TECHNIQUES OF CHEMISTRY

VOLUME VI

INVESTIGATION OF RATES AND MECHANISMS OF REACTIONS

Third Edition

Edited by

GORDON G. HAMMES

PART II

INVESTIGATION OF ELEMENTARY REACTION STEPS IN SOLUTION AND VERY FAST REACTIONS

TECHNIQUES OF CHEMISTRY

ARNOLD WEISSBERGER, *Editor*

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INTRODUCTION TO THE SERIES

Techniques of Chemistry is the successor to the Technique of Organic Chemistry Series and its companion—Technique of Inorganic Chemistry. Because many of the methods are employed in all branches of chemical science, the division into techniques for organic and inorganic chemistry has become increasingly artificial. Accordingly, the new series reflects the wider application of techniques, and the component volumes for the most part provide complete treatments of the methods covered. Volumes in which limited areas of application are discussed can easily be recognized by their titles.

Like its predecessors, the series is devoted to a comprehensive presentation of the respective techniques. The authors give the theoretical background for an understanding of the various methods and operations and describe the techniques and tools, their modifications, their merits and limitations, and their handling. It is hoped that the series will contribute to a better understanding and a more rational and effective application of the respective techniques.

Authors and editors hope that readers will find the volumes in this series useful and will communicate to them any criticisms and suggestions for improvements.

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ARNOLD WEISSBERGER

PREFACE

Ten years have passed since the previous edition of this volume. During this time fast reaction techniques have become almost routine tools in the investigation of reaction mechanisms in solution. Many advances in technology have been made, and the results of numerous experimental studies have been published. Although Part II of the new edition is confined to a discussion of fast reaction techniques, it has been difficult to keep the volume to a manageable size. However, an attempt has been made to include all of the major techniques currently in use.

As in any multiauthored volume, the presentation varies from chapter to chapter. In general, the emphasis is on the principles of the experimental methods, together with a description of the equipment. A comprehensive review of experimental results is not presented, although illustrative examples are discussed. All of the chapters are new and bear little resemblance to those in the previous edition, even though some of the titles are unchanged.

It is hoped that this volume will provide both the novice and the experienced investigator with the information needed for the application of fast reaction techniques to new problems and that it will encourage future development of experimental techniques.

I am greatly indebted to the authors and to the staff of the publishers for their cooperation in producing a timely volume. I especially wish to acknowledge the encouragement and advice of Dr. A. Weissberger, the editor of this series.

March 1973
Ithaca, New York

GORDON G. HAMMES

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Chapter 1

INTRODUCTION

Gordon G. Hammes

During the past decade, the use of fast reaction techniques for studying chemical reaction mechanisms in liquid solutions has become commonplace. No chemical reaction today is labeled "immeasurably fast," since current experimental methodology extends to reactions with half-times approaching molecular vibrations and rotations. Much of the impetus for the remarkable developments that have occurred in chemical kinetics is due to vastly improved instrumentation. Modern electronics provides an extraordinary range of sensitivity and time resolution. The use of computers for signal averaging, for the processing of large amounts of data, and for control of experiments has given further versatility and sensitivity.

The improvement in experimental capabilities has been paralleled by an increased sophistication in mechanistic chemistry. The specification of a chemical mechanism now requires a knowledge of all the individual or elementary steps in the overall chemical reaction and an understanding of each elementary step on a molecular basis. This molecular concept of mechanism has been pursued in both gas and liquid phases by detailed studies of the individual elementary reactions. In liquid solutions, the studies have involved electron and proton transfer, ligand substitutions, hydrogen bonding, solvation, and many other reactions. More complex processes such as enzyme catalysis and regulation and acid–base catalysis of organic reactions have been studied in terms of their elementary steps.

Only with fast reaction techniques can elementary processes be fully resolved, and this volume is concerned with exactly how this can be done. An introductory survey of the methods now available is given in terms of their principles and limitations. With any fast reaction technique, two problems arise: first, the reaction has to be initiated, and second, the time course of the reaction must be observed.

