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REVIEW ARTICLE

The temperature-jump technique for the study of fast reactions in solution

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Abstract. The temperature-jump technique is one of the most widely used methods for measuring the rates of those chemical reactions in solution which are so fast that reaction is complete as soon as the reagents are mixed. The reaction mixture is allowed to come to equilibrium, and then the equilibrium is perturbed by a rapid temperature rise, produced by Joule, microwave or laser heating. The rate at which the system adjusts to the new equilibrium is monitored by observation of the intensity of light absorbed or fluoresced, or by the electrical conductivity. Rate constants as fast as the theoretical limit of 10^{10} dm^3 mol^{-1} s^{-1} are measurable. The review considers the limitations on the performance of temperature-jump apparatus and gives examples of systems studied.

1. Introduction

A topic of major concern to chemists and biochemists is the description at the molecular level of the ways in which one substance is converted into another. The principal tool used for this purpose is the measurement of the rate of the reaction, and the way this rate depends on such factors as temperature, concentration or solvent.

The oldest, and still the most common method of measuring the rate of a reaction in solution is to mix solutions of reagents and observe what happens to the mixture. This technique is subject to the limitation that mixing requires a finite time. Even in the most sophisticated mixers, mixing cannot be achieved in much less than a millisecond, and a large number of reactions are known to be complete within this time. An estimate of the maximum rate is provided by the theory of diffusion-controlled reactions. According to this theory, molecules of reagents A and B move at random through the solvent, and reaction to give a molecule of product C occurs wherever a molecule of A collides with a molecule of B. The rate of formation of C on the macroscopic scale is given by the second-order rate equation

$$\mathrm{d[C]}/\mathrm{d}t = k_2[\mathrm{A}][\mathrm{B}]$$

where [A] is the concentration of A, etc. Diffusion theory (North 1964) gives an approximate equation for k_2:

$$k_2 = 4\pi r_{\mathrm{AB}} D_{\mathrm{AB}}/1000 N_0 \ \mathrm{dm^3\ mol^{-1}\ s^{-1}}$$

where r_{AB} is the sum of the radii of molecules A and B, in cm, D_{AB} is the sum of their diffusion coefficients, in cm^2 s^{-1}, and N_0 is the Avogadro number. Substitution of typical numerical values for small molecules in dilute aqueous solution at room temperature gives a value of k_2 of around 10^{10} dm^3 mol^{-1} s^{-1}. Since it is experimentally inconvenient to use solutions of concentrations much less than 10^{-5} mol dm^{-3}, reactions occurring at the diffusion-controlled limit will be complete in much less than a millisecond.

The temperature-jump technique enables reactions to be studied whose rates are up to the diffusion-controlled limit. The reagents are mixed, and allowed to come to equilibrium, producing a solution containing finite concentrations of both reagents and products. This equilibrium is dynamic: the rate of production of C is equal to the rate of its breakdown to give A and B again. The position of equilibrium, i.e. the relative concentrations of A, B and C, depends on the temperature, as given by the Van't Hoff isochore:

$$\mathrm{d}\ln K/\mathrm{d}t = \Delta H^{\ominus}/\mathscr{R}T^2$$

where $\Delta H^{\ominus}$ is the standard enthalpy change of the reaction, T is the absolute temperature, $\mathscr{R}$ is the gas constant, and K is the equilibrium constant, defined by

$$K = [\mathrm{C}]/[\mathrm{A}][\mathrm{B}].$$

If the temperature is raised abruptly, the new value of K is such that the concentrations are no longer appropriate. Chemical reaction takes place until the new equilibrium concentrations are established. For a small perturbation the rate of change of concentration at time t is proportional to the concentration change necessary to reach equilibrium. Integration of the rate equation gives

$$[\mathrm{C}]_t - [\mathrm{C}]_\infty = ([\mathrm{C}]_0 - [\mathrm{C}]_\infty)\exp(-t/\tau)$$

