Malaysian Journal of Chemistry, 2024, Vol. 26(3), 36-41
(Special Issue: *The 4th International Conference on Recent Advancements in Science and Technology*)

Optical Sensing Material Based on p-Toluidine Immobilised in Polyvinyl Alcohol (PVA) Film for Detection of Hydroxymethylfurfural (HMF) in Honey

Mashri Ahmed Yahia[1], Che Wan Zanariah Che Wan Ngah[1], Lee Yook Heng[2] and Musa Ahmad[1]*
[1]Faculty of Science &Technology, Universiti Sains Islam Malaysia (USIM), 71800 Bandar Baru Nilai, Negeri Sembilan DK, Malaysia
[2]Faculty of Science & Technology, Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor DE, Malaysia
*Corresponding author (e-mail: andong@usim.edu.my)

The heating process, particularly at high temperatures, can destroy vitamins and enzymes in honey and lead to the production of hydroxymethylfurfural (HMF), therefore reducing the quality of honey. HMF is practically absent in fresh honey, but concentrations increase with the temperature and time of heating, as well as due to improper storage and adulteration. This research work was designed to fabricate simple optical sensing materials for HMF detection based on immobilised p-toluidine in polyvinyl alcohol (PVA) film supported on filter paper. The reflectance intensities of the immobilised reagent were measured at a wavelength range of 250 - 500 nm using a spectrophotometer.

The photostability and reproducibility of the optical sensing material were good, with relative standard deviation (RSD) values of 1.9% and 2.8%, respectively, and exhibited a linear response across a wide range of HMF concentrations from 1×10^{-3} M to 7×10^{-3} M. The optimum response of the sensing material was obtained at pH 3.0. The interference study revealed no effect on the presence of glucose and fructose. The results revealed a great agreement between the fabricated optical sensing material and the high-performance liquid chromatography (HPLC) standard methods, with correlation coefficient (R^2) and slope value at 0.99 and 1.02, respectively. The optical sensing material had good potential use in the quantitative determination of HMF in industrial honey with LOD 0.09 and LOQ 0.27.

Keywords: Optical sensing material; hydroxymethylfurfural; honey; p-toluidine; PVA

Received: October 2023; Accepted: December 2023

HMF is usually absent in fresh honey, but its concentration tends to increase due to inappropriate thermal treatment or unsuitable storage conditions [1, 2]. The maximum HMF level acceptable in honey is 40 mg/kg.

HMF determination is normally conducted spectrophotometrically using direct absorption or by the preparation of a derivative, and the absorbance is recorded. The earlier Winkler method was modified by White and Sicilia [3], who recorded the absorbance at a wavelength of 284 and 336 nm using the spectrophotometer.

The liquid chromatographic (LC) methods are preferable for reliable and accurate estimation of the furanic compounds present in many food products. This technique helps to determine HMF and furfural levels specifically. It does not require the formation of any coloured derivatives, as the furfurals have the ability to absorb strongly at a wavelength of 280 nm. Many studies choose 280 nm as their detection wavelength as it lies between the absorption maxima for HMF (284 nm) and for furfural (277 nm). HMF displays a band at the wavelength of 284 nm (18,000 molar absorptive), with a low-intensity band at 230 nm. The reverse phase LC (RP /LC) techniques are widely used for the determination of HMF.

PVA is water soluble and a non-toxic synthetic polymer characterised by its chemical and physical properties. It is one of the polymers that can form thin films [4]. PVA is widely used in many applications like packaging, systems controlling drug delivery, polymer recycling, and membrane preparation. Many studies have reported solubility and the different mechanisms that result in the crystalline and swollen PVA along with its gel-forming capacity [5]. The immobilised p-Toluidine on PVA is usually used to support the formation of the HMF sensor.

In this work, spectrophotometric reagent p-T has been immobilised in PVA supported by filter

---

†Paper presented at the 4th International Conference on Recent Advancements in Science and Technology (ICoRAST2023)

paper for use as the sensing phase in reflectance detection for HMF.

EXPERIMENTAL

Chemicals and Materials

All the chemicals, reagents, and solvents were of analytical grade and were used without further purification. The samples of honey were acquired from a farmer in Green Mountain in Libya.

