

ESC4

Advanced kinetics approaches to  
unravel protein structure and function

# Experimental methods to address time resolved reaction kinetics

*Prof. Stefano Gianni*

# Experimental methods to address chemical kinetics

- Mixing techniques

- Relaxation techniques

## *A Method of Measuring the Velocity of Very Rapid Chemical Reactions.*

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(From the Physiological Laboratory, Cambridge.)

(Communicated by Prof. J. N. Langley, F.R.S.—Received June 26, 1923.)

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### § 1.—*General Principles and the Two Essential Conditions.*

In devising methods for determining the velocity of any chemical reaction there are two experimental problems which invariably arise: (1) To arrange that the chemical system under investigation be made initially unstable in a period of time that is negligibly short in comparison with that taken by the chemical reaction. (2) To record from time to time the stages reached by the system (during its passage from the initial unstable state to the final stable condition wherein the several reacting substances are in chemical equilibrium) by means of methods which take a negligibly short time in comparison with that taken by the chemical reaction.

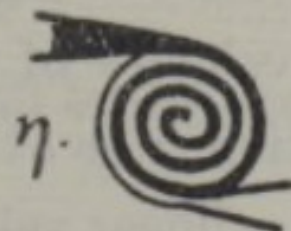
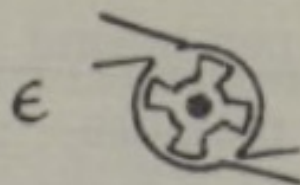
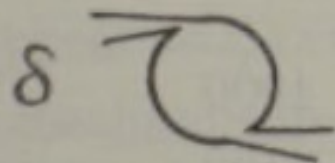
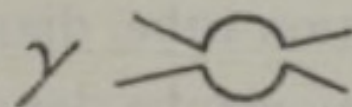
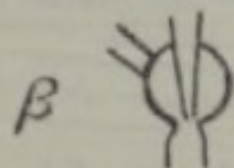
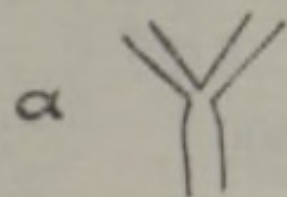
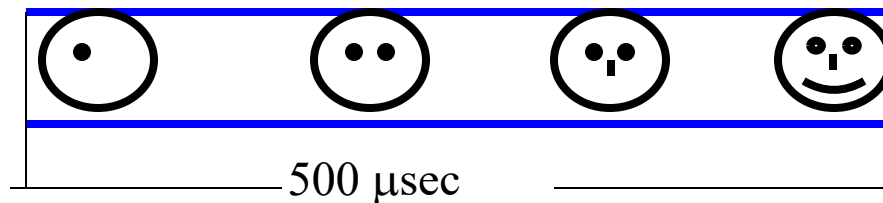
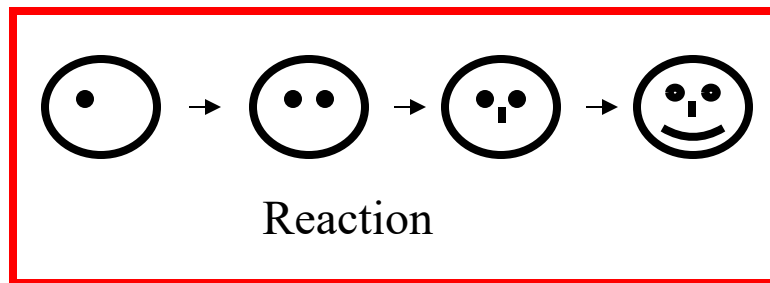
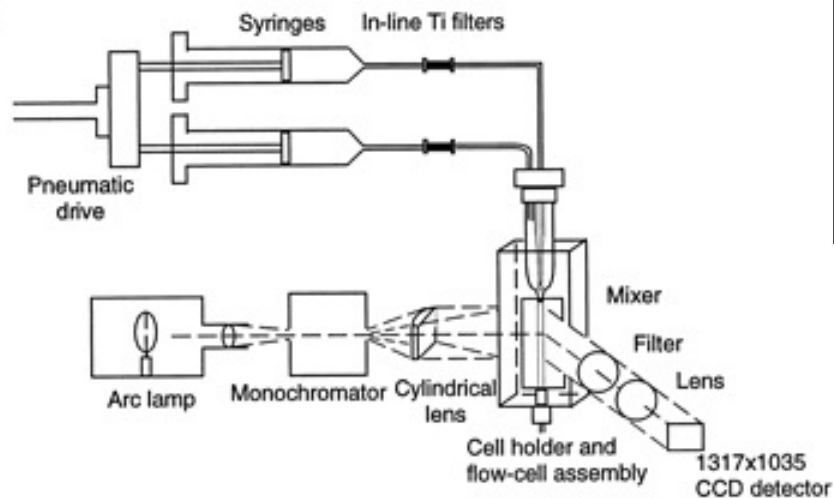


FIG. 1.