where $[\mathrm{C}]_t$, $[\mathrm{C}]_0$ and $[\mathrm{C}]_\infty$ are the concentrations at time t and the initial and final concentrations respectively, and τ is the relaxation time. The relaxation time is related to the rate constants for the reactions setting up the equilibrium. For the scheme

$$\mathrm{A} + \mathrm{B} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C},$$

$$\tau^{-1} = k_{-1} - ([\mathrm{A}]_\infty + [\mathrm{B}]_\infty)k_1,$$

so that a plot of $([\mathrm{A}]_\infty + [\mathrm{B}]_\infty)$ against τ^{-1} gives values of k_1 and k_{-1}. A useful check is provided by

$$K = k_1/k_{-1}.$$

If $K = 10^5$ dm^3 mol^{-1}, $[\mathrm{A}]_\infty = [\mathrm{B}]_\infty = 10^{-5}$ mol dm^{-3} and k_1 is the diffusion-controlled rate of 10^{10} dm^3 mol^{-1} s^{-1}, then $\tau = 3\ \mu$s, showing that the heating time must be of the order of microseconds if the fastest reactions are to be studied.

It often happens that a chemical system contains more than

one equilibrium, the equilibria being coupled together. Each equilibrium is associated with a relaxation time. For example, for the system

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} D$$

there are two relaxation times. If it is assumed that the first is much shorter than the second.

$$\tau_1^{-1} = k_{-1} + k_1([A]_\infty + [B]_\infty)$$

$$\tau_2^{-1} = k_{-2} - k_1([A]_\infty + [B]_\infty)/\{1 - k_1([A]_\infty + [B]_\infty)\}.$$

More complex equations are obtained if τ_1 is similar to τ_2 or if more equilibria are involved (Bernasconi 1976).

It is not only the magnitude of $\Delta H^\ominus$ which determines the amplitude of the relaxation. The equilibrium must be poised in such a way that a small change in K produces a significant change in concentration of one of the species. This is often expressed in terms of the Γ factor

$$-\Delta c = \Gamma \Delta K/K$$

where c is a species concentration. For the simple association $A + B \rightleftharpoons C$, for the special case where $[B]_\infty \gg [A]_\infty$

$$\Gamma = K[A]_\infty[B]_\infty/(1 + K[B]_\infty).$$

This relationship shows that the largest amplitude relaxations are observed for systems for which K is large but in which there are still appreciable concentrations of the reagents at equilibrium. The temperature-jump method cannot be used for reactions which proceed to completion.

It can be seen that the problems of design of a temperature-jump apparatus centre on two areas; the method of producing a sufficiently rapid temperature rise, and the method of monitoring the consequent rapid change in concentration.

2. Production of the temperature-jump

2.1. Joule heating

Joule heating is by far the most common method for producing a rapid temperature rise. A capacitor, of capacitance C, is charged to a high voltage V, then discharged through the sample solution, of resistance R, by the triggering of a spark gap. Typically C is 0.01 μF and V 20 kV, giving a 2 J energy pulse, sufficient to raise the energy of 0.1 cm^3 of aqueous sample solution by 5 K. This is a convenient magnitude of temperature rise, large enough to produce an appreciable signal but not so large as to cause the perturbation to be nonlinear. Combination of formulae in the previous section shows that for the simple association $A + B \rightleftharpoons C$, with B in excess,

$$\frac{\Delta[A]}{[A]_\infty} = \frac{k[B]_\infty}{1 + K[B]_\infty} \frac{\Delta H^\ominus \Delta T}{\mathscr{R} T^2}.$$

If typical values of K (1000 dm^3 mol^{-1}), $\Delta H^\ominus$ (−50 kJ mol^{-1}) and T(300 K) are taken, [A] is seen to increase by 17% for a 5 K temperature rise.

