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# Supersaturation in sickle cell hemoglobin solutions*

(sickle cell disease/hemoglobin S gelation/protein self-assembly/nucleation kinetics/protein solubility)

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**ABSTRACT** **The kinetic inhibition of the gelation of hemoglobin S is compared to the change in hemoglobin S solubility, when the solubility is altered by carbon monoxide, pH, or urea. By means of a new technique, the delay time and the extent of gelation are measured on the same sample. The delay time, $t_d$, is found to be proportional to a high power (30–40) of the hemoglobin S solubility. Together with the previously reported concentration dependence, this result demonstrates that the rate is proportional to a high power of the supersaturation, $S$, defined as the ratio of the total hemoglobin S concentration to the equilibrium solubility. The results obey the supersaturation equation $t_d^{-1} = \gamma S^n$, where $\gamma$ is an empirical constant (about $10^{-7}$ sec$^{-1}$) and $n$ is about 35. The supersaturation equation can successfully account for observations on the kinetics of cell sickling and is therefore used to estimate the increase in the delay time for sickling necessary to produce significant clinical benefit to patients with sickle cell disease.**

In previous papers we reported initial results on the kinetic and thermodynamic behavior of deoxyhemoglobin S gelation (1, 2). When gelation was induced by temperature jumps, there was a delay period during which no observable polymerization took place (1, 3, 4). The delay time ($t_d$) was found to depend inversely on an extraordinarily high power (about 30) of the deoxyhemoglobin S concentration ($c_t$) (1), and to be extremely sensitive to changes in temperature (1, 3). We also found that over a limited temperature range the delay time was proportional to a very high power of the equilibrium solubility (1, 2), where the solubility ($c_s$) was taken as the concentration of deoxyhemoglobin S in the supernatant after sedimentation of the polymers by high-speed ultracentrifugation. The concentration and temperature dependence of the delay time could then be summarized to first order by a single empirical equation:

$$1/t_d = \gamma S^n; \qquad S \equiv c_t/c_s \qquad [1]$$

where $\gamma$ is an experimental constant. By analogy to crystallization or condensation processes, the ratio of the total initial concentration, $c_t$, to the solubility, $c_s$, was called the supersaturation ratio, $S$. The form of this "supersaturation equation" could be justified theoretically on the basis of our proposed nucleation-controlled polymerization mechanism (1).

Using the supersaturation equation, we carried out a series of calculations on the influence of physiological variables on the delay time of intracellular gelation *in vivo*. The results of these calculations led to the kinetic hypothesis that the rate of intracellular gelation relative to the capillary transit time of the red cell is a major determinant of clinical severity in sickle cell disease (1, 5). A crucial *assumption* in these calculations was that the supersaturation equation would hold approximately, no matter how the solubility was changed. Experimental data, however, on the influence of physiological variables, such as oxygen concentration and pH, on the delay time and solubility were lacking.

In this paper we report the results of experiments in which the solubility was varied by changing pH, carbon monoxide saturation, or urea concentration at constant total hemoglobin S concentration and temperature. In each case we have found that the delay time is proportional to a very high power (30–40) of the solubility, confirming the supersaturation equation. The experimental methods employed in this investigation are described in considerable detail, since they provide a convenient way of studying the kinetics and thermodynamics of gelation, and should also be useful as a sensitive method in screening for potential therapeutic inhibitors.

## MATERIALS

The enormous concentration dependence of the rate of gelation requires the selection of a hemoglobin S concentration giving a conveniently measurable delay time. Either red cell lysates or purified hemoglobin S may be used. For the studies presented here, hemoglobin S was purified by chromatography on DEAE-cellulose as previously described (1). As sample containers we have used quartz electron paramagnetic resonance tubes (PQ701 Wilmad Glass Co., Buena, N.J.), which permit us to perform thermodynamic and kinetic measurements on the same sample. These tubes have a precision bore of 3.43 ± 0.013 mm and a volume of about 370 μl when cut to a length of 45 mm. They were chosen for their small volume and their ability to withstand centrifugal fields in sedimentation experiments up to 250,000 × $g$ with only infrequent breakage. Their high optical quality and constant pathlength also permit them to be used as cuvettes in concentration determinations from the near infrared absorption spectrum and in turbidity measurements of the delay time (see below).

