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Research Article

Visual Detection and Determination of Melamine Using Synthetic Dyes

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We have used spectroscopic technique for the detection of melamine. The effect of melamine on the colour as well as the pH of bromophenol, methyl red and alizarin red dye solutions was examined at different mole ratios. It is found that we observe color transition and the absorption maxima for bromophenol were at 598 nm, while for methyl red, and alizarin red-S dye they are at 520 nm and 423 nm, respectively. We observe an increase in the absorption intensities at 598 nm with increase in the concentration of melamine in bromophenol blue dye. The absorption intensities at 520 nm decreases and new peak at 420 nm emerges in methyl red dye-melamine mixture. While the absorption intensities at 420 nm decreases and 520 nm peak emerges in alizarin red S dye-melamine at higher mole ratios. The results indicate that we can choose the appropriate dye of suitable range to detect the concentration of melamine from 3 to 206 mg dm^{-3}. The results demonstrate possible use of the simple method for the qualitative and quantitative detection of melamine in adulterated food samples.

1. Introduction

Melamine is a weak organic base with the chemical formula $C_3N_6H_6$ which contains 67% of nitrogen mass. Melamine in combination with formaldehyde produces melamine resin and has been widely used as fire retardant for the release of nitrogen when burned [1, 2]. Melamine foam has also been employed as a colourant, superplasticizer, polymeric cleansing product, insulator and so forth [3]. In early 1950 and 1960s, melamine was used as nonprotein food source for ruminants and also as source of nitrogen for food crops [4]. Development of dairy industries in last few decades has resulted in the promotion of adulterating the food products across the world with an ulterior motive to gain higher profits [5]. Several thousand people die every year due to the consumption of adulterated food. One of the classic examples is the sudden death of infants and pets across the world in 2007 and 2008 due to the adulteration of infant milk powder and pet food with melamine [6]. One of the most widely used methods to detect the protein content in the samples is by using Kjeldahl and Dumas test. In this test, nitrogen content will be estimated to obtain information about the protein content [7]. Melamine contains higher percentage of nitrogen content and this promoted the food industries to adulterate the food products with melamine illegally to enhance the apparent nitrogen content in the milk powder during the estimation of protein levels [8]. Alternative techniques used for the detection of melamine are HPLC, GC, MS, IR, Raman, Zone electrophoresis, electrospray ionization, and so forth. The above techniques can detect the melamine concentration up to parts per million (ppm) range [9–15]. Major limitation is the cost of the equipment; it demands highly skilled labor and is economically and practically not feasible for routine analyses [16]. Also detection of parts per billion (ppb) or even parts per trillion (ppt) levels of melamine by advanced analytical techniques can also generate false-positive results. To overcome the above limitations, development of analytical methodologies enables in situ detection and estimation of organic contaminants involving simple sample preparation and measurement procedure. Colorimetric methods have been reported to use gold- and silver-based nanoparticles as

Table 1: The concentration range of different dye solutions.

Dye	Concentration range of melamine ($mg\ dm^{-3}$) can be detected
Bromophenol blue	3–30
Alizarin red-S	10–50
Methyl red	41–206.8

probes, crown ether-assembled gold nanoparticles, citrate-capped gold nanoparticles, and so forth [13, 17–21]. These visual methods are simpler, do not require expensive instrumentation, and have practical application for the detection of melamine but gold- and silver-based reagents are expensive. By using appropriate pH indicator and adjusting the pH of the medium, a simple and effective spectrophotometric method has been developed. The safety limit for infant milk products in US, China, and Europe has been set at 2.5 mg/kg. If the melamine intake exceeds the safety limit then it will severely damage kidneys. Hence in this work, we report on the use of dye solution such as bromophenol, methyl red, and alizarin red-S as pH indicator for the detection of melamine in the range of 3–206 $mg\ dm^{-3}$. The increase in the melamine causes shift in the pH of the test system thus leading to color change. The change in colour of melamine-dye (bromophenol, methyl red, and alizarin red-S) solution is proportional to the amount of melamine present in the solution and hence a simple and economical method for the qualitative and quantitative determination of melamine has been reported.

