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Thermochromic latent pigment based Time Temperature Indicators for perishable goods

Daniela Galliani, Luca Mascheroni, Mauro Sassi, Riccardo Turrisi, Roberto Lorenzi, Alberto Scaccabarozzi, Natalie Stingelin and Luca Beverina

Luca Beverina, corresponding author

Department of Materials Science and CNR-ISTM, University of Milano-Bicocca

Via R. Cozzi 55, I-20125

luca.beverina@unimib.it

Daniela Galliani, Luca Mascheroni, Mauro Sassi, Riccardo Turrisi, Roberto Lorenzi, Alberto Paleari,

Department of Materials Science and CNR-ISTM, University of Milano-Bicocca

Via R. Cozzi 55, I-20125

Alberto Scaccabarozzi, Natalie Stingelin

Imperial College London

Exhibition Road

London SW7 2AZ, UK

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Food safety has always been a major concern for mankind. The wide availability of fresh perishable goods characteristic of modern society only made this issue more relevant as the spontaneous spoilage of such goods – milk might probably be the best everyday life example – due to inappropriate handling and storage, particularly during long distance delivery, is a treat even more serious than adulteration. Therefore, the European Commission ruled that shipping and storage of milk requires the strict respect of definite temperature limits. For pasteurized milk, rigorous storage at 4° C is required, with a maximum tolerated thermal exposure of + 9 °C for short periods of time (less than 3 hours).^[1] This is important because the product shelf life is based upon the assumption that the good has been handled at the prescribed temperature at all times. The customer has so far, however, not the means to track

the temperature history. He/she can only verify the storage temperature at the moment of purchase, without capability to get information on the previous handling of the good.^[2]

As such, there is a relevant technological interest towards the development of simple and low-cost smart labels that can be integrated into packages giving information on their whole thermal history. The current Time Temperature Indicator (TTI)^[3,4] devices include two classes: a) Integrated circuits featuring data loggers and radio frequency identification chips,^[5] and b) simpler colour indicators relying on chemical reactions such as enzymatic reactions,^[6,7] dye diffusion,^[8] polymerization reactions,^[9] controlled dewetting processes,^[10] shape memory liquid crystals,^[11] and colloidal structures comprised of silver layers grown around gold nanorods.^[12] TTIs of the first class provide detailed information on the thermal history but are too expensive for everyday perishable goods. The second class is inexpensive and attractive for the packaging industry,^[13] provided that the optical contrast shown by the label cannot be tampered with. In terms of sensitivity, the chronochromic behavior of an ideal smart label should match the rate of growth at ambient temperature of the bacteria responsible for dairy products spoilage. *Escherichia coli*, the most common bacterial strain affecting fresh goods, multiplies according to an exponential law. The bacterial population doubles every 1 h at 30°C and every 6 h at 20°C. Assuming an ambient temperature in the range of 20-25°C, a suitable label should provide a clear signal to be read by the naked eye in around 3 hours.^[12]

We here describe a new class of irreversible thermochromic molecular materials whose chemical transformation - when deposited as thin films - from a colourless state to a strongly colored one depends upon temperature, chemical substitution and substrate. The selection of the appropriate active molecule/substrate combination provides sensitivity to different targeted time/temperature regimes, coherent with the sensitivity limits just described.

The key process providing the required irreversible color change is connected with the “latent pigment” approach.^[14] This process enables to induce the reversible transformation of organic pigments – like diketopyrrolopyrroles (as the red 254 pigment shown in scheme S1)^[15] and

