HIGH PRESSURE CHEMISTRY

1. Introduction

The use of high pressure in synthetic and mechanistic chemistry in which the chemical system is in the liquid state is the subject of this article. It covers the reasons for the use of pressure, the types of pressure application, the technology and methods employed for successful pressure application, examples of results, and the value of the information obtained from these studies. In many cases in this article, the liquid will be in the form of a homogeneous solution containing chemical or biochemical reagents, and not on pure liquids. In other applications, particularly of industrial relevance, the effect of pressure on some colloidal materials is described.

High pressure technology for liquid systems can range from a relatively straightforward form of autoclaving to elaborate, highly specialized techniques involving rapid experimental observation following mixing of two liquids under pressure. Spectroscopic methods in which detection is also *in situ*, ie, the sample is monitored while it is under pressure, are also used. It is convenient to separate high pressure investigations into different types; the technology required is often different depending on the type of investigation, although there can be some overlap. These types are presented below.

1.1. A Pressure Cycle Involving System or Product Distribution Change. The first category of study is when the objective or expected outcome of pressurizing a sample or a chemical reaction is that after decompression the product is different from the sample prior to compression. It can also be different from the product obtained by allowing a reaction to proceed at ambient pressure. The product can also be different from that obtained by either catalysis, or nonconventional methods, such as microwaves or ultrasound. Obviously, the pressure variable is only of value if the product difference is materially or synthetically useful. Other favorable aspects of pressure application can be faster reaction times, increased yields, favorable isomer distribution, reduction of unwanted pathways that produce wasteful by-products, inactivation of biological spoilage organisms, or an economic processing benefit. Monitoring *in situ* may provide information that could lead to the manipulation of reaction conditions such as magnitude of pressure applied, or duration of pressure application, yielding even further improvement in desirability of product(s). The primary focus here is on characterization of materials or product(s) following decompression, and exploiting their value. In fact, trial- and- error runs of pressure magnitude and duration are usually conducted to establish optimal conditions, since this can often be achieved more easily than devising an *in situ* monitoring approach. In some areas of study, whether or not a system can be run semicontinuously under pressure rather than as a batch process is an issue. In production based, large-scale activities, this may be critical although one can envisage greater technical complexity in semicontinuous methods, but these would be favored in some high pressure fields, such as the food industry and possibly the pharmaceutical industry.

1.2. Organic Chemistry Reactions: Mechanism. The second category of high pressure chemistry studies involves primarily organic chemistry reactions. Reactions that have been thoroughly investigated at ambient pressure are subsequently studied at various pressures. A principal objective is to determine as many features of the reaction mechanism as possible, thus enabling a tuning of the reaction conditions to yield a more favorable product outcome. Since organic chemistry reactions can be grouped into series of similar reactions (ie, reactions of members of homologous series), often similar mechanisms prevail for related reactions so valuable information can be extrapolated throughout a given series. High pressure practitioners are frequently able to obtain further insight into the reaction mechanism than can be obtained from ambient pressure studies. Many of the reactions in this category are carried out in organic solvents and it is important to understand and possess knowledge of the effect of pressure on the physical properties of the solvents employed, for appropriate experimental design. In many examples here, the reactions proceed at conventional time ranges, which can be seen later to mean reaction times of many minutes or hours. In addition to organic reactions, some organometallic catalytic chemistry can be included in this section, except in some cases *in situ* methods have been applied to obtain information about the mechanism with the aim of driving the reaction to be more efficient and to generate suitable product(s) (1,2).

1.3. Inorganic Chemistry Reactions: Mechanism. A third category involves many inorganic reactions whose mechanisms are sought by using the pressure variable in addition to other variables, eg, solvent, concentration, ionic medium (strength and different electrolytes), and temperature variations. Often the scale of the system is small using materials not available in large quantities, and the interest is more in the realm of academic research, but basic knowledge acquired can often have potential exploitation value in the future. The rapidity of many reactions of transition metal complexes has stimulated the development of measurement technology and technically sophisticated instruments have been constructed. They are often built in individual investigator's laboratories and workshops, and are not widely available and commercial offerings are scarce. Bioinorganic chemistry and biochemical reactions can be included in this category. The effect of pressure upon them is mainly for the purpose of mechanistic understanding. However, the effect of pressure can have a complementary role to structural and spectroscopic methods involving the site of reaction, eg, in metalloproteins, the active site in enzyme catalysis, reactions in a cascade of biochemical processes or in protein folding. Thus, there are implications for understanding erroneous biochemical processes. High pressure studies have also aided in bringing an understanding of properties of reagents that are suitable for use in medical imaging. In the reactions considered here, the significant feature is that upon decompression and conclusion of the reaction the system will be identical to that obtained if the reaction had proceeded to an end point or a position of equilibrium without the application of pressure.

1.4. Pressure: Units and Magnitude. The choice of magnitude of pressure or range of pressures in a particular application is dictated often by the specific properties of the system and by the specifications, ie, the pressure rating and range of the appropriate available apparatus or instrument. Of course, the experimental design will also be governed by expectations of the

outcome or hypothesis regarding the results that will ensue from application of pressure. The pressure range in purely mechanistic studies such as in category three is usually up to 200 MPa [0.1 MPa (0.1 megapascal) = 1 bar, and 1 atm = 1.01325×10^5 Pa). For reasons that will become evident, higher pressures than 200 MPa can complicate interpretation of results and often pressures no higher than 150 MPa are needed to extract maximum information. In synthetic chemistry and in some organic reaction chemistry of the second category, much higher pressures, up to 2000 MPa, are employed, although sometimes an equivalent result can be obtained employing a lower pressure for a longer time. In synthetic chemistry, where no *in situ* monitoring is practiced, the nature of the apparatus can permit use of pressures at higher levels. Commercially, available equipment for processing materials is usually designed for operating at pressures up to ~700 MPa. For most purposes, the measurement of pressure need not be highly accurate or precise because a pressure measurement that deviates 1 or 2% percent from the actual value has only a minimal effect on the mechanistic analysis or synthetic product.

2. Basic Principles and Practical Aspects

The physical properties of liquids are influenced by pressure (3). These properties include boiling point, melting point, viscosity, density, dielectric constant, conductivity, and compressibility. The effect of pressure on the viscosity of a liquid should be recognized, particularly in the case of a reaction in a solvent if the reaction contains one or more very rapid steps, ie, the reaction is subject to diffusion control. It then may be difficult to apportion the experimentally observed effect of pressure between the effect on the chemical reaction and on the solvent viscosity. The melting point of many organic solvents is increased by pressure, eg, benzene has a melting point of 5.5°C at atmospheric pressure that increases to ~100°C at 500 MPa. Water by contrast has a melting point of −9°C at 100 MPa. The solubility of solids in liquids is usually decreased by increase in pressure. When the solvent is water, allowance must be made for the change in pH resulting from the effect of pressure on the ion product constant of water. Likewise, the pK_a values of weak acids and the pK_b values of weak bases that may be used as buffer agents or as reactants can depend on pressure. Consequently, the effect of pressure on physical or chemical properties must be taken into account when designing high pressure experiments. The effect of pressure on the physical properties of matter has been discussed elsewhere (3).

Compressing liquids causes an increase in temperature. Typically organic solvents, having lower heat capacities, experience a more significant temperature increase than water, under comparable pressures. Undesirable heating effects can be minimized by increasing pressure incrementally in steps with adequate cooling by thermostated circulating fluid around the sample container.

However, stepwise compression can in some cases lead to a change in the overall outcome of pressure application as compared with proceeding to the required value of elevated pressure in one step (4). From a practical standpoint, it usually takes longer to reach a required operating pressure then it does to permit “instant” decompression. There may be consequences of rapid cooling of the

sample from the thermally equilibrated temperature of operation at elevated pressure, upon rapid decompression. Ideally (although not necessary in many applications), pressurizing equipment should have settings of megapascal (MPa)/s or min (capable of selection by an operator) for each activity to regulate the system and provide reproducible cycles. The importance of these factors has been observed, eg, in pressure treatment of certain colloids (4–6).

Compression of liquids gives rise to a much smaller volume reduction than compression of gases. Therefore, the potential level of hazard is much reduced in-high pressure practice if the total system is liquid based. Failure of a component in the system that is supposed to maintain hydrostatic pressure will quickly lead to liquid leakage, and rapid overall decompression. Therefore the safety regime is much less rigorous than it would be in practice with compressed gases. However, if elevated temperatures or toxic liquids are employed, extra precautions must be taken to prevent liquid leakage reaching operators and appropriate training must be provided. For liquid-phase systems involving large volumes (eg, up to 300 dm^3) in industrial scale processes, special arrangements to isolate operators from high pressure components must be in place. If compressed gases are used to bring about elevated hydrostatic pressure, then a very rigorous safety regime must be followed.

The means to achieve pressure, ie, the pressurizing device, and the materials chosen to house the sample, depend in part on the magnitude of pressure required, the type of sample, and the hypothesis about the likely events occurring in the system upon pressure application. In addition, in which category the system or reaction falls will determine the selection of apparatus or instrument. Several aspects of these considerations have been discussed in Ref. 7. The emphasis in Ref. 7 was in the context of relatively small samples, where the experiment in each case was a mechanistic investigation. Nevertheless, these considerations are generally applicable.

3. Theoretical Aspects

For materials processing and some organic synthetic–preparative studies, there is no underlying theory regarding the effect of pressure. Rather, a successful result mostly arises from a combination of precedent from the literature, exploratory, and trial-and-error approaches with the chemistry system at issue, and intuition. Where no *in situ* monitoring occurs, the product that emerges following high pressure treatment can be analyzed and characterized by the same or similar methods that would be used when necessary to characterize the reactants. Clearly, only if the reactants were prepared by the investigator, rather than they were obtained as high grade commercial materials, would characterization be needed. The same methods would be used as if the products were obtained by reaction at ambient pressure. Isolation of products would be carried out by "work-up" procedures that are standard in organic chemistry. Product analysis would be carried out using normal spectroscopic techniques including infrared (ir), mass, ultraviolet (uv)/visible (vis), and nuclear magnetic resonance (nmr) spectroscopies, beside melting or boiling point measurement, and

chromatographic procedures. Structural determination by X-ray diffraction methods might be pursued in some cases. Turbidity measurements, light scattering techniques, or microstructure imaging techniques could be employed additionally, for processes where the product is in the liquid phase but not homogeneous, eg, colloids or emulsions.

For systems or reactions where the mechanism is sought and *in situ* measurements are acquired, then a fundamental thermodynamics and kinetics theoretical framework relating to pressure effects must be followed. An important aspect of chemical kinetics is establishment of the rate law for a particular chemical reaction (8). One method of determining the rate law is the initial rate law method. The initial rate (R) of reaction, the change in concentration of a reactant, or product over a short-time interval at the beginning of the reaction, is determined for various initial concentrations of one reactant. All other reactants are maintained at a constant concentration, and the temperature is maintained at a constant value during these measurements. This allows the dependence (the order) of the rate of reaction upon the concentration of the reactant whose concentration is being varied to be determined. The process can be repeated to establish the order for the other reactants. The order with respect to a given reactant is most frequently one, two, or zero. The concentration is rarely measured directly, as this is often more complicated than obtaining the value of change of a physical property of a reactant or product (eg, light absorbance). It is preferable, and usually the case that the property in question is linearly related to the concentration (of a reactant or product). The rate law for a reaction between A and B takes the form:

$$R = k[A]^a[B]^b \tag{1}$$

where a and b are the order of reaction with respect to [A] and [B]. The proportionality constant k, the rate constant, is the important kinetic parameter in high pressure kinetics practice. The disadvantage of the initial rate method is that it generates a rate law that may only be guaranteed for the initial part of the reaction, although often the rate law is applicable for an extended part of the reaction. A more satisfactory way of generating the rate law is by the integrated rate law method. In this approach, the concentration or suitable physical property of a reactant or product is measured for most of the duration of a reaction. The profile is then fitted to integrated forms of possible rate equations. It is important for potential mechanistic information from high pressure studies that the rate law be simple, or that the design of the experiments be effectively converted from a complex rate law into a simpler one. (In some chain reactions, the complexity of a rate law can be useful in unraveling mechanistic steps such as product inhibition or catalytic steps.) Details of methods for establishing rate laws, and in some cases interpreting them, may be found in Refs. 9–16.

Once the rate law has been determined, the mechanism can be probed by several approaches, such as varying the pH in aqueous systems, the solvent, the ionic strength, the electrolytes used to maintain a given ionic strength, testing possible catalysts, or varying the temperature. How these variables affect reaction rates has been addressed in many publications (9–16). The effect of temperature is included here since the consequences of pressure variation on

reaction rate constants can often be matched with temperature effects on the same parameters in mechanistic diagnosis.

The theoretical basis for understanding and interpreting the effects of temperature and pressure on kinetic parameters is embodied in transition state theory (17–21). The theory, described and formulated in the 1930s, shows that the rate constant is proportional to the exponential of the negative value of the free energy of activation, leading to the relationship often known as the Eyring equation, which relates the rate constant to the temperature (T) of reaction:

$$k = (\mathrm{k}T/h)\ \exp\ (\Delta S^{\neq}/R)\ \exp\ (-\Delta H^{\neq}/RT) \tag{2}$$

where k is the Boltzmann constant, h is Planck's constant, R is the ideal gas constant, and $\Delta S^{\neq}$ and $\Delta H^{\neq}$ are, respectively, the entropy and enthalpy of activation. The latter are obtained from the intercept and slope, respectively, of a plot of $\ln(k/T)$ versus $1/T$. A theory of the effect of pressure on reaction rates was first developed by van't Hoff over a century earlier (22). The treatment in terms of transition state theory is based on the effect of pressure upon an equilibrium constant; (9,23,24) there is assumed to be an equilibrium (equilibrium constant, $K^{\neq}$) between the activated state and the reactant state, leading to equations 3–6.

$$(\delta\ \ln)\ K^{\neq}/\delta P)_T = -\Delta V^{\neq}/RT \tag{3}$$

and since

$$k = (KT/h)K^{\neq} \tag{4}$$

$$(\delta\ \ln\ \mathrm{k}/\delta P)_T = -\Delta V^{\neq}/RT \tag{5}$$

The parameter $\Delta V^{\neq}$ is the difference between the partial molar volumes of the transition state and the initial state, and is known as the volume of activation. This parameter may be determined by applying the integrated form of this equation (eq. 6), following determination of the reaction rate constant at several pressures (k_0 is the rate constant at zero pressure, in effect for all intents and purposes at ambient pressure).

$$\ln\ k = \ln\ k_0 - (\Delta V^{\neq}/RT)P \tag{6}$$

If a plot of $\ln\ k$ versus P is linear, the volume of activation can be determined from the slope, and linearity implies that the volume of activation is independent of pressure over the range employed. Rate acceleration with increased pressure means a negative volume of activation, and a positive volume of activation results from rate reduction with an increase in pressure. If the plot is not linear, then the pressure dependence of the volume of activation can be obtained by several possible treatments of the data (24–26). Here, emphasis is on obtaining and interpreting volumes of activation that are independent of pressure.

Restricting the pressures employed to < 200 MPa in chemical reaction studies usually results in linear plots, and hence a volume of activation independent of pressure, and thus avoiding unneeded complications. The necessity to use pressures up to this value could reasonably be queried. That is, why not reduce technical difficulty by using pressures of a few atmospheres, eg, up 10 MPa? Many reactions do not experience significant rate retardation or acceleration upon pressure application, and therefore a pressure range up to 100, 150, or 200 MPa is usually required to enable an acceptable level of precision on the volume of activation to be obtained. An examination of compilations of $\Delta V^{\neq}$ values in the literature indicates that often values are not much $> \pm$ 30 cm^3/ mol (although for some organic reactions, volumes of activation of 50 cm^3/mol have been reported) and often are much smaller (27). To put this in perspective, this means that a pressure of 100 MPa for a reaction in which $\Delta V^{\neq}$ is $\pm$ 30 cm^3/mol brings about a factor of $\sim$3.5 of acceleration or retardation relative to the rate constant at 0.1 MPa. Obviously, smaller values of the volume of activation result from smaller effects of pressure on kinetic parameters.

