



# Novel multifunctional molecular recognition elements based on molecularly imprinted poly (aniline-co-itaconic acid) composite thin film for melamine electrochemical detection

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## ABSTRACT

The combination of copolymerization and molecularly imprinting technology provides functional materials with improved properties and can enhance the number of binding sites than the individual monomer. In this work, molecularly imprinted poly (aniline-co-itaconic acid) (MI-PANI-PIA) composite thin film was synthesized on glassy carbon electrode (GCE) by using an in-situ electropolymerization method using melamine (MA) as a template. The formation of the thin film was monitored by electrochemical methods and characterized by Fourier transform infrared (FTIR) spectrophotometry and scanning electron microscopy (SEM). Solvent extraction of the template generated the binding cavities in the polymer matrix which fit the target in size, shape and functionality. After the rebinding of MA, the proposed sensor shows a linear range between  $0.25 \times 10^{-9}$  M to  $100 \times 10^{-9}$  M, limit of quantification (LOQ) of  $5.98 \times 10^{-11}$  M and limit of detection (LOD) of  $1.79 \times 10^{-11}$  M respectively. The composite film showed high affinity towards MA through multiple non-covalent interactions, good reproducibility and stability with RSD 4.02% and 4.67% respectively. The developed sensor has an interesting potential for real sample testing applications with good recovery ranging from 95.87% to 101.5%. The new sensing composite material has good sensitivity and selectivity due to the synergistic effects of multi-functionality from the two polymers, porous thin film and imprinting effect. To this end, the MI-PANI-PIA can be regarded as a potential functional material for the chemical sensor development in future.

## 1. Introduction

Molecular recognition based on the natural molecular receptors like proteins is one of the most significant phenomena of nature, and is a common event in living organisms [1–3]. These recognition elements have been used for sensor development for long time and their practical applications was challenged by factors such as instability in harsh environments, high cost and incompatibility with modern nanofabrication [4–6]. In the attempt to search for an alternative to these natural molecular recognition elements, molecularly imprinted polymers (MIPs) have been emerged and got higher in popularity. MIPs are artificial molecular recognition elements which can be synthesized from both organic and inorganic precursors. Each of the imprint's recognition cavities generated after extracting the template has specific interaction in shape, size, and functional groups [7].

MIP copolymers can be prepared by employing more than one

functional monomer to modify the capability and compatibility of the product in different media depending on the feed ratio of hydrophilic/hydrophobic monomers used. Each functional monomer is able to recognize a different functional group of the template molecule and the resulting product offers increased selectivity. However, thorough study is required for the selection of the appropriate monomers and their combining advantages in preparing MIPs with multiple functional monomers [8]. Thus imprinted copolymers or composites become the promising materials for molecular recognition elements preparation for various applications. MIP copolymers were reported for quercetin [9], uracile and thymine [10,11] and diosgenin [12]. Electrochemical sensor based on imprinted organic-organic composite or copolymers thin films were also constructed for the detection of glutamic acid [13], ciprofloxacin hydrochloride [14], ascorbic acid [15], 4-nonyl-phenol [16] and atropine sulfate [17]. This implies that the copolymers are highly sensitive and selective towards the target template which may

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attribute to the presence of different functional groups from the two or more monomers cooperated in the formation of binding sites.

Molecularly imprinted conducting polymers (MICPs) are interesting functional materials for the development of electrochemical sensors where the flow of electrons or diffusion of the analyte is very important [18]. These functional materials are promising because of their ease fabrication, low cost, stability and tailor made functionality [19,20]. MICPs combines the enhanced selectivity arising from highly specific binding sites of molecular imprinting together with the properties of conducting polymers, thus creating highly sensitive and selective platforms for analyte detection [21]. From organic conducting polymers, polyaniline (PANI) is unique in its wide range of electrical, electrochemical, electroluminescence, optical and anticorrosion applications, and good stability, chemical and electrochemical preparation [22–26]. Electropolymerization of PANI can be done either at constant current (galvanostatic), constant potential (potentiostatic) or potentiodynamic (potential scanning/cycling or sweeping) method. Standard electrochemical technique, which employs a cell containing three electrodes (a working electrode, a counter electrode and a reference electrode) commonly, generates the best films. This technique enables one to perform polymerization and doping simultaneously [27,28]. Furthermore, the properties of PANI can be modified through copolymer or composite forming by incorporating different functional materials. There is no literature report on the PANI based molecularly imprinted copolymer film on glassy carbon electrode (GCE) for melamine (MA) detection using basic monomers aniline and acid monomer itaconic acid respectively. MA is a common milk adulterant posing health threat on infants and adults and needs strict monitoring using suitable methods [29,30]. Herein, we report the fabrication of new molecularly imprinted poly (aniline-co-itaconic acid) copolymers films (MI-PANI-PIA/GCE) by electrochemical polymerization technique using MA as an imprint molecule. In the this work, particular emphases was given to thorough investigation of the following parameters that are affecting the rebinding of MA by the MI-PANI-PIA/GCE sensor: (i) the molar ratio of the two functional monomers (aniline and itaconic acid) and MA template molecule, (ii) scan rate and scan cycles, (iii) pH of acetate buffer solution (ABS), (iv) the washing and incubation time and (v) detection of different concentration of MA. For comparison, the non-imprinted sensor was developed following the same procedures but without the addition of MA molecule. This sensor was designated as NI-PANI-PIA/GCE. The selectivity of the fabricated sensor to MA was investigated using the interfering molecules such as acetoguanamine (AGA) and diaminomethylatrazine (DMT) in addition to casein and glycine. The modified electrodes or thin films were determined by cyclic voltammetry, Fourier transform infrared (FTIR) and scanning electron microscopy (SEM). Square wave voltammetry (SWV) was applied to determine the specificity, selectivity and sensitivity of the films towards melamine (MA).