The standard HMF solution of 3×10^{-2} M was prepared by dissolving 0.094 g of HMF in 25 mL deionised water. Eight serial dilutions in deionised water were prepared from standard HMF solution to the measure of HMF solution with concentration in the range of (1–8) $\times10^{-3}$ M.

The preparation of p-toluidine standard stock solution of 3×10^{-2} M was accomplished by dissolving 0.080 g of p-toluidine in 25 mL deionised water. Serial dilutions of p-toluidine in deionised water were performed to prepare the solution with different concentrations of p-toluidine in the range of (1–8) $\times 10^{-3}$ M.

The homogeneous stock solution of PVA was prepared by dissolving 2 g of PVA in 100 mL deionised water. Overnight heating of the PVA stock solution was performed until a temperature of 80°C was reached [6].

The immobilised p-toluidine in PVA was prepared using 1.5 mL of p-toluidine in 2.5 mL PVA, mixed with phosphate buffer solution (pH 3.0). The mixture was stirred continuously using a magnetic stirrer for 15 minutes until a homogenous solution was achieved. A total volume of 0.3 mL of the mixture of p-toluidine in the PVA solution was pipetted into the petri dish. Then, a paper filter strip was immersed into this solution to form a membrane. Next, the paper filter strip was dried by placing it in the refrigerator overnight.

Characterization Methods

A Mettler Toledo pH meter was used for pH measurement. Measurement of reflectance was conducted by employing a UV-VIS spectrophotometer (model Perkin Elmer, US). Ocean Optic software was used to control the instrumental parameters.

The optimisation of the pH of the optical chemical sensing materials was conducted in the pH range of pH 2 - 8. The photostability of the sensing materials was studied by measuring the reflectance every 30 minutes for 6 hours with wavelength in the range of 250 - 500 nm. The reproducibility of the sensing material was studied by conducting seven batches of experiments when the concentration of HMF was fixed at 3×10^{-3} M.

The calibration graph of HMF concentration was established by plotting a graph of reflectance against HMF concentration in the range of 1×10^{-3} to 9×10^{-3} M. The reflectance measurements were recorded in the wavelength range of 250 nm – 500. The interferences from glucose and fructose commonly present in honey were evaluated based on the comparison of the reflectance readings obtained in the presence of the interfering compounds and HMF. The interference studies were investigated using 3×10^{-3} M HMF. The tolerance limit was set as the amount of interfering compounds causing ±5% error in the determination of HMF.

The results for HMF concentration determination in several honey samples using the developed sensing material were compared with the results from the standard HPLC method. The honey was buffered using phosphate buffer solution; 1 g of the raw honey in deionised water 100 mL (1/100 w/v) was prepared and used as stock solution. The HMF standard curve for HPLC analysis was prepared by serial solutions of HMF with concentrations ranging from 0 to 6×10^{-3} M.

RESULTS AND DISCUSSION

The reaction between HMF and p-toluidine produced a yellow-coloured Schiff base, with a possible chemical reaction displayed in Figure 1.

Figure 2 illustrates the reflectance spectra of immobilised p-toluidine in PVA support by filter paper before and after the reaction with HMF. The Schiff base, which has been formed, gives maximum reflectance at 350 nm due to the changes in colour of the immobilised p-toluidine from colourless to yellow.

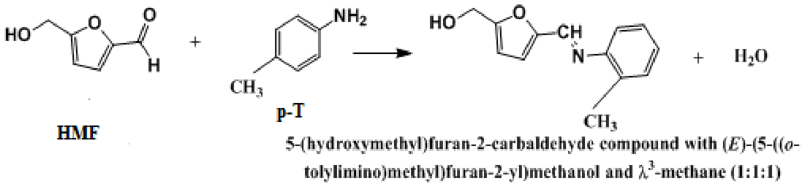

Figure 1. Possible reaction between HMF compound and p-toluidine.

