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Development of formaldehyde detection method using onion juice as chromogenic agent

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ABSTRACT

This work reports an initial study on the use of natural compound, i.e. onion juice, as a novel environmental-friendly chromogenic agent to determine the concentration of formaldehyde. The method is based on the reaction of formaldehyde with yellow onion juice to produce a pink solution which absorb strongly at 514 nm. Factors affecting the extent of reaction between formaldehyde and onion juice such as temperature, pH of onion juice and reaction time were optimized using the response surface methodology approach via the Box–Behnken design. The experimental values obtained were close to the predicted values indicating good approximation of the model. The optimum response was obtained by heating the onion juice at 60˚C, pH 4.9, reaction temperature of 103˚C and reaction time of 17 min. A linear calibration curve was obtained using formaldehyde concentrations ranging from 0.5 to 15 mg L^{-1}. This study demonstrates the potential application of onion juice as a simple, safe and environmental friendly technique for quantitative determination of formaldehyde from various sources such as drinking water and aquaculture products.

Keywords: Formaldehyde; Detection; Onion juice; RSM; Chromogenic

1. Introduction

Formaldehyde (HCHO) is a colourless, flammable gas at room temperature. It has a pungent, distinct odour and may cause a burning sensation to the eyes, nose and lungs at high concentrations [1]. Formaldehyde has been classified as most serious hazardous waste and may reasonably be anticipated to be a human carcinogen by U.S. Department of Health and Human Services [1]. Therefore, the guideline for the acceptable daily intake (ADI) of formaldehyde as set by EPA is 0.2 mg/kg body weight, as potential adverse health effects will be manifested for intake of formaldehyde more than ADI [2].

There are many available methods for formaldehyde detection. The methods include chromatographic, colorimetric, fluorimetric technique as well as spectrophotometric technique. A spectrophotometric technique may provide a rapid and simple formaldehyde detection method. However, the existing spectrophotometric methods make use of toxic reagents, expensive, time consuming and have low detection limits [3]. Colorimetric methods are relatively slow

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and have limited sensitivity [4], the gas chromatography and HPLC methods are very expensive and may be interfered by many carbonyl substances, including acetaldehyde and acetone [5]. Fluorimetric method is specific, non-destructive and quantitative and allows the continuous detection, however, the requirement of large, complex, and expensive instrumentation limits the method for routine applications [6]. Most of the detection methods require expensive and large apparatus, a large amount of samples, long sample preparation, long reaction time, requires toxic reagent and are not environmental friendly.

Therefore, in this study, a safe detection reagent for formaldehyde determination was proposed based on the reaction of formaldehyde with yellow onion juice. The intensity of pink coloured complex was recorded using a spectrophotometer at 514 nm. This method will not compete for the use of onion in food as the amount of onion extract used is small. The optimization of the condition for formaldehyde detection method was carried out using response surface methodology (RSM) by Box–Behnken design. In this paper, a method to detect formaldehyde was developed which is safe, inexpensive and simple to use.

2. Materials and methods

2.1. Reagents

Analytical grade reagents and deionized water were used in all sample preparations and experiments.

An aqueous formaldehyde stock solution, 1,000 mg/L, was prepared by diluting 2.5 mL of 37% (w/v) stock formaldehyde (QReC) solution to 1 L with deionized water. The working standard solutions (0–15 ppm) were prepared from the stock solution.

2.2. Instrumentation

The absorption spectrum was recorded using UV–vis spectrophotometer (Shimadzu UV-1601PC) from 400 to 600 nm. For absorbance measurements, a UV–vis spectrophotometer (HACH DR 5000) was used at maximum wavelength of 525 and 514 nm. Colour measurement was recorded using a colour spectrophotometer (Hunter Lab, ColorFlexEZ).

2.3. Preparation of onion extract

Onion juice was prepared by homogenizing 500 g of peeled yellow onion bulb with 500 mL of deionized water in a kitchen blender. The onion juice was adjusted to the required pH (3.5–6.5) using 1 M acetic acid and incubated at different temperatures (50–150˚C). The onion extract was then filtered and centrifuged to remove the pulp before further use.

2.4. Formaldehyde calibration curve

To establish a standard calibration curve, a series of standard formaldehyde solution at concentrations of 0.5–15 mg/L were prepared. Then, 2 mL of onion extract was added to 1 mL of standard formaldehyde solution in a tube, and the mixture was shaken vigorously for 15 s using vortex mixer (Thermolyne Type 37600, Sybron). The mixture was placed in a water and oil bath at temperatures ranging from 50 to 150˚C. The reaction time was varied from 5 to 35 min. Then, the absorbance was recorded at 525 nm using UV–vis spectrophotometer (HACH DR 5000).