2. Experimental Section

2.1. Materials. Bromophenol blue, methyl red, alizarin red-S sodium salt, and melamine were procured from commercial sources (SD-Fine Chemicals, India) and used without purification.

2.2. Sample Preparation. Stock solution of dye (bromophenol blue, methyl red, and alizarin red-S) was prepared by weighing known quantities of dyes and was made up to one litre using distilled water separately. In case of methyl red, we have used 60% water and 40% ethyl alcohol as solvent mixture. The concentrations of bromophenol dye, methyl red, and alizarin red-S solutions used for the experiments are given in Table 1.

Into a series of 100 mL volumetric flasks, 50 mL of dye solution was added to melamine solution (50 mL) of different concentrations (see Tables 2, 3, and 4). The mixtures were stirred well and the absorbance values were measured in the range from 335 nm to 1000 nm. The concentrations of melamine are in the range from 7.0909×10^{-5} M to 1.64×10^{-3} M and the pH of the above solutions was recorded using glass electrode.

3. Characterization

Melamine was characterized using Bruker-D8 Advanced powder X-ray diffractometer with Cu Kα source (λ = 1.5418 Å, scan rate 2° min^{-1}; steps-0.05°; scan range-10–65° 2θ) was used to determine the crystal structure. Elico 157 mini UV-visible spectrometer was used to measure the absorbance spectra of different solutions.

4. Results and Discussion

Indicators are weak acids or weak bases whose conjugate base/conjugate acid exhibits different colour with change in the pH

$$\mathrm{H(anion)} + \mathrm{H_2O} \longleftrightarrow \mathrm{Anion^-} + \mathrm{H_3O^+} \quad (1)$$

Several factors affect the absorbance, that is, pH, ionic strength, concentration, volume of the solution, and so forth, of which pH plays an important role in most of the analytical methods especially in case of acid-base reactions which occur in aqueous medium. In view of this, the indicator must be accordingly selected to change colour when the pH of the test solution either increases or decreases. Indicators such as bromophenol blue, methyl red, and alizarin red-S were chosen as indicators and the structures are shown in Figure 1. Bromophenol blue ($C_{19}H_{10}Br_4O_5S$ or 3′,3″,5′,5″, tetrabromophenol Sulfophathalein) is a redox indicator dye which shows colour transition in the range from 3 to 6. The colour of bromophenol blue solution is yellow at pH 3 and exhibits bluish purple colour at pH > 4.6. Bromophenol blue exhibits pK_a values at 3.6, 3.85, and 4.0 with absorption maxima at 422 nm, 436 nm, 529 nm, and 598 nm in the visible region [22]. Methyl red, $C_{15}H_{15}N_3O_2$, is an azo dye which exhibits colour changes from red at pH 4.4 to yellow at pH 6.2. They have pK_a values at 2.3, 2.5, 4.95, and 5.06 [23]. Alizarin red-S is classified under anthraquinone dye which changes its yellow colour at pH 3.5 to red at pH 6.5. They exhibit two pK_a values at 4.5 and 11 [24]. The absorption maxima are exhibited at 423 nm, 546 nm, and 596 nm, respectively. The concentration of melamine has been examined for different mole ratios. The pH of the different concentrations of dye solutions in contact with melamine at different mole ratios leads to an increase in the pH due to the basic nature of melamine (see Tables 2–4). The concentration of melamine using different types of dyes (bromophenol blue, methyl red, and alizarin red-S dye) has been examined at different mole ratios. Thus a visual color change observed is proportional to the amount of melamine thus causing the change in the equilibrium resulting in a higher or basic pH.