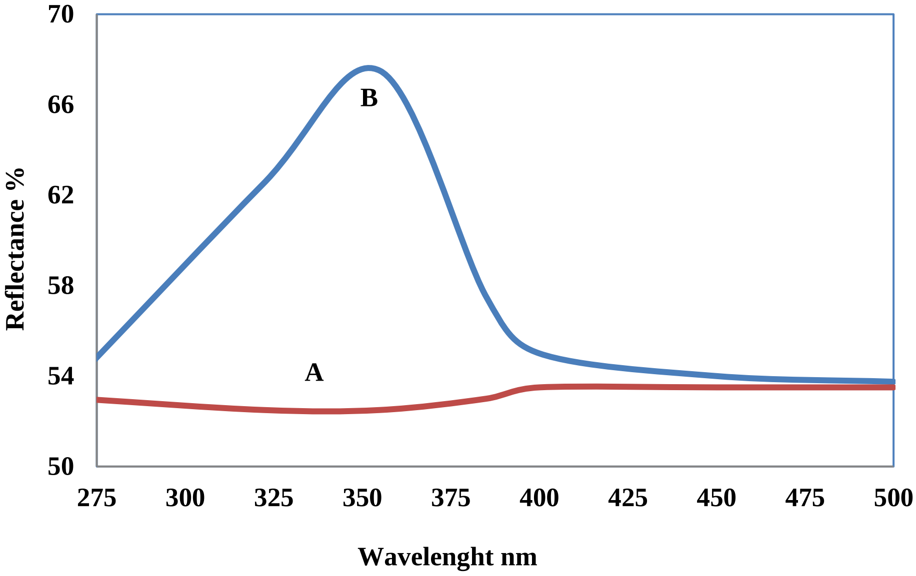

Figure 2. The reflectance spectra of immobilised p-toluidine alone in PVA (A) and after reaction with HMF (B).

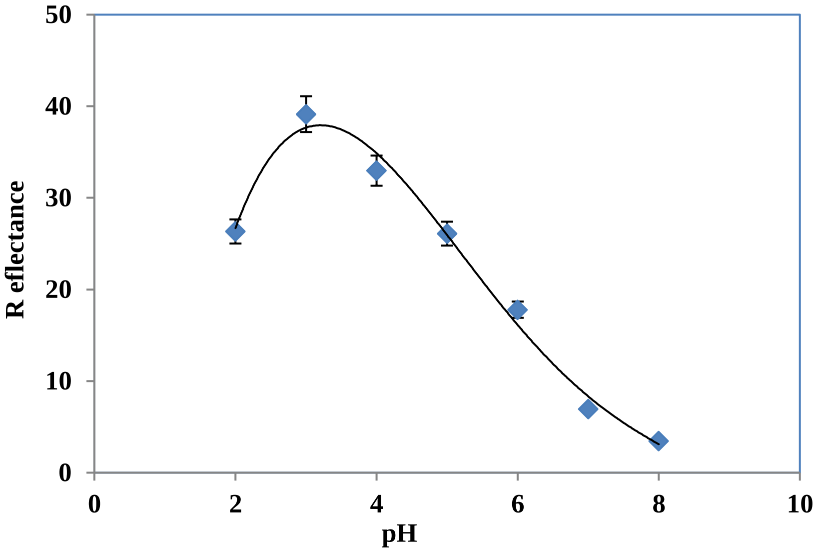

Figure 3 The effect of pH on the immobilised p-T in PVA supported by filter paper upon reaction with 3 x 10^{-3} M HMF.

As portrayed in Figure 3, the optimum response was obtained at pH 3.0. The optimum pH is found to be the same as the optimum pH in solution work. This indicated no significant effect on the functional group in the p-toluidine involved in the Schiff base formation during immobilisation. The same exploration and trend of results has been previously reported by Ahmad *et al.* [7].

The effect of different concentrations of immobilised p-toluidine upon reaction with HMF has also been investigated. The use of a higher concentration of p-toluidine allowed more reactions to occur. Thus, a higher reflectance signal was observed (Figure 4). The reflectance signal ultimately became a plateau as almost all immobilisation sites have been fully occupied by the HMF compound. The same phenomena have been previously explained by Abdullah *et al.* [8].

