International Journal of Basic and Applied Chemical Sciences ISSN: 2277-2073 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jcs.htm
2014 Vol. 4 (1) January-March, pp.23-36/Peerzada et al.
Research Article

STUDIES ON THE INTERACTION OF RADICAL SCAVENGING DRUGS WITH ACETAMINOPHEN BASED OSCILLATORY CHEMICAL REACTION

***G. M. Peerzada, Momina Bashir, Nadeem Bashir and Sayima Riyaz**
Department of Chemistry, University of Kashmir Hazratbal Srinagar-190006 J&K India
**Author for Correspondence*

ABSTRACT

Chemical oscillations have been reported for a wide range of organic substrates like aliphatic acids, phenols, ketones, sugars and many more. The present investigation aims to explore the possibility of the compounds as substrates in oscillatory chemical reaction which have some medicinal value. Further the interaction of such a system with some radical scavenging drugs as perturbants will be of vital importance both kinetically as well as mechanistically. Such interactions will also serve as prototype examples for understanding the interaction of drugs in vivo. As such the reaction system undertaken for study is cerium catalyzed bromate driven oscillatory chemical reaction with acetaminophen (paracetamol) as the substrate molecule in aqueous sulfuric acid medium. The choice of the acetaminophen as substrate is owing to its wide usage as analgesic and antipyretic drug. The system was optimized with respect to initial reagent concentrations in order to study the kinetics of acetaminophen with the radical scavenging drugs like Glutathione, Ccaptopril and N-Acetylcysteine. Complex, long time series periodic oscillations are reported in an unstirred batch reactor (UBR) at constant temperature (35 ± 0.1^0C) for the aforesaid system. The drugs were injected into the system at appropriate time viz. initially at t = 0 s, just after induction period and during the onset of oscillations in order to explore the possible interactions of these perturbants with the generated intermediates. The oscillatory parameters have been found to be the function of the initial concentrations of the aforesaid BZ reagents. The various oscillatory parameters such as time period (t_p), induction time (t_{in}), frequency (ν), amplitude (A) and number of oscillations (n) were derived for the parent reaction system. The aqueous H_2SO_4 was found as a better reaction medium because, the system exhibited wider oscillation window in this medium.

Keywords: *Drugs, Acetaminophen, BZ Reaction, Quenching*

INTRODUCTION

The study of BZ reaction has gained considerable momentum in the past three decades. The reactions show spatio-temporal structures such as redox oscillations of the catalyst and travelling waves (Field *et al.,* 1985, Epstein *et al.,* 1998, Field *et al.*, 1972, 1974, Zhabotinsky *et al.*, 1964, Zaikin *et al.,* 1970). Unusual properties of reagents in far-from-equilibrium conditions and the prevalence of instability where small changes in initial conditions may lead to amplified effects have been well documented. Applications of oscillating chemical reactions in analytical chemistry have grown substantially in the last few years (Gao *et al.*, 2005, Yang *et al.,* 2002, Rocio *et al.*, 2000, Ramaswamy *et al.,* 1983). Owing to these reasons there have been a steady effort and increasing interest in expanding the family of BZ oscillators. Understanding the onset of these exotic phenomena in chemical systems has provided important insight into the formation of similar behaviors in nature (Winfree 2000, Kadar *et al.,* 1998). This has also allowed scientists to explain the intricate and complicated behaviors underlying these phenomena. The controlling parameters of nonlinear phenomena in bio-systems are often out of reach, whereas chemical systems can be easily manipulated through adjusting the initial reagent concentration of each reagent, temperature, or flow rate in a continuously flow stirred tank reactor (CSTR) (Ungvarai *et al.,* 1989). The overall process of BZ is the oxidation of an organic substrate such as citric acid or malonic

acid by an oxidizing agent in a strongly acidic solution with or without catalyst. Catalyzed systems contain a metal ion catalyst such as Ce (III/IV), Fe(II/III), and Mn(II/III) and an aliphatic or aromatic organic substrate which is oxidized and brominated by bromate ion. Uncatalyzed systems do not contain metal ion but possess more reactive aromatic compounds like polyphenol and polyaniline derivatives (Ramaswamy *et al.*, 1983). The mechanism of such reactions is explained in detail by Field, Koros and Noyes (FKN) in 1972.

In the present investigation, acetaminophen is chosen as the organic substrate in the BZ reaction to study its kinetic behavior with respect to initial concentrations of other reagents in the reaction mixture, comprising of inorganic bromate as oxidant and Ce (IV) as catalyst in aqueous sulfuric acid medium (Ungvarai *et al.*, 1989, Hegedus *et al.*, 2000, Nogueira *et al.*, 2012). Acetaminophen, also known as paracetamol, is a nonsteroidal anti-inflammatory drug with potent antipyretic and analgesic actions but with very weak anti-inflammatory activity (Botting 2000, Mehring 1893). The compound has sufficient solubility in aqueous acid medium and exhibits dynamic behavior over a wide range of concentration. The compound is widely prescribed as analgesic and antipyretic drug to human beings throughout the world. As we know that in a BZ reaction there is generation of radical intermediates and reactive oxygen species. This is the reason for optimizing an acetaminophen based BZ reaction system and then choosing some specific medicinally important, radical scavenging, sufficiently water soluble and orally administered drugs like glutathione, captopril and N-acetylcysteine for kinetic studies in vitro. Thus, present study can act as a prototype example in understanding the mechanism of action of acetaminophen (Anderson 2008) and its interaction with aforesaid drugs and similar biologically important molecules like drugs, antioxidants, etc.

MATERIALS AND METHODS

The reagents used were acetaminophen 99% (Sigma Aldrich; AR), potassium bromate 99.6% (Merck), Cerium (IV) sulfate monohydrate 98% (BDH), sulfuric acid 98% (Merck), glutathione 99% (Sigma Aldrich; AR), captopril 99% (Sigma Aldrich; AR), N-acetylcysteine 99% (Sigma Aldrich; AR).These reagents were used without any further purification owing to their high degree of purity and all the solutions were prepared in de-ionized double distilled water.

