On the Viscosimetric Assay of Enzymic Activity and Rate Constants

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The viscosity of solutions of polymeric homologous substances has been the object of much interest during the last few decades, and many formulas have been suggested. A review of this subject has recently been made by Ewart [1] to which the reader is referred.

As early as 1887 Arrhenius [2] put forward a formula giving the relationship between the relative viscosity η_r of a solution and the concentration c of the solute. We may write it in the following form

$$\eta_r = A^c \tag{1}$$

where A is a constant.

Berl and Bütler [3] found that the logarithmic form

$$\log \eta_r = Kc \tag{2}$$

where K is a constant, was applicable to solutions of starch nitrates.

Staudinger and Heuer [4] found that there exists a simple relationship between viscosity, solute concentration and molecular weight, valid for dilute solutions:

$$\eta_{sp} = K_m c_{gm} M \tag{3}$$

where η_{sp} = the specific viscosity,
c_{gm} = the concentration in basic moles per litre,
K_m = the viscosity-molecular weight constant, and
M = the molecular weight.

Staudinger [5] also called attention to the fact that the constant in equation (2) is proportional to the molecular weight, and Hess and Sakurada [6] showed that Staudinger's formula (equation 3) can be regarded as the first approximation of the logarithmic formula, if natural logarithms are used, i. e.

$$\lim_{c \to 0} \frac{\eta_{sp}}{c} = \lim_{c \to 0} \frac{\ln \eta_r}{c} \qquad (4)$$

The formula

$$\ln \eta_r = K_m c_{gm} M \qquad (5)$$

will be referred to here as the modified Arrhenius-Staudinger formula *.

When the viscosity is used for the determination of molecular weights and for the characterization of depolymerization processes, it must be observed that the limes value (4) is the value required. By increasing concentration, the values of $(\ln \eta_r)/c$ generally differ less from the limes value than do the values of η_{sp}/c, and values of $(\ln \eta_r)/c$ can frequently be used instead of the corresponding limes values. If necessary, a calculation of the limes value may be performed by Mead and Fuoss's method [9], who give the formula

$$\frac{\ln \eta_r}{c} = [\eta] - \beta [\eta]^2 c \qquad (6)$$

where $[\eta]$ = the intrinsic viscosity [10], and
β = a constant, which is characteristic for the solute.

The present author [11] has previously deduced ** a formula for the viscosimetric assay of enzymic activity:

$$A = c_s^2 \cdot \frac{d \frac{1}{\eta_{sp}}}{dt} \qquad (7)$$

where A = the activity of the enzyme in the reaction mixture,
c_s = the concentration of the substrate in grams per gram of solution in the reaction mixture, and
t = the time in seconds.

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* There are two logarithmic expressions for viscosity which are sometimes confused, especially as both may be written log η/c. One is $(\log \eta_r)/c$, discussed above, and the other is log (η_{sp}/c), proposed by Bungenberg de Jong, Kruyt and Lens [7] and extensively used by Staudinger [E.g. 8].

** The equation (5) in that paper inludes an approximation: the number of basic molecules N is substituted for the number of linkages actually present between the basic molecules.

The deduction was based on Staudinger's formula (equation 3). If, however, we apply the modified Arrhenius-Staudinger formula (equation 5), we get

$$A = c_s^2 \cdot \frac{d\dfrac{1}{\ln \eta_r}}{dt} \tag{8}$$

The present author [12] has recently deduced some equations for the rate constant k of the depolymerization of high-polymer substances and for the degree of depolymerization a. These equations are also based on Staudinger's formula, and the same arguments can be applied as to the enzyme assay. In cases where the modified Arrhenius-Staudinger formula is valid, we thus get the following equations, corresponding to the equations (8), (10), and (13) in that article respectively:

$$k = 2K_m c_{gm} M_0 \frac{d\dfrac{1}{\ln \eta_r}}{dt} \tag{9}$$

$$k = 2K_m c_{gm} M_0 \frac{\dfrac{1}{\ln \eta_r}}{t} \tag{10}$$

$$a = 2K_m c_{gm} M_0 \frac{1}{\ln \eta_r} \tag{11}$$

The functions c/η_{sp} and $c/\ln\eta_r$ are thus of special interest for substances for which Staudinger's formula and the modified Arrhenius-Staudinger formula are valid respectively.

