

## A Contribution to the Theory of Periodate Oxidation of Starch\*

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### Introduction

It has been shown in a previous paper<sup>1</sup> that it is possible to give an approximate estimate of the degree of polymerization (DP) of the amylose and amylopectin components of randomly degraded starch by quantitatively oxidizing whole (unfractionated) starch by means of periodate. The present paper represents a more generalized theoretical treatment of the oxidation of whole starches, both degraded and undegraded, by periodate. It will be shown that this treatment leads to the following results.

1. Both the upper and the lower limit for the DP of amylose and the lower limit for the DP of amylopectin may be found for any starch; if certain additional assumptions are made, the upper limit for the DP of amylopectin may be found as well.

2. The extent of degradation of a randomly degraded starch may be found.

3. For starch, which has been randomly degraded to a sufficient extent, the DP values of both components may be found with fairly satisfactory accuracy.

It will be assumed that the starch is a mixture of amylose and amylopectin and that the DP of amylose is not greater than that of amylopectin. It will further be assumed that the proportion of amylose in the starch, the amount of formic acid evolved on quantitative oxidation of whole starch by periodate, and the repeating chain unit of the amylopectin component of the original (undegraded) starch may be found by experiment and are thus known. All other values will be obtained by calculation. A fractionation must admittedly precede the determination of the repeating chain unit of the amylopectin component in the original starch; this involves, however, only *one* fractionation operation on each *kind* of starch, and the experimental error caused by imperfect fractionation and the determination itself is negligible. This is quite unlike the direct

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determination of DP on the separate fractions by the conventional physico-chemical methods, when the error involved may be of several orders of magnitude.<sup>1</sup>

### The DP Limits of the Components

On quantitative periodate oxidation 2 and 1 mole of formic acid is evolved from each reducing and nonreducing end anhydroglucose unit (AGU), respectively, of both molecular species. It has been shown<sup>1</sup> that this leads to the fundamental equation

$$F = 3w/N^A + [2(1 - w)]/N^B + (1 - w)/n \quad (1)$$

where  $F$  is the number of moles of formic acid evolved per AGU of oxidized starch,  $w$  is the weight fraction of amylose in the starch,  $n$  is the DP of the repeating chain unit of the amylopectin component, and  $N^A$  and  $N^B$  are the number-average DPs of the amylose and amylopectin components, respectively. As has been shown,<sup>1</sup> it is permissible to treat each component as homogeneous with respect to its DP, even though it is in fact a polymer homologous mixture.

If we put

$$M = F - (1 - w)/n \quad (2)$$

the equation may be rewritten as

$$M = 3w/N^A + 2(1 - w)/N^B \quad (3)$$

Since

$$N^B \geq N^A \quad (4)$$

we may write

$$M \leq 3w/N^A + 2(1 - w)/N^A$$

and

$$M \geq 3w/N^B + 2(1 - w)/N^B$$

and, on the other hand, since  $2(1 - w)/N^B \geq 0$ ,

$$M \geq 3w/N^A$$

Solving for  $N^A$  and  $N^B$  we get

$$3w/M \leq N^A \leq (2 + w)/M \leq N^B < \infty \quad (5)$$

The upper DP limit for amylopectin may be found only if additional evidence is available to the effect that the DP of *amylose* is not less than a number  $N$  which is itself larger than the lower limit value given by eq. (5); that is, if

$$3w/M < N \leq N^A$$

In view of eq. (3), we obtain

$$M \leq 3w/N + 2(1 - w)/N^B$$

whence

$$N^B \leq 2(1 - w)/[M - (3w/N)] \quad (6)$$

### Extent of Degradation

The extent of degradation  $\alpha$  is defined as the ratio of the number of bonds broken,  $V$ , to the total number of bonds. Thus, for amylopectin

$$\alpha = V/(N^B - 1) \quad (7)$$

To find  $\alpha$ , we must first find the repeating chain unit  $n_\alpha$  of the amylopectin degraded to the extent  $\alpha$ . By definition

$$n = N^B/t \quad (8)$$

where  $t$  is the number of nonreducing ends AGUs in an amylopectin molecule having a DP equal to  $N^B$ .