The redox potential from the BZ oscillator was recorded by the Cyberscan Dual channel/pH/ ion conductivity meter (Eutech Instrument; Model PC 5500). The dynamics of the reaction was monitored with a Platinum electrode as an indicator electrode and a Saturated Calomel Electrode (SCE) as reference electrode. One half cell contained the reaction mixture under investigation with platinum electrode dipped into it, and the another half cell contained 2.5×10^{-4} mol L^{-1} potassium chloride solution with SCE dipped into it. The two half cells were connected through a salt bridge containing saturated solution of potassium nitrate (Merck) in agar-agar. The thermostatic conditions were achieved by using a water bath (ADVENTEC-SRS266PA) set up at desired temperature i.e. 35 ± 0.1^0C. This temperature was chosen in order to have observation nearer to human body temperature. All the solutions used were first kept under thermostatic conditions for 20 minutes to acquire the desired temperature. Then 2 mL each of Ce(IV) and acetaminophen were mixed in one reaction cell and the reaction started by injecting 2 mL of potassium bromate into this reaction mixture from the top. The total volume of the reaction mixture was 6 mL in a 100 mm long reaction vessel with a diameter of 25 mm. For each trial the electrodes and the salt bridge tips were washed thoroughly with double distilled water. The redox potential was recorded only after the addition of BrO_3^- into the reaction mixture. The injection of radical scavenging drugs as perturbants was chosen at three stages using different concentrations of the same in order to understand the kinetics and mechanism. The three stages chosen were: a) initially at t = 0 s, when the BZ reagents were mixed together, b) just after the induction period, when there is generation of critical bromosubstrate concentration and c) during the oscillations when the autocatalysis process is going on and the formation and consumption of radical intermediates and reactive oxygen species like HOBr, $HBrO_2$ and $BrO_2^{\cdot}$ takes place in the reaction system.

RESULTS AND DISCUSSION

According to Orban and Koros (1978), oxidation of phenol or aniline derivative with acidic bromate results in the formation of quinoid and bromine as intermediates. However, our substrate is substituted aminophenol derivative which shows an ample number of monotonic periodic oscillations within the narrow window of acetaminophen/bromate concentrations. It has been observed that on addition of bromate to the acetaminophen-cerium solution, the solution becomes colored with intermittent florescent behavior, indicating some photochemical behavior probably due to the formation of quinoid type species. However, this greenish florescence diminishes with the passage of time due to the consumption of organic substrate. The appearance of the greenish color initially results in fluorescence which is due to the formation of critical bromosubstrate concentration during induction period. As indicated by the platinum electrode, there is a large excursion of potential on addition of bromate to the reaction mixture. After an initial quick rise, the reaction shows a significant induction period before the periodic oscillatory behavior is seen in the time versus potential graph. The induction period directly depends on the initial concentration of bromate and acid. A typical result with optimal oscillation parameters is shown in figure 1 with $[acetaminophen]_0$ = 0.06 mol L^{-1}, $[BrO_3^-]_0$ = 0.08 mol L^{-1}, $[Ce^{4+}]_0$ = 0.0055 mol L^{-1} and $[H_2SO_4]_0$ = 1.3 mol L^{-1}.

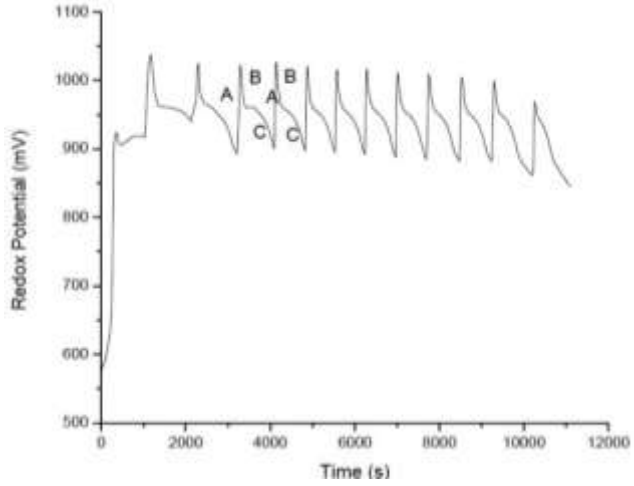


Figure 1: Typical Oscillating profile of the system (showing regions A, B and C) containing [acetaminophen] = 0.06 mol L^{-1}, $[Ce^{4+}]$ = 0.0055 mol L^{-1}, $[BrO_3^-]$ = 0.08 mol L^{-1}, $[H_2SO_4]$ = 1.3 mol L^{-1} at 35 ^{0}C.

Table 1: Variation in [Acetaminophen] having other species such as $[Ce^{4+}]$ = 0.0055 mol L^{-1}, $[BrO_3^-]$ = 0.08 mol L^{-1}, $[H_2SO_4]$ = 1.3 mol L^{-1}, and Temperature = 35 $^\circ$C

[Acetaminophen] (mol L^{-1})=	Induction Period (s)	Number of oscillations	Amplitude (mV)	Time Period (s)
0.04	150	3	99.6	3825.00
0.05	140	6	112.3	1391.00
0.06	140	11	127.0	1080.00
0.07	140	7	141.0	814.28
0.08	140	2	105.0	438.33

Table 1 shows the variation in oscillatory parameters at different concentrations of acetaminophen keeping the initial concentrations of other reacting species constant at 35 ± 0.1 ^{0}C. From the data it is apparent that with increase in the concentration of acetaminophen, the induction period decreases first and then remains constant thereby showing no or very meager dependence of [acetaminophen] on induction period. However, the time period shows a continuous decrease with increase in [acetaminophen]. The

number as well as the average amplitude of oscillations first increase and then decrease. The observation seems plausible because the system bifurcates into an oscillatory regime only after the accumulation of a desired amount of the intermediate species and the bromo-organic species. With increase in the $[acetaminophen]_0$, the rate of formation of bromoderivative increases and hence there should be shortening of the pre-oscillatory period, i.e. the induction period, but it remains unchanged. This may be due to the fact that the concentration of critical bromo-organic derivative remains constant in all the reaction systems despite of increasing the initial [acetaminophen] in them. Further, the time period of the oscillations increases as the reaction proceeds and ultimately the system damps out because it is a closed one. It is also observed that with change in the concentration of acetaminophen, the fluorescence behavior also changes.

