Time Capsule: An autonomous sensor and recorder based on diffusion-reaction

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Abstract:

We describe the use of chemical diffusion and reaction to record temporally-varying chemical information as spatial patterns without the need for external power. Diffusion of chemicals acts as a clock, while reactions forming immobile products possessing defined optical properties perform sensing and recording functions simultaneously. The spatial location of the products reflects the history of exposure to the detected substances of interest. We refer to our device as a time capsule, and show an initial proof of principle in the autonomous detection of lead ions in water.

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Fundamentally, all methods for recording temporal information involve a transformation of time-varying signals into signals that vary in space. From Edison's phonographs, magnetic tape recorders, to CDs and DVDs, the clock is given by the mechanical movement of the recording medium (e.g. magnetic tape), such that incoming signals at different times are recorded at different locations of the medium.[1] The actual recording involves a change in the state of the material. Electronic and solid-state memory devices do not involve mechanically moving parts. They still require a source of power, and involve, in the most general sense, the movement and storage of charges in different capacitors or transistors when signals arrive at different times.[2] More recently, synthetic biology has allowed the genetic engineering of *E. coli* for recording single exposure to chemical stimulants by arresting cell division and activating cell division to cause an elongation in cell length.[3] The time signal is transformed into spatial information in the form of the length of the cell.

We hypothesize that the movement of chemicals in space, as governed by Brownian motion and diffusion down a concentration gradient, can also serve as a clock for recording. Irreversible reactions leading to a change in the state of matter (e.g. precipitation from soluble reactants) designed to occur at the diffusion front of the chemicals can perform the recording function.[4] The reaction can be chosen to be specific to the substance of interest ("analyte"). Here, instead of using chemical gradients for extracting electrons as used in batteries, we use them for new functions—timing, sensing, recording, and reporting information. The key advantage of this scheme is that diffusion down a gradient is "free" and does not require external power to operate, so long as a concentration gradient is present. It also allows the direct transduction of biochemical signals into optical readout without the need for mechanical or electrical inputs.[5]

A few commercial products, such as self-expiring visitor badges, have used the diffusion of inks for the indication of time.[6-8] When the expiration time is reached, an additional message (e.g. "Expired") appears on the badge. The timing is based on the diffusion of ink from a bottom layer to the top layer through an opaque intermediate layer. Only when the ink reaches the top does the hidden message become visible. This method has been engineered to operate from minutes to days.[6-8] Recently, a similar principle has been used in timing paper-based diagnostic assays.[9] These applications show that diffusion is a fairly robust indicator of time without the need for external power. No work thus far, however, has demonstrated its use for *recording* temporal information. Here, we show the use of diffusion-reaction for transforming, and thereby recording, temporal dynamics into spatially varying patterns. The concept loosely resembles that of tree rings, where the position of the rings indicates the history of the growth conditions of the tree. We refer to our device as a time capsule as it captures the history of the substance of interest.

Figure 1a shows the design of the time capsule. The device consists of a one-dimensional porous substrate through which chemicals diffuse. Apart from the left and the right ends, the substrate is sealed in poly(dimethylsiloxane) (PDMS). The left end is in direct contact with a reservoir where a "timer reactant" is pre-loaded. This reservoir is sealed in PDMS except at the contact with the porous substrate, from which the timer reactant diffuses in the $+x$ direction. The right end of the porous substrate is open to the environment that may contain analyte at unknown times. If the analyte is present at time t, it diffuses into the porous substrate in the $-x$ direction. As this diffusion front meets the diffusion front of the timer reactant, an irreversible reaction—precipitation here—occurs. The reaction product is immobile and forms a visible band within the porous substrate. The time that the analyte was present can then be derived from the position and the profile of the precipitation band.

We demonstrate the principle of the time capsule for the detection of lead ions in water. We used potassium chromate ($K_2CrO_{4(aq)}$, 50 mM) as the timer reactant, and lead nitrate ($Pb(NO_3)_{2(aq)}$, 5 mM – 20 mM) as the analyte. We chose this combination of chemicals as the precipitate lead chromate ($PbCrO_{4(s)}$) has a low solubility (0.2 mg/L in water).[10] We note that other ions (e.g., silver, mercury) will also precipitate with chromate. In this first proof of concept, we focus on demonstrating a novel time recording function. As such, we have chosen a simple precipitation reaction. Future work will employ chemistries with increased specificity towards analytes of interest, for example, by using developed methods in immunoprecipitation, and agglutination assays (also see ESI S8).