## 2. Materials and methods

### 2.1. Chemicals and reagents

Aniline (ANI) (for synthesis ACS reagent, > 99.5%, Merck) was used as a functional monomer and distilled under reduced pressure before use and stored at 4 °C in deep freeze. Melamine (MA) (sym-triaminotriazine pure, AR) used as template or imprint molecule was obtained from Sigma-Aldrich. Itaconic acid (puriss AR, ≥99%, HPLC grade) was obtained from Sigma-Aldrich and used as functional monomer. Casein (AR) and glycine(AR) from Merck, HCl (AR, 38%), dimethyl sulfoxide (AR), acetonitrile (AR), methanol (99.8%), ethanol extra pure, acetone (AR), nitric acid (ACS reagent, 70%), hydrochloric acid (ACS reagent, 37%), sodium acetate (AR, ACS), acetic acid (AR-p-test), sulfuric acid (98%, AR), sodium hydroxide (AR), potassium ferrocyanide extra pure, potassium ferricyanide (AR), potassium chloride (AR), acetoguanamine (AR, 98%), diaminomethylatrazine (AR, 98%) and deionized water.

### 2.2. Apparatus and measurements

Electrochemical experiments were performed with a CHI660D electrochemical workstation (CHI Instruments, USA) in a conventional three-electrode cell system. The working electrode used was a glassy carbon electrode (GCE). A platinum electrode was applied as the auxiliary electrode, and an Ag/AgCl (in saturated KCl solution) electrode served as reference electrode. The sample solutions were purged with purified nitrogen for at least 15 min to remove oxygen prior to the beginning of a series of experiments.

### 2.3. Electrode surface preparation

Glassy carbon electrode (GCE) (model CHI104, 3-mm diameter) was mechanically polished before each experiment with 1, 0.3- and 0.05- $\mu\text{m}$  slurry of gamma alumina powder, respectively, rinsed thoroughly with doubly distilled water between each polishing step, then washed successively with 1:1 nitric acid, 1:1 ethanol, and doubly distilled water in an ultrasonic bath, and dried in air. The cleaned electrode was characterized in 5 mM of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  solution containing 0.1 M KCl by CV between the potentials of  $-0.6$  to  $+0.6$  V at the scan rate of 100 mV/s for several cycles and sharp peaks for oxidation and reduction were obtained. Then the electrode surface was activated in 0.5 M  $\text{H}_2\text{SO}_4$  by CV between the potential range of  $-0.5$  to  $+2.0$  V at the scan rate of 100 mV/s for several cycles until stable voltammogram was obtained after rinsed with deionized water. After effective cleaning and characterization, the electrode was rinsed with deionized water, blown with nitrogen and dried at room temperature (25 °C). After this, the electrode was ready for surface modification by MIPs via electropolymerization method.

### 2.4. Synthesis of melamine imprinted and non-imprinted thin films

Electropolymerization of molecularly imprinted composite thin film was carried out from the acetate buffer solution (50 mM + 0.1 M KCl) containing 0.1 M aniline, 0.1 M itaconic acid, and 0.01 M melamine by the application of the potentiodynamic CV technique through the sweeping potentials between  $-2.0$  V and  $+2.6$  V at the scan rate of 100 mV/s for 10 cycles on cleaned GCE. After rinsing with water to remove excess monomers and physically adsorbed molecules, the MA template was extracted by using dimethyl sulfoxide (DMSO) and acetic acid (4:1, v/v) in acetate buffer solution.

