Development Of A Colour-Changing Patch To Detect Temperature Fluctuation

A

Dissertation submitted
In partial fulfilment of the requirement for the degree of

Masters of Science

In

Biochemistry

Submitted by

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CANDIDATE'S DECLARATION

I, Anindita Ghosh, hereby declare that the work being presented in the thesis entitled "Development of Colour-changing Patch to Detect Temperature Fluctuation" in partial fulfilment of the requirement for the award of the degree of Masters of Science in Biochemistry and being submitted to the School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, is my work carried out during the period January 2023 to June 2023 under the supervision of Dr Diptiman Choudhury, Assistant Professor, School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala. No part of the matter embodied in this thesis has been submitted to any other university or institute for the award of any degree.

DATE: 28 July 23

PLACE: Patiala.

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This is to certify that the above statement made by the candidate is correct and true to the best of my knowledge.

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CERTIFICATE

This is to certify that the thesis entitled "Development of Colour-changing Patch to Detect Temperature Fluctuation", is submitted by Ms Anindita Ghosh, in partial fulfilment of the requirement for the award of the degree of Masters of Science in Biochemistry to School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, is an authentic record of the work carried out by her under our supervision. The content of this thesis has not been submitted for the award of any other diploma or degree.

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ACKNOWLEDGEMENT

I take this opportunity to express gratitude to Dr. Diptiman Choudhury, Associate Prof., faculty of the department of the School of Chemistry and Biochemistry, my mentor during the dissertation project, for his supervision and encouragement leading to the unhindered completion of the project work and for giving me this opportunity to do my summer internship under her expert guidance.

I would like to extend my sincere thanks and appreciation to Sunidhi Sharma (PhD Scholar), Komal Atrri (PhD Scholar) and Deepinder (PhD Scholar) who have helped and guided me throughout the project irrespective of the circumstances which made it possible to complete the whole experiment. My deepest regards for the help provided in terms of infrastructure and their quick response to overcome any problems within the project's tenure.

I convey my sincere gratitude to my PG Coordinator Dr. Kamaldeep Paul, Professor, faculty of the department of the School of Chemistry and Biochemistry, for giving me unconditional support and encouragement throughout this project, and Dr. Satnam Singh, head of the department of School of Chemistry and Biochemistry, for giving me this opportunity and exposure to explore more about research field.

Last but not least I would like to thank my family, Komal Verma and Anjali Sharma for supporting me in many ways throughout the project.

CONTENTS

Lists of Figures

Lists of Tables

Lists of Abbreviation

pH: Potential of Hydrogen

^{o} C: Degree Celsius

Temp.: Temperature

CMC: Carboxymethyl Cellulose

PEG: Polyethylene glycol

1. ABSTRACT

It is not possible to visually determine if a drug has degraded or been exposed to high temperatures because degradation is not related to its expiration date. Therefore, there is a need to create a product that can differentiate visibly between degraded drugs and others. In this study, we are developing a cost-effective patch that can detect temperature fluctuations and be placed on top of the drug label. The patch will be pH-sensitive and temperature-sensitive, changing color when exposed to higher temperatures. This color change will help distinguish degraded drugs from others. The experiment involves simple chemical reactions, including the absorption of evaporated water and the detection of pH changes by a pH indicator. The setup consists of four layers: a hydrogel layer to absorb water, NaOH paper, a thymol blue patch, and a wax slack layer separating the first two layers. When the setup is exposed to high temperatures, water evaporates from the hydrogel layer and is absorbed by the NaOH paper. This causes both the NaOH paper and the thymol blue patch to become wet, resulting in a pH change that changes the color from yellow to blue. As a waste paper management strategy, extracted cellulose from office waste paper is used to synthesize CMC hydrogel blend as a water absorbent layer.

2. INTRODUCTION & LITERATURE REVIEW

2.1 Introduction

Various drugs are sensitive to temperature also known as thermolabile drugs, especially protein drugs. Protein drugs are highly unstable in high temperatures and start to degrade when exposed (Dan Katzki, 2013). It is reported that protein drug starts degrading when exposed to temperatures higher than 25ºC (YC Chen, 2017; B Leader, 2008).

According to a review paper by Loftsson et al. (2014) published by the Arthritis Foundation, it has been found that temperature-sensitive drugs can lose their effectiveness and, in rare cases, become toxic when exposed to fluctuations in temperature, humidity, or sunlight for extended periods of time. This poses a significant problem, particularly in urgent situations where the use of such drugs is necessary. Improper storage can lead to the administration of degraded drugs, which not only fail to have any therapeutic effect on the patient but may also cause harm if they have become toxic. It is important to note that the degradation of these drugs is not necessarily indicated by their expiration date, making it difficult to visually determine their quality. Consequently, there is a need to develop a product that can visually distinguish between degraded drugs and those that remain unaffected.

In this study, the we aim to develop a cost-effective and efficient method for creating a temperature-sensitive patch that can be applied to drug labels. The patch will be designed to change color when exposed to elevated temperatures, allowing for easy differentiation between degraded drugs and others. The experiment relies on basic chemical reactions, such as the absorption of evaporated water and the detection of pH changes using a pH indicator. The setup consists of four layers: a hydrogel layer to absorb water, a NaOH paper layer, a thymol blue patch, and a wax slack layer separating the first two layers. When the setup is exposed to high temperatures, the water in the hydrogel layer evaporates and is absorbed by the NaOH paper, causing both the NaOH paper and the thymol blue patch to become wet. Consequently, the thymol blue patch detects the pH change and changes color from yellow to blue.

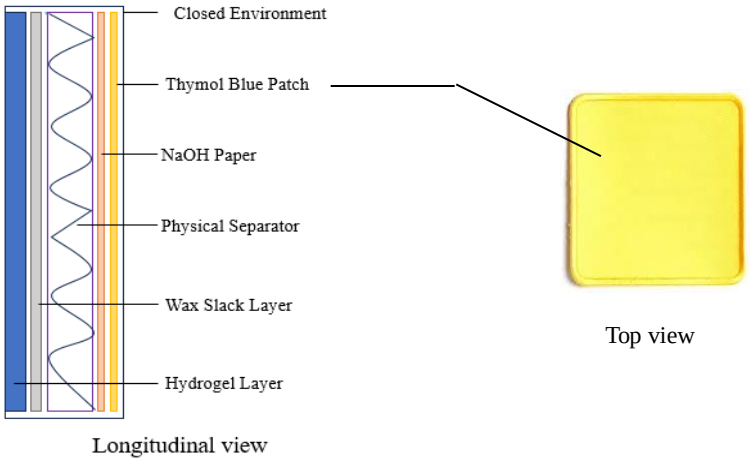

Fig 1: Figure representing the finals setup of pH-sensitive and temperature-sensitive colour-changing patch

2.2 Literature Review

In order to determine the degradation or exposure to high temperatures of a drug, visual inspection alone is insufficient as degradation is not necessarily correlated with the drug's expiration date. Consequently, there is a need to create a product that can effectively distinguish degraded drugs from those that are not.

The inspiration for this experiment is derived from a scholarly article titled "Thermochromic ink as a smart indicator on cold product packaging – review" (Thamrin et al., 2022). In this study, Thamrin et al. employed thymol blue and NaOH paper within food packaging to detect the presence of moisture. The researchers observed that the intensity of the blue color of thymol blue, upon reacting with NaOH paper, served as an indicator of moisture content. Specifically, a higher intensity of blue color corresponded to a greater amount of moisture. This preliminary test aimed to detect moisture content in cold food packaging. While pH indicators exhibit a permanent color change, thermochromic ink can revert to its original form or color when the temperature is lowered, as the temperature threshold for permanent change in thermochromic ink exceeds 70°C (Friškovec, 2010). Consequently, we decided against using thermochromic ink due to its reversible reaction and high cost.

In this study, the use of pH paper as a standalone product was found to be limited due to its patented nature. As a result, an alternative approach was taken by formulating ink using thymol blue, with slight modifications to the ink formulation of Cyan blue (Causley et al., 1989). Thymol blue, a greenish-brown or brownish-red crystalline powder, is a key component of pH paper. It is soluble in alcohol and dilute alkaline solutions, but insoluble in water. Thymol blue exhibits a color transition from red to yellow within the pH range of 1.2 to 2.8, and from yellow to blue within the pH range of 8.0 to 9.6 (Zaggout, F. R, 2006). The thymol blue ink was prepared and adjusted to a pH of 6.8, resulting in a bright orangish-yellow color. Upon reaction with NaOH, the thymol blue ink undergoes a structural change, leading to a transition from yellow to blue.

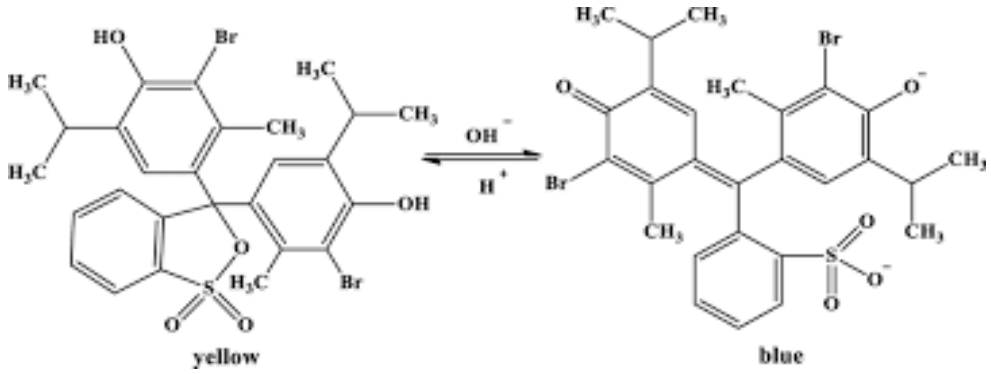

Fig 2: Figure representing the structural change occurring in thymol blue structure upon reacting with NaOH

In office and academic environments, there is a significant amount of paper waste. To address this issue, we have implemented a waste management strategy in our product development process. Specifically, we have focused on synthesizing a hydrogel from a cellulose derivative known as CMC hydrogel blend. The choice to use CMC hydrogel blend is due to its superabsorbent properties, allowing it to absorb large amounts of liquid. The degree of swelling of the hydrogel can be tailored, ranging from 100% to 5000%, depending on the degree of substitution of CMC, the extent of crosslinking with CA, and the addition of PEG. Furthermore, the hydrogel remains stable at temperatures of 60°C.