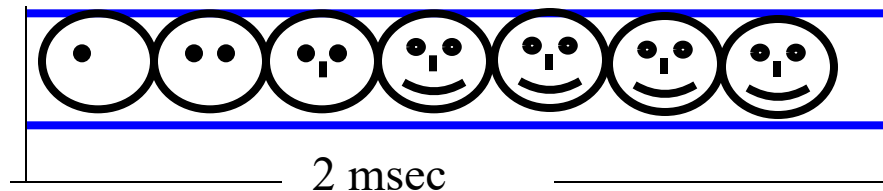
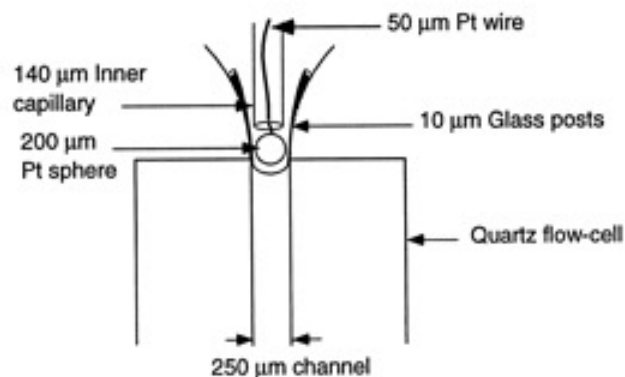
# The (home made) Capillary Continuous flow (40 $\mu\text{sec}$ )

A.



High flow rate

B.



Low flow rate

## Physical Instruments for the Biologist

Detlev W. Bronk  
Associate Editor in Charge of this Section  
Johns Hopkins University, Baltimore, Maryland

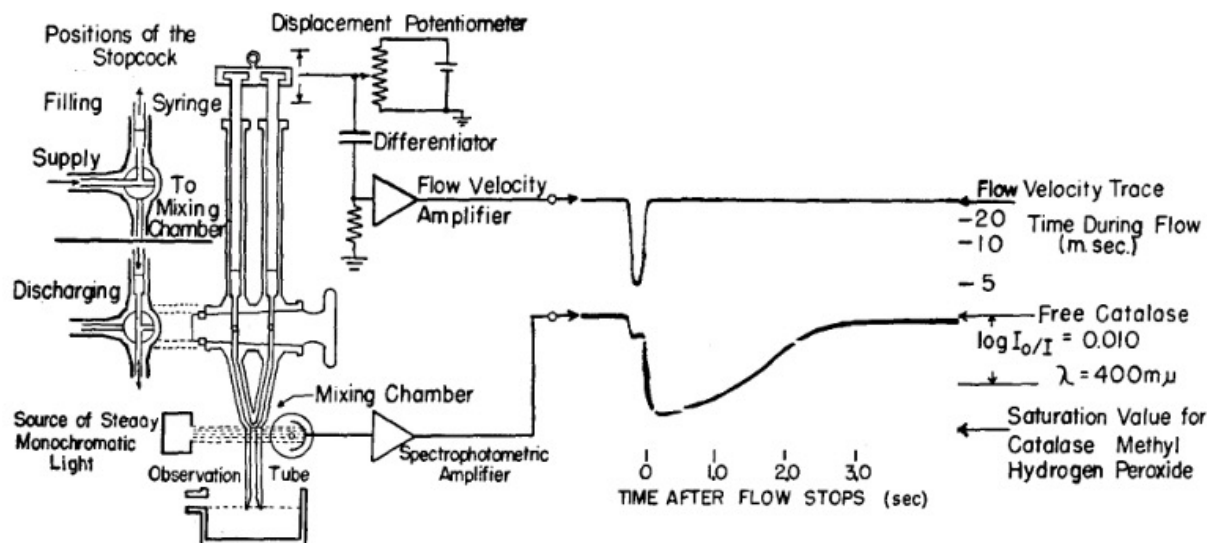
### Rapid and Sensitive Spectrophotometry. I. The Accelerated and Stopped-Flow Methods for the Measurement of the Reaction Kinetics and Spectra of Unstable Compounds in the Visible Region of the Spectrum