The reaction features giving rise to particular values of the volume of activation are, eg, bond formation, bond deformation, or bond breakage in the transition state, and processes partly developed in the transition state leading ultimately to ionization or charge neutralization. From assessing the literature, estimates have been made of typical volume contributions that these features cause (28,29). These reaction features fall broadly under the term of intrinsic volume changes, although where charge neutralization, charge dispersal, or features involving increase or decrease in polar character occur in forming the transition state, they also induce volume changes of the solvent. Solvent molecules surrounding a metal ion are constricted in their volume relative to free solvent molecules. Hence, any process leading to one or more solvent molecules leaving the influence of the charge upon the metal ion will cause a reduction in solvent electrostriction and a volume increase. Conversely, an oxidation–reduction process leading to charge development in the transition state would give rise to an increase in solvent electrostriction. In the absence of other effects, a decrease in the volume of the molecular species in the transition state relative to the initial state occurs. Ideally, the measured volume of activation can be divided into two contributions, intrinsic and solvation.

$$\Delta V^{\neq} = \Delta V^{\neq}_{\text{intr}} + \Delta V^{\neq}_{\text{solv}} \tag{7}$$

In many situations, it is difficult to attribute the contribution each component makes to the experimental total. However, in solvent exchange reactions, where the solvation component is essentially negligible, the experimental value of the volume of activation can be used in a reasonably facile manner for mechanism diagnosis. In oxidation–reduction reactions, there is scope for further theoretical development since measured volumes of activation can be compared with those estimated from extension of electron-transfer theory for ambient pressure kinetics to elevated pressure kinetics.

In suitable cases, when a reaction can be studied as a function of pressure in both forward and reverse directions, the volume of activation can be determined for the reactions in both directions. This enables ΔV, the reaction volume to be

obtained, since

$$\Delta V = \Delta V^{\neq}_{\text{forward}} + \Delta V^{\neq}_{\text{reverse}} \tag{8}$$

This easily happens, only when a reaction is represented by a simple rate law. Since standard thermodynamics provides

$$V = (\delta G/\delta P)_T \tag{9}$$

and

$$\Delta V = (\delta \Delta G/\delta P)_T \tag{10}$$

and

$$\Delta G = -RT \ln K \tag{11}$$

The reaction volume (eq. 12) can be obtained providing the equilibrium constant can be determined at several pressures.

$$(\delta \ln K/\delta P)_T = -\Delta V/RT \tag{12}$$

The significance of determining the reaction volume is that the volume profile for the reaction can be established, and compared with and complements the free energy, enthalpy, and entropy profiles, thus characterizing the reaction thoroughly. This is considered an important objective for investigators of kinetics and mechanisms.

If determination of the equilibrium constant as a function of pressure or the volumes of activation for the reaction in both directions is precluded by the properties of the system, another possible route to obtaining components of the volume profile could be available. The partial molar volume of a dissolved substance can in principle be obtained from density measurements. Excellent densitometers are available commercially and these operate on the principle of a sound wave whose frequency depends on the density of the solution. The method depends on measuring the density over a range of concentrations yielding apparent molar volumes. These values are extrapolated to zero concentration to yield the partial molar volume. It is also important to add some more practical information briefly for the benefit of new investigators in the field to ensure appropriate use of equation 13. The concentrations needed for these experiments are often much higher than are used in the relevant kinetic experiments, and solubility, scarcity, and stability of the solute can limit the extent to which this approach can be taken. It is of absolutely paramount importance that the temperature of measurement is precisely and accurately known and exactly reproducible and controlled, because the density of moderately dilute solutions is very sensitive to temperature. In addition, the equation developed to link the density of solutions to the apparent molar volume of a solute involves a subtraction of the density of a solute solution from the density of solvent. This is a very small difference. The temperature control in available instruments is excellent. Obviously, highly pure, outgassed solvents are required and the method is not

suitable for use with volatile solvents or mixed solvents.

$$\phi = (MW/d) - ((d - d_0)/d_0) \times (1000)/c \quad (13)$$

where ϕ is the apparent molar volume of a solution of molar concentration c and density d, d_0 is the density of solvent, and MW is the molecular weight of the solute. The partial molar volume of the solute is obtained by extrapolating the apparent molar volumes to zero concentration.

If either or both of the partial molar volumes of reactants and products are known, and one or both of the volumes of activation are known, then the volume profile can be put onto an absolute rather than relative volume scale. Reaction volumes may also be obtained by dilatometric methods.

When experimental circumstances permit, it would be customary to obtain volume parameters under discussion at 25.0°C. When this is not possible, there is not a substantial significantly different magnitude of volume parameters. The basis for the temperature dependence of either the volume of reaction or the volume of activation resides in the Maxwell equations. This aspect of thermodynamics has been dealt with in some detail (30). The consequence is that volume parameters obtained from measurements at 25 ± 20°C do not have significantly different values. Hence, they have the same usefulness for diagnosis of mechanism and for cross-comparison with parameters obtained at not exactly the same temperature.

4. Apparatus, Techniques, and Methods in High Pressure Chemistry and Related Fields

The choice of apparatus or instrument depends on the system being studied, and whether the pressure activity is related to bringing about change in one or more substances, synthesis, or mechanistic determination according to general classifications. It is in the first category that complete commercial units are available, and a number of companies provide standard units or can to an extent modify their basic product line to customer need. The selection of equipment described does not involve endorsement or advocacy, but to an extent reflects the availability of details of a supplier's product line.

4.1. Equipment for High Pressure Processing. For industrial scale high pressure processing, Avure Technologies (Vasteras, Sweden and Kent, Washington) have developed vessels from 2- to 320-dm^3 capacity for batch or continuous processing. The QUINTUS press technology vessels are prestressed using spring steel wire around the cylinder. The vertically orientated cylinder has axially sliding end closures and a patented safety liner. Connections for the pressure media and contents and for the sensors for temperature and pressure are located in the end closures. A closure manipulator permits opening and closing of the pressure vessel (see Fig. 1). A cycle time of ~5 min can be achieved for 35-dm^3 volume of material compressed to 600 MPa and decompressed, excluding any required holding time and loading and unloading the sample. Process temperature (4–35°C) is measured by a thermocouple and the pressure is measured by a pressure transducer. The pressure cycle data (pressure, hold time,

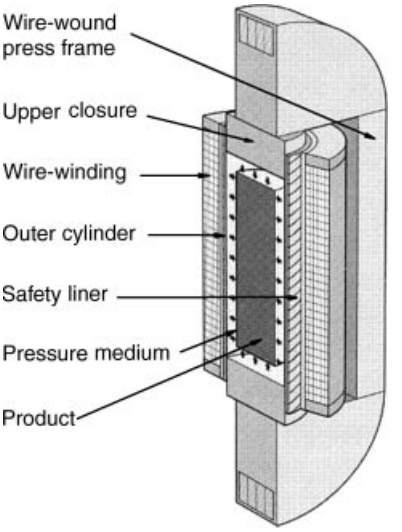


Fig. 1. Schematic cutaway of the QUINTUS pressure vessel. (Courtesy of Avure Technologies.)

temperature) can be recorded. For a 215-dm^3 pressure vessel, the pressure regime is the same, but the comparable cycle time is ~7 min and the upper operating temperature is 25°C. The intensifier pumps needed to generate the required pressures are also from Avure Technologies. Water is the pressurized fluid. This development has found considerable use in the beverage and food industry, where products can be pressure treated in suitable packaging. Although the medium of the samples is water and the pH range is normally close to neutral, there is no need for contact of the sample with the metal of the high pressure vessel, as inert plastic containers are used to hold the sample. The advantages of high pressure treatment are (*1*) reduction of spoilage organisms without using chemical preservatives; (*2*) retention of characteristics of fresh products; and (*3*) elimination in some cases of heating for (bacterial) safety purposes. The technology can also be applied to emulsions or colloids whose organization or microstructure can be changed as a result of pressure induced physical or physicochemical kinetics, or gel formation for matrices used in a host of personal products and foods.

Stansted Fluid Power provides a range of high pressure processors that have found application for research in various scientific fields or routine purposes in the food industry. However, there are modules where small sample volumes can be used and research samples that have been changed by a pressure cycle can be analyzed following decompression. Depending on the system, the properties may still be changing after decompression and kinetics of the re-establishment to a stable or equilibrium state can be monitored. The processors operate on the plunger press principle and incorporate what is termed a Mantled Barrel construction. The pressure barrel is a duplex construction with a super stainless steel liner that is prestressed by shrink fitting into a 2.5% NiCr alloy

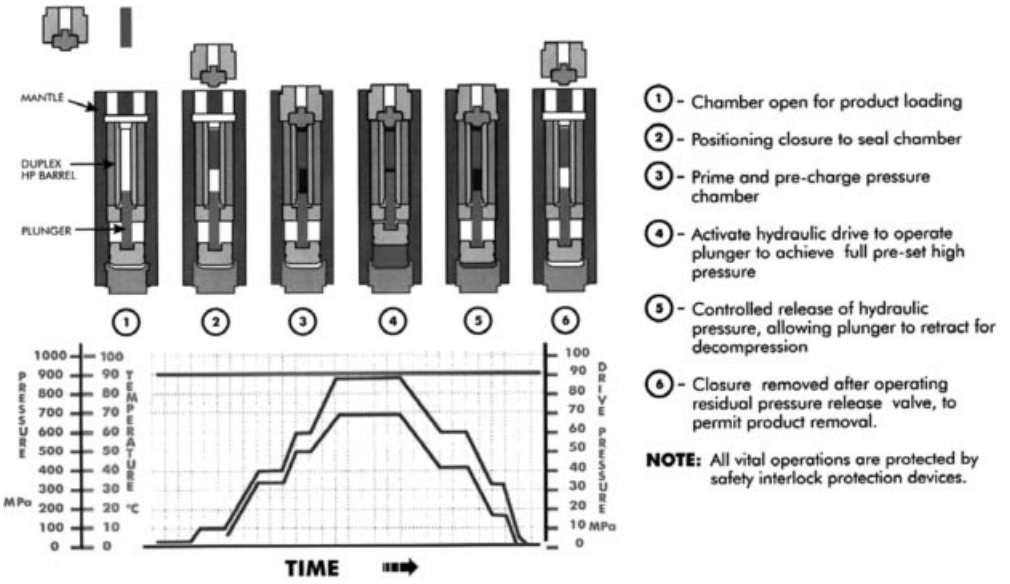


Fig. 2. Schematic of pressure apparatus. (Courtesy of Stansted Fluid Power.)

outer body. The high pressure barrel is confined within a mantel, thus minimizing an undesired consequence of component failure. The pressurizing fluid is water or an aqueous solution. Pressure is generated by displacement of the plunger into the working chamber. Decompression occurs when the plunger is retracted, and thus only one working stroke per pressure cycle is applied, which prolongs seal life. An operating cycle is schematically shown in Fig. 2.

A partial list of high pressure vessel suppliers includes: Elmhurst Research; Alstom; Engineered Pressure Systems, Inc.; and Resato International BV.

4.2. Organic Chemistry. Organic chemistry reactions that have been pressure treated are divided into two sections. The first has one or more of the following objectives. Improvement is sought in synthetic procedure, in a better yield, in production of a better ratio of desired to less desired or unwanted products, in avoidance of use of costly catalysts, in a lower temperature regime, or, in simply accelerating the reaction (31,32). The second section covers practical issues associated with mechanistic studies of organic reactions, although there can be considerable overlap with the former section since some of the same improvement objectives pertain (27,28,33,34).

Synthesis. Although an examination of the contents of a pressure vessel during a pressure cycle could be informative, practice in this area of chemistry involves usually decompression and characterization of product(s). This is not to say that there is not a step-by-step approach of experiments to define optimal conditions and reach a preliminary conclusion regarding the mechanism that can lead to further improvement. A typical apparatus is of the piston-cylinder type, in which the high pressure chamber is a vertical steel cylinder, sometimes surrounded by external jackets that can be closed and sealed at the top and the piston from which pressure is applied is sealed at the lower end of the cylinder.

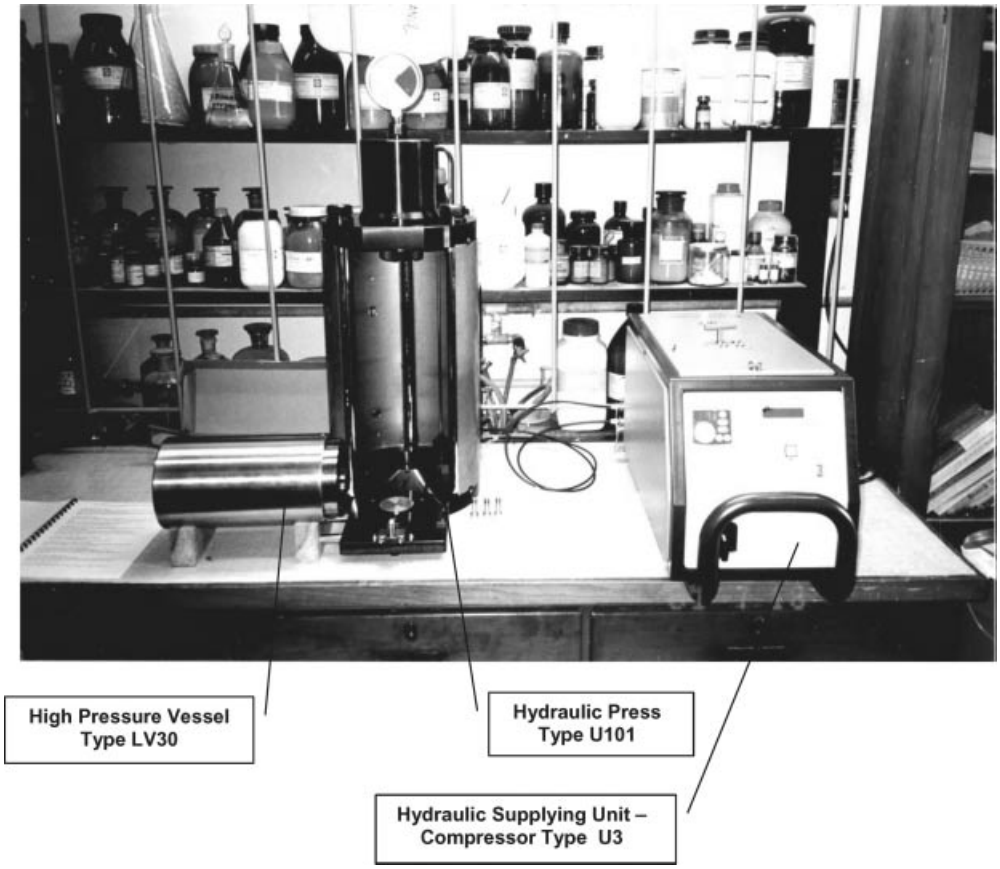


Fig. 3. Preparative high pressure apparatus. (Courtesy of Unipress, Warsaw, Poland.)

Clearly, proper sealing of the piston is a key necessity of any version of this general type of apparatus or indeed of all high pressure equipment, for efficiency of operation. Possible options for sealing are metal rings, special O-rings, or Teflon stoppers with O-rings. Thermostating can be achieved by circulating temperature-controlled fluid through the external jacket, or immersing the apparatus in a thermostat bath. Although investigators may construct in-house their own version of a piston-cylinder high pressure apparatus, complete high pressure systems are available from, eg, Unipress (see Fig. 3).

For many applications pressures up to 1500 MPa are commonly used. Operation should be relatively straightforward providing applied pressure is maintained constant.

Mechanistic Studies. Organic synthesis could be carried out in this next generation of apparatus, especially if sampling during synthesis is important. The basic principle is again a piston-cylinder, but with the important addition of a valve for release of aliquot samples, for analysis while the sample is under pressure (see Fig. 4). Aliquot sample removal at suitable time intervals as the

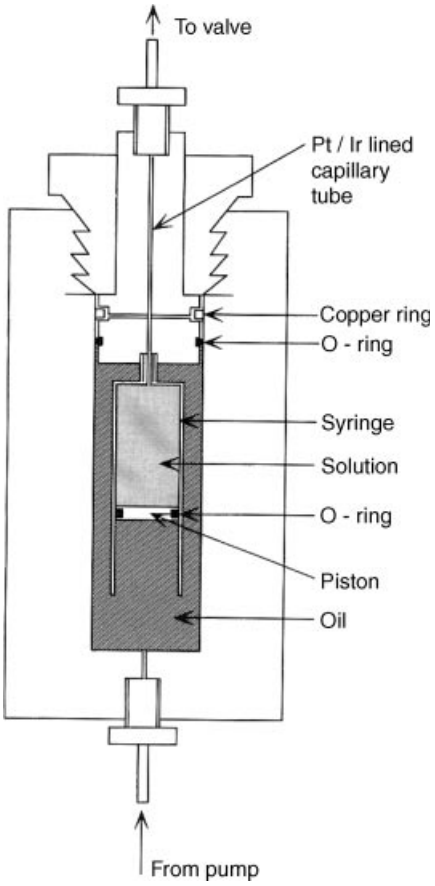


Fig. 4. An example of a piston-cylinder high pressure apparatus.

reaction progresses is essential if reaction kinetics are to be studied. The introduction of a sampling valve that can be built into the sealing lid of the apparatus increases the technical complexity, as the piston must be able to move within the cylinder at the time of sample removal to account for the volume reduction (see Fig. 5). In many apparatus, the pressure rated valves are manually operated. Sample suppliers are SITEC or Aminco/Newport Scientific.

