Two Different Kinetic Approaches for the Determination of Number-average Molecular Weight of Amylose

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Two Different Kinetic Approaches for the Determination of Number-average Molecular Weight of Amylose

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Two different kinetic methods (exo-enzyme kinetic (I) and polarographic o-phenylenediamine (II) methods) for the determination of number-average molecular weight ($\bar{M}_n$) of amylose have been developed.

I. A theory has been presented which describes the time course of exo-enzyme-catalyzed hydrolysis of linear high polymer substrates accompanied by a competitive inhibition by products. The theory predicts that there is a linear relationship between $t/p$ and $p$, where $p$ is the concentration of product at time $t$, and that the slope $s$ of the plot of $t/p$ versus $p$ is proportional to the $\bar{M}_n$ of the polymer substrate when a given amount of substrate is taken. Based on the proportionality between $s$ and $\bar{M}_n$, a kinetic method was proposed to determine $\bar{M}_n$ of linear polymer substrate by using an exo-enzyme hydrolyzing the polymer.

The theory was applied to the hydrolytic reactions of various fractions of amylose of different degrees of polymerization catalyzed by crystalline exo-enzyme glucoamylase of *Rhizopus delemar*. A good agreement was obtained between the $\bar{M}_n$ values determined by the present kinetic method and those determined by osmotic pressure and reducing end-group measurements.

This method is especially useful for the determination of $\bar{M}_n$ of amylose having larger $\bar{M}_n$ values for which conventional methods would fail to give reliable data.

II. The time course of the polarographic limiting current development due to quinoxalines formed in the reaction of maltodextrin with o-phenylenediamine at pH 10 and at 100°C was studied. The development of the limiting current was found to be of zero order in the early reaction period and the current at the 20 min. reaction time was proportional to the molar concentration of the terminal carbonyl group, i.e. to the reciprocal $\bar{M}_n$ of maltodextrins. Taking maltodextrin (anhydroglucose units AGU=12.6±0.1) as standard, $\bar{M}_n$'s of various fractions of amylose and maltodextrins were obtained. They are in good agreement with those by other methods. This method is characteristic of the availability for a wide range of $\bar{M}_n$.

INTRODUCTION

Determination of number-average molecular weight ($\bar{M}_n$) of amylose and maltodextrin is usually made by the measurement of osmotic pressure$^{1,2}$ or by end-group analysis (for example, reducing power measurement by Somogyi-Nelson method).$^{3-8}$ For all of these methods, the observable quantity, from which the $\bar{M}_n$ is determined, decreases with increasing molecular weight of the sample for a given amount of the sample taken. It is practically impossible or very difficult to obtain reliable data for amylose having molecular weights more

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than ca. 500,000. For amylose of higher average molecular weight, therefore, a new method which is essentially different from the conventional ones may be needed.

Recently in our laboratory two different kinetic methods for this problem have been developed: The one is based on a kinetic analysis of the time course of consecutive liberation of glucose from the non-reducing end of amylose catalyzed by exo-amylase. This will be termed exo-enzyme kinetic method.91 The other is also based on a kinetic follow of the time course of the polarographic reduction wave due to quinoxalines which are produced from the reaction of the reducing end of amylose and o-phenylenediamine. This will be termed polarographic o-phenylenediamine method.10 The enzyme kinetic method is especially useful for amyloses of higher molecular weight for which conventional methods would fail to give reliable data, while the polarographic method is characteristic of the availability for a wide range of $M_n$, by using only common reagents.

Of the kinetic approach of this kind, only one method utilizing the over-oxidation by periodate has been known,11 but the method itself does not seem very reliable because of the difficulty of controlling the conditions.

In this paper principles of our two methods and their applications to amylose will be briefly reviewed.

EXO-ENZYME KINETIC METHOD93

a) Principle

Consider an exo-enzyme-catalyzed hydrolysis of a linear high polymer consisting of a number of monomer unit $P$, in which the exo-enzyme $E$ attacks the substrate polymer at its particular end to produce the monomer product $P$. In general, the initial state of substrate is an ensemble of linear polymers of various degree of polymerization (DP). Let $S_i$ represent the substrate molecule having DP = $i$, and $S_n$ be the substrate molecule having the highest DP, $n$, in the initial ensemble of substrate. Each step of degradation of a substrate molecule involves the Michaelis-Menten mechanism,12 and considering the occurrence of competitive inhibition by product $P$, the reaction scheme can be written as follows:

