

## Frequency Control of an Oscillatory Reaction by Reversible Binding of an Autocatalyst

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We demonstrate a novel mechanism for controlling the frequency, amplitude, and existence of chemical oscillations. Using a ferrocyanide-iodate-sulfite reaction, we show that a chemical buffer can increase or decrease the frequency of oscillations. Furthermore, the same buffer can completely suppress or, conversely, induce oscillations when added to the appropriate composition. This simple buffering scheme can provide a practical method for controlling the oscillations of a variety of chemical and biological systems. [S0031-9007(99)08396-9]

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The speed and efficiency of many chemical and biochemical systems depend strongly on the *pH* of the reaction environment. Optimization of these reactions is often achieved by regulating the hydrogen ion concentration with a *pH* buffer [1,2]. Although the maintenance of a stable *pH* is desirable in many applications, in some cases, such as drug delivery, the controlled periodic variation of *pH* is preferred. For example, novel drug-delivery systems take advantage of membranes with *pH*-dependent permeabilities [3–5]. Controllable oscillations in *pH* would provide a mechanism for accurately timed drug release.

Past theoretical studies of a simplified model of glycolysis suggest that buffering one of the reacting species can reduce the frequency of oscillations in the system. [6,7]. However, this analysis is limited to a two-variable system and requires an unlimited quantity of buffer. Here we employ the mixed Landolt reaction to demonstrate, both experimentally and numerically, that buffering a reacting species can (i) decrease or *increase* the frequency of *pH* oscillations, (ii) reduce the amplitude of oscillations, and (iii) completely suppress or *induce* oscillations.

Oscillations in the mixed Landolt reaction arise from iodate oxidation of sulfite in the presence of ferrocyanide in a continuously fed stirred tank reactor (CSTR) [8]. Hydrogen ion is an autocatalytic species in the reaction and thus exhibits a large and brief increase in concentration during each cycle. To control the oscillations, we introduce a phosphate buffer, which reversibly binds hydrogen ions. The phosphate buffer is well suited as a modulator of the oscillations because it does not participate in reactions other than acid-base equilibria, and its buffering range coincides with the *pH* range of the oscillations. By varying the concentration of buffer and the monohydrogen/dihydrogen phosphate ratio, we can tune the frequency and amplitude of the oscillations.

Experiments were carried out in a thermostated well-stirred reactor (volume 20.2 ml) at a constant temperature of 40 °C. The reagents were fed into the reactor by a Rainin peristaltic pump without premixing. The input concentra-

tions of reagents were 0.075*M* KIO<sub>3</sub>, 0.09*M* Na<sub>2</sub>SO<sub>3</sub>, and 0.022*M* K<sub>4</sub>Fe(CN)<sub>6</sub> · H<sub>2</sub>O (all analytical grade, Aldrich). Unless otherwise stated, the input concentration of sulfuric acid was 0.005*M*. The mean residence time was fixed at 500 s throughout the experiments. The progress of the reaction was followed with a combined *pH* glass electrode, and the reaction was monitored by an Orion SA 720 *pH* meter.

For our numerical simulations, we employ the model of the mixed Landolt reaction suggested by Rábai, Kaminaga, and Hanazaki [9] (RKH model). The reaction scheme, including phosphate acid equilibria, rate laws, and rate constants, is given in Table I. Mass balances in the CSTR configuration are written as

$$\frac{dc_j}{dt} = f_j(c_1, \dots, c_n) + k_0(c_{j,0} - c_j). \quad (1)$$

Here  $c_j$  is the concentration of the  $j$ th species,  $f_j$  is the function describing its reaction kinetics by combining rate laws  $R_i$  of the composite reactions in Table I,  $k_0$  is the reciprocal of the mean residence time, and  $c_{j,0}$  is the input concentration of the  $j$ th component. In the buffer-free system, the total number of species  $n$  is 10; in the system with phosphate buffer,  $n = 12$ . For all simulations, residence time and input concentrations are identical to those used in the experiments.

In both the experiments and the simulations, we add solutions of pure Na<sub>2</sub>HPO<sub>4</sub>, pure NaH<sub>2</sub>PO<sub>4</sub>, or mixtures of Na<sub>2</sub>HPO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub> to the CSTR input. Figure 1 illustrates the frequency and amplitude changes in the presence of small amounts of phosphate buffer. The addition of 0.003*M* Na<sub>2</sub>HPO<sub>4</sub> buffer causes a decrease in the frequency of oscillations (Fig. 1b), while the addition of 0.006*M* NaH<sub>2</sub>PO<sub>4</sub> causes an increase in the frequency of oscillations (Fig. 1c). In both cases, the amplitude of oscillations is reduced by the addition of the buffer. When the concentration of monohydrogen or dihydrogen phosphate buffer is increased, the frequency and amplitude changes become more pronounced (Fig. 1d).