The temperature increase follows the exponential decay law, having time constant $\frac{1}{2}RC$. It is necessary to dissolve inert salt in the sample solution to obtain an acceptably low value of R. In a typical sample cell, in which the electrodes are 6 mm diameter and separated by 10 mm, a 0.1 mol dm^{-3} salt solution produces a resistance of about 400 Ω, so that the heating time constant is about 2 μs. With careful design of the sample cell to minimise R, heating time constants as low as 175 ns have been observed (Reich and Sutter 1977).

An improved shape of heating pulse is obtained if the discharge is from a coaxial cable rather than a simple capacitor. Short-circuiting one end of a coaxial cable gives a rectangular pulse with a clearly defined end. Hoffman (1971) uses a 5 m length of cable charged to 100 kV to produce a temperature rise of 10 K in a 40 mm^3 sample in only 80 ns. However, although technically feasible, heating pulses of less than 1 μs duration are not commonly used. Too rapid heating of the sample produces shock waves which give spurious signals due to cavitation and transient changes in refractive index. Furthermore it is difficult to screen a sensitive detector from the electrical noise produced by a 100 kV discharge.

The variation in the rate of a reaction with changing pressure provides useful information about the transition state of the reaction, and methods of obtaining these data are of considerable current interest. A high-pressure sample cell, for use up to 200 MPa, which can be placed in a standard Joule heating apparatus has been described by Doss *et al* (1982). Liphard (1979) describes temperature-jump apparatus capable of operation at 200 MPa in which a 50 ns heating pulse is produced by the discharge of a 5 m length of coaxial cable.

2.2. Microwave heating

Joule heating has the limitation that a sample solution of fairly high electrical conductivity must be used, thereby ruling out almost all non-aqueous solvents. Microwave heating has the much less stringent requirement that the solvent molecules have a permanent dipole moment. The sample cell is mounted within a resonant waveguide cavity, at a point where the change in electric field is greatest. A pulse of microwave energy is supplied by a magnetron. The apparatus is necessarily more complex and expensive than for Joule heating, and the amplitude of the temperature jump is usually less, being limited by economic considerations. Caldin and Crooks (1967) describe an apparatus in which a 100 kW magnetron, operating at 3 cm wavelength, is used to heat 150 mm^3 of chlorobenzene by 0.4 K in 1.2 μs. Microwave heating does provide a particular advantage in that the heating pulse may readily be applied repetitively, so that the signals from a large number of pulses may be collected and averaged. Buchwald and Ruppel (1971) average the signal from 1200 pulses to reduce the noise by a factor of 25. It is of course necessary to circulate the sample solution continuously through the cell to prevent an accumulative temperature rise. The use of signal averaging is also described by Aubard *et al* (1979) and by Caldin and Field (1982a, b). The main disadvantage in the study of small relaxations is that artefacts, due, for example, to transient refractive index changes also induced by the temperature rise in the sample solution, may also be brought up out of the noise.

A limitation on the applicability of the temperature-jump technique is that the relationship between the equilibrium constant and the concentrations of the species involved must be such that the equilibrium is poised. If the equilibrium lies too far on one side, the temperature rise will not produce a measurable concentration change. However a more suitable value of the equilibrium may be obtained by working at a temperature well away from ambient. This technique is especially valuable if non-aqueous solvents, which can be cooled to low temperatures without freezing, are used, as is permitted with microwave heating. Caldin and Field (1982 b) study the formation of the charge-transfer complex between iodine and imidazole in chlorobutane at −64°C for this reason.

2.3. Laser heating

At first sight, the Q-switched laser would appear to be the ideal energy source, readily delivering 20 J in 20 ns. There are, unfortunately, problems in the conversion of this optical energy into thermal energy. Water and other common solvents are transparent in the visible and near ultraviolet, the regions of the spectrum in which most lasers emit. If a dye, absorbing at the appropriate wavelength, is dissolved in the solvent, it is found

there is a limiting rate at which energy can be absorbed. Each dye molecule can only absorb one photon and takes several nanoseconds to return to the ground state in which it is ready to absorb another photon. This rate is less than the power output of a Q-switched laser (Caldin *et al* 1971). Dye conversion is only feasible if the laser is used in the non-Q-switched mode. Caldin *et al* (1971) use a ruby laser emitting at 694 nm to give temperature rises of 5 K in 100 μs with vanadyl phthalocyanine as dye absorber. Caldin *et al* (1973) exploit the great advantage of laser heating, the complete decoupling of the energy source from the sample cell, to design a high-pressure temperature-jump apparatus. The sample solution is pressurised to 300 MPa, and the laser pulse is admitted through a sapphire window.