2.5. Experimental design for RSM

The parameter affecting reaction of formaldehyde with onion extract was optimized using RSM. Statistica 8.0 (Statsoft, USA) was employed for calculations related to response surface optimization. RSM was employed to investigate the optimum conditions for formaldehyde detection using onion extract. The formaldehyde concentration used in the reaction was 15 mg/L. The optimum conditions of the four variables were determined using a Box–Behnken design. Box and Behnken [7] suggested how to select points from the three-level factorial arrangement, which allows the efficient estimation of the first- and second-order coefficients of the mathematical model. These designs are more efficient and economical then their corresponding 3^k designs, mainly for a large number of variables. The variables are as follows: The heating temperature for onion juice (X_1), pH of the onion juice (X_2), reaction temperature (X_3) and reaction time (X_4). The levels of the evaluated variables are presented in Table 1.

3. Results and discussion

3.1. Optimization by RSM

The objective of a RSM approach is to optimize the parameters affecting formaldehyde reaction with onion extract and to attain the best system performance with small number of experiments. The design matrix for the Box–Behnken study was generated using four parameters at three levels resulting in a total of 27 experiments. Box–Behnken design (3^k factorial) and data observed for the response variables of formaldehyde reaction is listed in Table 2.

Table 1
Experimental levels employed for Box–Behnken design

Variable	Coded variable		
	(−1)	(0)	(+1)
Heating temperature for onion juice (°C)	50	100	150
pH of onion juice	3.5	5	6.5
Reaction temperature (°C)	50	100	150
Reaction time (minutes)	5	20	35

3.1.1. Interpretation of regression analysis

The variables that predominantly affect formaldehyde detection by onion juice are heating temperature for onion juice (X_1), pH of the onion juice (X_2), reaction temperature (X_3) and reaction time (X_4). Analysis of variance (ANOVA) was used for statistical testing of the model in the form of linear terms and the interaction between the variables. The significant of regression can be evaluated by the ratio between the media of the square of regression (MS_{reg}) and the media of the square of residuals (MS_{res}) and by comparing these variation sources using Fisher distribution (F test). Thus, a statistically significant value for this ratio must be higher than the tabulated value for F [8]. From Table 3, the F value of 19.01945 for model was higher than the tabulated value ($F_{(14,12,0.05)} = 2.6371$), indicating that the mathematical model fitted well to the experimental data at 95% confidence level [8]. The regression analysis indicated that X_3, X_1X_3 are highly significant ($p < 0.05$) in this model. This fact was also confirmed by a reasonable coefficient of determination ($R^2 = 71.26\%$).

Based on the ANOVA results, a Pareto chart of main effects was produced. Bar lengths were proportional to the absolute value of the estimated effects, which helps in comparing the relative importance of effects. Factor bars, which exceed graphically the 95% significant line (at $p = 0.05$), exert a statistically signifi-

Table 2
Box–Behnken design and observed responses

Run	Heating temperature for onion juice (°C) X_1	pH of onion juice X_2	Reaction temperature (°C) X_3	Reaction time (min) X_4	Response (A_{525})
1	50	3.5	50	5	0.003
2	50	3.5	100	35	0.145
3	50	3.5	150	20	0.093
4	50	5.0	50	35	0.031
5	50	5.0	100	20	0.321
6	50	5.0	150	5	0.145
7	50	6.5	50	20	0.064
8	50	6.5	100	5	0.080
9	50	6.5	150	35	0.000
10	100	3.5	50	35	0.062
11	100	3.5	100	20	0.113
12	100	3.5	150	5	0.089
13	100	5.0	50	20	0.256
14	100	5.0	100	5	0.190
15	100	5.0	150	35	0.000
16	100	6.5	50	5	0.104
17	100	6.5	100	35	0.126
18	100	6.5	150	20	0.000
19	150	3.5	50	20	0.230
20	150	3.5	100	5	0.169
21	150	3.5	150	35	0.000
22	150	5.0	50	5	0.195
23	150	5.0	100	35	0.215
24	150	5.0	150	20	0.000
25	150	6.5	50	35	0.101
26	150	6.5	100	20	0.114
27	150	6.5	150	5	0.109