TABLE I. RKH scheme of mixed Landolt reaction.

Irreversible reactions	
$\text{IO}_3^- + 3\text{HSO}_3^- \rightarrow \text{I}^- + 3\text{SO}_4^{2-} + 3\text{H}^+$	
$\text{IO}_3^- + 5\text{I}^- + 6\text{H}^+ \rightarrow \text{I}_2 + 3\text{I}_2 + 3\text{H}_2\text{O}$	
$\text{I}_2 + \text{HSO}_3^- + \text{H}_2\text{O} \rightarrow 2\text{I}^- + 3\text{H}^+ + \text{SO}_4^{2-}$	
$\text{I}_2 + 2\text{Fe}(\text{CN})_6^{4-} \rightarrow 2\text{I}^- + 2\text{Fe}(\text{CN})_6^{3-}$	
$2\text{Fe}(\text{CN})_6^{3-} + \text{SO}_3^{2-} + \text{H}_2\text{O} \rightarrow 2\text{Fe}(\text{CN})_6^{4-} + \text{SO}_4^{2-} + 2\text{H}^+$	
Equilibria	
$\text{SO}_3^{2-} + \text{H}^+ \leftrightarrow \text{HSO}_3^-$	
$\text{Fe}(\text{CN})_6^{4-} + \text{H}^+ \leftrightarrow \text{HFe}(\text{CN})_6^{3-}$	
$\text{I}_2 + \text{I}^- \leftrightarrow \text{I}_3^-$	
Buffer equilibria	
$\text{H}_2\text{PO}_4^- + \text{H}^+ \leftrightarrow \text{H}_3\text{PO}_4$	
$\text{HPO}_4^{2-} + \text{H}^+ \leftrightarrow \text{H}_2\text{PO}_4^-$	
Rate laws	
$R_1 = k_1[\text{IO}_3^-][\text{HSO}_3^-][\text{H}^+] + k'_1[\text{IO}_3^-][\text{HSO}_3^-]^2$	
$R_2 = k_2[\text{IO}_3^-][\text{I}^-]^2[\text{H}^+]^2$	
$R_3 = k_3[\text{I}_2][\text{HSO}_3^-]$	
$R_4 = k_4[\text{I}_3^-][\text{Fe}(\text{CN})_6^{4-}]$	
$R_5 = k_5[\text{SO}_3^{2-}][\text{Fe}(\text{CN})_6^{3-}] + k'_5[\text{HSO}_3^-][\text{Fe}(\text{CN})_6^{3-}]$	
Rate and equilibrium constants	
$k_1 = 2.0 \times 10^4 M^{-2} s^{-1}, k'_1 = 30 M^{-2} s^{-1}$	
$k_2 = 8.1 \times 10^8 M^{-4} s^{-1}$	
$k_3 = 2.3 \times 10^7 M^{-1} s^{-1}$	
$k_4 = 4.0 \times 10^3 M^{-1} s^{-1}$	
$k_5 = 0.03 M^{-1} s^{-1}, k'_5 = 0.03 M^{-1} s^{-1}$	
$K_6 = 1 \times 10^7 M^{-1}$	
$K_7 = 1000 M^{-1}$	
$K_8 = 720 M^{-1}$	
$K_9 = 166 M^{-1}$	
$K_{10} = 1.45 \times 10^7 M^{-1}$	

The addition of monohydrogen or dihydrogen phosphate buffer can also result in qualitative changes in the oscillatory dynamics, i.e., suppression or induction of oscillations. When the hydrogen ion concentration of the input  $\text{H}_2\text{SO}_4$ ,  $[\text{H}^+]_0$ , is  $0.01M$ , oscillations are completely suppressed by the addition of  $0.004M$   $\text{Na}_2\text{HPO}_4$  (Fig. 2a). A much larger amount of  $\text{NaH}_2\text{PO}_4$  (at least  $0.02M$ ) is required to achieve the same effect. Conversely, the addition of phosphate buffer can induce oscillations when the buffer-free system is in a steady state. When  $0.003M$   $\text{Na}_2\text{HPO}_4$  is added to the reaction with  $[\text{H}^+]_0 = 0.012M$ , the steady state is destabilized and oscillations emerge (Fig. 2b).

