both from direct evidence[10] and because of the excellent agreement of theory and experiment for amylose.[14,15] (See also the discussion in Chapter I and in Section 7 of Chapter II of ref 4.) The second assumption of neighbor-independent reactivities $k_i$ is less safe and we shall return to this question after comparing the agreement of our theoretical results for guaran and locust bean gum with experiment (section IV).

Before we can attempt this comparison we must take two steps. First, we shall develop a model to take account of the consumption of periodate by the galactopyranosyl side stubs (section II). Then we shall argue that $a = 0.5$ is a good approximation for galactomannans (section III).

The last section of this article, section V, contains a discussion of the results achieved. A delicate question that we have to answer in section V is whether we have found *the* sequential structure of locust bean gum and guaran. We anticipate that due to the large experimental uncertainties in the difficult methylation analysis used to measure the reaction ratio $d(t)$[6,7] our determination of the Markovian probabilities contains very large uncertainties. Hence, we make no strong claim about the actual sequential structure of these copolymers.

A satisfactory result is that theory is consistent with experiments. For instance, the agreement of the theoretical and experimental values for the periodate consumption by locust bean gum is very good. Thus, our analysis adds two new cases to the list of polymer reactions that can be described by the model of nearest-neighbor cooperative effects. [Previous successes were quaternization of poly(4-vinylpyridine) by *n*-butyl bromide,[16] periodate oxidation of amylose[14,15] and of nigeran and isolichenan,[17] and second periodate oxidation of amylose.[4]] As late as 1975 a well-known textbook[18] characterized the applicability of the model of nearest-neighbor cooperative effects as very limited in practice. The situation has changed now, partly because the solution of this model for copolymers[1-4] has expanded the potential domain of applicability but also because Dr. Painter's recognition of periodate oxidation of polysaccharides as a promising domain of application for the model in question is proving to be correct.

## II. Periodate Oxidation of Galactopyranosyl Groups

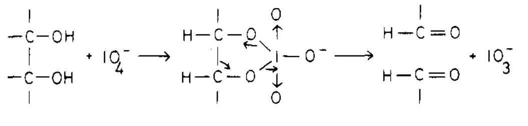
We remind the reader that the "monomer" species called 1 units actually are branched mannose groups

α-D-Gal*p*
1
↓
6
β-D-Man*p*

(see also Figure 1a).

The galactopyranosyl group contains hydroxyl groups on three consecutive carbon atoms (2, 3, and 4); as such, it will consume 2 mol of periodate and can be formally regarded as a kind of dimer, which is capable of showing nearest-neighbor cooperative effects. There are, however, two complications: (a) The reactivities of the two oxidizable sites are not even approximately equal; it is an experimental fact that cleavage between positions 3 and 4 is much faster than cleavage between positions 2 and 3 (ref 19). (b) Formation of the protective hemiacetal is not, in this case, a fast reaction, compared to the rate of oxidation; that is to say, oxidation of the second site can take place before a state of equilibrium is reached between the oxidizable, aldehydic form and the unoxidizable, hemiacetal form.[20]

The chemical reason for these effects is fairly well understood.[19,21] When the periodate ion reacts with a vicinal diol, the first step is rapid formation of a cyclic ester (II), which then decomposes with formation of a dialdehyde and liberation of an iodate ion:

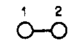


(I) (II)

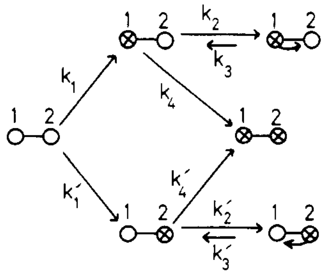
Formation of ester II requires the two oxygen atoms of diol I to come very nearly into a plane, which means that the concentration of II, and, hence, the rate of oxidation, is profoundly sensitive to any steric hindrance, opposing the planar arrangement. When the diol is present as part of a six-membered ring, as it is in an unreacted pyranose sugar, the steric hindrance is rather severe, but it is much more severe when the two vicinal hydroxyl groups are on opposite sides of the ring (i.e., trans) than when they are on the same side (i.e., cis). This is probably the main reason for fact (a) mentioned above (see ref 19 for certain qualifying remarks).

The same principles explain fact (b); when the diol is present in an acyclic chain, the steric hindrance to the planar arrangement is much smaller, and for a monooxidized pyranose sugar, consumption of a second mole of periodate is several orders of magnitude faster than that of an intact pyranose ring. It turns out that the rate is, in fact, of the same order of magnitude as the rate of formation of the unoxidizable cyclic hemiacetal.[20] It is therefore not surprising that the protective effect of hemiacetal formation can safely be assumed to be instantaneous for a polymer like amylose, whereas complications arise when there is more than one oxidizable site in a given monomer unit.

We are thus led to studying the model in eq 18



for the reaction of a "dimer"



(18)

where the numbers 1 and 2 denote the cis, trans configuration, respectively. A cross means an oxidized bond and the curved arrow symbolizes the formation of the protective hemiacetal. The rate constants for the different processes are $k_1$, $k_2$, $k_3$, $k_4$ and $k_1'$, $k_2'$, $k_3'$, and $k_4'$.

Using the symbol 0 for an unoxidized and unprotected unit, the symbol 1 for an oxidized unit, and −1 for an unoxidized and protected unit, the densities, $q$, of the different states of reaction of the dimer at time $t$ will be represented by $q(0,0;t)$, $q(1,0;t)$, $q(1,-1;t)$, $q(0,1;t)$, $q(-1,1;t)$, and $q(1,1;t)$. The kinetic equations describing the reaction model eq 18 are:

$$\frac{dq(0,0;t)}{dt} = -(k_1 + k_1')q(0,0;t) \tag{19}$$

$$\frac{dq(1,0;t)}{dt} = -(k_2 + k_4)q(1,0;t) + k_1q(0,0;t) + k_3q(1,-1;t) \tag{20}$$

$$\frac{dq(1,-1;t)}{dt} = -k_3q(1,-1;t) + k_2q(1,0;t) \tag{21}$$

$$\frac{dq(0,1;t)}{dt} = -(k_2' + k_4')q(0,1;t) + k_1'q(0,0;t) + k_3'q(-1,1;t) \tag{22}$$

$$\frac{dq(-1,1;t)}{dt} = -k_3'q(-1,1;t) + k_2'q(0,1;t) \tag{23}$$

the last quantity, $q(1,1;t)$, being determined through the normalization

$$q(0,0;t) + q(1,0;t) + q(1,-1;t) + q(0,1;t) + q(-1,1;t) + q(1,1;t) = 1 \tag{24}$$

For the solution of the kinetic eq 19–24 and for a discussion of the special case when the formation of the protecting hemiacetals is instantaneous, we refer the reader to ref 4. Here, we only give the solution of eq 19–24 with the usual initial condition of completely unreacted dimers at $t = 0$. They are:

$$q(0,0;t) = e^{-(k_1+k_1')t} \tag{25}$$

$$q(1,0;t) = \frac{k_1}{\lambda_- - \lambda_+}\left\{\frac{\lambda_+ + k_2 + k_4}{\lambda_- + k_1 + k_1'}[e^{-(k_1+k_1')t} - e^{\lambda_-t}] - \frac{\lambda_- + k_2 + k_4}{\lambda_+ + k_1 + k_1'}[e^{-(k_1+k_1')t} - e^{\lambda_+t}]\right\} \tag{26}$$

$$q(1,-1;t) = \frac{k_1}{k_3}\frac{(\lambda_+ + k_2 + k_4)(\lambda_- + k_2 + k_4)}{\lambda_- - \lambda_+}\left\{\frac{1}{\lambda_- + k_1 + k_1'}[e^{-(k_1+k_1')t} - e^{\lambda_-t}] - \frac{1}{\lambda_+ + k_1 + k_1'}[e^{-(k_1+k_1')t} - e^{\lambda_+t}]\right\} \tag{27}$$

where the abbreviations $\lambda_-$ and $\lambda_+$ are defined by

$$\lambda_\pm = \frac{-(k_2 + k_3 + k_4) \pm [(k_2 + k_3 + k_4)^2 - 4k_3k_4]^{1/2}}{2} \tag{28}$$

The expressions for $q(0,1;t)$ and $q(-1,1;t)$ are obtained by the substitutions $k_2 \rightarrow k_2'$, $k_3 \rightarrow k_3'$, and $k_4 \rightarrow k_4'$ in eq 26–28.

