

# Nonlinear dynamics in closed biological and chemical systems

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*Abstract:* In cell free extracts of yeast, glycolysis exhibits nonlinear temporal behaviour, in response to the infusion rates of metabolisable sugars. Such bistability in closed systems is difficult. In the closed system, the glycogen induced nonlinear behaviour in cell free extracts of *Saccharomyces carlsbergensis* are described and the control characteristics are evaluated. In closed chemical systems involving acidic bromate, reactions exhibit both linear and nonlinear oxidation kinetics depending on the nature of the participating reactants. Taking the appropriate examples, the nonlinear and the steady state behaviour in the acidic bromate systems is discussed.

## INTRODUCTION

In the reactive systems with constant entropy, equilibrium position will be in the direction of lowest energy and in the systems of constant energy, the equilibrium position will be in the direction of highest entropy. When both the energy and entropy change, the equilibrium shifts in the direction of minimum free energy. To understand what is nonlinear dynamics, it necessary to know what linear dynamics is? A typical thermodynamically favoured reaction exhibits the linear dynamics. It proceeds with decrease in  $\Delta G$ , i.e. the continuous decrease in the reactant concentrations and increase in product concentrations. In the process some intermediates may reach a maximum or maintained in steady state and finally deplete. Nonlinear reactions also work with in the thermodynamic laws, but exhibit temporal behaviour in the consumption of their intermediates before finally reaching equilibrium. Such temporal behaviour can be followed by monitoring any physical property of the respective intermediate.

The nonlinear behaviour occurs in some systems under certain conditions, due to the competitive and autocatalysed reactions and through the feed back controls which influence the rates with limit behaviour. In the open systems with constant flux, self sustained oscillations can occur indefinitely, but closed systems are unique in generation of temporal phenomena. In this report we discuss the results from our studies on the batch biological and chemical systems involving single addition of all the reagents and compare with the open systems, where appropriate.

## RESULTS AND DISCUSSION

### Nonlinear dynamics in biological systems

Since the discovery of glycolytic oscillations, considerable work has been done on the effect of glycolytic flux on the temporal behaviour. In *in vivo* systems of yeast cells, the membrane limits the flux through the cell. The cell free extracts provide the homogeneous medium to investigate the inherent enzyme controls, without the restriction of membrane transport. Therefore, an extensive experimental work and mathematical modelling have been done on the influence of glucose infusion on the sustained oscillations in the cell free extracts of yeast, which is grown aerobically. In such open systems, the flux is determined by the infusion rate. The range of glucose infusion rates, which induce oscillatory behaviour, the role of phosphofructokinase and the influence of infusion rates on the amplitude and frequency of oscillation were well documented in

the literature (ref. 1). A single addition of glucose to the cell free extract does not induce sustained oscillations. Glycogen, a polysaccharite and natural substrate in the biological systems is introduced phosphorolytically into the glycolytic chain gives as example of closed biological system. It results in higher ATP yield per glucosyl unit compared to glucose which is converted to G-1-P at the expense of ATP (Fig.1).

