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ORIGINAL PAPER

Opto-chemical micro-capillary clocks

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Abstract Opto-chemical capillary clocks are presented that are based on the measurement of a colored segment in a microchannel (a capillary). Color is created by a chromogenic chemistry involving the oxidation of a (virtually colorless) leuco-dye. Poly(ethylene glycol) (PEG) is used as a solvent, and indigo and thioindigo (in their reduced leuco forms) act as oxygen-sensitive dyes. The clock is started by removing one seal at the end of the capillary. A visible color change occurs as air diffuses into the microchannel due to an irreversible color reaction. The length of the colored segment is proportional to the time elapsed. PEGs of different average molar mass affect the diffusion rate of oxygen in the microchannel and thereby affect the rate of the migration of the color front. Both temperature and relative humidity exert a strong effect. Six types of such clocks are described that enable times to be determined in the range from 1 day to 6 months, possibly of even decades.

Keywords Optical timer · Chromogenic chemistry · Indigo · Oxygen · Clock · Poly(ethylene glycol)

Introduction

Optical timers are capable of detecting elapsed time, for example in case of food whose packaging has been opened. Such timers undergo a change in color over time that is displayed as a visually perceptible signal [1]. They can be based on chemical or physical processes [2]. These may be divided into two categories [3]. The first requires indicators that measure not only time but also take into account the actual cumulative thermal exposure of the product [4–8]. The second is based on methods in which the thermal sensitivity is minimized and the timer works much like a timepiece, giving a visual indication of the time elapsed [9–13].

Time-temperature indicators (TTIs) are probes that respond to the combined effects of time and temperature [14]. They are used in the food and pharmaceutical industry which help to determine safe storage conditions and freshness of the products [15–18]. Examples for commercially available TTIs include (a), the so-called *Fresh-Check*® (www.freshcheck.com) that is based on color change as a result of solid state polymerization of substituted monomers; (b), the *Vitsab*® (www.vitsab.info) that is based on the time-temperature dependence of enzymatic reactions; (c), the *OnVu*® time-temperature indicator (www.onvu.com) that is based on organic crystals that are activated by UV light first to become dark blue and then gradually change to lighter color as time passes [1].

Gohil [19] as well as Hu and Loconti [20] have reported on a timer consisting of a receptacle that contains a defined quantity of an oxidized redox dye and an effective quantity of a reducing agent in an alkaline medium. The conversion of the oxidized redox dye into its reduced leuco form [21] occurs within the sealed receptacle. Oxygen, on entering the receptacle, reacts with the reduced dye to give a visible color change [22]. The predetermined color transition time interval is precisely controllable. Lewis [23, 24] describes timers that consist of a generally laminar structure having an upper reactive layer which includes one or more reactive materials capable of reacting with oxygen to create a color change over time and a lower oxygen impermeable layer. The reactive layer is constructed such that the oxygen permeability of the layer is higher towards the periphery of the device but lower towards the center. This construction

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yields a gradual color transition from the rim to the center depending on the time that has elapsed.

Biritz [25] has reported on a timer based on the chromogenic reaction of a free radical of green color with oxygen to form a colorless peroxide [26–28]. This device consists of a hermetically sealed container comprising the colored oxygen-sensitive material as a lamination over a paper surface. The container has a transparent window with a pinhole-sized opening at one end of the device to allow for the ingress of air. The opening is covered by a removable tape. Once the tape is removed, air can diffuse in to start the decolorization of the green dye. The process slowly proceeds towards the other, i.e. unexposed end. The endpoint is reached once there is no color difference between the two ends. This device indicates periods of time without being affected by ambient temperature in the range from 5 to 70 °C.

Another timer that remains virtually unaffected by normal variations in temperature and humidity was reported by Lilly et al. [29]. It employs a silicone oil that slowly moves up a porous strip (by a kind of capillary action). One side of the strip is printed with an oil-soluble ink, while the other is unprinted. As the silicone oil moves up the strip, it contacts the ink and causes a dye in the ink to migrate from the printed side to the unprinted side, thus providing a measurable color front moving up the strip.