\[
\begin{align*}
E+S_i & \xrightleftharpoons[\kappa^{-1}]{\kappa^+1} ES_i \\
E+P & \xrightarrow[\kappa^{-y}]{\kappa^+y} EP
\end{align*}
\]

\[
\begin{align*}
E+S_n & \xrightarrow[\kappa^{-1}]{\kappa^+} ES_n \\
E+P & \xrightarrow[\kappa^{-y}]{\kappa^+} EP
\end{align*}
\]
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where ES, \((i=2^-n)\) is the intermediate enzyme-substrate complex formed between the enzyme and the substrate molecule \(S_i\), EP is the enzyme-product complex, the product being a competitive inhibitor, and \(k's\) are the rate constants specified in the scheme. For simplicity, the rate constants \(k_{+1}, k_{-1}\) and \(k_{+2}\) are assumed to be independent of DP of the substrate molecule.\(^*\)

The steady-state treatment leads to the \((n-1)\) rate equations for \(S_i\) \((i=2\) to \(n)\) and one for \(P\) as follows:

\[
x_i/dt = KV(x_{i+1} - x_i)/(1 + K \sum_{i=2}^{n} x_i + Kp
\]

and

\[
p/dt = KV(\sum_{i=2}^{n} x_i + 2x_i)/(1 + K \sum_{i=2}^{n} x_i + Kp)
\]

where \(x_i\) and \(p\) are the molar concentrations of \(S_i\) and \(P\), respectively, and \(K, V\) and \(Kp\) are the reciprocal Michaelis constant, the maximal velocity and the reciprocal inhibitor constant, all expressed in molar concentration, respectively, which are given by

\[
K = k_{+1}/(k_{-1} + k_{+2})
\]

\[
V = k_{+2}e_0
\]

\[
Kp = k_{+p}/(k_{-p})
\]

where \(e_0\) is the enzyme concentration.

Except for the latest phase of reaction where an appreciable number of substrate molecule have been converted into product \(P\) and an appreciable amount of \(S_2\) is present, it is reasonable to approximate the sum \(\sum_{i=2}^{n} x_i\) equal to the total molar concentration of substrate present in the initial ensemble of substrate, \(X^o\), and to neglect \(2x_2\) compared with \(X^o\). By using the approximation, Eq. (3) reduces to

\[
p/dt = KVX^o/(1 + KX^o + Kp)
\]

which will hold except for the latest phase of reaction.

Upon integration, Eq. (5) leads to

\[
KVX^o = (1 + KX^o)p + (Kp/2)p^2
\]

Dividing Eq. (7) by \(p\), we have

\[
KVX^o(t/p) = (1 + KX^o) + (Kp/2)p
\]

Thus the plot of \(t/p\) against \(p\) will give a straight line with a slope \(s\), which is given by

\[
s = Kp/2KVX^o
\]

\(^*\) It was found in the glucoamylase-catalyzed hydrolysis of linear substrates,\(^{10}\) that Michaelis constant \(K_m\) decreases and \(k_{+2}\) increases with DP up to DP=ca. 15, but both of the parameters are scarcely dependent on DP for substrates having DP higher than this value. However, the assumption made here that the rate constants \(k_{+1}, k_{-1}\) and \(k_{+2}\) are independent of DP of substrate down to DP=2 does not affect the final results seriously, since we are not interested in the latest phase of reaction where appreciable amounts of smaller substrates will be present.

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Let \( c \) be the substrate concentration expressed in gram per 100 ml. solution, \( \bar{M}_n \) of the initial ensemble of the polymer substrate, is given by

\[
\bar{M}_n = \frac{M_1 x_0}{\Sigma x_0} = 10c/X^0
\]

(9)

where \( M_1 \) and \( x_0 \) are the molecular weight and the molar concentration of the substrate \( S_i \) present in the initial ensemble of substrate. Insertion of Eq. (9) into Eq. (8) leads to

\[
s = K_p \bar{M}_n/20KVC \propto M_n
\]

(10)

Now it is seen that \( s \) is proportional to \( M_n \) of substrate when a given amount (weight) of substrate is taken. Therefore, it is possible to determine the \( M_n \) of a polymer substrate ensemble by measuring the slope \( s \) and comparing it with that obtained for a standard polymer substrate ensemble whose \( M_n \) is already known.

b) Results

Glucoamylase of \textit{Rh. delemar} is one of typical exo-enzymes which catalyzes the complete hydrolysis of starch producing glucose from its non-reducing end.\textsuperscript{14,15} The product glucose has been known to be a competitive inhibitor of this enzyme.\textsuperscript{16,17} Moreover, the enzyme is so stable that no inactivation has so far been observed at all for the period of reaction. The time courses of hydrolytic reaction of several fractions of potato amylose (AF series) and those of partially degraded amylose (DF series) catalyzed by crystalline glucoamylase at 25°C and pH 4.5, were followed by the determination of glucose by the modification of Somogyi-Nelson's method.\textsuperscript{30}

The linear relationship between \( t/p \) and \( p \) predicted by Eq. (7) was checked by plotting \( t/p \) versus \( p \) in Figs. 1 and 2.