A relatively large reservoir with a volume of 70 μL was used for the timer reactant to ensure that its concentration is constant over the course of the experiment (2 hours). We fabricated our device in PDMS using standard soft lithography (see ESI for fabrication details).[11, 12] The porous substrate, through which the timer reactant and the analyte diffused, was generated by filling the PDMS microchannel (width × height = 1 mm × 30 μm) with 0.5 % agar gel. This water-permeable substrate was chosen as the analyte and the timer reactant were both in aqueous phase. **Figure 1b** shows the expected evolution of the concentration profiles of timer reactant, analyte, and precipitate over time. The profiles were calculated using the *Transport of Diluted Species* module in COMSOL Multiphysics (COMSOL 4.4, Stockholm, Sweden). For this calculation only, the timer reactant (50 mM) and the analyte (10 mM) were set to be constant over the entire duration. Precipitate formed and accumulated where the diffusion fronts of the timer reactant and the analyte met. **Figure 1c** shows a prototype of the device consisting of two independent channels having lengths of 5 mm and 10 mm respectively. These lengths are chosen to facilitate the recording and direct observation of chemical signals over a duration of two hours.

To increase the operational duration of the device, a porous substrate with increased length or decreased porosity leading to decreased diffusion coefficients can be used. The rationale for including two channels will be discussed and illustrated later in Figure 3. **Figure 1d** shows an experimental setup to characterize the prototype. It was immersed in deionized water, which would be replaced with solutions of lead nitrate at various times. In actual applications, the device could be potentially dispersed in a flow, for example, to detect the location of lead contamination. For analysis, we imaged the formation of the precipitate under a stereoscope onto a CMOS camera (Infinity 2.0, Lumenera). We used MATLAB to extract the intensity profiles of the precipitates. We also performed numerical simulations in COMSOL Multiphysics to complement some experiments.

We first characterized the ability of the time capsule to record a single event. The timer reactant ($CrO_4^{2-}{}_{(aq)}$) started diffusing into a 10-mm long porous substrate at $t = 0$. The device was initially immersed in deionized water, and was later exposed to a fixed $Pb^{2+}{}_{(aq)}$ concentration (10 mM) for $t \geq t_{Pb}$, where t_{Pb} indicates the start time of the lead exposure. **Figure 2a** shows a series of images of precipitation bands formed in the porous substrate observed at $t = 2$ hours, for $t_{Pb} = $ 0, 10, 20, 30, 40, 50, and 60 minutes respectively. The positions of the precipitation bands were clearly distinguishable for different analyte histories (i.e., different t_{Pb}). ESI movie S1 shows a time-lapse movie of the formation of the precipitation bands. While the diffusion of a single component follows simple Fick's law, the combined diffusion-reaction equation is not simple to generalize. The dynamics of band formation and the band profile can be predicted qualitatively, however, based on the instantaneous local concentration gradients of the timer reactant and the analyte (see ESI S7). For simplicity of analysis, we used the position of the left edges only to indicate the location of the bands for subsequent discussions. To quantify the left edge, we

inverted the colormap of the recorded images, such that the bands appeared white and the substrate appeared black (intensity = 0). Since the concentration profile of the precipitate and the corresponding intensity profile within the band were not uniform (see Figure 1b), we identified the location of the left edge x_{band} as the location where the intensity of the precipitation band reached 1 % of the maximum intensity of each individual band. **Figure 2b** shows the left edge position x_{band} as a function of t_{Pb}, the time when $Pb^{2+}_{(aq)}$ was first introduced. The relationship was approximately linear over the range of t_{Pb} tested. The temporal sensitivity of the device could then be found from the slope of this graph to be ~ 20 μm/min (linear fitting; R^2 = 99.3%). From the slope and the height of the error bars (≤ 50 μm; obtained from 5 experiments), a temporal resolution of 5 minutes was calculated. Although this resolution is low compared with state-of-the-art electronic counterparts, our device could find potential applications where changes in chemical signal are slow, for example, in the detection of heavy metal contaminations in environmental samples. We also note that in our simple design, the chromate would continue to diffuse out of the device at prolonged operation. Future design would include methods to sequester the chromate and prevent its diffusion into the environment.