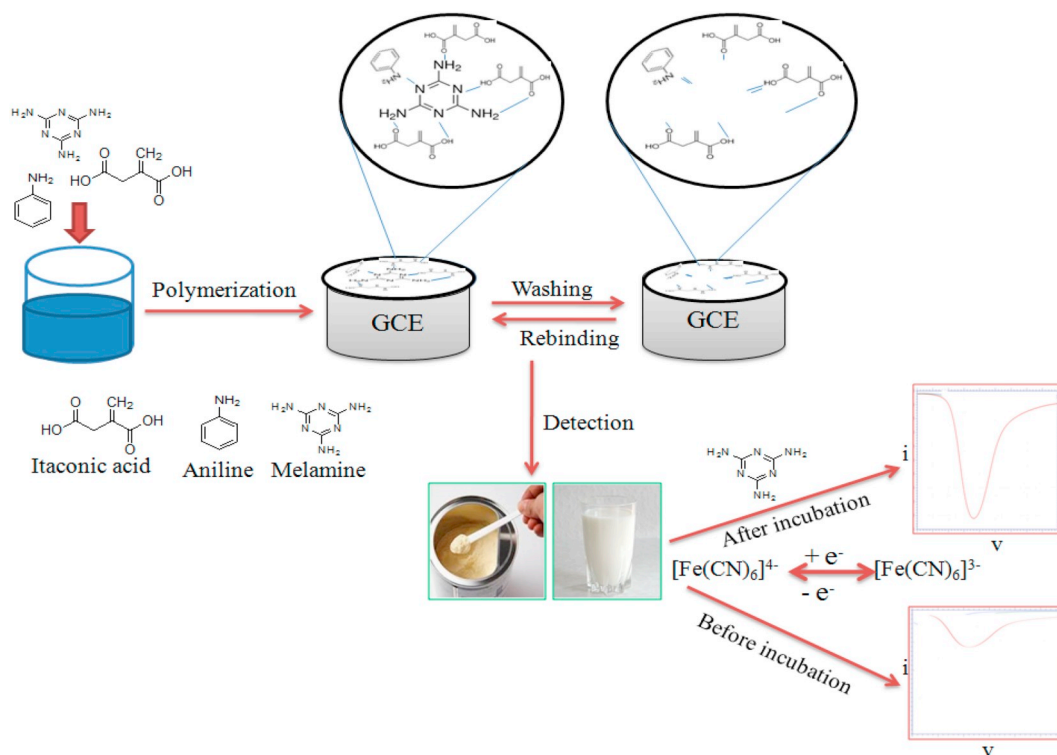
### 2.5. MI-PANI-PIA film characterization and analyte measurement

Both MI-PANI-PIA-GCE and NI-PANI-PIA-GCE were characterized by using electrochemical methods, FTIR and SEM to confirm the formation of the polymer film on the surface of the electrode. Removal of the template created cavities with specific binding sites for MA in shape, size and functional unit complementarities. After the template was removed, the resulting sensor was used for the detection of MA by using SWV technique. Finally, the proposed sensor was applied for real samples analysis. All the solutions used here were fully degassed with nitrogen gas prior to the electrochemical measurements.

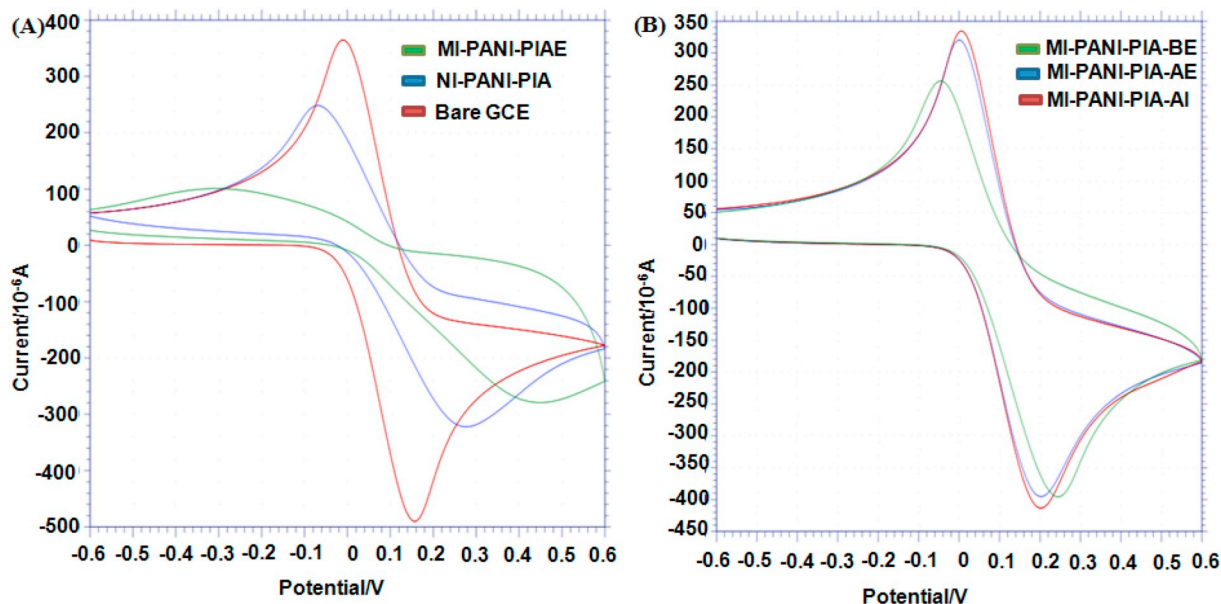
## 3. Results and discussion

### 3.1. Electrochemical polymerization and characterization of composite thin films

MIPs are highly cross-linked polymers with high binding capacity and selectivity to the target molecules and they are used in chemical sensor development, in drug delivery, purification and separation [8]. In the present work, MI-PANI-PIA composite thin film was developed (Scheme 1) on GCE to detect MA in artificial and complex milk samples. The intention of synthesizing the copolymers was to bring together



**Scheme 1.** General preparation of MA imprinted poly (aniline-co-itaconic acid) copolymer thin film modified GCE through electropolymerization, template removal and rebinding of MA by the sensor.