Let us define the following three quantities that are experimentally measurable:[5]

$$F(t) = q(1,1;t) \tag{29}$$

$$A(t) = q(1,0;t) + q(1,-1;t) + q(0,1t) + q(-1,1;t) \tag{30}$$

$$P_0(t) = 2 - 2q(0,0;t) - q(1,0;t) - q(1,-1;t) - q(0,1;t) - q(-1,1;t) \tag{31}$$

$F(t)$, the fraction of doubly oxidized galactopyranosyl residues, can be measured as the relative number of moles of formic acid released under periodate oxidation. $A(t)$, the fraction of singly oxidized galactopyranosyl residues, can be found as the relative number of moles of formaldehyde developed. $P_0(t)/2$ is the fraction of oxidized sites (each dimer, i.e., galactopyranosyl group, has two oxidizable sites). $P_0(t)$ equals the relative number of moles of periodate consumed.

The ratio $R = \{q(0,1;t) + q(-1,1;t)\}/\{q(1,0;t) + q(1,-1;t)\}$ can be measured by gas chromatography,[19] the result for galactose being that $R$ is negligibly small, implying that for the case in question $k_1' \ll k_1$.

The quantities $F$, $A$, and $P_0$ are not independent of each other. The equations

$$P_0(t) = A(t) + 2F(t) \tag{32}$$

and

$$A(t) + F(t) + q(0,0;t) = 1 \tag{33}$$

are self-evident. Using eq 25 one gets:

$$A(t) + F(t) = 1 - e^{-(k_1+k_1')t} \tag{34}$$

The total consumption of periodate per mole of hexose (here mannose and galactose), $P(t)$, by a galactomannan having a proportion $p_1$ of branched mannose units is found from the degree of oxidation of the mannose skeleton, $y(t)$, and the periodate consumption, $P_0(t)$, by the galactopyranosyl residues as

$$P(t) = \frac{p_1}{1 + p_1}P_0(t) + \frac{1}{1 + p_1}y(t) \tag{35}$$

Obviously, it is not possible to distinguish by experimental techniques between the periodate consumed by the galactopyranosyl and by the mannopyranosyl residues. Hence, we must evaluate $P_0(t)$ on the rhs of eq 35 indirectly by means of eq 32 by first matching the theoretical values for $A(t)$ and $F(t)$ with the experimental ones as given in ref 5 and 8.

Before doing so, we remind the reader that the time variable, $t$, appearing in our equations is the ideal time scale corresponding to an idealized experiment where the reagent's concentration is kept constant through steady replenishing. The real time scale, $t^*$, is a function of $t$ given by[4,14]

$$t^*(t) = \int_0^t ds \frac{c_1}{c_1 - c_2y(s)} \tag{36}$$

$c_1$ and $c_2$ being the initial concentration of periodate and the concentration of hexose, $y(t)$ being the extent of reaction *defined as the relative number of reacted monomers*. This is important, since for guaran and locust bean gum we use the symbol $y(t)$ to designate the proportion of reacted mannose units. With the last definition, the transformation $t^*(t)$ from ideal time to real time is different from eq 19. Arguing as before,[4,14] we find

$$t^*(t) = \int_0^t ds \frac{c_1}{c_1 - c_2P(s)} \tag{37}$$

We recall that $P(t)$ is defined as the total consumption of periodate by the galactomannan per mole of hexose.

Now, the experimental results being given in terms of pairs of values of $t^*$ and $P(t^*)$ one should think that the procedure to be applied is for a set of values of the ideal time parameter, $t$, to evaluate the corresponding values of $y(t)$, eq 7 and 13, $P_0(t)$, eq 31, $P(t)$, eq 35, and $t^*$, eq 37. However, this procedure can hardly be applied for the following reasons: (a) The number of parameters to be adjusted is high, thus making it very awkward to simultaneously match three quantities, viz., $P_0(t)$, $P(t)$, and $t^*$. Additionally, (b) use of eq 37 greatly increases the computing time, both by making necessary a much higher number of the time-consuming computations of expression $y(t)$, eq 7–11 and eq 13, and by demanding a higher numerical precision in the evaluation of these equations, in order that $t^*$ be determined with sufficient accuracy.

There is a simple way to avoid these difficulties. Equation 37 can be inverted to give the ideal time scale as a function of the experimental time, $t^*$:

$$t(t^*) = \int_0^{t^*} ds^* \left[ 1 - \frac{c_2}{c_1} P(s^*) \right]$$

or

$$t(t^*) = t^* - \frac{c_2}{c_1} \int_0^{t^*} ds^* \, P(s^*) \qquad (38)$$

Integrating the experimental curve for the periodate consumption $P(t^*)$, the transformation from $t^*$ to $t$ is found once and for all. Not only can $P_0(t)$ and $P(t)$ now be matched to experiment *separately*, but in addition the computing time is reduced by at least a factor of 10.[23]

The crucial point is whether the accuracy in finding $t$ by integrating the experimental curve for $P(t^*)$, eq 38, is satisfactory. The answer is affirmative because a random error of, say, ±1% in $P(t^*)$ will to a certain extent be averaged out by the integration and, additionally, the factor $c_2/c_1$, which is necessarily less than unity, diminishes the error still further. For a check we have computed the ideal time scale for periodate oxidation of amylose[11] according to eq 38. The results agreed very well with previous ones[14] found by simultaneous matching of the theoretical values for $y(t)$ and $t^*(t)$ to experimental values.

The procedure used to integrate the experimental curve of periodate consumption [cf. eq 38] consisted of fitting polynomials to the data. Once an acceptable fit was found, the integral in eq 38 followed in an elementary manner. In ref 4 the reader will find tables for conversion of times for locust bean gum and guaran.

With these preliminaries settled, we now return to the question of accounting for the oxidation of the galactopyranosyl residues by means of the theoretical results in eq 25–30. Ishak and Painter[5] have measured the liberation of formic acid, $F$, and of formaldehyde, $A$, on oxidation of guaran by periodate. Since $k_1' \ll k_1$, the theoretical model in eq 25–30 depends essentially on four parameters $k_1$, $k_2$, $k_3$, $k_4$. It is easy to see that

$$A(t) \simeq k_1 t + \theta(t^2)$$

$$F(t) \simeq k_1 k_4 t^2 + \theta(t^3)$$

These equations could in principle be used to determine the reactivities $k_1$ and $k_4$ from small $t$ results, but this possibility is only a theoretical one, since there is at the present time no way of obtaining accurate experimental results with realistic periodate concentrations for very small times (of the order of 1 min or less).

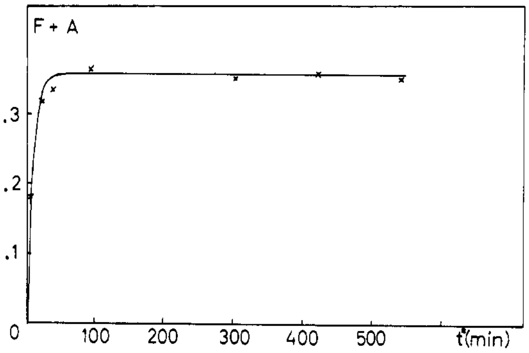


**Figure 2.** Optimum least-squares fit of the function 0.36–0.36 exp($-k_1t$) to the experimental results[5,8] for $A + F$.

Table I
**Optimum Least-Squares Fit of the Function 0.36–0.36 exp($-k_1t$) to the Experimental Results[5,8] $A + F$**

| real time $t^*$, min | ideal time $t$, min | exptl $A + F$ | theory $A + F$ |
|---|---|---|---|
| 5 | 4.79 | 0.180 | 0.170 |
| 20 | 17.45 | 0.318 | 0.325 |
| 35 | 29.07 | 0.334 | 0.353 |
| 90 | 69.26 | 0.364 | 0.360 |
| 300 | 213.9 | 0.354 | 0.360 |
| 420 | 294.1 | 0.361 | 0.360 |
| 540 | 373.8 | 0.354 | 0.360 |

It is still possible to make an accurate determination of $k_1$, however. Equation 34 shows the way. First, we note that for guaran $A$ and $F$ are measured as liberated moles of formaldehyde and formic acid per mole of hexose residue. Since there are 36% galactose out of 100% hexose, the proper normalized version of eq 34 reads:

$$A(t) + F(t) = 0.36 - 0.36e^{-k_1 t} \qquad (39)$$

where we approximated $k_1 + k_1' \simeq k_1$. The big advantage of eq 39 is that it is valid for any time $t$. Hence, a least-squares fit of the function $0.36(1-e^{-k_1t})$ gives the value of $k_1$. Figure 2 and Table I show the optimum fit, corresponding to

$$k_1 = 0.134 \text{ min}^{-1} \qquad (40)$$

and a total root mean square (rms) deviation of 0.024 corresponding to an average error of 0.009. To judge the fit, we remark that the experimental uncertainties are greatest in $A$, viz., about ±0.02.