Several conditions must be met by both the apparatus and by the properties of the homogeneous system being studied. These are

1. The time taken to withdraw the sample must be very rapid compared with the rate of progress of the reaction.
2. The analytical method for measuring directly or indirectly the concentration of reactant or product must be very rapid compared with the rate of progress of the reaction.
3. The relevant property of the sample is being measured at ambient pressure, rather than at the high pressure of the reaction. The error of the measurement is negligible providing conditions *1* and *2* prevail.

Fig. 5. A typical manual high pressure valve.

4. The pressure in the cylinder is reduced as soon as the sampling valve is opened. Therefore, the pressure application system must have the capability of restoring the pressure to that of the presample withdrawal level as soon as the sampling valve is closed. This must be carried out very rapidly compared with the rate of reaction progress.

Although this type of apparatus is not particularly difficult to construct, suitable seals of the piston must be devised. It is essential that the piston is not so well sealed that it cannot move upon opening the sampling valve, but at the same time it must be sufficiently strongly held that it can resist permitting expulsion of the whole sample upon opening the sampling valve. One type of construction uses a stainless steel cylinder and a solid Teflon piston with Viton-rubber O-rings in grooves in the piston. A possible limiting factor is that research samples that are not available in unlimited quantities are not readily studied, as the total volume of solution needed is about 0.1 dm^3. From the conditions 1–4 it is apparent that reactions taking hours rather than fractions of an hour are conveniently studied. Time must be allowed to elapse for a return to the measuring temperature after pressure induced warming of the sample. Thus, there is need for efficient thermostating arrangements. For reactions that might be sensitive to metals, the direct contact of the stainless steel cylinder with the reaction solution should be avoided and suitable checks of any potential contamination should be exercised. Filling, sealing, and valve operation of such

an apparatus can require more training for operators than for many other methods of high pressure study. The method of analysis of aliquot samples is invariably uv/vis spectrophotometry. Close scrutiny of the contents of the sampling cuvette must be undertaken to ensure no microscopic bubbles persist. One advantage of this type of apparatus, unlike several reported below, is that it does not compel dedication of an instrument (eg, a spectrophotometer) to the high pressure mode.

4.3. Inorganic Reactions. Among inorganic reactions that have benefited from being studied over a range of pressures are some of those of coordination compounds of the transition metals. More recently, solvent exchange reactions of some main group metal ions and increasingly of the lanthanide or first row of the inner transition metals, the latter elements possessing up to 14 *f* electrons, have also been the subject of high pressure studies (35,36). Whereas many organic reactions are not particularly rapid, and often require elevated temperatures, the inorganic reactions considered here can span reaction times from 10^{10} to 10^{-10}s at ambient temperature. Water exchange on aqua europium(II) and on aqua copper(II) are examples of very rapid processes (35,36). Ligand substitution reactions of chromium(III) and cobalt(III) complexes can be quite slow, ie, not necessarily requiring special rapid reaction methods, and water substitution on aqua iridium(III) can take years (35,36). Binding of small molecules to bioinorganic macromolecules have also been included here, many of these are also in the subsecond time range. Photochemically initiated reactions can also be studied using the techniques and apparatus to be described here; however, other specialized sources should be consulted for the types of photochemical reactions that have been treated by pressure (37,38). Here, there are additional demands upon the techniques for studying such reactions at high pressure. The investigators' interests are almost exclusively mechanistic and sometimes the materials are in limited supply meaning that the scale of apparatus involves volumes in the cubic centimeter (cm^3) range. Although for slow reactions of materials in plentiful supply, the piston-cylinder with sampling valve type of apparatus described earlier has been employed (39,40). Clearly, if the objective of using the pressure variable is mechanistic, then *in situ* or aliquot sampling is mandated.

Slow Reactions. It is customary in kinetics studies to divide reactions into somewhat arbitrary categories of conventional time range reactions (half-life of greater than a few minutes), and rapid reactions. But for high pressure kinetics the division has to be altered slightly, because once pressure is applied following reaction initiation, there is a delay period during which the reaction restores to the desired temperature of measurement. A reaction in the "awkward" time window can be studied by the simple expedient of slowing it down by lowering the measurement temperature. However, if the time window cannot be suitably positioned by temperature change, a mixing system has been developed (41). The reactants are contained in compartments separated only by a thin membrane that can be broken by a magnetic field activation of a mixing bar following temperature and pressure equilibration. Thus the reactants are then mixed; this approach has given excellent results for reactions of half-lives greater than $\sim$5 s, but too rapid for the piston-cylinder type of apparatus or other techniques where pressure is applied subsequent to reactant mixing. In many cases,

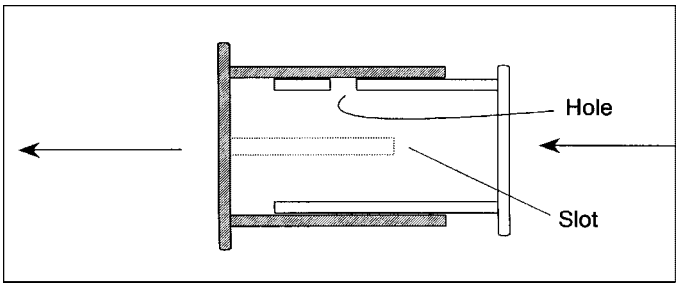


Fig. 6. Schematic presentation of a "pill-box" optical cell for measurements in a high pressure optical cell. The slot and hole allow the pill-box cell to be filled and extra liquid to be released on closing the cell.

for rapid reactions the experimental method succeeds by applying pressure on the reactants before they are mixed and the reaction initiated.

Much of the significant technical development of cells and apparatus for monitoring chemical reactions at high pressures occurred during the 1970s and 1980s. More recently, there have been more than incremental improvements in high pressure methods such as in nmr spectroscopy. In all areas, substantial improvement in data acquisition and processing has made a more efficient research regime and has provided the opportunity to undertake the resolution of more complex kinetic processes. High pressure cells for reaction kinetics were designed to mirror the methods most commonly used in ambient pressure kinetics. Although statistics have not been compiled, a cursory examination of kinetics literature indicates that spectrophotometry is the most widely used reaction monitoring method, specifically uv/vis absorbance measurements. In principle, optical rotation, circular dichroism, or fluorescence measurements could be made if the reacting system possessed appropriate properties. The cell compartment of most commercially available spectrophotometers is sufficiently spacious to allow modification for high pressure operations. The most commonly used optical cell for high pressure measurements in this category, and for conventional time range measurements, is the "pill-box" cell (see Fig. 6) (42). This is constructed from two concentrically cylindrical components, one of which "push-fits" into the other. At both ends are optically flat windows through which at one end, light of appropriate wavelength is incident and transmitted light following absorption is sent from the other window to the detector. Compression of the cell results in the two windows coming slightly closer, but allowing uniform pressure throughout the sample. A small aperture exists that enables the cell to be filled with solution by a syringe and needle technique. The cell is sealed by rotating one component with respect to the other and is placed in the enclosing steel module, schematically shown (see Fig. 7), containing quartz or sapphire windows aligned with those of the pill-box cell. The pressurizing fluid, typically water or *n*-heptane, is introduced, the outer cell is sealed and pressure is applied from the external pressurizing system. Other types of cells have been designed and one especially for handling oxygen or moisture sensitive samples has been described (see Fig. 8) (24). However, the pill-box cell appears to be the most enduring for

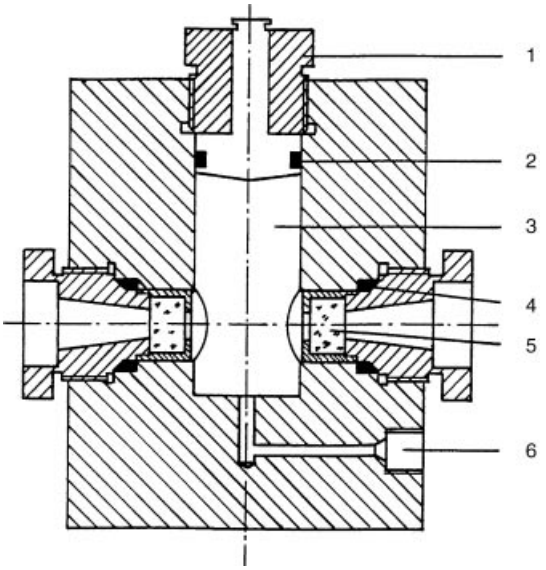


Fig. 7. Schematic view of a two-window high pressure cell to accommodate the pill-box optical cell for pressures up to 200 MPa: 1 is a pressure plug; 2 is an O-ring; 3 is the reaction compartment; 4 is a Δ- and O-ring; 5 is a sapphire window; 6 is the pressure connection.

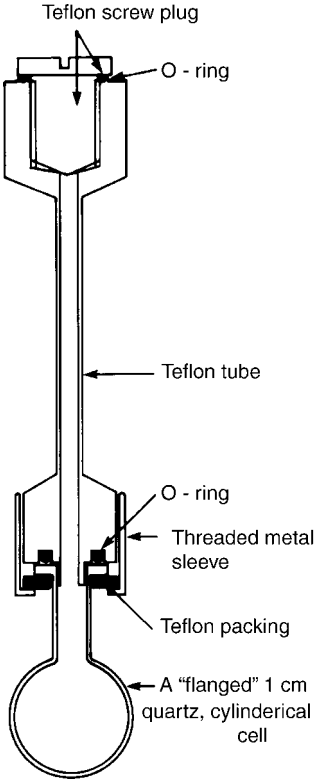


Fig. 8. A completely sealed optical cell for oxygen or moisture sensitive samples.

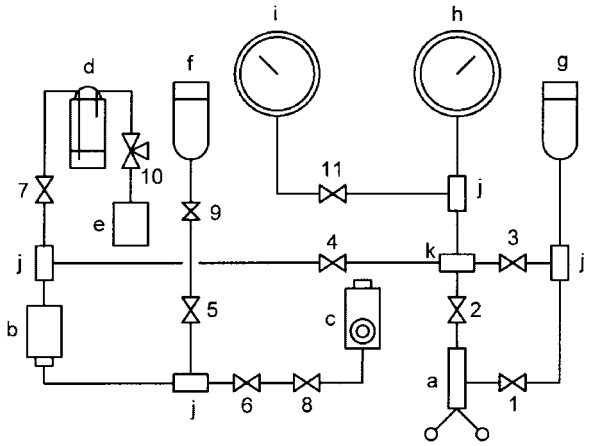


Fig. 9. Schematic diagram of the high pressure unit with separator and high pressure cell for measurements up to 400 MPa (31). Components: high pressure valves, 1–9, 8 (optional), 11(optional); glass valve (for evacuation of the separator), 10; T-connections, j; cross-connections, k; manometers, h, i (optional); Teflon separator, b; thermostated high pressure cell with two sapphire windows (NOVA SWISS, 400 MPa), c; high pressure pump (NOVA SWISS, 400 MPa), a; oil reservoir, g; secondary pressure medium reservoir, f; oil reservoir for vacuum pump, d.

samples not sensitive to oxygen and indeed is often used for aqueous solutions. A schematic setup of a recent incarnation for slow reactions and that extends the pressure range to 400 MPa and the wavelength range into the near ir, is shown (see Fig. 9) (43). Another design that does not use a pill-box type cell and has been used widely, incorporates the observation chamber within the pressurizable container itself is shown (see Fig. 10) (44). In the latter example pressure is applied directly to the reacting solution. Clearly suitable tests must be carried out to ensure that the steel container does not contaminate the reactant solution.

Although the vast majority of high pressure kinetics measurements in this category have used dual beam spectrophotometry as the monitoring method, attention may be drawn to a conductivity detection system (45). The experimental method would proceed independently of the monitoring method, typically as follows; the first pressure applied would customarily be 5 or 10 MPa and if reproducibility has been established about five replicate runs at that pressure would be conducted. Subsequently, comparable numbers of kinetics runs at pressures of 25, 50, 75, 100, 125 MPa would follow. The accumulated data would be a sufficient minimum with which to attempt to determine the volume of activation, although individual preferences on exact pressure range and pressure values can be variable. Obviously, the pressure range used would need to be compatible with realistic limits of the properties and rates of the reacting system. Note that the high pressure cell is often unsuitable for measuring the rate constant at ambient pressure. The latter is most easily carried out employing standard ambient pressure methods and comparing the rate constant with that obtained from extrapolating the ln k versus P plot to ln k at 0.1 MPa.

Rapid Reactions. Mixing Methods. The critical factor is designing and constructing a method of mixing two reactant solutions rapidly so that the mixed

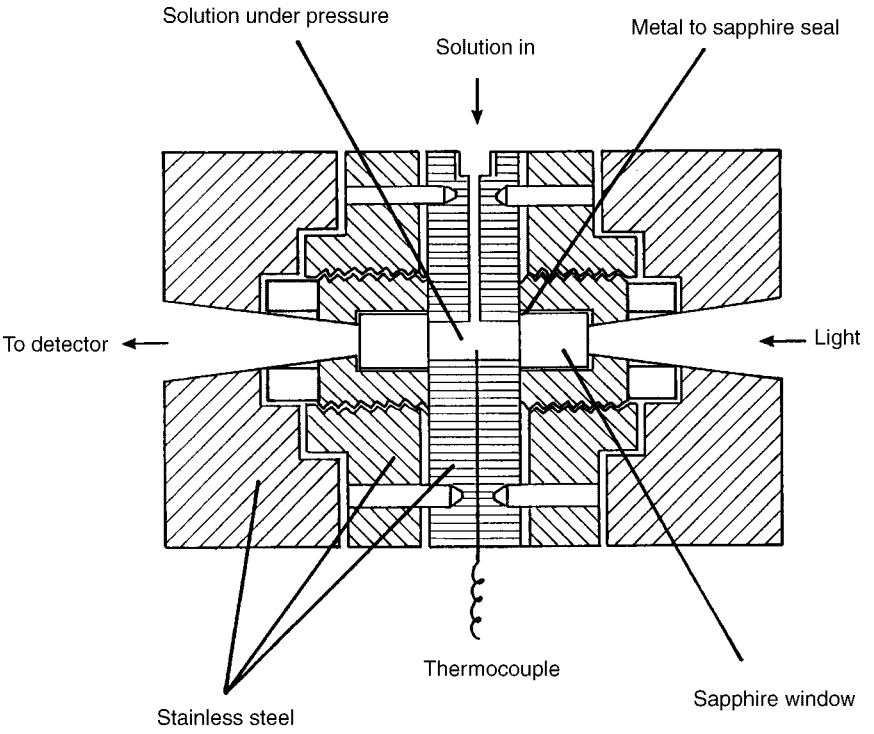


Fig. 10. High pressure cell in which pressure is applied directly to the sample solution (39).

solution has a homogeneous composition. Rapid monitoring of the mixed, reacting solution is subsequently required. A detailed description of the principles behind rapid mixing that leads to flowing together of the reactant solutions, uniformity of the solution in the observation chamber has been provided and this includes delineation of the need for turbulent flow and the critical value of the velocity of the flowing solutions (46). Originally, the flow method developed some 80 years ago was a continuous flow method, (47) and was briefly succeeded by an accelerated flow method (48). There was recognition that stopping the mixed solution flow rapidly and immediately observing the contents of the post-mixing chamber would improve the time resolution of the method and reduce solution volumes. The key development of a rapid stopping piston can be attributed to Gibson and co-workers (49–51). In fact, many stopped-flow (sf) devices as they became known trace their origin to this design. The reactant solutions held in small ($\sim$2 cm^3) syringes are driven manually or by a mild gas pressure through a specially designed mixer containing four tangentially arranged jets. The observation cell typically 1.0 or 2.0 cm in optical path length and 2-mm diameter, is immediately past the mixing chamber at right angles to the flow direction. The mixed solution is led to a syringe that brings the flowing solution to a rapid stop. The volume of each flow can be adjusted, but originally was

usually $\sim$0.15 cm^3 per reactant solution. Observation is triggered by a microswitch activated upon stopping the flow. The mixed, stopped solution can be expelled subsequently by use of a valve, meaning that $\sim$8–10 aliquot replicates can be flowed together before refilling the reactant syringes is required. A sf spectrophotometer based on the Gibson model, and another fundamentally based on the Caldin/Canterbury model became available commercially [Durrum Instruments, later Dionex (no longer on the market) and Hi-Tech Scientific, respectively]. Some very early variants have been described (46). Other versions have been developed, eg, by Applied Photophysics, Leatherhead, and by Bio-Logic Science Instruments. Note that almost all of the pioneering work in developing flow-mixing apparatus for studying fast reactions, technology now in widespread use by chemists, was actually carried out by biochemists.