Diekmann et al. [30] reported on timers “comprising a housing enclosing a first fluid reservoir at least partially filled with a first fluid, a substrate comprising a plurality of channels, a second fluid reservoir in fluid communication with at least one channel of the substrate and a barrier located between the first fluid reservoir and the second fluid reservoir”. The device is designed such that it can provide an indication of the progress of the fluid as it moves through the channels of the substrate over time. Timestrip® (www.timestrip.com) offers commercially available time indicators or time-temperature indicators based on the properties described before.

We are reporting here on another kind of opto-chemical clock. The detection scheme is based on an oxygen-sensitive leuco-dye [31, 32] embedded in a matrix of poly(ethylene glycol) (PEG) [33] contained in a micro-capillary. A visible color change occurs as a result of the diffusion of oxygen into the open distal end of a glass microchannel and subsequent reaction of the leuco-dye with oxygen. A linear relationship is mostly observed between the length of the visible segment and the time elapsed. The pace of migration of the front of the colored segment between leuco-dye and oxidized dye can be adjusted by using PEGs of different average molar masses.

Experimental

Chemicals Indigo, poly(ethylene oxide) (with an average molecular weight of 600 kD) were purchased from Sigma (www.sigmaaldrich.com). Sodium hydroxide solution (1 M), sodium dithionite and poly(ethylene glycol) (2 kD) were from Merck (www.merck-chemicals.com), thioindigo from TCI Europe (www.tcieurope.eu), and poly(ethylene glycol) (20 kD) from Serva (www.serva.de). All chemicals were used as received. All manipulations were performed under an atmosphere of dry nitrogen gas (from Linde; www.linde-gase.de).

Instruments The port diameter of the glass microchannels (average diameter 1.1 mm) was determined with a Leica DM RE microscope (www.leica-microsystems.com). The pictures of the microchannels were acquired with a Canon Ixus 110 IS digital camera (www.canon.com) equipped with a 1/2.3 inch 12-megapixel CCD.

Preparation of the capillary clocks Solutions of leuco-indigo and leuco-thioindigo, respectively, in concentrations of approximately 3 mM were made by reduction of the respective vat dye [34] with an alkaline solution of sodium dithionite (20 mM) under an atmosphere of nitrogen at 95 °C. The clear, lightly yellow solution of the leuco-dye was mixed with the respective PEG to give a homogenous, viscous “cocktail” which was stirred for about 30 min under nitrogen atmosphere at temperatures between 75 and 95 °C. The hot cocktail was sucked into one end of a Pasteur pipette with elongated tip capillary by applying low pressure at the other end, and then cooled to room temperature under nitrogen. Data on the PEG used, mass concentrations, stirring temperature of the cocktail, dyes, and codes of the materials are given in Table 1. Each PEG was used in combination with two leuco-dyes (leuco-indigo and leuco-thioindigo) to give the materials listed in Table 1. Unless otherwise specified, all experiments were performed at room temperature (approximately 20 °C) and ambient humidity.

Table 1 Materials and experimental conditions for making the opto-chemical clocks, and respective codes

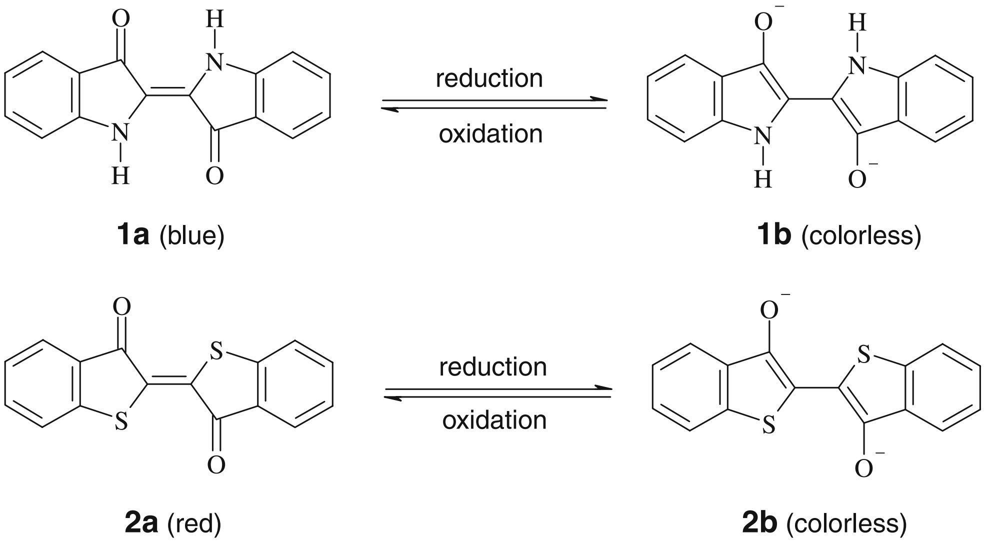
PEG[a]	PEG[b] [g/mL]	Temperature [°C]	Leuco-dye[c]	Code
PEG2	1	75	LI	LI-PEG2
PEG2	1	75	LTI	LTI-PEG2
PEG20	1	85	LI	LI-PEG20
PEG20	1	85	LTI	LTI-PEG20
PEG600	0.5	95	LI	LI-PEG600
PEG600	0.5	95	LTI	LTI-PEG600