![Graph](image-url)

**Fig. 1.** \( t/p \) versus \( p \) plots for AF series.

- ○ : AF I-1 \( M_n = 1,650,000 \)
- △ : AF I-2 \( M_n = 1,100,000 \)
- ● : AF II \( M_n = 360,000 \)

The arrow shows the point of 70% hydrolysis.
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The linearity of $t/p$ versus $p$ plot is seen to hold up to the extent of hydrolysis ca. 70%, above which the curve tends to deviate upwards, i.e., the rate becomes lower than that expected from Eq. (7). Except for this latest phase of reaction, however, good linearity between $t/p$ and $p$ has been proved to hold in accordance with the theory. It is necessary to see whether or not the slope of the linear part of the plot is proportional to $M_n$. Since no reliable value of $M_n$ has been obtained for some fractions of AF series having $M_n$ higher than 500,000, the viscosity-average molecular weight $\bar{M}_v$ will be used in place of $M_n$ to check the proportionality. It may be reasonable to assume that $\bar{M}_v$ is approximately proportional to $M_n$ for these subfractions. Figure 3 shows the plot of the slope $s$ obtained from the linear part of the plot in Fig. 1 against $\bar{M}_v$ for AF series. For DF series, the $M_n$ determined by the end-group analysis was plotted against the slope $s$, as seen in Fig. 4.

Figures 3 and 4 show that there is good proportionality between the slope $s$ and $\bar{M}_v$, and $s$ and $M_n$, respectively. It is possible, therefore, to determine the

- **Fig. 2.** $t/p$ versus $p$ plots for DF series.
  - $\circ$: DF 2 $\bar{M}_v=33,000$
  - $\triangle$: DF 3 $\bar{M}_v=20,000$
  - $\bullet$: DF 5 $\bar{M}_v=13,000$

- **Fig. 3.** Relationship between the slope $s$ and the viscosity-average molecular weight $\bar{M}_v$ for AF series (AF I-1, I-2, I-3 and II).

- **Fig. 4.** Relationship between the slope $s$ and the number-average molecular weight $M_n$ determined by end-group analysis for DF series (DF 2, 3 and 5).
$\bar{M}_n$ of an unknown amylose sample by comparing the slope $s$ with that of a standard amylose sample whose $\bar{M}_n$ is known. Two fractions AF I-1 and DF 2 were chosen as the standard samples for the determination of $\bar{M}_n$ of various amylose fractions by the present kinetic method. Both the osmotic pressure measurement and the end-group analysis gave consistent values of $\bar{M}_n$ for each of these two standard samples.

The procedure for determining $\bar{M}_n$ of an amylose sample by the present method is as follows: Under the same reaction conditions with respect to the substrate concentration $c$ (gram of dried amylose per 100 ml.), the enzyme concentration, pH and temperature, the hydrolytic reaction of the sample and that of the standard sample catalyzed by glucoamylase are followed by a suitable method to obtain the product concentration $p$ as a function of time $t$. From the plot of $t/p$ versus $p$, the slope $s$ is obtained for both samples (see Figs. 1 and 2). The $\bar{M}_n$ of the sample is determined from that of the standard sample, $\bar{M}_n^\circ$, and the slope $s$ of the sample according to the following equation

$$\bar{M}_n = \bar{M}_n^\circ \times \frac{s}{s^\circ}$$

where $s^\circ$ is the slope obtained for the standard sample. The $\bar{M}_n$'s of various fractions of amylose determined in this way are listed in the fifth column of Table 1, together with the ratio of the slopes $s/s^\circ$ in the last column. The error involved in the determination of $\bar{M}_n$ by this method is determined by the error in the evaluation of the slope $s$, which is usually 10%. Thus the final error in $\bar{M}_n$ may be 20% or less, so far as $\bar{M}_n^\circ$ is correct. For the purpose of comparison, the $\bar{M}_n$ and the $\bar{M}_n$ determined by the osmotic pressure measurement and the end-group analysis are also included in Table 1.