The application of CMC hydrogel blend includes its ability to adsorb and release proteins, making it suitable for use as a carrier for protein-based drugs in drug delivery systems. Additionally, it can be utilized in DNA scaffolding.

Initially, cellulose is obtained from waste paper through acid hydrolysis, and subsequently, carboxymethyl cellulose (CMC) is synthesized using the extracted cellulose through alkylation and esterification processes. CMC, a derivative of cellulose, is commonly found in the form of Carboxymethyl Cellulose sodium salt. CMC has various applications in different fields, including electronics, pesticides, leather, plastics, printing, ceramics, and the daily-use chemical industry. Additionally, CMC can be utilized for the temporary relief of burning, irritation, and discomfort caused by dryness of the eye resulting from exposure to wind or sun.

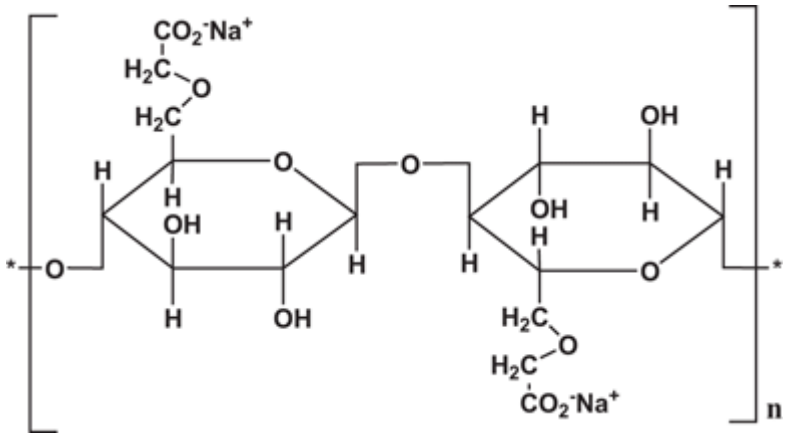

Fig 3: Fegure representing the structure of Carboxymethyl Cellulose sodium salt

2.3 Research gaps

There is currently no effort being made to develop color-changing codes for drugs, similar to what is being done in the food packaging industry. There is a lot of research being conducted on the development of thermochromic ink codes in food packaging, such as QR codes or interactive designs that change color when exposed to higher temperatures or for a specific duration. However, there is a lack of similar efforts in the pharmaceutical industry. As mentioned earlier, thermolabile drugs can degrade due to temperature fluctuations, leading to reduced effectiveness or, in rare cases, toxicity that can harm patients. Therefore, a solution is needed for this issue.

2.4 Hypothesis

The pH indicator undergoes a visible alteration in color upon exposure to a change in pH. Therefore, it is hypothesized that by establishing a connection between the evaporation of water and temperature, it may be possible to achieve a color change at a specific temperature.

2.4 Objective of the study

- To develop a setup that allows for optimal conditions for the reaction to occur.
- To analyze the color change and duration of the reaction in order to understand how it works.
- To prevent any unwanted reactions that could interfere with the main reaction.
- To discover a cost-effective and efficient method for creating a patch that changes color based on temperature sensitivity.

3. MATERIALS AND METHODS

3.1. Material used

3.1.1 Hydrogel used

The hydrogel used here is Sodium Polyacrylate brought from Bangalore Fine Chemicals,
Empirical formula: $(C_3H_3NaO_2)n$,
Catalogue no. 9419

3.1.2. Wax used

The wax used for this experiment is palm wax.

3.1.3. Oil used

The oil used in this experiment is Sundrop Sunflower oil, marketed by Agro Tech Food Ltd. And is brought from a local grocery store.

3.1.4. pH paper used

The pH paper used is Qualigens full range indikorm strips by Thermo Fisher Scientific (I) Pvt. Ltd, catalogue no. 10140

3.1.5. Thymol blue used

The thymol blue used is Thymol blue indicator by Loba Chemie Pvt. Ltd, Empirical formula: C27H30O5S, molecular weight: 466.59 g/mol, catalogue no. 06306

3.1.6. Paper used

5 g of office paper is used.

3.2. Preparation of NaOH paper

A 0.1 N sodium hydroxide (NaOH) solution was prepared by dissolving 4 grams of NaOH pellets in 80 mL of distilled water, and the final volume was adjusted to 100 milliliters (Revilla Sai Vamshi, 2021). Wattman paper was cut to match the dimensions of a 35 mL Petri plate. Subsequently, the cut paper was immersed in the NaOH solution and subjected to drying in a hot air oven at a temperature of 20 degrees Celsius for a duration of 10 minutes. This dipping and drying process was repeated twice to ensure consistent and even dispersion of the solution on the paper.

3.3. Setup to observe the absorption of evaporated water

In the initial phase of the experiment, various configurations are tested to investigate the absorption of evaporated water and determine the optimal setup. The setup consists of three components: a layer designed to absorb water, NaOH paper, and a pH-sensitive color-changing patch. A 100mm Petri plate is utilized and sealed with parafilm to create a closed system. The reaction occurring within this closed system can be described as follows:

- Initially the NaOH paper will absorb the evaporated water from the water-absorbent layer
- This wet NaOH paper results in changing the pH of the colour-changing parch hence the colour change is observed.

3.3.1 Preliminary tests

3.3.1.1 Setup I

In this experimental configuration, a layer of wet tissue serves as the water absorbent component, while a colour-changing patch is created using methyl orange. The methyl orange patch is prepared by immersing Wattman filter paper in a solution of methyl orange and subsequently drying it. These components are arranged in a concentric circle pattern on a 100mm Petri plate, with the wet tissue forming the outermost layer, followed by the methyl orange patch and a NaOH paper patch, as deFigted in the accompanying diagram. The Petri plate is then sealed using parafilm

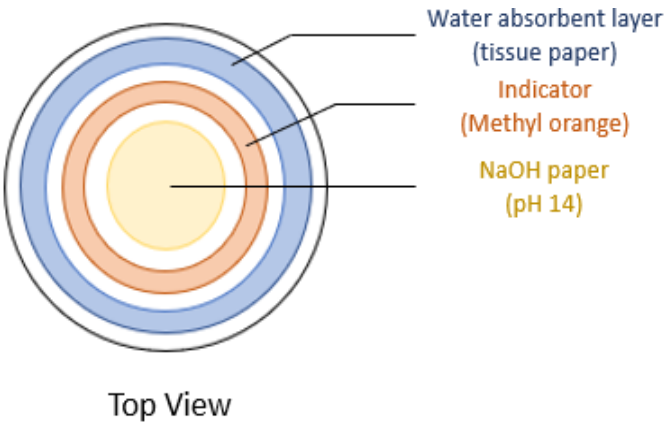

Fig 4: The figure represents the top view of setup I to determine the absorption of evaporated water

3.3.1.2 Setup II

The configuration of this arrangement is identical to setup I, with the exception that the Methyl orange patch is positioned above the NaOH paper. Both components are suspended above the wet tissue paper, which serves as a water-absorbent layer. This arrangement is facilitated by the use of threads, as deFigted in the diagram.

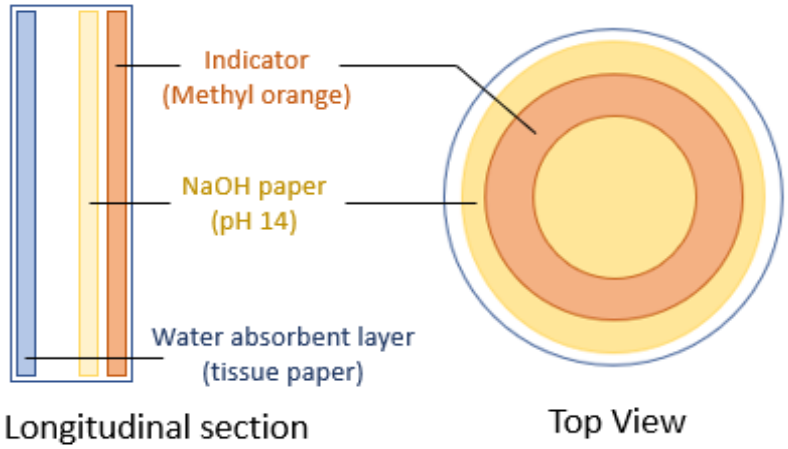

Fig 5: The figure represents the longitudinal and top view of the arrangement of setup II for the determination of absorption of water vapour.

3.3.2 Final test

3.3.2.1 Setup III

Similar to setup II, this setup contains the components, which include Naoh Paper, pH paper as colour changing patch and wet tissue as water absorbent layer. The NaOH paper and pH paper are suspended above wet tissue paper using thread as support, as represented in the diagram.

