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Amine Sensors

Solid Polymer Substrates and Coated Fibers Containing 2,4,6-Trinitrobenzene Motifs as Smart Labels for the Visual Detection of Biogenic Amine Vapors

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Abstract: Attempts to polymerize trinitrobenzene derivatives (TNB) have been fruitless so far. Accordingly, polymers containing TNB have not been exploited in spite of their envisaged potential applications. Here, we describe two ways for preparing polymers with TNB moieties thus overcoming the previously reported polymerization impairments. We also report on the exploitation of the materials, both obtained as tractable transparent films and coated fibers, as smart labels for the visual detection of amine vapors. More precisely, amines in the atmosphere surrounding the sensory materials diffuse into them reacting with the TNB motifs forming highly colored Meisenheimer complexes, giving rise to development of color and to the naked eye sensing phenomenon. This is the case of highly volatile amines, such as trimethylamine, produced in food spoilage, specifically in the deterioration of fish or meat, for which the color development of the smart labels can be used as a visual test for food freshness.

The development of chemical sensors, or chemosensors, with optical output is a topic of current interest. Envisaged applications in food, healthcare, household, and homeland security are fostering the research on this field. This is especially true with sensors with chromogenic response, that is, in which the presence of a target chemical induces a color change, so that the human eye acts as detector.[1] Furthermore, if the sensor is a polymer, the material can be processed in different and manageable shapes as smart labels, for instance, as films or textiles.[2] Among target molecules, biogenic amines (BAs) are of special interest, specifically if they can be detected in the vapor phase, because their increase in packaged food, especially fish and meat, is related with the food freshness.[3] BAs occurs in food spoilage because bacteria enzymes decarboxylize amino acids. Samples of BA are spermine, tryptamine, spermidine, histamine, tyramine, β-phenylethylamine, cadaverine, putrescine, ethylenediamine, and trimethylamine. The latter originates in the reduction of trimethylamine-*N*-oxide (TMAO) by spoilage bacteria, such as *Pseudomonas*.[4,5] Accordingly, we undertook the preparation of sensory polymers that give rise to visual color change upon interaction with BA vapors.

Our recent preparation of colorimetric sensory materials for nitro-explosives based on polymers containing amino sensing motifs[6] made us think the other way round, the naked eye recognition of BAs based on polymers that contain trinitrobenzene subunits (TNB).[7] The sensing and recognition phenomena come from the formation of Meisenheimer complexes.[8] In this work we prepared a methacrylamide monomer containing the trinitrophenyl core *N*-(2,4,6-trinitrophenyl)methacrylamide (**NPhMA**), and carried out without success different attempts of thermally and photochemically initiated radical polymerization under conventional conditions and using different comonomers. The initial fiasco in the polymerization of the monomer was in agreement with the known radical scavenging capabilities of TNB units, which prevent polymerization even in very low ratio in copolymerization mixtures, as initially described by Wiley and Behr in the 1950s.[9,10] The phenomenon was ascribed by Kadoma et al. to the reaction of the nitro groups with the growing radicals resulting in chain termination.[11] The presence of TNB-containing monomers also inhibited cationic and anionic polymerization.

Therefore, our initial efforts were directed to overcome the above-mentioned difficulties to prepare sensory materials, followed by their study in the naked eye or colorimetric recognition of amines.

We initially tackled the preparation of the materials by firstly preparing a polymer membrane with benzene units (**M1**), coming from *N*-phenylmethacrylamide (**PhMA**), followed by its nitration to render a material containing the TNB sensory motifs as sensory membrane **M1 sen** (Section S2 in the Supporting Information). More precisely, the nitration was carried out with the conventional sulfonitric mixture with the peculiarity that the solid was a membrane swelled in this mixture. This methodology has the advantage of overriding the polymerization inhibition capability of TNB-containing monomers. However, the inherent difficulties associated to polymer modifications, spurred in the solid state, gave us only partial control on membrane reproducibility.