The melamine is alkalescence with the pK_a of 5 and has no absorption in the range of 335 to 1000 nm, while the bromophenol blue, methyl red, and alizarin red-s dye exhibit absorbance peaks at 598 nm, 520 nm, and 400 nm, respectively. The stoichiometric ratio of melamine-dye mixtures is shown in Tables 2–4. The concentrations of dye solution are fixed and the melamine concentration is increased. Increase in the concentration of melamine leads to the increase in the pH of the solution from 4 to 6.8 (see Tables 2–4). Visual detection of the colour changes may not provide accurate information about the colour transitions (see Figures 2, 3, and 4). Hence it is suitable to carry out the acceptable sensitivity for the determination of melamine by spectrophotometric estimation.

Table 2: Variation in the mole ratio of bromophenol blue dye and melamine solutions and pH.

Mole ratio of bromophenol blue and melamine (total volume—100 mL)	Concentration		pH
	Bromophenol blue (M)	Melamine (M)	
Melamine	—	2.37×10^{-5} ($3\ mg\ dm^{-3}$)	6.18
Bromophenol blue	2.37×10^{-5} ($15\ mg\ dm^{-3}$)	—	4.9
1 : 1	2.37×10^{-5} ($15\ mg\ dm^{-3}$)	2.37×10^{-5} ($3\ mg\ dm^{-3}$)	4.92
1 : 2	2.37×10^{-5} ($15\ mg\ dm^{-3}$)	4.75×10^{-5} ($6\ mg\ dm^{-3}$)	4.95
1 : 4	2.37×10^{-5} ($15\ mg\ dm^{-3}$)	9.51×10^{-5} ($12\ mg\ dm^{-3}$)	5.24
1 : 6	2.37×10^{-5} ($15\ mg\ dm^{-3}$)	1.268×10^{-4} ($16\ mg\ dm^{-3}$)	5.55
1 : 8	2.37×10^{-5} ($15\ mg\ dm^{-3}$)	1.9029×10^{-4} ($24\ mg\ dm^{-3}$)	5.73
1 : 10	2.37×10^{-5} ($15\ mg\ dm^{-3}$)	2.378×10^{-4} ($30\ mg\ dm^{-3}$)	5.85

Table 3: Variation in the concentration of melamine and methyl red dye solutions and pH.

Mole ratio of methyl red and melamine (total volume—100 mL)	Concentration		pH
	Methyl red (M)	Melamine (M)	
Melamine	—	7.909×10^{-5} ($10\ mg\ dm^{-3}$)	6.32
Methyl red	7.909×10^{-5} ($10\ mg\ dm^{-3}$)	—	5.03
1 : 1	7.909×10^{-5} ($10\ mg\ dm^{-3}$)	7.909×10^{-5} ($10\ mg\ dm^{-3}$)	5.36
1 : 2	7.909×10^{-5} ($10\ mg\ dm^{-3}$)	1.781×10^{-4} ($20\ mg\ dm^{-3}$)	5.68
1 : 3	7.909×10^{-5} ($10\ mg\ dm^{-3}$)	2.372×10^{-4} ($30\ mg\ dm^{-3}$)	5.89
1 : 4	7.909×10^{-5} ($10\ mg\ dm^{-3}$)	3.163×10^{-4} ($40\ mg\ dm^{-3}$)	6.06
1 : 5	7.909×10^{-5} ($10\ mg\ dm^{-3}$)	3.954×10^{-4} ($50\ mg\ dm^{-3}$)	6.21

Table 4: Variation in the mole ratio of melamine and alizarin red dye solutions and pH.

Mole ratio of alizarin red and melamine (total volume—100 mL)	Concentration		pH
	Alizarin red (M)	Melamine (M)	
Melamine	—	3.29×10^{-4} ($41.5\ mg\ dm^{-3}$)	6.19
Alizarin red-S	3.29×10^{-4} ($41.5\ mg\ dm^{-3}$)	—	4.49
1 : 1	3.29×10^{-4} ($41.5\ mg\ dm^{-3}$)	3.29×10^{-4} ($41.5\ mg\ dm^{-3}$)	5.18
1 : 2	3.29×10^{-4} ($41.5\ mg\ dm^{-3}$)	6.58×10^{-4} ($83\ mg\ dm^{-3}$)	5.46
1 : 3	3.29×10^{-4} ($41.5\ mg\ dm^{-3}$)	9.87×10^{-4} ($124.5\ mg\ dm^{-3}$)	5.65
1 : 4	3.29×10^{-4} ($41.5\ mg\ dm^{-3}$)	1.31×10^{-3} ($165.2\ mg\ dm^{-3}$)	5.71
1 : 5	3.29×10^{-4} ($41.5\ mg\ dm^{-3}$)	1.64×10^{-3} ($206.8\ mg\ dm^{-3}$)	5.82