Unfortunately, the three reactivities $k_2$, $k_3$, and $k_4$ cannot be determined by simple methods. One has to vary these parameters until a reasonable fit is found. On the basis of chemical considerations, Painter expected $k_2$ and $k_4$ to be 50–100 times larger than $k_1$. The contribution of the side reaction described by $k_1'$, $k_2'$, $k_3'$, and $k_4'$ was set equal to or less than 1% and a fit with $k_2$ and $k_4$ in the range 5 to 30 min$^{-1}$ was attempted in accordance with Painter's expectation. A fit was considered acceptable if the rms deviation from experimental results was equal to or less than the experimental uncertainty of about ±0.01 for $F$ and ±0.02 for $A$. The best fit was found for

$$k_2 = 23 \text{ min}^{-1}; \quad k_3 = 0.007 \text{ min}^{-1}; \quad k_4 = 6.4 \text{ min}^{-1} \qquad (41)$$

with a rms deviation per result of 0.008 for $F$ and 0.014 for $A$. Figure 3 shows the results.

The reader should note that $k_3$ turns out to be three orders of magnitude less than $k_2$ and $k_4$. It is even about 20 times less than $k_1$. Thus, while the rate of formation of the hemiacetals is much larger than the rate of the first attack on galactose, the rate of the inverse process, $k_3$, seems to be very small. If this is also true for the hemiacetals forming between mannose units, the basis for describing the cooperative effect in terms of protection

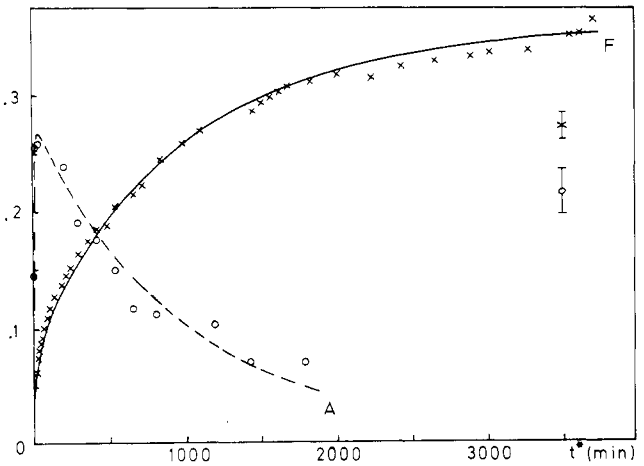


**Figure 3.** Experiment vs. theory in formic acid (F) and formaldehyde (A) liberation by periodate oxidation of guaran. The bars indicate the average experimental uncertainty.

constants would seem less firm. Indeed, as shown in ref 4, the description in terms of protection constants is possible when $k_2 \to \infty$ *and* $k_3 \to \infty$, subject to the conditions $k_2/k_3$ = constant, $k_1$ = constant, and $k_4$ = constant. Without having exhaustively investigated this question, we believe that the way out of the dilemma is the low sensitivity of the model. It turned out to be possible to describe the experimental results for galactose residues with different sets of parameters $k_2$ and $k_4$ of the order of 1 to 30 min$^{-1}$ by *roughly* keeping the ratios $k_2/k_4$ and $k_2/k_3$ constant. Thus, one may tentatively draw the conclusion that what really matters is the ratio of these reactivities once $k_2$ and $k_4$ are much larger than $k_1$. The values of $k_2$ and $k_4$ were chosen in the range given in eq 41 because, in agreement with Painter's expectation of their order of magnitude, they turned out to correspond to an, albeit very flat, optimum of agreement.

## III. The Partial Protection by Oxidized Unbranched Mannose

It is important to realize that while both types of units will, when oxidized, give hemiacetals, branched mannose (unit type 1, see Figure 1a) leaves only one possibility for hemiacetal formation: the two aldehydes at positions 2 and 3 that arise by periodate oxidation will each form a hemiacetal linkage with the closest hydroxyl group on the neighboring mannopyranosyl residue (interresidual hemiacetal formation). This explains why oxidized 1 units protect their neighbors completely against oxidation.[5] The situation is different for oxidized unbranched mannose, unit type 2 (see Figure 1b). Here, there is a hydroxyl group in the same unit, viz., at position 6. Hence, intraresidual hemiacetal formation by the aldehydes that arise at positions 2 and 3 with the hydroxyl group at position 6 will compete with interresidual hemiacetal formation with hydroxyl groups on neighboring mannopyranosyl residues. This means that oxidized 2 units will only exert *partial* protection against oxidation.

We now assume that intraresidual hemiacetal formation is dominant. This means that at any time either the aldehyde at position 2 or the one at position 3 will form an intraresidual hemiacetal linkage, the other aldehyde forming an interresidual hemiacetal linkage. Hence:

$$(a + \tilde{a})/2 = 0.5$$

and, remembering the discussion in section I, we can set

$$a = \tilde{a} = 0.5 \tag{42}$$

The reader should note that it could make a difference for the protection constant whether the oxidized unit has an unoxidized or an oxidized neighbor. In both cases there will be hydroxyl groups to form a linkage; in the first case there are the hydroxyl groups of the neighboring mannose residues, and in the second the hydroxyl groups arising from hydration of the aldehydes. However, we make the simplifying assumption that the value of the protection constant is not changed by this situation and refer to amylose[4,14,15] where the same mechanisms are at work: The agreement between theory and experiment for amylose seems to indicate that the strength of the protective mechanism is independent of whether or not the protecting unit has an oxidized neighbor.

It is important to keep in mind that if the assumption of dominance of intraresidual hemiacetal formation does not hold, then $(a + \tilde{a})/2$ must be less than 0.5. This is the case for amylose, where $(a + \tilde{a})/2 = 0.23$. There seems to exist some evidence that this does not happen for the mannose skeleton in galactomannans. The evidence, which is indirect, comes from the fact that second oxidation after completed first oxidation of both guaran and locust bean gum gives essentially the maximum theoretical oxidation limit. We refer to the next section, where this point will be discussed further.

Altogether, the assumption $a = \tilde{a} = 0.5$ greatly simplifies the process of matching theory with experiment. We shall see that this assumption leads to reasonable agreement between theory and experiment. It is difficult to judge to what extent the results confirm the assumption, since we have not tested it against other values of $a = \tilde{a} < 0.5$.

It would be very desirable to eliminate this uncertainty by making a measurement of $a$ and $\tilde{a}$ in short mannan chains (short, because long mannan chains are insoluble in water).

## IV. Sequential Analysis of Guaran and Locust Bean Gum

**A. The Second Oxidation.** By stopping the initial ("first") oxidation after some time, $t_0$, and eliminating the protecting hemiacetal linkages by reduction, it is possible to carry out a new reaction called "second" oxidation.[10-12,24] Three types of mannose units are now present in the polymer: reduced, oxidized mannose (oxidized, but nonprotecting), unoxidized mannose, and unreduced, oxidized mannose (oxidized and protecting). From previous results[4,25] we know that the oxidized and nonprotecting units may slightly enhance the reactivity of unreacted neighbors. In the following we shall ignore this effect. It is then possible to obtain the reactivity ratio $k_2/k_1$ by comparing the initial reaction rates for the second oxidation of guaran and locust bean gum that have been first oxidized until essentially all the galactose side stubs have reacted. (This experiment is feasible, since the galactopyranosyl residues get fully oxidized while the mannan chain does not.) If a proportion $\alpha_i(\beta_i)$ of unoxidized $i$ units is present when the first oxidation is stopped in guaran (locust bean gum), the initial reaction rate in the second oxidation of guaran will be

$$K_g = \alpha_1 k_1 + \alpha_2 k_2 \tag{43}$$

and the corresponding initial rate for locust bean gum

$$K_l = \beta_1 k_1 + \beta_2 k_2 \tag{44}$$

The proportions $\alpha_i, \beta_i$ can be obtained from the degree of oxidation, $y_{\mathrm{exptl}}$, and the reaction ratio, $d_{\mathrm{exptl}}$, at the end of the first oxidation. Hence measurement of $K_g$ and $K_l$ will determine $k_1$ and $k_2$.[28] Because of the possible activatory effect by reduced mannose units, the values of $k_1$ and $k_2$ determined in this way may be somewhat inaccurate. It is reasonable, though, to expect that this activatory effect will not influence the *ratio* $k_2/k_1$ to the same extent. Hence, we shall work with the result[28]

$$k_2/k_1 = 1.4 \pm 0.1 \tag{45}$$

and we perform the absolute determination of $k_1$ and $k_2$ by matching the theoretical results with the initial values for the oxidation of locust bean gum.