For a phosphate buffer containing a mixture of  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$ , we observe three different effects: (i) a frequency increase when the average rate of  $\text{H}^+$  release from dihydrogen phosphate is larger than the average rate of  $\text{H}^+$  binding by monohydrogen phosphate, (ii) a frequency decrease when this ratio is opposite,

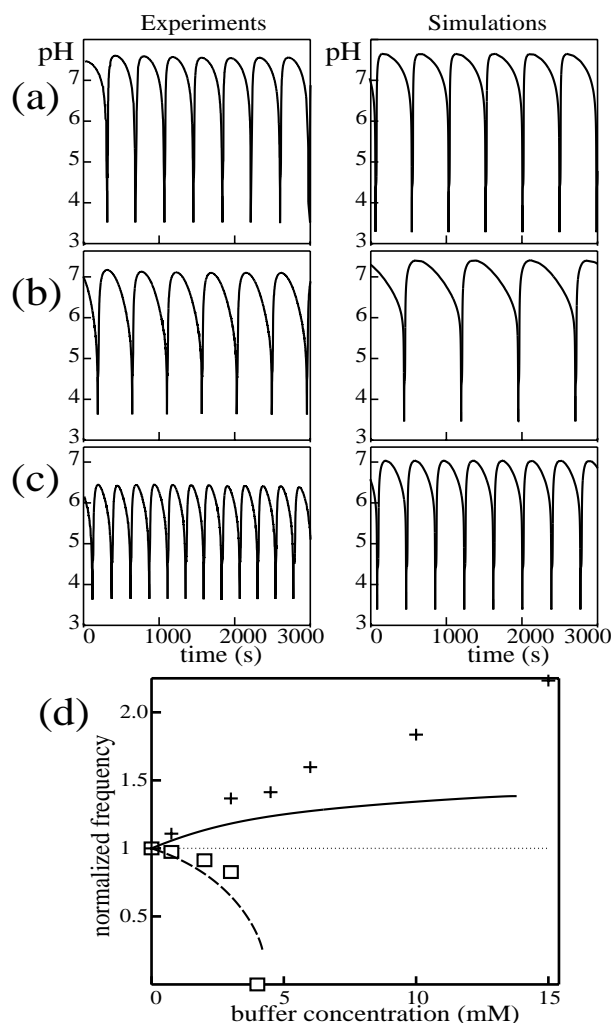


FIG. 1. Effect of phosphate buffer on oscillatory dynamics—experiment and simulation. (a) Oscillations without added phosphate buffer; (b) decrease of frequency in the presence of  $0.003M$   $\text{Na}_2\text{HPO}_4$ ; (c) increase of frequency in the presence of  $0.006M$   $\text{Na}_2\text{H}_2\text{PO}_4$ ; and (d) frequency dependence on the amount of added phosphate buffer. Lines represent simulations; crosses and squares correspond to experimental results. The addition of monohydrogen phosphate is indicated by the dashed line and squares; the addition of dihydrogen phosphate is indicated by the solid line and crosses. Frequencies are normalized to the basic frequency  $f_b$  of buffer-free oscillations.

and (iii) no change in frequency when the rates are approximately equal. The amplitude of oscillations is reduced in all cases. For example, an equimolar mixture of  $0.003M$   $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$  does not affect the frequency, but it does reduce the amplitude of oscillations.

Using experimental data, we construct a map of the oscillatory domain in the parametric space of monohydrogen phosphate concentration versus dihydrogen phosphate concentration for  $[\text{H}^+]_0 = 0.01M$  (Fig. 3a). In the absence of buffer, the reaction is oscillatory. Hence the addition of buffer can modulate or suppress oscillations. Numerical simulations reveal the structure and shape of the oscillatory domain in detail (Fig. 3b). The relative amounts

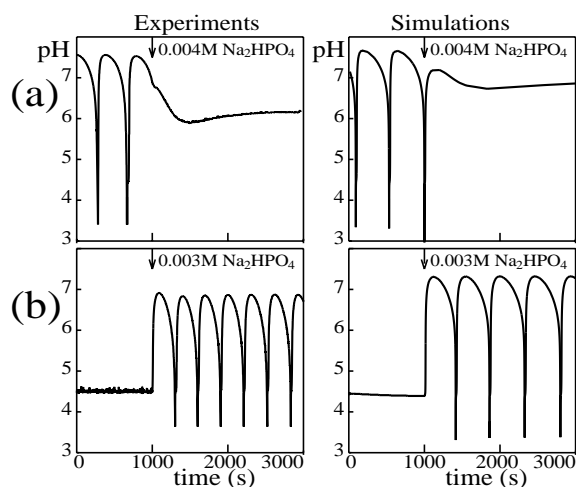


FIG. 2. Suppression and induction of oscillations—experiment and simulation. Arrows indicate the initiation of the phosphate buffer addition. (a) For  $[H^+]_0 = 0.010M$ , a small amount of monohydrogen phosphate buffer suppresses the oscillations. The reaction stabilizes in a high- $pH$  steady state. (b) For  $[H^+]_0 = 0.012M$ , a small amount of monohydrogen phosphate buffer destabilizes the low- $pH$  steady state, and oscillations are induced.