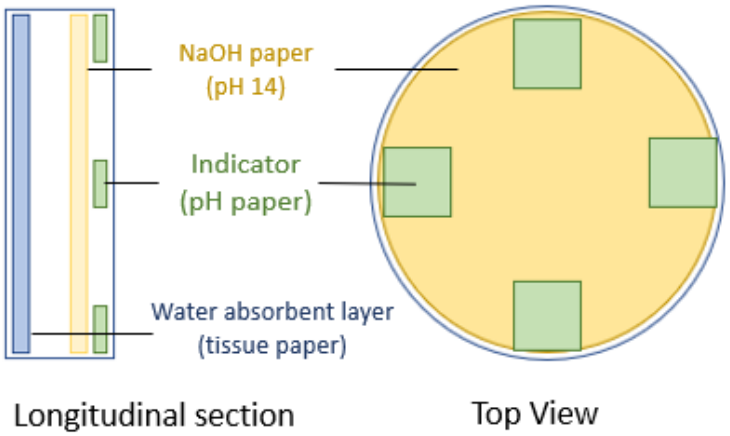

Fig 6: The figure represents the longitudinal and top view of the arrangement of setup III for the determination of absorption of evaporated water.

3.4 Determination of water absorbent layer

This experiment was conducted to ascertain the optimal water-absorbent layer. The desired attribute of a water-absorbent layer is its ability to retain sufficient water until the drug's expiration date. Hydrogels, particularly Sodium Polyacrylate, are renowned for their water-absorbing properties and were employed in this study. The objective was to determine the superior water-absorbent layer between wet tissue paper and hydrogel.

In this experiment, the experimental arrangement closely resembles that of setup III, wherein the pH strip and NaOH paper are suspended above the water-absorbent layer. Two distinct sets are prepared, one utilizing wet tissue paper and the other employing hydrogel as the water-absorbent layer, as deFigted in the diagram. To create a confined environment, a 35 mm plate is employed and subsequently sealed with parafilm once the components have been arranged within the plate.

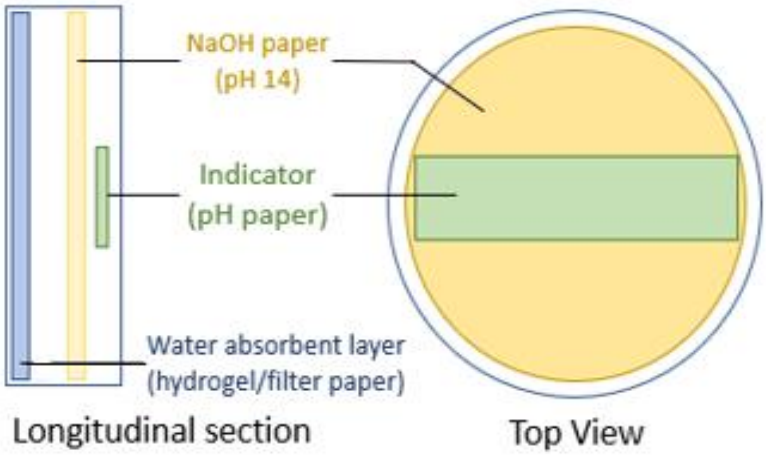

Fig 7: The figure represents the longitudinal and top view of the arrangement of setup for the determination of water absorbent layer

3.5 The process of making the ink

For this experiment, we are using Thymol Blue, a component of pH paper, which gives a red colour, yellow colour and blue in acid, neutral, and basic pH, respectively (Moore Z., 2017). Thymol blue is used as it gives drastic colour change with the change in pH and is dissolvable in ethanol which is easily available.

3.5.1 Preliminary ink samples

3.5.1.1 Sample Ink I

Initially, 100mg of thymol blue is dissolved in 5 mL of ethanol. The solution is then poured into a fountain pen to check if it has the fluidity required to stamp on paper.

3.5.1.2 Sample Ink II

Following the protocol with slight modifications, we have prepared the ink stock by adding 250 mg of thymol blue, 3.6 mL of Triethanolamine, and 5 mL of ethanol. The ink is prepared by diluting the ink stock by adding 1.2 mL of ETDA dissolved in deionized water and 1.6 mL of 1N HCL in 1 mL of ink stock. HCL was added to achieve the required pH.

3.5.2 Final Ink Sample

We prepared the ink stock by dissolving 456 mg of thymol blue in 3.6 ml of Triethanolamine, and 10 mL of ethanol was added. To attain the desired pH of the ink, it is diluted by adding 1.5 mL of HCL in 1 mL ink stock.

3.6. Prevention of condensation

The condensation of water at low temperatures is a well-known phenomenon. This occurrence can be visually observed through a change in color when test sets are exposed to lower temperatures. To prevent interference from water absorption, it is necessary to establish a physical barrier between the water-absorbent layer and the NaOH paper. Natural water repellents such as wax and oils have been employed to create this barrier. Wax, known for its high melting point, and oils, which possess a fluid nature due to their low melting points (Xiao, J., 2016), were chosen for this purpose. However, using wax in its original state would impede water evaporation even at high temperatures, while the use of oils would disrupt the fundamental workings of the experiment. In this particular experiment, palm wax with a melting point of 78 oC (Ahmad, N., 2011) and Sundrop Sunflower refined oil with a melting point of 6 oC were utilized. Various ratios of these substances were mixed, and their melting points were measured using a manual melting point set-up.

3.6.1 Determining the melting point of Wax : Oil ratios.

The melted wax is combined with oil in various ratios ranging from 1:1 to 1:35 in order to achieve a final volume of 1mL in an Eppendorf tube. Each Eppendorf tube is labeled according to the specific ratio used. The Eppendorf tubes are then heated and agitated to ensure a homogeneous dispersion of wax in the oil. Subsequently, they are stored in a cold environment until solidification occurs. Using a capillary tube, samples are extracted from each Eppendorf tube. Next, a beaker with a volume of 250 mL is filled to one-fourth of its capacity with water and heated on a heat plate. The capillary tube is attached to a thermometer in a manner that positions the end containing the sample at the same level as the thermometer's tip. The

thermometer, along with the attached capillary tube, is then immersed in the heated water. The capillary tube is observed until it becomes completely transparent in the water, and the corresponding temperature is recorded.

3.6.2 Determining the working wax ratios

1:3, 1:32, and 1:35 wax: oil ratios are used as the wax layer on top of the hydrogel layer. A thin layer of half-frozen wax is poured on top of the hydrogel layer in a 35mm plate and is evenly spread to provide full coverage. After coating all the plates, the plates are exposed to temperatures 25^{o}C, 30^{o}C, 35^{o}C and 40^{o}C respectively for 1h interval each.

3.7. Determination of wax layer thickness

The determination of the thickness of the wax layer is crucial due to its impact on the subsequent reaction. If the wax layer is excessively thick, it will remain stagnant on the hydrogel layer after melting, thereby preventing the exposure of the hydrogel layer and hindering the reaction. Conversely, if an insufficient amount of wax is poured, the hydrogel layer will not be adequately covered, leading to the observation of water condensation at lower temperatures. Consequently, it is imperative to ascertain the appropriate thickness of the wax layer.

3.7.1 Preliminary tests

1mL, 1.5 mL and 2 mL of wax are poured on top of the hydrogel layer in a 35mm plate and spread uniformly using a spatula for all 1:15, 1:17, 1:18, 1:19 and 1:20 Wax: Oil ratios. The plates are exposed to temperatures 25^{o}C, 30^{o}C, 35^{o}C and 40^{o}C respectively for 1h interval each and observation is noted.

3.7.2 Final test

0.6 mL, 0.7 mL and 0.8 mL of wax are poured on top of the hydrogel layer in a 35mm plate and spread uniformly using a spatula for all 1:15, 1:17, 1:18, 1:19 and 1:20 Wax: Oil ratios. The

plates are exposed to temperatures 25^{o}C, 30^{o}C, 35^{o}C and 40^{o}C respectively for 1h interval each and observation is noted.

3.8 Synthesis of CMC hydrogel blend

In order to develop a proficient waste management approach for this particular product, cellulose has been derived from discarded office paper and subsequently modified to produce Carboxymethyl cellulose. The resulting CMC hydrogel blend exhibits stability at a temperature of 60 degrees Celsius, rendering it suitable for utilization as a highly efficient water-absorbing layer within this patch.

3.8.1 Extraction of cellulose

Cellulose can be obtained from office waste paper through a process known as acid hydrolysis. In this method, 5 grams of office waste paper is first shredded and then combined with 100 mL of a 7.4% NaOH solution. The resulting mixture is stirred for a period of 6 hours to facilitate the dissolution of cellulose. Subsequently, the solution is filtered and subjected to titration using a 3 M H2SO4 solution until the pH reaches 5. Following this, the sample is centrifuged at a speed of 8000 rpm for a duration of 10 minutes, and the supernatant is discarded. To eliminate any residual ink, the resulting pellet is washed with a combination of H2O2 and ethanol, and subsequently dried

3.8.2 Synthesis of CMC

In an experimental setting, a 10 mL solution containing NaOH and Cellulose was prepared with a molar ratio of 4:1. The solution was placed in a water bath and stirred for a duration of 1 hour at a temperature of 35 oC. Additionally, a separate solution consisting of NaOH and Chloroacetic was prepared with a molar ratio of 4:3. This solution was then diluted with 35 mL of 95% ethanol and subsequently added to the previously mentioned solution. The combined solution was then subjected to a water bath for a duration of 1 hour and 30 minutes at a temperature of 75 oC. After cooling the solution to room temperature, it was titrated against a concentrated solution

of HCL until a neutral pH was achieved. The resulting solution was then centrifuged at a speed of 6000 rpm for a duration of 20 minutes and subsequently dried.