The challenge for adapting this technology to high pressures required the reactant solutions to be compressed and thermally equilibrated prior to mixing. A further aspect was to be able to ensure that the reactant syringe-piston and stopping syringe-piston could move synchronously and efficiently under pressure. Effectively sealing components in a high pressure sf device might not be any more difficult than in an ambient pressure device as the pressure would be the same in all directions. Sometimes component sealing could be a problem at ambient pressure at temperatures $>20^\circ$C away from ambient, although in a Hi-Tech Scientific version the immersion of the seal-less observation chamber applicable to low temperatures as well (for nonaqueous solvents) avoids the problem.

A high pressure stopped-flow apparatus (hpsf) was first reported by Heremans and co-workers some 25 years ago (52), and in the following few years other versions were described (53–58). While the reactant and stopping syringes were mounted either horizontally or vertically in ambient pressure sfs, the flow device itself is mounted vertically in most hpsf. An important feature of hpsf is that the flow device itself is completely immersed in pressurizable fluid in a container. The solutions can be introduced to the reactant syringes (but not mixed) and the device can be introduced to the container, pressurizing fluid added, the pressure compartment sealed, and pressure applied. Optical observation can be monitored if the sf device is mounted so that incident light can be transmitted through the cell and collected by a rapidly responding photomultiplier, as in ambient pressure sfs. Fiber optics may be attached to the windows in the container. Optically transparent liquids are used for pressurizing fluid, eg, *n*-heptane. There are several significant differences from ambient pressure sfs. In many versions of hpsfs there is a "stop" syringe, but no actual physical stopping plate. The tightness of the three pistons themselves in their respective barrels brings about a stop to the flow once the pressure on the reactant pistons brought about by, eg, a step motor, is removed. In the example illustrated below, this can be achieved using barrels of the syringes and the pistons from Kel-F and adjustable tightness of the Viton O-rings held in grooves within the pistons. Another question that needed addressing was how could the experimental protocol become less labor intensive. If for every half a dozen replicate runs the system had to undergo decompression and new solutions introduced and the compression cycle repeated, covering a minimal pressure range would be very time consuming and possibly require new reactant solutions to be

prepared periodically and often. The remedy has been found whereby smaller reactant volumes per reaction initiation can be used together with a larger volume mixed solution receiver syringe. In favorable circumstances, ~35 reaction initiations can be conducted before refilling is required. This enables five measurements at each of six pressures, typically at 5, 25, 50, 75, 100, 125 MPa to be carried out and generates a minimum set of kinetic data for determining a volume of activation from a ln k versus P plot. Different methods of initiating a reaction have been reported. In some cases, the stepping motor is inside the pressure container and is operated by an electrical signal from outside the pressure container (53), while in other cases the device for initiating the reaction is outside the pressure container (56,57). Disadvantages of hpsfs are the signal-to-noise ratio is generally lower than in sfs, and more limiting, the dead time, the time delay from when the theoretical zero time of the reaction occurs until meaningful measurement of the monitoring signal can take place, is much longer in a hpsf. In an efficient sf, this dead time is ~2 ms, and can vary around this ideal, depending on the observation chamber path length. However, in hpsf this time can be up to 20–30 ms, increasing by an order of magnitude the half-life of reactions that can be studied by hpsf. This factor is aggravated if a reaction is accelerated considerably by increasing pressure. A schematic of a widely used hpsf is shown in Fig. 11 (53). Commercial versions of hpsf are available, one from Hi-Tech Scientific is based on the Merbach design and is illustrated in Fig. 12.

The dead time or dead time plus delay time has been shortened compared with an earlier version of an hpsf (59). The remedy that seems to be most effective in bringing dead time in an hpsf closer to that of ambient pressure sfs is to equip the apparatus with a push–pull coupler. This device, which was reported in an earlier version (55), has been refined as described in a later publication (60). The device operates by pulling the receiver piston simultaneously with pushing the reactant sample syringes and has the effect of achieving a dead time of 2–3 ms for a reaction at 50 MPa varying slightly with the gas pressure applied to the actuator mechanism. Furthermore, this latest hpsf (see Fig. 13) also available commercially from Hikari High-Pressure Machinery, Co., Ltd. is constructed with materials that permit it to be employed in studies using organic solvents or strongly acidic media.

One hpsf, the design and construction of which was participated in by one of the authors, and both authors have used this apparatus since the first version was reported and more recently with its updated version (59), is chosen for more detailed description. A general schematic of the sf unit is Fig. 11. The syringe barrels push fit to seal into the central block containing the observation chamber and quartz windows. Filling of the reactant solution syringes and a small quantity of solvent in the receiver syringe is an acquired art if any air bubbles present are to be eliminated. The central block is held in a steel frame, which prior to introducing into the high pressure container vessel supports the vessel lid and stepping motor. Taut springs hold the assembly in a rigid fashion and alignment is made so that upon introduction into the container the windows in the outer container align exactly with the windows of the cell block. The total vertical assembly is about the height of an A4 sheet of paper. An appropriate quantity of the pressurizing fluid is added just prior to inserting the assembly

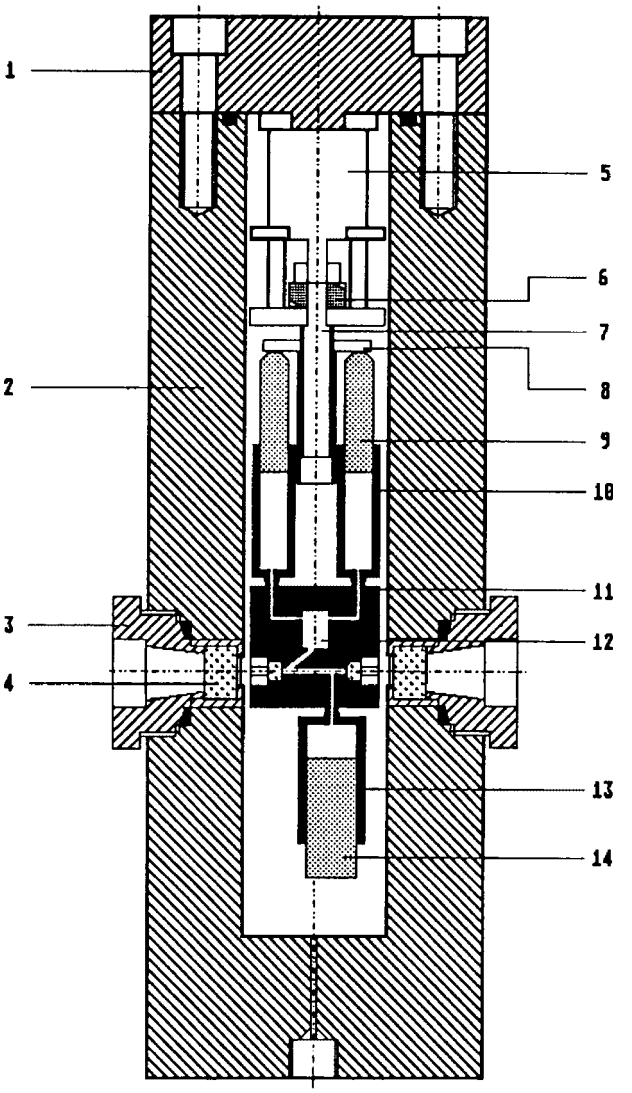


Fig. 11. Schematic presentation of a hpsf unit: 1 - lid to overall unit; 2 - outer vessel; 3 - window holder; 4 - quartz windows; 5 - electric motor; 6 - motor actuator; 7 - stopped-flow unit positioning rod; 8 - syringe driving plate; 9 - drive syringe (inner); 10 - drive syringe (outer); 11 - block holding windows, mixer and syringe attachment points; 12 - mixing jet; 13 - stop syringe (outer); 14 - stop syringe (inner).

into the container, the container vessel is then sealed and through operation of valves and a pump system pressure is applied. The desired value of pressure can be applied and noted from the Bourdon-type gauge slaved to the pressure in the vessel. Temperature control is exercised by pumping thermostatically controlled fluid through copper tubing surrounding the cylindrical vessel. A thermal equilibration time of 20 min is a minimum to ensure restoration to the required temperature following the heating phase induced by compression. Valves used

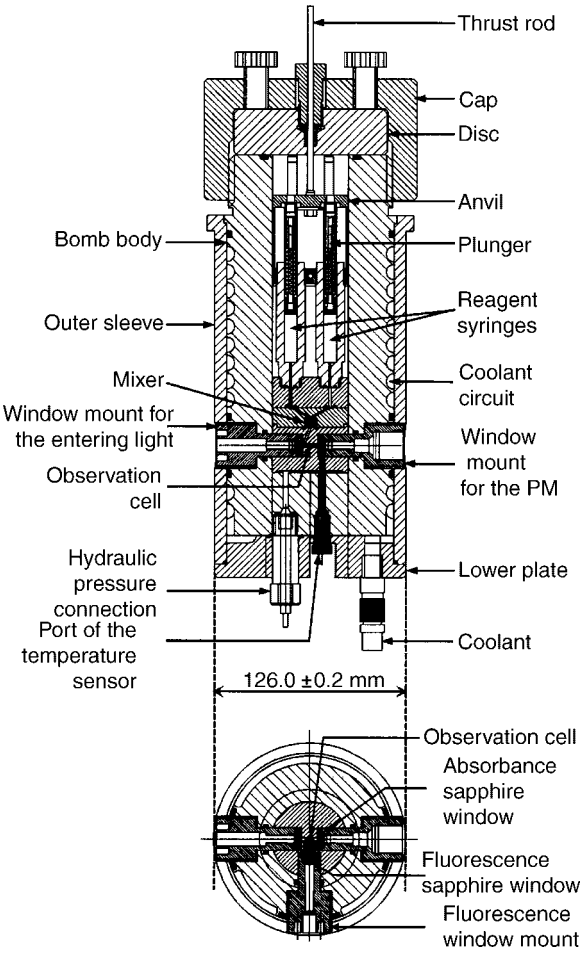


Fig. 12. Schematic presentation of the commercially available Hi-Tech HPSF-56 hpsf unit.

in these and other similar apparatus are standard, manually operated cone valves that must be chosen bearing the needed pressure rating. They are available from a variety of sources including Aminco (Newport Scientific) and SITEC. Other developments in hpsf technology are contained in additional literature, see, eg, Refs. 61–64.

Relaxation Methods. Other methods may need to be introduced when a reaction is very rapid so that it cannot be monitored by the sf method. It was recognized ~50 years ago that when a reaction is faster than it is physically possible to mix liquid-phase reactants, that the solution resides in premixing the reactants to form an equilibrium state, and then perturbing the equilibrium state rapidly by a physical probe (65). The latter is one upon which the position of equilibrium depend, eg, on temperature, pressure, electrical impulse, photochemical impulse, pulse radiolysis, or ultrasound wave. Following rapid pertur-

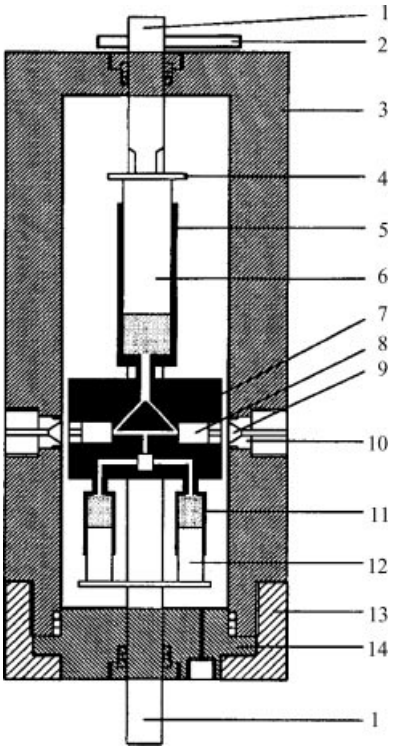


Fig. 13. Cross-sectional diagram of high pressure vessel and sf unit. (1) Rod made of AISI 316, (2) lever to revolve rod, (3) high pressure vessel, (4) push–pull rod, (5) receiver syringe, (6) receiver–syringe piston, (7) mixer and optical cell block, (8) quartz, (9) sapphire window, (10) sapphire window holder, (11) reactant syringe, (12) reactant-syringe piston, (13) high pressure vessel lid holder, (14) high pressure vessel lid.

bation, the system then "relaxes" to an equilibrium state, and the relaxation process can be monitored and kinetic parameters thereby derived. These relaxation methods as they are known have been described in detail and the applicable time ranges tabulated (46,66–68). Note that most are single impulse methods; ultrasound provides a continuous periodic pulse. A general impression from the literature is that the temperature-jump and flash photolysis methods have been most widely applied at ambient pressure, and are discussed briefly, together with pulse radiolysis and the approach in adapting them to the high pressure mode. The temperature-jump method requires administering a very rapid, of the order of a few microseconds (μs), temperature increase to a solution at equilibrium. For aqueous solutions, this can be achieved by discharge of the energy from a condenser energized from a voltage in the 20,000–30,000-V range. The cell containing horizontally positioned optical windows is made of an inert plastic (eg, nylon or Kel-F), and also contains two vertically mounted electrodes, the upper one may be part of the cell lid. In the early cell, the electrodes were shaped somewhat similar to the end of a dumbbell, and were finely polished, and the solution must contain electrolyte of concentration 0.1 mol/dm^3 or higher and be degassed.

Unless these conditions are fulfilled, successful experiments are not possible. An early version of this type of cell contained $\sim$5 cm^3 of solution and $\sim$1 cm^3 of which was heated by $\sim 8^\circ C$ (69,70). Thermostating of the cell by circulating fluid was at a temperature $8^\circ C$ below the temperature at which kinetics measurements were required. Within $\sim$20 ms after the temperature pulse, convection currents within the cell commence, signaling that the kinetics observations should be completed. The magnitude of the relaxation signal will depend in part on the value of ΔH° (the enthalpy change of the reaction) relating to the temperature dependence of the equilibrium constant of the reaction, and on the extinction coefficients of the chromophore groups being employed. The relationship is

$$d \ln K/dT = \Delta H^\circ/RT^2 \tag{14}$$

In some instances, where no suitable chromophores are present in either products or reactants, an acid–base colorimetric indicator can be added to unbuffered solutions to follow proton-transfer reactions that are part of the relaxation process (71). Proton-transfer reactions of the acid–base indicators themselves are regarded as very rapid compared with any proton-transfer processes intrinsic to the relaxation, and so do not complicate the kinetic analysis. Later temperature-jump cells have used smaller reaction volumes and required smaller temperature jumps and lower voltages. When the system is not aqueous based and does not contain an electrolyte, and therefore is not a conducting medium, other methods of causing a temperature jump must be applied. These have included laser pulses (72), and J. Crookes, P. A. Tregloan, and E. F. Caldin at the University of Kent (U.K.) in the 1970s experimented with microwave energy. Whether the system is studied at ambient or high pressure the difficulty with this and other perturbation methods is identifying with which reaction is the relaxation signal associated. It is also customary to be confronted with reduced signal-to-noise ratios and lowered reproducibility with these methods than for direct mixing systems.

Adapting the temperature-jump method to elevated pressures poses less of a challenge than for the sf method since there are no moving parts and the solution is already at equilibrium upon cell filling. Providing the power source can be supplied without difficulty, then the high pressure adaptation, at least for optical monitoring, should be similar in principle to that for slow reactions providing fast detection capability is present. A cell-enclosing pressure vessel containing optical windows and ports for the power source and pressurizing fluid will be adequate. Several versions were developed $\sim$20–30 years ago, the first by Tregloan and co-workers (72). Their design included cells for either conductometric detection or optical monitoring. The cells contained a movable piston to allow transduction of the pressure from the pressurized fluid to the solution being examined. Since one of their primary research interests at the time was proton transfer and quantum mechanical tunneling in aprotic solvents, "joule" heating could not be used and the temperature-jump was provided by a laser source (73). In an electrical discharge apparatus, a Teflon membrane was incorporated in the cell to allow transmission of pressure to the sample. Again strict degassing of solutions is required for successful operation and avoidance of cavitation subsequent to the temperature increase. Figures 14 and 15 illustrate an

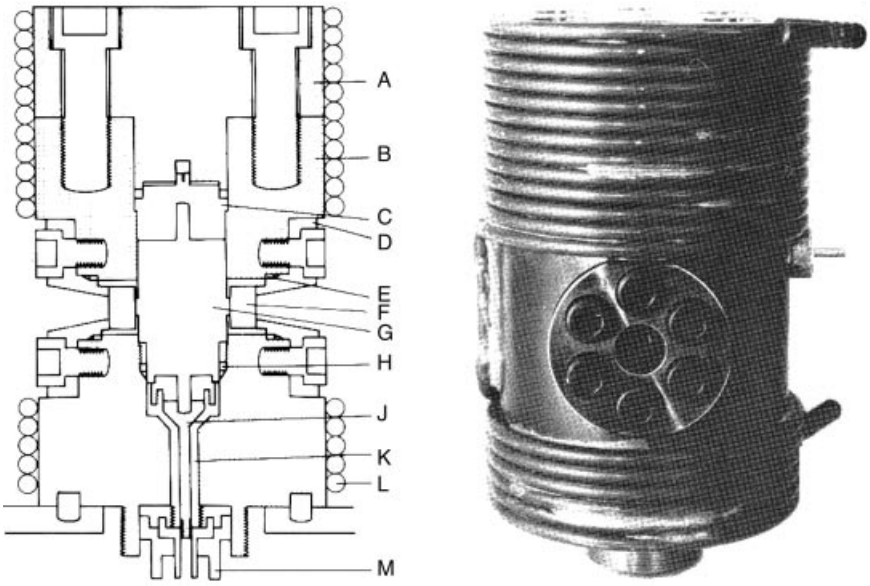


Fig. 14. High pressure cell for T-jump experiments: A – vessel lid, B – vessel body, C – steel piston, D – window support, E - Δ and O ring, F – sapphire window, G – space for T-jump cell (see Fig. 15), H - Δ and O ring, J – steel high voltage connector, K – insulation material, L – circulation coil, M – high voltage plug.

example of a high pressure temperature-jump apparatus (74). The schematic of the cell enclosing apparatus for high pressure application is presented in Fig 14, and Fig. 15 shows detail of the sample cell itself. The selection does not imply superiority over other designs, (75,76) but rather that this apparatus has been used frequently and is experimentally familiar to the authors. The keys to the figures make the overall apparatus quite self-explanatory. An important point to recognize in applying relaxation methods is that the relaxation signal, if only one is observed, can be fitted to a single exponential function, providing the initiating jump (temperature in this case) does not remove the system too far from the equilibrium position. In other words, a higher temperature jump can give rise to a larger amplitude signal, but then the relaxation kinetics will not necessarily be a first-order approach to equilibrium, thus complicating the data analysis. It is also not always obvious to which process the relaxation signal represents.

