


Irreversible temperature indicator based cellulose membranes conjugated with leuco-dye pigment

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Abstract

This research focuses on the development of thermochromic membranes made of cellulose acetate (CA) for temperature monitoring of sensitive food products. Two dual TC membranes developed for the control of different temperature ranges were formulated using a three-component system: a leuco-dye membrane (crystal violet lactone, CVL) integrated with an acidic membrane containing the color developer (salicylic acid) and the acidic solvent with different melting points (decanoic acid, DA, or methacrylic acid, MA). The CVL membrane, together with the DA membrane, showed an irreversible color change when exposed to 35°C, which was facilitated by the melting of DA. The CVL membrane also underwent an irreversible color change when exposed to 15°C together with the MA membrane. The membranes were characterized in detail using scanning electron microscopy. The evaluation of color changes, reproducibility, specificity, and stability ensured the practical suitability of these membranes. Overall, this innovative approach has proven to be a reproducible, sustainable, cost-effective method to produce irreversible colorimetric temperature sensors. These sensors have significant potential for applications in the food and pharmaceutical industries and offer a promising way to improve product safety and quality.

KEYWORDS

cellulose, colorimetric, double membranes, irreversible, Leuco-dye, temperature monitoring

1 | INTRODUCTION

Food safety and quality are global challenges that everyone must face. Ensuring that all food available for consumption is completely safe is an unrealistic goal,

especially due to the extensive processing methods that have evolved over the years. Nevertheless, we can take measures to control certain factors that affect food quality and safety, in particular the presence of pathogenic bacteria, fungi, and viruses on food.^{2–5} A considerable number

of these factors are directly influenced by food storage conditions, including external factors such as storage temperature and humidity.⁶ Consequently, cases of food poisoning are often due to transportation conditions that deviate from ideal storage conditions, particularly in terms of temperature monitoring. To solve this problem, innovative intelligent and active packaging systems have been developed to effectively regulate various factors and ensure food quality.⁷ Active packaging plays a crucial role in monitoring important variables such as temperature, pH,^{1,8} gasses,⁹ and humidity.^{10–13} Overall, it actively contributes to creating optimal conditions for extending the shelf life of food. This dynamic interaction between the packaging and the food itself plays a crucial role in maintaining and improving food quality.¹⁴

According to Chen et al., smart packaging consists of active and intelligent packaging systems that provide consumers with more accurate information about the condition of the food and have a protective effect on the food. Smart packaging can track a food product by analyzing and controlling the internal or external environment and provide information for the entire food supply.¹⁵ To control a factor like temperature, there are many different sensors in the literature^{15–17} based on crystal liquids (CL),^{18,19} inorganic materials,^{20,21} polymers,^{22–24} or dyes.^{25–28} An interesting work reported by Wu and colleagues describes a sensor that using CL for temperature monitoring.²⁹ CL sensors offer excellent sensitivity, versatility, and real-time response, making them suitable for various applications. However, they can have limitations under extreme temperature conditions, require calibration, have a viewing angle dependence that should be considered when selecting a sensor for a particular application, and are generally reversible. Another interesting research is the work described by Pirsá and Shamusí, who developed a smart packaging with a temperature indicator sensor based on a conductive nanostructure of cellulose-polypyrrole-ZnO films to control and estimate the storage temperature of chicken thighs legs.³⁰ These sensors are suitable for temperature monitoring because they can change their color reversibly or irreversibly when exposed to a certain temperature, which is due to a change in molecular structure. They offer unique ways of possibilities for detecting temperature changes and responding to them with a color change. However, they also have disadvantages, such as problems with environmental stability, limited response time, variations in sensitivity, dependence on viewing angle, complicated manufacture, and toxicity of the material. Despite these drawbacks, they provide visual signals for temperature variations and have specific applications.^{31,32} As for irreversible thermochromic materials that respond to low temperatures, few works based on

low temperature monitoring are described in the literature. Some irreversible sensors are based on polymers as irreversible thermochromic sensor.