When  $V$  bonds are broken in an amylopectin molecule,  $V$  new nonreducing end AGUs are formed, so that the total number of nonreducing end AGUs in all the fragments will be  $(t + V)$ , whereas the total number of AGUs will remain unchanged. Thus, in view of eqs. (7) and (8):

$$n_\alpha = N^B/(t + V) = N^B/[(N^B/n) + \alpha(N^B - 1)]$$

or

$$n_\alpha = n/(1 + \alpha n) \quad (9)$$

very approximately, since  $N^B \gg 1$ .

The extent of degradation  $\alpha$  may now be calculated. For starch, randomly degraded to the extent  $\alpha$ , we may write, in analogy with eq. (1):

$$F_\alpha = 3w/N_{\alpha^A} + 2(1 - w)/N_{\alpha^B} + (1 - w)/n_\alpha \quad (10)$$

where  $N_{\alpha^A}$  and  $N_{\alpha^B}$  are the respective DPs of degraded amylose and amylopectin, and  $F_\alpha$  is the formic acid evolved (in moles per mole AGU) by the starch degraded to the extent  $\alpha$ . (In the previous paper<sup>1</sup> it had been assumed, as a first approximation, that  $n_\alpha = n$ .)

It is known from the theory of random degradation<sup>2</sup> that:

$$\alpha = 1/N_{\alpha^A} - 1/N^A = 1/N_{\alpha^B} - 1/N^B \quad (11)$$

Subtracting eq. (1) from eq. (10) and substituting (9) for  $n_\alpha$ , we get

$$\alpha = (F_\alpha - F)/3 \quad (12)$$

### The DP Limits of the Degraded Components

Let us put

$$M_\alpha = F_\alpha - (1 - w)/n_\alpha \quad (13)$$

and write eq. (10) as

$$M_\alpha = 3w/N_{\alpha^A} + 2(1 - w)/N_{\alpha^B} \quad (14)$$

In view of eq. (4) we have

$$N_{\alpha^A} \leq N_{\alpha^B} \quad (15)$$

By juxtaposing eqs. (14), (15) and (3), (4), we obtain immediately, by analogy with eq. (5),

$$3w/M_{\alpha} \leq N_{\alpha^A} \leq (2 + w)/M_{\alpha} \leq N_{\alpha^B} < \infty \quad (16)$$

We shall immediately see, however, that these limits can be further narrowed. In view of eq. (11) we have

$$\begin{aligned} N_{\alpha^B}/N_{\alpha^A} &= (\alpha + 1/N^A)/(\alpha + 1/N^B) = 1 + (1/N^A - 1/N^B)/(\alpha + 1/N^B) \\ &\leq 1 + 1/\alpha N^A \quad (17) \end{aligned}$$

But, according to eq. (5):

$$N^A \geq 3w/M$$

and therefore also

$$1/\alpha N^A \leq M/3w\alpha$$

It follows from the above inequality and from eq. (17) that

$$N_{\alpha^B} \leq N_{\alpha^A}(1 + M/3w\alpha)$$

By applying this inequality to eq. (14) in the manner in which eq. (4) has been applied to eq. (3), we obtain, after certain transformations

$$N_{\alpha^A} \geq 3w/(M + 3\alpha w) \quad (18)$$

and

$$N_{\alpha^B} \leq 1/\alpha \quad (19)$$

From eqs. (3), (9), (12), and (13) it may be shown that

$$M_{\alpha} = M + \alpha(2 + w) \quad (20)$$

and, since  $w < 1$ ,

$$M_{\alpha} > M + 3\alpha w$$

and therefore

$$3w/M_{\alpha} < 3w/(M + 3\alpha w) \quad (21)$$

In view of eqs. (16), (18), (19), and (21) we finally obtain the narrowed intervals

$$3w/(M + 3\alpha w) \leq N_{\alpha^A} \leq (2 + w)/M_{\alpha} \leq N_{\alpha^B} \leq 1/\alpha \quad (22)$$