A second relaxation method is the pressure-jump method (77). The shift from the position of equilibrium upon a pressure-jump depends on the change of volume, ΔV, associated with the reaction, according to

$$d \ln K/dP = -\Delta/RT \tag{15}$$

Pressure-jumps occurring in ~0.1 ms, from, eg, 5 to 0.1 MPa, can be brought about by puncturing a diaphragm that is part of the pressurized cell. The relaxation kinetics can be followed conductometrically. Adaptation of the pressure

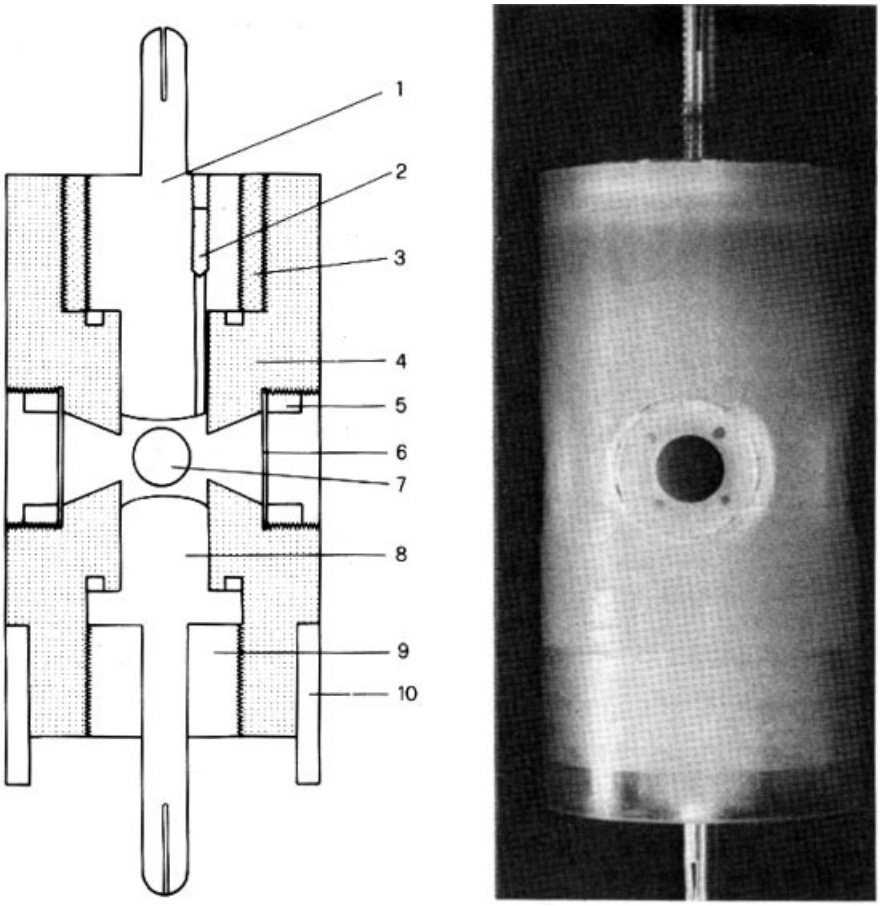


Fig. 15. T-jump cell: 1 – upper electrode, 2 – deaeration hole, 3 – electrode thread support, 4 – Kel-F body, 5 – membrane support, 6 – Teflon membrane, 7 – optical window, 8 – lower electrode, 9 – threaded bolt, 10 – support ring.

jump method to high pressure studies requires that the system under study can be compressed to 150–200 MPa and then reducing the pressure by increments and studying the relaxation at each reduced pressure. Commercial units or components of pressure jump apparatus are not available, and only a very few efforts of using this relaxation approach for high pressure kinetics have been reported. Pressures up to 150 MPa were used in measurements in an early study (78). Later the technical details of a high pressure, pressure jump system making use of pressure jumps of 13 MPa at various pressures up to 100 MPa were reported (79). Other approaches to obtaining the pressure dependence of rate constants have been described (80,81). The mostly dormant recent period of applying high pressure, pressure jump technology reflects the difficulty of experimental practice and lack of versatility of the method in so far as not as many chemical systems are as amenable to study by this technique relative to some other techniques.

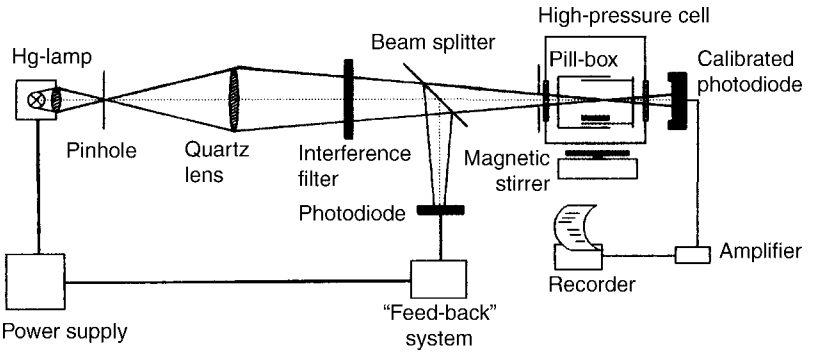


Fig. 16. Apparatus for high pressure photochemistry.

Photochemical reactions in the conventional time range can be studied as a function of pressure using a pill-box cell or one modified for the excitation energy to be incident as well as for the detection windows, in a very similar arrangement as that described above for thermal reactions followed spectrophotometrically. The early impediment to studying rapid photochemical processes either unidirectional or as light induced relaxation reactions was removed upon the development of a fast (10-μs) flash method (82). Laser flash photolysis apparatus for rapid reactions is quite similar to that for slow reactions except for rapid detection and data acquisition, and high pressure adaptation is also similar (37,38) (see Fig. 16).

Radiation Methods. Pulse radiolysis can be regarded as analogous to flash photolysis in radiation chemistry; a pulse of high energy radiation in the form of a beam of electrons or X-rays is the equivalent reaction initiation method. Again the reaction initiation time needs to be very rapid (ns) since the radiation energy is estimated or calculated to generate free radicals whose subsequent reactions are often extremely rapid. Clearly, these energy sources and the accompanying monitoring equipment are not common laboratory currency. Consequently, there are few facilities available for studying the kinetics of the relevant chemical processes, and even fewer facilities where the method has been devoted to high pressures. For high pressure studies a critical issue is delivering sufficient intensity of radiation to the sample as the high pressure container's windows and pressure fluid can effect a reduction of energy (83). This problem has been solved by designing a cell window that has a steel disk containing honey-combed holes (see Fig. 17) (84). Providing the energy source is suitable and the monitoring system (usually uv–vis spectroscopy) is capable of following very rapid reactions useful results can be obtained. The pressurizing system associated with the pulse radiolysis equipment is reasonably standard. One further critical factor for both ambient and high pressure pulse radiolysis experiments is identifying the chemical processes that lead to the effects observed following the pulse.

Electrochemical Methods. Interest in various oxidation–reduction reactions and their mechanisms has stimulated the development of high pressure adaptation of ambient pressure apparatus or instruments. For example, self-exchange electron-transfer reactions can be studied in appropriate cases by

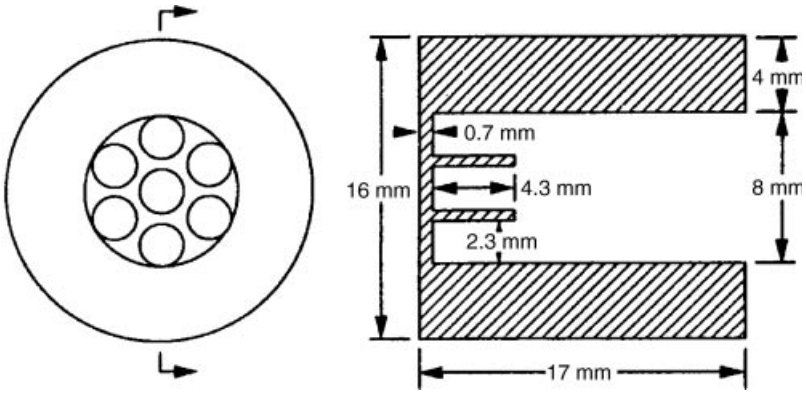


Fig. 17. Schematic diagram of the electron beam window for high pressure pulse radiolysis. The electron beam enters from the right side.

high pressure nmr spectroscopy. Other self-exchange reactions and other redox reactions can be studied by high pressure electrochemistry. Additional interest is generated in comparing some of these processes in aqueous and nonaqueous media, and also comparing or contrasting homogeneous and heterogeneous electron transfer. Furthermore, there is scope for comparing experimental results with data calculated from extending Marcus-Hush theory (85,86) for ambient pressure redox processes to the same reactions at high pressures. Recent authoritative reviews on the subject of electrochemistry at elevated pressures preclude the need to describe the design and construction of apparatus in detail (87–90). The method requires the measurement of the rate constant as a function of pressure for electron transfer of a given redox couple at an electrode. Determination of the volume of activation for the self-exchange of the redox couple from the volume of activation derived from the measurement of rate constants at the electrode is not completely straightforward and the references cited should be consulted for the steps in that process. Only a few pressure cells have been described and the method is restricted to expert specialists in the field (91–93). A cell consists of a Teflon body encompassing three electrodes, one is the working electrode, a second is the counterelectrode, and the third is a reference electrode. The reference electrode is designed to allow ionic contact and to allow compression of the solution in the reference compartment. Overall the cell has a movable piston to accommodate compression of the reaction medium. The cell is assembled in a pressure vessel and suitably designed electrical connections are in place (see Fig. 18). The pressurizing fluid, typically hexane, is separated from the oil of the hydraulic pump via a piston separator vessel. Pressure is monitored by, eg, a Bourdon gauge, while cell temperature is controlled by thermostated water in an enclosing jacket-vessel. It has been found that the best results are obtained by employing alternating current voltammetry. Pressures up to 200 MPa are usually applied, and five or six different pressures are normally sufficient to obtain satisfactory results.

In addition to these techniques, several highly specialized high pressure methods have been established. This technology has not been used widely, nor

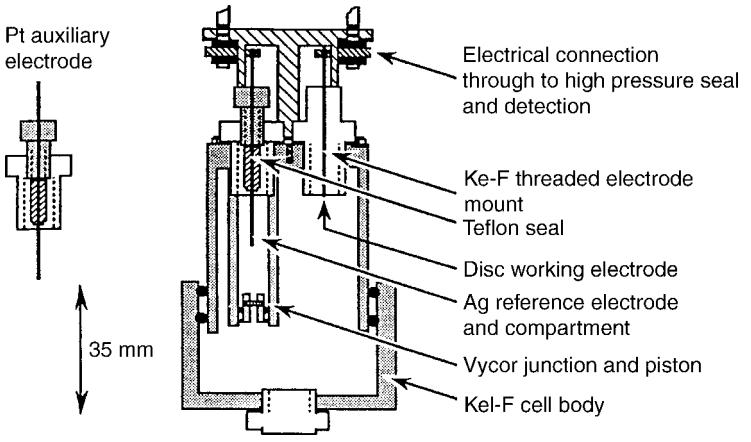


Fig. 18. High pressure electrochemical cell.

indeed applied very much to chemistry itself, but rather more to examine properties of substances and to investigations in biochemistry. Several projects involving neutron radiation on materials at high pressure are ongoing (94). Both liquids and gases are studied under the umbrella of the ISIS neutron radiation programme (94). In many applications involving uv–vis spectrophotometry, an optical path length of 1 cm is a convenient dimension allowing for reasonable signal changes and concentration levels of solutes that avoid solubility problems and do not run a risk of violating the Beer-Lambert law. However, for turbid solutions such a dimension would not permit sufficient light to be transmitted to enable adequate measurements to be made. Accordingly for the purpose of studying how colloidal dispersions respond to pressure an optical cell with a 0.2-cm path length has been developed for pressures up to 600 MPa (see Fig. 19).

Against a background of public health issues, understanding unfolding and refolding of proteins particularly *in vivo* has become a priority. Molecular chaperones help to ensure that a protein has a correct final three-dimensional (3D) assembly. However, if mistakes occur, proteins assume unnatural conformations and this can cause any one of a family of diseases known as amyloidoses in which misfolded proteins accumulate and form aggregates. High pressure (by means of the diamond anvil cell) Fourier transform infrared (ftir) (95,96) and fluorescence spectroscopies (96) have been employed in protein-folding investigations. In addition, the volumes of activation for the protein folding processes in the presence of denaturants have been determined using pressure-jump and hpsf methods (97). Other high pressure methods for protein folding studies include a high pressure jump method, in conjunction with a small angle synchrotron X-ray scattering technique (98) and high pressure Raman spectroscopy (99). Elastic and inelastic X-ray diffraction experiments upon liquids have been carried out in a special high pressure cell capable of being operated over a wide temperature range (100).

4.4. High Pressure nmr Spectroscopy. Ambient pressure nmr spectroscopy ranges from the routine measurement for structural characterization

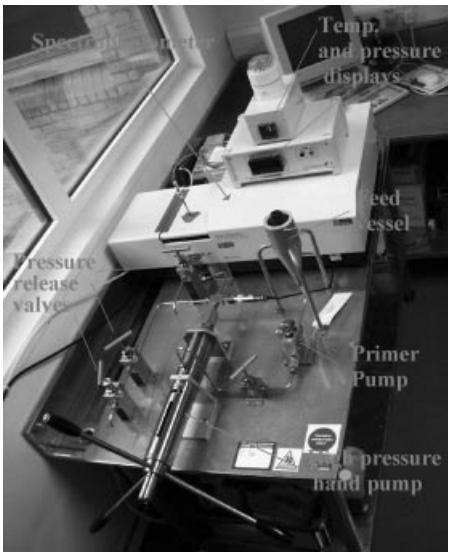


Fig. 19. High pressure apparatus for an optical cell for colloidal liquids rated at 700 MPa. (Courtesy of Unilever Research.)

of small, usually organic molecules to complement other methods such as ir spectroscopy, elemental analysis, and chromatographic procedures to extremely sophisticated nmr spectroscopy to gain insight into structures of macromolecules such as proteins. The information from the nmr spectrum of a solution of the sample can be compared with the structure in the solid state obtained by X-ray crystallographic methods. The structure may not always be available, but if it has been determined the two methods are complementary. The vast preponderance of nmr spectra obtained are of samples in solution, although under suitable technical conditions (magic angle spinning) the spectra of solid samples can be acquired. Historically, nmr spectroscopy was applied to obtain ^{1}H spectra of organic compounds dissolved in deuterated solvents, but rapidly other nuclei such as ^{13}C and ^{31}P were employed. Whether magnetically active nuclei can be employed depends on the percent abundance of the particular isotope and on the sensitivity to detection (101,102). In some cases, the relevant nucleus can be enriched to improve resolution and precision. The progress in improving the resolution and sensitivity levels for detection with instrumental development can be described as spectacular, as nmr spectrometer instruments operating at up to 500 MHz are routinely in use. From the perspective of kinetics, if a reaction has an appropriately developing or declining resonance signal and the reaction is not too rapid, then relatively standard procedures could be used to determine the rate law and kinetic parameters. However, nmr spectroscopy would not be the method of choice unless properties of the system were such that other techniques could not be exploited, since modern high resolution nmr spectrometers are very expensive to purchase and operate. Within transition metal coordination chemistry, understanding of important ligand substitution reactions can be greatly

enhanced by knowledge of solvent exchange on a fully or partially solvated metal ion. Pioneering experiments on the kinetics of water exchange on some first row transition metal aqua ions were conducted by monitoring the exchange of $H_2{}^{17}O$ between coordinated and bulk water molecules (103). The spectra were acquired as a function of temperature and this permitted, after considerable data treatment, the calculation of kinetic parameters. Developing this method for high pressures (hpnmr) became a goal for investigators of reaction mechanisms, such as solvent exchange (36), for transition metal catalysis studies (1,2), and other processes, eg, conformation changes, involving macromolecules (104) and more recently to applications in supramolecular chemistry (105). Constructing a probe that can be introduced into a nmr spectrometer presents a significant technical challenge as the sample tube will not be spinning and all the materials of construction must be those that do not interfere with the magnetic resonance measurements being undertaken. Early work on high pressure nmr (106,107) was followed by a burst of activity in the 1970s and 1980s with suitable sample cells being constructed for the ever-advancing new generations of nmr spectrometers (108–114). Several aspects of these original developments have been reviewed (115,116). Several different high pressure probes have been constructed and to an extent the spectrometer becomes dedicated to the high pressure mode (117,118); therefore there is an economic factor involved and a comprehensive and sustained plan of research using hpnmr is needed as justification. Consequently, the number constructed is limited to a few university investigating groups who possess the expertise and workshop facilities required. Clearly, the early work referred to above is now mostly of historical interest and much of it relates to spectrometers with electromagnets. For current practice with superconducting magnets, the later references should be consulted. Some examples of probes that have been developed are better illustrated with explanatory legends than described (see Figs. 20 and 21). The pressurizing system is relatively standard and not very different from that used for conventional time range uv–vis spectrophotometry. Successful construction of the probe is a delicate and difficult task.