3.8.3 Synthesis of CMC hydrogel blend

A CMC hydrogel is prepared by dissolving 1.8 g of carboxymethyl cellulose (CMC) in 100 mL of distilled water, followed by thorough stirring until complete dissolution is achieved. Subsequently, 200 uL of polyethylene glycol (PEG) is introduced into the CMC solution and stirred for a duration of 20 minutes. Following this, 10 g of citric acid is added to the solution and allowed to react for a period of 20 minutes. Subsequently, a volume of 10 mL of the resulting mixture is transferred into 100 mm petri plates and subjected to a hot air oven at a temperature of 40 oC for a duration of 24 hours, followed by an additional 24 hours at 80 oC. This sequential process leads to the formation of a CMC hydrogel.

3.9 Working of CMC hydrogel blend

This series of experiments aims to investigate the desorption affinity of absorbed water molecules from hydrogel materials. In certain cases, water molecules can become tightly bound to hydrogel, resulting in minimal water loss. The objective is to assess the potential of these hydrogels as a viable alternative to sodium polyacrylate hydrogel in patch applications.

3.9.1 Determining the efficiency of CMC hydrogel blend

This experimental configuration is identical to setup III, aimed at investigating the occurrence of evaporation at temperatures of 25oC, 30oC, 35oC, and 40oC. However, in this instance, the sodium polyacrylate hydrogel has been replaced with a blend of MC hydrogel.

3.9.2 Determining the working of the patch using CMC hydrogel blend as a water-absorbent layer

The experimental configuration closely resembles setup III, with the exception that the water absorbent layer consists of a blend of CMC hydrogel. Additionally, varying amounts of slack wax, specifically in ratios of 1:15, 1:17, 1:18, 1:19, and 1:20, are employed in different sets. Subsequently, the sets are subjected to different temperatures, specifically 25°C, 30°C, 35°C, and 40°C, respectively, and the outcomes are subsequently observed.

4 RESULTS

Results for determining the absorption of evaporated water

- Results of preliminary tests

- for setup, I, no absorption of water or colour change is observed in any of the tests over the span of 2 hours at intervals of 20 min and 30^0 C, 35^0 C and 40^0 C respectively.

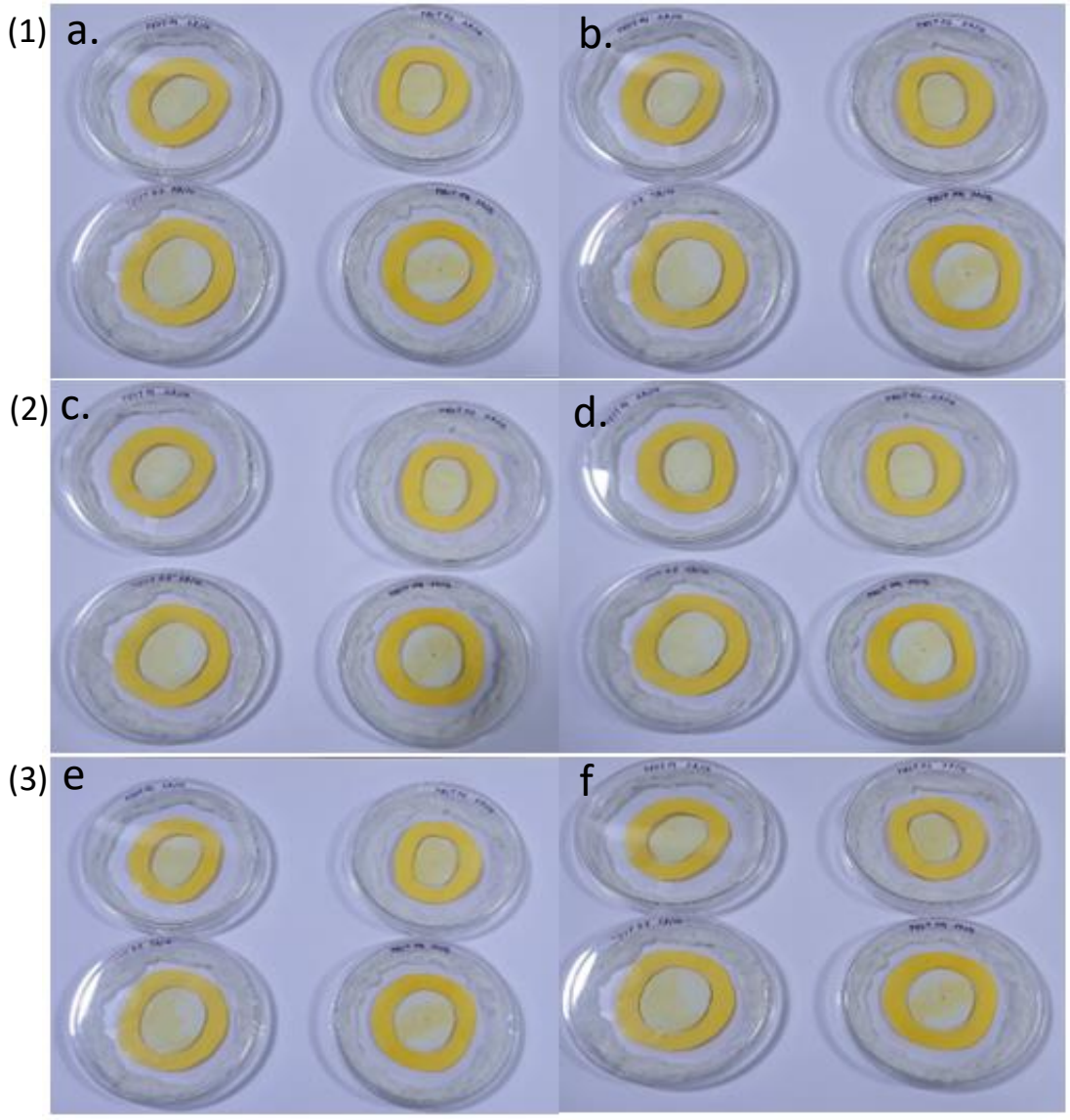

FIG 8: The figures (a to f) represent the observation made in the span of 2h at 20 min intervals respectively. (1), (2) and (3) represents the observation taken at temperature 30^0 C, 35^0 C and 40^0 C respectively.

- For setup II, water absorption is observed but no colour change is seen as methyl orange in the test.

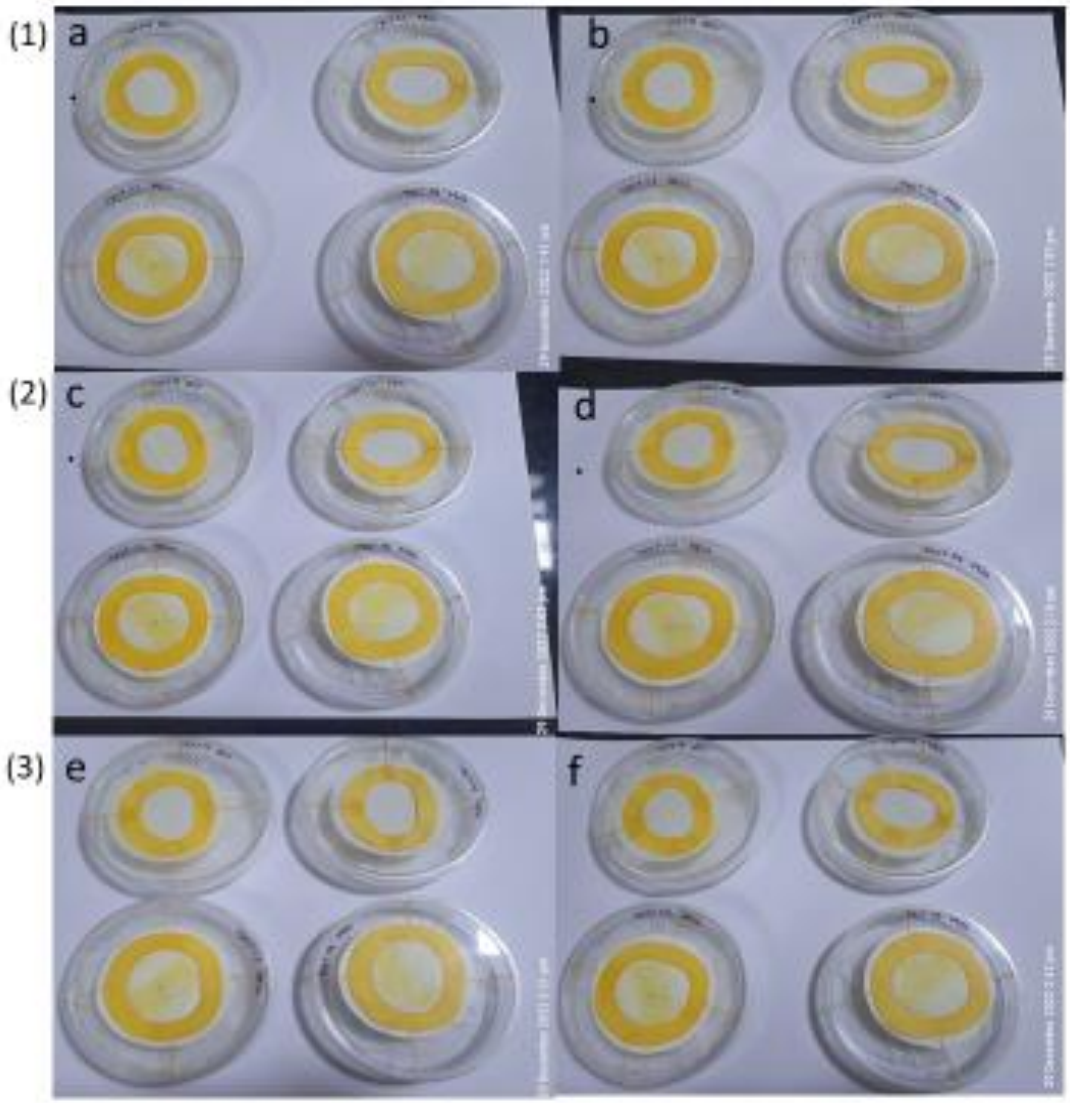

FIG 9: The figures (a to f) represent the observation made in the span of 1h min intervals respectively. (1), (2) and (3) represents the observation taken at temperature 30^0 C, 35^0 C and 40^0 C respectively.