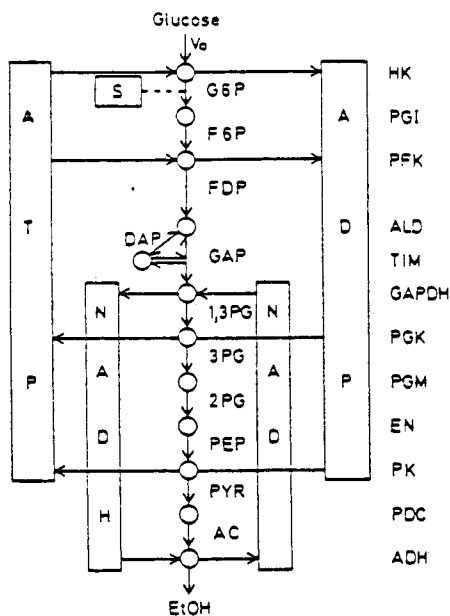


Fig. 1. The glycolytic pathway

#### ABBREVIATIONS :

S = Substrate  
 G-6-P = Glucose-6-phosphate,  
 F-6-P = Fructose-6-phosphate,  
 DAP = Dihydroxyacetone phosphate,  
 GAP = Glyceraldehyde-3-phosphate,  
 1,3-PGA = 1,3-Diphosphoglycerate,  
 3-PGA = 3-Phosphoglycerate,  
 2-PGA = 2-Phosphoglycerate,  
 PEP = Phosphoenolpyruvate,  
 PYR = Pyruvate,  
 AC = Acetaldehyde  
 EtOH = Ethanol  
 HK = Hexokinase  
 PGI = Phosphoglucosomerase,  
 PFK = Phosphofructokinase,  
 ALD = Aldalose,  
 GAPDH = Glyceraldehydephosphatedehydrogenase,  
 TIM = Triosephosphateisomerase,  
 PGK = Phosphoglyceratekinase,  
 PGM = Phosphoglyceromutase,  
 ENO = Enolase,  
 PK = Pyruvatekinase,  
 PDC = Pyruvatedecarboxylase,  
 ADH = Alcoholdehydrogenase,  
 ADK = Adenylatekinase,  
 ATPase = Adenosinetriphosphatase,  
 ATP, ADP & AMP = Adenosintri-, di and monophosphates,  
 NAD = Nicotinamide-adeninedinucleotide (Oxidised),  
 NADH = Nicotinamide-adeninedinucleotide (Reduced),

The effect of glycogen on the path way is studied by adding 95 mM of glycogen (in glucosyl units) and monitoring the levels of various metabolites and adenylates (Fig. 2a and b).

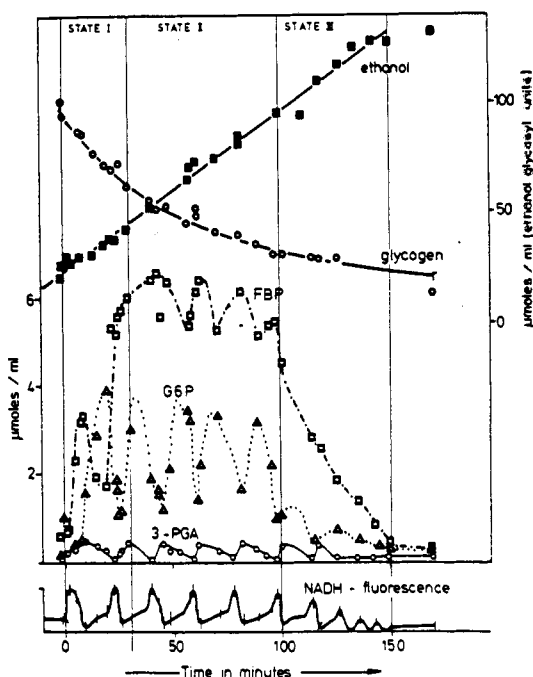
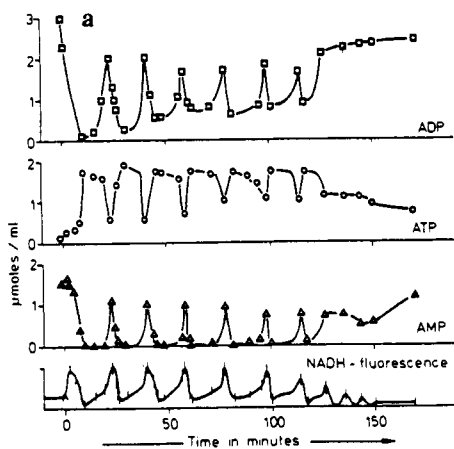


Fig. 2. Nonlinear dynamics of glycolytic system in yeast extract with glycogen (95 mM in glucosyl units). a. Phase relations between NADH and adenylates, b. Oscillations in NADH, temporal profiles of key metabolites, glycogen consumption and ethanol production rates.

Figure 2a shows the temporal behaviour NADH, G-6-P, FDP and 3-PGA (ref. 2). The rate of glycogen decreased exponentially, but ethanol flux remained the same. Finally 80-85% of the glycogen consumed was accounted as ethanol. Figure 2b shows that NADH, AMP and ADP are in a phase opposite to ATP. The exponential breakdown of glycogen and high levels of G-6-P and FDP suggest that no control exist in the upper part of glycolysis. The increased initial concentrations of glycogen (200 mM) increased the rate of its consumption proportionately, while the temporal behaviour was observed for longer duration, the ethanol flux (0.59  $\mu\text{mol}/\text{min}$ ) is not affected. Appearance of sustained glycolytic oscillations show the AMP-activation of phosphofructokinase is nevertheless effective and PFK reaction acts as the controlling step owing to its allosteric properties (ref. 2). These results confirm that although PFK controls the oscillatory behaviour through AMP activation, the glycolytic flux is regulated by GAPDH (ref. 2 and 3). This hypothesis is checked by three different experiments. Initially, the effect of added FDP, on the rate of ethanol production