<table>
<thead>
<tr>
<th>Fraction number</th>
<th>$\bar{M}_n$</th>
<th>Osmotic pressure</th>
<th>End-group</th>
<th>Enzyme kinetic</th>
<th>$s/s^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF I-1</td>
<td>1,650,000</td>
<td>-</td>
<td>-</td>
<td>1,520,000</td>
<td>3.80</td>
</tr>
<tr>
<td>AF I-2</td>
<td>1,100,000</td>
<td>-</td>
<td>-</td>
<td>920,000</td>
<td>2.29</td>
</tr>
<tr>
<td>AF I-3*</td>
<td>460,000</td>
<td>400,000</td>
<td>420,000</td>
<td>400,000</td>
<td>1.00*</td>
</tr>
<tr>
<td>AF II</td>
<td>360,000</td>
<td>210,000</td>
<td>300,000</td>
<td>270,000</td>
<td>0.68</td>
</tr>
<tr>
<td>AF III-1</td>
<td>540,000</td>
<td>-</td>
<td>-</td>
<td>480,000</td>
<td>1.19</td>
</tr>
<tr>
<td>AF III-2</td>
<td>270,000</td>
<td>220,000</td>
<td>-</td>
<td>200,000</td>
<td>0.50</td>
</tr>
<tr>
<td>DF 2**</td>
<td>57,400</td>
<td>33,000</td>
<td>33,300</td>
<td>33,000</td>
<td>1.00**</td>
</tr>
<tr>
<td>DF 3</td>
<td>33,300</td>
<td>-</td>
<td>20,000</td>
<td>19,000</td>
<td>0.58</td>
</tr>
<tr>
<td>DF 5</td>
<td>15,200</td>
<td>-</td>
<td>13,400</td>
<td>12,000</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* The fractions AF I-3 and DF 2 were used as standard samples of known $\bar{M}_n$ in the determination of $\bar{M}_n$ by the present method for fractions AF and DF, respectively.

Table 1 shows good agreement between $\bar{M}_n$ determined by the present method and that determined by osmotic pressure and or end-group measurements, demonstrating the utility of the present kinetic method. The $\bar{M}_n$'s of some fractions (AF I-1 and I-2) which had not been determined before the present method
Kinetic Approaches for the Determination of Molecular Weight of Amylose became available, since osmotic pressure measurement and end-group analysis could not give any reliable data for them. It may be worthwhile to emphasize that in contrast to the conventional methods the present method is more accurately applicable to the samples having higher molecular weights, since the slope $s$ is larger and more sensitive to $M_n$ for the samples with higher $M_n$ as seen from Figs. 3 and 4. This is the greatest advantage of the present method over the conventional ones. Furthermore, the dependence of the slope $s$ on $M_n$ is larger for lower substrate concentration $c$, which means that we need only a small amount of sample for the determination. (In the present study, 10 to 25 mg. of amylose was used per each run). The applicability and utility of the present method by the use of exo-enzyme kinetics has thus been demonstrated for the determination of $M_n$ of amylose fractions by using glucoamylase exo-enzyme for a wide range of $M_n$.

**POLAROGRAPHIC O-PHENYLENEDIAMINE METHOD**

a) Principle

Reactions of o-phenylenediamine (OPD) with reducible sugars and even with amylose yielded various quinoxalines, which showed well-defined polarographic reduction waves at the same potential. For example, quinoxaline waves derived from glucose are shown in Fig. 5. The wave heights were proportional to the concentrations of glucose. Time courses of the formation of quinoxalines from the reaction of maltodextrins with OPD were then followed by measuring the total limiting current under definitively chosen conditions: the concentration of

![Fig. 5. Quinoxaline waves derived from glucose. OPD $5 \times 10^{-4} M$; Carbonate buffer pH 10; Heated at 100°C for 1 hr. under $N_2$. Concn. of glucose: Curve (1) $5 \times 10^{-4} M$, (2) $4 \times 10^{-4} M$, (3) $3 \times 10^{-4} M$, (4) $2 \times 10^{-4} M$, (5) $1 \times 10^{-4} M$.](image)
OPD 0.1M, sodium sulfite 0.3M in carbonate buffer pH 10.0 (0.2M) at 100°C±0.1°C. Sodium sulfite was added so as to suppress unfavorable side reactions. The maltodextrins for which anhydroglucose unit (AGU) had been known by the end-group determination, were tested, and the results are presented in Fig. 6.