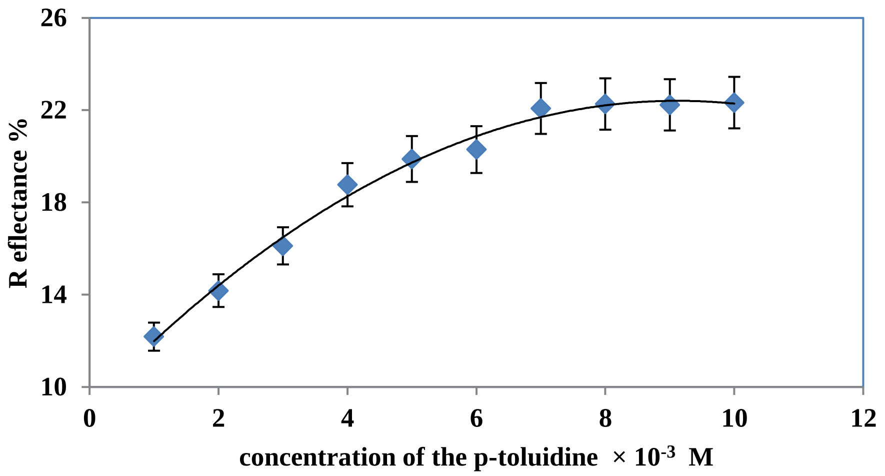

Figure 4 The effect of p-toluidine concentration used during immobilisation on the sensor response was fixed at 3×10^{-3} M with buffer solution at pH 3.0.

The study also indicated that the immobilised p-toluidine was discovered to be very photostable with no indication of photobleaching when continuously observed for a duration of 6 hours. The RSD value of 1.9% was obtained, which indicates acceptable photostability of the immobilised p-toluidine. Ahmad et al. [7] have reported a similar photodecomposition trend for using a complex of zinc dithionite immobilised on XAD 7 for a continuous monitoring period of 5 hours. The immobilised p-toluidine was also found to be reproducible for the determination of HMF concentration (3×10^{-3} M) with an RSD value of 2.8%.

The sensing material response curve, as displayed in Figure 5, indicates that the reflectance signal seems to increase significantly with the rise in HMF concentrations. However, at higher HMF concentrations, this response slowly levelled off and reached saturation at 7×10^{-3} M HMF. This suggests that higher HMF concentration resulted in a greater number of reactions between the immobilised reagent and analyte molecules, which could be observed by the generation of a higher signal. However, a plateau was reached after the analyte molecules completely occupied all the available sites within the immobilised p-toluidine. The concentration of p-toluidine was fixed at 7×10^{-3} M for the experiments since this concentration gives the highest signal. The HMF concentration was varied within the range of 1 to 10×10^{-3} M. Under the optimised conditions, the calibration curve was obtained, as shown in the inset. The same trend, NH_3, at the response curve, was also reported by Tan *et al.* [9] in their work on sensors based on immobilising cobalt (II) ions onto high-capacity Dowex HCR-W2 microspheres.

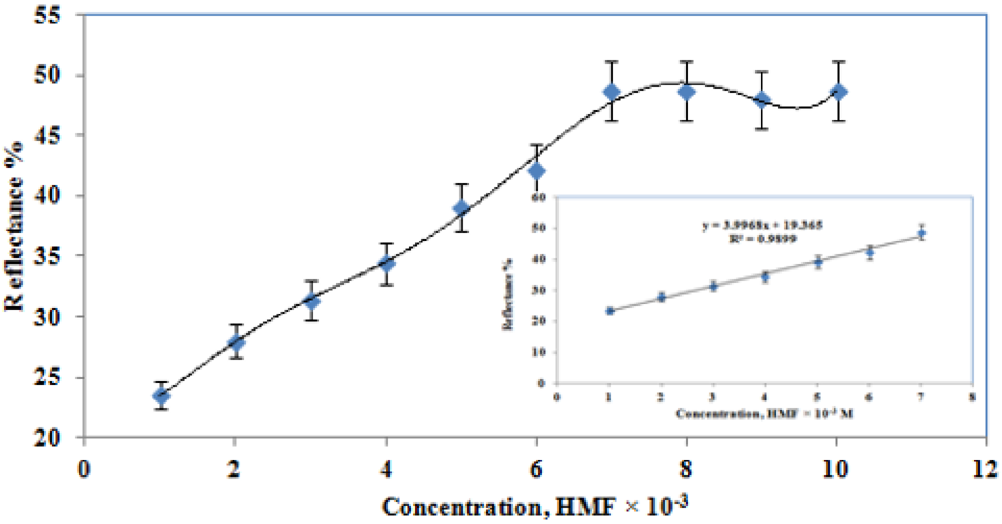

Figure 5. The dynamic response range of the sensing material to different HMF concentrations and the linear range of the sensing material towards different concentrations of HMF (inset).