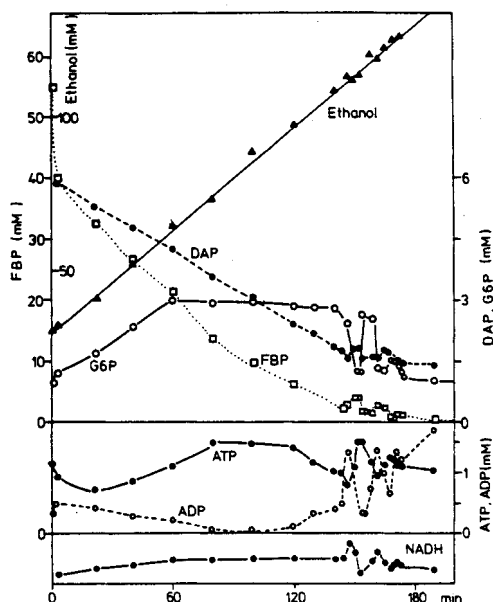


Fig. 3. Effect of FDP (60 mM) on glycolysis. FDP consumption and ethanol production rates and levels of FDP, G-6-P, DAP and adenylates

was studied. With the addition of 60 mM FDP, there was a rapid increase in DAP, but after the initial drop, FDP depleted at a constant rate (Fig. 3). Interestingly, as expected, the ethanol flux remained fairly the same (0.56  $\mu\text{mol}/\text{min}$ ) and 90% of FDP was accounted for as ethanol. To know whether the ethanol flux restriction observed during the glycogen consumption is either limited by the enzyme capacity or regulated by the enzyme as per the energy need of the system, the ethanol flux was studied using trehalose as substrate. While glycogen gives 3 ATP per glucosyl unit, trehalose similar to glucose gives 2 ATP per unit. Under otherwise identical conditions, trehalose (100 mM) gave 1.5 times higher ethanol flux of 1.0  $\mu\text{mol}/\text{min}$ , compared with the glycogen (0.64  $\mu\text{mol}/\text{min}$ ) as substrate. This confirms that glycolytic flux in the cell free yeast extract is controlled by the energy need of the system in the lower pathway, while temporal behaviour is controlled by PFK in the upper part. This observation is also supported by the observed increases in the ethanol flux from 0.59 to 0.68  $\mu\text{mol}/\text{min}$  by increasing the ATPase activity in the system by addition of apyrase, an ATPase (ref. 2).

### Nonlinear dynamics in closed chemical systems

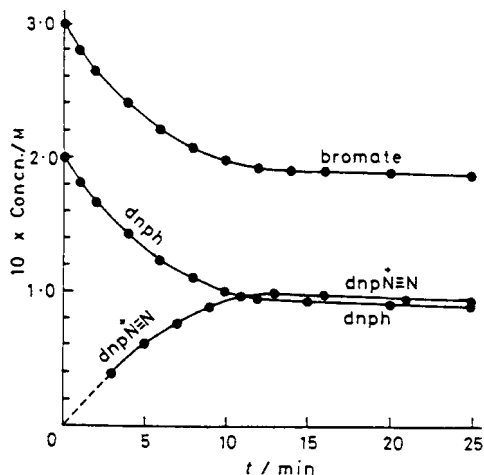


Fig. 4. Kinetic curve.  $[\text{H}^+] 3.14 \text{ M}$ ,  $[\text{dnpnh}] 2.0 \times 10^{-4} \text{ M}$  &  $[\text{BrO}_3^-] 3.0 \times 10^{-4} \text{ M}$ .

In open systems, such as an isothermal continuously stirred reactor with constant feed, self sustained oscillations can be generated for indefinite duration (ref. 5). The temporal behaviour occurs, when the bistability exists in the system, i.e. the possibility of switching of two or more intermediates between the two critical levels (ref. 6). The peculiar ability of bromate to generate oscillatory behaviour during the oxidation of certain organic substrates, has drawn considerable attention towards the elucidation of the Belousov-Zhabotinskii (BZ) reaction mechanism. It is a much studied reaction (ref. 7). Based on the understanding of the BZ system, various new oscillatory systems have been designed (ref. 8). Depending on the nature of the substrate and pH conditions, due the generation of various highly reactive bromo and oxybromo species in the system, bromate reactions exhibit intricate chemistry, both linear

and nonlinear phenomena. The profiles of kinetic curves of selected reactions of acidic bromate with different organic substrates are shown. Figure 4 shows the linear/steady state profile of the reaction between bromate and 2,4-dinitro-phenylhydrazine (dphn). The dphn gets oxidised to diazonium ion, which couples with the unreacted dphn to form an azo compound (ref. 9). Figure 5 illustrates the nonlinear dynamics, an oscillatory behaviour (emf *versus* time) exhibited by the gallic acid (GA)-acidic bromate system with ferroin as catalyst (ref. 10).