Inference: As NaOH paper is suspended above the wet tissue paper, therefore it is easy to absorb the evaporated water. The working pH of methyl orange is 3 – 4.5 and the pH of this reaction is 14 as we are using 0.1M NaOH, hence no colour change is seen.

- For setup III, Both water absorption and colour change are observed in the test over 2 hours at intervals of 10 min and 30^0 C, 35^0 C and 40^0 C respectively..

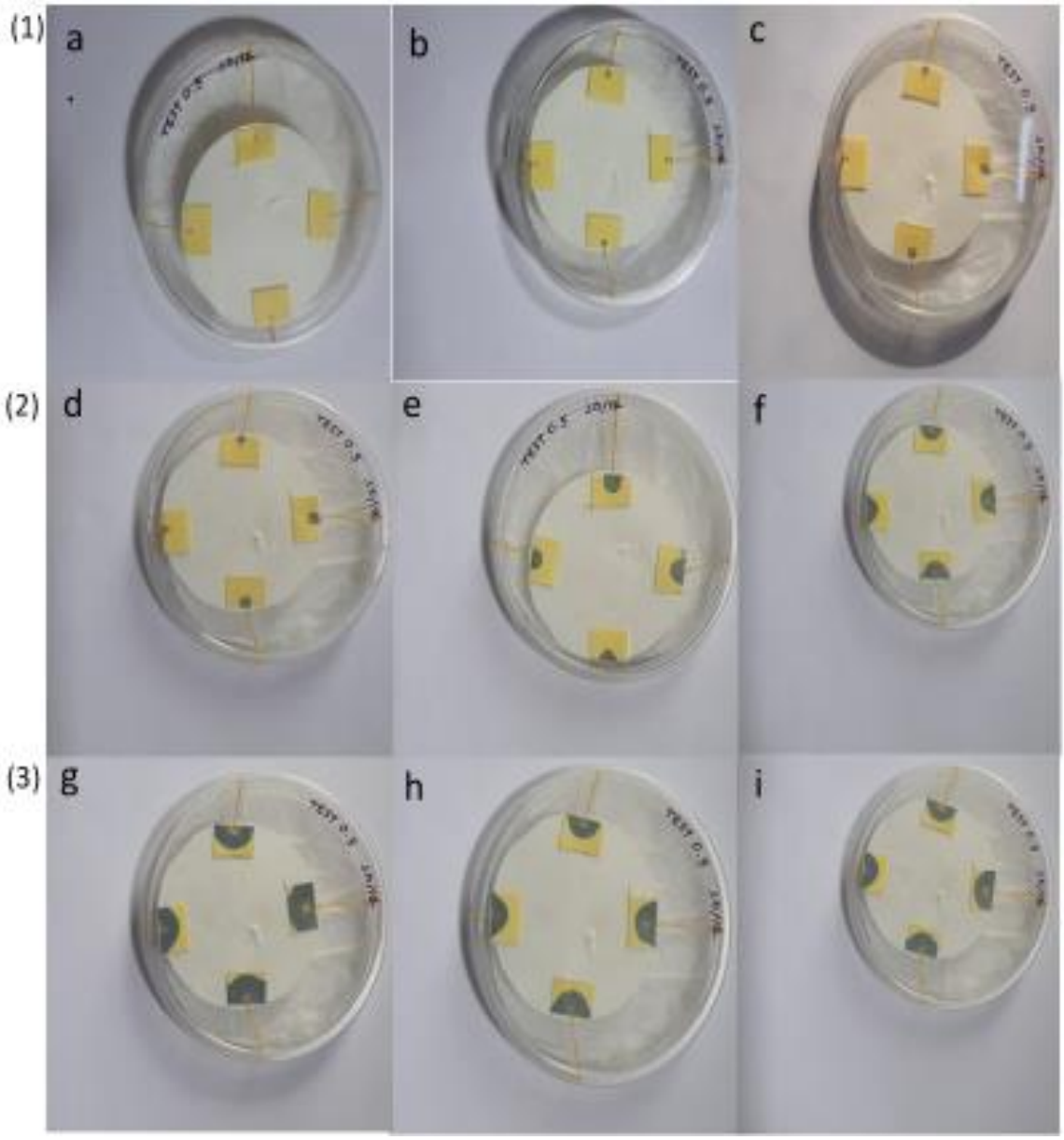

FIG 10: The figures (a to i) represent the observation made in the span of 2 hours 30 mins. (1), (2) and (3) represents the observation taken every 20 min at a temperature of 25^0 C, 30^0 C, 35^0 C and 45^0 C respectively.

Result for determining water absorbent layer

- From the test sets we can conclude that hydrogels are more efficient as a water-absorbent layer than wet tissue paper

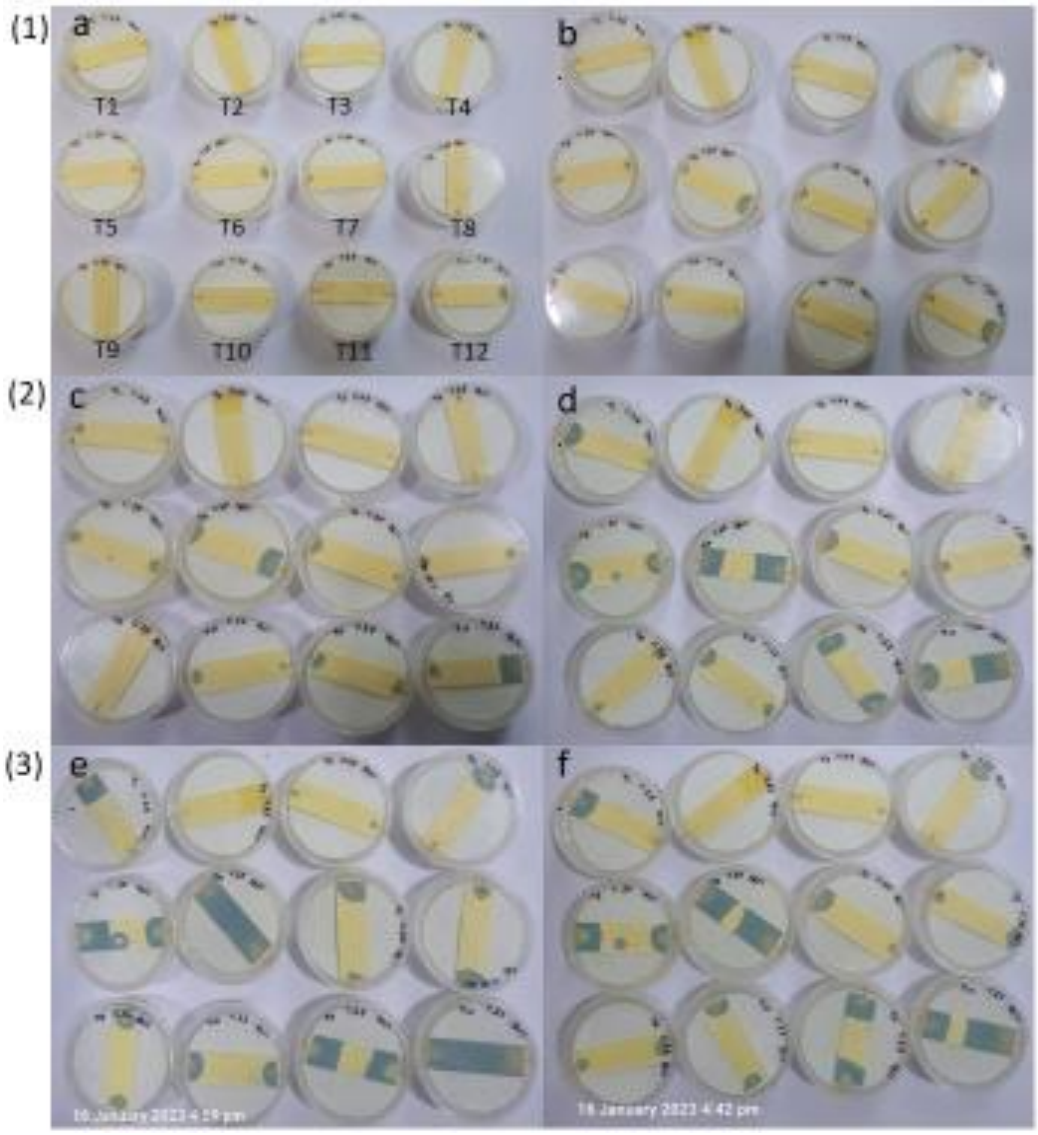

FIG 11: The figures (a to f) represent the observation made in the span of 2 h at 15 min intervals respectively. (1), (2) and (3) represents the observation taken at a temperature 30^0 C, 35^0 C and 40^0 C respectively

TABLE 1: The table represents the number of sets and water absorbent used in it i.e., out of 12 sets, each set of 6 tests contains hydrogel and wet tissue paper respectively to determine a better absorbent layer.