Let us take as the approximate values of  $N_{\alpha^A}$  and  $N_{\alpha^B}$  the mid-values of the limit range, i.e.,

$$\bar{N}_{\alpha} = 1/2[3w/(M + 3\alpha w) + (2 + w)/M_{\alpha}] \quad (23)$$

and

$$\bar{N}_{\alpha^B} = 1/2[(2 + w)/M_{\alpha} + 1/\alpha] \quad (24)$$

The respective relative errors  $\delta N_{\alpha^A}$  and  $\delta N_{\alpha^B}$  will be

$$\delta N_{\alpha^A} \leq |N_{\alpha^A} - \bar{N}_{\alpha^A}|/[3w/(M + 3\alpha w)] \leq [(M + 3\alpha w)/3w]^{1/2} \\ [(2 + w)/M_{\alpha} - 3w/(M + 3\alpha w)] = M(1 - w)/3wM_{\alpha} \quad (25)$$

and

$$\delta N_{\alpha^B} \leq |N_{\alpha^B} - N_{\alpha^B}|/[(2 + w)/M_{\alpha}] \leq [M_{\alpha}/(2 + w)]^{1/2} \\ [1/\alpha - (2 + w)/M_{\alpha}] = M/[2\alpha(2 + w)] \quad (26)$$

The values of  $M$ ,  $M_{\alpha}$ , and  $\alpha$ , which appear in the above formulae, can be calculated from the experimental values  $w$ ,  $n$ ,  $F$ , and  $F_{\alpha}$  by the use of eqs. (3), (9), (12), and (13).

In view of eq. (20) it can be deduced from eq. (25) and (26) that the relative errors  $\delta N_{\alpha^A}$  and  $\delta N_{\alpha^B}$  diminish with the increase in  $\alpha$  or  $w$  or both.

Finally, if additional evidence is available to show that

$$N^A \geq N > 3w/M$$

we may deduce the following inequalities

$$[3w + 2(1 - w)/(1 + 1/\alpha N)]/M_{\alpha} \leq N_{\alpha^A} \leq (2 + w)/M_{\alpha} \leq N_{\alpha^B} \\ \leq [3w(1 + 1/\alpha N) + 2(1 - w)]/M_{\alpha} \quad (27)$$

This relationship will yield more satisfactory results than eq. (17), as will be seen from the numerical example that follows.

### Numerical Example

A sample of undegraded starch is known to contain 20% of amylose and 80% of amylopectin, the latter having a repeating chain length of 21 AGUs. The sample was found to evolve, on quantitative periodate oxidation, 1 mole of formic acid per 25 AGUs. It was then randomly degraded, when the quantity of formic acid evolved from the degraded unfractionated material was 1 mole per 20 AGUs. Thus  $w = 0.2$ ,  $F = 1/25 = 0.0400$ ,  $F_{\alpha} = 1/20 = 0.0500$ , and  $n = 21$ .

The DP limits for both components may be found from eq. (5). According to eq. (2):

$$M = 0.0400 - (1 - 0.2)/21 = 0.00190$$

$$3 \times 0.2/0.00190 \leq N^A \leq (2 + 0.2)/0.00190 \leq N^B < \infty$$

or

$$315 \leq N^A \leq 1158 \leq N^B < \infty$$

If it is known that  $N^A$  is greater than, say, 500, we may substitute  $N = 500$  in eq. (6), when

$$N^B \leq 2(1 - 0.2)/[0.00190 - (3 \times 0.2)/500] = 2286$$

The extent of degradation is obtained from eq. (12):

$$\alpha = (0.0500 - 0.0400)/3 = 0.00333$$

The DP limits of the degraded components may be found from eq. (22). According to eq. (20),

$$M_\alpha = 0.00190 + 0.00333(2 + 0.2) = 0.00923$$

$$M + 3\alpha w = 0.00190 + 3 \times 0.2 \times 0.00333 = 0.00390$$

$$3 \times 0.2/0.00390 \leq N_{\alpha^A} \leq (2 + 0.2)/0.00923 \leq N_{\alpha^B} \leq 1/0.00333$$