Figures 5–7 illustrate the change of absorption spectra of melamine-dye mixtures at different mole ratios with the dye concentration fixed. The colour of the bromophenol blue dye solution is violet and in presence of melamine at higher concentrations changes to navy blue (see Figure 5). The absorption maxima for bromophenol blue dye solution are observed at 598 nm and increase with increase in the melamine concentration (1 : 10) (see Figure 5). In case of methyl red dye, the colour of the solution is light red and its intensity increases when large concentration/quantity of melamine is added (see Figure 6). We observe decrease in the intensity of peak at 520 nm and new peak emerges at 420 nm when methyl red and melamine are mixed in different mole ratios (see Figure 6) (melamine : methyl red ratio 1 : 5). In case of alizarin red-S dye solution, the peak was observed at 400 nm and shifts to 423 nm; a new peak at 520 nm emerges (see Figure 7). We have evaluated the change in the absorbance in different dye solutions with increase in the melamine concentration, while alizarin red dye exhibits yellowish orange and changes to wine red in presence of melamine (see Figure 7). Figures 8, 9, and 10 show UV-visible spectroscopic response of different types of dyes with melamine at different ratios. A strong linear correlation was obtained by the absorbance of the methyl red dye solution with increase in the melamine concentration at λ = 520–527 nm and the correlation coefficient was 0.99 (see Figure 11), while in case of alizarin red-S it is not linear in nature. The change in the absorbance of bromophenol with increase in the concentration of melamine shows contrasting data compared to methyl red dye-melamine solutions (see Figures 12 and 13). Thus we observe significant change in the colour of dye with changes in the melamine concentration.

5. Conclusion

A simple spectrophotometric method for the determination of trace quantities of melamine in aqueous solution has been

