

## TURBIDITY AS A CRITERION OF COAGULATION

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### ABSTRACT

The use of opalescence as criterion of coagulation in aggregating systems is examined. A rapid method of estimating the rate of particle growth is described.

### INTRODUCTION

In the study of aggregating or coagulating macromolecular systems, the degree of solution turbidity, or opalescence, has been used often as a criterion for the extent of the reaction. For example, in the realm of biological macromolecules, one finds this method applied to a vast variety of molecules, such as the caseins (1, 2), myosin (3), ovalbumin (4), the TMV protein (5),  $\beta$ -lactoglobulin (6), and tomato bushy stunt virus (7). In the initial stages of aggregation, when the particle size is small compared to the wavelength,  $\lambda$ , of the light used ( $r < \lambda/10$ ,  $r$  = particle radius), the Rayleigh law of scattering (8) is obeyed and the turbidity is a direct measure of the degree of association.

By combining the Rayleigh law of scattering with Flory's statistical treatment of the particle size distribution at any time in condensation polymerization (9) and Smoluchowski's theory of the kinetics of diffusion-controlled coagulation (10), Oster (7) has shown that, in the initial stages of coagulation, the optical density is directly proportional to time, whereas its rate of change with time is proportional to the square of the concentration of the aggregating component. In later stages of coagulation, however, when  $r > \lambda/10$ , these simple relationships are no longer applicable and the turbidity-particle size relation must be analyzed in terms of the Mie theory of light scattering (11). The purpose of the present paper is to extend Oster's treatment to the region beyond Rayleigh scattering and to describe a simple method for obtaining from turbidimetric measurements at least an estimate of the rate of particle growth with time in a coagulating system.

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### THEORETICAL

According to La Mer (12-14), the intensity of scattered light,  $I$ , at any value of  $r/\lambda$  can be expressed by the general equation:

$$I = K' N r^2 (r/\lambda)^y = K'' \frac{1}{r} \left(\frac{r}{\lambda}\right)^y, \quad [1]$$

where  $N$  is the number of particles in the scattering volume,  $K'$  and  $K''$  are constants independent of  $r$  and  $\lambda$ , and  $y$  is a constant which varies from a value of 4.0 (Rayleigh scattering) to  $-2.2$  in a complicated fashion and can be calculated from the Mie theory of light scattering. In a coagulating polydisperse system the turbidity,  $\tau$  can be expressed at any time,  $t$ , as

$$\tau = K \sum_x N_x V_x^{2/3} \left(\frac{V_x^{1/3}}{\lambda}\right)^y; \quad V_x = xV_0; \quad [2]$$

where  $N_x$  is the number of particles of degree of polymerization  $x$ ,  $V_x = \frac{4}{3}\pi r_x^3$  is their volume,  $K$  is a proportionality constant, and  $V_0$  is the volume of the initial aggregating particles. Flory (9) has shown that when the total mass of the associating species in the system is kept constant,

$$N_x = N_0(1 - p)^2 p^{x-1}, \quad [3]$$

where  $N_0$  is the initial number of particles, and  $p$  is the fraction of all functional groups reacted after a given time,  $t$ . Then, for any given value of  $y$ ,

$$\tau = \frac{KN_0 V_0^{(y+2)/3}}{\lambda^y} \sum_x p^{x-1} (1 - p)^2 x^{(y+2)/3}, \quad [4]$$

and

$$\frac{d\tau}{dt} = \frac{KN_0 V_0^{(y+2)/3} (1 - p)^2}{\lambda^y} \frac{d}{dt} \sum_x p^{x-1} x^{(y+2)/3}. \quad [5]$$

Furthermore,  $p = \frac{1}{2}kt/(1 + \frac{1}{2}kt)$ , where  $k$  is the rate constant of the second-order (15) condensation polymerization or coagulation reaction.

Equations [4] and [5] describe completely the dependence of the turbidity of a coagulating system on the extent of the reaction over the entire course of the reaction, i.e., for any value of  $r/\lambda$ . Their usefulness depends, however, on a knowledge of  $y$  at any time and on the ability to evaluate the sum in these equations.