The major difficulty with laser heating is the need to have the correct absorbance for the sample solution. If the absorbance is too low, only a small proportion of the laser energy is used to heat the sample in the detection region. If the absorbance is too high, the laser light is all absorbed within a few mm depth of penetration into the sample, causing a steep temperature graident. If a dye is used, the absorbance can be readily adjusted to the optimum value, around 0.5 cm^{-1}. Water is transparent in the visible and opaque in the infrared, but there is a narrow region around 1.4 μm where the intrinsic absorbance is suitable. Laser radiation of this wavelength is produced by a neodymium laser whose output, at 1.06 μm, is shifted by the Raman effect in liquid nitrogen to 1.41 μm, where H_2O has an absorption of 10 cm^{-1}. Since D_2O is transparent at this wavelength, H_2O–D_2O mixtures of suitable absorbance may be prepared. Turner *et al* (1972) describe such an apparatus in which 2 J are delivered in 20 ns. Aubard *et al* (1977) describe a similar apparatus. The laser heating pulse enters along approximately the same axis as the spectrophotometric monitoring light beam, but at sufficient of an angle to avoid the monitoring photomultiplier. An extremely high light level is required to obtain a high signal-to-noise level at the high bandwidth used. This is achieved by boosting the lamp, a 150 W Xe arc by a 400 A current pulse from a capacitor bank for approximately 1 ms. The noise level is only 10^{-3} of an absorbance unit, extremely low for the fast response time of 10 ns.

The iodine laser is an extremely promising energy source for temperature-jump studies (Holzwarth 1979). This laser emits at 1.315 μm, where the absorbance of H_2O is 1 cm^{-1}. Although it has been little used so far, it will probably become the standard laser for this pupose.

3. Monitoring the concentration change

3.1. Spectrophotometric detection

Spectrophotometric detection is the most popular method for monitoring the relaxation. It is specific, sensitive and capable of following very fast changes. The absorbance A of the sample is related to the concentration, c, of the absorbing species by the Beer–Lambert law:

$$A = \lg I_0/I = \varepsilon c l$$

where I is the intensity of light passing through the sample, I_0 the corresponding intensity for a non-absorbing sample, ε is the molar absorbance of the absorbing species and l is the optical path-length. Monochromatic radiation in the 200–700 nm wavelength region is used, a wavelength being chosen at which only one species absorbs. Deviations from the linear dependence of the absorbance on the concentration are found if the light is not truly monochromatic and ε varies over the spectral range used. However for small changes in concentration, as typical for temperature-jump studies, the nonlinearity is not perceptible. The intensity of the light transmitted is monitored by a photomultiplier, and the signal voltage is displayed on an oscilloscope or stored in a data-capture system as a function of time from the initiation of the jump.

Differentiation of the Beer–Lambert law shows $\Delta V/V$, the relative signal change during relaxation, is linearly proportional to Δc, the change in concentration of absorbing species, if $\Delta c/c$ is small.

$$\Delta c = \frac{\Delta A}{\varepsilon l} = \frac{-2.303}{\varepsilon l}\frac{\Delta I}{I} = \frac{-2.303}{\varepsilon l}\frac{\Delta V}{V}.$$