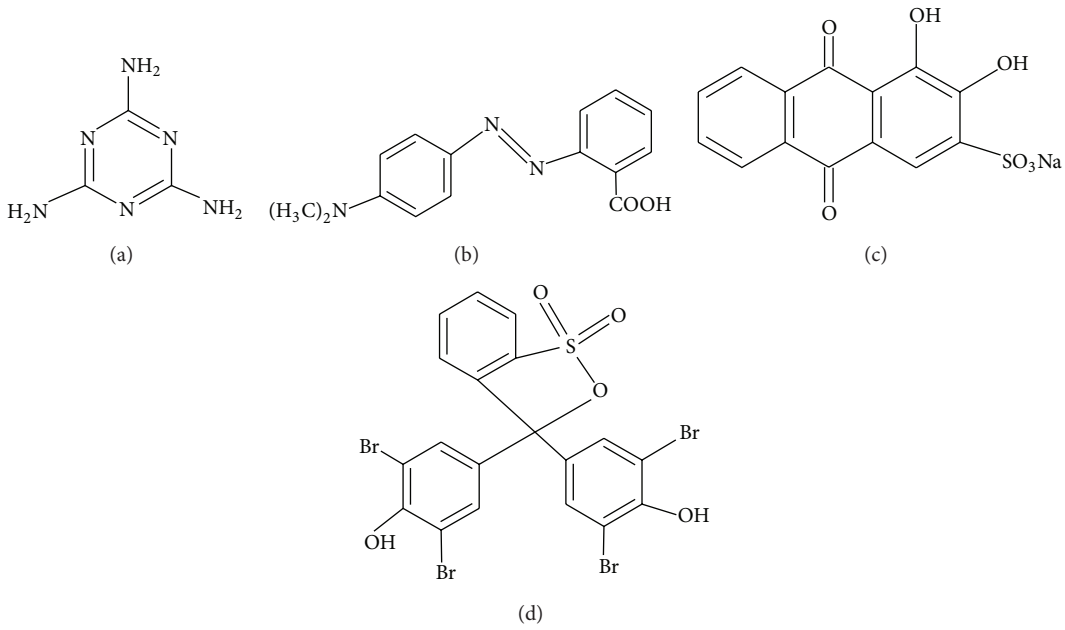

Figure 1: Structures of melamine and dyes.

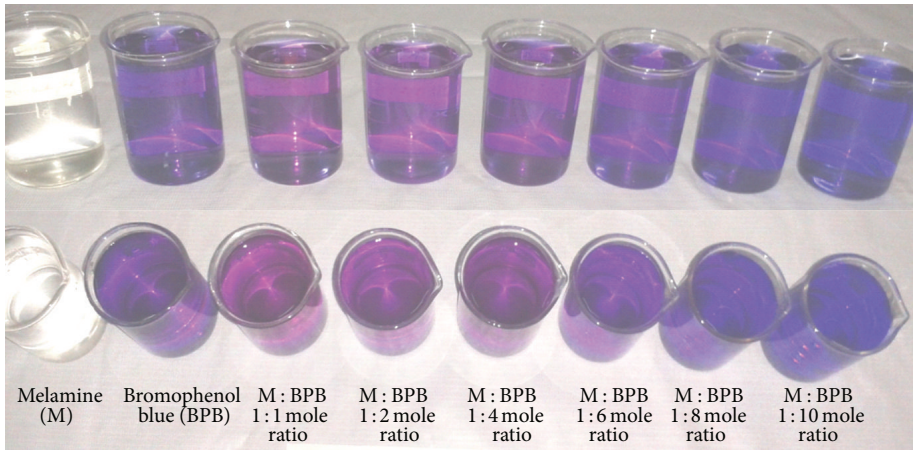

Figure 2: Melamine and bromophenol blue solution mixtures at different mole ratios.

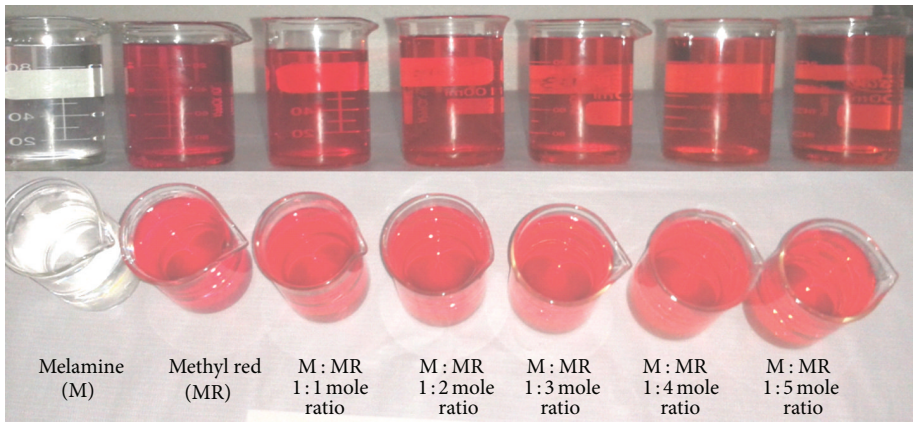

Figure 3: Melamine and methyl red solution mixture at different mole ratios.