The second oxidation results provide a very important clue on the sequential structure of guaran and locust bean gum. In fact, experiments show[5,7,8] that the second oxidation limits $y^{(2)}(\infty,\infty)$ of fully first oxidized guaran and locust bean gum are very close to 100%. Taking account of the experimental uncertainty, it seems safe to eliminate all those sets of parameters that lead to $y^{(2)}(\infty,\infty) < 98.5\%$ for being in disagreement with the facts. The general theory for second oxidation of copolymers after an arbitrary extent of the first reaction is fairly straightforward but complicated.[26] The theory becomes simple, however, when the first oxidation has been completed. (This corresponds to taking the limit $t_0 \to \infty$ for the first oxidation.) After a completed first oxidation, the unoxidized units occur as singlets and doublets bounded by at least one oxidized 1 unit. The probability for every such situation can be found in an elementary way in terms of the limits of the extents of reaction $y_1(\infty)$ and $y_2(\infty)$ and, by application of the principle of independence, of the quantities $\nu_{ij}(\infty)$ and $\tilde{\nu}_{ij}(\infty)$. For instance, the probability of occurrence of the singlet 1*11* is given by

$$p_1 p_{11} p_{111} P_\infty\{\ldots-1^*-1-1^*-\ldots\}$$

$P_t\{\ldots-1^*-1-1^*-\ldots\}$, defined according to the rules of ref 3 and 4, can in principle be expressed in terms of $y_1(\infty)$ and $y_2(\infty)$ but it is not necessary to do so at this stage. As a different example, the probability of occurrence of the doublet 1*121* is given by

$$p_1 p_{11} p_{112} p_{121} \nu_{21}(\infty) \tilde{\nu}_{11}(\infty)$$

The process of the second oxidation is now simply the reaction of isolated singlets and doublets. Multiplying the number of reacted units for each singlet or doublet type by its probability of occurrence, the following result is obtained in an elementary way (Appendix A):

$$\begin{aligned} y^{(2)}(\infty;t) = {} & y(\infty) + F_1(1 - e^{-k_1 t}) + F_2(1 - e^{-k_2 t}) + \\ & F_3(1 - e^{-2k_1 t}) + F_4\left\{2 - \frac{k_1}{k_1 + k_2} - \frac{k_2}{k_2 + k_1(1-a)} e^{-k_1 a t} + \right. \\ & \left. \left[\frac{k_2}{k_2 + k_1(1-a)} + \frac{k_1}{k_1 + k_2} - 2\right] e^{-(k_1 + k_2)t}\right\} + \\ & F_5\left\{2 - \frac{k_1}{k_1 + k_2} - \frac{k_2}{k_2 + k_1(1-\tilde{a})} e^{-k_1 \tilde{a} t} + \right. \\ & \left. \left[\frac{k_2}{k_2 + k_1(1-\tilde{a})} + \frac{k_1}{k_1 + k_2} - 2\right] e^{-(k_1+k_2)t}\right\} + F_6\left\{2 - \right. \\ & \left. \frac{1}{2-a} e^{-k_2 a t} - \frac{1}{2-\tilde{a}} e^{-k_2 \tilde{a} t} + \left[\frac{1}{2-a} + \frac{1}{2-\tilde{a}} - 2\right] e^{-2k_2 t}\right\} \end{aligned} \tag{46}$$

where the coefficients are defined by the equations:

$$F_1 = p_1 - 2F_3 - F_4 - F_5 - y_1(\infty) \tag{47}$$

$$F_2 = p_2 - F_4 - F_5 - 2F_6 - y_2(\infty) \tag{48}$$

$$F_3 = p_1 p_{11} p_{111}{}^2 \nu_{11}(\infty) \tilde{\nu}_{11}(\infty) \tag{49}$$

$$F_4 = p_1 p_{11} p_{112} p_{121} \nu_{21}(\infty) \tilde{\nu}_{11}(\infty) \tag{50}$$

$$F_5 = p_1 p_{11} p_{112} p_{121} \nu_{11}(\infty) \tilde{\nu}_{21}(\infty) \tag{51}$$

$$F_6 = p_2 p_{21} p_{122} p_{221} \nu_{21}(\infty) \tilde{\nu}_{21}(\infty) \tag{52}$$

For our purposes, the $t = \infty$ limit of eq 46 ("double-infinite" oxidation limit) suffices. One obtains:

$$\begin{aligned} y^{(2)}(\infty;\infty) = 1 - p_1 p_{11} & \left\{ p^2{}_{111} \nu_{11}(\infty) \tilde{\nu}_{11}(\infty) + \right. \\ & \left. \frac{k_1}{k_1 + k_2} p_{112} p_{121} [\nu_{11}(\infty) \tilde{\nu}_{21}(\infty) + \nu_{21}(\infty) \tilde{\nu}_{11}(\infty)] \right\} \end{aligned} \tag{53}$$

Equation 53 could have been obtained directly by observing that out of the unoxidized configurations at the end of the first reaction, only the doublets 1*111*, 1*121*, and 1*211* will not get completely oxidized during the second oxidation.

**B. The Analysis.** We now follow the strategy mentioned in section I to determine those values of the three independent conditional probabilities $p_{11}$, $p_{111}$, and $p_{222}$ that are compatible with the experimental results

$$y(p_{11},p_{111},p_{222};\infty) = y_{\text{exptl}}(\infty) \tag{54}$$

$$d(p_{11},p_{111},p_{222};\infty) = d_{\text{exptl}}(\infty) \tag{55}$$

$$y^{(2)}(p_{11},p_{111},p_{222};\infty;\infty) \geq 98.5\% \tag{56}$$

We use the previously assumed values

$$k_2/k_1 = 1.4 \tag{57}$$

and

$$a = \tilde{a} = 0.5 \tag{58}$$

The theoretical values [the lhs of eq 54–56] do not depend on $k_1$ and $k_2$ separately, nor do they depend on the two-side protection constant $c \equiv c_{22}$ (assumed larger than zero), see ref 3 and 4.

We exhibit in Figures 4 and 5 the results for guaran ($p_1 = 56.25\%$, $y_{\text{exptl}}(\infty) = 0.59 \pm 0.01$, $d_{\text{exptl}}(\infty) = 1.75 \pm 0.15$).

The reader should notice that the curves stop at $p_{11} \simeq 0.625$. This is because the conditional probabilities get unphysical. Figure 5 implies that only values of $p_{11}$ larger than or equal to 0.6 are able to meet the criterion of yielding $y^{(2)}(\infty;\infty) \geq 0.985$, and barely so. Figure 4 shows that for $p_{11} \geq 0.60$ the uncertainty in $p_{111}$ and, especially, in $p_{222}$ originating from the experimental error in $d(\infty)$ is at its minimum. Since values of $p_{11}$ larger than 0.625 are unphysical the relevant parameter space for guaran as found from Figures 4 and 5 will be given by the shaded regions in Figure 4.

Having found that, with $k_2/k_1 = 1.4$ and $a = \tilde{a} = 0.5$, the criteria in eq 54–56 are scarcely met because of $y^{(2)}(\infty;\infty)$ barely reaching the value of 98.5%, it is natural to ask whether the hypothesis of $(a + \tilde{a})/2 = 0.5$ (section III) is valid, i.e., whether *smaller* values of $(a + \tilde{a})/2$ could lead to a more satisfactory fulfilment of the criteria in eq 54–56. Since also for $y^{(2)}(\infty;\infty)$ the main dependence of $a$ and $\tilde{a}$ is through the combination $(a + \tilde{a})$, we set $a = \tilde{a}$. Figure 6 shows the results for $y^{(2)}(\infty;\infty)$ as a function of $a$ for $p_{11} = 0.6$ and for values of $p_{111}$ and $p_{222}$ yielding $y_{\text{th}}(\infty) = 0.59$ and $d_{\text{th}}(\infty) = 1.75$ and 1.9, respectively (the results for $d(\infty) = 1.6$ were essentially the same as for $d(\infty) = 1.75$). Figure 6 shows clearly that values of $a$ less than 0.5 fail to meet the criterion in eq 56. On the other hand $a$ cannot be larger than 0.5 (section III). Thus our results substantiate the hypothesis $a = \tilde{a} = 0.5$. But the reader should note that our conclusion has been reached for $k_2/k_1 = 1.4$.