quinacridones^[16] - into soluble dyes by means of the protection of its hydrogen bond forming functionalities. As this is usually done by reaction of the pigment with di-tert-butyl dicarbonate, the resulting tert-butylcarbonate (tBOC) protection is both thermal and acid labile, with the evolution of CO₂ and isobutene (Scheme S1).^[17] Importantly, note that due to the involved (minor) change in the optical gap and (more importantly) aggregation state, the latent pigments are in general irreversible thermochromic materials, as we have recently shown in the context of organic solar cells.^[15] Once applied to a very peculiar class of pigments - the 1,3-squaraines^[18] shown in **Scheme 1**- the optical gap variation associated with the latent pigment process becomes extreme, turning the blue pigment into a colourless molecule. This reaction provides the kind of dramatic colour change required for smart labels directly readable by the naked eye and it is thermally irreversible (see below, particularly regarding to the discussion of Figure 2), thus ensuring a reliable reading by the customer. In details, Scheme 1 shows that the treatment of squaraines **1** and **2** with di-tert-butyl dicarbonate and 4-*N,N*-dimethylaminopyridine (DMAP) leads to the formation of betaines **3** and **4**. Since the addition of DMAP to the squarylium double bond breaks the typical cyanine conjugation of the dye, said betaines have no visible absorption. It should be noted, though, that under analogous experimental conditions, DMAP alone did not react with squaraines. Possibly, such unusual reactivity is a consequence of the severe twisting induced by the introduction of the four tBOC bulky groups in close proximity to the squarylium core. Such distortion makes the squarylium double bond more reactive towards the nucleophile addition. The pristine squarylium dye can be quantitatively recovered by thermal treatment or by addition of trifluoroacetic acid, according to the well know acid catalyzed cleavage of tBOC groups.

TGA analyses (Figures S1 and S2 of the Supporting Information) show that the thermal cleavage of bulk samples of latent squaraines **3** and **4** requires around 140 °C, irrespective to the substitution pattern. Such temperature is clearly out of the relevant range for TTIs. Conversely, trifluoroacetic acid catalyzed room temperature conversion in solution is

essentially instantaneous and definitely too fast to be useful for TTIs. Deposition of latent squaraines as thin films on substrates possessing different surface area and chemical nature provides the key to adjust sensitivity to the required time/temperature regime. In fact, **Figure 1a** shows (red dots) the evolution as the function of time at 80°C (well below intrinsic cleavage temperature as assessed by TGA) of the absorption spectrum peak intensity @ 638 nm (See Figures S3-5 of the Supporting Information for the whole spectra and different temperatures) of a wire bar coated thin film of derivative **3** on a microscope slide. The peak intensity increases exponentially in the first 5 minutes, eventually slowing down to linear growth after 8 minutes. We interpreted the data by taking into account the acidity of the silica surface silanole groups. The quick response in the first 5 minutes is thus connected with the first layer of **3** in direct contact with the silica surface. Indeed, we repeated the experiment on a silanized microscope slide (See Supporting information for silanization conditions and figure S9 for the whole spectra) obtaining the data shown by the black squares of Figure 1a. The data gave a satisfactory linear fit all over the investigated time range, moreover the slope ($2.5 \cdot 10^{-3} \text{ min}^{-1}$) is similar to the one obtained by fitting the linear regime of the red trace ($2.0 \cdot 10^{-3} \text{ min}^{-1}$). As the silanization process removes the surface silanoles, the data confirm that the exponential regime of the red trace is due to the surface induced acid catalysis, only affecting the portion of the layer intimately connected with the surface. The two data sets of Figure 1a also show that surface acidity can be used as a tool extend the material sensitivity to temperature regimes very far from those expected on the basis of the TGA analyses, provided that very high surface area substrates are selected. In this case in fact most of the deposited material will respond according to the surface induced behavior.