The quartz tubes were loaded under nitrogen with 300 μl aliquots of the cold, concentrated hemoglobin S solution, to which were added 30 μl of a 0.5 M buffered sodium dithionite solution and 30 μl of additive (e.g., an inhibitor) in the same buffer. Dithionite reduction of oxygen required pH measurements of the final solutions. (We have found that experiments on spontaneously deoxygenated lysates and lysates deoxygenated with dithionite give similar kinetic results.) To assure reproducibility in the final deoxyhemoglobin S concentration of ± 0.1%, Chaney-adapted Hamilton syringes were used. The tubes were then quickly sealed with pressure caps (Wilmad no. 521-PC) and immediately immersed in ice water with intermittent vigorous mixing to obtain a homogeneous deoxyhemoglobin S solution. After mixing, the caps were coated with (Glyptal) varnish at room temperature to provide an oxygen-impervious seal. The tubes were stored in nitrogen-filled containers and continuously rotated in a 1° C refrigerator. Thorough mixing is necessary to eliminate small concentration in-

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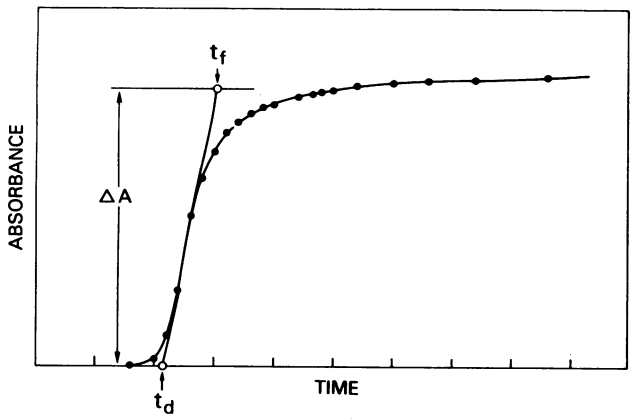


FIG. 1. Time course of the gelation of purified deoxyhemoglobin S. The solid curve shows a typical measurement of the optical density at 800 nm versus time for a deoxyhemoglobin S solution. The cylindrical electron paramagnetic resonance tube is taken from an ice bath, preheated for 1 min at the desired temperature, and inserted into a rectangular cuvette in a Gilford spectrophotometer thermostatted at the same temperature (±0.1°). The sample tube is supported in the cuvette with Teflon spacers that accurately center the tube in the light beam. The beam is masked to prevent light from reaching the detector (Hamamatsu R446, S-20 response) which has not passed through the sample. The cuvette contains water to provide approximate refractive index matching with the cylindrical tube. Some samples show a slow initial optical density increase of less than 20% of the main optical density change. For delay times less than 2 min, the optical density–time curve shows a sharp maximum, followed by a shallow minimum prior to a slow increase in turbidity. This "overshoot" may result from the formation of polymers that are longer than their equilibrium length (8).