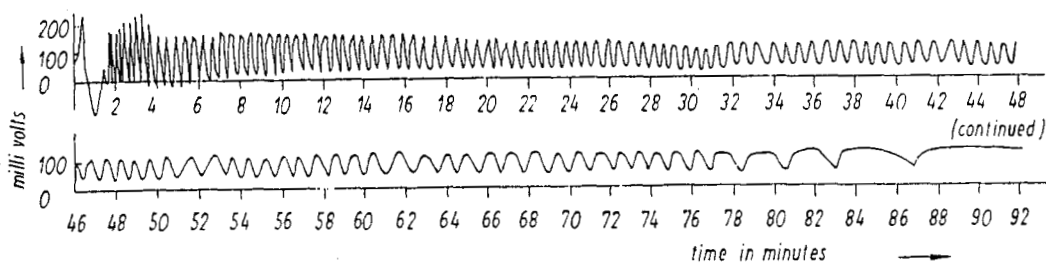


Fig. 5. emf *versus* time. [GA] 0.04M, [BrO<sub>3</sub><sup>-</sup>] 0.1M, [H<sup>+</sup>] 2.5 M and [ferroin] 10<sup>-3</sup> M. 35°C.

Figures 6a and b show the temporal behaviour in *m*-nitrophenol (mnp) and bromate system. In absence of a catalyst it exhibits highly damped chaotic oscillations while the presence of a catalyst facilitates the sustained oscillations over longer duration (in absence of presence of Mn(II) catalyst (ref. 11).

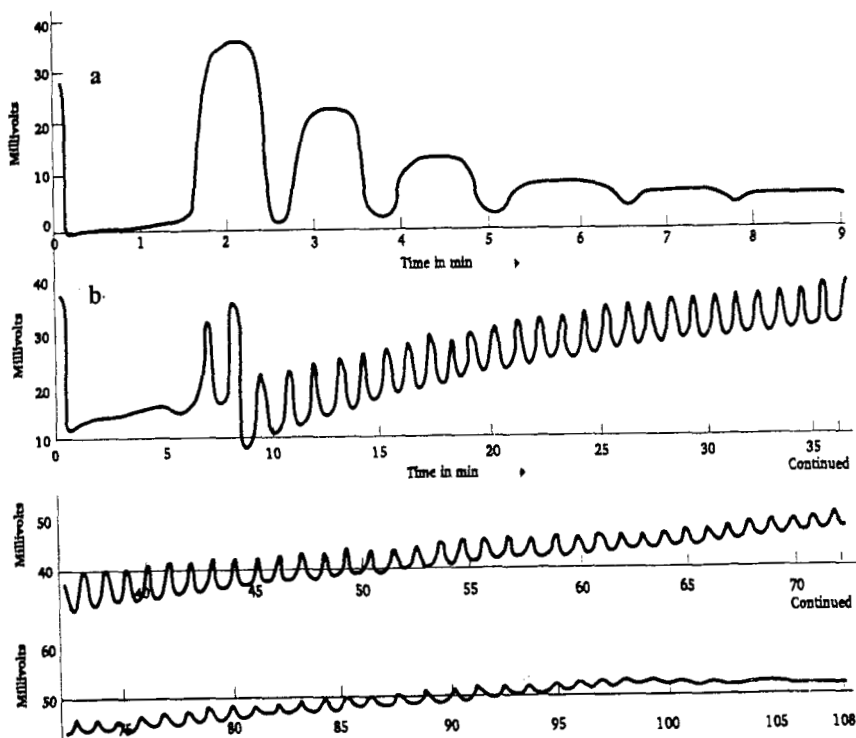
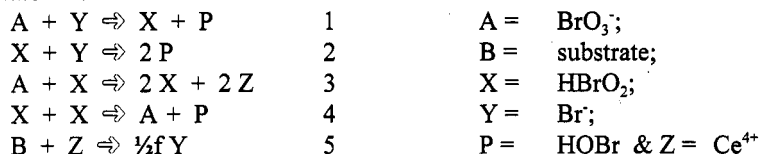


Fig. 6. a - Uncatylased reaction. emf *vs* time plot. [H<sup>+</sup>] 0.38 M, [mnp] 0.012 M, [BrO<sub>3</sub><sup>-</sup>] 0.03 M, and [ferroin] 10<sup>-3</sup> M. b- Mn(II) catalysed plus Mn(II) 5.6 x 10<sup>-6</sup> M.