The electronic noise voltage, N, is mainly shot noise from the photomultiplier, which is given by (Reich and Sutter 1977)

$$\frac{V}{N} = \left(\frac{i_k}{2eB}\right)^{1/2}\left(\frac{\delta - 1}{\delta}\right)^{1/2}$$

where i_k is the photomultiplier cathode current, e the charge on the electron, B the electronic bandwidth and δ is the gain per dynode. B must be large enough for the monitoring of fast signals, which means that the magnitude of i_k is crucial if an adequate value of V/N is to be obtained. A 50 W tungsten-filament quartz-halogen lamp is commonly used for radiation in the range 350–700 nm, and a xenon arc is used for the deeper ultraviolet. The monochromator slit is opened up to give an intense light beam through the sample. It is common practice to have a preamplifier of unit gain and low output impedance, e.g. an emitter-follower, close to the photomultiplier to ensure that the stray capacitance is due solely to the photomultiplier itself and not to connecting cables. Typically an anode load of 50 kΩ is used, which gives a photometer rise-time of 1 μs for 20 pF stray capacitance. A cathode current of 0.1 μA may be achieved. Under these conditions a value of 1400 for V/N is found. Only a few dynodes, e.g. four, are used for electron multiplication and a gain of 1000 is typical, to give a total signal of 5 V with less than 5 mV noise. This signal is the 'light-to-dark' signal, V. The relaxation signal, ΔV, is rarely more than 10% of V, and may be much less in unfavourable circumstances, which is why a high value of V/N is essential.

The magnitude of $\Delta V/N$ depends on the absorbance A. If A is too large, V, and hence ΔV, is too small. If A is too small the solution is so dilute that Δc, and hence ΔV is small and the large value of V produces a large value of N. Differentiation of the noise equation shows that $\Delta V/N$ is at a maximum when $A = 2/\ln 2$, i.e. 0.868. In practice one aims to use solutions in the absorbance range 0.5–1.0.

For many systems of interest none of the species involved absorb in a convenient region of the spectrum, but a pH change is produced by the relaxation. It is then common practice to add an indicator, whose absorption spectrum in the visible changes with pH. The colour change is due to a proton-transfer reaction which is much faster than the reaction to be studied. The observed relaxation then shows two steps: a fast initial change due to the proton transfer followed by a slow change due to the reaction under study. To prevent the initial step taking the signal out of the range of the data-capture system, or off the oscilloscope screen, it is usual to include an autobias circuit which grounds the signal for a preset time, say 10 μs, after the temperature rise.

Infrared signals are so weak that it is extremely difficult to follow fast relaxations by infrared spectrophotometry. An unusual apparatus has been developed by Möller and de Maeyer (1982) in which the sample, only 8 μl in volume, is spread on the surface of a multi-reflector germanium plate. The temperature of the plate is raised by several degrees in about 50 μs by capacitor discharge, and the sample is heated by thermal conduction from the plate. The penetration depth of the infrared radiation used to monitor the relaxation is only 0.5 μm and multiple reflections are needed to enhance the sensitivity of the detection. The detector

is a Hg/Cd/Te photoconductor, cooled by liquid nitrogen to reduce Johnson noise.

3.2. Fluorimetric detection

A small proportion of molecular species are capable of storing absorbed radiation for a few nanoseconds and then re-emitting it. The wavelength of the re-emitted radiation is longer, by 100–200 nm, than that of the absorbed radiation, since some energy is lost during storage of thermal modes. This phenomenon is known as fluorescence. The ability of a molecule to fluoresce depends partly on its structure (fluorescence is only seen for large, rigid molecules with extensive electron delocalisation) and partly on the local environment. A molecule which is fluorescent when free in aqueous solution may not fluoresce if tightly bound to a macromolecule at a site from which water is excluded. Fluorimetric detection is thus often used for the study of enzyme–substrate reactions, or other reactions of biochemical interest where a small molecule is attached to a protein (Rigler and Ehrenberg 1973). A macromolecule may have a fluorescent group attached to it either intrinsically (e.g. proteins) or by design, and changes in the overall shape, or conformation, of the macromolecule cause changes in its fluorescent behaviour.