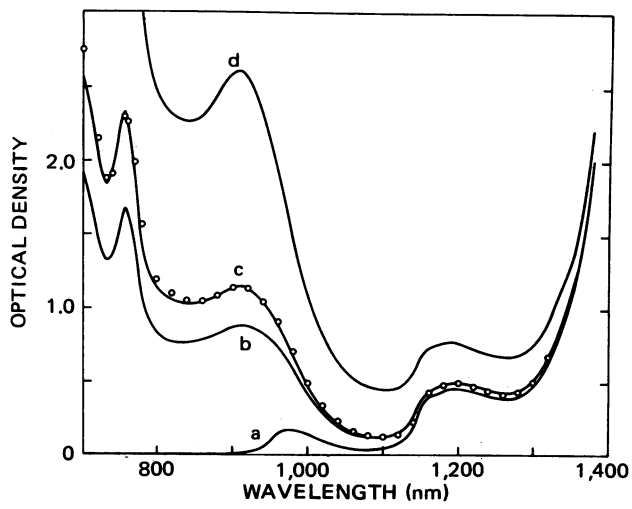


FIG. 2. Optical absorption spectra of deoxyhemoglobin S in electron paramagnetic resonance tubes measured with a Cary 17 recording spectrophotometer (slit width < 0.4 mm) on a 25.8 g/dl sample in 0.15 M potassium phosphate, 0.05 M sodium dithionite, pH = 7.15. No reference was used. Spectra are of: (a) buffer blank; (c) ungelled sample at 11° with $t_d$ > 24 hr; (b) supernatant at 20.0°, after centrifuging gel at 20.3° for 3 hr at 140,000 × *g*; (d) corresponding pellet. The points were obtained from a spectrum measured at 15.0° on a 2 g/dl solution (too dilute to gel) in a 10.0 mm rectangular cuvette, and normalized at 910 nm. The temperature dependence of the optical density at 910 nm was measured to be less than 0.1%/°C in the range 8–25°. Prior to measurement of the supernatant spectrum, the tube was inverted several times to remove concentration gradients.

homogeneities which cause considerable irreproducibility in the kinetic measurements.

## RESULTS AND DISCUSSION

**Kinetic Measurements.** The kinetic measurements utilize the well-known property of hemoglobin S solutions that gelation is thermodynamically favored at higher temperatures up to about 40° (2, 6). Consequently, the rate of gelation can be studied by heating a solution from a temperature where hemoglobin S is completely soluble to some elevated temperature at which it will eventually gel (1, 3, 4). The tubes containing thoroughly mixed samples were quickly removed from an ice bath, preheated for 1 min in a constant temperature bath, and then inserted into a thermostatted cuvette of a spectrophotometer. The progress of the reaction was followed by measuring the optical density as a function of time. With this method, delay times from 3 to 5000 min could be measured accurately. The shortest time is limited by the time required for thermal equilibration of the sample. The turbidity that results from gel formation produces an increase in the apparent optical density (4). Because these concentrated solutions are opaque to visible radiation, the optical density was monitored at 800 nm, which is a minimum in the absorption spectrum of deoxyhemoglobin S (see below and Fig. 2). Rigorous temperature control is important because of the large activation energy for the gelation reaction, which increases with decreasing temperature and near 15° is about 120 kcal/mol (500 kJ/mol).

Fig. 1 shows a typical progress curve. The progress curve can be summarized by a simple graphical construction that yields three parameters: the delay time ($t_d$), the final time ($t_f$), and the amplitude (ΔA). We have obtained similar progress curves using the linear birefringence, calorimetric, and water proton magnetic resonance techniques (1, 7). The important parameter is the delay time, the reciprocal of which is defined as the rate of the reaction. In contrast to the amplitude, which varies from 0.5 to 0.03 optical density units for the shortest and longest delay times, respectively, the observed values of the shape parameter, $(t_f - t_d)/t_d$, while showing considerable scatter, are relatively constant with increasing delay time. This indicates that the progress curves have approximately the same shape despite the fact that the rates vary by a factor of over 1000 (1).

**Thermodynamic Measurements.** Once gelation was complete, as judged from the turbidity progress curve, the equilibrium properties of the resulting gel were characterized using a centrifugation technique similar to the one initially described by Bertles *et al.* (9). After the polymers had been sedimented, the concentration of hemoglobin S in the supernatant ($c_s$), the concentration of hemoglobin S in the pellet ($c_p$), and the volume fraction of the pellet ($v_p$) were measured. Mass conservation requires that these variables be related by:

$$c_p v_p + c_s(1 - v_p) = c_t. \qquad [2]$$

The volume fraction, $v_p$, was determined by measuring the height of the pellet on a dissecting microscope equipped with a graduated scale. To obtain $c_s$, $c_p$, and $c_t$, the near infrared absorption spectrum was measured directly on the quartz tubes.