If the expression $1/\eta_{sp}$ is used in those cases where the expression $1/\ln\eta_r$ would be correct, we get appreciable deviations at high viscosities, especially in calculations of molecular weights, and polymerization and depolymerization degrees. In calculations of enzymic activity and rate constants, however, where the derivative is considered, the error is less disturbing, as the depolymerization is carried out for a period when $1/\eta_{sp}$ is practically a linear function of the time. The curve $1/\eta_{sp}$ has one asymptote, since

$$\lim_{\ln \eta_r \to 0} \left(\frac{1}{\ln \eta_r} - \frac{1}{\eta_{sp}} \right) = \tfrac{1}{2} \tag{12}$$

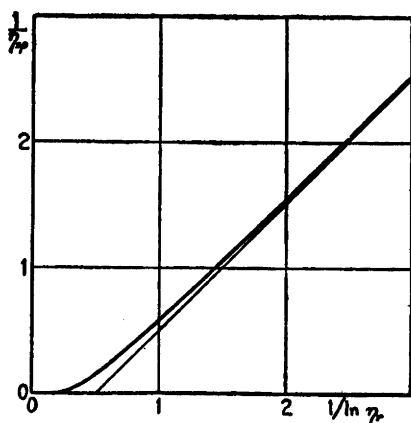and both the curve and the asymptote are given in Fig. 1.

Fig. 1. $1/\eta_{sp}$ as a function of $1/\ln \eta_r$.

SUMMARY

1. The modified Arrhenius-Staudinger formula $\ln \eta_r = K_m c_{gm} M$, where η_r = the relative viscosity, K_m = the viscosity-molecular weight constant, c_{gm} = the concentration in basic moles per litre, and M = the molecular weight, is used for the deduction of formulas for the assay of enzymic activity and the determination of rate constants.

2. For the enzymic activity A, the following formula is given: $A = c_s^2 \cdot d(1/\ln \eta_r)/dt$, where c_s = the substrate concentration in grams per gram of solution, and t = the time in seconds.

3. For the rate constant k, the following formula is given:
$k = 2K_m c_{gm} M_0 \cdot d(1/\ln \eta_r)/dt$, where M_0 = the basic molecular weight.

4. For the degree of depolymerization a, the following approximative formula is given: $a = 2K_m c_{gm} M_0/\ln \eta_r$.

REFERENCES

1. Ewart, R. H. *Advances Colloid Sci.* 2 (1946) 197.
2. Arrhenius, S. *Z. physik. Chem.* 1 (1887) 285.
3. Berl, E., and Bütler, R. *Z. ges. Schiess- u. Sprengstoffw.* 5 (1910) 82.
4. Staudinger, H., and Heuer, W. *Ber.* 63 (1930) 222.
5. Staudinger, H. *Z. physik. Chem.* A 153 (1931) 391.
6. Hess, K., and Sakurada, I. *Ber.* 64 (1931) 1183.

7. Bungenberg de Jong, H. G., Kruyt, H. R., and Lens, J. *Kolloid-Beihefte* **36** (1932) 429.
8. Staudinger, H., and Heuer, W. *Z. physik. Chem.* **A 171** (1934) 129.
9. Mead, D. J., and Fuoss, R. M. *J. Am. Chem. Soc.* **64** (1942) 277.
10. Kraemer, E. O. *Ind. Eng. Chem.* **30** (1938) 1200.
11. Hultin, E. *Svensk Kem. Tid.* **58** (1946) 281.
12. Hultin, E. *Acta Chem. Scand.* **3** (1949) 465.