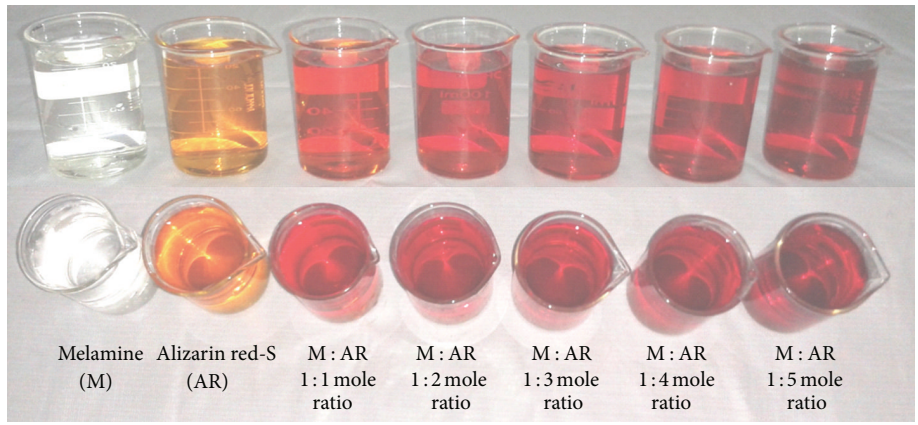

Figure 4: Melamine and alizarin red solution mixture at different mole ratios.

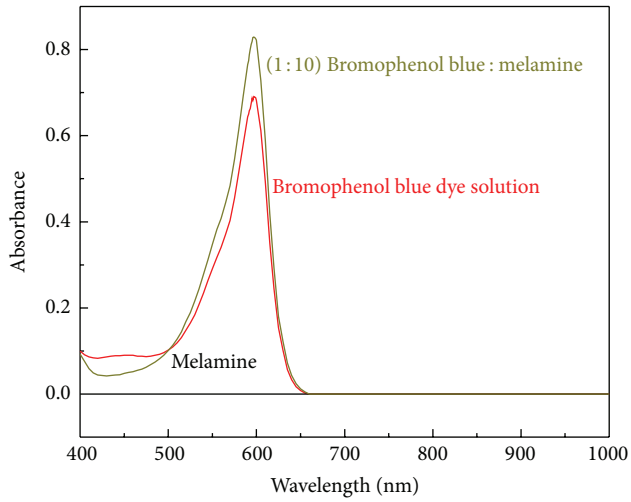

Figure 5: Absorption spectra of melamine, bromophenol blue dye, and mixture of melamine and bromophenol blue solutions at 1 : 10 mole ratio.

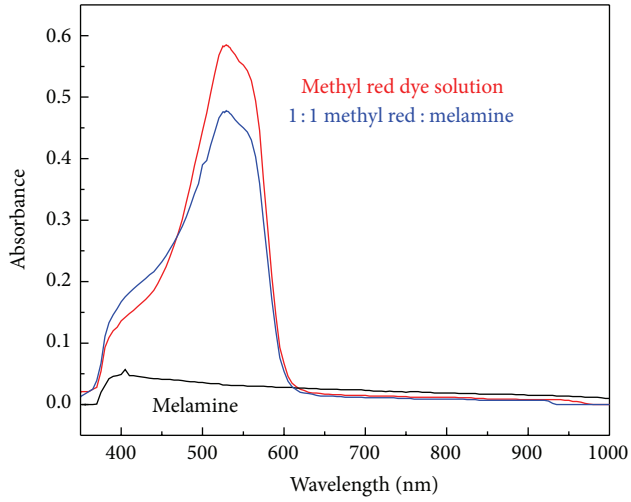

Figure 6: Absorption spectra of melamine, methyl red dye, and mixture of melamine and methyl red dye solutions at 1 : 1 mole ratio.