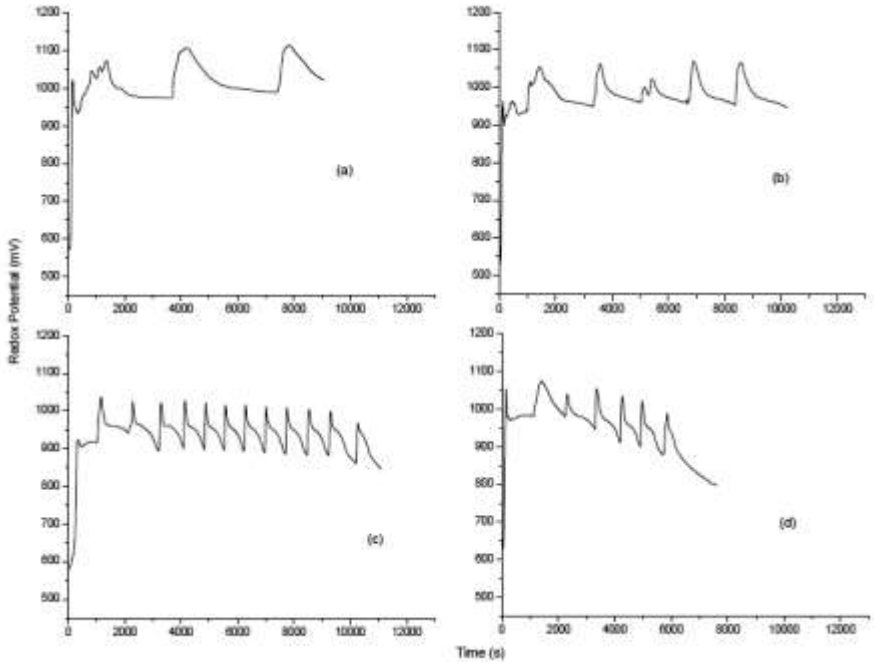


Figure 2: Potential (mV) verses time(s) plots showing variation of oscillatory characteristics for the system containing $[BrO_3^-]$ = 0.08 mol L^{-1}, $[Ce^{4+}]$ = 0.005 mol L^{-1} and $[H_2SO_4]$ = 1.3 mol L^{-1}, [Acetaminophen]: (a) 0.04 mol L^{-1}, (b) 0.05 mol L^{-1}, (c) 0.06 mol L^{-1}, and (d) 0.07 mol L^{-1}. Temperature = 35 ^{0}C.

Figure 2 shows the dependence of the oscillatory characteristics on $[acetaminophen]_0$. This dependence of the oscillation characteristics such as amplitude and time period is justified on the basis of the FKN mechanism (Field *et al.*, 1972, 1974). According to this mechanism, the overall BZ reaction may be divided into the following three processes: consumption of bromide ion (process A), autocatalytic reaction of bromous acid with oxidation of catalyst (process B), and organic reaction with reduction of catalyst (process C).

$BrO_3^- + 2Br^- + 3H^+ \rightarrow 3HOBr$ (A)

$BrO_3^- + HBrO_2 + 2M_{red} + 2H^+ \rightarrow 2HBrO_2 + 2M_{ox} + H_2O$ (B)

$2M_{ox}$ + Substrate + Bromoderivative $\rightarrow f Br^-$ + $2M_{red}$ + other products (C)

On the basis of this mechanism, the typical oscillatory profile of this system (Figure 1) has been split over three regions wherein process A is represented by the region in which the potential increases rapidly. The region corresponding to process B shows rapid decrease in potential whereas the region representing the process C shows a slow decrease in potential.

Table 2: Variation in [BrO_3^-] having other species such as [Acetaminophen] = 0.06 mol L^{-1}, [Ce^{4+}] = 0.0055 mol L^{-1}, [H_2SO_4] = 1.3 mol L^{-1}, and Temperature = 35 °C

[BrO_3^-] (mol L^{-1})	Induction Period (s)	Number of oscillations	Amplitude (mV)	Time Period (s)
0.05	790	3	91.0	226.66
0.06	140	11	127.0	1080.00
0.07	280	6	126.0	1050.00
0.08	140	9	123.7	1080.00
0.09	160	4	119.0	1043.00
0.10	160	2	*	*

Table 2 depicts the magnitude of oscillatory parameters at different concentrations of bromate. It is found that with increase in $[BrO_3^-]_0$, the induction period decreases up to 0.08 mol L^{-1} and then it shows an increasing trend. This unusual trend can be attributed to inhibitory effect of the $HBrO_2$ (autocatalytic process), wherein Br^- competes with $HBrO_2$ for bromate and the autocatalytic process would not start until $[Br^-]$ drops to a certain critical value. It is assumed that with increase in the initial bromate concentration, there is faster accumulation of bromoderivative and hence a shorter induction period is observed (Mehring, 1893). But with further increase in the bromate, the $[Br^-]$ increases quickly which requires longer time for it to reach the threshold value, leading to the increase in induction. It is found that the high [bromate]/[acetaminophen] ratio (0.08/0.06) is required to observe the oscillations owing to the reason that dibromo and even tribromo derivatives of acetaminophen are formed on its bromination. The bromination is mostly feasible on the benzene ring, whereas the bromination of acetyl side chain cannot be even ruled out. The time period of oscillations first increases up to 0.08M and then decreases. The increase can be due to the increase in concentration of M_{ox} from process B, which takes more time to get reduced in process C. However, any further increase in $[BrO_3^-]$ limits the formation of M_{red} because of lesser concentration of organic substrate in process C. The number and the amplitude of the oscillations first increase and then decrease with the increase in the concentration of bromate following the FKN mechanism.

Table 3: Variation in [Ce^{4+}] having other species such as [Acetaminophen] = 0.06 mol L^{-1}, [BrO_3^-] = 0.08 mol L^{-1}, [H_2SO_4] =1.3 mol L^{-1}, and Temperature = 35 °C

[Ce^{4+}] (mol L^{-1})	Induction Period (s)	Number of oscillations	Amplitude (mV)	Time Period (s)
0.0030	280	6	118.5	1600.00
0.0040	160	6	117.6	1485.00
0.0050	140	9	125.7	1080.00
0.0055	140	11	127.0	1080.00
0.0060	150	8	128.6	994.28
0.0070	140	8	131.5	1017.50
0.0080	160	6	130.0	1178.30

Table 3 shows the oscillatory dynamics with varying $[Ce^{4+}]$. With increase in $[Ce^{4+}]$, the induction period first decreases sharply up to 0.0055 mol L^{-1} and then shows a steady increase. The time period also shows more or less similar trend. This may be attributed to the combined effect of processes B and C involving $[Ce^{4+}]/[Ce^{3+}]$ dependence on the autocatalytic process, leading to the formation of $HBrO_2$. The amplitude of the oscillations initially showed an increasing trend with increase in $[Ce^{4+}]_0$ but later on, it decreases with increase in $[Ce^{4+}]_0$, of course, the number of oscillations decrease. The oscillation window for Ce^{4+} is much wider as compared to other metal ions for the system

Acidic medium in BZ reaction is as important as are the other reacting species (Srivastava *et al.*, 1991). The H^+ ion accounts for the protonation of the acetaminophen and at higher acid concentrations it will lead to breakage of acetate group from aminophenol moiety. This reactive protonated species acts as a good nucleophile for bromide ion to form the bromoderivative (Ganaie *et al.*, 2009, 2010). The data

reported in table 4 reveals that at 1.3 mol L^{-1} H_2SO_4 the oscillatory parameters of the system are well pronounced. Although, other acids like nitric acid, perchloric acid and orthophosphoric acid were also tried as media, but in sulfuric acid, oscillations have been observed over a wide range of concentrations of the medium (Ganaie *et al.*, 2011). The number and amplitude of oscillations was significant in 1.3 mol L^{-1} of H_2SO_4 (Figure 3)

