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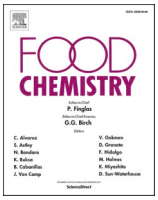

A novel polymeric fluorescence sensor based on acrylated citric acid for detection of melamine adulteration: Application in milk powder

Neşe Taşci [a,b], Soner Çubuk [a,*], Ece Kök Yetimoğlu [a], Memet Vezir Kahraman [a]

[a] Chemistry Department, Faculty of Arts and Sciences, Marmara University, 34722 Istanbul, Turkey
[b] Department of Chemistry, Gebze Technical University, 41400 Kocaeli, Turkey

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ABSTRACT

Melamine selective acrylate citric acid (ACA) based polymeric membrane sensor was prepared by radical polymerization method and the sensor was characterized. The sensor showed a selective fluorescent response to melamine ($\lambda_{ex}/\lambda_{em}$ = 388/425 nm). The sensor response is linear in the concentration range of 3.96×10^{-9} to 7.93×10^{-8} mol L^{-1}, the optimum pH value is 6.0 and response time is less than 1 min. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 2.32×10^{-10} mol L^{-1} and as 7.74×10^{-10} mol L^{-1}, respectively. The sensor showed great selectivity towards melamine in the presence of a large number of molecules and ions. The performance of sensor was also confirmed by determining of melamine in milk powder sample and the results were compared with HPLC results and acceptable results were obtained. As a conclusion, the results revealed that the proposed sensor is an interesting alternative for melamine determination.

1. Introduction

Food adulteration is the production of foodstuffs in violation of the legislation or permitted specifications. With adulteration, unfair gain is achieved by adding a foreign or cheap substance to the natural content of a product and replacing some or all of a valuable component in its content. Quality products are mixed with less quality products to reduce the cost and offered for sale. The adulteration of foods is an extremely important issue, as it can affect consumer confidence as well as the economic burden, and more importantly, it can affect consumer health. Some adulteration carries serious risks to public health and/or the environment. In our rapidly developing world, with the globalization of food supply and rapid distribution chains, consumers are vulnerable to this fraud as food adulteration increases. For this reason, it has become an extremely important issue to develop a method for the easy and fast detection of adulteration in foods.

Melamine (Mel; 1,3,5-triazine-2,4,6-triamine; $C_3H_6N_6$) is a white, powdered, odorless, nitrogen containing, triazine derivative, organic compound which is extensively used in the chemical industries (Gao et al., 2012). It has a wide range of industrial uses, including plastics, cleaning products, flame retardants, laminates, adhesives, fertilizers, resins and foams (Wang et al., 2010; Xu et al., 2009). It has a high nitrogen concentration of 66.6% by mass, relative to proteins. Adding 1% melamine to food causes an artificial increase of more than 4% in protein content. For this reason, it is added to many foods such as milk, milk powder, animal feed and baby food, illegally and deliberately to increase its protein content. There are many studies on this subject in the literature (Barreto et al., 2021; Hong et al., 2017; Serdiuk et al., 2010). Standard methods such as Dumas and Kjeldahl methods are available to calculate protein content. In these methods, the nitrogen content is measured, and the protein content is calculated using the appropriate nitrogen to protein conversion factors. However, these methods do not allow the protein to be accurately distinguished from other nitrogen-containing chemicals. Therefore, this adulteration is not easily detected by routine analysis.

Melamine has low toxicity, but at high concentrations it hydrolyzes to cyanuric acid, which results in the formation of insoluble melamine-cyanurate crystals, also known as kidney stones. There are several findings that prolonged and high dose exposure to melamine causes an increase in the incidence of bladder stone formation and bladder tumors. For example, in 2008, cases of urinary tract problems and deaths were reported in many infants in China, due to the illegal mixing of melamine into infant formula (European Food Safety Authority (EFSA), 2008; He et al., 2021). To avoid all these problems, determining the amount of melamine in foods has become a very important issue for ensuring food safety and protecting public health. For this reason, limitations on daily

* Corresponding author.
E-mail address: sonercubuk@marmara.edu.tr (S. Çubuk).

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intake limits for melamine have been introduced. For example, the daily intake of melamine by WHO (World Health Organization) and FDA (US Food and Drug Administration) has been determined as 2.5 mg kg^{-1} (2.5 ppm) for adults and 1.0 mg kg^{-1} (1 ppm) for children (Food and Drug Administration of USA, 2008; World Health Organization, 2008).

Various instrumental analysis methods are used for melamine determination, including HPLC (high performance liquid chromatography) (Haghi et al., 2018; Mohebi et al., 2020), LC-MS (Liquid chromatography-mass spectrometry) (Liu et al., 2020; Tkachenko et al., 2015), SERS (Surface-enhanced raman spectroscopy) (Tang et al., 2020; Zhang et al., 2019) and GC-MS (gas chromatography-mass spectrometry) (Malečková et al., 2020; Mıao et al., 2009). Although some of these methods stand out for their high precision and accuracy, many of them have disadvantages such as expensive equipment and complex instrumentation, difficult applicability in the field, the need for well-trained staff and time-consuming sample preparation. Considering the high demand for melamine determination as well as the proposed approaches, the need for a fast, simple and environmentally friendly analysis method emerges. Fluorimetric sensors stand out as an alternative method to the methods used in melamine determination with their ease of use, short response time, low detection limit, reproducibility, high sensitivity and selectivity.