Mergu and Son designed and synthesized polydiacetylenes and investigated their irreversible thermochromic properties at low temperatures.³³ Garreau et al. presented a planar to non-planar conformational transition in thermochromic polythiophenes in a spectroscopic study.³⁴ Inorganic materials were also used.^{35,36} An interesting work is described by Liu et al. who investigated the thermochromic behavior of yttrium-substituted bismuth oxides.³⁵ Furthermore, Irene et al. described the thermochromic properties of a photochromic, layered biogenic zirconium bisphosphonate compound. In the field of dyes, only a few works based on anthocyanins as colorants have been described.^{33,37}

A leuco-dye (LD) is an organic thermochromic material that can exist in two different forms, a colorless form and a colored form (leuco-form) that results from the opening of a specific ring in its structure.³¹ The color change is usually reversible, that is the color returns to its original color by heating and cooling. To develop a reversible or irreversible system with an LD, a three-component mixture of a color former, a color developer, and a solvent is usually used.²⁶ Although the reversibility of thermochromism of LDs has been extensively studied, their irreversible behavior has, to our knowledge, never been investigated.

Cellulose membranes are an emerging technology used in various fields such as the food and pharmaceutical industries as they are easy to produce, inexpensive, and highly efficient.³⁸ Various temperature-sensitive membranes can be found in the literature, such as the porous nanofiber membrane described by Kim et al. This work reports a thermochromic sensor based on LD, such as polyoxymethylene melamine, anchored on nanofibers to measure body temperature in real time.³⁹ They obtained promising results, however; it is a multistep work that lacks simplicity. In addition, He and coworkers developed a reversible thermochromic membrane (TM) based on micro/nano-encapsulated phase change materials such as TM which can change its color from blue to colorless with increasing temperature between 15 and 38°C, representing a potential application as a wearable temperature sensor.⁴⁰ Another example of the use of TM is provided by Jeon and co-workers, who developed a biocompatible TM for fever detection by mixing a thermochromic pigment and a thermoplastic polymer in different ratios, which can change its color with increasing temperature.⁴¹

Since cellulose acetate (CA) is a biodegradable polymer with hydrophilic properties that can be easily processed into membranes, this type of material was chosen

as a carrier for our TM in this work.⁴² Overall, the main objective of this work was to prepare a bilayer membrane with CA as polymer matrix and acetone as solvent, which is able to show irreversible color changes at certain temperatures, for example 15 and 35°C, respectively. To achieve this, a three-component system containing a LD (crystal violet lactone, CVL), a color developer, and a solvent was used. To control the color change at each temperature, two different membranes were prepared: one containing the CVL (CVL membrane) and one containing the color developer and solvent (acid membrane). When the two-layer membranes reach the target temperature, a phase change of the solvent takes place in the acidic membrane, causing the CVL membrane to dissolve. This dissolution leads to a color change as the CVL membrane interacts with the acid present in the acidic membrane and an irreversible color change is subsequently observed. In general, this work underlines the potential of CA as a biodegradable polymer to produce temperature-sensitive membranes that can be used in the food and pharmaceutical industries.

2 | EXPERIMENTAL SECTION

2.1 | Material and reagents

Acetone 99% was purchased from JMGS, acetate cellulose, decanoic acid (DA), and CVL were purchased from sigma Aldrich. D-sorbitol was purchased from VWR. Salicylic acid (SA) was purchased from Panreac Química SA. Methacrylic acid (MA) was purchased from Merck. Glycerin (Gly) was purchased from JMGS.

2.2 | Apparatus

The thermochromic behavior of the membranes was studied by applying a specific temperature in a Nabertherm oven. Color transition was controlled using a handmade darkroom with LED light and images were captured using a smartphone. The surface morphology of all membranes was examined with a scanning electron microscope (SEM) using a microscope (Phenomworld, Phenom Pro X) with an accelerating voltage of 15 keV. Prior to SEM analyzes, the membranes were coated with a 10 nm thick gold layer.