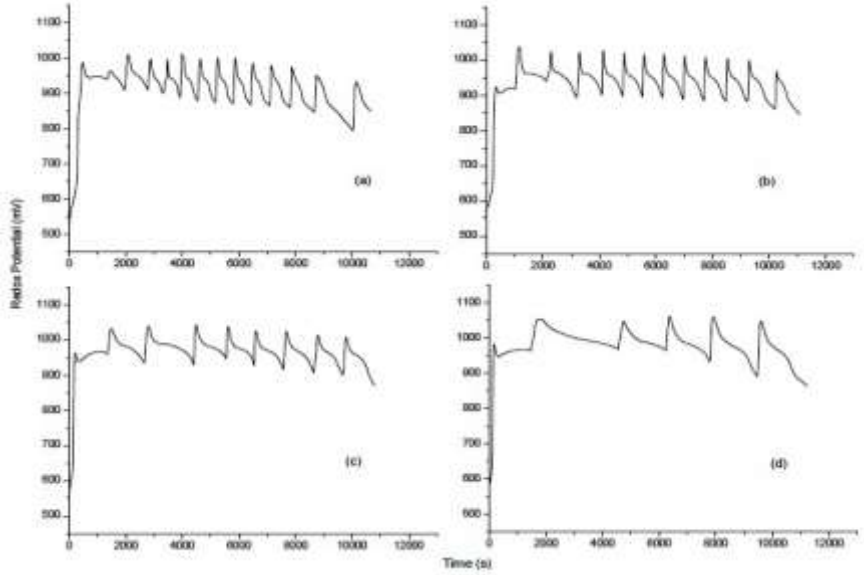


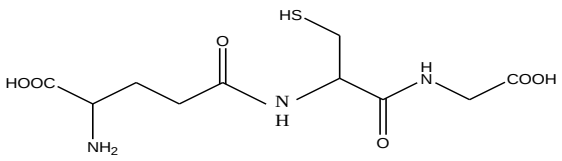
Figure 3: Potential (mV) verses time(s) plots showing variation of oscillatory characteristics for the system containing [Acetaminophen] = 0.06 mol L^{-1}, [BrO_3^-] = 0.08 mol L^{-1} and [Ce^{4+}] = 0.0055 mol L^{-1}, [H_2SO_4]: (a) 1.0 mol L^{-1}, (b) 1.3 mol L^{-1}, (c) 1.5 mol L^{-1}, and (d) 2.2 mol L^{-1}. Temperature = 35 ^{0}C

The induction period shows continuous decrease, whereas the time period shows increase with increase in the concentration of H_2SO_4.

Table 4: Variation in [H_2SO_4] having other species such as [Acetaminophen] = 0.06 mol L^{-1}, [BrO_3^-] = 0.08 mol L^{-1}, [Ce^{4+}] = 0.0055 mol L^{-1}, and Temperature = 35 $^\circ$C

[H_2SO_4] (mol L^{-1})	Induction period (s)	Number of oscillations	Amplitude (mV)	Time Period (s)
0.8	1020	9	94.22	842.0
1.0	480	10	115.25	745.3
1.3	140	11	127.00	1080.0
1.5	210	9	125.00	1061.0
2.0	130	9	120.50	1085.0
2.5	140	4	100.00	1357.0

In order to monitor the radical scavenging drugs the system was taken with the optimized concentrations like, $[AP]_0$ = 0.06 mol L^{-1}, $[KBrO_3]_0$ = 0.08 mol L^{-1}, $[Ce(IV)]_0$ = 0.0055 mol L^{-1}, [H_2SO_4] = 1.3 mol L^{-1} at 35 ± 0.1^{0}C for further kinetic studies with radical scavenging drugs. Figures 4, 5 and 6 show the effect of glutathione as perturbant at different stages of reaction like at t= 0 s, just after induction period and during the oscillations respectively.



GLUTATHIONE (G)

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It is observed that with increase in concentration of glutathione initially from 0.01 to 0.04 mol L^{-1}, first there is increase in induction period and then it exhibits quenching at higher concentrations as shown in Figure 4.

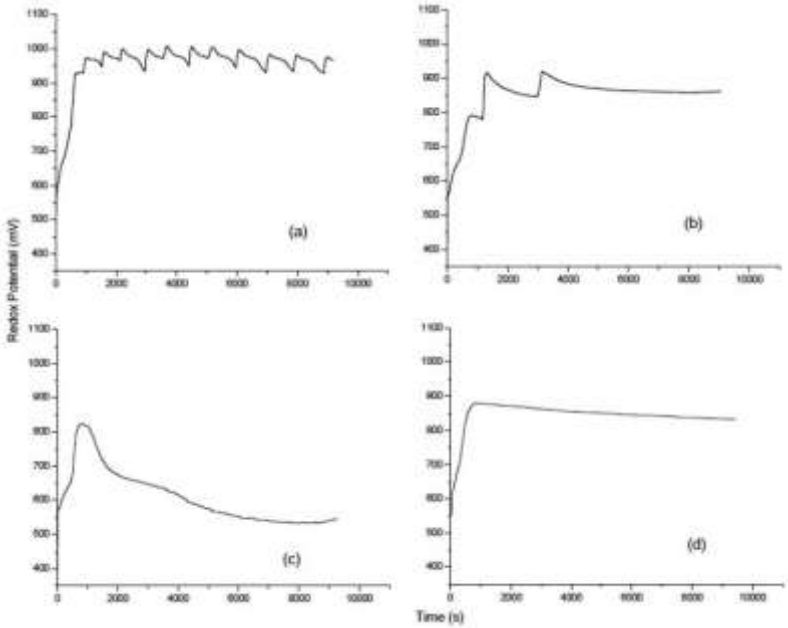
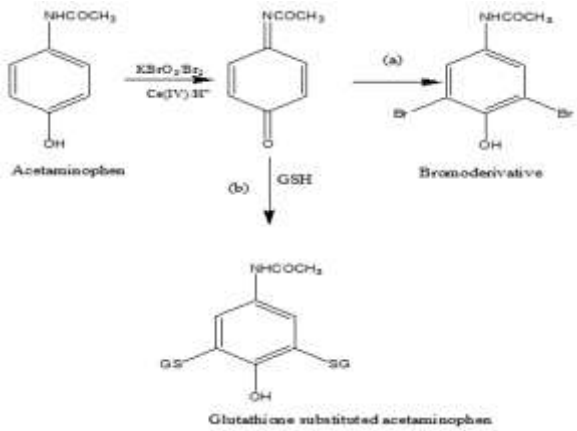