Table 3
ANOVA for experimental results

	SS	df	MS	F	p
Model	0.14167	14	0.089034	19.01945	<0.05
X_1	0.003838	2	0.001919	0.409890	0.67267
X_2	0.024928	2	0.012464	2.662589	0.11041
X_3	0.060363	2	0.030181	6.447373	0.01254
X_4	0.016140	2	0.008070	1.723941	0.21972
X_1X_2	0.000120	1	0.000120	0.025645	0.87543
X_1X_3	0.027876	1	0.027876	5.954788	0.03114
X_1X_4	0.001212	1	0.001212	0.258824	0.62015
X_2X_3	0.000477	1	0.000477	0.101884	0.75507
X_2X_4	0.002033	1	0.002033	0.434391	0.52229
X_3X_4	0.004682	1	0.004682	1.000128	0.33702
Residual	0.056174	12	0.004681		
Total SS	0.195449	26			
R^2	0.7126				

cant influence on the results [9]. The Pareto chart (Fig. 1) demonstrates that square term X_3 (reaction temperature) provides the most significant effect for formaldehyde detection method followed by the linear-linear interaction term for X_1X_3 and square term X_2 (pH). The empirical second-order polynomial model obtained with RSM for the reaction of formaldehyde with onion extract was established as follows:

$$\begin{aligned} Y = &-1.1136 + 0.002165X_1 + 0.28995X_2 \\ &+ 0.008798X_3 + 0.01170X_4 + 0.000003X_1^2 \\ &- 0.02726X_2^2 - 0.0000325X_3^2 - 0.0001526X_4^2 \\ &- 0.00004355X_1X_2 - 0.0000199X_1X_3 \\ &- 0.0000138X_1X_4 - 0.00008681X_2X_3 \\ &- 0.0005975X_2X_4 - 0.0000272X_3X_4 \end{aligned} \quad (1)$$

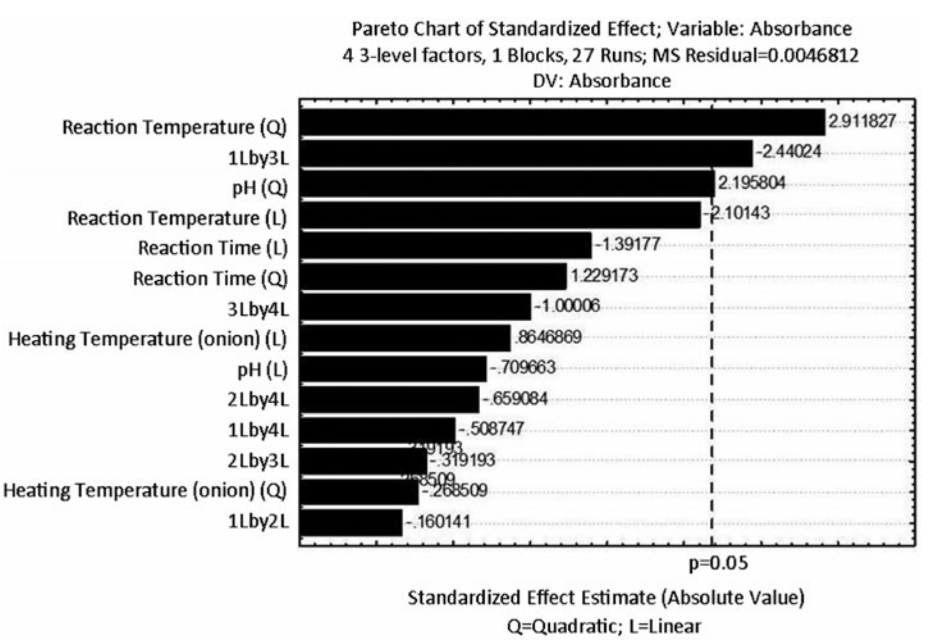

Fig. 1. Pareto chart of the variables.