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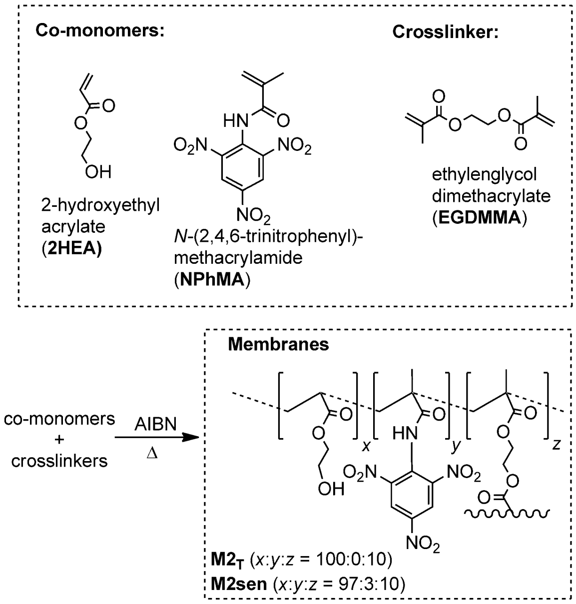
For this reason we undertook a polymerization study of monomer mixtures containing **NPhMA** increasing the polymerization temperature beyond reasonable when using AIBN as thermal initiator, which has a half-life of 6 min at 101 °C (and estimated value of 38 s at 120 °C).[12] Thus, we carried out what we call a flash polymerization at 120 °C and succeeded in obtaining the sensory membrane **M2 sen** (Scheme 1 and Section S2 in the Supporting Information). For testing the approach to this flash polymerization, we carried out polymerizations at conventional temperatures with the mixture of co-monomers used for preparing **M2 sen**, excluding **NPhMA**, that is, 60–80 °C, and compared the thermal and mechanical properties of films prepared at temperatures up to 150 °C (films **M2**$_T$, Scheme 1). Surprisingly, both mechanical and thermal properties were fairly similar (Section S2 and Table S1 in the Supporting Information), as well as the water swelling percentage (WSP). The properties showed negligible dependence on the polymerization temperature, and this result is not in agreement with the expected deterioration of the properties according to the steady-state radical polymerization theory. The preparation of different batches of **M2 sen** showed good reproducibility of spectroscopic characteristics and properties. The FTIR spectra showed the signals corresponding to the nitro groups (Figure S5 in the Supporting Information). On the other hand, the nitration process of **M1** to render **M1 sen** greatly lowered the WSP, probably because of side reactions that increased the crosslinking density, for example, by condensation of hydroxyl end groups to render ether linkages, which is in agreement with the increment in the char yield at 800 °C observed thermogravimetrically. In parallel, the handability of **M1 sen** was impaired by a brittleness increase.



Scheme 1. Monomers and chemical structure of membranes **M2**$_T$ (polymerization temperatures (T) = 60, 80, 100, 120 and 150 °C) and sensory membrane **M2 sen** (polymerization temperature = 120 °C).

The procedure followed to prepare **M2 sen** was also used to obtain the smart textile (**Tsen**) based on white cotton fabric. Thus, the fabric was coated with the monomers by immersion, and after wringing, the coating was polymerized in oxygen free atmosphere at high temperature (120 °C).

Previously to the preparation and characterization of the sensory materials, the colorimetric sensory behavior toward biogenic amines of monomer **NPhMA** was analyzed visually and by UV/Vis. Pale yellowish solutions of **NPhMA** in acetone turned reddish upon adding the amines (Figure 1). The color intensity and hue were dependent both on the **NPhMA** and on the amine type and concentration. On the other hand, the response, as derived from the UV/Vis spectra (Figure S7 in the Supporting Information), was insensitive to the type of amine, in terms of spectral patterns, with differences in absorbance probably because of variations in the nucleophilicity and number of amino groups per target molecule, that lead to different concentration of Meisenheimer complexes.