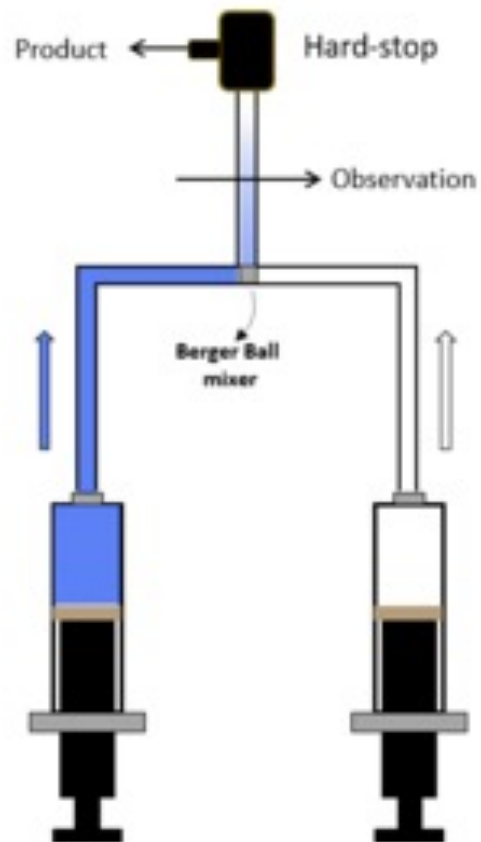
BRITTON CHANCE\*  
*Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania*  
(Received March 6, 1951)

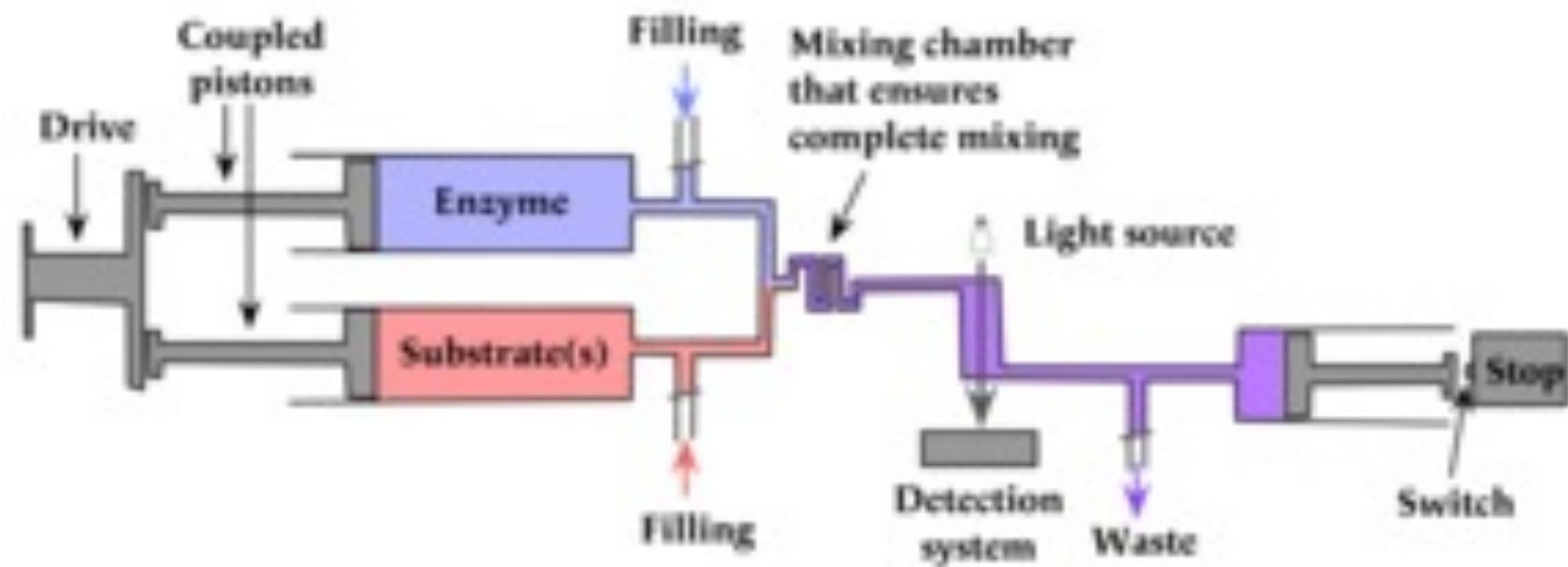
The present form of the accelerated and stopped flow methods for the measurement of the reaction kinetics and spectra of intermediate compounds in biochemical reactions is described. This apparatus gives satisfactory records with only a few tenths of a cubic centimeter of  $2 \times 10^{-3} M$  iron enzyme solution and covers a time range from a few milliseconds to several minutes. The wavelength range is 370 to 600  $m\mu$ , and the spectral interval is 7.5  $m\mu$ . The error in optical density measurement is about  $1 \times 10^{-6}$  corresponding to a signal-to-noise ratio of  $5 \times 10^4$  for a response time of 1 sec over the wavelength range of 380–580  $m\mu$ . The present performance and range of usefulness considerably exceed that reported previously.