2.3 | Preparation of the membranes

In this study, a double-layer TM was developed for irreversible temperature monitoring at 15 and 35°C,

respectively (Figure 1). For this purpose, three different membranes named membrane crystal violet (MCVL), membrane decanoic acid (MDA), and membrane methacrylic acid (MMA) were prepared. The MCVL consisted of a mixture of the color developer (CVL), Gly as plasticizer, and CA as a polymer matrix. The MDA membrane, contained the solvent DA with a melting point close to 31.5°C, and CA. The MMA was prepared similarly to the previous membrane except the solvent composition. In this case, we used MA with a melting point close to 15°C.

Specifically, for each membrane, 2.5 g CA was dissolved in 40 mL acetone. In addition, the MCVL membrane was prepared using Gly and 0.25 g CVL. The MDA and MMA membranes contained the color developer (SA, 1.5 g) but differed in the solvent used. The MDA was prepared by mixing 2.5 g DA with 0.3 g sorbitol, whereas the MMA was prepared with 2.5 g MA. During preparation, the respective compounds of each membrane were mixed in acetone with magnetic stirring. After thorough mixing, the solution was transferred to a Petri dish and dried for about 30 min, evaporating the acetone and leaving a well-formed membrane. For the development of MCVL, CA was added to acetone and the mixture was kept at 70°C for 10 min under stirring at 1000 rpm. After cooling, CVL was added to the resulting mixture and stirred again at 1000 rpm, but this time for 30 min. Then, 10 drops of liquid Gly were added to the CVL mixture, and the solution was stirred one more time at 1000 rpm for 24 h. Finally, 3 mL of the final mixture was added to a Petri dish and after evaporation of the acetone (30 min).

To prepare MDA, all reagents were mixed and stirred for 24 h at 1000 rpm on a stir plate. After 24 h, 3 mL of the resulting solution was placed in a Petri dish to allow the acetone to evaporate at room temperature for approximately 30 min until the membrane was formed. The same procedure was used for the MMA. Finally, the MCVL is assembled with the membrane (MDA or MMA) by clear tape.

3 | RESULTS AND DISCUSSION

3.1 | Preparation of the two-layer membrane

In general, the main challenge of this work is to develop new thermochromic formulations that allow color change at low temperatures according to the requirements of packaged foods, while providing an irreversible response to ensure the thermal stability of the product.