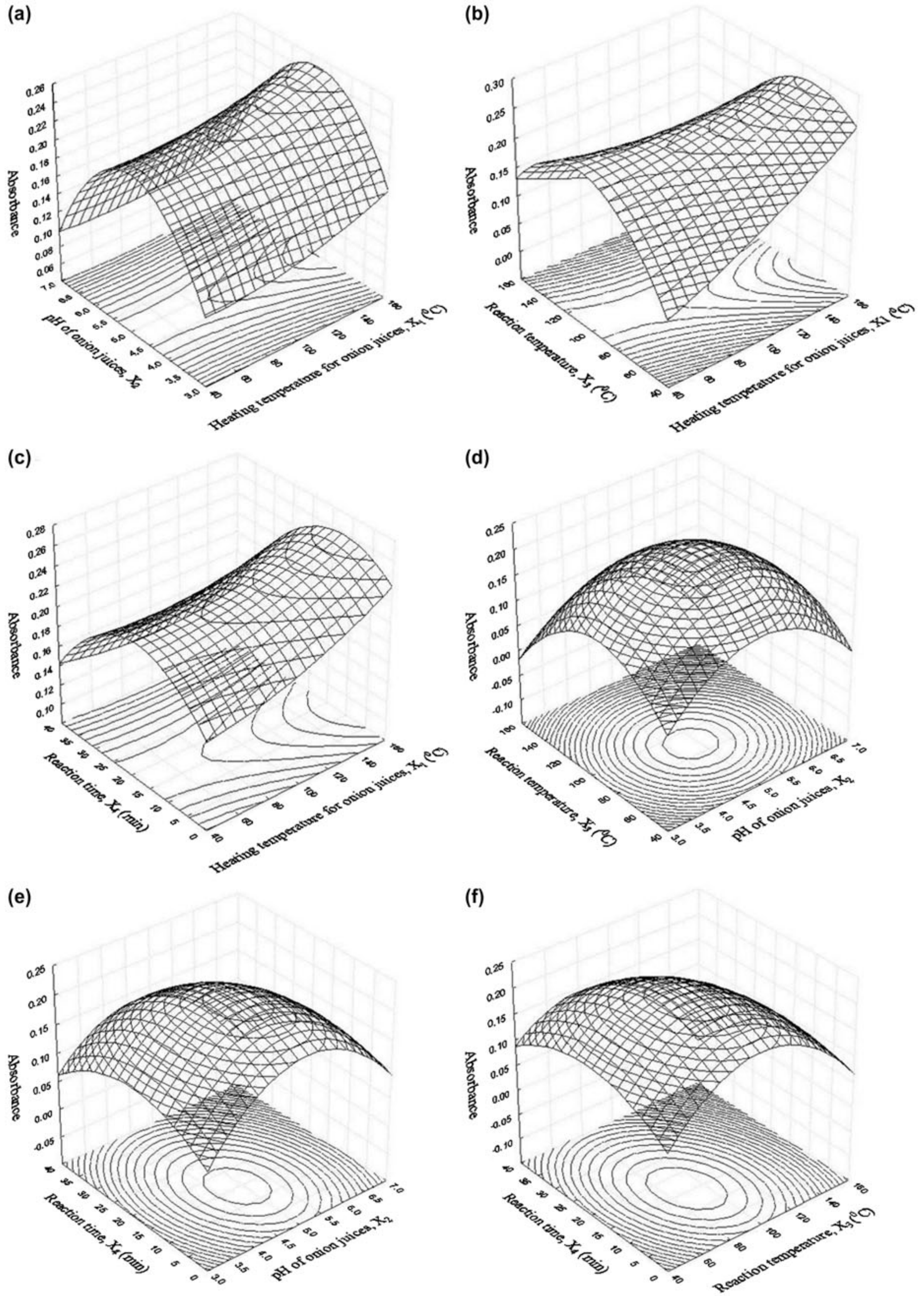

Fig. 2. The 3D response surface plots for the effects of parameters affecting formaldehyde determination by Box–Behnken design. (a) X_1X_2; (b) X_1X_3; (c) X_1X_4; (d) X_2X_3; (e) X_2X_4; (f) X_3X_4.

3.1.2. Interpretation of response 3D surface plots

In order to understand the effects on independent variables and their interaction on the formaldehyde reaction, three-dimensional (3D) surface plot and contour plots were constructed as shown in Fig. 2. Fig. 2 shows the interaction between heating temperature of onion juice (X_1), pH of onion juice (X_2), reaction temperature (X_3) and reaction time (X_4).

The predicted optimization values were calculated using the second-order polynomial equation (Eq. (1)). The coordinates of the maximum points were calculated through the first deviate of the mathematical function, which describes the response surface and equates it to zero [8]. The optimum conditions given by the model are as follows: heating temperature for onion juice of 60°C, pH of 4.9, reaction temperature of 103°C and reaction time of 17 min.