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BRITTON CHANCE

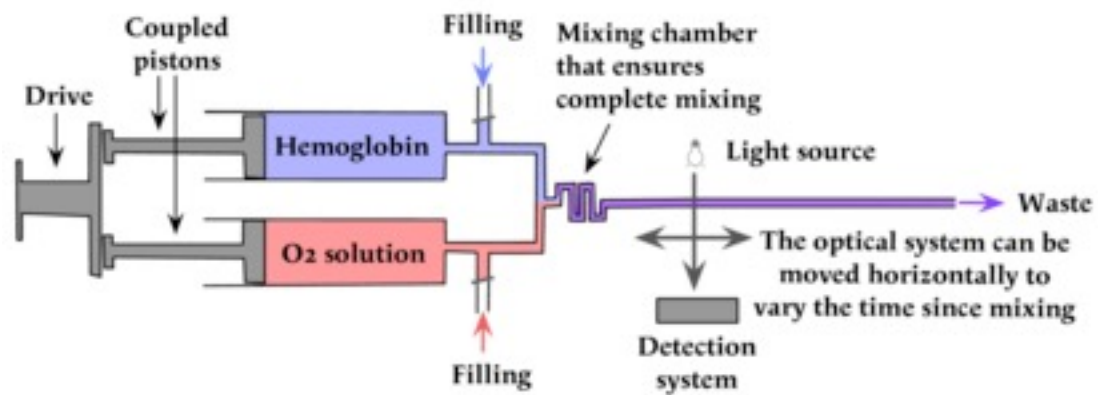






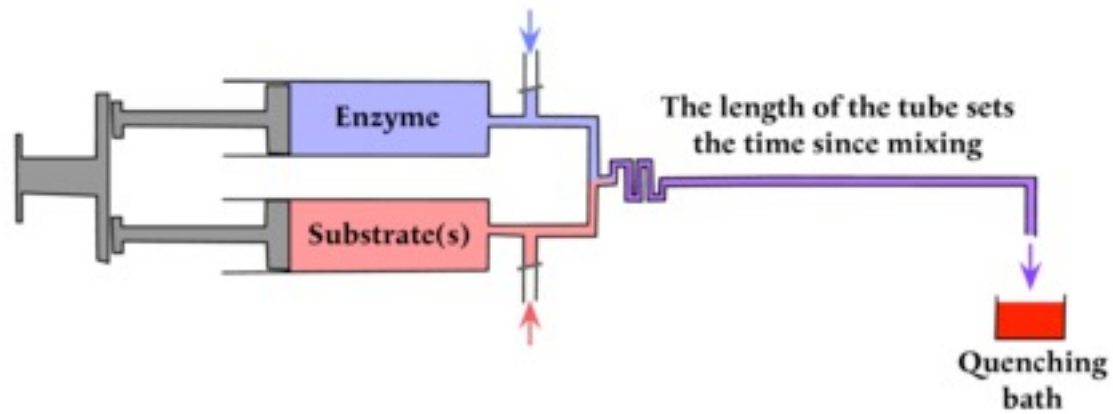


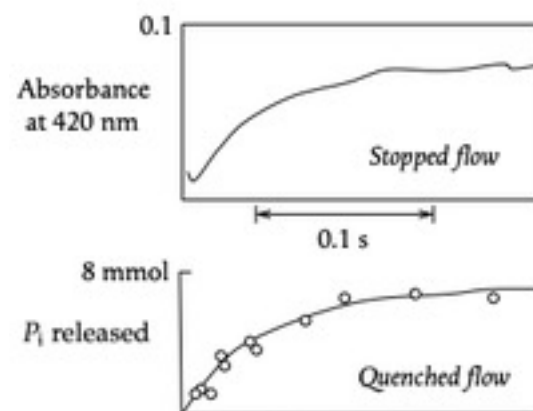




## Quench flow principle

*Freeze the reaction at a given time point*





## ELEMENTARY STEPS IN ENZYME REACTIONS (as Studied by Relaxation Spectrometry)

By MANFRED EIGEN, *Göttingen, Germany* and GORDON G. HAMMES, *Cambridge, Massachusetts*