The exponent  $y$  can be determined very simply by carrying out the measurements at a number of wavelengths (12, 16, 17) since, according to Eqs. [1] and [2],

$$\left(\frac{\partial \ln \tau}{\partial \ln \lambda}\right)_r = -y. \quad [6]$$

Whereas the summation in Eqs. [4] and [5] cannot be carried out readily

for fractional powers of  $x$ , these have been examined for particular values of  $y$  which yield integral exponents of  $x$ .

For  $y = 4$ , Rayleigh scattering applies, and Eqs. [4] and [5] reduce to those derived by Oster. For  $y = 1$ ,

$$\tau = \frac{KN_0 V_0}{\lambda} \quad \text{and} \quad \frac{d\tau}{dt} = 0, \quad [7]$$

i.e., scattering is independent of particle radius, and a maximum is reached in the turbidity as a function of time. For  $y = -2$

$$\tau = KN_0 \lambda^2 \frac{1}{1 + \frac{1}{2}kt};$$

$$\frac{d\tau}{dt} = -\frac{kKN_0 \lambda^2}{2(1 + \frac{1}{2}kt)^2}; \quad [8]$$

i.e., the scattering intensity decreases with time, the turbidity being directly proportional to the number of particles,  $N_t$ , present at time  $t$ , and inversely proportional to the cube of particle radius, i.e., to the particle mass, since (10, 18)

$$N_t = \frac{N_0}{1 + \frac{kTN_0}{3\eta} t}, \quad [9]$$

where  $k$  is Boltzmann's constant,  $T$  is the thermodynamic temperature, and  $\eta$  is the viscosity of the medium. Thus, as  $y$  varies from a value of 4.0 to  $-2.0$ , the turbidity changes from being directly proportional to particle mass (or extent of aggregation) to being inversely proportional to that quantity.

#### RESULTS AND DISCUSSION

In order to verify the validity of the above relations, turbidimetric measurements were carried out as a function of time and wavelength on a series of coagulating sulfur sols, these being systems for which La Mer and co-workers had calculated the relation between the values of  $y$  and  $r/\lambda$  (12).

Two types of sulfur sols were used. The first sols were generated by addition of water to methanolic solutions of sulfur. (The methanol-water system was chosen as solvent-precipitant since the two have essentially the same refractive index, which eliminates complications from multicomponent effects (19, 20)). The second system of sols was prepared by reacting equimolar aqueous solutions of sodium thiosulfate and sulfuric acid, which is the same system as that used by La Mer and co-workers and by Oster. In the first system, the total mass of coagulating species is constant; in the

second one it changes with time as sulfur is constantly generated in the chemical reaction. In the latter case, Eq. [1] becomes

$$I = K'' \left( \int_0^t \frac{dg}{dt} \right) \frac{1}{r} \left( \frac{r}{\lambda} \right)^y, \quad [1a]$$

where the integral represents the mass of coagulating material,  $g$ , produced at time  $t$  and must be determined from the kinetics of the chemical reaction.

The turbidity measurements were carried out on a Zeiss model PMQ II spectrophotometer,<sup>2</sup> as a function of wavelength.

The results obtained with two sols of the first type are presented in Fig. 1a and b. The first sol was prepared by addition of twice the volume of distilled water to a half-saturated methanolic solution of sulfur. The time dependence of the optical density (or turbidity) at all wavelengths is quite typical for a coagulating system, the optical density essentially reaching a plateau after 15–20 minutes. Examination of the wavelength dependence of scattering at various time intervals, however, shows that, as the reaction progresses, the exponent  $y$  changes from a value of 4 in the initial stages to 2.8 after 25 minutes. Thus, the flattening out of the turbidity *vs.* time curve reflects not the reaching of a steady state but a change in the dependence of turbidity from proportionality to  $M$  to proportionality to  $M^{0.6}$ , where  $M$  is the average particle weight. Turbidity values that would have been obtained after 25 minutes had the proportionality to  $M$  remained valid are shown by the circles drawn above each curve, assuming that the turbidity at 700 m $\mu$  is correct. These points show clearly that the coagulation reaction is far from having reached a steady state even though the rate of change of turbidity has flattened out.