In this study, a UV-cured polymeric membrane sensor containing acrylated citric acid (ACA) units, which was synthesized for the first time in this study, was prepared for the determination of melamine. There is no information in the literature about the synthesis of this substance and its use in the determination of melamine. The effect of pH value and time on the fluorescence intensity of the prepared polymeric sensor, the interaction of the sensor with melamine and the change in fluorescence intensity in the presence of interference species were investigated. Melamine was successfully determined in milk powder samples under optimum operating conditions decided by using the prepared polymeric membrane sensor. With this study, a novel method for melamine determination was defined by proposing for the first time an ACA-based reusable polymeric sensor (use at least 250 times by just washing with distilled water). This method stands out as it is a more sensitive, simple and fast technique compared to other fluorescent methods used for the determination of melamine.

2. Experimental

2.1. Chemicals and materials

All chemicals used in this study were analytical reagent-grade and used without prior purification. Trimethylolpropane triacrylate (TMPTA), poly (ethylene glycol) diacrylate (PEGDA) used as monomer in the preparation of the melamine sensor and 1-Hydroxy-1-methylethyl phenyl ketone (Darocur 1173) used as photo initiator were purchased from Merck (Bo-Ga, Turkey).

Citric acid (CA), acryloyl chloride (AC), tetrahydrofuran (THF), used for the synthesis of acrylated citric acid (ACA) monomer, and trichloroacetic acid (TCAA), used to precipitate proteins in milk powder, were purchased from Merck. Likewise, melamine (99% purity) was obtained from Sigma Aldrich (Bo-Ga, Turkey).

Acetic acid (CH_3COOH), sodium acetate trihydrate ($NaCH_3COO{\cdot}3H_2O$), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$), dipotassium hydrogen phosphate (K_2HPO_4), hydrogen chloride (HCl) and potassium chloride (KCl) used for the preparation of buffer solutions that will be used to keep the pH of the working environment constant during operation was purchased from Sigma Aldrich (Bo-Ga, Turkey).

Working standard solutions of melamine were prepared in acetate buffer and the pH of the working medium was adjusted regularly using a digital pH meter and Merck's standard buffer solutions. All water used during the study was purified using Millipore Direct-Q (Merck Millipore, Labor Teknik-Turkey) water purification system.

2.2. Instruments

Structural and optical properties of developed polymeric membrane sensor were investigated by nuclear magnetic resonance spectrometers (^{1}H NMR), fourier transform infrared spectrometer (FT-IR) and fluorescence spectrophotometer.

The ^{1}H NMR spectrum of ACA was recorded using the Bruker Avance 500 MHz spectrometer after this monomer was resolved with dimethyl sulfoxide (DMSO) solvent. FT-IR measurements of the sensor and ACA were taken using a Perkin Elmer Spectrum 100 ATR-FTIR spectrometer (4000–400 cm^{-1}).

The optical characterization study of the melamine selective polymeric membrane sensor was performed with Varian Cary Eclipse Fluorescence Spectrophotometer by measuring samples in a 1 cm path length cuvette (at room temperature).

2.3. Synthesis of acrylated citric acid monomer (ACA)

The acrylation reaction of citric acid was carried out by modifying the acrylation reaction in the literature (Constantin et al., 2014) The synthesis of acrylated citric acid monomer (ACA) to be used in the preparation of the melamine sensor was performed as follows (Fig. 1 (A)). 21.014 g of citric acid was weighed into a three-necked reaction flask, 40 mL of THF was added on it, and then it was homogenized by stirring at room temperature. After adding 10.5 g of AC dropwise over approximately 3 h to the reaction flask, the reaction mixture was stirred at room temperature for 24 h under N_2 gas to acrylate the citric acid. The temperature was then raised to 50 °C and the reaction mixture was stirred at this temperature for a further 2 h. To remove the salt formed from the reaction medium, at the end of the period, the reaction mixture was filtered while hot through filter paper and washed with 30 mL THF. Excess THF remaining in the reaction medium was removed by evaporation on the rotary. It has been observed that the acrylation process of the obtained material has been successfully performed from the obtained FT-IR and ^{1}H NMR spectra.