**Fig. 1.** CV curves recorded during polymerization (A) and after polymerization in 0.01 mM MA and then in 5 mM [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> solution containing 0.1 M KCl at 100 mV/s.

different functional units such as  $-N-$ ,  $-NH-$ ,  $-C=O$  and  $-OH$  to interact with MA in multiple ways (Scheme 1). The decrease in peak current after surface modification of the GCE indicates that the surface was changed from pure conductor to semiconductor. Compared to non-imprinted, the imprinted film shows high current response (Fig. 1A) attributing to the presence of the template induced electron flow and mass transport into the system. The as prepared composite film showed lower peak current in comparison to the washed and incubated film/electrode (Fig. 1B). This change in current response of the electrode shows the successful modification of the surface with film that can

recognizes the template.

Spectroscopic characterization of the composite film was performed using FTIR between the frequency ranges of 4000–500  $\text{cm}^{-1}$  under transmittance mode (Fig. 2). The broad peaks positioned at 3569 and 3021  $\text{cm}^{-1}$  are due to the stretching vibration of  $N-H$  and  $CH_2$  groups of MI-PANI and MI-PIA respectively. The peak at 2115  $\text{cm}^{-1}$  is assigned to  $C=N$  stretching vibration of secondary amine of MI-PANI backbone while that at 1640  $\text{cm}^{-1}$  attributes to the hydrogen bonded COOH group in MI-PIA. Skeletal vibration of quinone and benzene rings are responsible for the peaks at 1528 and 1392  $\text{cm}^{-1}$  respectively. The

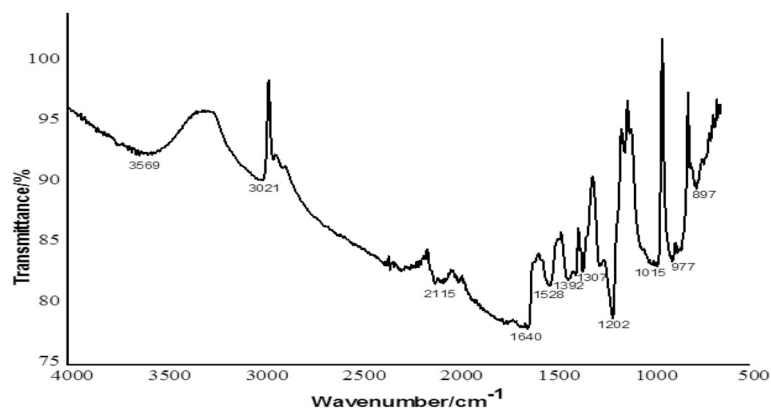


Fig. 2. FTIR spectrum of MI-PANI-PIA composite film.

formation of bipolarone is observed from the peak at  $1307\text{ cm}^{-1}$  but the one at  $1202\text{ cm}^{-1}$  is due to C–N stretching vibrations.

The peaks at  $1015\text{--}977\text{ cm}^{-1}$  and  $897\text{ cm}^{-1}$  belongs to C–H bending and C=C bending vibrations in aromatic rings. The PANI-PIA composite was formed in the form of amine salt by the electrostatic attraction between the amine and carboxylic groups. The various peaks observed from the FTIR result confirm the copolymerization of MI-PANI and MI-PIA in the presence of the template [31–33].

The morphology of MA imprinted composite films was evaluated by SEM. The morphology of MI-PANI and MI-PANI-PIA was characterized by SEM. The shape of the prepared films was seen in Fig. 3(A) and (B). Fig. 3(A) is a SEM of the MI-PANI. It shows that PANI was coated on the GCE by CV and has uniform appearance. Fig. 3(B) is a SEM image of the MI-PANI-PIA film. It can be seen the surface of GCE was fully covered by MI-PANI-PIA which seems highly porous uniformly distributed over the surface. It is clear that the morphology of MI-PANI-PIA is much more different from that of MI-PANI film indicating that the composite polymer was successfully formed.

### 3.2. Optimization of the parameters for MI-PANI-PIA sensor preparation

The functional monomer to template ratio affects the affinity and ability of the MIP films upon rebinding process [34]. The ratio of ANI and IA that form the imprinted copolymer to rebind melamine was optimized by taking different ratios of the functional monomers. At first, the amount of ANI functional monomer was varied while keeping that of IA constant. The current resulted from the rebinding experiment was recorded and revealed that the performance of the sensor greatly varies. The peak current decreases slowly while increasing the concentration of the monomer aniline. Next, the amount of IA acid was increased gradually

while keeping ANI and MA constant. The formulation that gave high peak current is 1:1:0.1 (ANI: IA: MA) and this ratio was selected as optimum feed ratio (Fig. 4A). MA is an electroactive species that can enhance the flow of electrons at the interface between the solution and electrode surface. The more MA rebind to the film, the higher the current signals confirming that there are specific binding sites formed on the polymer films. All these changes in the current densities are the confirmation of the formation of the imprinted composite thin film on the surface of the GCE which have the capability to recognize and quantify the template.