The near infrared absorption spectra of a deoxyhemoglobin S solution prior to gelation and of the supernatant and pellet phases resulting from sedimentation of a gel are shown in Fig. 2. The spectrum has a sharp peak at 758 nm and a broader band with a maximum at 910 nm. At wavelengths longer than the minimum at 1090 nm, the absorption arises almost completely from the vibrational spectrum of water. To account for variations in the optical imperfections in the tubes (<0.02 OD units) and contributions from turbidity, the following formula was

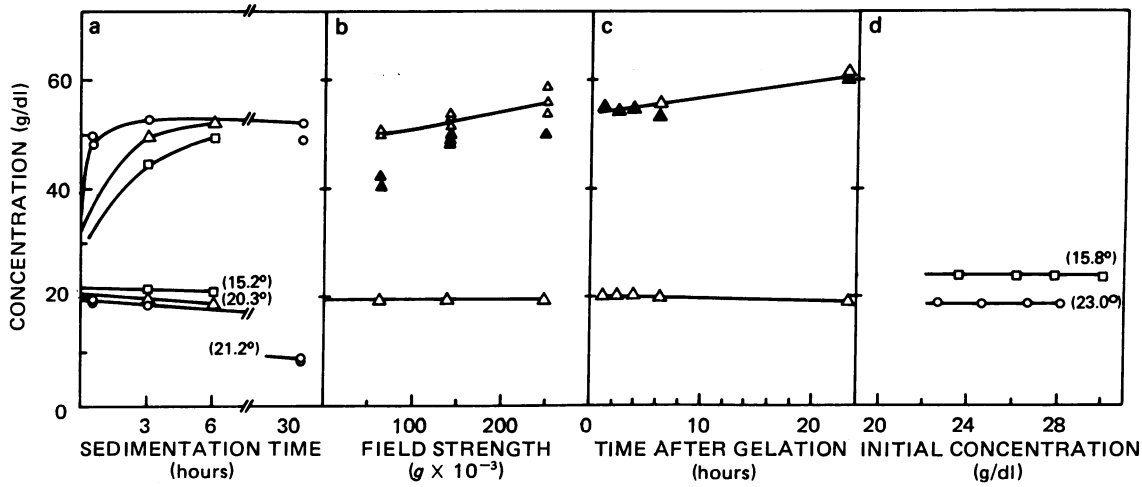


FIG. 3. Effect of variables in centrifugation for a 25.8 g/dl sample, 0.15 M potassium phosphate, 0.05 M sodium dithionite, pH = 7.15. Critically important to the sedimentation technique is the filling of the centrifuge buckets with a high density material such as glycerol to minimize tube breakage.

(a) Effect of time of centrifugation. The spectrophotometrically determined supernatant (lower curves) and pellet (upper curves) concentrations are plotted at (□) 15.2°, (△) 20.3°, and (○) 21.2° versus the time of sedimentation. The supernatant phase is cleared of polymer in less than 1 hr. Sedimentation for 30 hr depletes a considerable fraction (>50%) of the monomer from the supernatant phase, as expected from the known sedimentation coefficient of hemoglobin.

(b) Effect of centrifugal field. The concentration of the supernatant ($c_s$) and concentration of the pellet ($c_p$), determined both spectrophotometrically (△) and by conservation of mass using Eq. 2 (▲), are plotted versus the centrifugal field applied to a gel at 20°. Except for the lowest speed, the sample was centrifuged for 3 hr. At 60,000 × $g$ a minimum of 6 hr was required to produce a partial separation of monomer and polymer phases; one of the three samples did not produce any phase separation even after 6 hr. The measured solubilities in all experiments are essentially independent of field strength, the slight decrease with increasing $g$ most probably resulting from some sedimentation of monomer. The pellet concentrations measured spectrophotometrically (△) or calculated from Eq. 2 (▲) increase slightly with increasing centrifugal field. Treating the supernatant concentrations in terms of a pressure effect on the solubility equilibrium yields a negative volume change accompanying gelation of less than 0.001 liter/mole. This result is consistent with preliminary dilatometric observations that showed no volume change greater than 0.01 liter/mole, and justifies the conservation of volume assumed in Eq. 2.