One may still inquire whether other values of the ratio $k_2/k_1$ would lead to higher values of $y^{(2)}(\infty;\infty)$. Figure 7

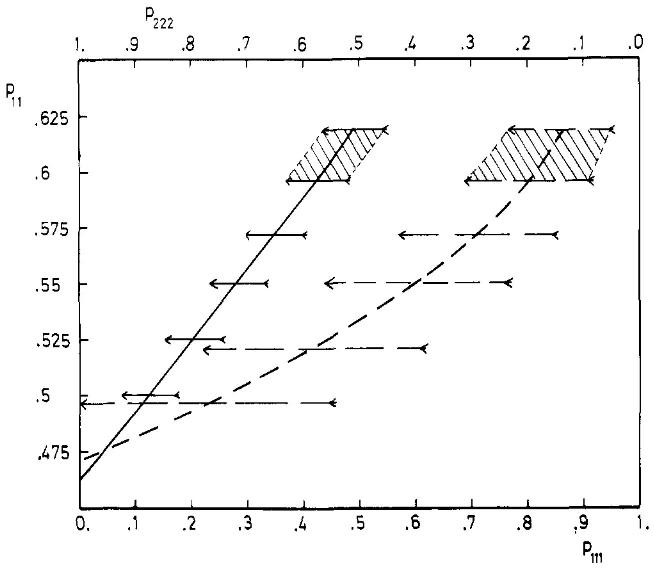


**Figure 4.** The values of $p_{11}$, $p_{111}$, and $p_{222}$ yielding theoretical results equal to the experimental ones for guaran: $y_{th}(\infty) = 0.59$, $d_{th}(\infty) = 1.75$. The reactivity ratio is $k_2/k_1 = 1.4$ and the protection constant is $a = \tilde{a} = 0.5$. The results are plotted as a projection on the $p_{11}$–$p_{111}$ plane (continuous curve) and as a projection on the $p_{11}$–$p_{222}$ plane (broken curve). The arrows show the extent of the uncertainty arising from the experimental error in the reaction limit $d_{exptl}(\infty) = 1.75 \pm 0.15$. The arrowhead corresponds to $d(\infty) = 1.9$ and the arrowfoot to $d(\infty) = 1.6$ ($y(\infty)$ being fixed to 0.59). One sees that the uncertainty is large for $p_{222}$, especially for low values of $p_{11}$. For the explanation of the shaded regions see the main text. The results for $y^{(2)}(\infty;\infty)$ are given in Figure 5.

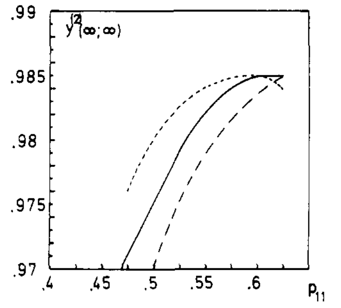


**Figure 5.** The dependence of the double-infinite oxidation limit $y^{(2)}(\infty;\infty)$ on $p_{11}$ for sets of values ($p_{11}$,$p_{111}$,$p_{222}$) yielding $y_{th}(\infty) = 0.59$ and (a) $d_{th} = 1.9$ (broken curve), (b) $d_{th}(\infty) = 1.75$ (continuous curve), (c) $d_{th}(\infty) = 1.6$ (stippled curve). Composition: $p_1 = 56.25\%$. Reactivity ratio: $k_2/k_1 = 1.4$. Protection constants: $a = \tilde{a} = 0.5$.

shows that for $k_2/k_1 > 1.4$, this is indeed so. Taking the upper limit 1.5 for the value of $k_2/k_1$, eq 45, one is able to satisfy the criteria in eq 54–56 with $y^{(2)}(\infty;\infty) = 98.8\%$. Thus, it appears possible that $k_2/k_1$ is in the range around 1.5. In this case, $a = \tilde{a}$ could be slightly less than 0.5 without violating the criterion in eq 56. In order to keep the speculation to a minimum, we shall use $k_2/k_1 = 1.4$ and $a = \tilde{a} = 0.5$ until new measurements supply additional evidence.

Using the same methods, the parameter space compatible with the experimental limits of oxidation $y_{exptl}(\infty)$, $d_{exptl}(\infty)$, and $y^{(2)}{}_{exptl}(\infty;\infty) \geq 98.5\%$ for locust bean gum can be found. Here, there are plenty of parameter sets yielding $y^{(2)}(\infty;\infty) > 99\%$. This is simply due to the low percentage of completely protecting units: $p_1 = 23.46\%$.

Finally, we attempt to match the experimental results for locust bean gum[7,8] and guaran[5,6,8] with parameters situated in the narrow regions of parameter space found in this way. One has previously to determine the absolute value of one reactivity, say $k_1$, and of $c$. It is convenient to work with the experimental values for locust bean gum which are first class in contrast to those of guaran which are less reliable, as is easily seen by plotting them. By matching the initial part of the oxidation curve we found

$$k_1 = 0.020\ \text{min}^{-1} \tag{59}$$

and with $k_2 = 1.4k_1$

$$k_2 = 0.028\ \text{min}^{-1} \tag{60}$$

The value of $c \equiv c_{22}$ was roughly estimated from an exponential least-squares fit to the asymptotic part of the oxidation (very large time values.) This protection constant was subsequently adjusted by matching the asymptotic part with our theoretical values for $P(t)$ and $d(t)$. We found

$$c = 0.01 \tag{61}$$

Figure 8 shows the result for locust bean gum ($p_1 = 23.46\%$) with a sequential structure described by the conditional probabilities

$$p_{11} = 0.30 \tag{62}$$

$$p_{111} = 0.60 \tag{63}$$

$$p_{222} = 0.95 \tag{64}$$

The total root mean square (rms) deviation between 39

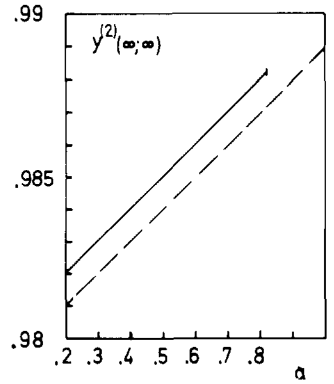


**Figure 6.** The dependence of $y^{(2)}(\infty;\infty)$ on $a$, for sets of values ($p_{11} = 0.60$, $p_{111}$, $p_{222}$) yielding $y_{th}(\infty) = 0.59$ and (a) $d_{th}(\infty) = 1.75$ (continuous line), (b) $d_{th}(\infty) = 1.9$ (broken line) ($p_1 = 0.5625$, $k_2/k_1 = 1.4$.) The continuous line stops where the conditional probabilities become unphysical.

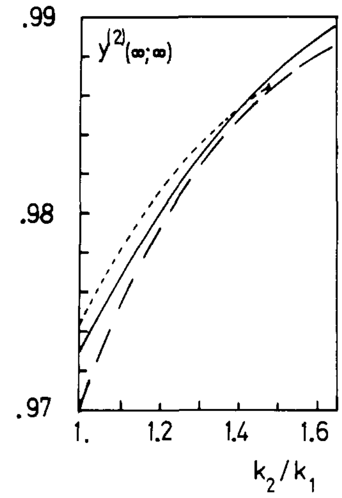


**Figure 7.** The dependence of $y^{(2)}(\infty;\infty)$ on $k_2/k_1$ for sets of values ($p_{11} = 0.6$, $p_{111}$, $p_{222}$) yielding $y_{th}(\infty) = 0.59$ and (a) $d_{th}(\infty) = 1.9$ (broken curve), (b) $d_{th}(\infty) = 1.75$ (continuous curve), (c) $d_{th}(\infty) = 1.6$ (stippled curve; this last stops where the probabilities become unphysical). One has $p_1 = 56.25\%$, $a = \tilde{a} = 0.5$.

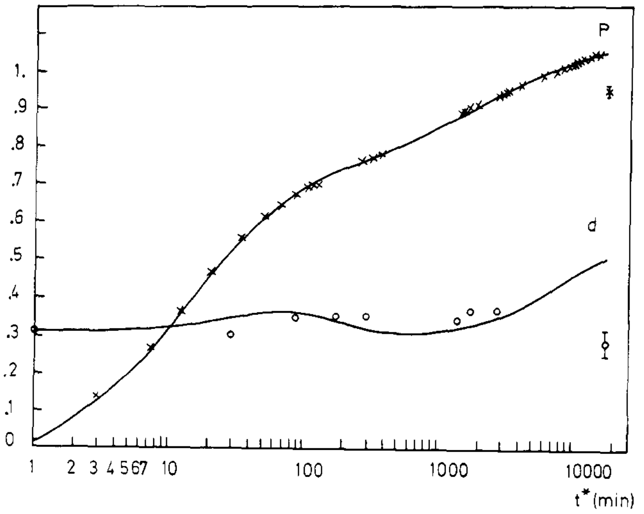


**Figure 8.** Theory and experiment for periodate consumption, *P*, and ratio of unoxidized units, *d*, for locust bean gum. The two points with bars are not experimental values but illustrate the typical experimental error. The parameters used were eq 57–64 and $t^*$ is the real time.