2.4. Preparation of melamine selective polymeric sensor

The polymeric membrane sensor to be used in the determination of melamine was prepared using the photopolymerization technique. Chemical structure of the polymeric sensor was given in Fig. 1(B). In the sensor formulation, PEGDA, ACA, and TMPTA were used as main polymer, functional monomer and crosslinker, respectively. Darocur 1173 was also used as a photoinitiator. In order to determine the optimum sensor formulation, sensors in different formulations were obtained by using different ratios of reactive monomer. The sensor formulations were prepared as follows: ACA reactive monomer was mixed with PEGDA, TMPTA and photoinitiator in a beaker until a homogeneous mixture containing different proportions of ACA reactive monomer was obtained. In this way, different formulations were obtained. Nitrogen gas was then passed for 15 min to remove any dissolved oxygen remaining in the system, and the prepared sensor mixtures were poured into a Teflon mold (WxLxD: 12 mm × 40 mm × 2 mm). The mixture transferred to the mold was cured at different curing times under high pressure UV lamp (Ultra Vitalux OSRAM 300 W, intensity 10 $mW.cm^2$, wavelength max. = 365 nm) for polymerization. The prepared polymeric membrane films were removed from the Teflon mold. Then it kept in deionized water for 24 h in order to remove the remaining monomers and photoinitiator without reacting on the films. It was then dried in a freeze dryer for 3 days. Formulations that exhibit undesirable conditions, such as late interaction with aqueous solution, curling, not completely drying, swelling too quickly in aqueous medium or dispersion in water or at different pH, were eliminated. The preparation procedure of the melamine sensor is given in Fig. 2. Formulations containing different amounts of reactive functional monomers were prepared. The percentages of monomers contained in these formulations

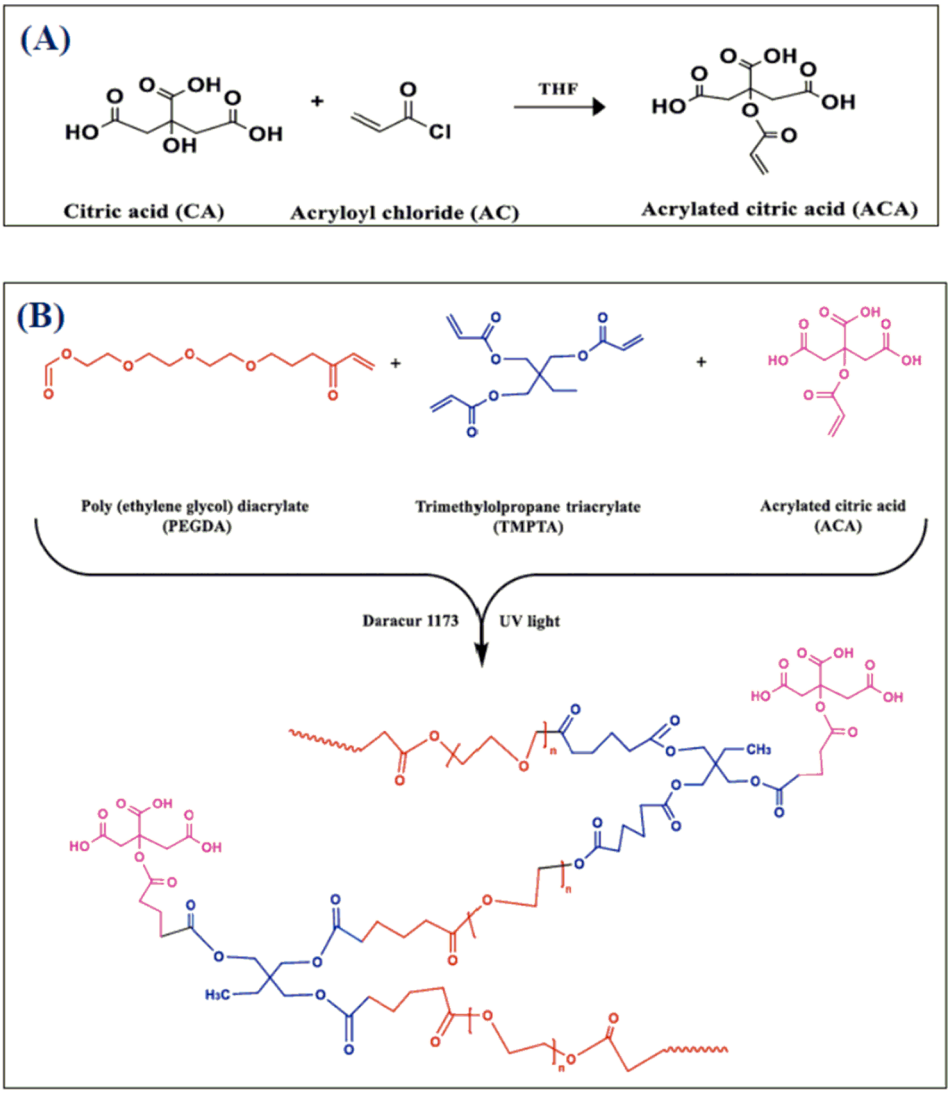

Fig. 1. (A) Synthesis of acrylated citric acid (ACA) monomer. (B) Chemical structure of the polymeric sensor.