Indeed, we stained standard silica gel TLC plates with derivative **3** and we characterized the time/temperature response of the obtained samples by UV-Vis reflectivity. **Figure 1b** shows the evolution of the reflectance spectrum at room temperature as the function of the elapsed time. For this particular derivative, the cleavage process does not involve major changes in

the squaraine aggregation state, as the shape of the spectrum is essentially unchanged. The value of the reflectance at 638 nm (the squaraine maximum of absorption) can thus be correlated with the concentration of the cleaved molecules. **Figure 1c** shows that in this case the whole dataset can be satisfactorily fitted with an exponential, according to the fact that essentially all of the absorbed material is affected by the acid catalysis. Even more importantly, the room temperature response is fast enough to make such stained TLC an attractive TTI prototype having a time/temperature response well suited for the monitoring of perishable goods the like of fresh milk. In particular, the duplication rate of *Escherichia coli* population at 25 °C is 2 h, exactly the same time required to halve our label original reflectivity value.^[12] Finally, **Figure 1d** shows that the acid catalyzed process has very remarkable temperature dependence. In fact, the as prepared film reflectance (red line) and the one recorded after 48 h at 4 °C (blue line), the fresh milk storage temperature, are essentially superimposable. Conversely, if the film is kept for 3 h at ambient temperature, the cleavage reaction can be clearly detected by the naked eye as a deep blue colour develops (black line). **Figure 1e** shows a series of pictures of the same TLC plate stained with **3** and taken at different times over a period of 3 h at room temperature. However, temperature sensitivity alone does not make a good TTI. Two additional features are also necessary: a) the process has to be irreversible: cooling down of the label should not influence the color in any way; b) the TTI color variations due to multiple expositions to ambient temperature should be additive. Explicitly, the final state of the device should be the same if for example a sample has been heated at 25 °C for 3 h continuously, or for three periods of 1 h each separated by a storage period at the appropriate 4°C temperature.

We thus prepared two twin samples of derivative **3** on silica gel and submitted them to different thermal histories and compared the results. Whiles the sample described in figure 2a was continuously kept at 25°C for 160 min, the one described in Figure 2b was kept at 25 °C for 40 min, cooled at 4 °C for 60 min and then brought back to room temperature for

additional 60 min. First of all Figure 2b shows that the thermochromic transformation is irreversible. In fact the sample absorption at 40 min and 100 min are essentially the same, the cooling down of the sample does not have any effect of the label coloration. Even more importantly, when the sample is brought back to room temperature the reaction proceed leading to a final reflectance value of 59 %, that is the very same value recorded for sample a) after 90 min at room temperature. The behavior of the TTI is thus additive as required.

Additional desired features can also be realized. The cleavage process is hardly noticeable, even for very prolonged storage periods, both for the pure latent squaraine powder and for solutions in a variety of solvents, including alcohols. It is possible to prepare active inks, whose thermochromic character is only displayed after printing. This could represent an advantage over other TTIs requiring the formulation of the thermochromic system in two or more distinct layers/components to be mixed prior to use.

Substitution pattern allows for further tuning of the time/temperature response. In fact, the substitution of the amine on the 4 position of the benzene rings with a third 'BOC protected hydroxyl functionality leads to both a sizeable high energy shift of the deprotected form optical gap and to a much slower deprotection reaction at ambient temperature. **Figure 3a** shows that the exposure to room temperature of a TLC plate stained with **4** as very little effect over a 1h period. The reaction becomes noticeable only after nearly one day. We monitored the process by taking pictures of the TLC plate at regular intervals over one week (**Figure 3b**), during which time a red-brown color clearly developed. Such label could find application for goods withstanding either longer storage time at ambient temperature or for the monitoring of higher temperatures (Figures S6-8 reports the complete characterization of **4** on glass at different temperatures).

The combination of **3** and **4** in a single smart label provides a multicolour sensing device capable to detect and record both long and short-term exposures: at short exposition time/low temperature **3** gets cleaved and the label turns blue; at long term exposure/high temperature, **4**

is cleaved leading to a greenish black label. The Supporting Information section contains time-laps videos demonstrating the colour evolution of pure **3** and combined **3/4** labels. We are actively working at the quantitative determination of the process activation energy as a function of the surface acidity. This is a somewhat complex task due to the nature of the substrate itself (it is difficult to have thickness estimate, required to get a concentration) and to the fact that the label is meant to work in reflectance, where a direct relationship between concentration and signal, particularly at very low and very high values could be problematic. The use of a gel formulation of latent squaraine and silica could enable to circumvent the problem, results will be published in due course.