A much more dramatic set of results is presented in Fig. 1b on a sol that had been generated by adding 2 parts of water to 3 parts of a quarter-saturated methanolic solution of sulfur. Here, at all wavelengths, a rapid increase in turbidity is followed by a slow decrease, the maximum in scattering occurring at progressively longer times and lower turbidity values with increasing wavelengths. As time passes, the scattered intensities at the various wavelengths reverse their order, indicating a change in the sign of  $y$ . This set of curves is, in general, quite similar to theoretical curves calculated by Casperson (21) for the dependence of scattering on particle radius and wavelength of light.

Values of  $y$  were determined at various times using Eq. [6] and these, in turn, were compared with theory via Eqs. [1], [4], [5], [7], and [8]. In this analysis, it was assumed that polydispersity was not very high—an assumption justified by the pastel shade backgrounds of the coagulating

<sup>2</sup> It is not implied that the U. S. Department of Agriculture recommends products of companies mentioned to the possible exclusion of others in the same business.

solutions (12). Since at the end of the experiment no precipitate had settled out nor was any particulate material detectable, the decrease in turbidity could not be due simply to a decrease in the amount of suspended matter.

The maximum in a scattering curve is found at the point where  $y = 1$  and the turbidity is independent of  $r$  (Eq. [7]). The scattering intensities at two nearest wavelengths measured at a time half-way between their maxima (Fig. 1b) were found to be in inverse proportion to the wavelengths, showing that  $y$  is in fact equal to unity in this region, as expected. This was further verified by comparing the values of  $y$  obtained from an analysis

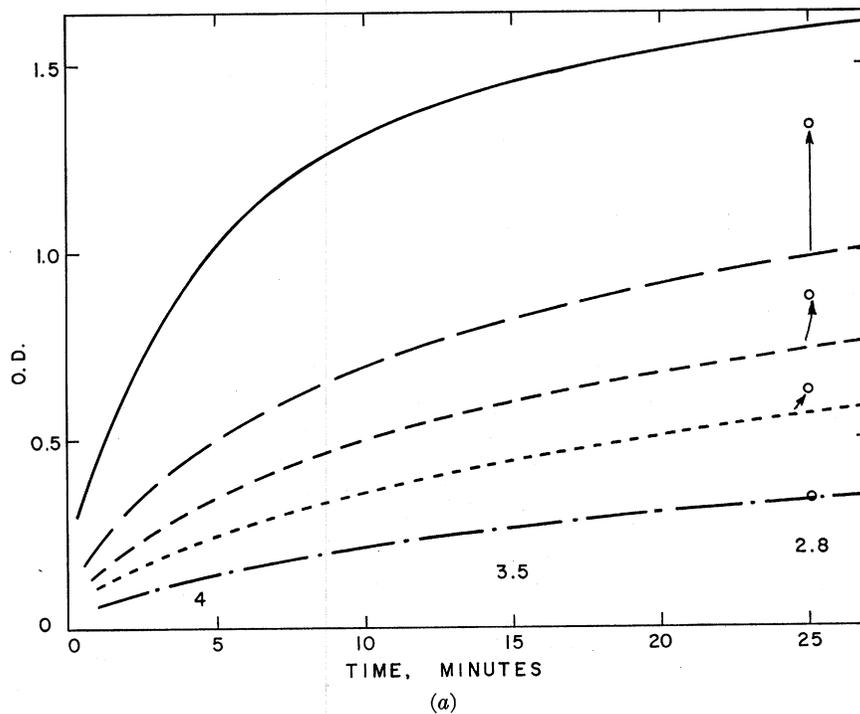


FIG. 1. Rate of change of turbidity with time of coagulating sulfur sols prepared by addition of water to methanolic solutions of sulfur. Solid line: 420  $m\mu$ ; long dashes: 500  $m\mu$ ; short dashes: 550  $m\mu$ ; dots: 600  $m\mu$ ; dot-dash: 700  $m\mu$ .

(a) Two volumes of water added to one volume of a half-saturated methanolic solution of sulfur. Numbers below the 700  $m\mu$  curve are values of  $y$  at various times. Circles at 25 minutes are theoretical values of optical density for  $y = 4$ , using 700  $m\mu$  point as base.

(b) Two volumes of water added to three volumes of a quarter-saturated methanolic solution of sulfur. Open circles are values of  $\bar{r}^3$  (expressed in  $m\mu \times 10^{-6}$ ) derived from intersections between turbidity curves; solid line is least squared relation,  $\bar{r}^3 = A + Bt$ , in accordance with kinetics of coagulation (10, 18).