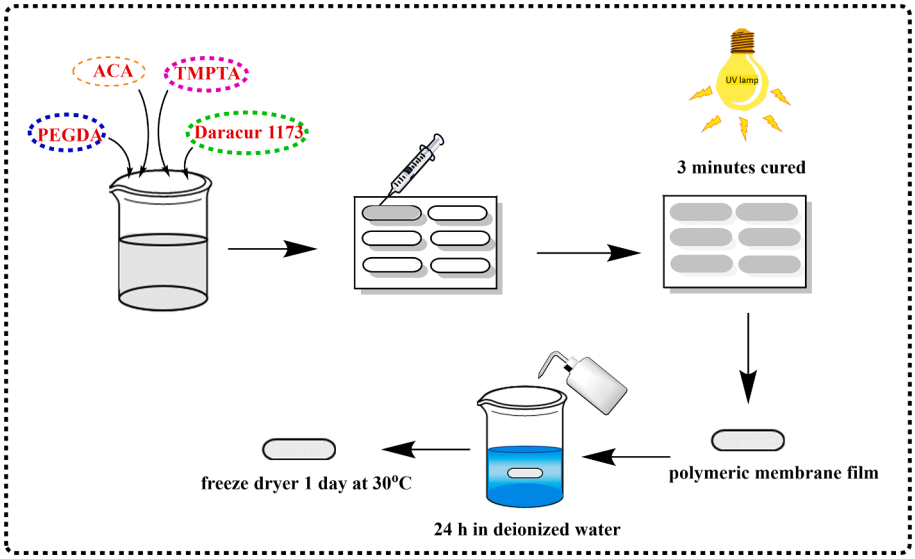

Fig. 2. Preparation procedure of the melamine sensor.

are given in Table S1. (Table S1 in the Supplementary Material).

2.5. Pretreatment of milk powder samples

Sample pretreatment for melamine is generally based on the principle of liquid extraction with a polar solvent. Organic solutions or acids (trichloroacetic acid, methanol, acetonitrile, hydrochloric acid, etc.) are used to extract the melamine in the samples and precipitate the protein to reduce the interaction caused by the sample matrix. The sensor developed in this study was used to determine the melamine in the milk powder. The milk powder samples were pretreated to eliminate protein and extract the analyte. 2 g milk powder sample was weighed and put into a 50 mL plastic centrifuge tube. 25 mL of 5% trichloroacetic acid (TCA) was added to the sample and it was extracted by shaking with vortex for 1 min. It was then kept in a sonic bath for 10 min. After mixing with vortex for 1 more minute, it was centrifuged at 4500 rpm for 15 min. The solution was filtered through a 0.45 μm filter for analysis and 5 mL of the filtrate was diluted with pH 6.00 buffer solution to a final volume of 10 mL for further analysis.

2.6. The proposed method for melamine determination

The solution obtained after the sample preparation step is placed in a 1.0 cm wide quartz fluorescent cell. Then, the melamine selective polymeric membrane sensor were placed in diagonal position in the quartz cell. Fluorescence measurements were taken at working conditions (λex/em = 388 nm/425 nm, pH:6.0) determined by the sensor developed to determine the amount of melamine in the solution obtained. The melamine concentration in milk powder samples was calculated using the calibration graph.

3. Results and discussion

3.1. Characterization of membrane sensor

3.1.1. FTIR spectrum of acrylated citric acid monomer (ACA)

The FT-IR spectrum of acylated citric acid (ACA) monomer obtained by acrylation of citric acid (CA) with acryloyl chloride (AC) was recorded (Fig. S1 in Supplementary Material). Weak peaks between 2981 and 2113 cm^{-1} in the spectrum were attributed to C—H symmetric and asymmetric stretching on CH_2 groups. The peaks at approximately 1720 cm^{-1} and 1630 cm^{-1} represent the C═O stretch band and the C═C– vibration band for acrylates, respectively (Gao et al., 2011). The peak of the –OH stretching vibration, seen at 3363 cm^{-1} in the citric acid (CA) spectrum, is not seen in the acrylated citric acid monomer (ACA) spectrum. This indicates that citric acid (CA) has been successfully acrylated.

3.1.2. ^1H NMR spectrum of acrylated citric acid monomer (ACA)

The ^1H NMR spectrum of the ACA monomer was recorded (Fig. S2 in Supplementary Material). Three centered quadruplets are seen in the spectrum, corresponding to cis, geminal, and trans protons, respectively. In the spectrum, peaks at 6.3–6.4 ppm correspond to cis proton, peaks at 6.02–6.12 ppm correspond to germinal proton and peaks at 5.77–5.85 ppm correspond to trans proton (Ramos-Lara et al., 2006). The peak seen around 4.1–4.2 ppm corresponds to $–CH_2$ protons.

3.1.3. FTIR spectrum of polymeric membrane sensor

The FTIR spectrum of the melamine selective polymeric membrane sensor was recorded. The FTIR spectrum of the F1 sensor, which was decided to be the optimum sensor formulation, is given in Fig. S3 in the Supplementary Material. In the spectrum, the peaks at 2967 cm^{-1} and 2877 cm^{-1} indicate the $–CH_2$ groups in ACA and TMPTA, and the peaks at 2159 cm^{-1} and 1250 cm^{-1} indicate the -C—O groups. The weak peak at 1976 cm^{-1} depicts the double bonds found in ACA, TPMTA and PEGDA. The peak at 1721 cm^{-1} shows the stretching of the C═O bond in the structure of ACA, TPMTA and PEGDA. C—H bond and C═O bond of methyl groups in TMPTA are seen at 1394 cm^{-1}. These results indicate that the polymeric membrane sensor has been prepared successfully.