In conclusion, we have shown that the application of the latent pigment approach to squaraines provides very versatile, programmable and simple TTIs to be used as cheap indicators for the correct handling and storage of perishable goods. The exploitation of surfaced induced acid catalysis provides a versatile tool to tune the reaction time/temperature profile in order to accord the label responsiveness to the specifications of the products to be monitored.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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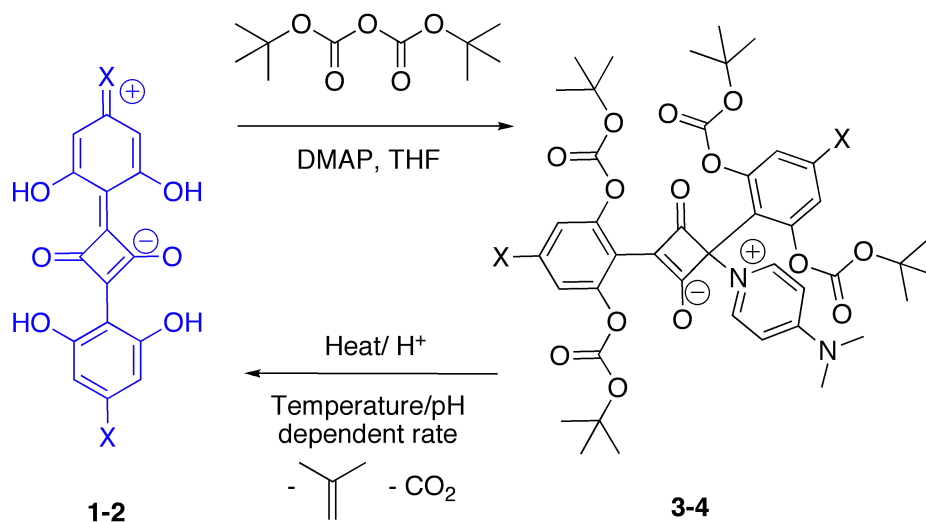
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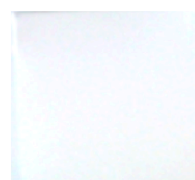
1,3 X = N(-CH₂-CH=CH₂)₂

2 X = OH

4 X = O^tBOC



TLC plate stained with 1



TLC plate stained with 3

Scheme 1.