Figure 4: Potential versus time plots for the BZ system containing [AP] = 0.06 mol L^{-1}, [BrO_3^-] = 0.08 mol L^{-1}, [Ce^{4+}] = 0.0055 mol L^{-1}, [H_2SO_4] = 1.3 mol L^{-1} at different injection concentrations of [GT]; (a) 0.01 mol L^{-1}, (b) 0.02 mol L^{-1},(c) 0.03 mol L^{-1}, (d) 0.04 mol L^{-1} at t = 0 s. Temperature = 35 ^oC

The time period and amplitude of oscillations, 606 s and 46 mV respectively for 0.01 mol L^{-1} glutathione, are smaller than the parent system confirming a reasonable interaction of glutathione with acetaminophen based BZ system. Lee in 2004 studied that the acetaminophen overdosing is the most frequent cause of acute liver failure in men (Lee 2004). Hence, the mechanisms of acetaminophen toxicity have been studied very intensively recently. According to Rousar *et al.*, acetaminophen toxicity is linked to reduced activity of glutathione reductase (GR) in vitro (Rousar *et al.*, 2010). It is a crucial enzyme in glutathione metabolism because it reduces glutathione disulphide (GSSG) back to the reduced form, glutathione. This enzyme is essentially important during oxidative stress, where the level of GSSG increases and the inhibition of GR could be a principal mechanism in acetaminophen toxicity. Thus increased glutathione concentrations paves way for increased interactions of it with the critical acetaminophen concentration causing delay in establishing the critical bromosubstrate concentration.



It is observed from Figure 5 that when glutathione is injected just after induction period, the system can be used to determine the concentration of glutathione by monitoring the dip in potential at the injection, as it follows a linear relationship with increased concentration (R^2 = 0.96). Also the oscillatory parameters like time period and amplitude showed a marked increase of 1023 s and 76 mV for 0.01 mol L^{-1} glutathione as compared to when glutathione was injected at t = 0 s. This can be due to lesser interaction of glutathione with the available [acetaminophen], as some portion of $[acetaminophen]_0$ is being used to form $[bromo\text{-}acetaminophen]_{crit}$. Further, there is first decrease and then a significant increase in the time period and amplitude of oscillations with increasing [glutathione] and later on, the oscillations get quenched. If the glutathione is injected after the commencement of oscillations, it shows a linear relationship of potential dip with increased [glutathione] from 0.01 to 0.04 mol L^{-1} (R^2 = 0.925) as depicted in Figure 6.

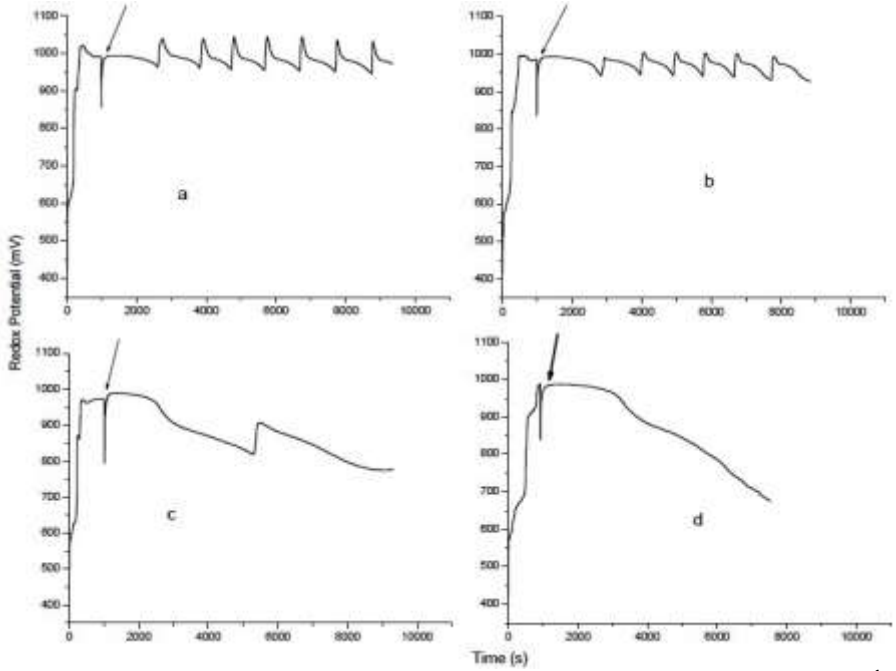
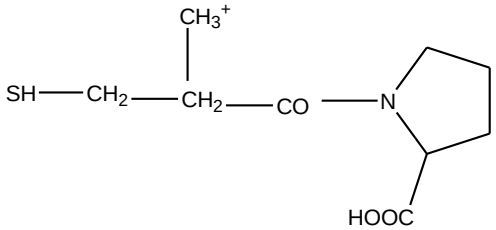


Figure 5: Potential versus time plots for the BZ system containing [AP] = 0.06 mol L^{-1}, [BrO_3^-] = 0.08 mol L^{-1}, [Ce^{4+}] = 0.0055 mol L^{-1}, [H_2SO_4] =1.3 mol L^{-1} at different injection concentrations of [GT]; (a) 0.01 mol L^{-1}, (b) 0.02 mol L^{-1}, (c) 0.03 mol L^{-1}, (d) 0.04 mol L^{-1} at induction period. Temperature = 35 ^oC.

Captopril or 1-[(2S)-3-mercapto-2-methyl-l-oxo-propyl]-l-proline is an angiotensin-converting enzyme (ACE) inhibitor used for the treatment of hypertension and some types of congestive heart failure. The presence of sulfhydryl moiety in captopril has led to its poor pharmacokinetic profile and several adverse effects, as it is orally taken and 70% of it is absorbed and bioavailable. So there are greater chances of this drug to interact with the other compounds present in stomach. Because of the presence of sulfhydryl group, this drug will react with the intermediates generated in situ in the BZ reaction, like HOBr, Br_2 and BrO_3^- and influence the established oscillatory parameters and thus helps in understanding the kinetic behavior in vitro.