3.2. Spectral properties of membrane sensor

Spectral characterization of the melamine selective polymeric membrane sensor was carried out using a spectrofluorometer. The change in fluorescence intensity was measured by wavelength scanning in the presence and absence of melamine. This step has been done for all sensor formulations. The results obtained by wavelength scanning with all prepared sensor formulations are shown in Fig. 3A.

Table S1 (Supplementary Material) contains the reactive monomer (ACA) amounts and their encodings. The amount of reactive monomer between F1-F4 is gradually increasing. As a result, more chemically and physically cross-links take place. It is thought that this causes interacts with melamine relatively difficult and does not cause an increase in fluorescence intensity. The optimum sensor formulation for melamine determination was found to be the F1 sensor (PEGDA 1.4 g, TMPTA 0.6 g, ACA 0.1 g and Darocur 0.06 g) from the fluorescence measurements taken with the sensors (Fig. 3A). The formulation (F1) with the maximum fluorescence intensity in Fig. 3A was chosen as the most suitable formulation and this formulation was used in the following stages of the study. In Fig. 3B, it is seen that the fluorescence intensity of the polymeric membrane increases in the presence of melamine and the excitation wavelength maxima 388 nm and emission wavelength maxima were determined as 425 nm. The optimum operating parameters for melamine determination have been determined (Table S2, supplementary material).

3.3. Optimization of the assay for melamine

3.3.1. Influence of the pH

To study the effect of the pH on the polymeric membrane sensor prepared for determination of melamine, the fluorescence intensity of the solutions containing 7.93×10^{-9} mol L^{-1} melamine prepared separately by using buffer solutions prepared according to the Britton Robinson (BR) method in the range of pH 1.0 to 8.0 were measured and plotted according to their pH values.

As seen in Fig. 4A, the fluorescence intensity increased from pH 1.0 to pH 6.0, reached the maximum intensity value at pH 6.0 and then started to decrease. According to these results, it was decided that the optimum pH value for the sensor should be pH 6.0. In the following parts of the study, pH 6.0 acetic acid / sodium acetate buffer system (AcB) was used.

It can be seen in Fig. 4A that the fluorescence intensity firstly increased from pH 1.0 to pH 6.0 and then began to decrease from pH 6.0.

3.3.2. Determining the response time

Sensor response time is affected by the pH of the environment and the concentration of melamine. To determine the response time, fluorescence intensity of 7.93×10^{-9} mol L^{-1} melamine prepared at pH 6.0 was measured every 10 s for 200 s. Fig. 4B shows the response time of the sensor with respect to time. While the fluorescence intensity is constant between 0 and 60 s, it is seen that it starts to decrease after 60 s. Therefore, it appears that the optimum response time for the sensor is in the range 0–60 s.

3.3.3. Calibration range, detection limit

The appropriate volumes were taken from the 7.93×10^{-7} mol L^{-1} stock melamine solution prepared in a buffer solution at the most appropriate pH value determined, and the final volume was 10 mL with this buffer solution and melamine solutions with different concentration values were prepared. Each measurement was made 3 times. The calibration graph is drawn by measuring the fluorescence intensity of these solutions separately and the related graphic is given in Fig. 5.

The most suitable working range was determined as range 3.96 ×

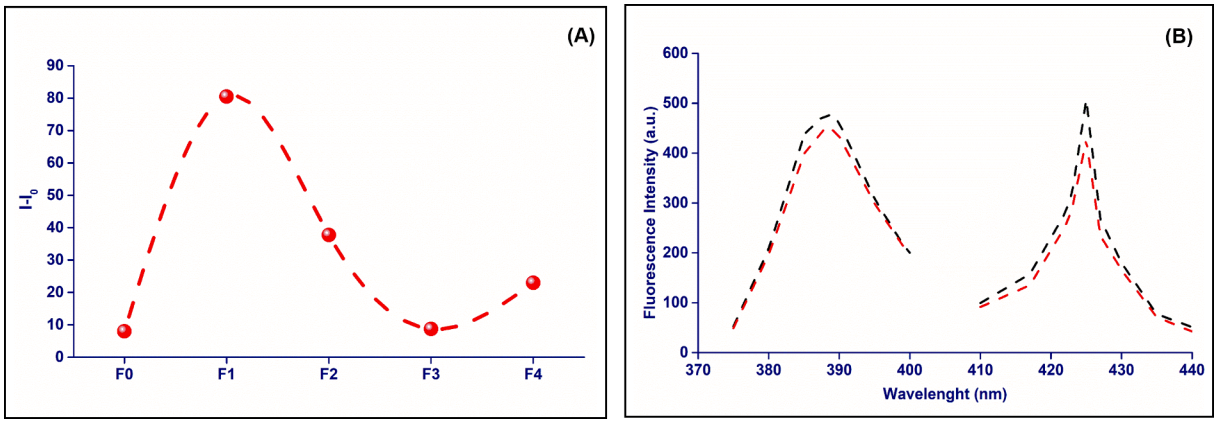

Fig. 3. A) The optimum sensor formulation. B) Excitation and fluorescence spectra of the polymeric membrane (F1) in the absence of melamine (black line) and in the presence of 7.93×10^{-9} mol L^{-1} melamine (red line); (λ_{ex} = 388 nm, λ_{em} = 425 nm; Slits, nm (ex/em): 5/5; pH: 6.0). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