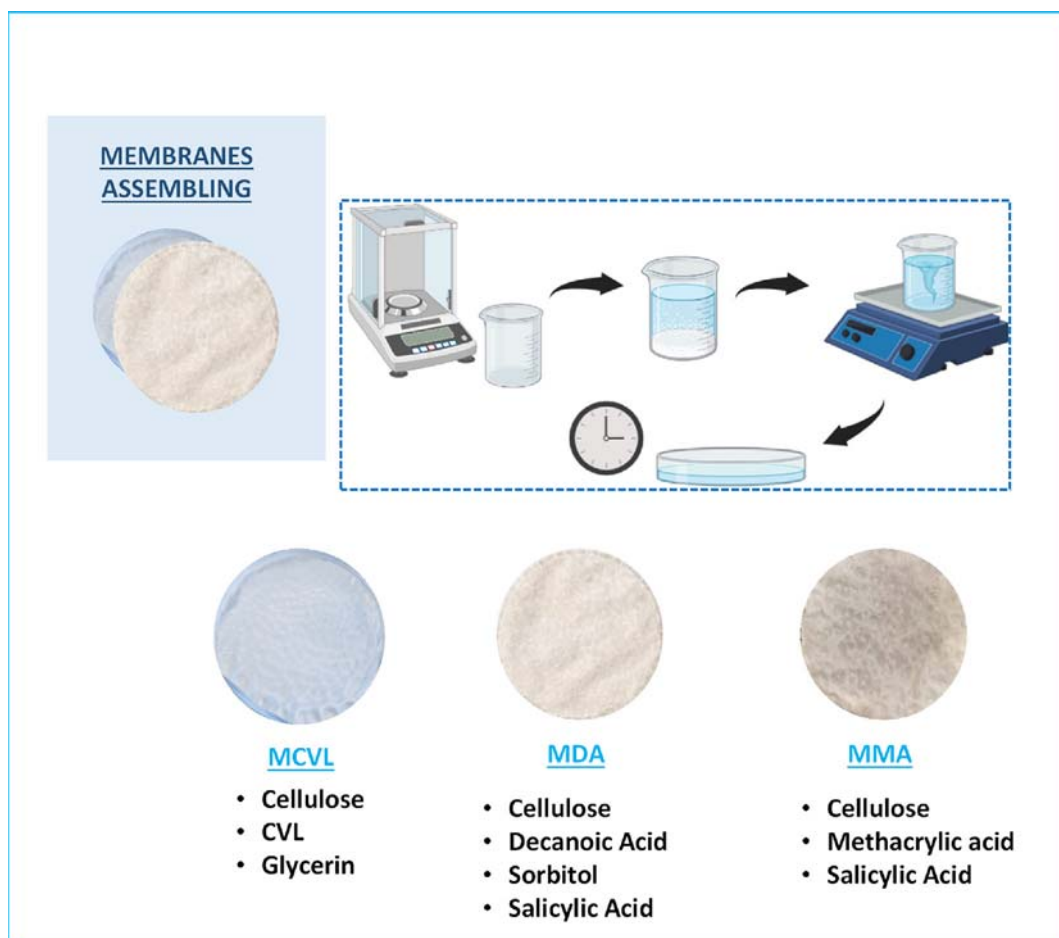


FIGURE 1 Membranes preparation. [Color figure can be viewed at wileyonlinelibrary.com]

For this purpose three different membranes were prepared: MCVL, MDA, and MMA, respectively.

Overall, these materials are based on LD consisting of three components: CVL, SA, and DA (at 35°C) or MA (at 15°C). In both cases, the observed color changed from light blue to dark blue. The color change as a function of temperature is mainly controlled by the melting point of the solvents (at 31.6 and 15°C). The presence of a color enhancer with acidic properties resulted in an irreversible color change of the system, since the melting of the solvent caused the dissolution of all components, and the acidic properties were maintained by the presence of SA even after cooling.

The mass ratio between CVL, SA, and a solvent (DA or MA) for the preparation of the thermosensitive material was investigated for different ratios of 1:6:50 and 1:6:100 and the better results in terms of kinetics and color development were observed for the higher amount of solvent. To understand the impact of the SA effect, we tested the 1:10:100 ratio, but no relevant changes in color development were observed. Furthermore, no dark blue color was observed with lower amounts of SA and the 1:6:100 ratio (0.25 g CVL; 1.5 g

SA; 2.5 g DA or MA) was selected for further measurements. Additionally, Gly was used in MCVL to improve the barrier properties against external agents. It also improves the mechanical properties of CA membranes. The addition of plasticizers such as Gly helps to increase the flexibility of the final product and improve its overall performance (Figure 1).⁴³ Sorbitol is incorporated into MAD and MMA to improve flexibility and barrier properties to external agents. This makes them more versatile and suitable for a wide range of applications.⁴⁴ Moreover, sorbitol is a small molecule that exhibits water resistance due to its lower affinity for water and stronger molecular interaction with CA.⁴⁵

3.2 | Surface characterization of the membranes

3.2.1 | Scanning electron microscopy

SEM plays a crucial role in the detailed study and analysis of surface features with high-resolution and three-dimensional images. Therefore, SEM analysis