In addition to the time of exposure t_{Pb}, the analyte concentration also affects the evolution of the precipitate profile and the position of the formed band. **Figure 3a** shows the position of the left edge of the precipitation band x_{band} as a function of t_{Pb}, at three concentrations of $Pb^{2+}_{(aq)}$ obtained from two channels having lengths of 5 mm and 10 mm respectively. As can be seen, the use of band position from a single channel would give inconclusive results about the concentration and the time of exposure. To decouple an unknown analyte concentration from an unknown time of exposure, we used an additional channel having a length of 5 mm. Figure 1c shows a prototype of this device. The use of two channel lengths allowed decoupling because

diffusion is a non-linear phenomenon, and the band position formed in a 5-mm channel was not proportional to that formed in a 10-mm channel. **Figure 3b** shows a surface plot generated by interpolating experimental data points shown in Figure 3a. The grayscale of the surface represents the concentration of the analyte. Each point on this surface was unique: each combination of $\{x_{\text{band,5 mm}}, x_{\text{band,10 mm}}\}$ gave a unique combination of $\{t_{\text{Pb}}, [\text{Pb}^{2+}_{(\text{aq})}]\}$. This surface can thus be used to look up unknown values of t_{Pb} and $[\text{Pb}^{2+}_{(\text{aq})}]$, given the band positions measured from the 5-mm and 10-mm channels respectively. Curve fitting to experimental data and the goodness-of-fit can be found in **ESI S6**.

Figure 4 shows that it is possible to use the time capsule to record multiple exposure events. To characterize the ability of our system in resolving multiple events, the device was exposed to $\text{Pb}^{2+}_{(\text{aq})}$ twice. The first exposure started at $t_{\text{Pb1}} = 0$ minute, for a duration of 40 minutes. The second exposure started at $t_{\text{Pb2}} = 45, 50, 60, 70, 80,$ and 90 minutes respectively. Figure 4a shows the results observed at $t = 2$ hours. The two exposures could be resolved only when they were separated by at least 5 minutes, below which the precipitation bands merged into a single profile. **ESI S2** shows the results when the second exposure was fixed and the timing of the first exposure varied. The measured resolution was also approximately 5 minutes. To account for increased complexity of the timing and concentration profile of the analyte, we expect that computational tools will be necessary to perform the analysis. Figure 4b shows our COMSOL simulation results of the formation of the two bands. The simulation implemented the diffusion-reaction equation (Eq. 1) for the timer reactant, analyte, and the precipitate respectively:

$$\frac{\partial}{\partial t} C_i = \frac{\partial}{\partial x}\left(D_i \frac{\partial}{\partial x} C_i \right) + R_i \qquad \text{(Eq. 1)}$$

where C_i, D_i and R_i represent the concentration, diffusion coefficient and reaction term of species i. The details of the simulation can be found in ESI S4. The simulations matched that of the experimental results with less than 2 % deviation in band positions.

Finally, we note that the effect of temperature on the precision of our device was not significant over a range of 4 °C to 37 °C. The dependence of diffusion coefficient on temperature can be estimated from the Einstein-Stokes relation $D = \frac{k_B T}{6\pi R\mu}$, where k_B is Boltzmann's constant, T is absolute temperature, R is the hydrodynamic radius of the diffusing species, and μ is the effective viscosity of the medium.[13] Based on this relation, a variation in temperature of 20 K around 293 K would result in a 6.8% variation in diffusion coefficient. The resulting variation in the precipitation band position should be smaller than this value, however, as the band position depends on the location where the counter-propagating diffusion fronts from the timer reactant and the analyte meet. Indeed, we found that experimentally, the position of the band changed by less than 3 % when the temperature changed from 4 to 37 °C (ESI S3).

Designing systems that are autonomous, self-powered, and capable of sensing the environment and reporting information is a significant engineering challenge. The system we described is an initial step in demonstrating the use of chemical diffusion-reaction for a new function, i.e. recording temporal dynamics via spatial pattern formation. Our passive, diffusion-reaction based system has multiple advantages: (i) It is simple to operate. (ii) It does not require electrical power to function and is immune to electrical interference. (iii) A chemical signal is translated directly into an optical output without involving mechanical moving parts or electronics. (iv) Diffusion-reactions are nonlinear; if designed correctly, the system can amplify small variations in input and lead to very sensitive results.[14] Although the operation duration of our current prototype is limited to 2 hours, it can be increased by using materials with lower

diffusivity, for example. The use of numerical simulations facilitates the extraction of more complex analyte dynamics. We expect our approach to complement its electronic counterparts, and could find potential applications where changes in chemical signal are slow, for example, in the detection of contaminations in environmental samples over long periods of time. Future work includes methods to trigger the release of the timer reactant to start the recording and to prevent it from draining into the environment, and methods to enhance the sensitivity of the approach.