3.2. Effect of heating temperature for onion juice

As shown in Fig. 2(a)–(c), it was found that the absorbance increases with increasing heating temperature for onion juice. Mathematical model obtained by optimization using RSM (Eq. (1)) suggested that the optimum temperature for onion juice was 60°C after considering the interaction of other factors affecting formaldehyde detection. Yellow onion juice was heated to inactivate alliinase enzyme that is naturally present in onion [10,11]. Alliinase enzyme was implicated not to be involved in the reaction, because the red coloured complex was observed at 60°C and above. It was reported that the activity of alliinase enzyme decreases at temperatures higher than 60°C [12]. Dong et al. [13] reported that alliinase did not contribute to the pinking of onion. Allin (S-(1-propenyl)-L-cysteine sulfoxide) alone in phosphate buffer (pH 5) was implicated to produce pink coloured complex from onion [13].

3.3. Effect of pH

The formation of pink-coloured complex increases as the pH of onion juice increased from 3.5 to 5 (Fig. 2(a,d,e)). The optimum pH was 4.9 as suggested by the model. The formation of pink-coloured complex decreases at pH 6.5. Thiosulphinates are known to be a colour developer for pinking of onion [14,15]. The level of thiosulphinates depends on the pH of the onion juices. Lee and Parkin [15] reported that the maximum thiosulphinates level in onion extracts was at pH 5. In addition, thiosulphinate was reported to be stable only at acidic environment [16]. The optimum pH obtained by other researchers ranged from pH 3–4 [17]. The slight difference in optimum pH between the present study and previous study may be due to the difference in the variety of onions used, the addition of formaldehyde and the use of acetic acid as acidifying agent [17].

3.4. Effect of reaction temperature and time on colour development

The effect of temperature on the absorbance was studied in the range of 50–150°C. The result demonstrated that the absorbance was lower than 0.05 when the reaction temperature was below 60°C and greater than 120°C (Fig. 2(b,d,f)). At 150°C, the solution turns black, with no pink coloured complex formation due to degradation of the complex at high temperature.

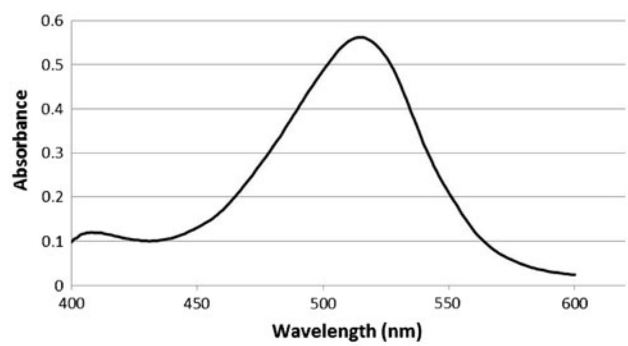

Fig. 3. Maximum wavelength for pink coloured complex.

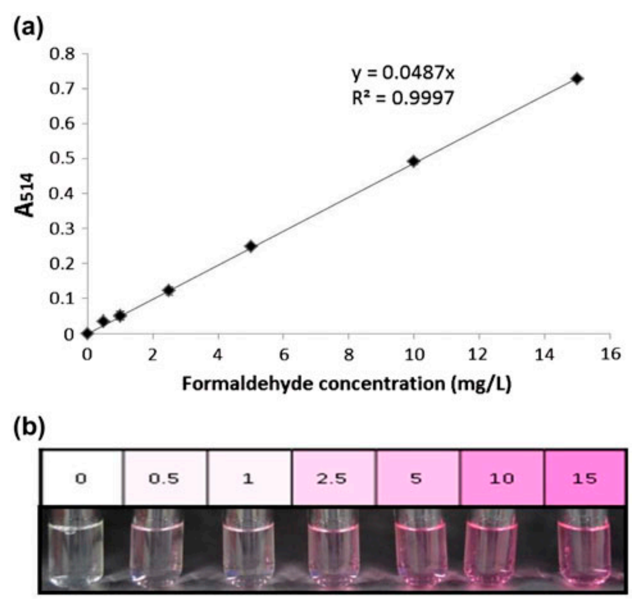

Fig. 4. Standard calibration curve (a) and standard reference colour card (b) for formaldehyde detection using onion juice.