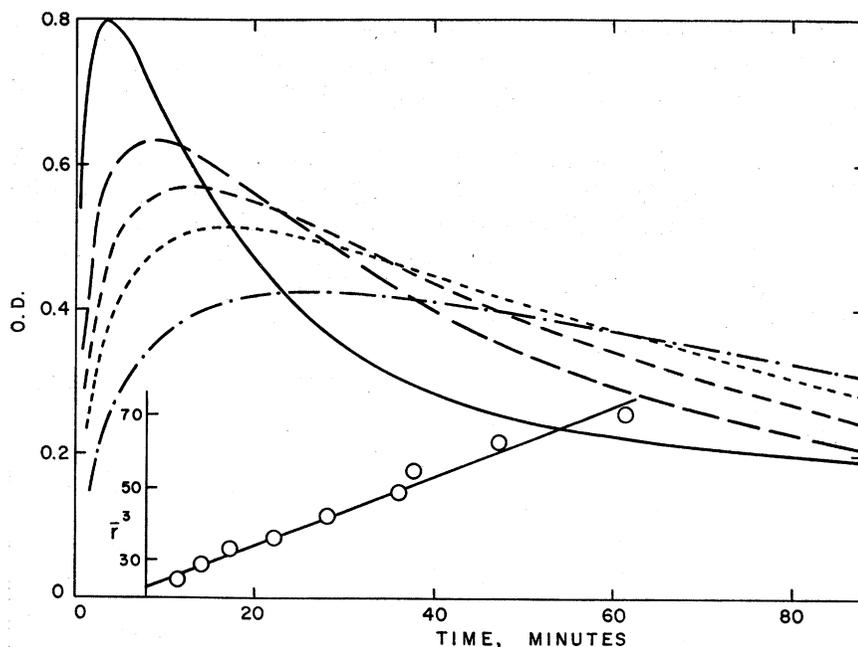


FIG. 1 (b)

of the intensities at two wavelengths at times corresponding to the attainment of the maximum at each. For example, comparing the turbidities at 500 and 550  $m\mu$ , at 9 minutes (where the maximum is attained at 500  $m\mu$ )  $y = 1.35$ , at 12.5 minutes (when the maximum is attained at 550  $m\mu$ )  $y = 0.65$ ; this is expected from Eq. [7]. As the experiment progresses beyond the maximum in turbidity, it is found that the scattering curves cross in a region in which both are decreasing; this is absolutely consistent with theory, since when  $y = 0$ , i.e. when scattering is independent of wavelength and two curves cross, the turbidity is inversely proportional to the particle radius, i.e., *the turbidity should decrease with further coagulation*. In the late stages of the process, at 80 minutes, the order of intensities at different wavelengths is reversed and  $y$  is found to be  $-1.03$ , i.e. turbidity varies as  $\lambda/r^2$ .

The present analysis of the turbidity data was verified further in the following way. In the case of sulfur sols, La Mer has shown that the point at which  $y = 0$  is attained when  $2\pi r/\lambda = 4$ . By means of the data of Fig. 1b, average values of the particle radius,  $\bar{r}$ , were calculated at each point of intersection between the turbidity curves, using the average of the wavelengths,  $\bar{\lambda}$ , i.e.,  $\bar{r} = 2\bar{\lambda}/\pi$ . Smoluchowski's kinetics of the coagulation give the result that the total number of particles at any time is inversely proportional to time (Eq. [9]). Since, for a constant mass of material, the number

of particles is inversely proportional to particle volume or to the cube of the particle radius, the last must vary directly with time ( $r^3 = A + Bt$ ). Plotting the calculated values of  $\bar{r}^3$  as a function of time (shown by the circles in Fig. 1b) resulted in a straight line with a standard deviation of the points of  $\pm 4\%$ . Thus, the particle dimensions (and, thus, extent of aggregation) derived from the present treatment of the turbidity data are absolutely consistent with the known kinetics of the process, further supporting the validity of the present analysis of the data.