Figure 1. Response of materials to the presence of amines (from left to right: blank, tyramine, tryptamine, spermine, spermidine, cadaverine, putrescine, histamine, trimethylamine, phenylethylamine, β-ethylenediamine): a) acetone solutions of **NPhMA** (a1: 1.9×10^{-4} M; a2: 9.4×10^{-4} M) upon adding a set or biogenic amines (concentration of each amine, top: 4.8×10^{-3} M, bottom: 6.3×10^{-3} M), b) discs cut from **M2 sen** upon dipping for 35 min in water (pH 10, sodium bicarbonate buffer 0.05 M) containing different amines (0.05 M); c) samples cut as squares from **Tsen** in atmospheres of different amines (sealed vials of 8.40 cm^3 in which 100 μL of a 0.3 M water solution of different amines were dropped at the bottom of the vial (resident time = 45 min, temperature = 25 °C).

However, we have centered our study in the detection of amine vapors, because these compounds are generated in the food spoilage and their visual detection is important for the consumer as well as for other fields related with food safety and with health and safety at work. More precisely, we have analyzed the color development of sensory materials upon entering into contact with atmospheres containing TMA (Figure 2). This amine is at gas phase at room temperature, and is usually used in water solution. We have mimicked the spoilage of packaged food in relation with the gradual increment of amine concentration in the packaging atmosphere, especially TMA for its high vapor pressure (Table S2 in the Supporting Information). Thus, the in-lab performance of the

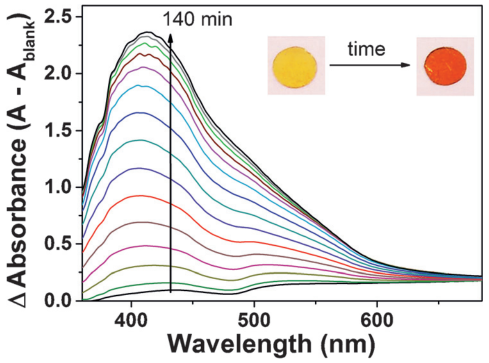


Figure 2. UV/Vis spectra along time of **M2 sen** in an empty cuvette (4.40 cm^3) upon adding 100 μL of a water solution of TMA 0.3 M at the bottom of the cuvette (temperature = 25 °C). The spectra were taken every 10 min. A blank film of **M2 sen** was used as reference. Inset: picture of **M2 sen** took before adding the amine and after 140 min of the addition.

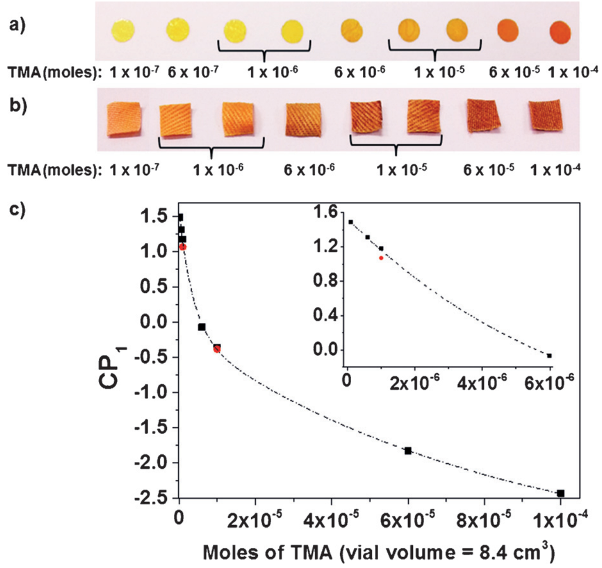


Figure 3. Titration of TMA vapors with discs obtained from **M2 sen** and smart textile squares cut from **Tsen**. a) and b) picture of discs of **M2 sen** and squares of **Tsen**, respectively, after being in contact with an atmosphere containing TMA. The number of moles of aqueous solution of TMA added to the chamber (vial of 8.4 cm^3) are indicated. c) Titration curve obtained by treatment of the digital picture data (CP$_1$ is the principal component of a PCA analysis using the red and green values of the RGB parameters that define the digital color of each disc). The titration curve corresponding to the textiles is depicted in the Supporting Information, Figure S10.