(c) Effect of time after gelation. The concentration of the supernatant, $c_s$, and concentration of the pellet, $c_p$, as determined spectrophotometrically (△) or by conservation of mass using Eq. 2 (▲) are plotted versus the time after completion of gelation at 20°, as judged from the turbidity progress curve (Fig. 1). No appreciable change in these parameters with time is observed, except for a very slight increase in pellet concentration. The data in this figure provide strong evidence that the interval between gelation and sedimentation does not affect the measured solubility. The same solubility is measured at 20° on samples that are first gelled at 30° and subsequently cooled to 20°, and on samples gelled at 20°.

(d) Effect of total concentration. The concentration of the supernatant ($c_s$) is plotted versus the total concentration ($c_t$) for samples that were gelled and centrifuged for 3 hr at 140,000 × $g$; (○) 23.0°, (□) 15.8°.

used to calculate the concentration of deoxyhemoglobin S:

$$c(\text{g/dl}) = 24.56\,[(\text{OD}_{910} - \text{OD}_{1090}) + 0.04]. \qquad [3]$$

This formula is based on determination of the extinction coefficients at 910 and 1090 nm relative to the assumed extinction, $\epsilon = 10{,}900\ \text{M}^{-1}\ \text{cm}^{-1}$ per heme at 542 nm for cyanmethemoglobin and a molecular weight of 16,100 per heme. The 0.04 OD correction term arises from the difference in water absorption at 910 and 1090 nm. In pellet spectra there is a considerable contribution to the total optical density from turbidity, amounting to 0.3 to 1.1 OD units (see Fig. 2). The scattering is a very weak function of wavelength, however, and the use of Eq. 3 for pellet spectra gives results in reasonably good agreement with $c_p$ calculated from $c_t$, $c_s$, and $v_p$ using Eq. 2.

The experimental criteria which must be satisfied for the measured $c_s$, $c_p$, and $v_p$ to represent equilibrium values are: (*i*) that the sedimentation of polymers is performed without any appreciable sedimentation of monomer, and (*ii*) that the measured values are independent of time. The results in Fig. 3a–c demonstrate that these criteria are indeed satisfied to a very good first approximation. Based on the results in Fig. 3a and b, we chose a standard protocol for centrifugation in which gelled samples were spun at 140,000 × $g$ for 3 hr. These conditions represent a compromise between the optimal conditions for producing a compact pellet of polymer without appreciable monomer sedimentation, and for minimizing the possibility of tube breakage.

A final thermodynamic criterion for our treatment of $c_s$ as a solubility is that both $c_s$ and $c_p$ are independent of the total concentration, $c_t$. Fig. 3d shows that this is the case in the range of supersaturation ratios ($c_t/c_s$) over which $c_s$ and delay times can both be measured on the same sample. The independence of $c_s$ from $c_t$ has also been reported by Magdoff-Fairchild *et al.* (10). Within the experimental errors of our technique, the pellet concentration is also nearly constant at 48–55 g/dl at pH 7.05–7.1. This range is higher than that previously reported by us (2) under slightly different conditions, using a less precise technique that overestimates $v_p$. The present results are comparable to an earlier estimate of 57 g/dl by Bertles *et al.* (9)