Particularly for solvent exchange processes, converting acquired data into meaningful kinetic parameters is not a trivial exercise. Nevertheless solvent exchange kinetics studies at high pressures on many solvento-metal ions both within and outside of the transition metal series have been conducted and the resulting volumes of activation served admirably for mechanism diagnostic purposes (36,119). Of particular note, recently very inventive efforts on solvent exchange kinetics at high pressures, usually on water exchange on the lanthanide ions, either fully or partially solvated, have been reported (35,36). The findings have led to the design of potential magnetic resonance contrast imaging agents (120–122). High pressure solvent exchange kinetics studies have stimulated several theoretical studies aimed at comparing or contrasting experimental results and supporting or not conclusions regarding the mechanisms (see, for example, Refs., 123–127). These experimental aspects of high pressure research have undergone substantial growth partly because of the potential medical relevance. It is well worth noting that in solvent exchange kinetics studies the method gives rise to signal broadening or signal coalescing depending on the temperature and pressure variations. The emerging kinetic parameters from

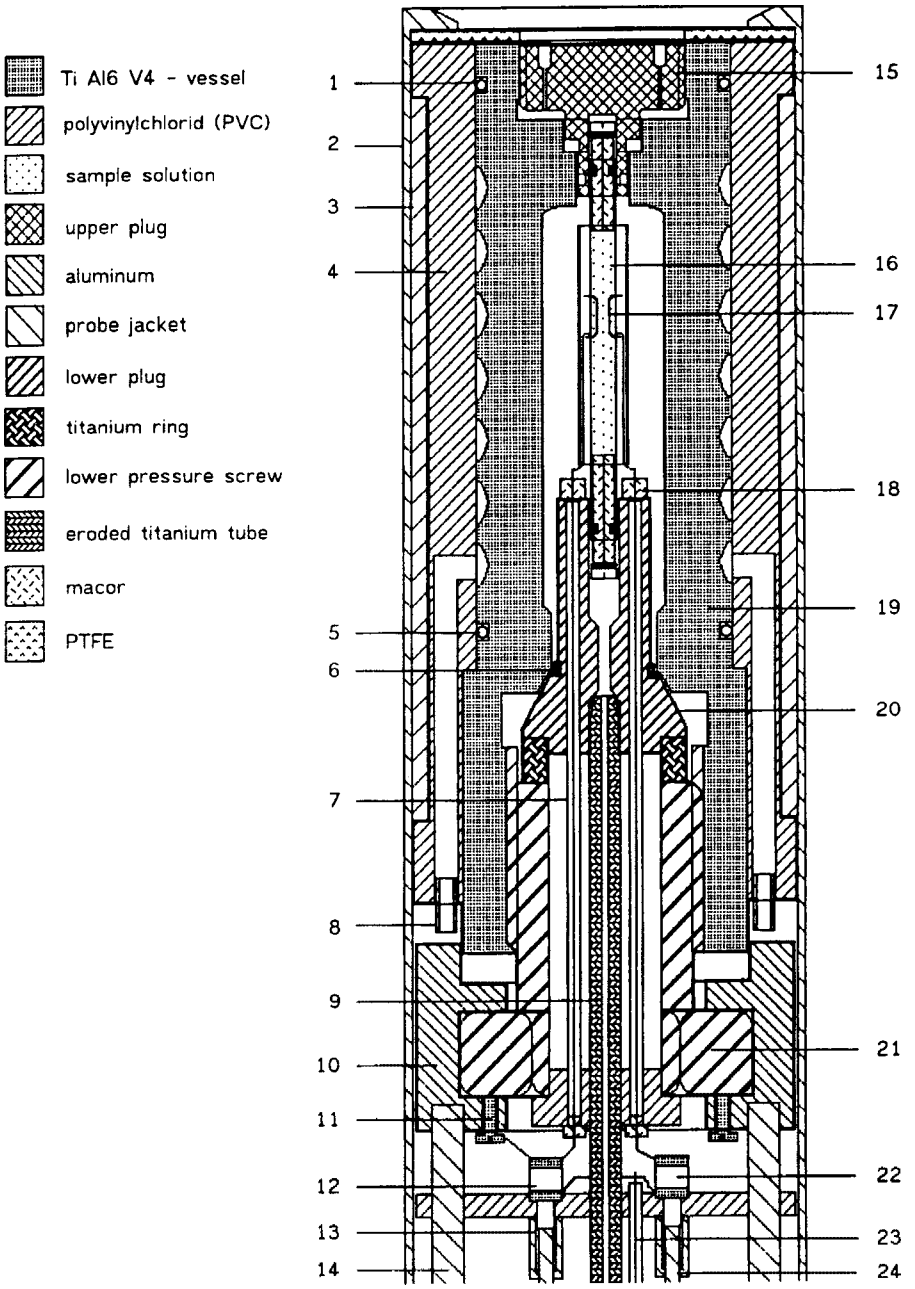


Fig. 20. Design features of a wide bore probe head for high pressure nmr (400 MHz) measurements: 1 - O-ring; 2 - probe jacket; 3 - thermal insulation; 4 - poly(vinyl chloride); 5 - O-ring; 6 - O-ring; 7 - semi-rigid coaxial cable; 8 - connection to thermostat; 9 - titanium tube; 10 - lid; 11 - screw; 12 - capacitor; 13 - capacitor holder; 14 - aluminum tube; 15 - upper plug; 16 - sample tube; 17 - saddle coil; 18 - Macor; 19 - TiAl6V4 vessel; 20 - lower plug; 21 - lower pressure screw; 22 - capacitor; 23 - coaxial cable; 24 - capacitor holder.

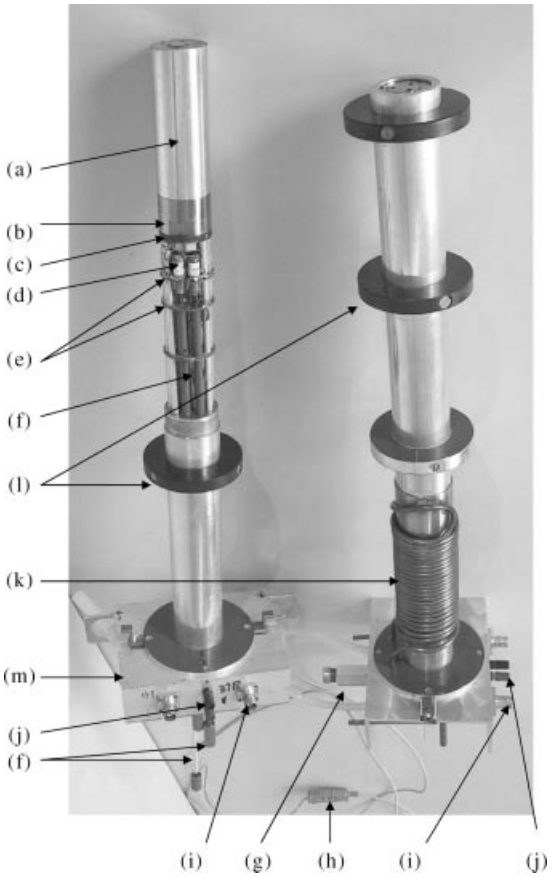


Fig. 21. Photograph of two narrowbore probeheads: (a) aluminum jacket sealing the double helix used for thermostating, (b) high pressure vessel, (c) platform carrying the autoclave, (d) capacitors, (e) capacitor platforms, (f) tuning rods, (g) high pressure connector, (h) thermocouple, (i) BNC connector, (j) Pt-100 connector, (k) copper tubing, (l) widebore adapter.

the data analysis can span an enormous time range, representing lifetimes of a single water molecule in the first coordination sphere of a metal ion from 10^{-10} to 10^{10}s (35,36). Obviously, this method represents a powerful tool for investigating the mechanisms of a very wide time spectrum of solvent exchange processes. Since there is no net reaction in solvent exchange, there is the satisfying knowledge for mechanistic analysis that the volume of activation does not contain a measurable contribution from electrostriction changes, as mentioned earlier.

4.5. Equilibrium Volume Parameters from Density Measurements. Probably the most convenient route to partial molar volumes of substances is from density measurements. The Anton Paar, range of density meters provides an opportunity to measure densities to an accuracy of 5×10^{-6}g/cm^3 (DMA 5000 instrument) with temperature controlled by a Peltier system ($\pm$ 0.01°C). The

principle of the measurement involves exciting the degassed liquid sample (1 cm^3), held in a U-shaped tube, electronically by a Piezo element. The continuous oscillation is at a characteristic frequency. Optical sensors record the oscillation period as the reciprocal of the frequency. The frequency is inversely proportional to the density of the sample. Measurements of the density of air and solvent at the same temperature are obligatory. Measurement times per sample can be as short as 1 min. The method of conversion of density measurements into partial molar volumes has been outlined earlier.

Reaction volumes for slow reactions can be measured directly by dilatometric techniques. The method involves confining the reaction solution to a thermostated, calibrated flask at the top or side of which is a capillary tube and measuring the change of level of the liquid in the capillary tube. This change of level can be related to the reaction volume. Diagrams of these relatively simple devices are shown in Ref. 24.

Readers can consult, in addition to the references cited, other review sources where the experimental aspects of high pressure studies have been presented. (3,7,24,35,36,83,128–130). The journals *High Pressure Research* and *Review of Scientific Instruments* are also frequently sources of reports of high pressure technology developments.

5. Selected Applications of High Pressure

Only a very few of the many applications of high pressure in chemistry, biochemistry, physics, and materials science are selected for discussion, but they should provide a sense of the wealth of information that can be obtained. Despite the technology challenge, the outcome can be very fruitful.

5.1. Commercial Products. A sample is changed after a pressure cycle. Pressure treatment of materials to effect change is not new (131,132), but only in the past decade has high pressure technology become established as one leading to commercial products (133,134). A whole range of food products has been treated by pressure cycles with the principal objective of removing or severely reducing spoilage microorganisms by pressure inactivation without heat treatment or addition of chemical preservatives (133). Typical pressures are 500–600 MPa for up to 3 min. The advantage of high pressure is extended safe product life, and retention of favorable characteristics that may be reduced by heat treatment. Data have been acquired as a function of pressure variation, pressure duration, pH, subsequent storage conditions, and other variables that indicate the magnitude of pressure inactivation of microorganisms in each of several products. Detailed results of test procedures are available from Avure Technologies. Other organizations such as Campden and Chorleywood Food Research Association, and Leatherhead Food Research Association, and the U.K. High Pressure Club for Food Processing, monitor the progress of this type of technology.

More fundamental studies on the effect of pressure on physicochemical characteristics of emulsions, colloids, and on the resulting microstructure have been conducted. Thus high pressure technology can be exploited to modify the properties of materials to generate matrices that can form the basis of a gelatinous product, eg,.

Example Study. Generating a gelatinous material is a frequent objective in several industries (eg, cosmetics, personal products, various types of foods). In the area of dairy product research, high pressure has been added recently to other traditional methods such as mild acidification or enzyme treatment for producing a gel or other desirable microstructure. The enzyme chymosin is the active component of the renneting process, the name given to the process inducing the microstructures that generate cheeses. An aqueous dispersion of skimmed milk powder (SMP) forms a colloid. Upon pressure treatment at ambient temperature, provided the pressure is >270 MPa, a suitable cosolute is present and, following pressure treatment, decompression is rapid, the colloid is converted to a gelled state (6). The latter can be characterized rheologically and by turbidity measurements. The final form of the gelled product is not obtained immediately following decompression, thus enabling the physicochemical kinetics of ultimate product formation to be monitored. Since the outcome of pressure treatment involves a subtle interplay between pressure magnitude, a medium effect and possibly a rapid cooling effect upon decompression, and the need to control and manipulate the product to a required microstructure, efforts to understand the mechanism were undertaken. From the measurements cited above and the finding of fluorescence enhancement following decompression, a hypothesis could be advanced (6). The principal protein component in SMP is casein in micellar form, made up mostly of four variants of casein held together by what is termed colloidal calcium phosphate. The micelle is stabilized in solution by hydrophilic residues on the external kappa-casein units. The effect of hydrostatic pressure is to dissociate the casein micelle into casein submicelles with the release of certain quantities of calcium ions and inorganic phosphate, P_I, species. The fluorescence enhancement, determined by titration with a fluorescent marker, indicates that pressure-induced dissociation of the casein micelle causes exposure of hydrophobic residues that were in the micelle interior originally.

$$\text{Casein micelle} + >270\text{MPa} \longrightarrow \text{casein submicelles} + Ca^{2+} + \text{Pi} \quad \text{(solute sucrose present)} \tag{16}$$

In addition, citrate ions are also released in more minor quantities. Following rapid decompression the sub-micelles rearrange themselves into a network gel, and P_i is reimmobilized (eq. 17, at 0.1 MPa).

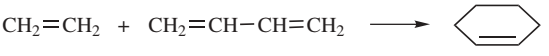
$$\text{Casein submicelles} + Ca^{2+} + P_i \longrightarrow \text{Casein gel} \tag{17}$$

The ^{31}P nmr spectroscopy measurements on the SMP sample before and after the pressure cycle showed three peaks, the principal one P_i in the serum phase, a signal identified as representing a phosphorus containing ester and a third that arises from a phospho-serine residue in the hydrophilic macropeptide of kappa-casein (5). The postpressure nmr spectrum was only marginally changed from the spectrum of the nonpressure treated sample, indicating that the reimmobilization of P_i was much faster than the kinetics of gel formation. The latter point was confirmed by ^{31}P hpnmr spectroscopy (5). Pressure steps up to

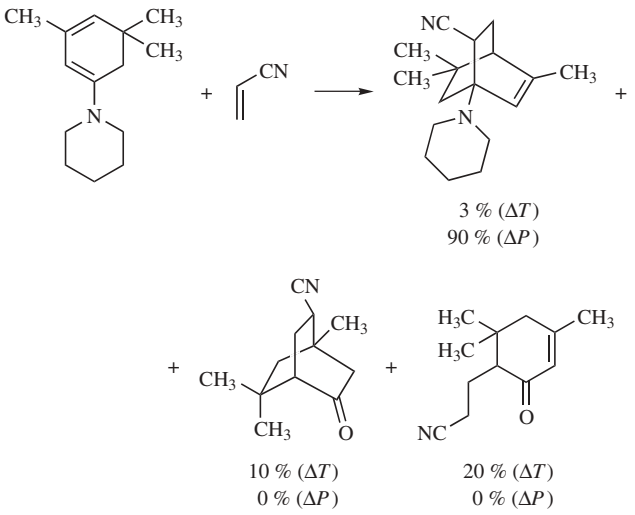
300 MPa showed progressive growth of the integrated area of the P_i peak, and subsequent equivalent pressure reduction steps showed an exact peak intensity reversal. Differences in the final microstructure of the postpressure treatment material were observed depending upon the increase in pressure in increments or in one compression application; analysis of these differences for natural casein and for artificial casein micelle assemblies is being undertaken (4). Understanding the mechanism is important in developing high pressure technology in the dairy industry where a gel could form the matrix in sub-areas such as ice cream.