smart labels, obtained as discs and squares from **M2 sen** films and **Tsen** textiles, respectively, with punchers or scissors, were studied by adding different amounts of 0.3 M TMA, from 1×10^{-7} to 1×10^{-4} mol, to nine sealed vials (8.40 cm^3) containing sensory discs at 25 °C (Figure 3). The pictures taken of the nine discs or squares allowed for the preparation of the titration curve by using the digital color data of each disc or square, as previously reported,[13] or by registering the UV/Vis spectra of the discs (the UV/Vis spectra of the discs and the derived titration curve can be found in the Supporting Information, Figure S9).

The proof of concept related with the performance of the labels were carried out with fresh fish (tuna) and meat (beef) bought in the local market and tray-packaged in facilities of the Biotechnology and Food Science Department at the University of Burgos (for conditions and pictures see Supporting Information, Section S3 and S7). Sensory discs cut from **M1 sen** and **M2 sen** were placed in the tray without physical contact with the food, and the color evolution along time was followed using a reference disc outside the packaging film. The study was carried out at constant temperature, both under and without refrigeration, 3 and 25 °C, respectively (Figure 4).

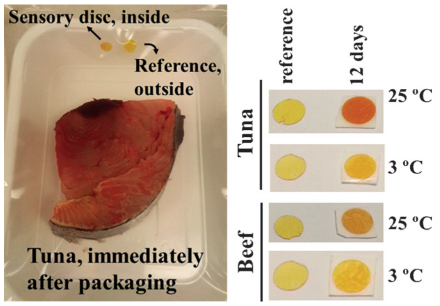


Figure 4. Color development of sensory discs cut from **M2 sen** inside refrigerated (3 °C) and non-refrigerated (25 °C) tray-packaged tuna and beef. The evolution along days can be seen in Supporting Information, Figure S12 and S13.

The spoilage of the food was accompanied by the darkening of the yellow sensory discs to orange or reddish, demonstrating that the material may encounter practical uses, for example, for visually informing the consumer of the freshness. As it has been pointed out, **M2 sen** has the advantage of precise control of the polymer structure, though the content of the sensory monomer is restricted to a low rate in relation with the other co-monomers. Alternatively, the reaction carried out in the parent **M1** membrane to render **M1 sen** limits the structure control, but affords a much higher content of TNB units, increasing the response and at the same time probably the applicability from a commercial viewpoint. The performance analysis of sensory material also require of the assessment of their stability, which can be evaluated from a mechanical, thermal, and ambient viewpoint. The mechanical and thermal stability was evaluated by strain–stress studies (universal testing machine) and thermogravimetic analysis (TGA), respectively (Supporting Information, Section S2.2., Figure S4 and Table S1). Regarding the stability of sensory membranes to ambient conditions (light, oxygen, and humidity), the different batches were prepared and stored between filter papers without special care, without differences in the sensing performance. Moreover, a disc cut form the recently prepared **M2 sen** was irradiated with UV and visible light for 50 h in a UV/Vis spectrophotometer by successive scans from 350 to 800 nm for 2 days in air (1 scan per hour). The collected spectra were virtually identical showing no-degradation of the TNB sensory motifs with light, even with UV irradiation (Supporting Information, Figure S6).

In short, trinitrobencene (TNB) motifs have been chemically incorporated into the polymer structure of transparent films and coated textiles to give smart labels that respond with a color change in presence of amines in its environment. The color change comes from the formation of Meisenheimer complexes between the TNB units and the amines. As TNB are well known polymerization inhibitors, two different approaches for the preparing the polymers were developed, by flash polymerization at unusual high temperature and by chemical modification of a parent polymer. Besides demonstrating ability to detect amines in the gas phase in the laboratory, these sensory materials have shown their potential in the visual indication of the spoilage of samples of tray-packaged fresh food, such as fish and meat.

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Keywords: amine sensors · biogenic amines · chemosensors · packaged food · polymers

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