The observed Belousov-Zhabotinskii type temporal behaviour is explained by the simple model called Oregonator by Noyes *et al.* (ref. 8):

## Oregonator model



In these reactions, bromide ion acts as control intermediate, bromous acid is as switched intermediate, and the catalyst is the regenerate species. While, these five equations form the basis for the chemistry of all the reactions involving acidic bromate, the rates at which various bromo and oxybromo species react with the reductant and respective reactive intermediates determine the dynamics of the reaction. This set of reactions together with the reactions and rates of the reducing substrates and their intermediates determine whether a reaction undergoes steady state kinetics or nonlinear dynamics.

Now, I show some examples of nonlinear dynamic behaviour, but with no oscillatory variations. Figure 7 illustrates the bromine formation profiles of 1-methyl-2-thiourea (MTU)-bromate reaction (ref. 12). Figure 8 shows depletion of acridine orange (AC) during its reaction with acidic bromate (ref. 13).

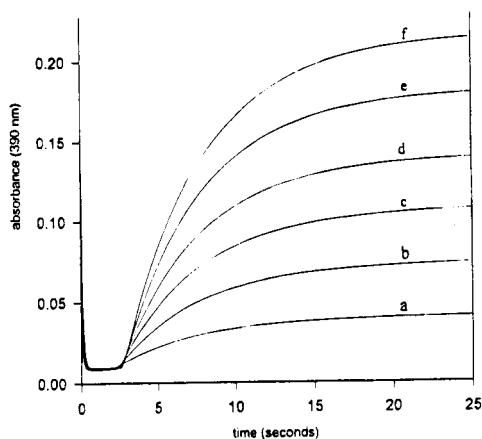


Fig. 7 Formation of  $\text{Br}_2$ . Kinetic plot.  $[\text{H}^+] 0.03 \text{ M}$ ,  $[\text{BrO}_3^-] 0.15 \text{ M}$  and  $[\text{MTU}]/10^{-3} =$  Curve a-0.5, b-1.0 M, c-1.5, d-2.0, e-2.5 & f-3.0

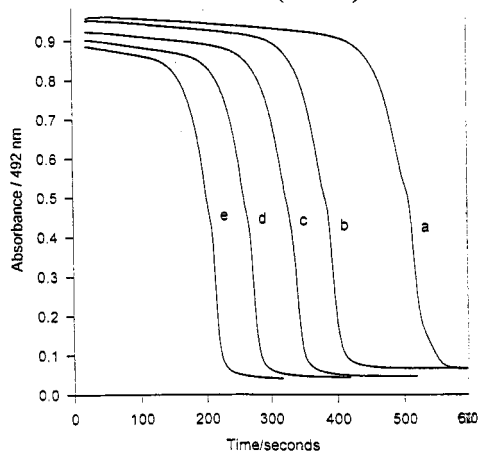


Fig. 8 AC-bromate reaction. Effect of  $[\text{H}^+]$   $[\text{AC}] 3 \times 10^{-5} \text{ M}$ ,  $[\text{BrO}_3^-] 8.4 \times 10^{-2} \text{ M}$ .  $[\text{H}^+] =$  Curve a-0.6, b-0.7, c-0.8, d-0.9 & e-1.0 M

Similar nonlinear behaviour is exhibited by the reaction of toluidine blue (TB) with acidic bromate. Figure 9 shows the repetitively scanned spectrum of TB (630 nm) and fig. 10 illustrates the effect of bromate variation on the dynamics of the reaction.

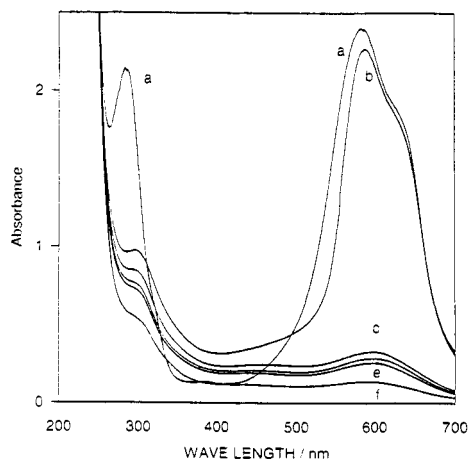


Fig.9. TB-acidic bromate reaction. Curve a: at 20 s b: 80 s, c: 140 s, d: 320 s, e: 500 s & f: 740 s.