5.2. High Pressure in Organic Synthesis. The advantage of high pressure over thermally initiated synthesis can be, in appropriate cases, a modification of the direction of the synthesis resulting in different proportions of the reaction products. There are many examples.

The Diels-Alder Cycloaddition Reaction. A Diels-Alder reaction is one between a conjugated diene (eg, 1,3-butadiene) and another compound containing a double bond, eg, ethene, the latter is known generally as a dienophile, (see eq. 18) (135). This particular reaction is quite slow, and is accelerated by pressure, whereas reaction between 1,3-butadiene and maleic anhydride proceeds at a convenient rate. A classical example of the value of high pressure in organic synthesis is the reaction of isophorone dienamine with acrylonitrile (see eq. 19) (136). At ambient pressure the yield of the desired product is 3%, and two other compounds in 10 and 20% yields, respectively, are prepared. Applying 1500 MPa at room temperature, the same reaction proceeds smoothly to generate the desired product with a 90% yield, a dramatic difference.



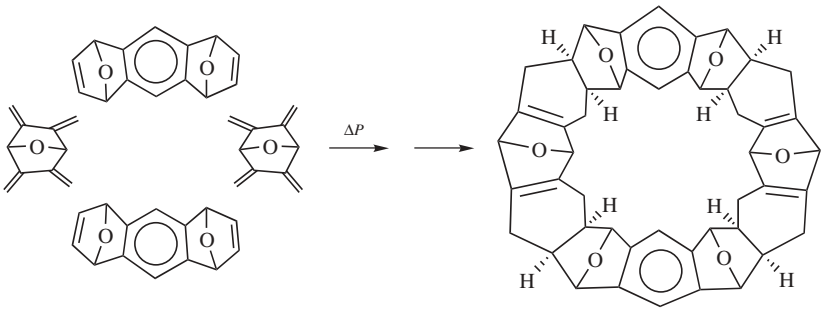
(18)



(19)

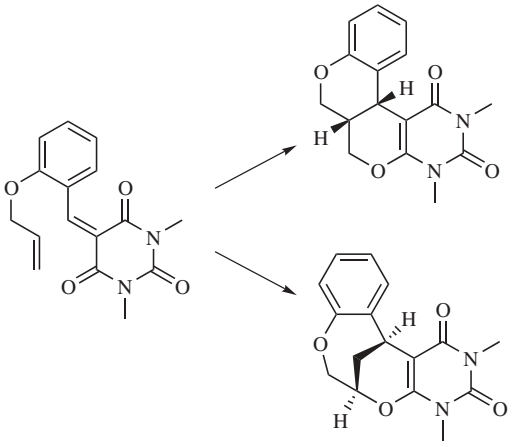
Preparation of Cyclic "Belt" Compounds. A more recent example of a successful use of high pressure in synthetic chemistry is the preparation of "belt" compounds from the diene and dienophile shown. At ambient pressure,

the yield was 2–3% and at 1000 MPa it was 30–35% (see eq. 20) (137,138). Other examples that are illustrative of the application of high pressure in preparative organic chemistry are 1,3-dipolar cycloadditions and Michael addition reactions (31).



(20)

5.3. Mechanistic Organic Chemistry. Processes that are accompanied by a decrease in volume such as carbon–carbon bond formation are accelerated by pressure ($\Delta V^{\neq} < 0$), whereas the reverse process, a homolytic cleavage of a carbon–carbon bond causes an increase in volume and the reaction rate is retarded by pressure ($\Delta V^{\neq} > 0$). One area of mechanistic organic chemistry, where high pressure can be exploited, is regioselectivity. This means that when two products of a reaction can be formed potentially, pressure can effect direction of the reaction exclusively or predominantly to one product. An example is the intramolecular hetero-Diels-Alder reaction of the benzylidenebarbituric acid derivative (see eq. 21) (139).

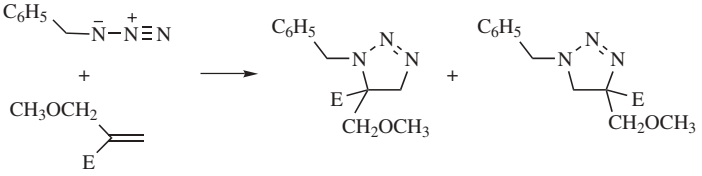


(21)

Two adducts may be formed, the ortho and the meta and the reaction has been performed in various solvents. The kinetics of the cycloaddition were monitored by ftir spectroscopy at various pressures and the reaction exhibited a pressure-dependent regioselectivity in favor of the ortho adduct. For example, for the reaction in acetonitrile, the ortho/meta ratio at 100 MPa was 5.54 and 6.33 at 400 MPa, and this ratio was 3.85 and 4.06 in toluene as solvent at the same

two pressures, respectively. A significant solvent effect upon the values of $\Delta V^{\neq}$ for formation of either adduct was observed, although the values themselves differed little for the two adducts for a given solvent. Volumes of activation were ca. -30 cm^3/mol for formation of both adducts in dichloromethane, 1-chlorobutane, and tetrahydrofuran (THF), but about one-half of those values in acetonitrile and toluene. This effect of solvent was not readily rationalized since toluene and acetonitrile are at opposite ends of the solvent polarity range. The volume profiles, obtained from measuring the partial molar volumes of the reactant and of the ortho product in four of the solvents were examined. The explanation is that the partial molar volumes of the product are the same in each solvent, but those of the reactant in toluene and acetonitrile are $\sim$10 cm^3/mol less than the values in THF and dichloromethane. Clearly, the partial molar volumes of the activated complex are about the same in each solvent. This study represents an excellent illustration of the value of high pressure kinetics in influencing regioselectivity. Furthermore, the development of complete volume profiles permits a solvent effect that otherwise would be difficult to comprehend, to be understood. The synthesis requires a succession of Diels-Alder reactions.

1,3-Dipolar cycloaddition reactions normally occur stereospecifically and retain the configuration in the dipolarophiles (see eq. 22). Their reaction rates are not very sensitive to solvent, and it is generally accepted that the mechanism is a concerted one (140,141). There are a few examples where the reaction mechanism is stepwise (142). Reaction volumes and activation volumes have been determined for some sample reactions; the former are typically -25 cm^3/mol and the latter are ~ -21 cm^3/mol, and taken together these values are indicative of a pericyclic reaction (143,144). This term is invoked when the transition state can be conjectured to possess aromatic character. The fact that the values of ΔV and $\Delta V^{\neq}$ are 5–10 cm^3/mol less than for Diels-Alder reactions has drawn comment, although it can be argued that the volume change could be related to ring size during cyclization. Indeed the volume decrease has been found to be larger for formation of a six-membered ring than for a five-membered ring (145).



(22)

80°C, 1 bar, 30 %:	50	:	50
25°C, 12 kbar, 83 %:	<5	:	<95

5.4. Inorganic Chemistry Reactions. *Solvent Exchange.* This type of reaction is invariably studied by nmr spectroscopy using a magnetically active atom in the solvent, although in principle in some cases it would be possible to study solvent exchange using radiochemical techniques. Solvent exchange is the most simple reaction studied herein, in that there is no net reaction and the reaction volume is zero, and nmr spectrscopy both at ambient and high pressure is the most widely employed method of study. Ironically, as indicated above, the

treatment of data using this method to obtain meaningful kinetic parameters is one of the more complex methods. The reaction is

$$MS_n^{m+} + S^* \longrightarrow MS_{(n-1)}(S^*)^{m+} + S \quad k_{ex} \tag{23}$$

Both S^* and S are isotopically different and are shown here as unidentate solvent ligands (almost always the case), n is the coordination number and m is the magnitude of charge. There are also solvent exchange studies on metal ions that are partly complexed with nonexchanging ligands, invariably bi- or higher dentate ligands. This can have profound consequences on the rate of solvent exchange compared with the fully solvated metal ion (146,147). For example, when hexaaqua copper(II) ion are substituted by some tripodal (four-coordinate) ligands (L), the coordination assumes a five-coordinate trigonal bipyramidal geometry, and the water exchange rate on $Cu(L)(H_2O)^{2+}$ is reduced by several orders of magnitude compared with the rate of exchange on the fully aquated copper(II) ion (146,147).

The following mechanistic classification system widely used in inorganic chemistry applies not only to solvent exchange systems (148,149). If the incoming solvent molecule becomes attached to the metal complex ion and thus increases the coordination number by one, the mechanism is designated A for associative. It is sometimes also termed limiting A as the extent of associativeness is 100%. Conversely, if the exchanging solvent departs from the solvated metal ion essentially completely before the bulk solvent molecule approaches, resulting in a coordination number reduction of one, then the mechanism is described as dissociative, or a D mechanism (limiting D). The rate constant for solvent exchange should increase with pressure for an A mechanism and give rise to a negative volume of activation, meaning the transition state has a smaller volume than the reactant state. Clearly, for a D mechanism, pressure increase decelerates the exchange rate and the volume of activation is positive. When water is the solvent, the limiting values of $\Delta V^{\neq}$ are estimated to be -13 and $+13$ cm^3/mol for the A and D mechanisms, respectively (150). If solvent exchange is perfectly "symmetrical", ie, the extent of the entering and leaving molecules is exactly matched, the mechanism is termed an interchange or I mechanism, with $\Delta V^{\neq}$ ideally zero. If the entering molecule has partly insinuated itself into the coordination sphere of the metal ion before the leaving molecule is actually leaving, the mechanism is termed I_a (interchange with associative character). The converse situation leads to an I_d mechanism. If the solvent is water, the volume of activation will have lower modulus values. Which mechanism a solvated metal ion follows may be determined by several factors: the charge on the metal ion, the radius of the solvated metal ion, the nature and bulkiness of the solvent molecule, the position of the metal ion in the Periodic Table and the outer electron occupancy.

The use of ^{17}O in H_2O to study the exchange of water on some aqua metal ions of the first transition series by nmr spectroscopy was pioneered by Connick and co-workers (103). Water exchange is rapid; of the metals investigated aquated Ni(II) was the slowest with k_{ex} of 3×10^4 s^{-1} at 25°C. One of the earliest studies of the effect of pressure on solvent exchange processes was on the exchange of dimethylsulfide and dimethyl ether on tantalum pentabromide ($TaBr_5$) (151). However, a study that attracted considerable attention was that in which the

volumes of activation for water exchange on several first-row transition metal ions in oxidation state 2 had been determined (152). Based on the $\Delta V^{\neq}$, values it was concluded that the mechanism of exchange was I_a for the aqua-metal ions up to and including Mn^{2+}, but thereafter the mechanism was I_d, a finding rationalized on the basis of changes in ionic radii and *d*-orbital electron occupancy. This result was consistent for the "I_d–group" with kinetic results from ambient and high pressure analysis of ligand substitution reactions. The mechanistic changeover was challenged on the basis of theoretical calculations that were purported to show that all these aqua-metal ions exchange water by an I_d mechanism (123). It was argued based upon further calculations that the mechanistic changeover is valid (124,125). High pressure kinetics studies of exchange of other solvents on these metal ions have been reported as have studies on trivalent cations and on monohydroxyaquametal ion species (35,36).

There is intrinsic interest in water and other solvent exchanges on the lanthanide series. However, the intensity of interest is multiplied, since some hydrated or partly hydrated lanthanide ions may have potential application as magnetic resonance imaging (MRI) agents in a medical context. A considerable amount of effort has been expended to establish the basic chemical properties of hydrated lanthanide ions or partially hydrated lanthanide ions, and these properties include the coordination numbers, stability of lanthanide complex ions, and the kinetics and mechanism of water exchange (35,36). The vast majority of the kinetic studies have been conducted using nmr spectroscopy and experiments at elevated pressures have been vital in determining reaction mechanisms. Following the establishment of the properties and defining parameters, suitable MRI agents can be designed. The criteria for a potential candidate MRI agent are that the most favorable metal species is gadolinium(III) and most of the coordination positions should be occupied by nonexchanging chelating ligands of a character that retains or promotes very rapid water exchange, since the latter condition ensures a desired high rate of proton relaxivity (35,36,120,153–156). The stability constant of the complex must be of sufficiently high magnitude that little free $Gd(aq)^{3+}$ is in solution, as it is toxic. The subject has been summarized in recent authoritative reports (35,36).

Ligand Substitution Reactions. An early example of the decisive power of high pressure kinetics in the conventional time range is the resolution of the mechanism of aquation of pentaammine complexes of Cr(III) and Co(III). The complexes studied, $Cr(NH_3)_5X^{3+}$ and $Co(NH_3)_5X^{3+}$, contain a neutral ligand X that is essential to avoid variable and unquantifiable changes in electrostriction if X is charged. For a series of X, the values of $\Delta V^{\neq}$ were found to be negative for Cr(III) and positive for Co(III) complexes, indicating from the sign and magnitude of the values, I_a and I_d mechanisms, respectively (157). This distinction was not possible from analysis of a variety of ambient pressure kinetics and other information.

Prior to the emergence of the report on the high pressure kinetics of water exchange and confirmation of mechanism, it had been argued from reactions of ligands with the hexaaqua nickel(II) ion, and to a lesser extent with hexaaqua cobalt(II) ions, that the rate of complex formation with these ions would be controlled by the rate and mechanism of water exchange, (158,159). Therefore

the volume of activation for ligand substitution should be positive (160,161).

$$M(H_2O)_6^{2+} + L \equiv M(H_2O)_6^{2+}, L \quad (24)$$

$$M(H_2O)_6^{2+}, L \longrightarrow M(H_2O)_5L^{2+} + H_2O \quad (25)$$

In this scheme, L is a unidentate ligand and M is either Ni or Co. The former step represents the formation of an outer-sphere complex, in which L is in the second coordination sphere of the metal ion. Subsequently, upon departure of a water molecule L enters the first coordination sphere and binds very rapidly. There is a small volume change arising from formation of the outer-sphere complex, but the major contribution is predicted (positive) to arise from the second step since the departing water molecule enlarges the volume of the transition state. If L is a bidentate ligand, then in most cases, the substitution of the first water molecule is the rate-determining and therefore the mechanistic determining step. Positive $\Delta V^{\neq}$ values were obtained for several ligands using laser high pressure T-jump or hpsf, supporting the predicted I_d mechanism. (160–163). Interestingly, the rates of formation of $Mn(bipy)^{2+}$ (where bipy = 2,2′-bipyridine) and $V(SCN)^{+}$ were accelerated by pressure and the mechanism of formation is I_a, consistent with the mechanistic changeover in water exchange in the first row of transition metal 2+ ions (56,164). A further point of interest is the effect of aqua-ion size on mechanism. The hpsf experiments showed that as expected the formation of $Zn(bipy)^{2+}$ [from the $Zn(aq)^{2+}$ ion and bipy] follows an I_d mechanism, but the formation of $Cd(bipy)^{2+}$ proceeds by an I_a mechanism, illustrating the improved access to the entering ligand allowed in the larger $Cd(aq)^{2+}$ ion, one period lower in the Periodic Table (165,166).

These relatively straightforward reactions reflect the history of development of the pressure variable in ligand substitution reactions. Many other reactions studied recently by high pressure methods have more complex chemistry, but are frequently important as models or directly relevant to biological processes. Reactions of complexes of the following metal ions, Fe(III), Co(III), Ru(II), Ru(III), Rh(III), Pd(II), Re(I), and Pt(II) (167) demonstrate the increasing value of the pressure variable in a wide range of mechanistic studies.

There has been a renaissance of interest in the chemistry of nitric oxide, NO, in the past decade. This is a consequence of the finding that NO is involved directly or indirectly in a multitude of biochemical processes and physiological functions (168). Reactions of NO with transition metal centers are important (169,170), and therefore it became incumbent upon investigators to examine the kinetics and mechanisms of these interactions, as initial, understood models for the natural processes. The iron(III) nitroprusside ion, $[Fe(CN)_5NO]^{2-}$ has been widely used pharmacologically for the provision of NO. The iron(II) analogue $[Fe(CN)_5NO]^{3-}$ is also of considerable interest. Formation of this species from pentacyano aqua complex ion, $[Fe(CN)_5H_2O]^{3-}$, and NO has been characterized kinetically yielding a markedly positive $\Delta V^{\neq}$ ($= +17.4$ cm^3/mol). This is clearly indicative of a dissociative mechanism (171). The product species is not particularly stable, but under appropriate conditions the reverse reaction could be studied, that is the loss of NO, also at elevated pressures and a positive value of $\Delta V^{\neq}$ was obtained ($+7.1$ cm^3/mol). Again this led to the conclusion of a

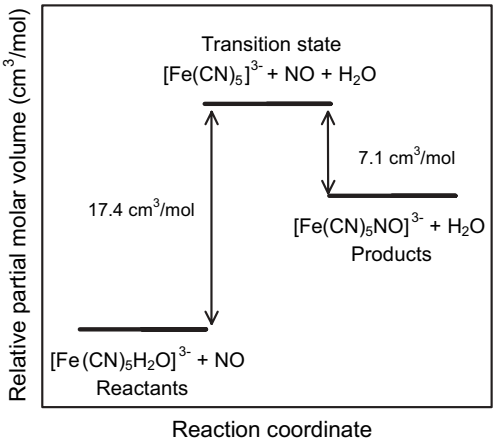


Fig. 22. Volume profile for the overall reaction $[Fe^{II}(CN)_5H_2O]^{3-} + NO \,?\, [Fe(CN)_5NO]^{3-} + H_2O$.

dissociative mechanism and a reaction volume of $+10.3$ cm^3/mol. A volume profile could be constructed (Fig. 22). It is important to emphasize that solutions containing NO must be very carefully controlled to prevent adventitious oxidizing impurities from yielding spurious results.