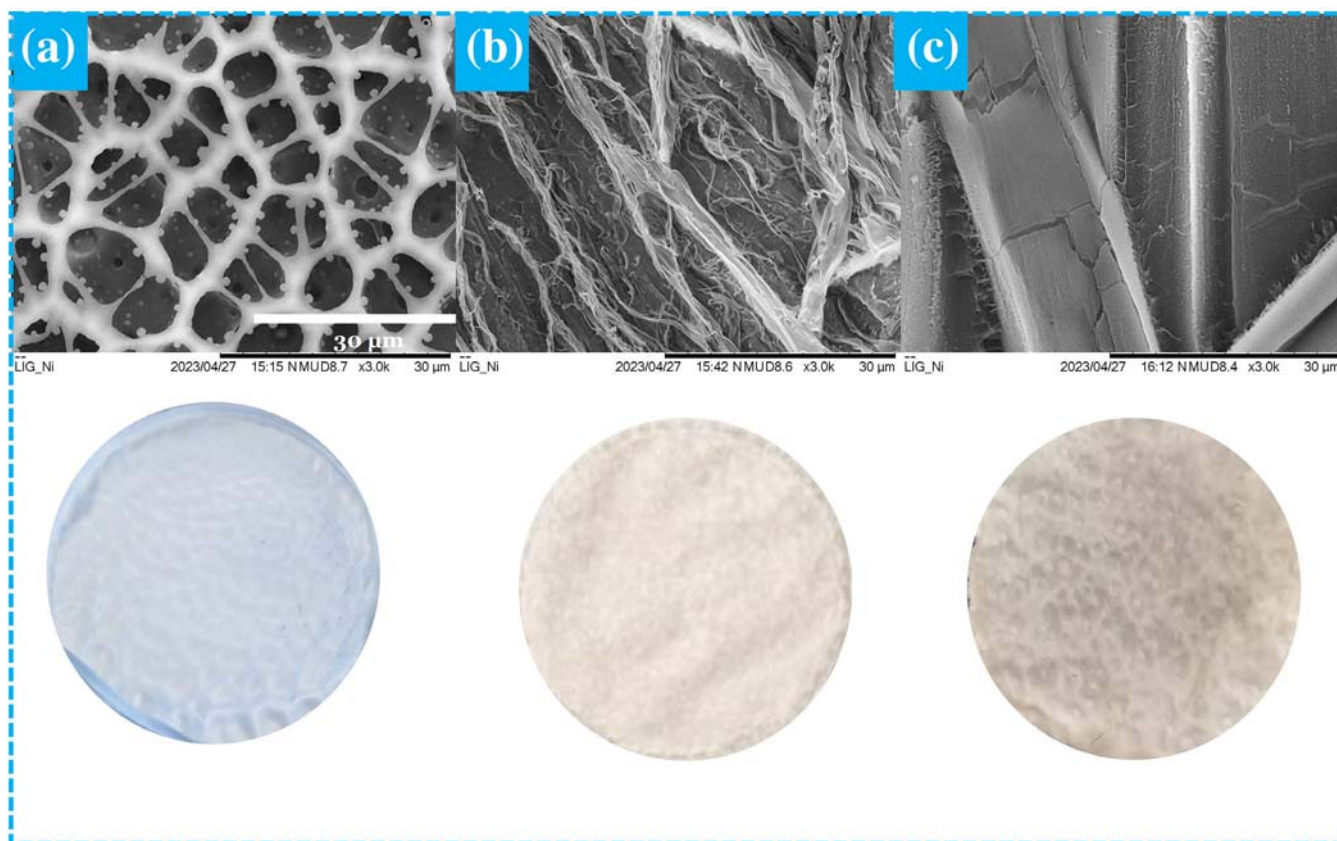


FIGURE 2 Scanning electron microscopy images for MCVL (a), MDA(b), and MMA (c). [Color figure can be viewed at wileyonlinelibrary.com]

of the different membranes prepared in this work was essential to understand the surface morphology such as porosity or roughness. Therefore, the following membranes were analyzed: MCVL, MDA, and MMA membrane, as shown in Figure 2.

According to Figure 2, the CVL membrane has a pore diameter between 4 and 10 μm . This porosity allows migration and contact of AD and AS, which are present in both MDA and MMA, upon temperature contact, resulting in a color change (dark blue) through the opening of the CVL ring. In contrast to MCVL, MDA, and MMA do not have a porous structure but a fibrous structure, as shown in Figure 2b,c.