A great advantage of fluorescence over transmittance is that zero concentration of species to be monitored gives zero signal, i.e. no light emission is observed at 90° to the incident excitation light, and so there is zero noise. Transmitted light, by contrast, is at a maximum for zero concentration of absorbing species, so that low concentrations are seen against a background of maximum noise. As a rough rule-of-thumb fluorescence is capable of measuring the concentrations two orders of magnitude less than for transmittance. In favourable circumstances, concentration changes as low as 10^{-9} mol dm^{-3} may be observed. The use of low concentrations may increase relaxation times to a more readily observed range of values.

Sample cell design is more critical for fluorescence than for transmittance observation. Wide-angle conical lenses are used as windows so that as much as possible of the emitted light may be observed by the photomultiplier. The xenon arc is a standard excitation light source.

3.3. Conductimetric detection

If a reaction involves a change in the number of ions, or the production of ions of different mobility, it may be followed by the change in electrical conductance. This change is swamped by the high conductance of the solution necessary for Joule heating, so that conductimetric detection is only feasible for microwave and laser heating. The sample cell forms one arm of a Wheatstone bridge, and the off-balance signal is monitored by an oscilloscope. The applied voltage must reverse polarity at regular intervals to prevent electrolysis, and either a square wave or sine wave at sufficiently high frequency may be used. A particularly interesting example of the use of this technique is the classical measurement of the rate of the primary reaction in aqueous solution

$$H^+ + OH^- \rightleftharpoons H_2O.$$

The relaxation time for pure water, heated through 0.35 K by a 2 μs pulse of 3 cm microwave radiation, was found to be 37 μs (Ertl and Gerischer 1961). This gives a value of 1.3×10^{11} dm^3 mol^{-1} s^{-1} for the ion combination reaction, the highest rate constant ever observed for a reaction in solution.

Conductimetric detection is in general little used for temperature-jump studies, partly because it is not applicable to Joule heating and partly because it is less specific than spectrophotometric detection. The use of conductimetric detection with laser heating has been described by Grubic *et al* (1981). An AC bridge, operating at 40 kHz, was used, and the envelope of the out-of-balance AC signal gave the reaction trace. The detection and heating systems were not well matched in this apparatus, since the temperature rise was produced in 30 ns, but the detector could not measure a relaxation time of less than 100 μs or so. A sub-nanosecond conductimetric detector is described by Beck (1979). Impedance-matching techniques are necessary to obtain the time resolution. The cell forms part of a 50 Ω line. A voltage pulse, 1 μs in duration, is applied from a 300 pF coaxial capacitor and the cell current is monitored as the voltage developed across a load resistor. A response time of about 100 ps is achieved. This detector was developed for use with a pulse-radiolysis apparatus, but would be very useful for a laser temperature-jump apparatus.

4. Stopped-flow/temperature-jump studies

A fairly common reaction scheme involves the irreversible decomposition of one of the components in a dynamic equilibrium

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} D.$$

The mixing of A and B results in a solution which eventually contains only D. Enzyme-catalysed reactions provide the best set of examples of this type. The enzyme and substrate bind reversibly to form a complex, which irreversibly breaks down to give the final products of the reaction. If k_2 is too large it is not possible to measure k_1 and k_{-1} in a simple temperature-jump apparatus, since reaction is complete in the time it takes to fill the sample cell. The time range of the apparatus may be extended by combining it with a stopped-flow system. Solutions A and B are held in separate syringes and driven through mixing jets into the observation cell of the temperature-jump apparatus by a push on the syringe pistons. The mixed solution flows on out of the cell into an empty syringe, pushing out the pistron. The piston is stopped by hitting a mechanical stop, fitted with a microswitch. The flow is stopped abruptly, leaving the temperature-jump cell filled with solution mixed only a few milliseconds previously. The closing of the microswitch triggers a delay circuit which in turn triggers the capacitor discharge after a preset time, chosen to allow C to have reached the desired concentration.