CAPTOPRIL

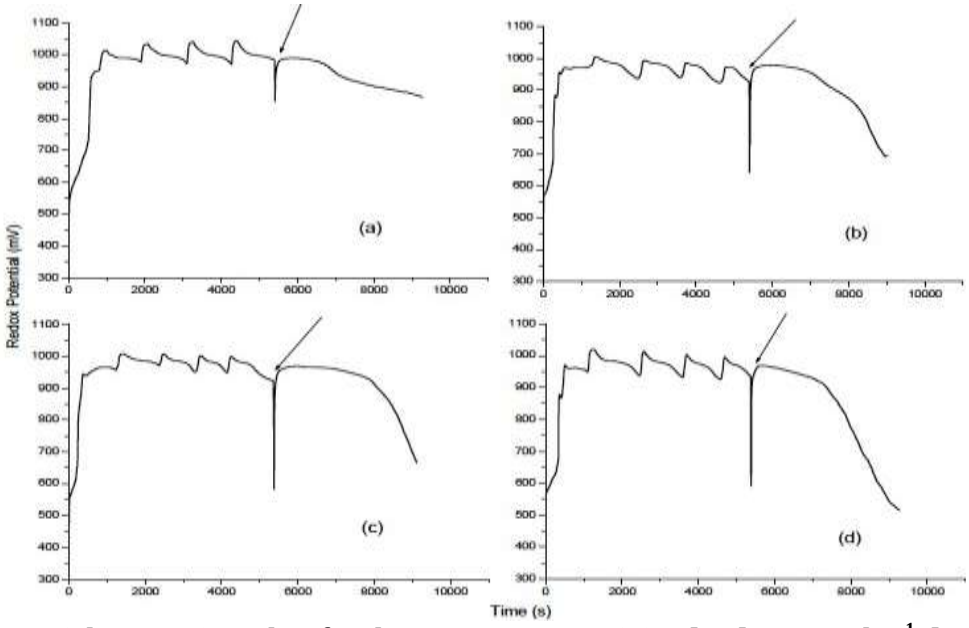
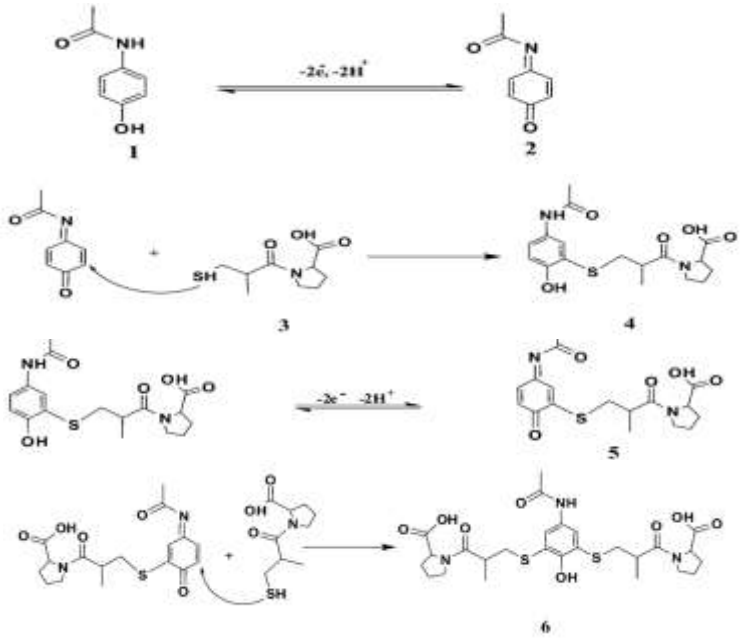


Figure 6: Potential versus time plots for the BZ system containing [AP]=0.06 mol L^{-1}, [BrO_3^-] = 0.08 mol L^{-1}, [Ce(IV)] = 0.0055 mol L^{-1}, [H_2SO_4] = 1.3 mol L^{-1} at different injection concentrations of [GT]; (a) 0.01 mol L^{-1}, (b) 0.02 mol L^{-1}, (c) 0.03 mol L^{-1}, (d) 0.04 mol L^{-1} after the commencement of oscillations. Temperature = 35 ^oC

El-Didamony *et al.*, (2010) studied the oxidation of captopril by bromine, generated in situ by the action of acid on the bromate-bromide mixture. They have proposed some schemes showing oxidation pathways.



Scheme: Interaction of acetaminophen with Captopril

Tammari *et al.*, in 2011 studied that acetaminophen is oxidized to its respective p-quinone-imine, which is attacked by captopril to form thioether with the rate constant of 3.1×10^4 L mol^{-1} s^{-1}. Hence, there is decrease in free [acetaminophen] with increasing [captopril] as is evident from Figures 7, 8 and 9.

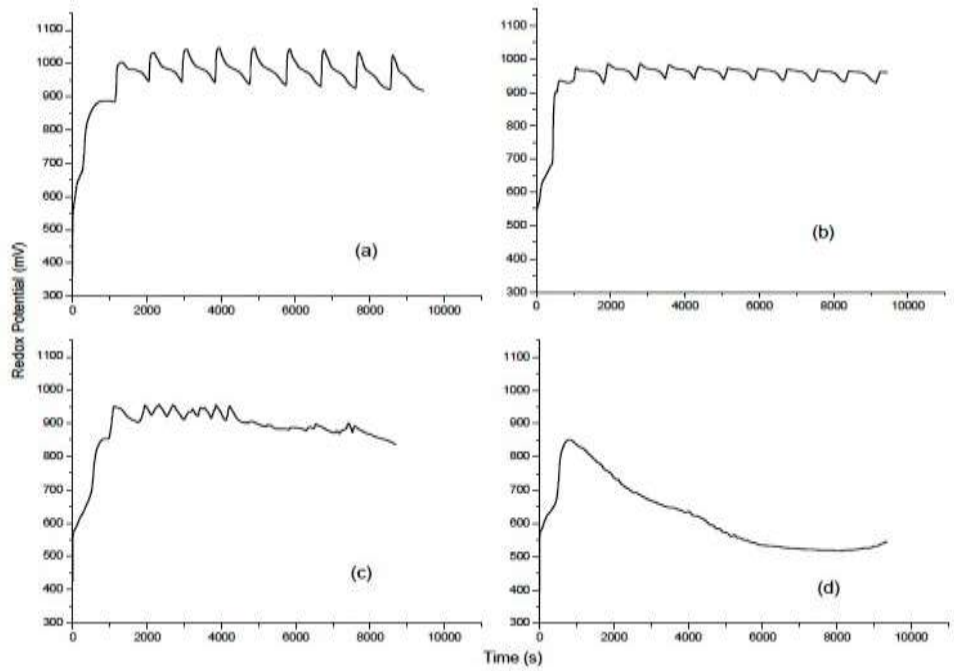


Figure 7: Potential versus time plots for the BZ system containing [AP] = 0.06 mol L^{-1}, [BrO_3^-] = 0.08 mol L^{-1}, [Ce (IV)] = 0.0055 mol L^{-1}, [H_2SO_4] = 1.3 mol L^{-1} at different injection concentrations of [Captopril]. (a) 0.01 mol L^{-1}, (b) 0.02 mol L^{-1}, (c) 0.03 mol L^{-1}, (d) 0.04 mol L^{-1} at t=0 s. Temperature = 35 ^oC