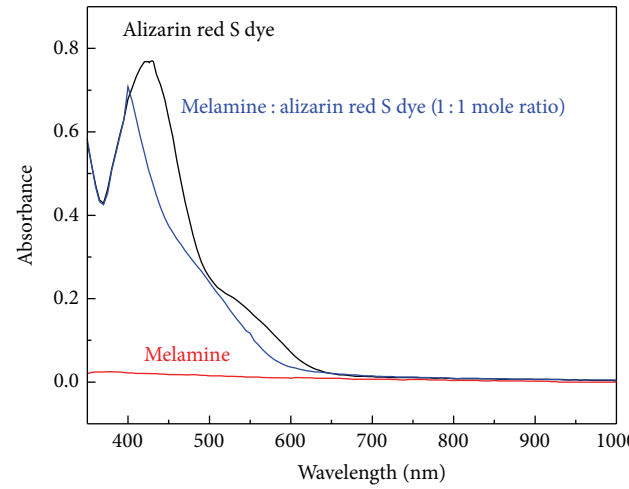

Figure 7: Absorption spectra of melamine, alizarin red dye, and mixture of melamine and alizarin red dye solutions at 1 : 1 mole ratio.

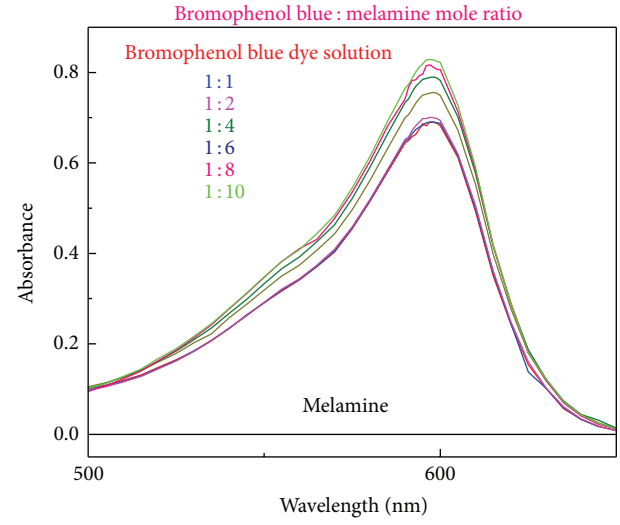

Figure 8: Absorption spectra of melamine, bromophenol blue dye, and mixture of melamine and bromophenol blue solution at different mole ratios.

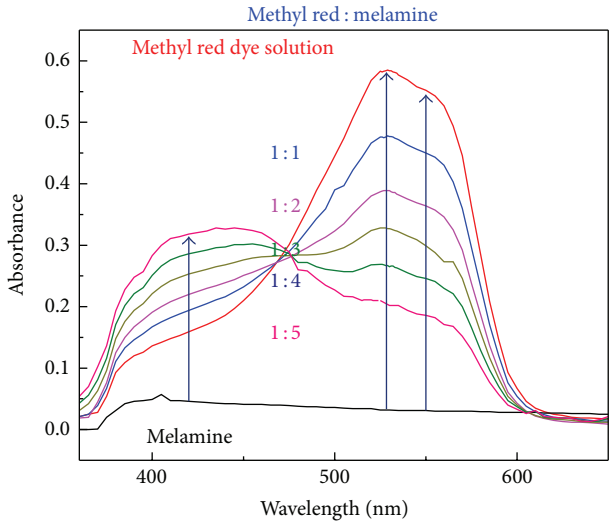

Figure 9: Absorption spectra of melamine, methyl red dye, and mixture of melamine and methyl red dye solution at different mole ratios.

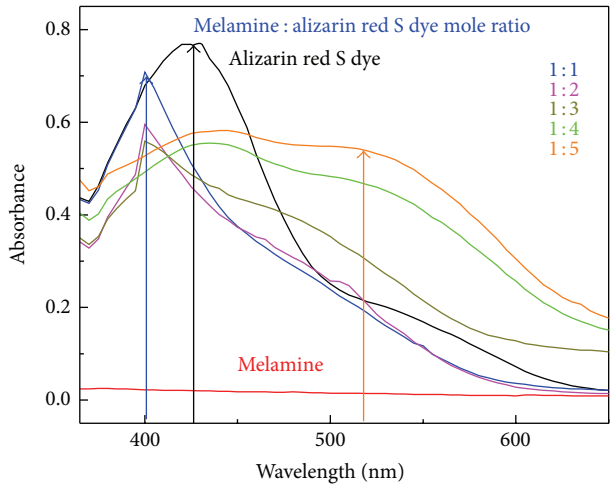

Figure 10: Absorption spectra of melamine, alizarin red dye, and mixture of melamine and alizarin red dye solution at different mole ratios.