The most obvious method of initiating a chemical reaction is to mix the reactants together. Indeed, *rapid mixing methods* are some of the most useful and important techniques available and have been utilized for many years. With modern technology, the initiation of reactions by mixing in milliseconds is almost routine, and tenths of milliseconds readily can be achieved. The direct method of following the course of the reaction is

to stop the flow and observe the concentration changes directly as equilibrium is achieved. A large variety of physical methods is used to follow concentration changes, although optical methods are probably the most widely used because of their great specificity, sensitivity, and time resolution. An alternative to the direct method is to set up a constant flow of material after mixing and to observe the concentrations at various distances from the mixing chamber. In this case, the competition between the flow transport and the chemical change establishes a stationary distribution of reaction products in the observation tube; thus the distance from the mixing chamber is directly proportional to the time after initiation.

Mixing is the most obvious means of perturbing a system and initiating the chemical reaction. However, rapid mixing methods have definite time limitations. In the group of direct methods called *relaxation methods*, an equilibrium or steady state is rapidly perturbed by changing an external parameter, such as pressure, temperature, or electrical field strength. Measurement of the time course of the reequilibration process provides a measure of the kinetics of the chemical reactions occurring in the system. Since mixing is not required for these processes, the time resolution is determined only by how fast the perturbation can be applied. In fact, the relaxation methods cover a time range from greater than one second to less than 10^{-10} sec.

In addition to the broad time range covered by these methods, the kinetic analysis of complex systems is considerably simplified because the external perturbations are always very small. This has the important consequence of linearizing all the rate equations needed to describe any reaction mechanism, and, therefore, the equilibration process is described by a discrete set of relaxation times readily related to the mechanism. The step perturbation techniques include the *temperature-jump method*, the *pressure-jump method*, and *electrical pulses*. The detection methods employed must be quite specific and sensitive: absorption and fluorescence spectrophotometry, polarimetry, and conductance measurements all are utilized. Periodic perturbations also are useful. In these cases, chemical relaxation processes cause a phase shift between internal and external parameters, and measurement of this shift or of the accompanying energy loss furnishes a measure of reaction dynamics. *Acoustical absorption* and *dielectric loss* are the most important methods of this type.

The absorption of energy by a system from radiation, light quanta, or accelerated particles, is the basis of both *flash photolysis* and *pulse radiolysis*. With these techniques, the rapid absorption of energy serves to produce unstable molecules (excited states or free radicals) generally very far from their equilibrium states. The fate of these molecules as the system proceeds to equilibrium can be studied directly, or the unstable molecules can be used

to initiate reactions of interest. In most cases, linearization of the rate equations is not possible because deviations from equilibrium are quite large. The rapid rise in laser technology has given considerable impetus to this area, both in generating rapid perturbations and in following the time course of the reactions. The time resolution of these methods is typically in the microsecond range, but nanosecond and even picosecond resolution has been achieved.

The combination of rapid mixing methods with relaxation or radiation pulse techniques permits the study of reactions of very reactive species, for example, unstable intermediates in chemical reactions. The perturbation of steady states of reaction intermediates has expanded considerably the range of reactions that can be studied.

A constant flux of radiation can be used to produce reactants that disappear through chemical reactions. A stationary concentration of reactants is achieved when the production and disappearance are balanced. This forms the basis of *photostationary methods.* By measuring quantum yields and the concentrations of photoproducts, relative rate constants can be measured. If one of the rate constants can be measured or estimated, absolute values of rate constants can be determined. This is also possible if the stationary state concentration of the reactant can be measured, either directly or indirectly. Since this is a competitive method, not involving time resolution directly, rate constants for very fast reactions can be measured even though the experiment is carried out over long time periods. If chemical reactions alter the fluorescence properties of an electronically excited molecule, the competition between fluorescence and the chemical reaction is reflected in the fluorescence spectrum or fluorescence lifetime. The measurement of *fluorescent transformations* is only useful when the chemical processes have characteristic time constants similar to fluorescence lifetimes, the accessible time range being from about 10^{-6} to 10^{-10} sec.