Table 1. The degree of interference at 3 × 10^{-3} M of compounds (glucose and fructose): with HMF.

Interfering compounds	(X) mean±SD (n=3)	(Y) mean±SD (n=3)	Percentage of interfering
Glucose	125.18±0.09	125.16±0.10	0.02.%
Fructose	125.00±0.09		-0.13%

% Interference/(x - y/y)/100%, where x is the average reflectance value for the mixed solution of HMF, y is the average reflectance value for the HMF sensor only

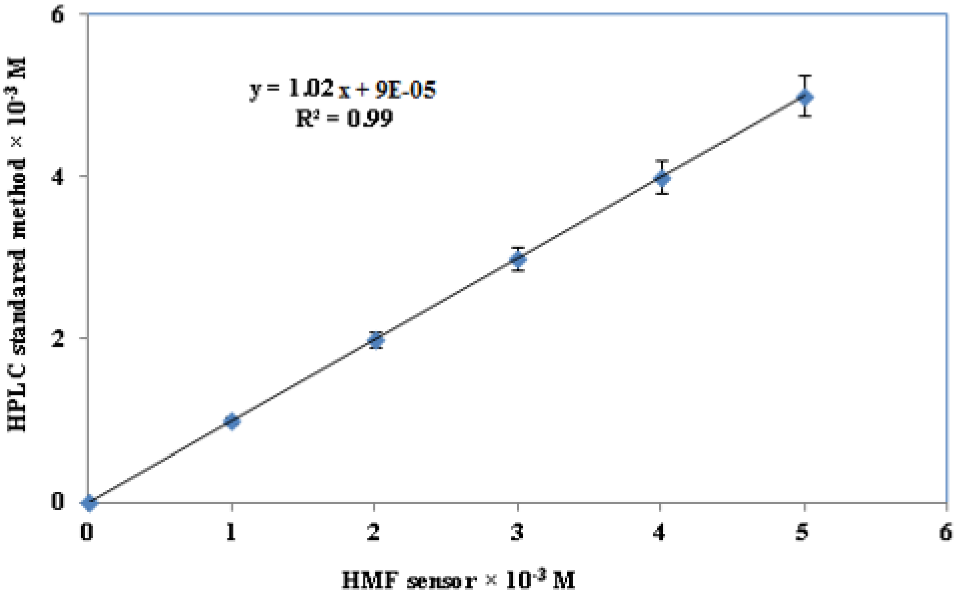

Figure 6. Comparison between the sensing material and standard HPLC methods in determining HMF concentration.

Fructose and glucose are the two major compounds found in honey. For this reason, these two compounds were studied to determine their potential interferences in the sensing material during the determination of HMF [10]. The sensing material response was recorded in the absence and the presence of interfering compounds, and the change in reflectance intensity was measured and tabulated in Table 1. It can be noted that the interfering compounds did not exhibit any significant interference since the percentage of interference is observed to be well below < 5%. The effect of both compounds on the methods was calculated using the equation as reported by Yusof and Musa [11].

The sensing material prepared in this study has been validated with a standard method of HPLC for the determination of HMF (Figure 6).

A straight plot with a slope value of 1.02 indicates that both methods show comparison results [12]. Table 2 provides the result of the comparative study of the developed sensing material and HPLC standard method. A statistical analysis (t-test) has been conducted. The t-value is calculated to compare the two means valves from the two sets of analysis. The calculated value of |t| is found to be less than the critical value, and the difference between the two methods is insignificant (< 5%). This indicated that the two mean values obtained from both methods were not significantly different and were in good agreement. Also, the f-value has been calculated, and the value obtained has no significant difference. The two methods are considered to give similar results.

Table 2. Comparison between the sensing material and standard HPLC methods in the determination of HMF.