Plate no.	Water absorbent layer	Plate no.	Water absorbent layer
T1	Hydrogel	T2	Filter paper
T4	Hydrogel	T3	Filter paper
T5	Hydrogel	T7	Filter paper
T6	Hydrogel	T8	Filter paper
T11	Hydrogel	T9	Filter paper
T12	Hydrogel	T10	Filter paper

Results for ink formulation

- For sample ink, I, colour change from yellow to blue is observed when exposed to temperatures 30^0 C, 35^0 C and 40^0 C respectively for 30 min intervals.

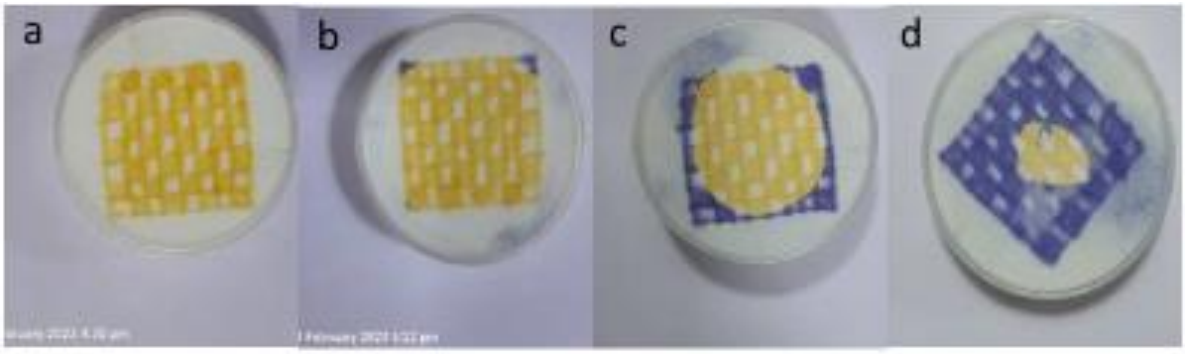

FIG 12: The figures (a to d) represent the colour change of the thymol blue patch in the span of 2 h at 30 min intervals at temperatures 25 ºC, 30 ºC, 35 º C and 40 º C respectively.

Inference: The evaporated water is absorbed by the NaOH paper which in turn is absorbed by the thymol blue patch resulting in colour change. The colour change happens due to a change in the pH from the slightly acidic pH of the thymol blue patch to the basic pH of wet NaOH paper.

- For sample ink II, the ink turned green upon long-term storage.

Inference: Sample ink II contained EDTA, as it degrades over time, it changes the pH of the ink to sightly basic resulting in the formation of green coloured complex and turning the ink green.

- For sample III, the ink remained the same upon long-term storage.

Inference: Due to the omission of EDTA, there is no change in the pH, therefore the colour of the ink remained the same. The addition of HCL provided extra stability to the ink formulation.

Results for prevention of condensation

- Results for determination of melting points of wax and oil ratios

The wax and oil ratios 1:30, 1:32 and 1:35 are selected as they have the melting point ranging from 35^{o}C to 37^{o}C.

TABLE 2: The table represents the average melting points of the wax and oil ratio

S. No.	Wax: Oil	Recorded Temperatures					Avg. Temp.
1	1:1	68	63	62	66	64	64
2	1:5	55	56	58	62	54	57
3	1:10	53	50	55	54	53	53
4	1:15	47	47	45	50	46	47
5	1:20	43	40	47	45	47	44.4
6	1:25	42	39	40	44	40	41
7	1:30	38	40	37	35	35	37
8	1:32	34	35	36	32	32	34.2
9	1:35	30	34	29	30	32	31

Inference: As the melting point of the wax: oil ratio 1:30, 1:32 and 1:35 is around 37^{o}C, these ratios were selected.

Results for the working wax: oil ratio

- In winters, the wax: oil ratio 1:30, 1:32 and 1:35 showed positive results but in summers no results were observed.

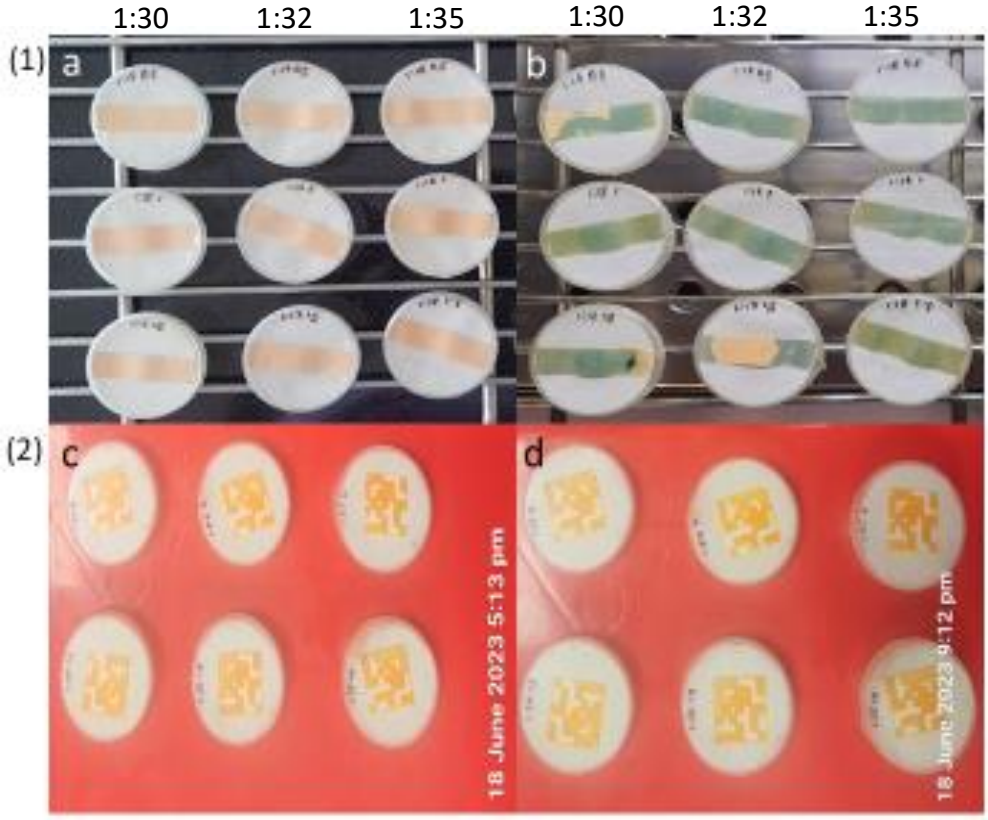

FIG 13: The figures (a to d) represents the reaction occurring in the span of 4h and (1) and (2) represent the winter and summer time respectively.

Inference: In winter, due to low temperatures wax: oil ratio 1:30, 1:32 and 1:35 froze instantaneously resulting in full coverage of the hydrogel layer. But in summers, due to higher temperatures, wax:oil ratios 1:30, 1:32 and 1:35 remained liquid which resulted in the seepage of the wax layer into the hydrogel forming a stagnant layer of wax hence no result was observed.

- Wax: Oil ratios 1:15, 1:17, 1:18, 1:19 and 1:20 showed positive results at room temperature.

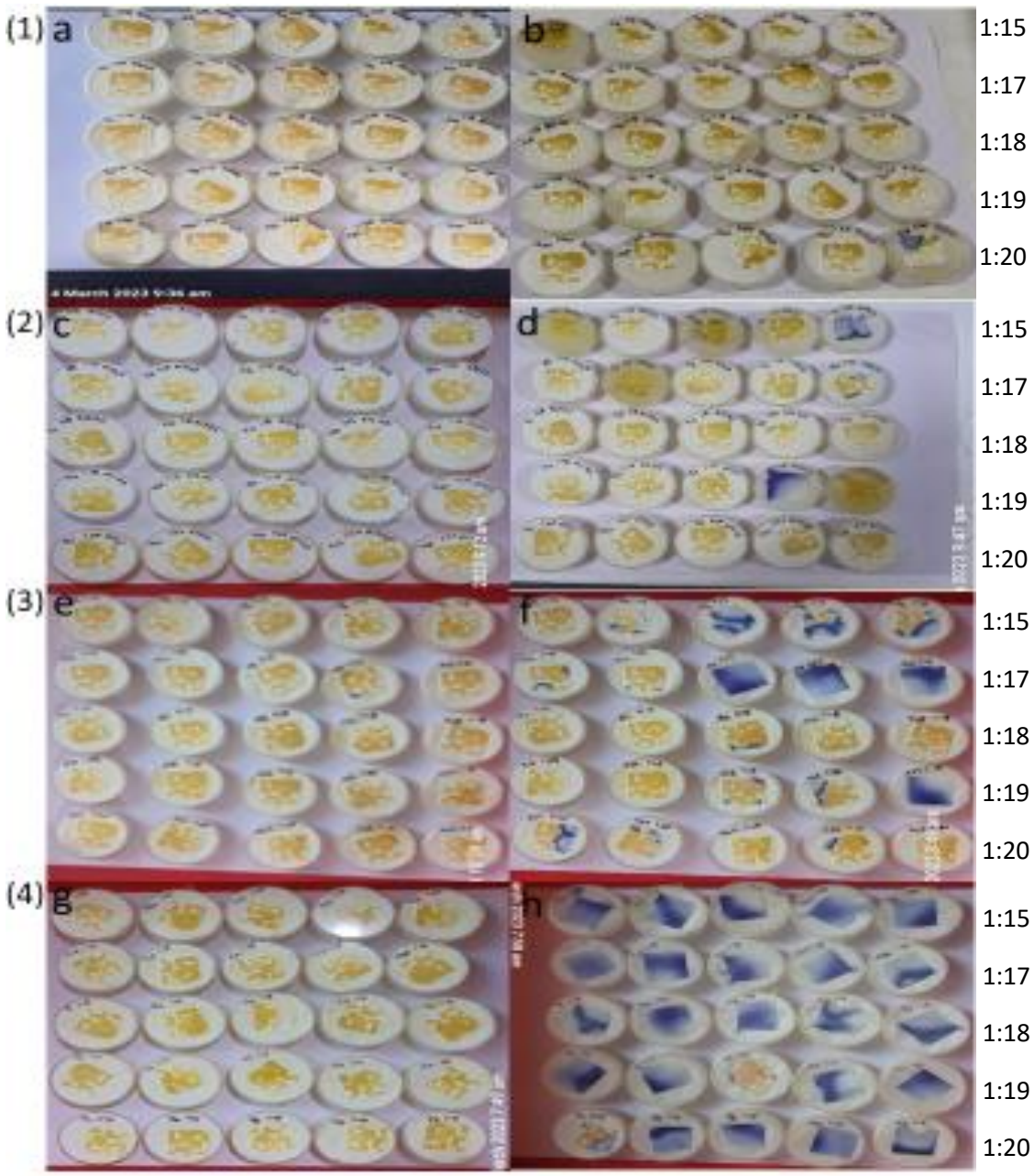

FIG 14: The figures (a to h) represents the reaction occurring in the span of 24h and (1), (2), (3) and (4) represent the temperatures 25^0 C, 30^0 C, 35^0 C and 40^0 C respectively.