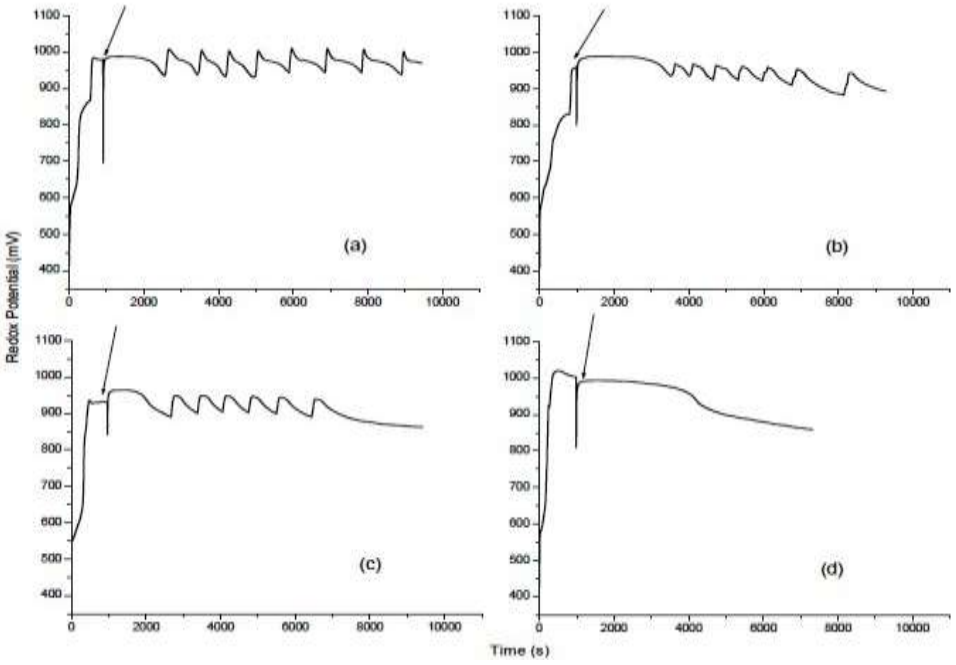


Figure 8: Potential versus time plots for the BZ system containing [AP] = 0.06 mol L^{-1}, [BrO_3^-] = 0.08 mol L^{-1}, [Ce(IV)] = 0.0055 mol L^{-1}, [H_2SO_4] = 1.3 mol L^{-1} at different injection concentrations of [Captopril]; (a) 0.01 mol L^{-1}, (b) 0.02 mol L^{-1}, (c) 0.03 mol L^{-1}, (d) 0.04 mol L^{-1} at induction period. Temperature = 35 ^oC

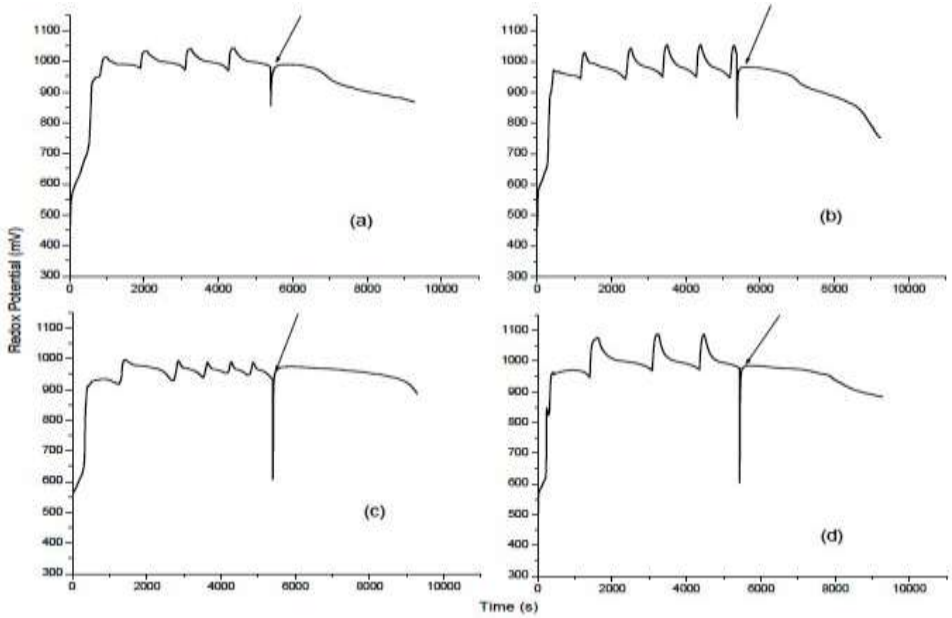
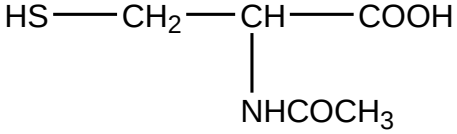


Figure 9: Potential versus time plots for the BZ system containing [AP] = 0.06 mol L^{-1}, [BrO_3^-] = 0.08 mol L^{-1}, [Ce(IV)] = 0.0055 mol L^{-1}, [H_2SO_4] = 1.3 mol L^{-1} at different injection concentrations of [Captopril]; (a) 0.01 mol L^{-1}, (b) 0.02 mol L^{-1}, (c) 0.03 mol L^{-1}, (d) 0.04 mol L^{-1} after the commencement of oscillations. Temperature = 35 ^oC

As we increased the concentration of captopril from 0.01 to 0.03 mol L^{-1}, when injected at t = 0 s, there is first increase in induction period, time period and amplitude of oscillations and then decrease, with a chaotic behavior associated with decrease in the number of oscillations as shown in Figure 7. However, when captopril is injected just after the induction time, it shows a linear relationship of potential dip with increase in [captopril] from 0.01 to 0.04 mol L^{-1} (R^2 = 0.9999). Here it can be due to enhanced interactions of captopril with the $[\text{bromo-acetaminophen}]_{crit}$ formed during the induction period. However, there is first increase in time period and amplitude of oscillations followed by larger decrease similar to glutathione, with increased [captopril] (Figure 8). The perturbation of this acetaminophen BZ system using captopril just after induction time can be helpful in determining the concentration of the same from various samples. The captopril when injected after the commencement of oscillations also shows a linear relationship of potential dip with increasing [captopril] (R^2 = 0.9751) as shown in Figure 9. Further, the reaction of captopril with the oxybromine species is evident from the change in oscillatory parameters observed for these systems as suggested by Sastry *et al.*, in 1998. But the change depends on the concentration and injection time of perturbant. Hence, the perturbations caused to this are due to reaction of acetaminophen with captopril, bromination of acetaminophen with simultaneous bromination of captopril in the acidic bromate-bromide mixture and interaction of oxybromine species with the thiol group of captopril.