Electrochemical methods represent another group of techniques for the measurement of reaction rates depending on the competition of the rate of a chemical reaction with a physical process. The physical process is the transport of electroactive species to the electrode, and the rate of any chemical reaction coupled to the electroactive species is potentially measurable. In order to utilize these methods, both the kinetic and mass transport equations must be solved for the method of interest. In principle, this is no problem; in practice, it means that the analysis of the experimental results must be done very carefully and critically. Properly used, electrochemical methods are a valuable addition to fast reaction techniques: a very wide time range is covered (from >1 sec to 10^{-8} sec), and a large variety of reactions can be studied.

Nuclear magnetic resonance and *electron paramagnetic resonance* are methods that monitor chemical reactions through the nuclear and electron spin states of molecules in a magnetic field. These methods have a unique advantage over direct metods in that virtual reactions not representing an overall macroscopic reaction can be studied, for example, proton or electron exchange between identical chemical species. However, the analysis is considerably more difficult than for direct methods, and generally it requires the use of a computer. The line widths of the resonance lines are determined by the lifetimes of different spin states, which in turn are related to the lifetimes of chemical states. For the simplest possible case, the frequency separation of resonance absorptions determines the time limits of the chemical processes that can be studied. For nuclear magnetic resonance, this typically involves times of 1 to 10^{-4} sec. With modern pulse techniques and paramagnetic probes, the time range can be expanded into the microsecond region. Since electrons have much higher resonance frequencies than nuclei, and correspondingly larger frequency separations between typical resonance lines, considerably shorter time constants can be detected, the typical range being 10^{-5} to 10^{-10} sec.

In conclusion, the various fast reaction methods and their approximate time ranges of applicability are presented in Fig. 1.1. At this point in time, the kinetics of almost any chemical transformation can be studied: it is only a matter of obtaining sufficient concentrations of the species of interest and choosing the proper experimental method.

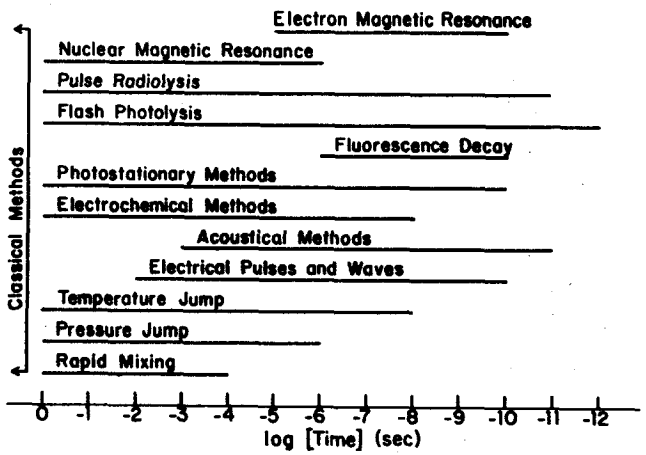

Fig. 1.1 Summary of fast reaction techniques and their time range of applicability.

Chapter II

RAPID FLOW METHODS

Britton Chance

1 INTRODUCTION

A determination of chemical structures and reaction mechanisms is basic to the general goal of biochemistry and biophysics—the delineation of biochemical and biophysical processes at the molecular level [1]. An essential aspect of this approach is the understanding of biocatalysis by step-by-step analyses of the total reaction mechanism.

The study of the overall reactions of enzymes and their substrates in the steady state provides indirect information on the intermediate compounds involved [2], but few data on the reaction sequence. For this reason, significant advances in the understanding of the mechanism of biochemical reactions have emerged from direct studies of enzyme intermediates in which the enzymes are used in substrate amounts and their intermediates, which may be short-lived, are identified by physical methods for rapidly initiating or perturbing the reaction steps. Rapid mixing of two reactants affords the most general and flexible perturbation for the kinetic study of enzyme reactions.