Sample cell design has proved to be more difficult than might appear at first sight. Successful apparatus of this type is rare, even though details of one was published fifteen years ago (Erman and Hammes 1966).

5. Applications

5.1. Introduction

A detailed discussion of applications would belong in a chemistry journal, but it is useful to give here an idea of the range of chemical reactions which have proved amenable to study by the temperature-jump technique.

5.2. Proton-transfer reactions

The first reactions to be studied by this technique were proton-transfers, of the type $AH + B \rightleftharpoons A^- + BH^+$. It was found that these reactions are diffusion-controlled in the exothermic direction if the proton transfer is from oxygen or nitrogen. The rates for carbon acids are many orders of magnitude slower (Crooks 1975).

5.3. Metal-complex formation

Metal ions react with molecules or anions to form complex ions which are often intensely coloured. The rate depends on the metal ion; some ions, e.g. Pt^{2-}, react slowly enough for complex formation to be followed by conventional spectrophotometry, while others, e.g. Ni^{2+}, Fe^{3-}, Mn^{2+} and Zn^{2+} form complexes

in the time scale accessible to temperature-jump studies. The rate-controlling step has been found to be the expulsion of a water molecule from the immediate vicinity of the metal ion, which explains why the rate depends on the metal ion, not on the complexing agent (Eigen and Tamm 1962).

5.4. Electron-transfer reactions

Electron-transfer, or oxidation – reduction, reactions do not lend themselves in general to study by the temperature-jump technique. The equilibria often lie too far to one side for a significant amplitude of relaxation to be observed. A recent example of a successful study is the oxidation of *N*-alkylphenothiazines by $Fe(CN)_6^{3-}$ (Pelizetti and Mentasti 1979). The data help in the understanding of the pharmacological properties of *N*-alkylphenothiazines.

5.5. Organic reactions

Many classic organic reactions, e.g. nucleophilic substitution on aromatic hydrocarbons, proceed via rapidly formed reaction intermediates. The kinetics of the formation of stable analogues of such intermediates, e.g. Meisenheimer complexes for nucleophilic aromatic substitution, has been studied, and new light shed on the reaction mechanism (Bernasconi and Terrier 1975).

5.6. Enzyme-substrate reactions

As mentioned in § 4 the pre-equilibrium between enzyme and substrate has been studied by the temperature-jump technique, and much information about enzyme function has been obtained. To obviate the problem of rapid decomposition of the enzyme–substrate adduct, many workers use an inhibitor rather than a substrate. An inhibitor molecule binds to the enzyme in the same way that a substrate molecule does but the resulting complex is stable and does not yield products of an enzyme-catalysed reaction. Complex reaction schemes have been encountered. In a study of aspartate aminotransferase no less than eight relaxation times were found and intepreted (Hammes and Haslam 1969). In general, substrates are found to bind to enzymes at the diffusion-controlled rate, but there are then slower conformation changes of the adduct before the final reaction to generate the product.

5.7. Base pairing in DNA

It is well known that the production of a new molecule of the genetic material, DNA, requires the pairing up of a long series of nucleotide bases, the sequence providing the genetic code. Two strands of polymer have to wind round each other to form the famous double helix. Temperature-jump studies on model compounds (Pörschke *et al* 1973) show that the rate-determining step is the formation of the third base pair, the rate constant being around 10^6 dm^3 mol^{-1} s^{-1}. Once three base pairs are formed the rest of the double helix is formed by a rapid 'zipping-up' process.

5.8. Micelle formation

Amphiphilic molecules, i.e. molecules with polar heads and long hydrocarbon tails, aggregate in solution into clusters of 50–100, called micelles. Micelles give amphiphilic compounds their detergent properties. The dynamic equilibrium between individual molecules and micelles is perturbed by a rise in temperature, so that the rate of micelle formation can be studied by the temperature-jump method (Lang and Eyring 1972). Relaxation times of a few milliseconds are found, but the interpretation in terms of rate constants is complex (Aniansson and Wall 1975).