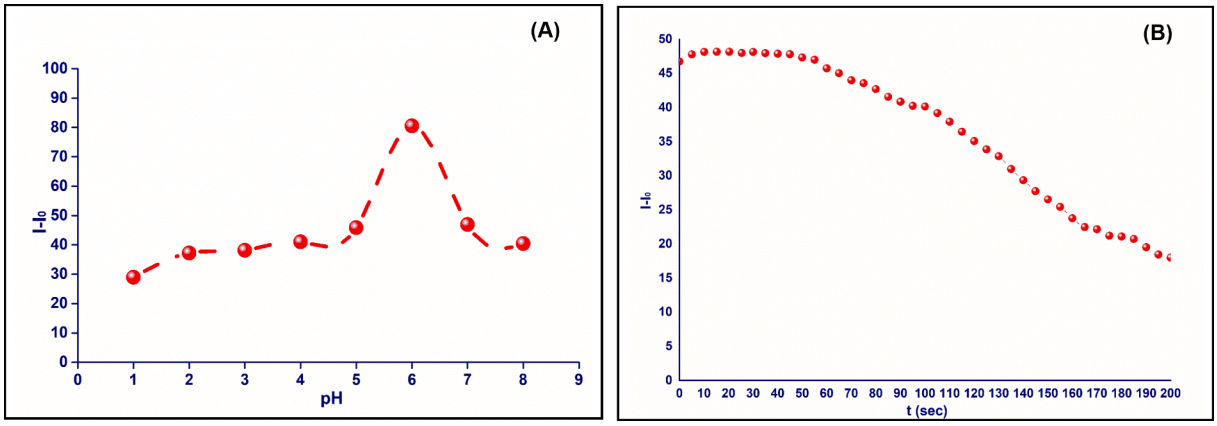

Fig. 4. A) Fluorescence intensities of the melamine sensor due to the pH effect. B) The change in fluorescence intensity of the melamine sensor with time for 200 s. (In the presence of 7.93×10^{-9} mol L^{-1} melamine; λ_{ex} = 388 nm, λ_{em} = 425 nm, Slits, nm (ex/em): 5/5; pH: 6.0).

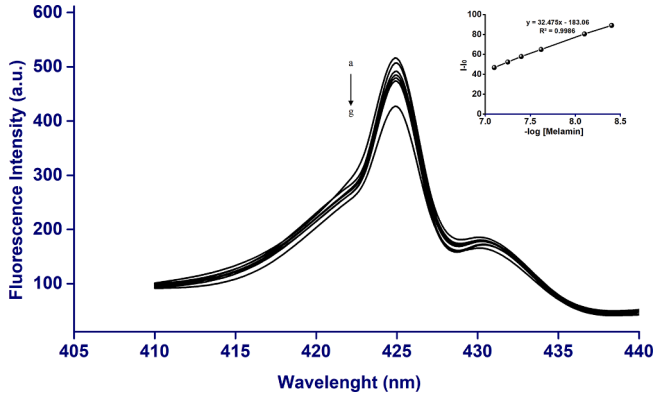

Fig. 5. Fluorescence spectra of the sensor in the different concentration of melamine. 0 (a), 3.96×10^{-9} mol L^{-1} (b), 7.93×10^{-9} mol L^{-1} (c), 2.38×10^{-8} mol L^{-1} (d), 3.96×10^{-8} mol L^{-1} (e), 5.55×10^{-8} mol L^{-1} (f), and 7.93×10^{-8} mol L^{-1} (g). The inset shows the calibration curve of the polymeric membrane ($\lambda_{ex/em}$ = 388/425 nm; Slits, nm (ex/em): 5/5; pH 6.0).

10^{-9}–7.93×10^{-8} mol L^{-1} by transferring the changing fluorescence intensities corresponding to the minus logarithm of the concentrations of melamine solutions prepared for the creation of the calibration graph. Since it was observed that the calibration graph deviates from linearity at concentrations higher than 7.93×10^{-8} mol L^{-1}, these values are not shown in the graph. To determine the limit of detection (LOD) and limit of quantification (LOQ) of the fluorescence sensor, the fluorescence measurements of 10 samples containing 7.93×10^{-9} mol L^{-1} melamine were taken under the determined optimum operating conditions. LOD and LOQ values were calculated using the standard deviation value (SD) and slope (m) of the calibration graph.

$$LOD = \frac{3SD}{m}$$

$$LOQ = \frac{10SD}{m}$$

Using the regression equation F = 32.475(-log(Melamine))-183.06 for the melamine sensor, the LOD value was found to be 2.32×10^{-10} mol L^{-1} and LOQ value was found to be 7.74×10^{-10} mol L^{-1} (n = 10, % RSD = 2.37).

3.3.4. *Selectivity of the polymeric membrane sensor*

One of the most important parameters for measuring a certain analyte in a given sample containing other interfering species is selectivity. A selective sensor only needs to detect the type to be detected, without being affected by the presence of other substances in the environment.