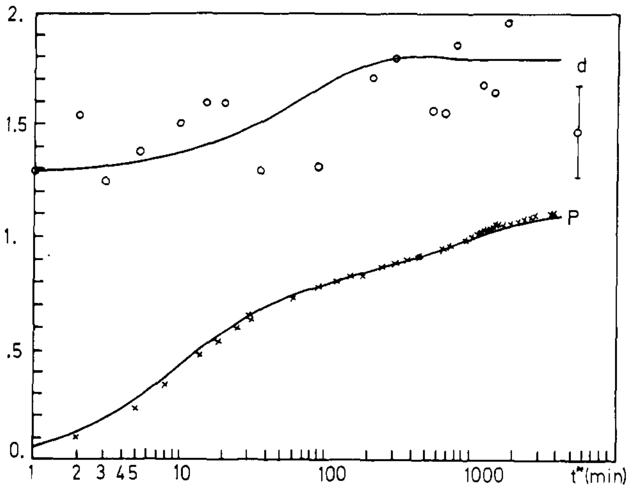


**Figure 9.** Theory and experiment for periodate consumption, *P*, and ratio of unreacted units, *d*, for guaran. The parameters are given by eq 57–61 and 65–67. The point with bars is not an experimental value but illustrates the typical experimental uncertainty in *d*. The size of the crosses indicates the experimental uncertainty in *P*. The time, $t^*$, is the real (experimental) time.

experimental points $P_{\text{exptl}}(t)$ and the theoretical ones is 0.038, corresponding to a rms deviation of 0.006 per result (the experimental uncertainty is somewhat less than 0.01). The total rms deviation between seven experimental values $d_{\text{exptl}}(t)$ and the theoretical ones is 0.079, corresponding to a rms deviation per result of 0.03 (the experimental uncertainty is about 0.045, or 15%, according to Painter). For the parameters used we find the theoretical result $y^{(2)}(\infty;\infty) = 99.3\%$. Altogether, the agreement is as good as for amylose.[4,14,15]

In comparison with locust bean gum the results for guaran are less satisfactory. Figure 9 shows these results. The parameters are $p_1 = 56.25\%$ and

$$p_{11} = 0.62 \tag{65}$$

$$p_{111} = 0.489 \tag{66}$$

$$p_{222} = 0.129 \tag{67}$$

In the range $t^* \leq 20$ min the theoretical results for *P* lie 2.5 times the experimental uncertainty *above* the experimental results. The results are in good agreement in the range 20 min $< t^* \leq 1000$ min. Finally, for $t^* > 1000$ *min* the theoretical results lie 1.5 times the experimental uncertainty *below* the experimental results for *P*. The rms deviation between theoretical and experimental values for *P* for altogether 37 points is 0.133, i.e., 0.022 per result. The experimental uncertainty for guaran cannot be as good as for locust bean gum; one would expect this experimental uncertainty to be in the average ±0.015.

Obviously, the experimental points for *d* scatter very much, about ±15% in the mean. The rms deviation between theory and experiment for altogether 16 results is 0.698, i.e., 0.175 or 12% per result.

Without having a rigorous proof, it appears to us impossible to get a substantially better agreement between theory and experiment for guaran with $k_2/k_1 = 1.4$, $a = \tilde{a} = 0.5$.

Thus, while the agreement between theory and experiment is excellent for locust bean gum, guaran fails to provide an agreement of similar quality, at least for the total periodate consumption *P*. However, even for guaran the agreement seems to be good enough to permit deduction of the approximate sequential structure.

More details can be found in a monograph[4] that will be sent upon request.

## V. Comments

The reasons for the difference in quality of agreement between experiment and theory for guaran and locust bean gum may become clearer on taking the following points into consideration:

(a) Although the theoretical results were gauged by setting $y_{\text{th}}(\infty) = y_{\text{exptl}}(\infty)$, one sees from Figure 9 that at the end of the experiment (here $t \simeq 3000$ min), the theoretical results for *P* for guaran lie definitely below the experimental points. The reason must be that $t = 3000$ min is not large enough. In fact, the kinetics of guaran and locust bean gum *must* develop on the same time scale. From Figure 8 one sees that even after 15 000 min (more than 10 days) the oxidation limit for locust bean gum has not been obtained. Hence, guaran too cannot get fully oxidized until after 10 days. The reason why the oxidation of guaran for $t \simeq 3000$ min proceeds extremely slowly is *not* that the oxidation is nearly finished but that the percentage of oxidizable configurations left is very low (much lower than for locust bean gum at corresponding times). Therefore, one must expect $y_{\text{exptl}}(\infty) > 0.59$, probably $0.60 \leq y_{\text{exptl}}(\infty) \leq 0.63$. (There is clearly no contradiction in expecting the oxidation limit to grow by about 3% in 12 more days and in stating that repeated measurements within 1 or 2 days in the asymptotic region seemingly give the same result.) The important point now is that with $k_2/k_1 = 1.4$ and $a = \tilde{a} = 0.5$ the criteria

$$y_{\text{exptl}}(\infty) \geq 0.60 \tag{68}$$

$$d_{\text{exptl}}(\infty) = 1.75 \pm 0.15 \tag{69}$$

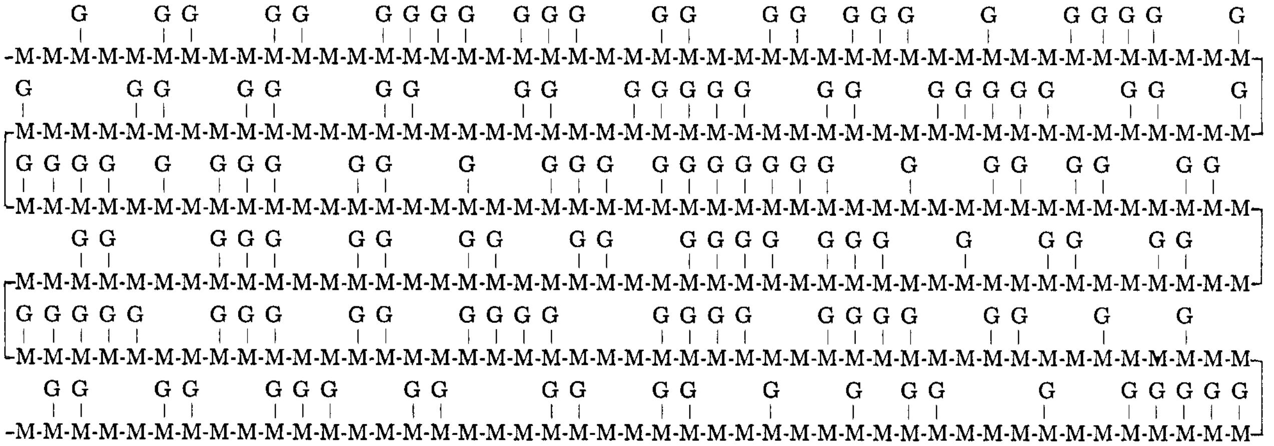
$$y^{(2)}(\infty;\infty) \geq 98.5\% \tag{70}$$

*cannot* be fulfilled (because of $y^{(2)}(\infty;\infty)$ getting smaller than 98.5%).

(b) From Figure 9 one sees that the experimental points lie significantly below the theoretical ones until $t^* \simeq 30$ min and above the theoretical values for $t^* > 1000$ min. One could expect that different ratios of $k_2/k_1$, or different values of the protection constant, *a*, may affect results in these regions in the right direction. There would be hope that the results for guaran may significantly change by varying $k_2/k_1$, while those for locust bean gum will not vary that much, because locust bean gum contains only 23% of 1 units (i.e., its behavior is to a greater extent determined by one unit type, viz., unbranched mannose). While

Scheme I

Scheme II



it is correct that by holding $y(\infty) = 0.59$ the ratio $k_2/k_1 = 1.5$ leads to increase in $y^{(2)}(\infty;\infty)$, see Figure 7, it proved impossible *both* to raise $y(\infty)$ enough to definitely improve the agreement of theory with experiment *and* to keep $y^{(2)}(\infty;\infty)$ above the critical value of 98.5%. Thus, the ratio $k_2/k_1 = 1.5$ does not lead to a substantially better agreement for guaran. Clearly, the double-infinite oxidation limit $y^{(2)}(\infty;\infty)$ plays a crucial role here, and the question arises how reliable is the evidence that $y^{(2)}(\infty;\infty)$ is close to 100% by 1.5% or less. While there exist many careful measurements by Painter and co-workers supporting that result the experiments are exacting and they consist of two subsequent measurements. It cannot be excluded that progress in experimental techniques could lead to lower values for $y^{(2)}(\infty;\infty)$ for guaran. Such a result would significantly influence the theoretical analysis.