A major advantage of our method is that five variables ($t_d$, $c_s$, $c_p$, $v_p$, and $c_t$) are determined on the same sample without opening the tube. The samples can then be used again to repeat either kinetic or sedimentation experiments at the same or different temperature. We have found that both the delay time and solubility are highly reproducible for periods of up to 1 month. No systematic variation in the solubility, the delay time, or the sample spectra with time was observed. Identical values of these parameters were obtained on samples that have been repeatedly gelled, centrifuged, and redissolved, and on samples that have never been centrifuged. Thirty control experiments on pure deoxyhemoglobin S samples yielded standard deviations in the observed delay times and solubilities of ±15% and ±0.5%, respectively. These errors are comparable, since the delay time is $n$ times more sensitive to small changes in the

Table 1. Inhibition of gelation by urea (20°), pH (20°), and carbon monoxide (26°)*

| | $c_t$ (g/dl) | $t_d$ (min) | $c_s$ (g/dl) | $c_p$ (g/dl) | $v_p$ |
|---|---|---|---|---|---|
| [Urea] (M) | | | | | |
| 0 | 28.1 | 6.0 | 20.8† | ‡ | 0.302† |
| 0.025 | 28.0 | 16.9 | 21.4 | 50.6 | 0.277 |
| 0.025 | 28.1 | 12.9 | ‡ | ‡ | ‡ |
| 0.050 | 27.9 | 55.2 | ‡ | ‡ | ‡ |
| 0.050 | 27.6 | 47.8 | 21.6 | 48.6 | 0.267 |
| 0.100 | 27.6 | 123 | 22.6 | 50.8 | 0.218 |
| 0.100 | 27.7 | 126 | 22.5 | 48.9 | 0.203 |
| 0.200 | 27.6 | 720 | 23.4 | 48.9 | 0.145 |
| 0.200 | 27.7 | 1160 | 23.4 | 48.6 | 0.149 |
| Measured pH | | | | | |
| 6.51 | 26.5 | 20.6 | 19.6 | 46.2 | 0.272 |
| 6.63 | 27.0 | 29.5 | 20.2 | 49.6 | 0.270 |
| 6.63 | 26.8 | 26.5 | 20.2 | 49.4 | 0.280 |
| 6.80 | 26.8 | 47.6 | 20.4 | 48.3 | 0.282 |
| 7.02 | 26.8 | 61.6 | ‡ | ‡ | ‡ |
| 7.02 | 27.1 | 49.0 | 20.7 | 53.7 | 0.203 |
| 7.11 | 26.8 | 172 | 20.8 | 56.5 | 0.159 |
| 7.32 | 27.2 | 208 | 21.3 | 57.2 | 0.178 |
| 7.32 | 28.2 | 50 | 21.4 | 57.0 | 0.195 |
| Initial CO % saturation | | | | | |
| 9.8 | 27.9 | 3.3 | 20.9 | 54.5 | 0.241 |
| 18.6 | 27.9 | 26.5 | 22.3 | 53.3 | 0.196 |
| 28.7 | 27.9 | 160 | 23.4 | 53.0 | 0.140 |
| 36.6 | 27.9 | 584 | 24.3 | 55.8 | 0.103 |

* In the four samples partially saturated with carbon monoxide $c_t$ and $c_s$ are taken as the sum of the deoxyhemoglobin and carbonmonoxyhemoglobin concentrations in the supernatant, while $c_p$ is the spectrophotometrically measured deoxyhemoglobin concentration in the pellet. Carbonmonoxyhemoglobin absorbance is very small at 910 nm, and has been neglected in the calculations.

† This value was taken from the data at pH 7.11.

‡ The sample tube broke during centrifugation.

solubility than are direct measurements of the solubility itself (Eq. 1) (1).

In previous studies gelation has most frequently been assayed by the determination of the "minimum gelling concentration" (MGC), usually defined as the minimum concentration of hemoglobin that produces cessation of flow inside a flask (11–14). The MGC values obtained are higher than the solubilities obtained under similar conditions by about 20–30%. The difference may arise from the delay time for the gelation reaction. Because a supersaturation of about 1.25 (see Eq. 1) is required for gelation to occur on a time scale of minutes, the observed MGC might be expected to be higher than the equilibrium solubility by about the same factor. The higher value might also arise from the requirement that a significant mole fraction of hemoglobin be polymerized in order to produce cessation of flow in the MGC flask.