The entire field of study of rapid biochemical reactions owes a great debt

to Hartridge and Roughton, who pioneered the investigation of the reaction of hemoglobin with oxygen [3]. These experiments, together with Millikan's subsequent studies on the reaction of myoglobin with oxygen [4], have served as models for the investigation of fast biochemical reactions involving ligand binding. The reaction of catalase or peroxidase with H_2O_2 later provided a similar model for enzymatic reactions involving labile enzyme–substrate intermediates [5, 6]. In addition to flash photolysis [7] and relaxation spectroscopy [8], rapid flow techniques have been developed for perturbing biochemical systems, and the three methods have become standard approaches to the elucidation of enzyme reaction mechanisms. Associated with these methods has been the development of appropriately fast physical techniques for measuring the results. At the same time, advances in the structural aspects of enzyme chemistry by X-ray crystallography [1], nuclear magnetic resonance [9], or spectroscopic probe [10] methods have afforded structural interpretations of kinetic phenomena.

Each one of the three rapid reaction techniques has its optimal time and concentration range [11]. The flow methods described here cover the range from 0·5 msec to 10 sec. In this time range, one of the three forms of the flow method (continuous flow) affords a unique "time clamp" during which intermediates are stabilized at time-invariant concentrations, and precise physical data can be obtained.

Principles and Types of Flow Apparatuses

The flow method affords a continuous supply of unreacted materials to the point of their mixing. Thus we have a reaction that is continuously in progress along an observation tube; the reaction time is stretched out spatially according to the flow velocity. The faster the flow velocity, the more the reaction "space" is lengthened:

$$t_m = \frac{S}{U_0}, \tag{2.1}$$

where S is the distance from mixing to observation and U_0 is the flow velocity.

Three basic categories of flow apparatus are identified by the velocity of the flow and the time of observation: (*a*) constant or continuous, (*b*) accelerated, (*c*) stopped. In the constant flow mode, the flow stream is maintained at constant velocity and the extent of reaction is measured at various distances from the mixing chamber, usually in a sequence of discharges. In the accelerated flow mode, the distance is constant and the flow rate is varied during a single discharge. In the stopped flow mode, used for "slower" reactions, the reaction of the mixed fluids has not started when they reach

the observation chamber and are stopped there. The methods are convenient not only for reactions that come to a readily determinable equilibrium, as in the case of relaxation techniques, but also for irreversible reactions and for those in which the presence of reaction products is undesirable. The fundamental principles of these types of flow apparatuses have been described in detail in previous editions [12, 13].

Two additional subcategories of flow methods that have developed rapidly are emphasized here, together with some applications to biochemical problems. The regenerative flow apparatus [13–15] provides for the recirculation of the reaction products into the mixer for the further injection of substrate; this mode is especially useful for the study of essentially irreversible reactions in which cylic responses, such as the formation and decomposition of an enzyme–substrate compound or the oxidation and reduction of electron carriers, are observed [15, 16]. The term "pulsed flow" apparatus [17] has been employed for those forms of the regenerative flow apparatus in which the concentration ratio of the injected reactants is large and the duration of the discharge is short; however, the term is not used extensively here.

Second, in a general class of apparatus that employs two mixers, two perturbations of the mixed reactants are possible [18–25]. Although we prefer and use here the term "multiple mixing," this method is often referred to as the "quenched flow" mode, since the purpose of the second mixer may be to stop the reaction rather than to perturb it further. Such apparatuses are often employed for reactions that show no change of physical parameters. The reaction is "quenched" in a time shorter than the reaction time, generally by the addition of a chemical reagent, although the apparatus discussed below [24] employs cooling for electron paramagnetic resonance applications. The reaction mixture is then subjected to appropriate chemical analysis to determine the extent of reaction.

Historical Development

Although the continuous flow method might be thought to be as old as the water clock, the hourglass, and the chip log, it appears that Rutherford in 1897 was the first to apply it to the measurement of reactions occurring in fractions of a second [26]. Probably having observed visually that the emission from ionized gases did not cease at the moment of stopping the irradiation, he seems to have been led quite naturally to "blowing air of a known velocity along a tube" and testing the conductivity "at different distances from the action of the rays" [26]. Employing various gas pressures to vary the flow velocity, Rutherford's experiments covered the time range from 0.13 to 2.4 sec. The particular principle of this apparatus was used by Hartridge and Roughton for their first kinetic measurements on the