As can be seen from the figure, the limiting current at given reaction time decreases with increasing AGU. The rate of the limiting current development seems to be almost of zero order for the initial reaction period, and to reach a saturated value after a sufficient reaction time, the value of the saturated limiting current being dependent on AGU of the sample.

In the sense to compare this rate of the zero order reaction at the initial reaction time, for convenience sake, the limiting current of the quinoxaline wave at the first 20 min. of the reaction was taken. As shown in Fig. 7, one may see a linear relation between the limiting current and the molar concentration or the reciprocal value of AGU of the fractionated maltodextrin, if the same amount of each sample is treated under the present experimental conditions. This would

<table>
<thead>
<tr>
<th>Maltodextrins</th>
<th>AGU</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>116.6±2.7</td>
</tr>
<tr>
<td>II</td>
<td>71.5±0.8</td>
</tr>
<tr>
<td>III</td>
<td>50.5±0.7</td>
</tr>
<tr>
<td>IV</td>
<td>38.4±0.5</td>
</tr>
<tr>
<td>V</td>
<td>28.4±0.4</td>
</tr>
<tr>
<td>VI*</td>
<td>12.6±0.1</td>
</tr>
</tbody>
</table>

* A crystalline product prepared according to Hizukuri et al.31)
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be more clearly demonstrated, if one may calculate the molar limiting current of each fractionated maltodextrin by dividing the observed limiting current by the molar concentration of the reducing end-group which can be obtained from the taken amount of sample and AGU in Table 2.

\[
\begin{align*}
\text{I: AGU} & = 116.6 \\
\text{II: AGU} & = 71.5 \\
\text{III: AGU} & = 50.5 \\
\text{IV: AGU} & = 38.4 \\
\text{V: AGU} & = 28.4 \\
\text{VI: AGU} & = 12.6
\end{align*}
\]

Fig. 8. Time dependence of molar limiting currents of maltodextrins. Reaction: the same as for Fig. 6.

In Fig. 8, the molar limiting current is plotted against the reaction time. The same treatment was also made for maltose and maltotetraose, and the results are presented together with those for maltodextrins, so as to facilitate the visualization of the nature of the reaction. The rate of the formation of quinoxalines at the initial period of the reaction time may be regarded as identical for all the maltodextrins in the same molar concentrations, whereas it seems difficult for oligosaccharides such as maltose or maltotetraose to find any agreement of the initial rate with those for maltodextrins even at a very early stage of the reaction. From the figure it may be said that, to apply the reaction to the determination of molecular size of maltodextrin or amylose, at least a standard specimen is necessary for which AGU has been carefully determined and moreover it should exceed at least 10.

The time required to reach the saturation value for the limiting current may depend on the AGU. The larger the AGU of maltodextrin, the longer the time for the completion of the reaction.

Fractionated amyloses were then examined in the same way as for the fractionated maltodextrins. Examples of the time course of quinoxaline formation from amyloses with different molecular size are presented in Fig. 9. Though the time course of a straight line of zero order reaction would be expected for each fractionated amylose, an appreciable curvature of each curve is still observed, while the limiting current at a very early stage is already enough large for measurement, indicating a high sensitivity for analytical purpose. Hence it
Fig. 9. Time courses of formation of quinoxalines from amyloses with high AGU. Molecular weights are given in Table 3. Reaction: amylose 100 mg/100 ml, other conditions are the same as for Fig. 6.

is considered that the comparison of the limiting current at a relatively early reaction time may be recommended for the practical estimation of the molecular size, i.e. $M_n$.

For polarographic measurements, limiting currents at $-1.0V$ (vs. S.C.E.) are recommended for both standard solution and the sample to be tested. $M_n$ can be obtained as

$$
M_n = M_n(st) \times \frac{H_s}{H_t} \times \frac{C_s}{C_t}
$$

where $H_t$ and $H_s$ are the limiting current of the standard sample and that of the sample to be tested, respectively, and $C_s$ and $C_t$ are the concentrations (mg %) of the standard sample and that of the sample to be tested, respectively. $M_n(st)$ is the number-average molecular weight of standard sample.

b) Results

In Table 3, $M_n$'s thus obtained with fractionated amyloses and maltodextrins are presented. The AGU of the standard maltodextrin used was 12.6. The limiting current was 1.42 $\mu$A, when the concentration was 10 mg %.