**Inhibitors of Gelation and the Supersaturation Equation.** In order to test the dependence of the delay time on the solubility, as predicted by Eq. 1, we measured the effect of known inhibitors—urea (6, 14), alkali (15), and carbon monoxide (16)—on the delay time and equilibrium properties of the gelation reaction. The results are shown in Table 1. In the range of urea concentrations from 0 to 0.2 M, the pellet concentration remains constant, while the solubility increases from 20.8 to 23.5 g/dl, the mole fraction of polymer formed decreases from 0.48 to 0.28, and the delay time increases by a factor of about 100.

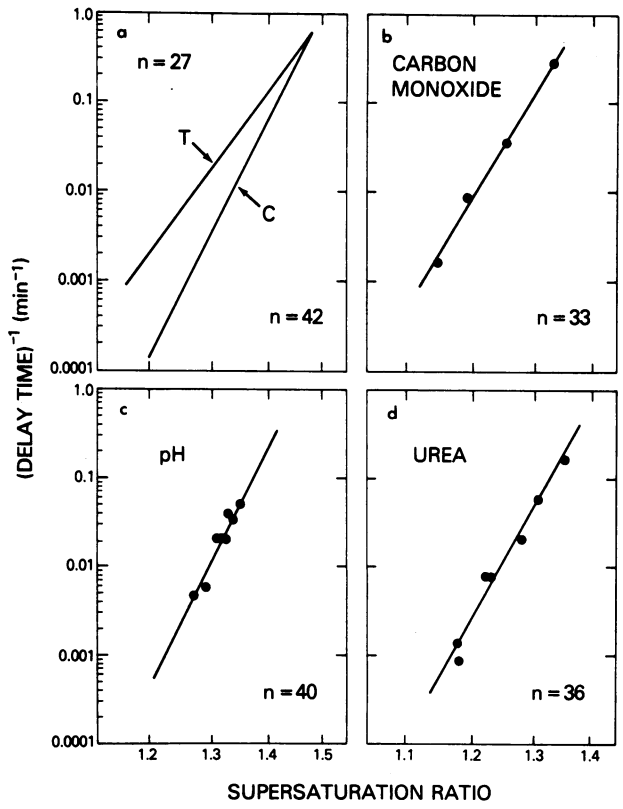


FIG. 4. Supersaturation plots. The reciprocal delay time ($1/t_d$) is plotted versus the supersaturation ratio ($S \equiv c_t/c_s$). Both scales are logarithmic. All samples are in 0.15 M potassium phosphate, 0.1 M sodium dithionite, pH = 7.1. Except for (a), each point represents the results of a single experiment. The figure shows the variation of the rate with changes in supersaturation (data from Table 1) produced by changing: (a) the sample concentration (C) and temperature (T); (b) the saturation of the sample with carbon monoxide; (c) the sample pH; (d) the concentration of urea. (a) Represents the results of a detailed study of the concentration and temperature dependence of the delay time which showed that the rate varied as the concentration raised to the 42nd power at all temperatures between 16° and 40°, but that the dependence of the rate on changes in solubility produced by increasing the temperature gave only a 27th power dependence on the supersaturation ratio. The solubilities determined at various temperatures are as follows: 11°, 23.9 g/dl; 15°, 21.4 g/dl; 20°, 19.6 g/dl; 25°, 17.8 g/dl; 30°, 16.9 g/dl; 35°, 16.6 g/dl; 40°, 16.8 g/dl; 45°, 17.3 g/dl. These data suggest that the requirement for precise temperature control for solubility determination could be relaxed by limiting experiments to the physiological temperature range (35–40°).