The time taken for electropolymerization is proportional to the number scan cycles. By controlling the number of scanning cycles during the electropolymerization process, the thickness of the polymer film can be easily adjusted [35]. Increasing the number of scan cycles increases the performance of the sensor (rebinding process) up to 10 cycles evidencing the deposition of polymer film with optimum binding sites (Fig. 4B). However, increasing the thickness does not guarantee the increased performance of the sensor. This can be seen from the curve that after scan cycle 10, the peak current from the rebinding experiment decreases. This might be due to the formation of non-uniform or non-accessible binding sites in the polymer matrices. Hence, the optimum scan cycle to fabricate the MI-PANI-PIA/GCE sensor was 10 and it was used during the fabrication of the sensor. The longer polymerization time may give more rebinding sites [36], however, in thicker films it is very difficult to extract the template and rebinding because of the deep interlock of many recognition sites [37].

To investigate the effect of scan rate on the rebinding potential of MI-PANI-PIA/GCE, different modified electrodes were fabricated employing different rates. Before and after template extraction as well as after rebinding in  $0.01\text{ mM}$  MA, the electrodes were characterized electrochemically by CV technique in  $5\text{ mM}$   $[\text{Fe}(\text{CN})_6]^{3-/4-}$  containing

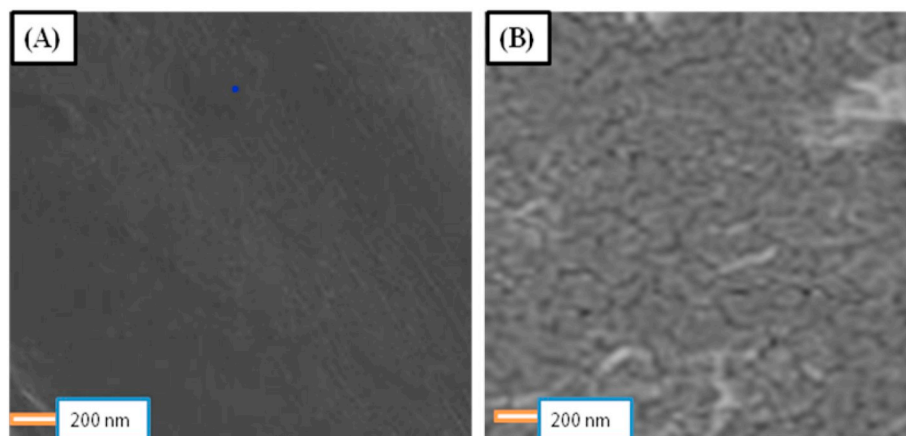


Fig. 3. SEM image of MI-PANI (A) and MI-PANI-PIA (B).