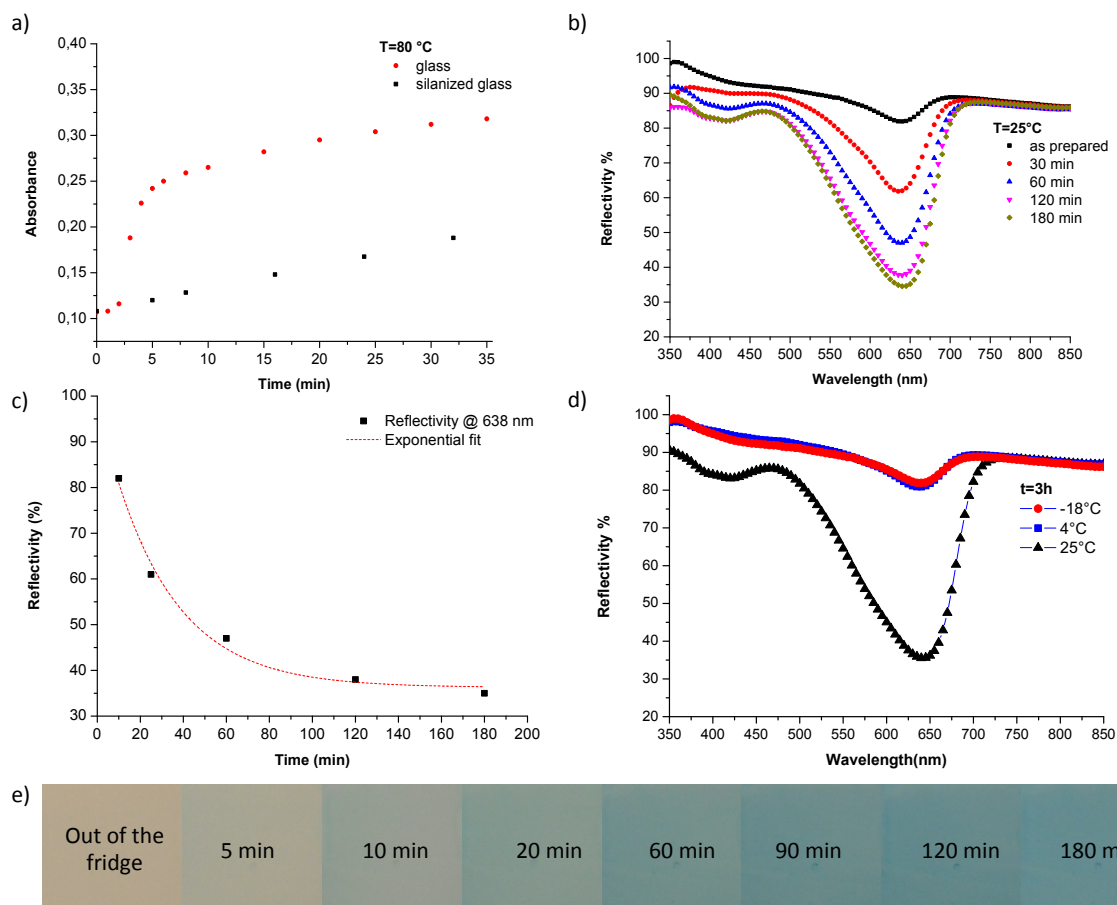


Figure 1. Reflectivity spectra of a thin film of **3** adsorbed on a TLC silica plate and stored for a) 3 h at -18°C (red), 4°C (blue) and 25°C (black); b) Stored at r.t. (25°C): as prepared (black), 30 s (red), 60 s (blue), 120 s (purple) and 180 s (brown). c) Reflectivity at 638 nm as a function of time for a thin film of **3** on a TLC plate at 25°C (black) and corresponding exponential fit (red dashed line); d) Absorbance @ 650 nm for a wire bar coated thin film of **3** on glass (red) and silanized glass (black) at 80°C ; e) pictures taken at different times of a **3** stained TLC plate at 25°C .