3.2.2 | Time–temperature indicator performance

The MCVL membrane proves suitable for both temperatures (15 and 35°C) once it contains a LD that undergoes a color change upon contact with the acidic membrane at certain temperatures. The MMA contains the color developer SA and MA as a solvent with a melting point of 15°C. When the MMA is combined with the MCVL

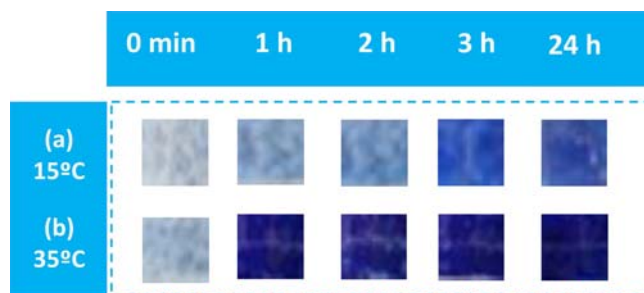


FIGURE 3 (a) MCVL and MDA membranes after junction (0 min) and after 1, 2, 3, and 24 h of 15°C exposure; (b) MCVL and MMA membranes after junction (0 min) and after 1, 2, 3, and 24 h of 35°C exposure. [Color figure can be viewed at wileyonlinelibrary.com]

membrane and exposed to a temperature of 15°C or higher, the MA dissolves the CVL in the MCVL membrane, resulting in a color change from light blue to dark blue (Figure 3a).

The MDA membrane contains the color developer SA and the solvent DA, which has a melting point of 31.6°C. When this membrane is combined with the MCVL membrane and exposed to 35°C, the DA melts and dissolves the CVL from the membrane, resulting in a color change

from light blue to dark blue. The color change was irreversible after 1 h (Figure 3b).

Overall, the results can be observed in Figure 3a when MCVL and MMA are combined and exposed to a temperature of 15°C within 24 h. Initially, (0 min) prior to exposure at 15°C the membrane appears almost colorless. Interestingly, a clear color gradient becomes visible within 24 h. The dark blue color becomes even more pronounced after 24 h at a temperature of 15°C. These promising results demonstrate the effectiveness of this double membrane as a time–temperature indicator.

Figure 3b shows the results for the assembly of MCVL and MDA and the subsequent exposure at 35°C. At the beginning, at room temperature, the double membranes have a light blue color. After being exposed at 35°C for 1 h, the color of the double layer membrane change to dark blue. This change is related to the fact that MDA contains DA, which has a melting point of 31.6°C. At a temperature of 35°C, DA undergoes a phase change that causes both DA and SA to open the CVL ring, resulting in the observed color change.

Overall, a faster development of the blue color can be observed in MDA compared with MMA. This could be related to the fact that DA dissolves earlier than MA when it meets a cellulose membrane at increasing temperature. This difference can be explained by several reasons. First, the molecular structure of DA, a long-chain fatty acid, favors its interaction with the cellulose membrane. In addition, the stronger hydrogen bonds that DA forms with cellulose increase its affinity for the membrane, which facilitates its dissolution. In addition, increasing the temperature increases the molecular movement, making the dissolution of DA in the membrane more efficient.

It is important to emphasize that the membrane has an irreversible behavior, as it retains the altered color even when exposed to 15 and 35°C for at least 10 days. This observation is supported by the stability tests described in the following section, which demonstrate the long-lasting color change.

Overall, both the MDA and MMA membranes, when assembled with the MCVL membrane and exposed to the respective temperatures, undergo a color change from light blue to dark blue, making them valuable tools for temperature-sensitive color change applications.

3.3 | Color stability study

A 10-day color stability study was conducted with CVL's double-layered membranes, consisting of MDA and MMA membranes. The membranes were exposed to temperatures of 15 and 35°C for 24 h. After exposure, the TM