(c) The quantity $P$ is much less sensitive to variations of the parameters than is the ratio $d$. Unfortunately, the experimental uncertainty in the difficult measurement of $d$ is so large that one cannot use this quantity for the fine adjustment of the parameters. Progress in the experimental techniques to determine the reaction ratio $d$ would be extremely important.

(d) Finally, the possibility that the presence of a galactopyranosyl group in a neighboring mannose could influence the reactivity of the unit in question should not be disregarded. If this were so, the reactivities $k_1$ and $k_2$ of our model would be *effective* reactivities depending on the polymer, i.e., being different for guaran and locust bean gum. We did find for guaran that with $k_1 = 0.016$ min$^{-1}$, $k_2 = 0.0224$ min$^{-1}$, and otherwise the same parameters and probabilities, the agreement between theory and experiment gets somewhat better. But the results are not so impressive that one could accept them as proof for the hypothesis.

Altogether, it seems that further studies on guaran, on short mannan chains, and on other galactomannans as well, in order to obtain more results for the oxidation curve, for $k_2/k_1$ and for $a$ would be very worthwhile. However, the data obtained in the pioneering work by Ishak and Painter[5,8] on the one hand and by Hoffman, Lindberg, and Painter[6,7] on the other provide excellent experimental material to compare with the theory. It seems probable that our results for the sequential structure of locust bean gum, eq 62–64, and guaran, eq 65–67, will prove to be reasonably correct also if new experiments alter the situation somewhat. As a rough estimate we expect the uncertainties in $p_{11}$, $p_{111}$, and $p_{222}$ to be *at least* of a size corresponding to the shaded areas in Figure 4. I.e., for guaran

$$p_{11} = 0.62 \pm 0.02 \qquad (71)$$

$$p_{111} = 0.49 \pm 0.05 \qquad (72)$$

$$p_{222} = 0.13 \pm 0.10 \qquad (73)$$

Finally, we present the complete results in terms of Markov probabilities, of doublet frequencies, $F_{ij} = p_i p_{ij}$, and of triplet frequencies, $F_{ijl} = p_i p_{ij} p_{ijl}$ in Table II.

Using a random number generator we produced representative pictures of locust bean gum and guaran. Using the symbols G and M for galactopyranosyl and mannosepyranosyl residues respectively, a typical portion of a chain of locust bean gum would be Scheme I, whereas a typical portion of the guaran chain would look like Scheme II.

The depicted portion of the chain for locust bean gum agrees with the qualitative sequential structure proposed by Courtois and Le Dizet[27] (see also p 274 or ref 9). For further comparison of our results we refer to an article

Table II
Sequential Structure of Locust Bean Gum and Guaran in Terms of Markov Probabilities and of Doublet and Triplet Frequencies (For Uncertainties See Main Text)

| | guaran | locust bean gum |
|---|---|---|
| Markov probabilities | | |
| $p_1$ | 0.5625 | 0.2346 |
| $p_{11}$ | 0.62 | 0.30 |
| $p_{22}$ | 0.51 | 0.79 |
| $p_{111}$ | 0.49 | 0.60 |
| $p_{211}$ | 0.83 | 0.17 |
| $p_{122}$ | 0.91 | 0.18 |
| $p_{222}$ | 0.13 | 0.95 |
| doublet frequencies | | |
| $F_{11}$ | 0.35 | 0.07 |
| $F_{12} = F_{21}$ | 0.215 | 0.165 |
| $F_{22}$ | 0.22 | 0.60 |
| triplet frequencies | | |
| $F_{111}$ | 0.17 | 0.04 |
| $F_{211} = F_{112}$ | 0.18 | 0.03 |
| $F_{122} = F_{221}$ | 0.19 | 0.03 |
| $F_{222}$ | 0.03 | 0.57 |
| $F_{121}$ | 0.02 | 0.13 |
| $F_{212}$ | 0.04 | 0.14 |

specially conceived for carbohydrate chemists.[28] But we do here emphasize again that we do not claim to settle the difficult question of the sequential structure of these galactomannans in this article (nor in ref 28). The conditional probabilities found for locust bean gum and guaran lead to agreement with today's available experimental data[5-8] but the richness in parameters of our model makes it difficult to estimate the effect of the fine adjustment of the reactivities, etc., that more refined data undoubtly will demand some future day. In fact, even with today's data we have no proof that our parameters lead to the optimum fit with experiment but we did try to optimize our results as far as one could do with our time consuming computer program.

Sequential analysis of copolymers can be carried out by several techniques, the most used one being nuclear magnetic resonance (NMR) spectroscopy.[29] However, heteropolysaccharides with a nonregular primary structure have proved to be very difficult to analyze by any method. It seems, though, that Grasdalen, Larsen, and Smidsrød have achieved a breakthrough in this field by obtaining through $^{13}$C NMR spectroscopy the full doublet frequencies and part of the triplet frequencies of alginate.[30] The triplet frequencies that are lacking refer to configurations having α-L-guluronate residues in the center, which give very weak NMR signals. Work to overcome this difficulty is in progress.

Our studies of guaran and locust bean gum also represent the sequential analysis of heteropolysaccharides having a nonregular primary structure. Similar studies of alginate by periodate oxidation are possible. Thus, there are good prospects for complementing NMR studies with our methods. On the other hand, Grasdalen and Painter[31] have been able to carry out an NMR analysis of doublet frequencies in guaran. Their preliminary results, $F_{11}$ = 30% and $F_{22}$ = 21%, are in reasonable agreement with ours (Table II), if due consideration is given to the uncertainties in both analyses.

Finally, sequential analysis by periodate oxidation may be applied to many other polysaccharides. In particular, there is no reason to doubt that this method will also be appropriate for other galactomannans.

**Acknowledgment.** Drs. P. C. Hemmer and T. Painter were always available as discussion partners, critical friends, and T. Painter also as an extremely helpful experimentalist. It is a great pleasure to acknowledge my indebtedness. This work has been supported by Nansenfondet, The Norwegian Research Council for Science and the Humanities (NAVF), and Norges Tekniske Høgskoles Fond to which all I am deeply indebted.

## Appendix A. The Second Oxidation after Completed First Oxidation of a Binary Copolymer

We list the probability of occurrence of the unreacted singlets at the end of the first oxidation, $t \to \infty$:

...–1*–1–1*–...

$$p_1 p_{11} p_{111} P_\infty\{\ldots\text{–}1^*\text{–}1\text{–}1^*\text{–}\ldots\} \tag{A1}$$

...–1*–1–2*–...

$$p_1 p_{12} p_{112} P_\infty\{\ldots\text{–}1^*\text{–}1\text{–}2^*\text{–}\ldots\} \tag{A2}$$

...–2*–1–1*–...

$$p_1 p_{12} p_{112} P_\infty\{\ldots\text{–}2^*\text{–}1\text{–}1^*\text{–}\ldots\} \tag{A3}$$

...–1*–2–1*–...

$$p_1 p_{12} p_{121} P_\infty\{\ldots\text{–}1^*\text{–}2\text{–}1^*\text{–}\ldots\} \tag{A4}$$

...–1*–2–2*–...

$$p_1 p_{12} p_{122} P_\infty\{\ldots\text{–}1^*\text{–}2\text{–}2^*\text{–}\ldots\} \tag{A5}$$

...–2*–2–1*–...

$$p_1 p_{12} p_{122} P_\infty\{\ldots\text{–}2^*\text{–}2\text{–}1^*\text{–}\ldots\} \tag{A6}$$

(For the notation see ref 3 or 4).