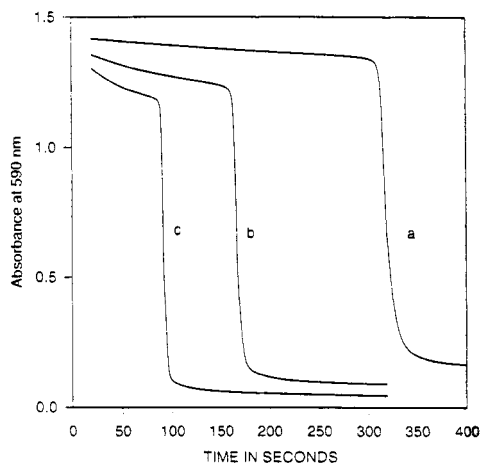


Fig.10. TB - acidic bromate reaction.  $[\text{H}^+] 0.4 \text{ M}$ ,  $[\text{TB}] 4 \times 10^{-5} \text{ M}$ ,  $[\text{BrO}_3^-]/\text{M}$  Curve a: 0.04, b: 0.08 & c: 0.12

All these reaction systems exhibit slow initial phase under steady state conditions and transition to nonlinear dynamics, which is a characteristic of autocatalysed reactions. A 16-step mechanism was proposed for the oxidation of acridine orange with acidic bromate. The reaction mechanism involves an intricate network of consecutive reactions, the rates of which influence the reaction behaviour significantly (ref. 13).

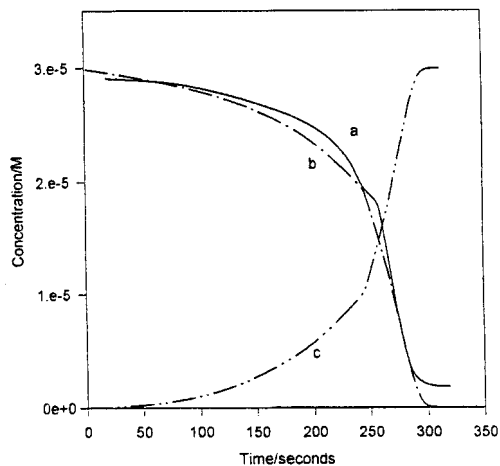


Fig. 11. Simulations. AC-bromate reaction.  
[H<sup>+</sup>] 2.0 M, [BrO<sub>3</sub><sup>-</sup>] 4 x 10<sup>-3</sup> M & [AC] 3 x 10<sup>-5</sup> M  
a: Expt. curve, b: Simu. curve & c: [Product].

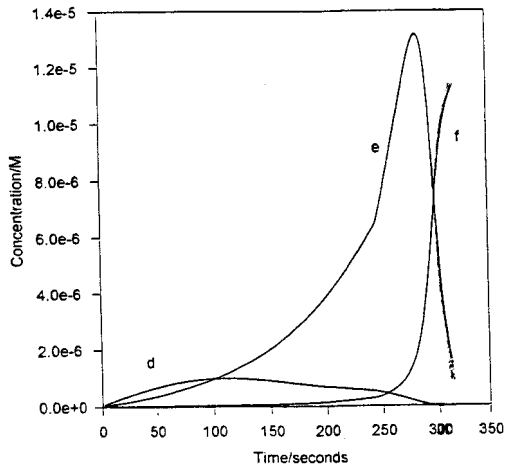


Fig. 12. Simulations. AC-bromate reaction.  
Curve d: HBrO<sub>2</sub>, e: Br & f: Br<sub>2</sub>

Computer simulations using the proposed mechanism were done using semi-implicate Runge-Kutta method devised by Kaps and Rendrop (ref.14). A fair agreement between the simulated and experimental curves was observed supporting the plausibility of the proposed mechanism (Fig. 11 and 12).

The insight into the complex behaviour of the bromate reactions is continuously enriching the understanding of the intricate chemistry and thermodynamics of the chemical, biochemical and biological systems that exhibit nonlinear phenomena. The studies on the nonlinear dynamics have helped in designing new oscillatory systems and in predicting the chaos in the various chemical and life processes.

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