[a] The number gives the average molecular weight in kilodalton (kD)

[b] g of PEG per mL of water

[c] *LI* leuco-indigo (Scheme 1, 1b); *LTI* leuco-thioindigo (Scheme 1, 2b)

Scheme 1 Chemical formulae of indigo (**1a**) and thioindigo (**2a**), and of the respective (colorless) leuco-forms (**1b, 2b**)



Results

Choice of materials Poly(ethylene glycol) (PEG), also known as poly(ethylene oxide) (PEO), poly(oxyethylene) (POE), or poly(oxirane) [35], is a linear neutral polyether soluble in water and most organic solvents such as toluene and chloroform. It is nontoxic and approved by the FDA for use as an excipient or as a carrier in pharmaceutical formulations, food, and cosmetics [36, 37]. It was also chosen because it is a good (polymeric) solvent for the leuco dyes and because its permeability of oxygen can be governed by proper choice of the average molecular mass. In fact, permeability increases strongly with the degree of polymerization.

Indigo and thioindigo are vat dyes that become water soluble in the presence of a suitable reducing agent. The reduced forms of indigo and thioindigo, respectively, are almost colorless—hence their names leuco-indigo [31, 32] and leuco-thioindigo. A visible color change occurs as a result of the reaction of oxygen with the leuco-dyes [34] that is schematically shown in Scheme 1. Other PEGs or other oxygen sensitive dyes may be used for creating different opto-chemical clocks. Figure 1 shows two typical pictures of such capillary clocks. The segments of LTI-PEG20 based clocks were separated with the help of an image-processing software GIMP 2.6.7. (www.gimp.org). In order to better identify the front between colored and colorless sections, the optical channels (RGB) of the digital pictures were separated.

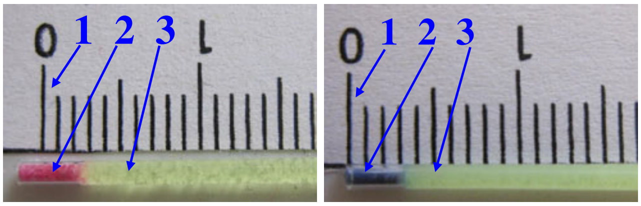


Fig. 1 *Left*: Picture of the clock LTI-PEG20. (1) mm scale, (2) thioindigo in the PEG matrix, (3) leuco-thioindigo in the PEG matrix. *Right*: Picture of the clock LI-PEG20. (1) mm scale, (2) indigo in the PEG matrix, (3) leuco-indigo in the PEG matrix

Capillary clocks based on 2-kD PEG Figure 2 shows three examples of opto-chemical clocks based on PEG with an average molecular mass of 2 kD with leuco-thioindigo. The colored segment is rather short (<1 mm) even after 6 months. Thus, an evaluation of the migration distance is hardly possible. These clocks obviously are suitable for indicating time ranges of several months to years, possibly even the typical lifespan of a human. Quantitative data on the materials used and the times covered are given in Table 2.