Acknowledgment

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Fig. 1. a) Design and principle of the time capsule. A reservoir containing timer reactant at a fixed concentration is connected to a porous substrate with known length and diffusion properties. The timer reactant diffuses through the substrate down its concentration gradient at time t_0. Analyte (at unknown concentration) present at t_1 diffuses into the substrate. Precipitation occurs when the diffusion front of the analyte meets that of the timer reactant. Analysis of the band position allows one to extract the history of exposure to the analyte. b) Evolution of concentration profiles over time calculated in COMSOL. See ESI S4 for simulation details and parameters. c) A prototype of the time capsule containing two independent channels with different lengths to allow decoupling of time of exposure and concentration of analyte (see text). Both channels are exposed simultaneously to the analyte. d) Scheme of the experimental setup to characterize the device.
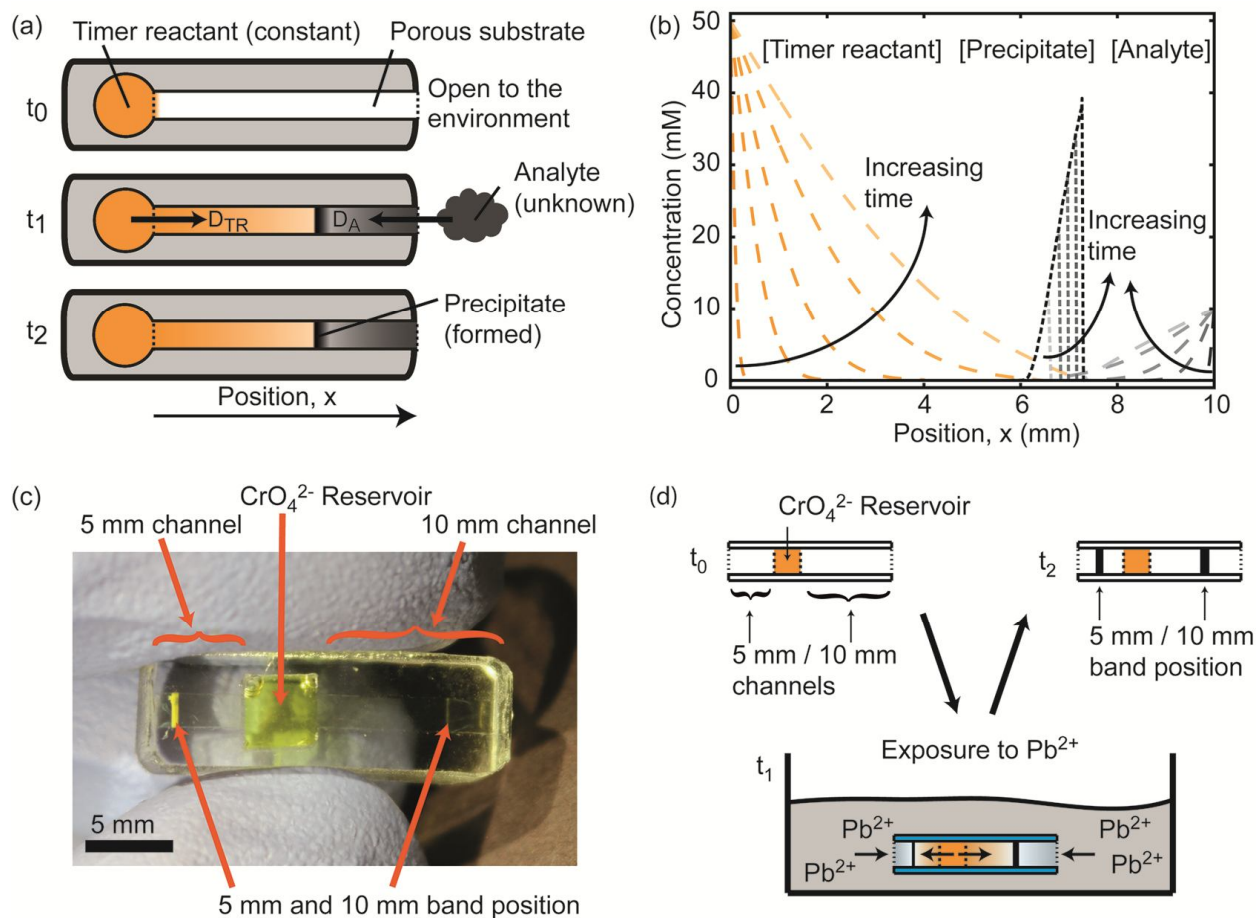
Fig. 2. a) Stereoscope images of seven independent 10 mm-channels that were exposed to 10 mM $Pb^{2+}_{(aq)}$ for different t_{Pb} . See text for experimental details. b) Left edge positions of the precipitation bands (x_{band}) as a function of t_{Pb}. A linear fit to the data gives a temporal sensitivity of 20 µm/min (R^2 = 99.3 %). The error in the measurement of x_{band} (n = 5) is less than 50 µm. The temporal resolution of the device is then calculated to be 5 minutes.