Using the principle of independence of unreacted neighbors and eq 20 and 21 of ref 3, the probability of occurrence of unreacted doublets reads:

...–1*–1–1–1*–...

$$p_1 p_{11} p^2{}_{111} \nu_{11}(\infty) \tilde{\nu}_{11}(\infty) \tag{A7}$$

...–1*–1–2–1*–...

$$p_1 p_{11} p_{112} p_{121} \nu_{21}(\infty) \tilde{\nu}_{11}(\infty) \tag{A8}$$

...–1*–2–1–1*–...

$$p_1 p_{11} p_{112} p_{121} \tilde{\nu}_{21}(\infty) \nu_{11}(\infty) \tag{A9}$$

...–1*–2–2–1*–...

$$p_2 p_{21} p_{122} p_{221} \nu_{21}(\infty) \tilde{\nu}_{21}(\infty) \tag{A10}$$

Obviously, $y_1(\infty)$ and $y_2(\infty)$ can be expressed in terms of these probabilities:

$$y_i(\infty) = p_i - p_i p_{i1} p_{1i1} P_\infty\{\ldots\text{–}1^*\text{–i–}1^*\text{–}\ldots\} - p_i p_{i1} p_{1i2} P_\infty\{\ldots\text{–}1^*\text{–}i\text{–}2^*\text{–}\ldots\} - p_i p_{i2} p_{2i1} P_\infty\{\ldots\text{–}2^*\text{–}i\text{–}1^*\text{–}\ldots\} - \sum_{m,n=1}^{2} (\delta_{mi} + \delta_{ni}) p_m p_{m1} p_{1mn} p_{mn1} P_\infty\{\ldots\text{–}1^*\text{–}m\text{–}n\text{–}1^*\text{–}\ldots\} \tag{A11}$$

The fraction of units that have reacted at the time $t$ after the second oxidation has started is given as the products of the probability of occurrence of the singlet or doublet times the average *number* of reacted units in the singlet or doublet. The average number of reacted units in a singlet $i$ after the time $t$ is:

$$1 - e^{-k_i t} \tag{A12}$$

and in a doublet $i - j$:

$$2 - \frac{k_j}{k_j + k_i(1 - a_j)} e^{-a_j k_i t} - \frac{k_i}{k_i + k_j(1 - \tilde{a}_i)} e^{-\tilde{a}_i k_j t} + \left\{ \frac{k_j}{k_j + k_i(1 - a_j)} + \frac{k_i}{k_i + k_j(1 - \tilde{a}_i)} - 2 \right\} e^{-(k_i + k_j)t} \tag{A13}$$

Using eq A11 to eliminate $P_\infty\{...-1^*-i-1^*-...\}$, $P_\infty\{...-1^*-i-2^*-...\}$, $P_\infty\{...-2^*-i-1^*-...\}$ one finally obtains, for the *total* fraction of oxidized units, the result in eq 46.

## References and Notes

(1) J. J. González and P. C. Hemmer, *J. Polym. Sci., Polym. Lett. Ed.*, **14**, 645 (1976).
(2) J. J. González and P. C. Hemmer, *J. Chem. Phys.*, **67**, 2496 (1977).
(3) J. J. González and P. C. Hemmer, *J. Chem. Phys.*, **67**, 2509 (1977).
(4) J. J. González, Avhandling for den tekniske doktorgrad, Trondheim, 1977.
(5) M. F. Ishak and T. Painter, *Acta Chem. Scand.*, **27**, 1268 (1973).
(6) J. Hoffman, B. Lindberg, and T. Painter, *Acta Chem. Scand., Ser. B*, **29**, 137 (1975).
(7) J. Hoffman, B. Lindberg, and T. Painter, *Acta Chem. Scand., Ser. B*, **30**, 365 (1976).
(8) T. Painter, unpublished.
(9) I. C. M. Dea and A. Morrison, *Adv. Carbohydr. Chem.*, **31**, 241 (1975).
(10) T. Painter and B. Larsen, *Acta Chem. Scand.*, **24**, 813 (1970); **24**, 2366 (1970).
(11) T. Painter and B. Larsen, *Acta Chem. Scand.*, **24**, 2724 (1970).
(12) M. F. Ishak and T. Painter, *Acta Chem. Scand.*, **25**, 3875 (1971).
(13) The value previously reported by J. Hoffman, B. Lindberg, and T. Painter, *Acta Chem. Scand., Ser. B*, **30**, 365 (1976), was $y(\infty) = 0.80$. However, a recent experiment by T. Painter (unpublished) shows that even after 11 days the reaction has not completely stopped although it proceeds very slowly. We have performed a least-squares fit analysis on these data and by extrapolation found a suggested oxidation limit of 86%.
(14) J. J. González, *Biophys. Chem.*, **2**, 23 (1974).
(15) O. Smidsrød, B. Larsen, and T. Painter, *Acta Chem. Scand.*, **24**, 3201 (1970).
(16) C. B. Arends, *J. Chem. Phys.*, **39**, 1903 (1963).
(17) P. C. Hemmer, unpublished.
(18) H. Morawetz, "Macromolecules in Solution", 2nd ed, Wiley, New York, N.Y., 1975.
(19) R. J. Yu and C. T. Bishop, *Can. J. Chem.*, **45**, 2195 (1967).
(20) M. F. Ishak and T. J. Painter, *Carbohydr. Res.*, **64**, 189 (1978).
(21) A. S. Perlin, *Adv. Carbohydr. Chem.*, **14**, 20 (1959).
(22) T. Painter, unpublished.
(23) Typical computation time with a UNIVAC 1121 for 40 points of the oxidation curve for, say, guaran is 5–10 minutes if one has previously found the ideal time scale by means of eq 38. If one had to find the transformation from ideal times a posteriori, as described in p 81 ff, a computation time of two hours per oxidation curve would be unavoidable.
(24) T. Painter and B. Larsen, *Acta Chem. Scand.*, **27**, 1957 (1973).
(25) P. C. Hemmer and J. J. González, *J. Polym. Sci., Polym. Phys. Ed.*, **15**, 321 (1977).
(26) J. J. González, unpublished.
(27) J. E. Courtois and P. Le Dizet, *Bull. Soc. Chim. Biol.*, **50**, 1695 (1968); **52**, 15 (1970).
(28) T. Painter, J. J. González, and P. C. Hemmer, *Carbohydr. Res.*, in press.
(29) See for instance F. A. Bovey, "High Resolution NMR of Macromolecules", Academic Press, New York, N.Y., 1972.
(30) H. Grasdalen, B. Larsen, and O. Smidsrød, *Carbohydr. Res.*, **56**, C11 (1977).
(31) H. Grasdalen and T. Painter, unpublished.
(32) All results on $y_1(t)$ and $y_2(t)$ are exact by 0.2% and were obtained by numerical computation of eq 7–10. Series (10) was truncated when the required accuracy was achieved and standard numerical integration routines were applied to evaluate eq 7. For details see ref 3 and 4.

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# Studies on the Mechanism of Alternating Radical Copolymerization: Quantitative Separation of the Participation of the Free Monomer and the Charge-Transfer Complex Monomer in the *N*-Vinylcarbazole–Diethyl Fumarate and *N*-Vinylcarbazole–Fumaronitrile Systems

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ABSTRACT: Described is the first example of the quantitative analysis of the degree of participation of the free monomer and the charge-transfer complex monomer in the 1:1 alternating radical copolymerization which was achieved by applying the method recently developed by us to the *N*-vinylcarbazole–diethyl fumarate and *N*-vinylcarbazole–fumaronitrile systems. The results show that the reaction of the free monomer contributes to the polymerization rate to a much greater extent than that of the charge-transfer complex monomer in both systems, although the degree of participation of each of them varies significantly depending on the total monomer concentration and on the monomer feed composition. The relative reactivity of the charge-transfer complex monomer to the free monomer toward growing polymer radicals and the termination process were estimated for the *N*-vinylcarbazole–diethyl fumarate system in which the copolymerization proceeds homogeneously.

The 1:1 alternating radical copolymerization has been an area of active research in free-radical polymerizations but the mechanism has been a subject of controversy. Two different mechanisms have been proposed to explain the 1:1 alternation in the resulting copolymer. One is the cross-reaction of free monomers whose transition state is considered to be more stabilized than that of the homoreaction due to either the difference in polarity between monomer pairs or charge-transfer interactions between a growing polymer radical and a monomer.[1,2] The other is the homopolymerization of a charge-transfer complex formed between monomer pairs, i.e., a charge-transfer complex monomer which is usually detected spectrophotometrically in many 1:1 alternating radical copolymerization systems.[3] However, both mechanisms are not capable of explaining satisfactorily polymerization features such as described below.

The initial rate of the 1:1 alternating radical copolymerization does not always maximize at the monomer feed molar ratio of 1:1 under a fixed total monomer concentration as is found in, e.g., styrene–maleic anhydride,[4] *N*-vinylcarbazole (VCZ)–diethyl fumarate (DEF), or VCZ–fumaronitrile (FN) systems.[5] This is inconsistent with the mechanism of the homopolymerization of the

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