Inference: As Wax:Oil ratios 1:15, 1:17, 1:18, 1:19 and 1:20 have higher melting points, they freeze readily at room temperature. The frozen wax layer provides better coverage over the hydrogel hence showing positive results.

Results for determining the thickness of the wax layer

For wax layer thickness of 1ml, 1.5ml and 2ml for the 1:15, 1:17, 1:18, 1:19 and 1:20 Wax:Oil ratios showed no colour change.

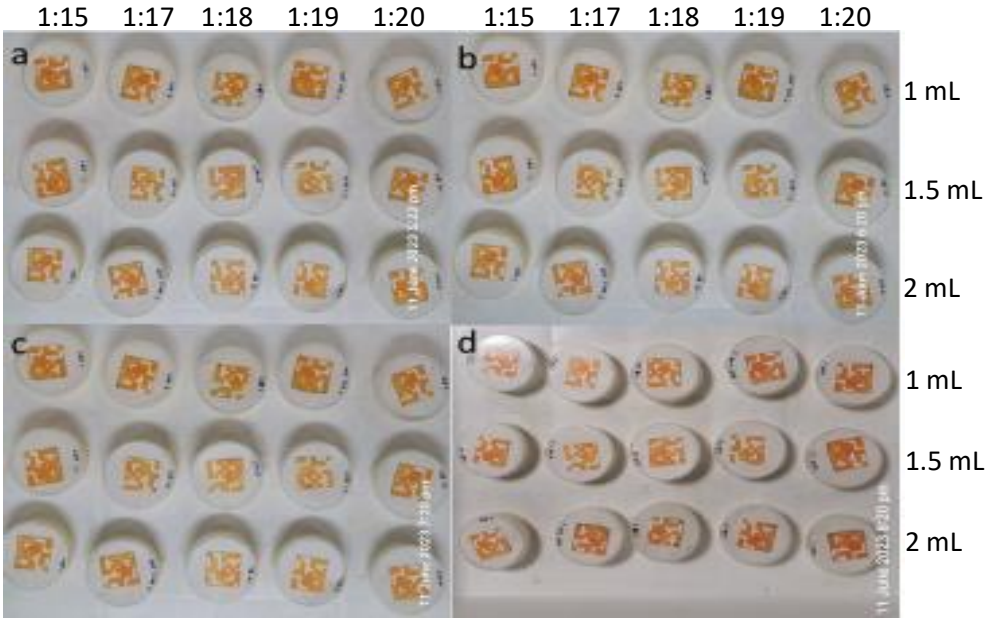

FIG 15: The figures (a to d) represent the reaction occurring in the span of 4h when exposed to the temperatures 25^0 C, 30^0 C, 35^0 C and 40^0 C respectively at the interval of 1h. The thickness of the slack wax layer is 1ml, 1.5ml and 2ml.

Inference: 1ml, 1.5ml and 2ml of wax layer is too thick as it remains stagnant on top of the hydrogel layer and prevents its exposure.

- For wax layer thickness of, 0.6 mL, 0.7 mL and 0.8 ml of wax layer for the 1:15, 1:17, 1:18, 1:19 and 1:20 Wax:Oil ratios showed positive results.

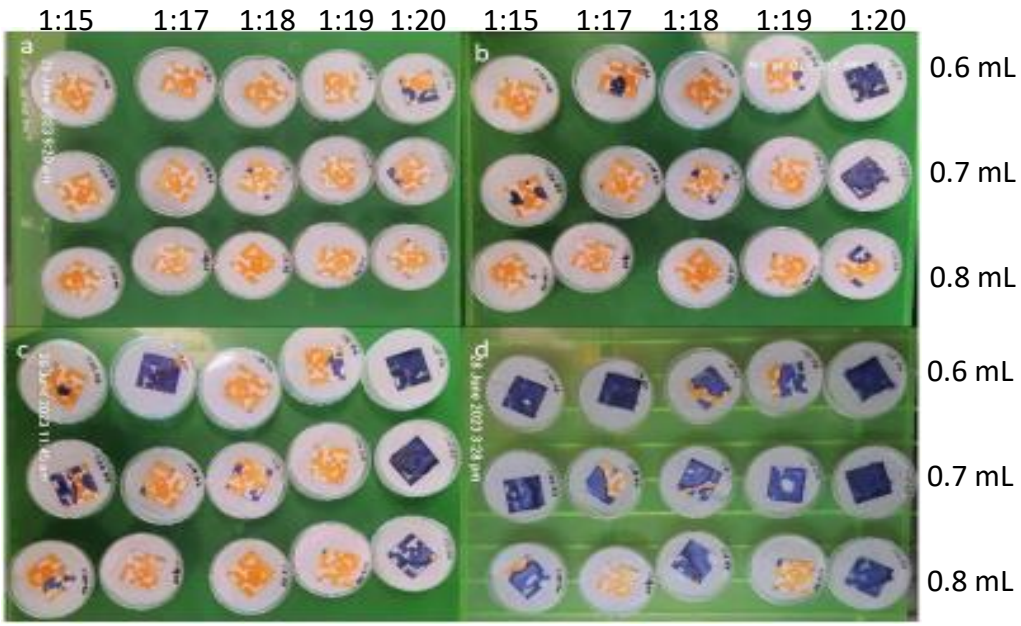

FIG 16: The figures (a to d) represents the reaction occurring in the span of 4h when exposed to the temperatures 25^0 C, 30^0 C, 35^0 C and 40^0 C respectively at the interval of 1h. The thickness of the slack wax layer is 0.6 mL, 0.7 mL, 0.8 mL.

Inference: 1ml, 1.5ml and 2ml of wax layer is too thick as it remains stagnant on top of the hydrogel layer and prevents its exposure.

Results for determining the efficiency of CMC hydrogel blend

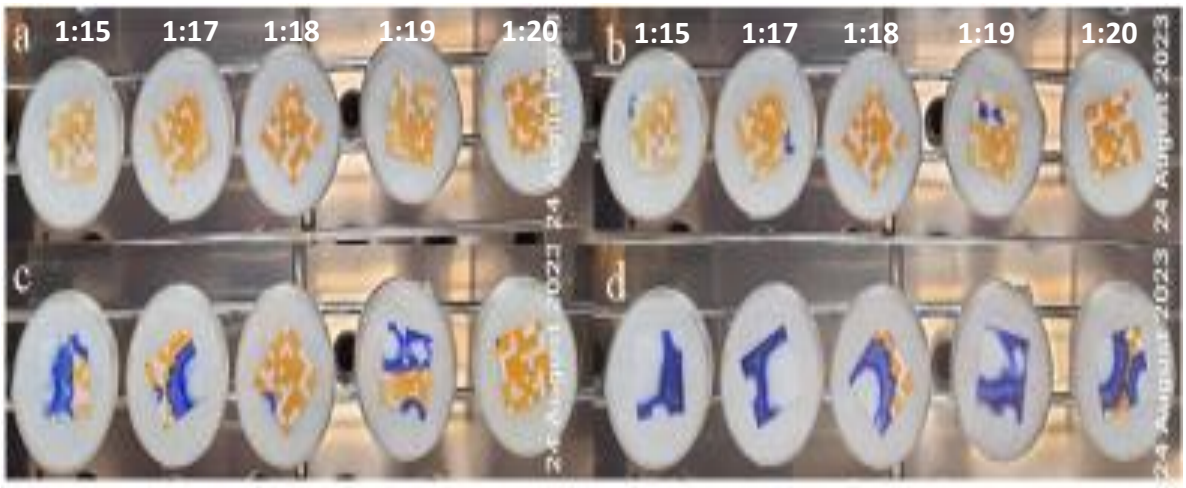

FIG 17: The figures (a to d) represents the reaction occurring in the span of 4h when exposed to the temperatures 25^0 C, 30^0 C, 35^0 C and 40^0 C respectively at the interval of 1h.