5.9. Phase changes

The transition from one phase of a substance to another occurs rapidly at the transition temperature, and may be studied by the temperature-jump technique. Strey *et al* (1982) study the separation of a homogeneous mixture of lutidine and water into two liquid phases as the temperature is raised. One phase forms as a dispersion of micrometre-size droplets in the other phase. The growth of these droplets is monitored by the light scattering they cause. Interpretation of the data is difficult, since the droplet radii are similar to the wavelength of the monitoring light, and the full Mie theory has to be employed. The apparatus previously described (Moller and de Maeyer 1982), with infrared detection, was developed to study phase changes in aqueous liquid crystals.

References

Aniansson E A G and Wall S N 1975
J. Phys. Chem. **79** 857–8

Aubard J, Meyer J J and Dubois J E 1977
Chem. Instrum. **8** 1–16

Aubard J, Nozeran J M, Levoir P, Meyer J J and Dubois J E 1979
Rev. Sci. Instrum. **50** 52–8

Bernasconi C F 1976 *Relaxation Kinetics* (New York: Academic) pp 20–36

Bernasconi C F and Terrier F 1975
J. Am. Chem. Soc. **97** 7458–66

Beck G 1979
Rev. Sci. Instrum. **50** 1147–50

Buchwald H E and Ruppel H 1971
J. Phys. E: Sci. Instrum. **4** 105–10

Caldin E F and Crooks J E 1967
J. Sci. Instrum. **44** 449–53

Caldin E F, Crooks J E and Robinson B F 1971
J. Phys. E: Sci. Instrum. **4** 165–9

Caldin E F and Field J P 1982a
J. Chem. Soc. Faraday trans. I **78** 1923–35

Caldin E F and Field J P 1982b
J. Chem. Soc. Faraday Trans. I **78** 1937–42

Crooks J E 1975 *Proton-Transfer Reactions* ed V Gold and E F Caldin (London: Chapman and Hall) pp 153–77

Doss R, van Eldik R and Kelm H 1982
Rev. Sci. Instrum. **53** 1592–5

Eigen M and Tamm K 1962
Z. Elektrochem. **66** 93–107

Erman J E and Hammes G G 1966
Rev. Sci. Instrum. **37** 746–50

Ertl G and Gerischer H 1961
Z. Elektrochem. **65** 629–33

Grubic M, Strey R and Teubner M 1981
J. Colloid Interface Sci. **80** 453

Hammes G G and Haslam J L 1969
Biochem. **8** 1591–8

Hoffmann G W 1971
Rev. Sci. Instrum. **42** 1643–7

Holzwarth J F 1979 *Techniques and Applications of Fast Reactions in Solution* (NATO Advanced Studies Series C—Mathematical and Physical Sciences: University College of Wales, Aberystwyth, 1978) ed. W J Gettins and E Wyn-Jones (Dordrecht: Riedel) pp 47–59

Lang J and Eyring E M 1972
J. Polym. Sci. A-2 **10** 88–99

Liphard K G 1979
Rev. Sci. Instrum. **50** 1089–92

Möller K O and de Maeyer L C M 1982
Rev. Sci. Instrum. **53** 1596–601

North A M 1964 *The Collision Theory of Chemical Reactions in Liquids* (London: Methuen) p 68

Pelizetti M and Mentasti E 1979
Inorg. Chem. **18** 583–8

Pörschke D, Uhlenbeck O C and Martin F H 1973
Biopolym. **12** 1313

Reich R M and Sutter J R 1977
Anal. Chem. **49** 1081–5

Rigler R and Ehrenberg M 1973
Q. Rev. Biophys. **6** 139–99

Strey R, Wagner J and Woermann D 1982
Ber. Bunsenges. Phys. Chem. **86** 306–11

Turner D H, Flynn G W, Sutin N and Beitz J V 1972
J. Am. Chem. Soc. **94** 1554–9

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