Capillary clocks based on 20 kD PEG Figure 3 shows examples of opto-chemical clocks based on 20 kD PEG with leuco-thioindigo (Fig. 3 left) and leuco-indigo (Fig. 3 right), and their kinetics are displayed in Fig. 4. Figure 4a shows the distance covered by visible color change of LTI-PEG20 over time. A linear relationship can be observed after 16 days. The linear equation ($y = 0.08x + 2.77$) found by linear regression (r^2=0.99) was obtained with averaged data of three different clocks. Figure 4b is a plot of length

Fig. 2 Picture of 3 LTI-PEG2 clocks based on leuco-thioindigo at 6 months after activation. Scale in mm

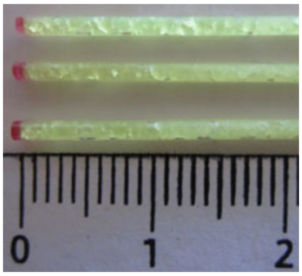


Table 2 Materials and properties of opto-chemical clocks in microchannels

Code	PEG[a]	PEG[b] [g/mL]	Leuco-dye[c]	Pace of frontline [mm/day]
LTI-PEG2	PEG2	1	LI	very slow
LI-PEG2	PEG2	1	LTI	very slow
LTI-PEG20	PEG20	1	LI	0.08
LT-PEG20	PEG20	1	LTI	0.15
LTI-PEG600	PEG600	0.5	LI	0.46
LI-PEG600	PEG600	0.5	LTI	0.63

[a] The number gives the average molecular weight in kilodalton (kD)

[b] In water

[c] *LI* leuco-indigo; *LTI* leuco-thioindigo

of the colored segment of the LI-PEG20 clocks over time. The plot is nonlinear at the beginning but converts into a linear relationship after 11 days. The linear equation ($y = 0.15x + 1.79$) found by linear regression (r^2=0.99) was again obtained by averaging data of three clocks. Table 2 gives quantitative data.

Capillary clocks based on 600 kD PEG As expected in view of the better permeability of this polymer for oxygen, the pace of the movement of the color front is much fast in this case. Data are given in Table 2, and the kinetics in Fig. 5. A linear relationship is found for both clocks already after 1 day. The linear relationship between elapsed time (x) and the length of the segment (y) can be described by $y = 0.46x + 2.95$; r^2=0.99 for LTI-PEG600 (Fig. 5a). The data are averages of three different clocks. Figure 5b shows the plot for LI-PEG600. Again, a linear relationship ($y = 0.63x + 2.31$) found by linear regression (r^2=0.99) was recorded by collecting the data of three different clocks.

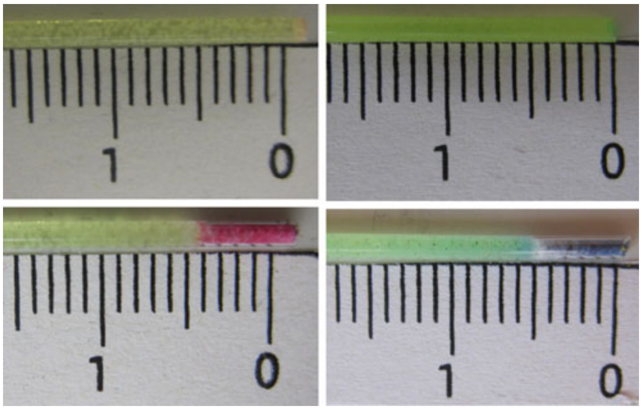


Fig. 3 *Left*: Pictures of the clock LTI-PEG20 based on leuco-thioindigo at day 0 and at day 54. *Right*: Pictures of the clock LI-PEG20 based on leuco-indigo at day 0 and at day 30. Scale in mm

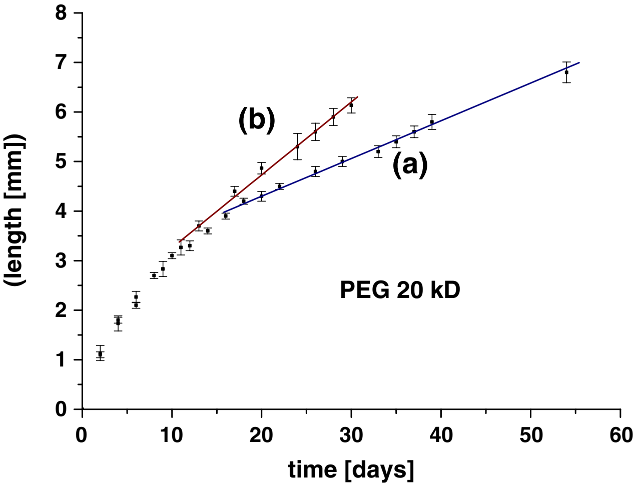


Fig. 4 Kinetics of the clocks based on leuco-thioindigo (**a**) and leuco-indigo (**b**) in a 20-kD PEG matrix