of monohydrogen and dihydrogen phosphate determine whether the frequency is increased or decreased. For most buffer compositions, high concentrations of buffer will suppress oscillations. However, we observe that, for a narrow range of monohydrogen/dihydrogen phosphate ratios, the reaction maintains oscillations in the presence of high concentrations of buffer. Furthermore, we note that, for certain buffer compositions (i.e., dihydrogen/monohydrogen phosphate ratio near 30:1—dotted line in Fig. 3b), oscillations will be alternately suppressed and induced as the buffer concentration is increased.

We also constructed maps of the monohydrogen/dihydrogen phosphate parameter space for systems in which oscillations are induced by the buffer solution (Fig. 4). In the absence of buffer, the mixed Landolt reaction can maintain a high- $pH$  steady state or a low- $pH$  steady state. In the high- $pH$  steady state ( $[H^+]_0 = 0.006M$ ), a buffer with only monohydrogen phosphate maintains the steady state; dihydrogen phosphate must be present to induce oscillations (Fig. 4a). On the other hand, when the buffer-free system is at the low- $pH$  steady state ( $[H^+]_0 = 0.012M$ ), the addition of buffer with some monohydrogen phosphate is necessary to induce oscillations (Fig. 4b).

Although it has been shown that the frequency of oscillations depends on the input sulfuric acid concentration [8], the observed frequency changes are not simply the consequence of the altered  $pH$  of the input sulfuric acid. A typical input  $H_2SO_4$  concentration is 5 mM ( $pH \approx 2$  at 40°C). Thus, the addition of monohydrogen phosphate ( $pK_2 = 7.16$  [1]), dihydrogen phosphate ( $pK_1 = 2.22$  [1]), or a mixture of both to the sulfuric acid will increase the  $pH$  of the input  $H_2SO_4$ . If the oscillation

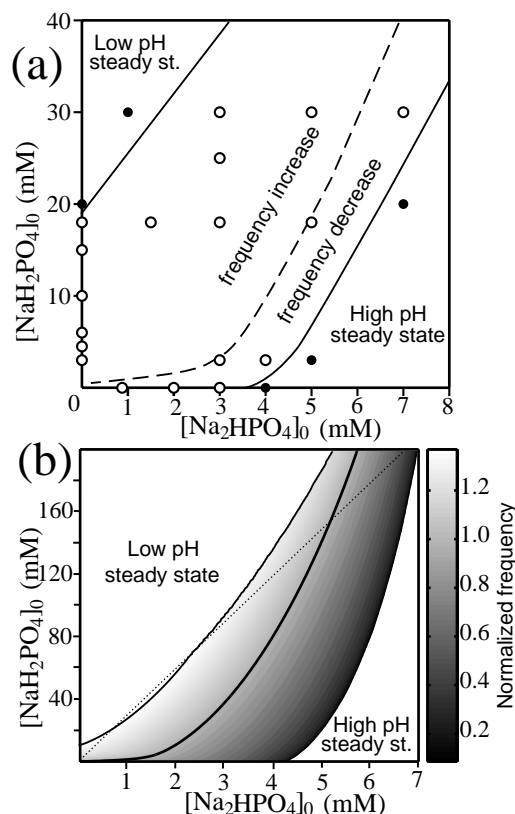


FIG. 3. Maps of the oscillatory region of the mixed Landolt reaction in the presence of phosphate buffer. The buffer-free system is oscillatory ( $[H^+]_0 = 0.010M$ ). (a) Experiment: addition of the phosphate buffer modulates the frequency (open circles) or suppresses oscillations completely (filled circles). Solid lines indicate estimated boundaries of the oscillatory domain. The dashed line indicates the estimated boundary between domains of frequency increase and decrease. (b) Simulations: addition of the phosphate buffer modulates the frequency (inside the gray-shaded region) or suppresses oscillation completely (outside the gray-shaded region). Frequencies are normalized to the basic frequency  $f_b = 0.123 \text{ min}^{-1}$ . Steady-state and oscillatory regions are separated by a Hopf bifurcation. For an explanation of the dotted line, see text.