That is,

$$154 \leq N_{\alpha^A} \leq 238 \leq N_{\alpha^B} \leq 300$$

Hence, from eqs. (23) and (24),

$$\bar{N}_{\alpha^A} = 196, \bar{N}_{\alpha^B} = 269$$

The relative errors are obtained from eqs. (25) and (26):

$$\delta N_{\alpha^A} \leq [0.00190 \times (1 - 0.2) \times 100]/(3 \times 0.2 \times 0.00923) = 27.4\%$$

$$\delta N_{\alpha^B} \leq (0.00190 \times 100)/[2 \times 0.00333 \times (2 + 0.2)] = 13.0\%$$

If it is known that  $N^A \geq 500$ , we may substitute  $N = 500$  in eq. (27):

$$173 \leq N_{\alpha^A} \leq 238 \leq N_{\alpha^B} \leq 277$$

whence  $\bar{N}_{\alpha^A} = 205$ ,  $\bar{N}_{\alpha^B} = 258$ ,  $\delta N_{\alpha^A} \leq 19.1\%$ ,  $\delta N_{\alpha^B} \leq 8.4\%$ .

## References

1. Schmorak, J., D. Mejzler, and M. Lewin, *J. Polymer Sci.*, **55**, 437 (1961).
2. Grassie, N., *The Chemistry of High Polymer Degradation Processes*, Butterworth, London, 1956, chap. 3.

## Synopsis

The mathematical theory of periodate oxidation of unfractionated starches was studied. The degrees of polymerization (DP) of the amylose and amylopectin components of the starch were considered to be unknown, whereas the proportion of amylose in the starch, the repeating chain length of the amylopectin component, and the amount of formic acid evolved on quantitative oxidation were considered to be experimentally ascertainable and thus known. The treatment thus reduces to an approximate solution of one equation with two unknowns. It is shown that certain inequalities are then valid, when the following information can be obtained: (1) the upper and lower DP limits of each component, (2) the extent of degradation of a randomly degraded starch, and (3) the maximum relative errors involved in the determination of the DPs of the components by the periodate method. A numerical example is given to show the application of the method in laboratory practice.

### Résumé

On a étudié la théorie mathématique de l'oxydation par le periodate d'amidons non-fractionnés. On a supposé que le degré de polymérisation (DP) des composants amylose et amylopectine de l'amidon soit inconnu. D'autre part on a considéré qu'on peut connaître par une détermination expérimentale la proportion d'amylose dans l'amidon, la longueur de l'unité periodique du composant amylopectique, et la quantité d'acide formique dégagée par oxydation quantitative. La méthode se réduit dès lors à une solution approximative d'une équation à deux inconnues. Il est montré que certaines inégalités sont valables et qu'on peut alors obtenir les informations suivantes: (1) les limites supérieures et inférieures du DP de chaque composant, (2) le taux de dégradation d'un amidon dégradé statistiquement, (3) les erreurs relatives maximales comprises dans la détermination du DP des composants par la méthode du periodate. On donne un exemple numérique pour montrer l'application de la méthode dans la pratique du laboratoire.

### Zusammenfassung

Die mathematische Theorie der Perjodatoxydation unfraktionierter Stärke wurde untersucht. Die DP's der Amylose- und Amylopectinkomponente der Stärke wurden als Unbekannte behandelt, während der Anteil an Amylose in der Stärke, die periodische Bausteinkettenlänge der Amylopectin-komponente und die bei der quantitativen Oxydation gebildete Ameisensäure als experimentell bestimmbar und daher bekannt betrachtet wurden. Die Behandlung reduziert sich somit auf eine Näherungslösung einer Gleichung mit zwei Unbekannten. Es wird gezeigt, dass gewisse Ungleichungen gültig sind: und die folgenden Größen dann bestimmt werden können: (1) oberer und unterer Grenzwert für DP jeder Komponente; (2) Ausmass des Abbaus beim statistischen Abbau von Stärke; (3) bei der Bestimmung von DP der Komponenten nach der Perjodatmethode auftretender maximaler relativer Fehler. Ein Zahlenbeispiel für die Anwendung der Methode in der Laboratoriumspraxis wird gegeben.

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