Eigen Manfred a Göttingen nel 1996



Premio Nobel per la chimica 1967

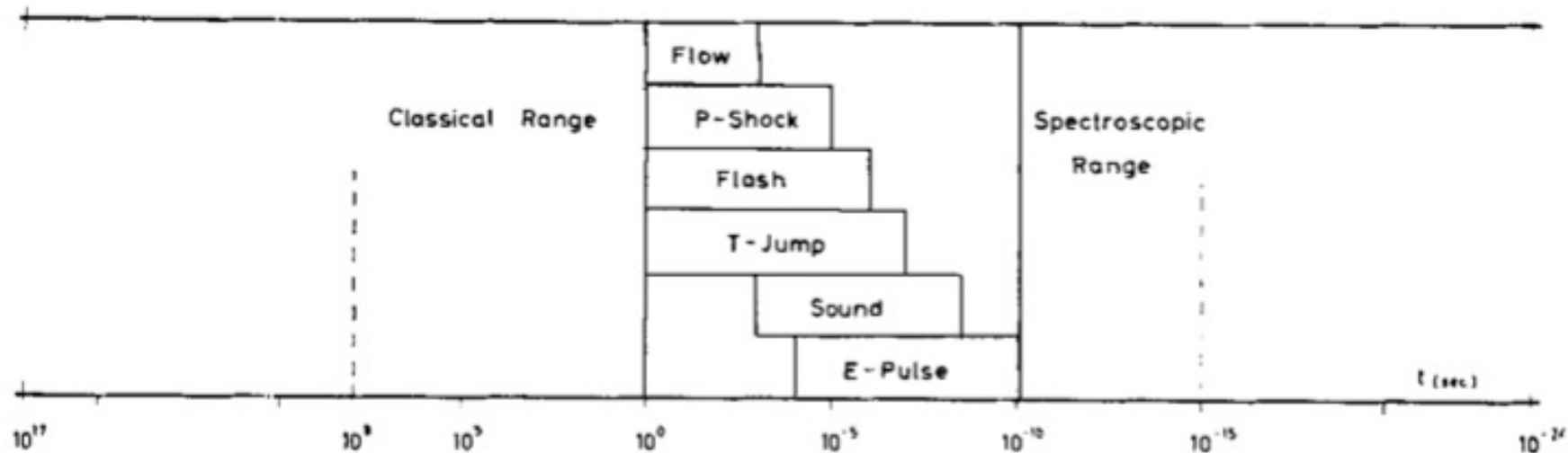


Fig. 1. Methods for the study of rapid reactions in solution and their time ranges of application.

Figure 11A.3 The temperature jump method

## Temperature Jump

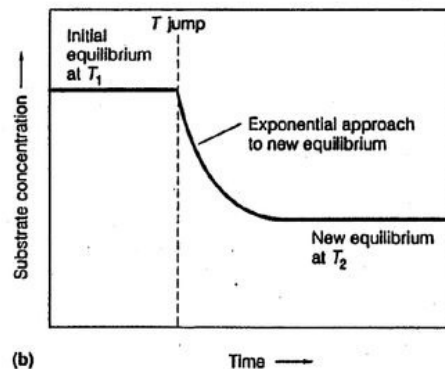
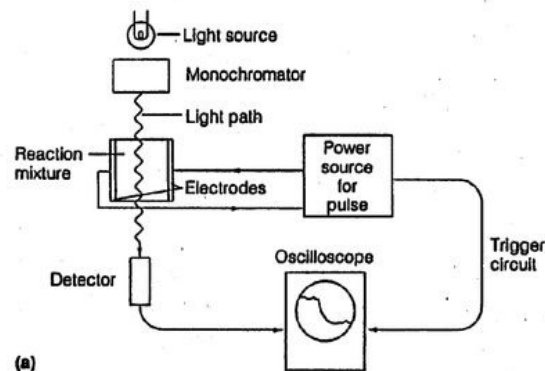
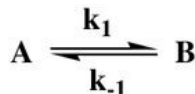
- For really fast kinetic processes (sub millisecond processes)
- Say reaction mixture is at equilibrium at  $T_1$  ...
- Quickly increase  $T_1$  to  $T_2$  by passing current between electrodes in the mixture, then watch relaxation to new equilibrium