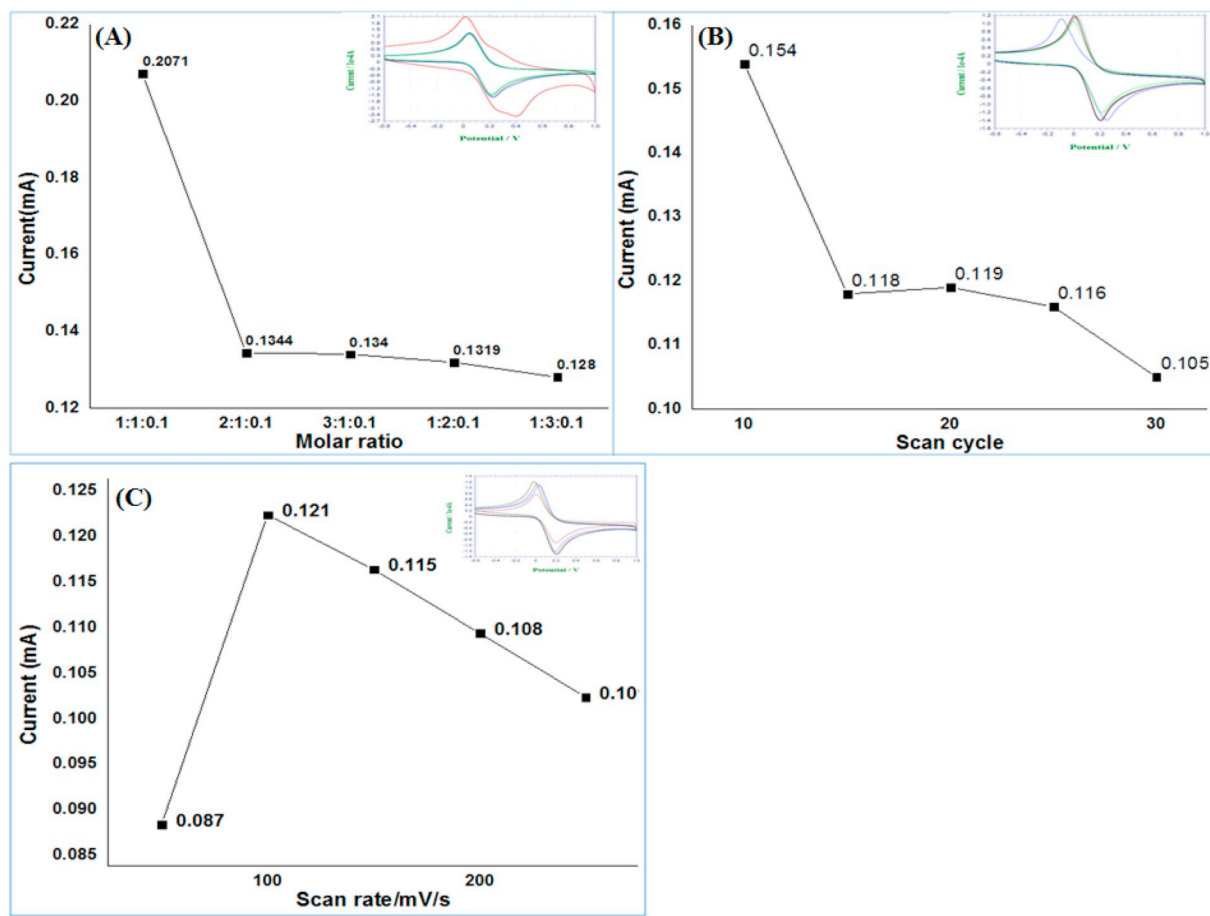


Fig. 4. Optimization of molar ratios of aniline: itaconic acid:melamine (A), scan cycles (B) and scan rates (C) and their CV curves after incubation in 0.01 mM MA and then in 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  solution containing 0.1 M KCl.

0.1 M KCl. The rebinding performance of the developed sensor increases until the scan rate of 100 mV/s but decreases after this value (Fig. 4C). The reason for this is due to the formation of non-uniform and less dense binding sites on the surface of the polymer with higher scan rates. This implies that increasing the scan rate does not necessarily increase the performance of the sensors since the primary focus here is the final performance. The scan rate of 100 mV/s was selected as an optimum rate to fabricate MIPANI-PIA/GCE.

### 3.3. Effect of pH, washing and incubation time

These parameters are very important determining factors to facilitate the interaction between the polymer film and the template, create accessible empty binding cavities and the uptake of the template during rebinding processes. SWV is the most advanced and highly sensitive electrochemical method suitable for the development of MIP based sensors. The effect of pH of the solution used for rebinding was evaluated by fabricating different electrodes using the same preparation procedures and methods. These electrodes were incubated in a solution containing MA (0.01 mM) but with different pH values and finally characterized by SWV between  $-0.3$  V to  $0.4$  V, at the amplitude of 25 mV and frequency of 20 Hz in  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  containing 0.1 M KCl. After evaluating the rebinding anodic peak current, the optimum pH for the MI-PANI-PIA/GCE was 7 (Fig. 5A). The pH = 7 was selected as the best for better performance of the sensor because it gave highest current that corresponds high amount of MA capturing by the polymer film through non-covalent interactions. This pH value favors the interaction between the polymer film and the template by creating favorable conditions implying that above or below this value the template or the

polymer undergoes change in structure or functionality. MIP modified electrode was prepared and optimized for the template extraction and rebinding process in 0.01 mM MA and characterized electrochemically in 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  containing 0.1 M KCl by CV technique between  $-0.6$  V -  $+0.6$  V at 100 mV/s for many cycles to obtain stable current-voltage curve. The imprinted polymer film was washed for 25 min with a solution of DMSO: acetic acid (4:1, v/v) in buffer in order to remove the template molecules and obtain selective recognition cavities.

The mixture of DMSO and acetic acid was used an extracting media. The acetic acid solution could weaken the binding of the template MA from the polymer matrices, and the DMSO solution could increase the solubility of MA and also maintain the swelling of the polymer matrix [38,39]. After template extraction, the current response decreases with increasing time indicating that the probe solution is not well permeating through the polymer or its flow is very slow [40]. The electrode was immersed in a new fresh solution after each step of the sensor polymerization, extraction, incubation and characterization procedures. The current response decreases until it reaches 25 min extraction then remain constant showing the complete removal of the template (Fig. 5B). The rebinding current intensity of MA by the modified electrode was high at 30 min because there is no significance change after this incubation time (Fig. 5C). This implies the saturation of the template uptake by the film. Therefore, 25 and 30 min were selected for the template extraction and incubation respectively.

### 3.4. Rebinding study, calibration curve and detection limit

The study of the effects of various concentration of MA on the rebinding ability of the MI-PANI-PIA/GCE sensor was performed by