Therefore, a systematic study was conducted to investigate this aspect of the proposed sensor. Firstly, ions and molecules that can interfere with melamine were determined (foreign ions: SO_4^{2-}, Ca^{2+}, Fe^{2+}, Cl^-, Co^{2+}, Mg^{2+}, Ba^{2+}, NH_4^+, Zn^{2+}; molecules: glucose, lactose, starch, citric acid, tartrate, ascorbic acid, citrate, and Vitamin B2). Then,

solutions were prepared in the ratio of melamine: foreign ions from 1: 1 to 1: 5000. Similarly, solutions containing ion mixtures (Mix 1) and molecular mixtures (Mix 2) with melamine: foreign ion and melamine: foreign molecule ratios from 1: 1 to 1: 5000 were prepared.

The melamine concentration was adjusted at 7.93×10^{-9} mol L^{-1} and then the foreign ions and molecules were added to measure the difference between the fluorescence response properties. Considering that the acceptable upper limit value may be the concentration value which there is a maximum 5% change in fluorescence intensity, the acceptable upper limits are determined for ions and molecules (Table S3 in Supplementary Material).

3.4. Determination of reversibility, stability and reproducibility of the sensor

Reversibility, stability and reproducibility are extremely important parameters for a sensor developed for use in any analyte determination.

To determine the reversibility of the proposed sensor in melamine determination, the fluorescence intensity of the sample containing 7.93×10^{-9} mol L^{-1} melamine was investigated by repeatedly measuring it under optimum operating conditions ($\lambda_{ex} = 388$ nm, $\lambda_{em} = 425$ nm; pH 6.0 AcB). It has been observed that washing with distilled water for only 30 s is sufficient for the regeneration of the sensor. It has been determined that the same sensor can be reused after being treated with pH 6.0 buffer solution for 30 s, and the fluorescence intensity difference between the 250th use and the first use of the regenerated sensor is acceptable with a low standard deviation of 2.77.

The short-term stability study of the sensor was determined by measuring the fluorescence intensity of the sample containing 7.93×10^{-9} mol L^{-1} (pH 6.0) melamine at certain time intervals (every 30 min, n = 7) using the same sensor. The standard deviation (SD) of the results was found to be 2.77. The results obtained show that the same sensor can be used for approximately 250 repetitive cycles without any change in fluorescence intensity and structure. In addition, to determine the long-term stability of the sensor, the fluorescence intensity of the sample containing melamine at the same concentration every month for 12 months was examined using the same sensor and it was determined that it remained stable for more than 6 months.

To examine the reproducibility of the sensor, 7 different sensors with the same formulation and prepared under the same conditions were used. The reproducibility of the sensor was examined by making fluorescence measurements of the sample containing 7.93×10^{-9} mol L^{-1} (pH 6.0 AcB) melamine with these seven different sensors (SD: 2.04). This result indicates that the sensor is reproducible for use in melamine determination.

3.5. Applicability of the developed sensor for melamine determination in real sample

3.5.1. Determination of melamine in milk powder samples

With the developed sensor, the milk powder samples that were pre-prepared under the optimum analysis conditions determined for the sensor were analyzed. The result obtained was compared with the HPLC analysis results of the same sample to determine the accuracy of our method. While the melamine content of the analyzed sample was 1.47×10^{-8} mol L^{-1} according to the HPLC result, it was found to be 1.44×10^{-8} mol L^{-1} as a result of three repeated measurements with the developed method. Student *t*-test was applied to determine the accuracy of the developed method;

$$t = \frac{|\overline{X} - \mu|}{SD / \sqrt{N}}$$

where; SD: standard deviation; μ: HPLC analysis result; $\overline{x}$ refers to the average of the three-replicate analysis results obtained with the developed sensor and N: number of analysis repetitions).

The t value calculated from the equation was found to be 0.06. The reference t value was determined to be 4.30 (with n = 2 freedom at a 95% confidence level). When comparing t_{exp} and t_{ref} it is seen that $t_{exp} < t_{ref}$. This result shows that the developed sensor can be used safely with high accuracy in the determination of melamine (Table S4 in Supplementary Material).

3.6. Method performance comparison of the proposed melamine sensor with other fluorimetric methods

A wide variety of fluorimetric methods are available in the literature for the determination of melamine. The comparison of proposed method with other determination methods reported in the literature for the determination of melamine is summarized in Table 1. Most of the methods reported in Table 1 need long pretreatment time. Some of the proposed sensors require an additional chemical to obtain fluorescent emission (Nascimento et al., 2015; Zhang et al. 2018), some are irreversible, some are not environmentally friendly, solvents such as ethanol (Qian et al., 2014), methanol (Feng et al., 2012), chloroform (Tang et al., 2013; Zhang et al., 2012) are needed, and some require very long analysis times (Vasimalai & Abraham John, 2013; Zhu et al., 2015). Compared to these methods, the proposed fluorescent sensor method has a linear range and LOD comparable to other methods in the literature. The proposed fluorescence sensor method stands out with its short analysis time (0–60 *sec*.), repeatability (at least 250 times with the same sensor), simplicity, cheapness and high selectivity. In addition, the results obtained using the prepared polymeric membrane sensor are in compliance with the threshold limit determined by WHO and FDA for melamine.