N-acetylcysteine (NAC) comes from the amino acid L-cysteine. Aminoacids are the building blocks of proteins. NAC has many uses in medicine. It is used to counteract acetaminophen and carbon monoxide poisoning. It is used in chest pain, bile duct blockade in infants, Alzheimers's disease, reducing levels of cholesterol, epilepsy and as antioxidant. It is also used in breaking di-sulfide bonds in mucus to liquefy it.



N-ACETYLCYSTEINE (NAC)

Research Article

The introduction of N-acetylcysteine intravenously in acetaminophen (paracetamol) overdose makes it to interact with the poisonous forms of acetaminophen formed in liver. When paracetamol is taken in large quantities, a metabolite called N-acetyl-p-benzoquinone imine (NAPQI) accumulates within the body, which reacts with key hepatic enzymes, resulting in severe liver damage and sometimes even death. NAC is a precursor in the formation of the antioxidant glutathione in the body and the thiol group confers radical scavenging effects. This tempted us to use AC as perturbant in the acetaminophen based BZ reaction, as it can directly quench the radicals generated in this reaction system in vitro.

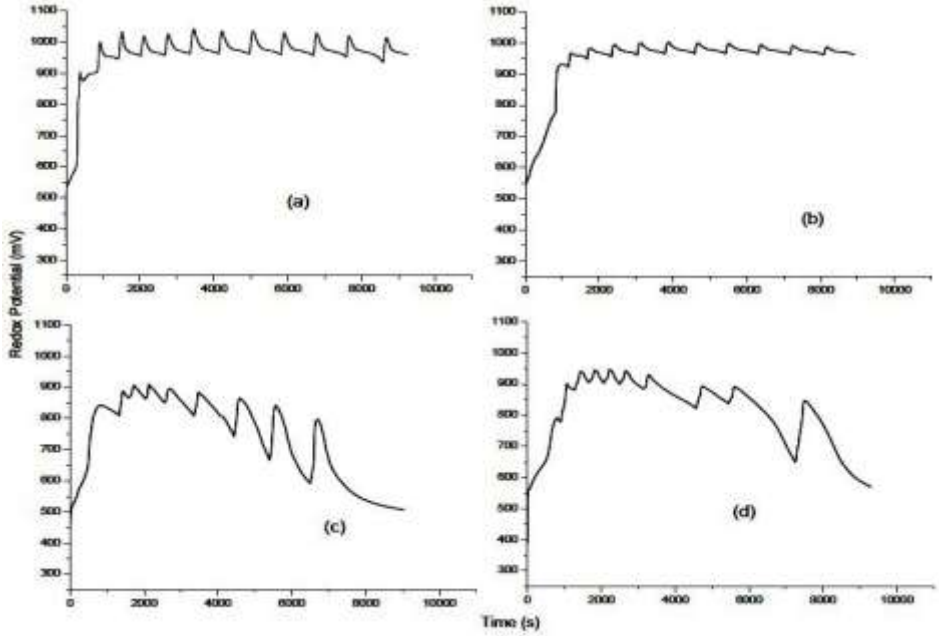


Figure 10: Potential versus time plots for the BZ system containing [AP] = 0.06 mol L^{-1}, [BrO_3^-] = 0.08 mol L^{-1}, [Ce(IV)] = 0.0055 mol L^{-1}, [H_2SO_4] = 1.3 mol L^{-1} at different injection concentrations of [N-acetyl cysteine]; (a) 0.009 mol L^{-1}, (b) 0.01mol L^{-1}, (c) 0.02 mol L^{-1}, (d) 0.03 mol L^{-1} at t= 0 s. Temperature = 35 ^oC

Figure 10 shows the effect of perturbant NAC at different concentrations and at injection time t=0 s. At t = 0 s, there is increase in induction period and amplitude of oscillations with increasing [NAC] from 0.009 to 0.04 mol L^{-1} and it shows a linear trend corresponding to R^2 = 0.986 and 0.953 respectively. However, there is decrease in time period and number of oscillations. It is also mentioned here that after a long time, the amplitude shows a marked increase with increasing time period for the same system at higher concentrations of NAC. This can be due to combined effect of acetaminophen and NAC acting as co-substrates and competing with the generated BZ intermediates as depicted in Figure 10. When NAC is injected just after the induction time, there is first increase and then decrease in time period, potential dip and amplitude of oscillations. However, the number of oscillations showed a continuous decrease with increasing [NAC] from 0.009 to 0.04 mol L^{-1}. The relatively larger oscillatory parameters at 0.01mol L^{-1} can be because of effective interaction of NAC with $[\text{bromo-substrate}]_{crit}$ accompanied by interactions with oxybromine species generated in situ. The larger potential dip at higher [NAC] and then subsequent quenching of oscillations shows potent antioxidant nature of NAC in this BZ system. Further, it is observed that NAC when injected after the commencement of oscillations, the potential dip experiences behavior just opposite to one observed when NAC is injected just after induction time. As we know from FKN mechanism that there is ample generation of oxy-bromine radicals/species viz. $BrO_2^{\cdot}$, HOBr, BrO_3^-, etc during oscillations than in induction period, the NAC will quench these radicals effectively resulting in decrease and then removal of oscillations from the system with increasing [NAC] injected during oscillations.

Acetaminophen: an analgesic acts as a potential substrate in a Belousov-Zhabotinsky reaction in aqueous sulfuric acid in presence of Ce^{4+} as a catalyst. It is observed that initial reagent concentration plays a critical role in bifurcation phenomenon. The concentration ranges of the different initial reagents indicate the narrow oscillation window for each reagent. The amplitude of oscillation is directly proportional to bromate concentration, i.e. concentration of Ce^{4+}/Ce^{3+} increases monotonically with increase in concentration of bromate. Oscillatory parameters seem to be much more sensitive to the concentrations of acetaminophen and bromate than that of cerium. Further, it was observed that with increase in initial concentration of any of the aforesaid reagents, the induction period is reduced. This can be due to the increased rate of formation of critical bromosubstrate. The time period of the oscillation increases with increase in initial concentration of bromate and that of the acid while as it decreases with increase in concentration of cerium and acetaminophen.
Regarding the effect of three radical scavenging drugs like glutathione, captopril and N-acetylcysteine, the mechanism and kinetics shows some similarity in using glutathione and N-acetylcysteine, as the later is the precursor in the formation of the former. However, the NAC is potent antioxidant than the glutathione. All these drugs when injected at t = 0 s and just after induction time can be used in determining their concentrations using Analyte Pulse Perturbation technique taking in account the potential dip or induction period, while as when used after the commencement of oscillations their antioxidant activity with respect to quenching of oscillations becomes apparent.

ACKNOWLEDGEMENTS
The authors are thankful to Department of Science and Technology, Government of India for providing grant in the form of FIST level-1 to the department. The authors also thank Head, Department of Chemistry University of Kashmir for the infrastructural facilities to carry out this work.

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