Redox Reactions. Kinetic studies of redox reactions of organic compounds in the presence of metal complexes can assist in understanding electron transfer mechanisms in organic molecules. Compounds termed N-substituted phenothiazines are important in biochemistry and pharmacology. The kinetics and equilibrium properties of reactions of a series of phenothiazines with hexaaquairon(III), in which the iron species is reduced to hexaaquairon(II) and the phenothiazine is oxidized to a cationic form, had been established (172). However the details of the mechanism became available following a high pressure kinetics study. The general reaction is (eq. 26).

$$R - (\text{phenothiazine}) + Fe(aq)^{3+} \equiv R - (\text{phenothiazine})^{+} + Fe(aq)^{2+} \qquad (26)$$

In the particular example when $R = CH_2CH_2CH_2N(CH_3)_2$, promazine, a hpsf study yielded $\Delta V^{\neq}$ values of -6.3 and -12.5 cm^3/mol for the forward and reverse reactions, respectively (173). The derived reaction volume of $+6.2$ cm^3/mol has been confirmed by calculation from the pressure dependence of the value of the equilibrium constant obtained from the variation of the uv/vis spectra of an equilibrium mixture as a function of pressure (173). The transition state species is thus compact; it was suggested that this could in part arise as a consequence of volume reduction upon formation of a precursor contact pair, with this factor being greater for the reverse than for the forward reaction. Markedly negative entropies of activation (172) indicated a significant electrostriction increase for the back reaction. Intrinsic changes regarding bond length changes in promazine during reaction were thought to be very small or negligible, and therefore solvation changes are the dominant contributors to volume

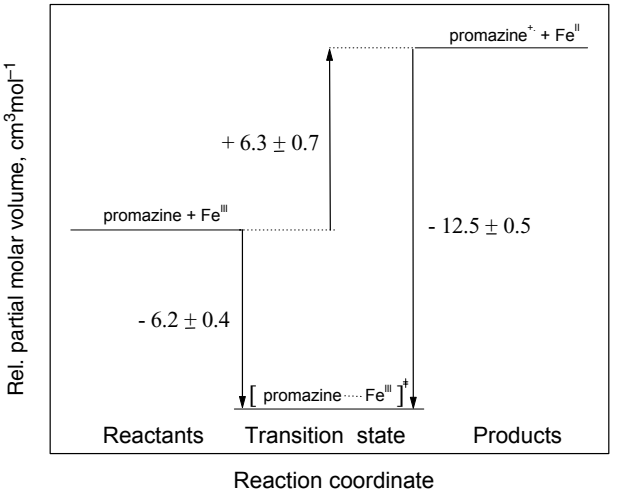
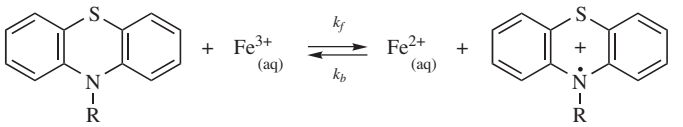


Fig. 23. Volume profile for the reversible redox reaction outlined in eq. 22.

changes for both directions of the reaction (see eq. 27 and Fig. 23).

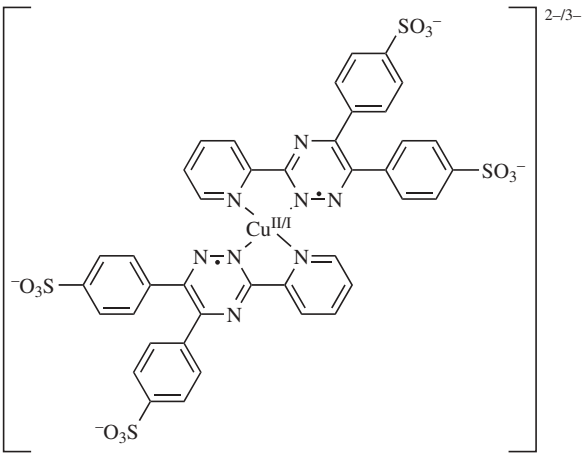


R = $CH_2CH_2CH_2N(CH_3)_2$ (27)

It has been known for some time that certain alkali metal ions and some other uni-charged cations catalyze the electron-transfer reaction between two redox partners that are both anionic. This occurs for both self-exchange reactions and for nonsymmetrical reactions (174–176). Only recently has a reasonably complete understanding been developed following the application of high pressure kinetics studies (177). Electron exchange between ferricyanide $[Fe(CN)_6]^{3-}$ and ferrocyanide $[Fe(CN)_6]^{4-}$ was studied principally by ^{13}C nmr spectroscopy. The counterion was K^+, an ion that catalyzes the reaction. Volumes of activation were determined for both the catalyzed and uncatalyzed pathways, the latter being conducted by sequestering the K^+ ions with agents such as a cryptand or a crown ether. It was concluded that the catalytic pathway was not only facilitated by the positive ion reducing the unfavorable interaction between the two negative species, but also involved the partial deaquation of the K^+ ion upon reaching the transition state. A full analysis of these results and the parallel results for other cations and considerations of ion-pairing have been presented (177).

Bioinorganic Reactions. The bioinorganic reaction focused upon is a redox reaction. Redox reactions can proceed by one of two distinct mechanisms, the inner- or the outer-sphere mechanisms. Simply stated the latter involves only electron transfer, whereas in the former a substitution reaction occurs

prior to electron transfer. Distinguishing between the two mechanisms from experimental results is not always straightforward. The subject has been thoroughly discussed (178). Fortunately, it was determined that for the reaction between an anionic complex of copper (I/II) and cytochrome *c*, the mechanism could be adjudged confidently to be an outer-sphere example (179). Cytochrome *c* (cyt *c*) is a hemoprotein in which the prosthetic group is a derivative of iron protoporphyrin IX. Cytochrome *c* is an efficient biological electron transporter, in which the iron fluctuates between the ferrous and ferric states. In many studies, including this one, the cytochrome *c* is derived from horse heart. The $Cu^{I/II}$ complex contains two ferrozine (fer) ligands (ferrozine is the bidentate ligand, bis(5,6-bis(4-sulfonatophenyl)-3-(2-pyridyl)-1,2,4-triazine). The copper(II) complex possesses square pyramidal geometry with the two ferrozine ligands in the equatorial plane, and the fifth, axial position is occupied by a water molecule, in aqueous solution, whereas by contrast the copper(I) complex has a tetrahedral arrangement of the two ferrozine ligands as judged from the evidence of uv–vis spectra and electrochemical properties. Each ferrozine possesses two negative charges, hence the charge on the copper(II) complex is 2– and is 3– on the copper(I) complex species (see structure **1**).



(**1**)

The reversible redox reaction that occurs between these partners can be written

$$\text{cyt } c^{II} + \text{Cu(II)(fer)}_2(H_2O) \equiv \text{cyt } c^{III} + \text{Cu(I)(fer)}_2 + H_2O \qquad (28)$$

It should be recognized that the actual charge on the cytochrome species is not 2 or 3, rather the species are shown conventionally in this manner, the II and III referring to the reduced and oxidized forms, respectively. The kinetics of the forward reaction, in the neutral pH region, conducted under pseudo first-order conditions [excess Cu(II) complex concentration], exhibited saturation kinetics, that is at high Cu(II) complex concentrations the observed rate constants assumed constant values. This is indicative of formation of a precursor complex

[sometimes termed outer-sphere (OS)]. The value of the equilibrium constant (K_{OS}) could be extracted from the kinetic results and was found to be 7.7×10^3/mol dm^{-3}. In addition, the electron-transfer (ET) rate constant, k_{ET}, was calculated to be 6.2 s^{-1}, and both of these parameters were obtained at 15°C. From a temperature dependence study of the kinetics of interaction between the reacting partners, values of ΔH_{OS} and ΔS_{OS} of -4 ± 8 kJ/mol and $+91 \pm 28$ J/mol K^{-1}, respectively, were obtained. These were interpreted as a small nonspecific interaction, but a significant entropy driven component in formation of the OS complex. The values of 85 ± 4 kJ/mol and -61 ± 13 J/mol K^{-1} for $\Delta H^{\neq}$ and $\Delta S^{\neq}$, respectively, for the actual electron-transfer step indicate a pronounced enthalpic requirement, but a structurally constrained transition state. From the kinetics results obtained at various pressures, ΔV_{OS} of $+0.8 \pm 1.3$ and $\Delta V^{\neq}_{ET}$ of $+8.0 \pm 0.7$ cm^3/mol were calculated. That the transition state is "early" along the overall reaction progress profile could be inferred from the fact that the overall reaction volume could be estimated to be $+30$ cm^3/mol (markedly $> +8$ cm^3/mol). A significant fraction of the reaction volume arises from departure of a coordinated water molecule (eq. 28) to the bulk solvent upon the reduction of the Cu(II) complex with coordination geometry and coordination number change from 5 to 4. From these arguments it was concluded that water loss occurs following transition state formation. Free energy and volume changes for the reaction are assembled in a combination three dimensional figure (Fig. 24).

Another recent bioinorganic electron transfer reaction that also featured cytochrome *c* is represented in eq. 29, where edta = ethylenediaminetetraacetate.

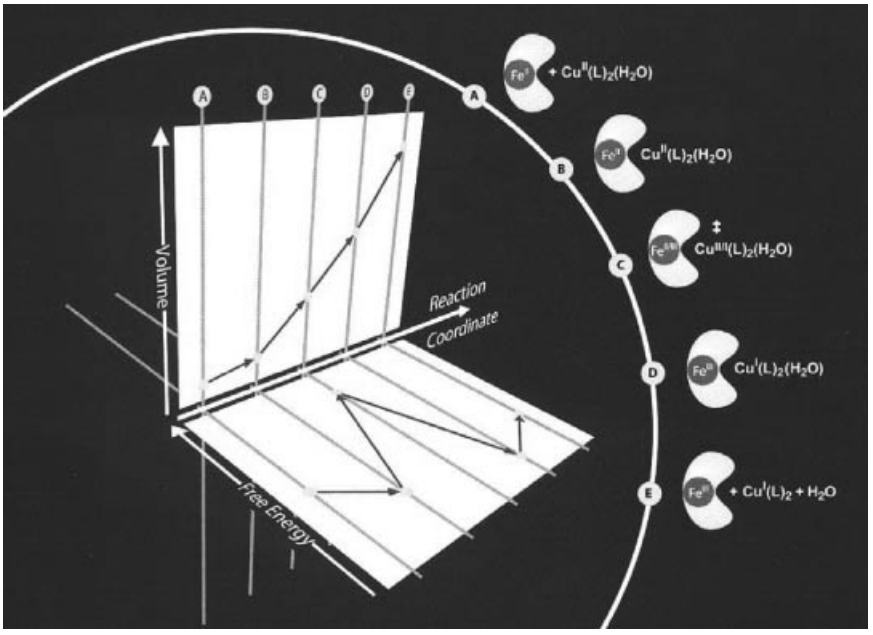


Fig. 24. Combined free energy–volume profile (3D) for the reversible redox reaction between cytochrome *c* and copper ferrozine (see eq. 28). Cyt $c^{II} + [Cu^{II} L_2]^{2-}$? Cyt $c^{III} + [Cu^{I} L_2]^{3-}$.

$$[Fe^{II}(edta)(H_2O)]^{2-} + \text{cyt } c^{III} \equiv [Fe^{III}(edta)(H_2O)]^{-} + \text{cyt } c^{II} \quad (29)$$

High pressure kinetics measurements and cyclic voltammetry measurements as a function of pressure yielded results that led to assembly of a volume profile (180), and also cast doubt on the previously proposed OS mechanism (181) as several of the findings were not inconsistent with an inner-sphere mechanism.

High pressure studies involving binding of small molecules to proteins, invariably metalloproteins have a lengthy history (182-184). Considerable additional insight into the binding mechanism and nature of the binding site has been a consequence of these studies. Where less progress has been possible is in providing additional mechanistic delineation in metalloenzyme catalyzed reactions. When a reaction is multistep, as is the case in enzyme catalyzed reactions, separation of observed effects of pressure on steady state or even transient state kinetics parameters into the contributions of the actual kinetic and equilibrium parameters of the enzyme catalyzed pathway is likely to be less than unequivocal. That is, a complete volume profile from the initial-to-final state in an enzyme catalyzed reaction is likely to be realized only in certain circumstances despite the range of high technology available. However, several investigations have been conducted, see, eg, Ref. 185. The first complete volume profile was reported for the zinc-containing enzyme carbonic anhydrase (186). Review of that report and some more recent high pressure enzyme kinetics studies [a cytochrome P450 and nitric oxide synthase (187), butyrylcholinesterase (188)] can point to the complexity of undertaking such investigations.

5.5. Other Applications. In addition to the colloid systems and materials processing systems noted above, it is worthwhile citing the use of high pressure in reactions where gases such as CO and syngas mixtures in solvents are used in hydroformylation reactions catalyzed by rhodium carbonyl clusters. Gas pressures up to 100 MPa have been used and both ^{13}C and hpnmr spectroscopy, and high pressure ir spectroscopy have been applied to identify the form of the cluster that is the actual catalytic agent (189). The specific technical aspects with respect to the hpnmr in this context have been presented (190,191). Another reaction involving gases under pressure in solvents is the copolymerization of styrene with carbon monoxide catalyzed by a palladium(II) complex, studied by hpnmr spectroscopy (192). These reactions are examples from organometallic chemistry.

6. Theoretical Studies

Efforts to calculate changes in reacting systems including calculating the volume of activation have been devoted mostly to water exchange reactions and electron self-exchange reactions, since these can be thought of as the simplest types. Various degrees of success have been achieved and satisfactory agreement with pressure-derived experimental parameters has been recorded in some cases, eg, as discussed above for water exchange on first-row transition metal ions in oxidation state two (124,125,152). More recent calculations have been on water exchange for the hexaaqua ions of rhodium(II) and iridium(III) (193).

The hypotheses used in setting up the calculations and relevant computational methods are presented in various publications (35,36,193).

7. Concluding Comments

High pressure is just one of several nontraditional methods of studying chemical and physical processes that have assumed increasing importance in the past few decades. High pressures and often high temperatures are needed in studying reactions and physical processes in supercritical fluids, although the pressure magnitudes are often less than in high pressure technology itself. Momentum is increasing in supercritical technology. In addition to facilitating selected chemical reactions, supercritical technology (194–196) is being widely explored in a range of environmental applications (197) and in the food industries. But just as the situation is with high pressure methods, amortizing costs is a factor in consideration before embarking on incorporating the technology into research and development or production. Other "extreme" conditions featuring in synthetic and environmental fields are ultrasound (198,199) and microwave energy (200). This article has examined the advantages of using high pressure in a variety of physical, physicochemical, chemical and biochemical applications, and has drawn attention to the commercial, less specialized and more sophisticated techniques that have been applied in these systems. Naturally, the presentation is not an exhaustive all-inclusive list of every piece of high pressure apparatus or of every application. It is clear that other than for non-*in situ* applications, assembling, and employing a "home-built" high pressure apparatus is not a trivial task and the value of doing so must be incontrovertibly, egregiously advantageous for processing, synthesis, or mechanistic purposes before proceeding. However, in many cases the efforts are worthwhile and the benefits rewarding and inestimable.

8. Acknowledgments

It is a pleasure to acknowledge many colleagues and members of the academic community who together with the invaluable, expert technical experience of their supporting staff have demonstrated the dedication that has enabled them to produce various generations and types of high pressure apparatus reported in this contribution. In addition, the authors are grateful to representatives of companies and organizations producing high pressure or related equipment who have responded graciously to requests for information on their products and permission to reproduce diagrams. The authors also wish to acknowledge their colleagues and collaborators who have served as coauthors on research publications, or who have made other written contributions in cited references.

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COLIN D. HUBBARD
RUDI VAN ELDIK
University of Erlangen-Nürnberg