$$\Delta [A]_{(t)} = (\Delta A)_{\text{tot}} e^{-t/\tau}$$

= relaxation time  $\rightarrow$  gives rate constant

$$1/\tau = k_1 + k_{-1}$$

For



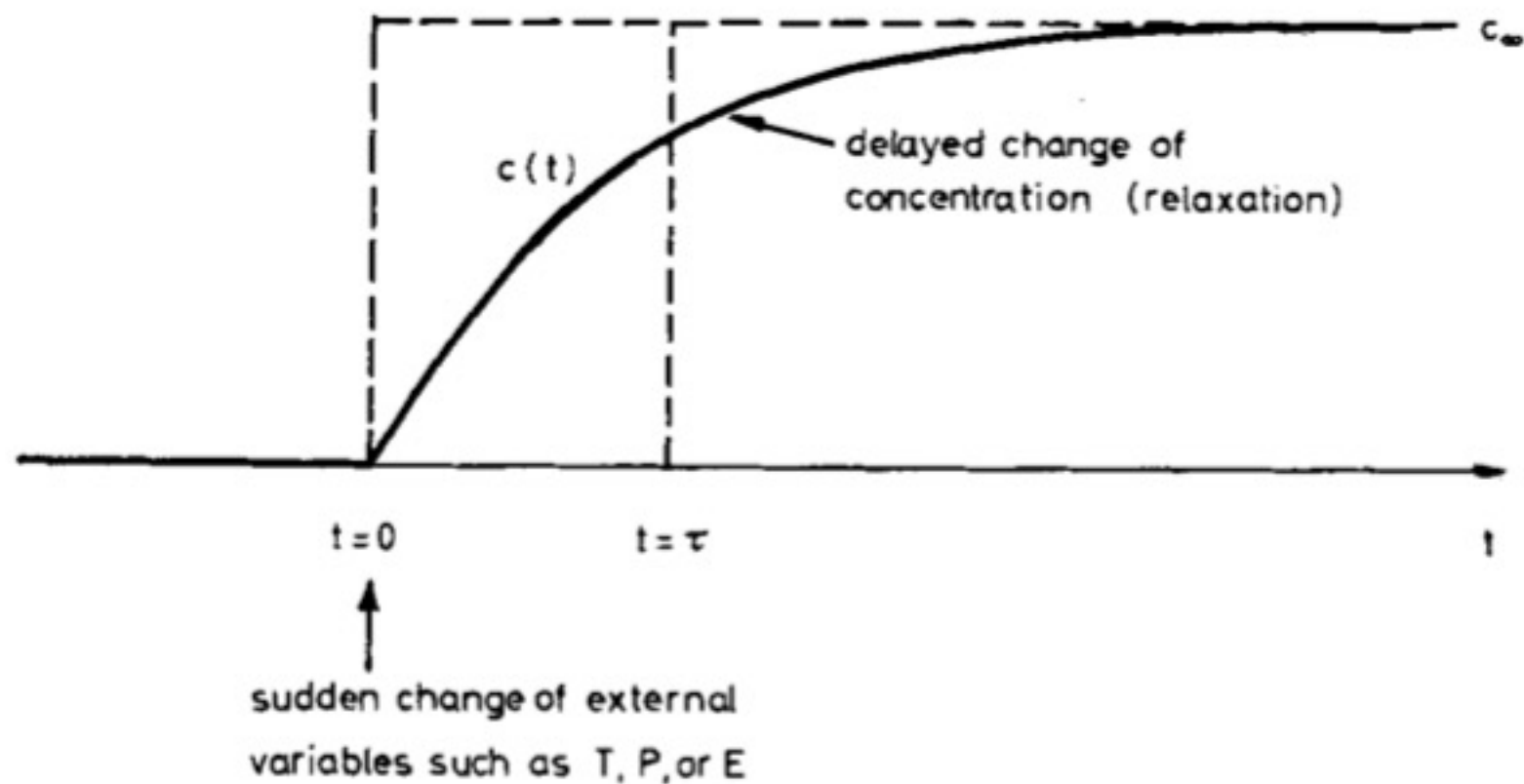


Fig. 2. Relaxational response to step function perturbation.

# Experimental methods to address chemical kinetics

-Mixing techniques pro's **VERSATILE** contro's **SLOW**

-Relaxation techniques pro's **FAST** contro's **LIMITED TO FEW CASES**