It is interesting to compare the data given in Table 2 for different clocks. Those based on 2 kD PEG are quite "slow", the length of the colored segment being very short. This is attributed to the much smaller permeability of the 2-kD PEG for oxygen compared to those of higher molecular mass [38]. In our estimation, they are suitable for timing over several months or even years (also considering the stability of indigo dyes).

Clocks based on 20 kD PEG cover the time frame from 2 weeks to several months. The frontline of LTI-PEG20 moves about half as rapid (0.08 mm per day) compared to LI-PEG20 (0.15 mm/day). Clocks based on 600 kD PEG exhibit the fastest pace and are suitable for determination of elapsed time between several days and a few weeks.

Effects of temperature and relative humidity These were studied with clocks based on the 600-kD PEG containing

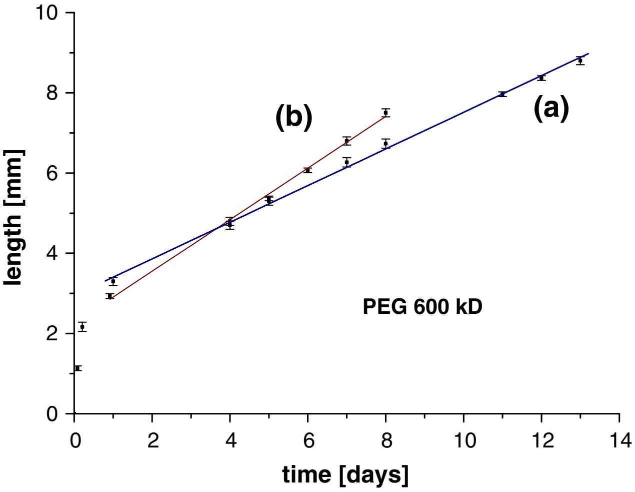


Fig. 5 Kinetics of the clocks based on leuco-thioindigo (**a**) and leuco-indigo (**b**) in a 600-kD PEG matrix

leuco indigo. A strong dependency on temperature is found for the pace of the frontline. In case of 4 °C and 60 °C (at ambient humidity), it is 0.29 mm per day and 0.55 mm per day, respectively, which is distinctly different from the 0.63 mm per day found at room temperature. Similarly and expectedly, relative humidity (RH) also exerts a substantial effect. At 0% RH (at 22 °C), the pace of the frontline is 0.09 mm per day only. The strong effect of both RH and temperature on the chromogenic reaction is obvious.

Effects of Photobleaching Indigo dyes are fairly stable but undergo some photobleaching on exposure to light [39]. We do indeed observe some bleaching of the blue (or red) zone under strong illumination with yellow or green light from a strong LED, but this leads to a slight reduction in the intensity of color, not to a shift in the length of the colored segment. Hence, photobleaching is of less concern.

Discussion

The opto-chemical clocks use well-known vat dye chemistries as a chromogenic reaction. This goes in parallel to capillaries that have been used as optical chemical sensors after having been coated with (reversible) indicator chemistry [40]. A highly significant color change can be detected visually and instrumental read-out is not required. The change in color is brought about by the reaction of oxygen with the leuco-dye embedded in PEG matrix in capillary. A frontline can be observed that separates the zones of the reduced and the oxidized form of the leuco-dye. The length of colored segments are linearly related to the time elapsed (after a certain delay) once the microchannel is exposed to air. The time frame of the clocks can be adjusted by varying (a), the polymer; (b), the dye; (c), the length of the capillary, and maximal timing can be adjusted to between 1 day and 1 year (if not longer). Conceivably, other oxygen sensitive dyes or other PEGs may be used for creating other clocks of this kind. Opto-chemical clocks in microchannels are a cost effective way to visually monitor elapsed time based on color transition. Main applications are in the food and pharmaceutical industry in order to determine storage time and the freshness of products.

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