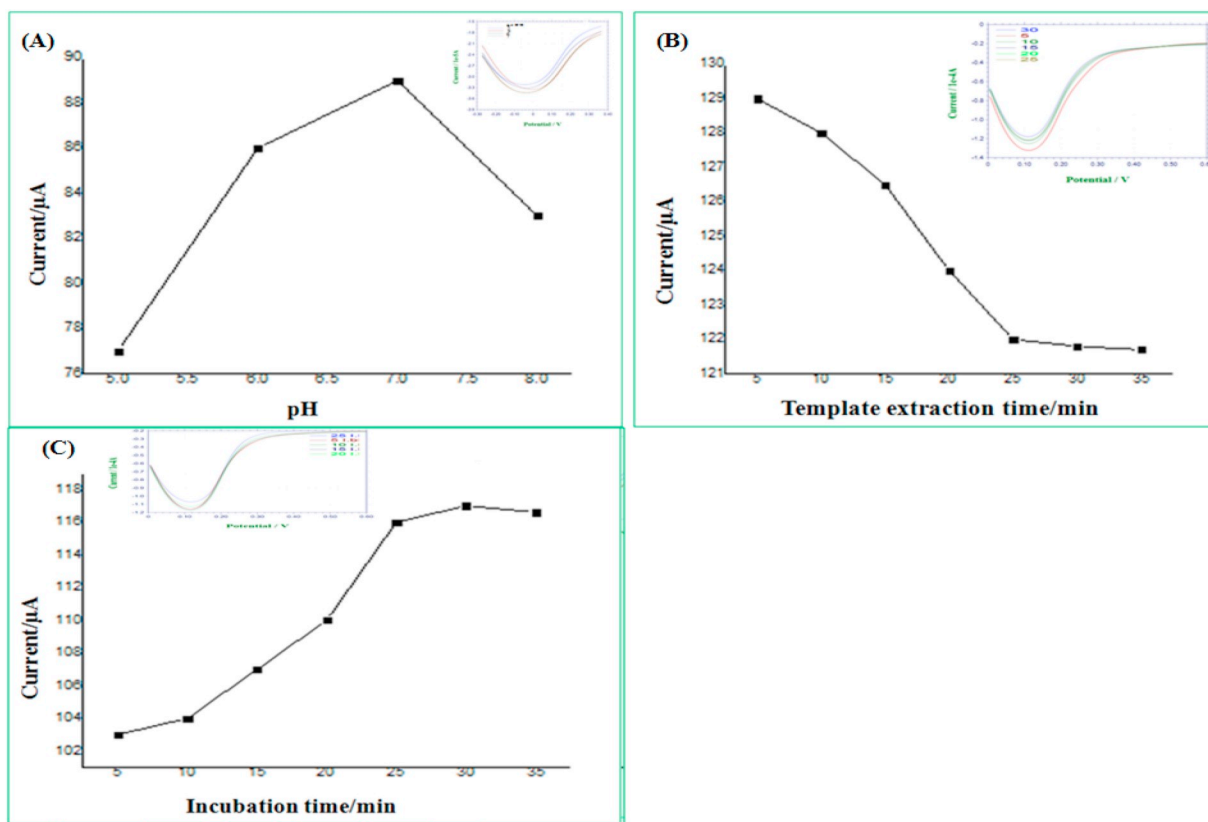


Fig. 5. Peak currents of optimized pH (A), template extraction time (B) and electrode incubation time (C) monitored by SWV between  $-0.3$  V to  $0.4$  V, at the amplitude of  $25$  mV and frequency of  $20$  Hz in  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  containing  $0.1$  M KCl.

measuring the blank (ABS) and various concentrations of the template. The linear range and calibration curves were drawn from the current response obtained by SWV for each concentration of MA. At the optimized conditions, the SWV peak currents were obtained after incubation of the electrode in various concentration of MA in  $5$  mM of ABS ( $\text{pH} = 7$ ). Calibration curve of MI-PANI-PIA/GCE incubated in different MA concentration ranges ( $0, 0.25, 0.5, 0.75, 1.0, 1.5, 20, 10, 20, 30, 50, 100$  nM) and evaluated by SWV in  $5$  mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  between the potential range of  $-0.3$  V to  $+0.4$  V, pulse amplitude  $25$  mV and frequency of  $20$  Hz (Fig. 6A). Fig. 6(B) shows the relationship between the peak currents measured by SWV and the negative logarithms of the corresponding MA concentrations. It can be noticed that the current response has a good linear relationship with logarithms of MA concentrations.

The linear regression equation was  $I(\mu\text{A}) = 71.397(-\log C) - 721.58$  in the concentration range of  $0.25$  to  $100$  nM and the correlation coefficient ( $R^2$ ) is  $0.99889$ . The limit of detection (LOD) of  $1.79 \times 10^{-11}$  M ( $S/N = 3$ ) and the limit of quantification (LOQ) of  $5.98 \times 10^{-11}$  M were achieved based on the  $\text{LOD} = 3S_b/m$  and  $\text{LOQ} = 10S_b/m$ , where  $S_b$  is the standard deviation of  $10$  blank measurements and  $m$  is the slope of the curve. To further verify the recognition selectivity, the efficiency of the imprinting effect was quantified by evaluating the imprint factor (IF) of the imprinted film. The average IF value for MA detected by the MI-PANI-PIA sensor was determined using the eq.  $\text{IF} = \Delta I_{\text{MIP}}/\Delta I_{\text{NIP}}$  [6]. Where,  $I_{\text{MIP}}$  represents the current of MI-PANI-PIA and  $I_{\text{NIP}}$  represents the current of NI-PANI-PIA which are directly proportional to the concentration of the bound template MA.

The average IF for the present sensor is  $2.98$  for the concentrations of  $0.5$  and  $0.25$  nM. A high increase of the current value with the increase in the concentration of MA is observed for due to the presence of selective binding cavities on the surface of MI-PANI-PIA/GCE for MA.