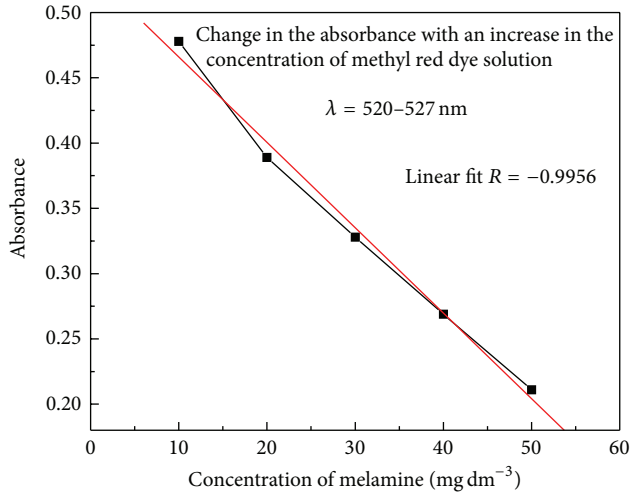

Figure 11: Linear absorbance as a function of concentration of melamine with methyl red dye.

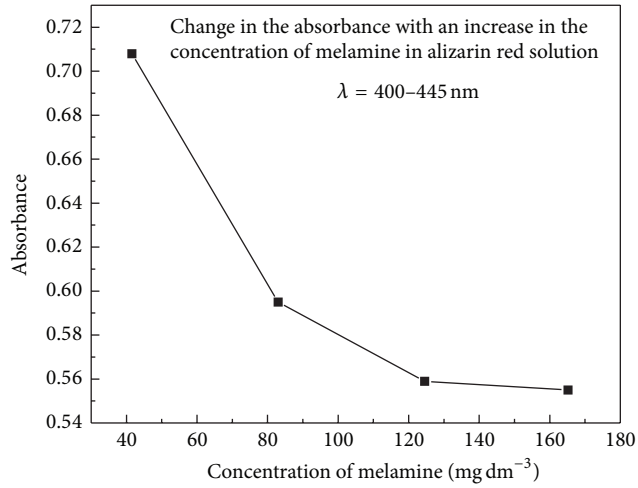

Figure 12: Absorbance as a function of concentration of melamine with alizarin red dye.

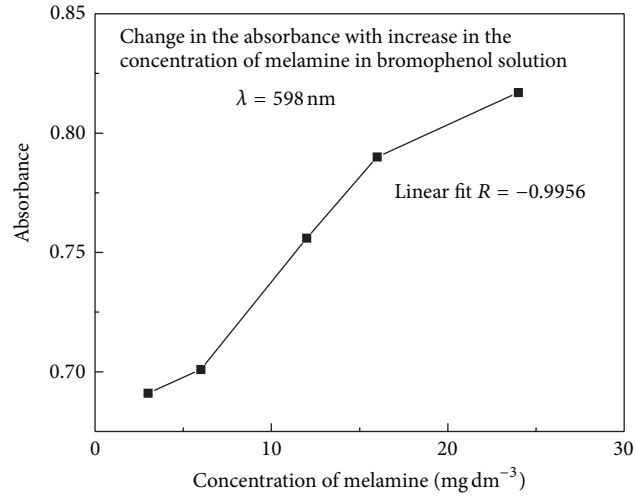

Figure 13: Linear absorbance as a function of concentration of melamine with bromophenol blue dye.

reported. The method involves interaction of melamine based on the acid-base reaction with different types of dyes. The colour change is due to the variation in the pH of the aqueous solution containing dye solution. The synthetic dyes can transform molecular recognition between the pK_a and their interaction with weak base melamine into the visual color change. The proposed method can be used for the detection of melamine in the range from 3 to 206 mg dm^{-3} using different dyes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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