frequency depended simply on the  $pH$  of the input  $H_2SO_4$ , then the addition of any phosphate buffer would decrease the frequency. However, as our results show, the frequency decreases only with the addition of monohydrogen phosphate, while the addition of dihydrogen phosphate increases the frequency. These effects are due to dynamic interaction of the buffer with the oscillatory reaction. The range of  $pH$  oscillations is approximately 3.5–7 (Fig. 1). Thus, the molecules of dihydrogen phosphate, with a low  $pK$ , release  $H^+$  ions as soon as they enter the reactor. This release of  $H^+$  ions enhances the autocatalytic phase of the reaction, and the frequency increases. On the other hand, the  $pK$  of monohydrogen phosphate is close to the maximum  $pH$  of the oscillations. When molecules of monohydrogen phosphate enter the reactor, they bind free  $H^+$ , resulting in a slower reaction during the autocatalytic phase and a frequency decrease.

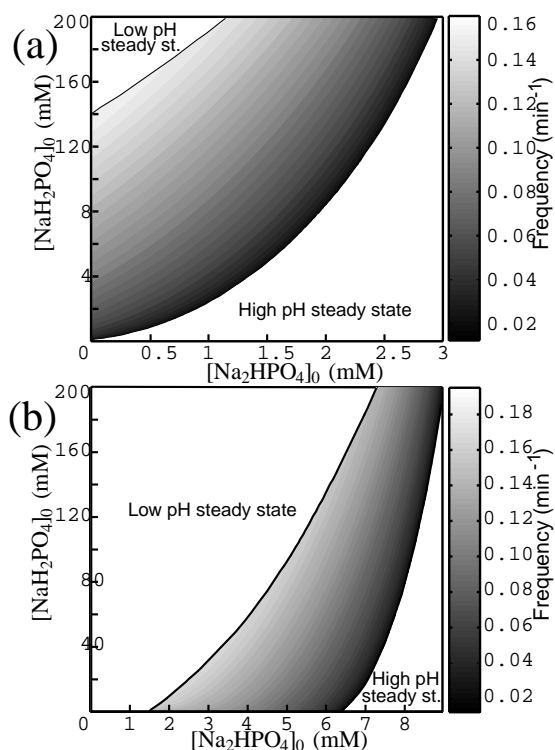


FIG. 4. Induction of oscillations by a phosphate buffer—simulation results. (a) The buffer-free system rests at high- $p\text{H}$  steady state ( $[\text{H}^+]_0 = 0.006M$ ). (b) The buffer-free system is at low- $p\text{H}$  steady state ( $[\text{H}^+]_0 = 0.012M$ ). Oscillations are induced inside the gray-shaded regions.

The observed amplitude changes are also the result of acid-base equilibria. Free hydrogen ions are released at the  $p\text{H}$  maximum and bound at the  $p\text{H}$  minimum. Thus, the maximum  $p\text{H}$  of the oscillations is reduced and the minimum is increased. We also observe that the buffer influences the maximum of  $p\text{H}$  oscillations more strongly than the minimum (Fig. 1). At high  $p\text{H}$ , the release of a small number of hydrogen ions from the bound form results in significant relative changes in  $[\text{H}^+]$ , but, at low  $p\text{H}$ , the binding of a similar number of hydrogen ions by the buffer is relatively insignificant in comparison with the concentration of hydrogen ions already in solution.

The analogous use of a passive load to modulate the frequency of an oscillatory system has also been examined in neurobiological systems. Studies of an oscillating neuron with injected current [10] or electrically coupled to a passive cell [11] revealed a monotonic (increasing) and a nonmonotonic (U-shaped) frequency dependence on the amount of the passive load. Our study shows that a chemical oscillator can display similar behavior: monotonic (either increasing or decreasing) or nonmonotonic dependence of the period on the concentration of added buffer. However, we show that it is not necessary to change the intrinsic properties of the oscillatory system to

obtain different frequency dependences. The different behaviors can be obtained by adjusting the composition of a multicomponent load.

Oscillatory chemical reactions often serve as convenient model systems for understanding the dynamics of more complex biological systems. Control of  $p\text{H}$  oscillations in the mixed Landolt reaction by buffering hydrogen ions is analogous to control of the cell division cycle (CDC) by reversible inhibition of cyclin (one of the CDC proteins). We have recently shown, through mathematical modeling, that the dynamics of the cell cycle can be manipulated by introducing a reversible inhibitor of one of the key species [12]. Thus, the experimental results presented here, combined with recent work by Gray *et al.* [13] concerning the design of a variety of cyclin-dependent kinase inhibitors, suggest a practical means for implementing our method to control cell division. Moreover, because our technique relies only on the buffering of one of the reacting species, it should be applicable to a variety of chemical and biochemical systems.

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