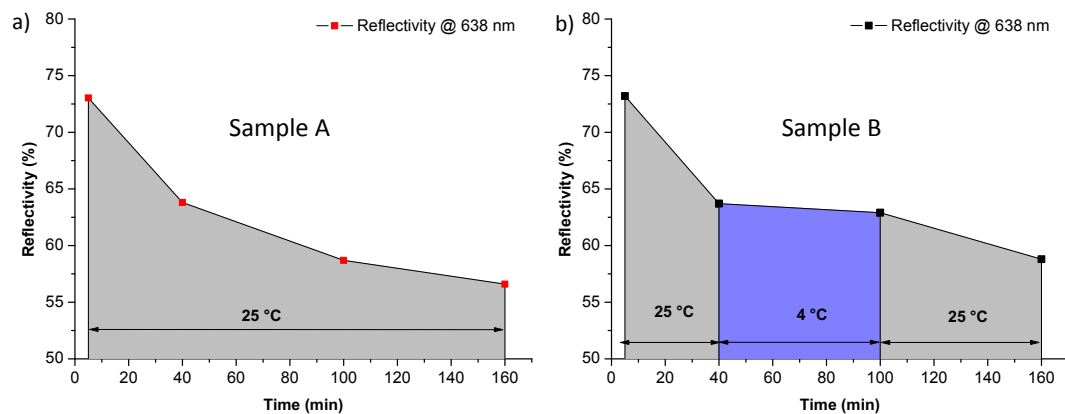


Figure 2. a) Reflectivity at 638 nm as a function of time for a thin film of **3** on a TLC plate at a constant temperature of 25 °C; b) Reflectivity at 638 nm of a twin sample maintained at 25 °C for 40 min, at 4 °C for 60 min and again at 25 °C for 50 min.

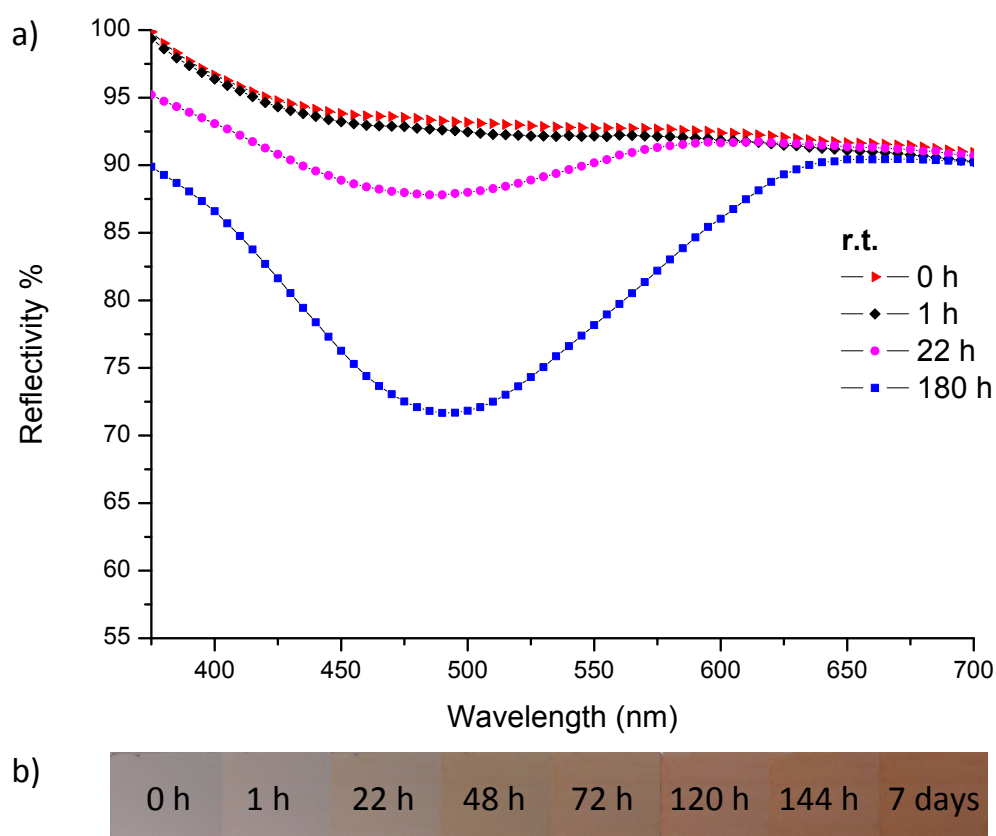


Figure 3. a) Reflectivity spectra of a thin film of **4** on a TLC silica plate as a function of time at r.t. (25°C); b) Pictures of the sample taken at different times during a week of exposure to ambient temperature.

The development of cheap and reliable time-temperature indicators (TTIs) could greatly improve customer's satisfaction with respect to the correct handling and storage of perishable goods. A series of new irreversible thermochromic squaraine dyes enables the preparation of simple TTIs whose time-temperature regime can be controlled by selection of the appropriate molecule/substrate combinations.

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ToC figure