4. Conclusions

With the proposed sensor, melamine in milk powder is analyzed in less than 1 min. In addition, the effect of various ions and molecules that can interfere and their mixtures on the determination of melamine were investigated and 3000 fold SO_4^{2-}; 5000 fold Co^{2+}, Cl^-, Mg^{2+}, NH_4^+, Zn^{2+}, glucose, starch, citric acid, citrate and lactose; 1000 fold Ca^{2+} and Ba^{2+}; 2000 fold Fe^{2+}, tartrate and ascorbic acid; 4000 fold Vitamin B2; It has been observed that melamine can be detected selectively even in environments where ion mixtures are 1000 fold higher and molecular mixtures 250 fold higher. For the regeneration of the prepared sensor, it is sufficient to wash it with pure water. Moreover, it can be used at least 250 more times, so it is cost-effective and easy to apply. It has a low detection limit compared to fluorescence sensors in the literature such as 1.60×10^{-11} mol L^{-1}. These analytical features of the polymeric membrane sensor developed for the determination of melamine make the proposed method for the detection of adulteration in milk powder attractive and it is thought that it will contribute to the prevention of bad results that this illegal situation may cause. In addition, the fact that acrylated citric acid, which is the main monomer in the structure of the developed sensor, was used for the first time in the determination of melamine makes the study unique and contributes to the literature in this respect.

CRediT authorship contribution statement

Neşe Taşci: Investigation, Methodology, Writing – original draft, Validation, Data curation. **Soner Çubuk:** Supervision, Investigation, Methodology, Writing – original draft, Validation. **Ece Kök Yetimoğlu:** Methodology, Validation, Project administration. **Memet Vezir Kahraman:** Investigation, Methodology, Writing – original draft, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial

Table 1
The comparison of fluorescence methods for the determination of melamine.

Methods	$\lambda_{ex/em}$ (nm)	pH	Concentration Range (mol L^{-1})	LOD (mol L^{-1})	Analysis time (min)	Reference
Fluorescence	350/438	8.0	4.99×10^{-8} – 4.99×10^{-7}	3.65×10^{-8}	5	Dai et al., 2014
Fluorescence	510/541	6.5	1.58×10^{-9} – 1.27×10^{-5}	4.76×10^{-9}	20	Feng et al., 2012
Fluorescence	380/ 561	6.5	7.5×10^{-9} – 3.5×10^{-7}	8.90×10^{-10}	30	Gao et al., 2012
Fluorescence nanosensor	320/520	6.0	5.00×10^{-9}–500.00×10^{-9}	4.20×10^{-10}	20	Li et al., 2020
Ratiometric fluorescence	350/ 445–595	7.0	0.27×10^{-6}–110.00×10^{-6}	90.00×10^{-9}	3	Lin et al., 2021
Fluorescence	400/550	7.0	1.00×10^{-8} – 4.00×10^{-6}	3.00×10^{-9}	30	Lu et al., 2015
Fluorescence quenching of Triton X-114	235/302	3.0	7.93×10^{-6} – 4.76×10^{-5}	6.34×10^{-6}	20	Nascimento et al., 2015
Fluorescence	365/ 525–625	7.4	2.00×10^{-6}–2.00×10^{-5}	6.80×10^{-7}	240	Niu et al., 2015
Fluorescence	355/382,	7.4	5.00×10^{-8} –5.00×10^{-7}	8.00×10^{-9}	5	Qian et al., 2014
Ratiometric fluorescence	980/ 542–654	6.8	1.58×10^{-7}–1.58×10^{-6}	8.80×10^{-9}	15	Shi et al., 2020
Fluorescent quanta- sensor	360/532	7.0	0.01×10^{-9}–60.00×10^{-6}	0.13×10^{-10}	25–30	Singh et al., 2018
Fluorescence	440/576	8.0	5×10^{-8} – 4×10^{-6}	1.00×10^{-8}	15	Tang et al., 2013
Fluorescence	520/759	7.4	1.00×10^{-10}–1.00×10^{-9}	1.00×10^{-14}	65	Vasimalai & Abraham John, 2013
Fluorescence	980/550	7.0	3.17×10^{-8}– 4.99×10^{-7}	1.82×10^{-9}	12	Wu et al., 2015
Fluorescence	252/370	3.0	8.00×10^{-10} – 8.00×10^{-8}	6.10×10^{-10}	40	Xiang et al., 2011
Fluorescence	290/545	7.2	7.9×10^{-6} –79.3×10^{-6}	1.00×10^{-10}	10	Yang et al., 2022
Fluorescence	450/553	8.0	7.93×10^{-8} – 7.93×10^{-7}	3.96×10^{-8}	40	Zhang et al., 2012
FRET between CPNs9 and Au NPs	365/522	7.0	9.99×10^{-8} – 1.49×10^{-6}	6.82×10^{-9}	10	Zhang et al., 2018
Fluorescence	350/670	8.0	1.00×10^{-7}– 6.0×10^{-6}	9.50×10^{-9}	5	Zhu et al., 2015
Fluorescence	**388/425**	**6.0**	**3.96×10^{-9} – 7.73×10^{-8}**	**2.32×10^{-10}**	**Less than 1**	**This work**

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133525.

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