Results of determining the working of the patch using CMC hydrogel blend as a water absorbent layer

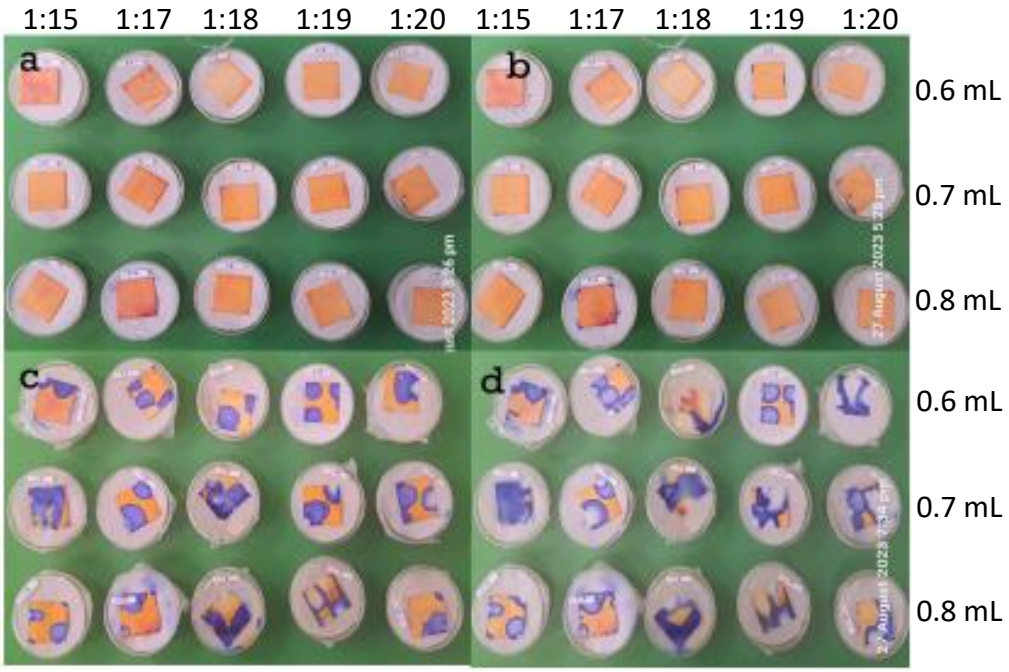

FIG 18: The figures (a to d) represents the reaction occurring in the span of 4h when exposed to the temperatures 25^0 C, 30^0 C, 35^0 C and 40^0 C respectively at the interval of 1h. The thickness of the slack wax layer is 0.6 mL.

Inference: The findings demonstrate comparable efficacy to sodium polyacrylate hydrogel in terms of its functional outcomes.

5 DISCUSSIONS

This experiment aims to develop a temperature-sensitive and pH-sensitive mechanism for detecting the degradation of a temperature-sensitive drug when exposed to elevated temperatures. The reaction involves the color change of the pH indicator, specifically the transition of thymol blue from yellow to blue, which occurs as the pH shifts from 6.8 to 14. The reaction components consist of a thymol blue patch, NaOH paper, and a water-absorbent layer. The temperature range for the experiment spans from 25°C to 40°C, as many temperature-sensitive drugs begin to degrade above 25°C.

The initial experiments were conducted to determine the appropriate setup and arrangement for the reaction to occur. In setups I and II, wet tissue paper was used as the water-absorbent layer, Methyl orange paper served as the pH indicator patch, and the reaction took place in a 100 mm petri plate. In setup I, where the components were arranged in concentric circles, no water absorption or color change of the pH paper was observed. This was because the evaporated water could not be absorbed unless it started to condense, which was not possible as the temperature continued to rise. On the other hand, in setup II, water absorption was observed because the NaOH paper and pH paper were suspended above the wet tissue paper, making it easier to absorb the evaporated water. However, no color change was observed as the working pH of Methyl orange was not within the appropriate range. Therefore, for the initial testing in setup III, the pH indicator was changed from Methyl orange to pH paper, while the water-absorbent layer remained as wet tissue paper. In this setup, both water absorption and color change were observed. This was because the NaOH paper and pH paper was suspended above the wet tissue paper using threads, which facilitated the absorption of evaporated water and consequently led to the observed color change.

From the following experiment onwards 35mm plates are used to stimulate a closed environment for the reaction.

Research has indicated that hydrogels possess superior water retention capabilities compared to paper. The purpose of the present study is to ascertain the more effective water-absorbent layer between hydrogel and wet tissue paper. The results of the experiment demonstrate that hydrogel

outperforms tissue paper in this regard, as evidenced by its ability to retain 3 mL of water when 20 mg of hydrogel is utilized, whereas 20 mg of tissue paper can only retain 1 mL of water. Consequently, the hydrogel is selected as the preferred water-absorbent layer for the subsequent stages of the experiment.

For this experiment, a distinct change in color was necessary to indicate the completion of the reaction. Initially, pH paper was utilized as it contains a wide range of indicators. However, the thymol blue indicator, which is a component of pH paper, was selected due to its pH range of 2 to 8 and its ability to produce a vivid color change, which was essential for the experiment. The ink formulation incorporating thymol blue was prepared using ethanol, triethanolamine, and EDTA with minor adjustments. It was observed that the ink turned green over time when EDTA was included in the initial formula, as EDTA degraded over time (Dimiev, 2023). Consequently, EDTA was excluded from the final ink formulation, and HCL was added for stabilization purposes. The pH of the ink was adjusted to 6.8, as this pH value produces a bright yellow color, ensuring that any subsequent changes in pH will result in a highly noticeable color change.

Water condensation is a commonly observed phenomenon at low temperatures, particularly during experimental procedures. To mitigate this issue, a layer of wax slack has been introduced between the hydrogel layer and NaOH paper to serve as a physical barrier. The melting points of various wax slack ratios were initially determined within the temperature range of 25°C to 40°C, and the appropriate ratios were selected accordingly. Based on the experimental findings, wax slack ratios of 1:30, 1:32, and 1:35 were found to have melting temperatures of 37°C, 34.7°C, and 31°C, respectively, falling within the desired working temperature range. However, it was observed that these ratios yielded positive results only during winter, as they easily froze upon contact with the hydrogel layer, forming a protective layer. Conversely, during summer, these ratios remained in a liquid state after pouring, resulting in the seepage of wax slack into the crevices of the hydrogel particles and leaving the top layer exposed. This led to a change in color even before reaching 25°C, and excessive pouring of wax slack resulted in no color change at all. Consequently, wax slack ratios of 1:15, 1:17, 1:18, 1:19, and 1:20 were selected, as they possessed higher melting points and remained solid at room temperature. These ratios exhibited positive results when exposed to higher temperatures. The selection of a wide range of wax slack ratios was necessary due to the varying degradation temperatures of temperature-sensitive drugs.

By utilizing ratios with different melting points, the appropriate ratio can be chosen based on the specific temperature requirements of the drug. For instance, if the drug has a high degradation temperature, a lower wax slack ratio should be employed, and vice versa.

As previously mentioned, the presence of an excessive thickness of the wax slack layer can impede the reaction by causing the excess wax to remain stagnant even when melted, thereby preventing exposure to the hydrogel layer. To address this issue, we have determined the appropriate thickness of the wax slack layer. In the initial experiment, we tested the reaction using wax slack layers of 1ml, 1.5ml, and 2ml, but no observable reaction occurred due to the excessive thickness of the wax slack layer. Consequently, in the subsequent experimental set, we utilized wax layers measuring 0.6 mL, 0.7 mL, and 0.8 ml. Based on the results obtained from this experimental set, it can be concluded that wax layers measuring 0.6 mL, 0.7 mL, and 0.8 ml effectively function as a physical barrier, yielding positive outcomes.

To establish an effective waste management strategy for this product, we initially developed a nano cellulose composite hydrogel by utilizing Gelatin, Alginate, and Polyvinyl alcohol as polymerizing agents. However, it was observed that these nanocomposite hydrogels were not stable at a temperature of 40 ^{o}C, as this exceeded their polymerization temperature, resulting in their dissolution in water. Consequently, we opted to employ a CMC hydrogel blend, which is synthesized from extracted cellulose derived from waste paper. This alternative demonstrated a thermal stability of 60 ^{o}C and proved to be a suitable replacement for sodium polyacrylate hydrogel, exhibiting efficient performance

6 CONCLUSION AND FUTURE PROSPECTIVE

This is a one-of-a-kind study, though many developments are going on regarding printable colour-changing designs on the packaging of food products indicating the degree of damage to the product due to improper storage. In this experiment, we have developed a pH-sensitive colour-changing patch with similar working but is cost-effective for temperature-sensitive drugs. When the drug is exposed to a higher temperature for a longer duration of time, the thymol blue patch will change colour due to the pH change inside it. The reaction happening inside the patch is, due to changes in the temperatures, the water inside the patch starts evaporating which is absorbed by the NaOH paper present above the hydrogel layer. The wet NaOH paper changes the pH of the Thymol blue patch from 6.3 to 14 hence the colour changes from yellow to blue.

Hydrogel is observed to have better water retention ability than wet tissue paper hence hydrogel is used as a water absorbent layer in experiments.

The ink formulation using thymol blue dissolving 459 mg of thymol blue in 3.6 ml of Triethanolamine, and 10 mL of ethanol was added by adding 1.5 mL of HCL in 1 mL ink stock with a pH of 6.8. The patch made using the ink works efficiently.

We know that water condensation happens at low temperatures. To avoid that, we added a slack wax layer to the hydrogel layer. From the experiment, we concluded that, slack wax of 1:15, 1:17, 1:18, 1:19 and 1:20 wax: oil ratios work as efficient barrier. Every drug has a different temperature at which it starts degrading, therefore different wax ratios can be used according to the requirement due to their difference in melting point. The 1:15 slack wax layer takes more time than 1:20 slack wax layer as 1:15 slack wax has a higher melting point.

The thickness of the slack wax layer also plays an important role as too thick to too little wax can hamper the reaction. From the experiment, we can conclude that 0.6 mL of wax works efficiently as a barrier

To establish an effective waste management strategy in this product, we opted to employ a CMC hydrogel blend, which proved to be an adequate replacement for sodium polyacrylate as a water-absorbent layer.

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