Urea thus has a marked inhibitory effect on the rate of gelation even at very low concentrations where the equilibrium properties are only slightly perturbed. If either the initial saturation with carbon monoxide or the initial pH is increased, the results in Table 1 show that the effect of these inhibitors on the rate of the reaction is much greater than, but proportional to, their effect upon the solubility. The carbon monoxide results are particularly noteworthy since the data on the composition of the pellet show that carbonmonoxyhemoglobin is almost completely excluded from the polymer. If the assumption is made that polymerization preferentially selects for the deoxy (T) quaternary structure of hemoglobin (16, 17), the low pellet saturation is in qualitative agreement with the low saturation of T state molecules predicted by variety of models which have been developed to explain the binding of carbon monoxide and oxygen to hemoglobin (18–20).

The data in Table 1 can be directly used to test the supersaturation Eq. 1. Fig. 4 shows the reciprocal delay time plotted

on a logarithmic scale versus the supersaturation ratio, $S \equiv c_t/c_s$ (also on a logarithmic scale). For calibration purposes we also show plots for the concentration and temperature dependence of a delay time (Fig. 4a). The urea results yield a straight line with a slope of 36, intermediate between the values observed for the concentration and temperature dependence. Similar slopes are observed for the pH and carbon monoxide data. The results in Fig. 4 are unmistakable. Although inhibition of gelation by urea, carbon monoxide, increasing pH, and decreasing temperature certainly act by different detailed molecular mechanisms, they all produce linear supersaturation plots with similar slopes. This result demonstrates the applicability of the supersaturation equation and shows that, at least for small perturbations, the kinetics and thermodynamics are intimately related. In forthcoming publications we shall discuss the effects of these inhibitors in detail and present a theory on nucleation-controlled polymerization that predicts the supersaturation equation (Ross *et al.*, in preparation; 21).

## CONCLUSION

The principal conclusion of our kinetic and thermodynamic studies (21) is that both the thermodynamics and the kinetics of gelation are controlled by the supersaturation ratio. The critical difference, however, is that the amount of polymer formed is roughly proportional to the supersaturation, whereas the rate of polymer formation is very much more sensitive, depending on the 30–40th power of $S$. We have shown that this behavior persists in the presence of inhibitors and in partially liganded hemoglobin S solutions.

Based on this result, the supersaturation equation can be used to estimate both intracellular delay times and the effect of inhibitors on these delay times from a knowledge of the total concentration, $c_t$, and the solubility, $c_s$. If we make use of the solubility data on solutions partially liganded with carbon monoxide to recalculate the delay time map for cell sickling at various oxygen saturations (1, 21), we find the essential features of the delay time map unchanged. A delay time of 1 sec is calculated for a cell having a hemoglobin S concentration of 32 g/dl and a fractional saturation of 40%. The predictions are capable of explaining reported data (22, 23) on the kinetics of cell sickling, which have shown concentration- and temperature-dependent delay times (21).

We have also used the supersaturation equation to estimate the increase in the delay time which would be required to produce a measurable change in the clinical course of the disease (21). For this calculation we use the minimum gelling concentrations of mixtures of hemoglobins (13, 24) to estimate changes in the solubility, and correlate the delay time expected for various sickle hemoglobinopathies with the clinical status (25, 26). The comparison suggests that an increase in the delay time by a factor of 20 may produce a detectable therapeutic effect, but that a factor of 100 may be required to produce a clear improvement in clinical status (21). This result casts doubt on the effectiveness of maintenance oral urea therapy (27–30; †), which our studies suggest could produce a maximum increase in the delay time of only a factor of 2. This conclusion has also been reached by May and Huehns (31) on the basis of oxygen equilibrium studies on intact red cells.

† A recent double blind study has shown that urea administered to patients intravenously is ineffective in treating sickle cell crises (29, 30). No controlled studies have yet been performed to test the efficacy of oral urea maintenance therapy.

We would like to thank Alan N. Schechter for helpful discussions.


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