A typical set of results obtained with the second type of sulfur sol is presented in Fig. 2. These results are qualitatively similar to those of Fig. 1b, with two striking exceptions. First, the maximal scattering intensities are equal at various wavelengths (use of higher concentrations of reagents led to an increase in the maximal intensities with increasing wavelength). Second, the intersections between the curves occur at times at which the intensity at the lower wavelength is increasing while that at the higher wavelength is decreasing. Furthermore, after passing the maximum, the decrease in turbidity with time is very slow. Analysis of this system is highly complicated, since coagulating material is constantly generated in the course of the reaction and the change in turbidity must reflect now two

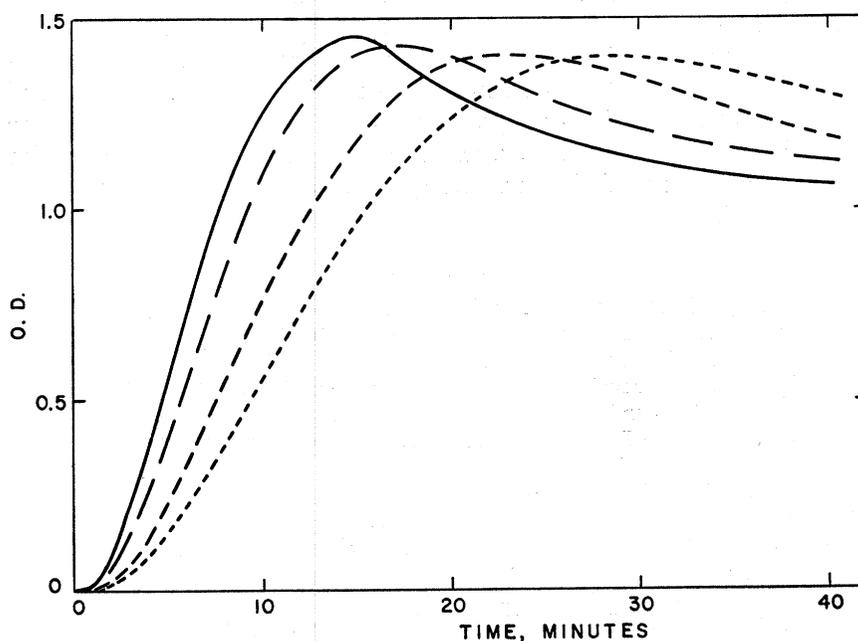


FIG. 2. Rate of change of turbidity with time of a coagulating sulfur sol generated by reaction of  $0.01 M \text{Na}_2\text{S}_2\text{O}_3$  and  $0.01 M \text{H}_2\text{SO}_4$ . Solid line:  $440 \text{ m}\mu$ ; long dashes:  $500 \text{ m}\mu$ ; short dashes:  $600 \text{ m}\mu$ ; dots:  $700 \text{ m}\mu$ .

sets of kinetics (Eq. [1a]). However, even though the total amount of matter in the system is increasing in addition to an increase in particle size, in this case as well, turbidity will either decrease with time or reach a plateau, leading potentially to the naive erroneous conclusion that a steady state has been reached in the system.

The present simple analysis of change in turbidity with time in coagulating systems points to the extreme care with which such measurements must be interpreted. It seems essential that in all such experiments measurements must be carried out at a number of wavelengths and the data examined carefully for changes with time of the exponent in the relation between optical density and wavelength. When this exponent falls from 4, the simple, often employed, analysis may no longer be used, but equations, such as [1], [4] and [5], based on the Mie theory of scattering must be applied. The present analysis points out some of the dangers in the use of opalescence as a criterion of coagulation, but it indicates also a rapid way of obtaining an estimate of the degree of coagulation of a system. This may be done by using the points of intersection between scattering curves obtained at a series of wavelengths and comparing them with theoretical curves calculated from the Mie theory, for the proper refractive index (22-25) as was done by La Mer for sulfur sols. This approach is only approximate, neglecting a number of factors such as polydispersity, secondary scattering, and a slight dependence of solute refractive index on wavelength; nevertheless, it can be used for the rapid screening of a number of conditions of coagulation of a given system. When precise measurements of particle sizes are desired in a monodisperse system, the method of choice remains that developed almost twenty years ago by La Mer and his school (12, 14, 26-28).

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