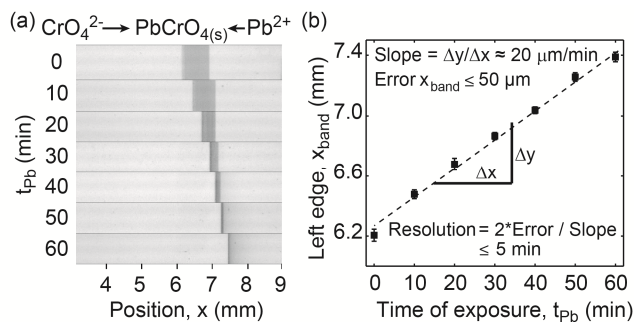
Fig. 3. a) Plots of the left edge positions of precipitation bands x_{band} as a function of t_{Pb} for three analyte concentration (5, 10, 20 mM) using channels of lengths 5 mm and 10 mm respectively. b) Interpolation of the data shown in 3a as a three-dimensional surface. To use this surface to look up unknown values of t_{Pb} and analyte concentration, a vertical line along the z-axis is drawn from the intersection of the measured values of $x_{band, 5mm}$ and $x_{band, 10mm}$ on the bottom plane. Intersecting this vertical line with the surface allows one to obtain the time of exposure (represented on the z-axis) and analyte concentration (represented in grayscale).
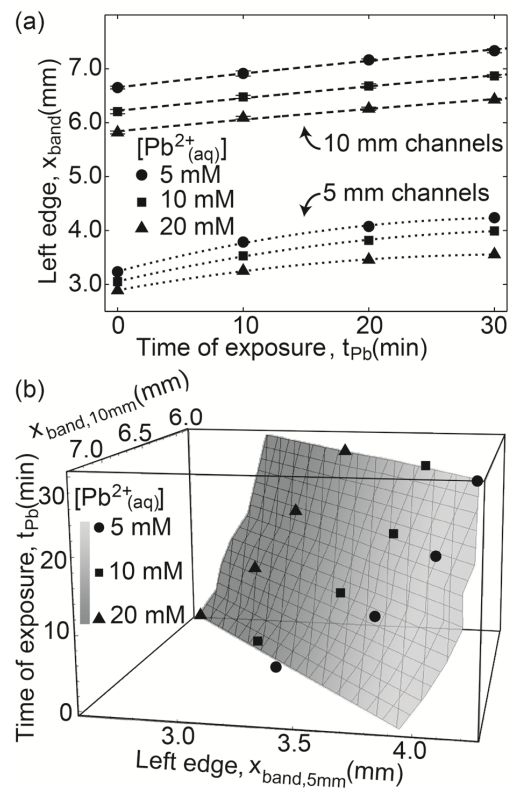
Fig. 4. Recording of multiple exposure events. a) Images of six independent 10 mm-channels after being exposed to two events occurring over the course of 2 hours. The times of exposure are indicated on the left and detailed in the text. The black lines overlaid on top of the images are intensity values extracted using MATLAB. Two events could be resolved if they are separated by 5 minutes. b) COMSOL simulation of the precipitation bands using conditions described in 4a. The simulations matched that of the experimental results with less than 2 % deviation in band positions.