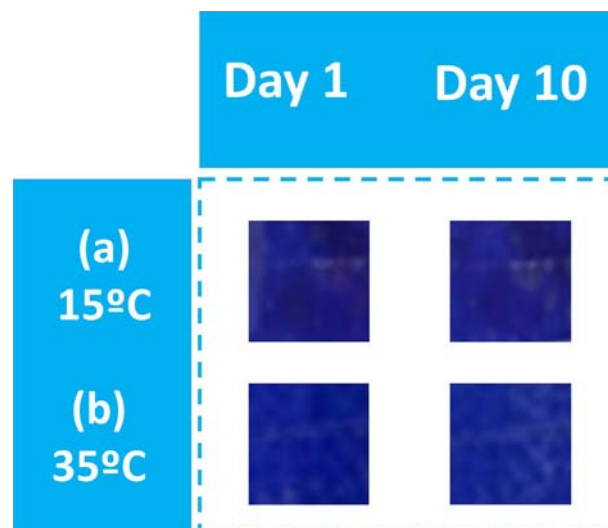


FIGURE 4 Results of the 10-day stability study of the double membrane of MCVL and MDA after 24-h exposure at 35°C. Results of the 10-day stability study of the double membrane of MCVL and MMA after 24-h exposure at 15°C. [Color figure can be viewed at wileyonlinelibrary.com]

was kept at room temperature and observed for 10 days. As can see in Figure 4a,b, the color of the membranes changes slightly after the 10-day exposure period. This is evidence of the irreversible nature and stability of this double-sensitive membrane.

Overall, Figure 4a shows the results of the color stability study performed with the irreversible double membrane of MCVL and MMA at a temperature of 15°C. This study was performed using the same methodology as the study with the irreversible double membrane at 35°C (Figure 4b), with both experiments performed in triplicate.

3.4 | Color transition temperature of membranes

This study was carried out with the double membranes MCVL and MDA or MMA to investigate the color transition of the ternary system at temperatures below the melting point of the solvent used. The aim of this study was to ensure that no color change occurs before 15 or 35°C. To achieve this, several membranes were prepared in triplicate and exposed to different temperatures, including room temperature, 8, –16, and 15°C for TM (15°C) and 8, –16, and 35°C for TM (35°C). The results obtained are shown in Figure 5.

According to (Figure 5a), temperatures below 15°C for TM (15°C) do not cause a color change to dark blue, contrary to what was observed after exposure at 15°C.

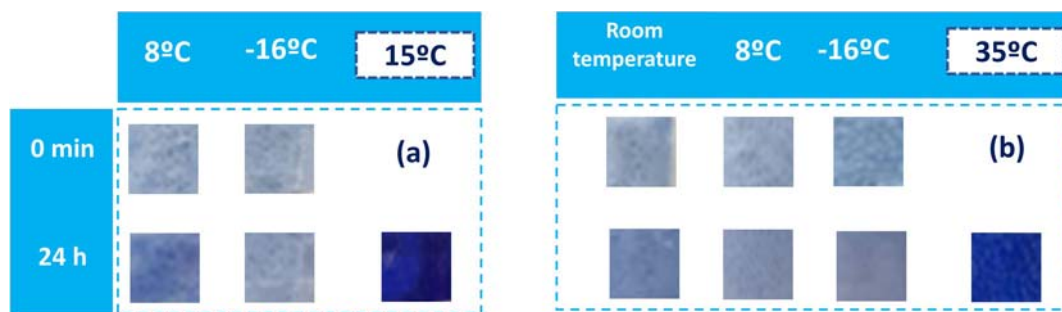


FIGURE 5 (a) Results of the thermal behavior test for the double membrane MCVL and MMA before (0 min) and after 24 h at 8 and -16°C and after 24 h at 15°C ; (b) results of the color change test for the double membrane MCVL and MDA before (0 min) and after 24 h at room temperature, 8, and -16°C and after 24 h at 35°C . [Color figure can be viewed at wileyonlinelibrary.com]

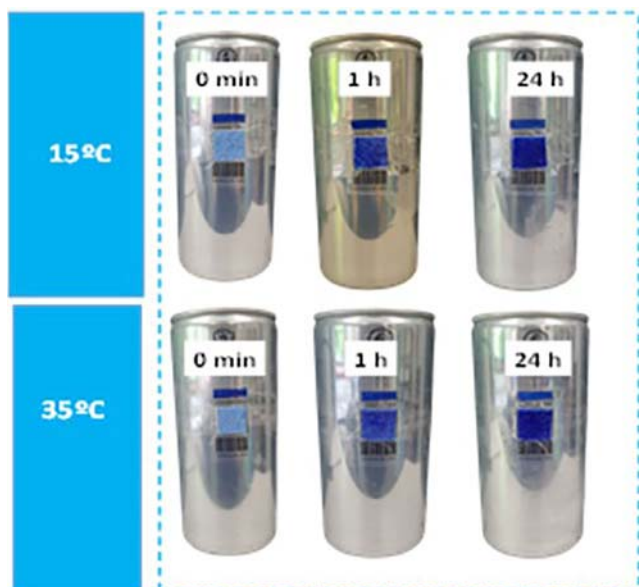


FIGURE 6 Application of double-layer membrane in aluminum cans. [Color figure can be viewed at wileyonlinelibrary.com]