Table 4
Colour measurement

(HCHO) mg/L	*L* (lightness)	*a* (red/green value)	*b* (yellow/blue value)	*c* (specifies chroma)	*h* (hue angle, °)	Colour
0	73.15	−1.12	2.67	2.65	115.63	Light Yellow
0.5	73.11	−0.77	2.59	2.47	108.80	Light Yellow
2.5	72.74	−0.03	2.40	2.20	91.47	Light Pink
5	72.42	1.18	2.09	2.28	58.44	Pink
10	71.50	3.42	1.48	3.79	22.32	Pink
15	70.06	6.35	1.02	6.60	9.90	Pink

The optimum reaction temperature was 103℃ as predicted by the model. The formation of the pink-coloured complex was found to be temperature-dependant; 50℃, >30 min and 103℃, 17 min (Fig. 2(f)).

3.5. Development of a safe reagent for formaldehyde detection

The detection is based on the reaction of active compounds in onion extract with formaldehyde. In this work, yellow onion extract was used as reagent due to the high amounts of thiosulphinates present [16]. The mechanism of the formation of pink-coloured complex from onion juice have been studied by other researchers [10,13,14,18]. The mechanisms are as follows; The first step represents the formation of thiosulphinates under the action of alliinase with onion juice. The second step corresponds to the production of a pigment precursor (PP) from the reaction between thiosulphinates and amino acid. While the last step involves the reaction between PP and naturally occurring carbonyl such as formaldehyde to form the pink-coloured complex. However, in this study, the mechanism of the formaldehyde detection using onion remains to be determined. The maximum wavelength of the pink coloured complex was 514 nm as shown in Fig. 3. The value obtained (514 nm) was slightly different from that reported by Dong et al. [13], and Lee and Parkin [15] (525 nm) due to the presence of formaldehyde in our mixture. The reaction of formaldehyde with thiosulphinates in onion extract resulted in a bathochromic shift.

3.5.1. Calibration graph

The reaction of onion extract with different concentrations of formaldehyde was carried out under the suggested conditions by RSM. The standard calibration curve obtained and the colour changes are shown in Fig. 4. The experimental results showed good linear relationship between absorbance and the formaldehyde concentrations with a correlation coefficient of 0.9997. The colour recorded was light yellow to pink in the concentration range of 0.5–15 mg/L, and a colour card was prepared based on the correlation. The colour change was not discernible at concentration of formaldehyde exceeding 15 mg/L due to deviations from Beer Lambert's Law which resulted in a nonlinear calibration curves.

3.5.2. Colour measurement

A series of standard formaldehyde solutions were prepared and reacted with onion extract under optimized conditions. The lightness (*L*), *a* and *b* values, chroma (*c*) and hue angle of the complex was measured using a colour spectrophotometer. Table 4 shows the colour measurement of pink-coloured complex. By increasing concentrations of formaldehyde, the *L*, *b* and *h* values decreases due to the changes of light yellow of onion extract to pink solution. This is also supported by the value of hue angle between 120° and 0°, which is yellow to red on the colour wheel. A sample with a hue angle of 0℃ is purplish-red, 90℃ is yellow, 180℃ is bluish-green and 270℃ is blue [19]. The positive values of *a* for formaldehyde concentrations of 5, 10 and 15 mg/L indicates that the

Table 5
Comparison of the results obtained by the proposed method and standard acetylacetone methods

(HCHO) (mg/L)	Proposed method (mg/L)	Acetylacetone method (mg/L)
2	1.95 ± 0.079	1.888 ± 0.188
7	8.049 ± 0.943	7.191 ± 0.009
12	11.846 ± 0.720	11.995 ± 0.245

solution is in the red region. The *a* coordinate denotes red when positive and green when negative, and *b* denotes yellow when positive and blue when negative. A low chroma (*c*) values indicates dull and achromatic colours [19].

3.5.3. Comparison of analysis by proposed method with conventional method

The proposed method was used in the analysis of three known concentrations of formaldehyde in deionised water (Table 5). The results obtained by the proposed method was compared with standard acetylacetone method [20] as reference, and a paired *t*-test confirmed their congruence at 95% confidence level. There is no significant difference between the two methods at 95% confidence level. The good agreement between these results indicates the successful applicability of the proposed method for the determination of formaldehyde. These results demonstrated that the proposed method was suitable for the rapid determination of formaldehyde.

4. Conclusion

In the present study, a safe method for formaldehyde detection has been developed using yellow onion juice as reagent. Four independent factors affecting formaldehyde detection, i.e. heating temperature for onion juice, pH of onion juice, reaction temperature and reaction time were successfully optimized by RSM in this work. The optimum response was obtained by heating the onion juice at 60˚C, pH of 4.9, reaction temperature of 103˚C and reaction time of 17 min. The proposed method is simple, inexpensive and safe to the environment.

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