Concentrations × 10^{-3} Mol L^{-1}	**Developed sensing material × 10^{-3} M mean±SD (n=3)**	**HPLC method × 10^{-3} M mean±SD (n=3)**	**t-value**	**f-value**
1	0.96±0.11	0.99±0.01	0.33	1.06
2	2.08±0.08	2.01±0.11	0.01	0.93
3	3.30±0.22	2.97±0.16	0.01	0.81
4	3.99±0.10	4.37±0.05	0.03	1.19
5	4.79±0.20	4.90±0.02	0.01	1.04
Honey real sample	1.97±0.04	2.25±0.01	0.02	1.30

Note: The critical value, $t_3 = 3.18$ (P=0.05) $f_{2,2} = 39$ (P=0.05)

CONCLUSION

The p-toluidine was successfully immobilised into PVA film support by filter paper to develop a sensing material based on reflectance measurement. The photostability and reproducibility were good, with RSD values of 1.90 and 2.83%, respectively, and exhibited a linear response across a wide range of HMF concentrations from 1×10^{-3} M to 7×10^{-3} M. The optimum response of the sensing material was obtained at pH 3.0. The interference study revealed no effect in the presence of glucose and fructose. The results revealed a great agreement between the sensing material and the HPLC standard methods, with correlation coefficient (R^2) and slope value at 0.99 and 1.02, respectively. The developed sensor had good potential use in the quantitative determination of HMF in industrial honey with LOD 0.09 and LOQ 0.27.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Faculty of Science & Technology, Universiti Sains Islam Malaysia, as well as the Chemical Sensors and Biosensors Research Group, Universiti Kebangsaan Malaysia, for the research facilities extended to Mashri Ahmed Yahia during his PhD research.

REFERENCES

1. Anklam, E. (1998) A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry*, **63**, 549–562.
2. Bath, P. K. and Singh, N. (1999) A comparison between Helianthus annuus and Eucalyptus lanceolatus honey. *Food Chemistry*, **67**, 389–397.
3. White Jr, J. and Siciliano, J. (1980) Hydroxymethylfurfural and honey adulteration. *Journal-Association of Official Analytical Chemists*, **63**, 7–10.
4. Chandy, T. and Sharma, C. P. (1992) Prostaglandin E1-immobilized poly (vinyl alcohol)-blended chitosan membranes: Blood compatibility and permeability properties. *Journal of Applied Polymer Science*, **44**, 2145–2156.
5. Paradossi, G., Lisi, R., Paci, M. and Crescenzi, V. (1996) New chemical hydrogels based on poly (vinyl alcohol*). Journal of Polymer Science Part A: Polymer Chemistry*, **34**, 3417–3425.
6. Charernsriwilaiwat, N., Opanasopit, P., Rojanarata, T., Ngawhirunpat, T. and Supaphol, P. (2010) Preparation and characterization of chitosan-hydroxybenzotriazole/polyvinyl alcohol blend nanofibers by the electrospinning technique. *Carbohydrate Polymers*, **81**, 675–680.
7. Ahmad, M., Hamzah, H. and Marsom, E. S. (1998) Development of an Hg (II) fibre-optic sensor for aqueous environmental monitoring. *Talanta*, **47(2)**, 275–283.
8. Abdullah, J., Ahmad, M., Heng, L. Y., Karuppiah, N. and Sidek, H. (2006) Stacked films immobilisation of MBTH in nafion/sol-gel silicate and horseradish peroxidase in chitosan for the Determination of phenolic compounds. *Analytical and Bioanalytical Chemistry*, **386**, 1285–1292.
9. Tan, L. L., Ahmad, M. and Heng, L. (2012) A novel optical ammonia sensor based on reflectance measurements for highly polluted and coloured water. *Sensors and Actuators B: Chemical*, **171**, 994–1000.
10. Castoldi, K., Milani, M. I., Rossini, E. L., Pezza, L. and Pezza, H. R. (2016) Flow injection analysis of 5-(hydroxymethyl)-2-furaldehyde in honey by a modified Winkler method. *Analytical Sciences*, **32**, 413–417.
11. Yusof, N. A. and Ahmad, M. (2002) A flow cell optosensor for lead based on immobilised gallocynin in chitosan membrane. *Talanta*, **58**, 459–466.
12. Miller, J. N. and Miller, J. C. (2010) Statistics and Chemometrics for Analytical Chemistry, Prentice Hall, England.