From these results, we can safely conclude that the irreversible color change of the bilayer membrane occurs specifically at temperatures close to 15°C , with no color change occurring at temperatures below this threshold.

The same method was used to investigate the irreversible behavior of the MCVL and MDA double membranes at 35°C (Figure 5b). The membranes were also examined for their color transition behavior at temperatures of 8 and -16°C and the comparison of color after 24 h at 35°C . Overall, no color change from light blue to dark blue was observed at temperatures below 35°C . It can therefore be concluded that the irreversible double membrane of MCVL and MDA is selective for the temperature of 35°C and that no color change occurs at temperatures below the melting point of the solvents used.

3.5 | Tests in water filled aluminum cans

The double-layered membrane was applied to the cans filled with water to test the kinetics of color development. The double-layer membrane was applied to acetate labels and then to the cans. It is interesting to note that the kinetics of color development are different for the membranes applied to the cans with the double-layered membranes when compared with the membranes applied alone (please see Figure 6). When the TMs are placed in the cans, no color change is observed at 15 and 35°C , but after 1 h of exposure, the color of the membrane changes to dark blue. This result could be attributed to the heat transfer between water and cans.

Overall, both samples showed a change in color (Figure 6) after 1 h and 24 h of exposure. This test shows that these cans can be used to monitor foods that need to be stored at a temperature of 8°C .

4 | CONCLUSION

In summary, this study successfully developed thermo-chromic double membranes based on CA for temperature monitoring in liquid beverages, especially milk in aluminum cans. The membranes were designed to undergo an irreversible color change at certain temperatures, providing visual indications of temperature changes during storage and transport and thus increasing food safety.

The TMs were produced using a three-component system consisting of a LD membrane (MCVL) and an acid membrane containing the color developer (SA) and the acid solvent (DA or MA) for different melting points and temperature monitoring. The MCVL membrane in combination with the MDA membrane irreversibly changed color when exposed to 35°C , which was facilitated by

the melting of the DA membrane and subsequent dissolution of the MCVL membrane. Similarly, the MCVL membrane irreversibly changed color when combined with the MMA membrane and exposed to 15°C. Overall, the results demonstrated the successful production of thermochromic double membranes with reproducible color changes at specific temperatures.

Reproducibility studies showed consistent color changes of the membranes, indicating their reliability in temperature monitoring. The membranes were stable and their color did not change below the melting point of solvents, further confirming their applicability for food safety. Overall, these cellulose acetate-based thermochromic double membranes offer a promising solution for temperature monitoring in liquid beverages and allow visual temperature monitoring during storage and transport. The introduction of such intelligent and active packaging systems can significantly contribute to ensuring food safety and quality worldwide.

AUTHOR CONTRIBUTIONS

Margarida Carvalho: Investigation (equal); methodology (equal); validation (equal); writing – original draft (equal). **Ana. P. M. Tavares:** Methodology (equal); writing – review and editing (equal). **Maria A. Marques:** Methodology (supporting); validation (supporting). **Joaquim J. Alves:** Methodology (supporting); validation (supporting). **Joana Figueira:** Investigation (equal); methodology (equal); writing – review and editing (equal). **Maria Morais:** Methodology (equal); writing – original draft (equal). **Joana V. Pinto:** Formal analysis (supporting); investigation (supporting); methodology (supporting); validation (supporting); writing – review and editing (supporting). **Felismina T. C. Moreira:** Conceptualization (lead); formal analysis (lead); investigation (lead); methodology (lead); validation (lead); writing – review and editing (lead).

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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