Comparing the present method with conventional ones, it may be at least emphasized that, though this polarographic OPD method needs a standard sample of fractionated maltodextrin (preferably of single species) for which $M_n$ has been determined by other method (the AGU should be above 10), the estimation of AGU of unknown amyloses can be easily done for all practically possible $M_n$’s, by using only one standard substance, while other conventional methods can cover only certain ranges of $M_n$ and most of them still require the standard substance and further their procedures are often tedious and time-consuming.

For the other kinetic methods, the kinetic follow of the reactions requires more or less colorimetric procedures, while the present polarographic method
Kinetic Approaches for the Determination of Molecular Weight of Amylose
demands no further addition of reagents into the reaction mixture and measure-
ments can be carried out directly only by transferring thereaction mixture into
the polarographic cell and taking the polarograms in a classical way.

In addition, the problem of the standard substance may be discussed. For
all the kinetic methods including the present method, some standard substance
is absolutely important. Whelan recommended maltotetraose but the substance
is not adequate for the present case as seen from Fig. 8. Maltodextrin (AGU =
12.6) used for the present investigation was substantiated as an excellent stand-
ard but the preparation of it may not be said very simple.

To solve this problem, the determination of the factor of the molar limiting
current of maltodextrin at 20 min. reaction time versus the limiting current of
glucose or maltose under fixed conditions may be worth to consider. The limit-
ing current of the quinoxalines from glucose or maltose after 60 min. reaction
time has been known to be stable and reproducible as reported elsewhere. If the
factor for the molar limiting current of maltodextrin at 20 min. reaction time
versus the stable and reproducible limiting currents of glucose or maltose has
once been obtained, the value might be used by other investigators with their
own experiments on glucose or maltose, irrespectively of the characteristics of
the capillary used in the present investigations. For such efforts, however,
more careful and repeated experiments will be demanded. This will be, there-

<table>
<thead>
<tr>
<th>Amyloses</th>
<th>Polaro.</th>
<th>Somogyi-N.</th>
<th>Enzyme</th>
<th>Viscosity</th>
<th>Osmotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF-1</td>
<td>1,500,000</td>
<td>1,700,000</td>
<td>1,100,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2</td>
<td>880,000</td>
<td>760,000</td>
<td>750,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-3</td>
<td>770,000</td>
<td>720,000</td>
<td>700,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-4</td>
<td>640,000</td>
<td>(600,000)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-5</td>
<td>350,000</td>
<td></td>
<td>400,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-6</td>
<td>260,000</td>
<td></td>
<td>220,000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Maltodextrins</th>
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<tr>
<td>I</td>
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<tr>
<td>IV</td>
</tr>
<tr>
<td>V</td>
</tr>
<tr>
<td>VI</td>
</tr>
</tbody>
</table>

* This fraction was used as standard for the enzyme kinetic method.
** A crystalline product used as standard for all the polarographic measurements.

The $M_n$ determination of amylopectine by the present polarographic method
may then be possible under the assumption that every amylopectin molecule has
no branch of $\alpha-1,6$ glucosidic linkage between the reducing end and the follow-
ing linear $\alpha-1,4$ glucosidic polymer, until the number of glucose unit correspond-
ing to this part surpasses at least 10.

(267)
S. ONO

Using the same maltodextrin (AGU=12.6) as standard, \( \bar{M}_n \) can be calculated from the limiting current at the 20 min. reaction time, for example, a preparation of amylopectin showed the limiting current of 0.044 \( \mu \)A, when the concentration was 150 mg per 100 ml. The obtained \( \bar{M}_n \) was 980,000.

CHARACTERISTICS OF THE TWO METHODS

As characteristics common for the both methods it may be emphasized that 1) amounts of samples required are very small, and 2) procedures are rapid and simple, though at least a standard sample of known \( \bar{M}_n \) must be available, (which is an inevitable condition for all the kinetic methods so far).

Some further characteristics for each method may be as follows:
Specific for the method I: 1) The method is especially useful for the higher molecular weight for which the conventional methods are difficult to give reliable data. 2) It is insensitive to impurities of lower molecular weights, which may cause large errors in the conventional methods. 3) The theoretical treatments may predict that by choosing an appropriate exo-enzyme, it may be applied generally to linear homo-polymers.
Specific for the method II: 1) The method is applicable for a wide range of \( \bar{M}_n \) which no other methods could cover. 2) Such a special caution as the check of the enzyme activity made in the method I, is unnecessary. 3) It can also be applied to other carbohydrates having a reducible end (i.e. amylopectin, glycogen etc.).

REFERENCES

Kinetic Approaches for the Determination of Molecular Weight of Amylose