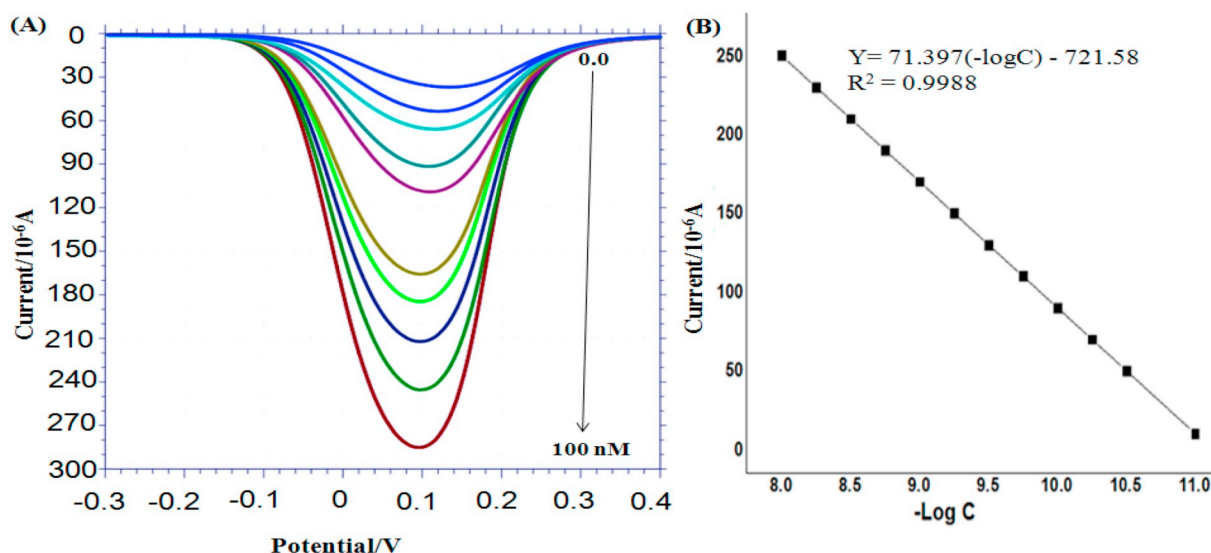
The higher concentration of electroactive MA molecules in MI-PANI-PIA film increases the electron transfer rate through doping and dedoping processes. For NI-PANI, due to the absence of the template during polymerization step, there are no binding sites and hence very low uptake of the template was observed. Thus the signal obtained for NI-PANI-PIA is significantly decreased and it may be attributed to the non-selective adsorption of the template on the surface.

### 3.5. Melamine detection mechanism by MI-PANI-PIA film

There are hydrogen bond donor/acceptor interaction between MI-PANI-PIA film and MA as well as  $\pi$ -donor/ $\pi$ -acceptor interaction between PANI moieties and MA molecules. The conductivity of the MI-PANI-PIA film is partly based on PANI, a conjugated polymer electron donor. This conductivity is increased when the concentration of MA molecules in the polymer increases which is reasoned out to be due to the electroactivity of the MA molecule. Electroactive dopants have a significant effect on the conductivity, morphology and stability of PANI based polymer films [41]. These properties depend on the molecular size and polar characteristics of the dopants. Furthermore, the delocalization of charges forms acceptor type sites and causes the events of electron transfer rates of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  to be increased when the amount of MA increases. This event has been supported by fluorescence quenching when MA is added [42] and through increase of electron transfer rate for MI-PANI-PIA film when concentration of nitrogen containing aromatic compounds increases [43].

### 3.6. Specificity and selectivity of the MI-PANI-PIA sensor

Specificity and selectivity are very important analytical criteria for the MIP sensors. To investigate the specificity of the fabricated sensor, bare GCE, NIPANI and MIPANI affinity towards  $0.01$  mM MA in terms



**Fig. 6.** Rebinding study of different concentrations of MA and calibration curve obtained for MI-PANI-PIA/GCE sensors by SWV for 0.0–100 nM MA under optimized parameters: extraction time 25 min, incubation time 30 min and pH 7.

of peak current intensity was performed (Fig. 7A). The detection ability of the sensor towards MA in the presence of compounds with similar in structure shows that the sensor is selective to the imprint molecule MA [44]. Briefly, the selectivity of the developed sensor was evaluated by the application of acetoguanamine (AGA) and diaminomethylatrazine (DMT) ( $3 \times 0.01$  mM each) and MA (0.01 mM).

After SWV measurements, no significant changes were observed when the interferents were applied to the sensing system developed (Fig. 7B). It is worthy to mention that the concentrations of AGA and DMT were three times greater than that of MA but their current responses are minimal. The high sensitivity of the sensor towards MA was achieved despite the high similarity between these three molecules. This proves the effect of imprinting and template extraction processes to obtain cavities with high affinity for the template. Similarly the casein protein and glycine amino acid existing in milk were also investigated for their effect on the sensor response to detect MA. Different amount (1.0 and 5.0  $\mu$ M) of each of them was used to add to the sample solution containing 0.01 mM MA and the current response of the sensor was evaluated. There was a decrease in current response of the sensor in the range of 0.98–1.21%. Therefore, addition of protein binding salts or removal of proteins with simple treatment is demanding. This indicates that there is weak interaction between the MA and the interferents but

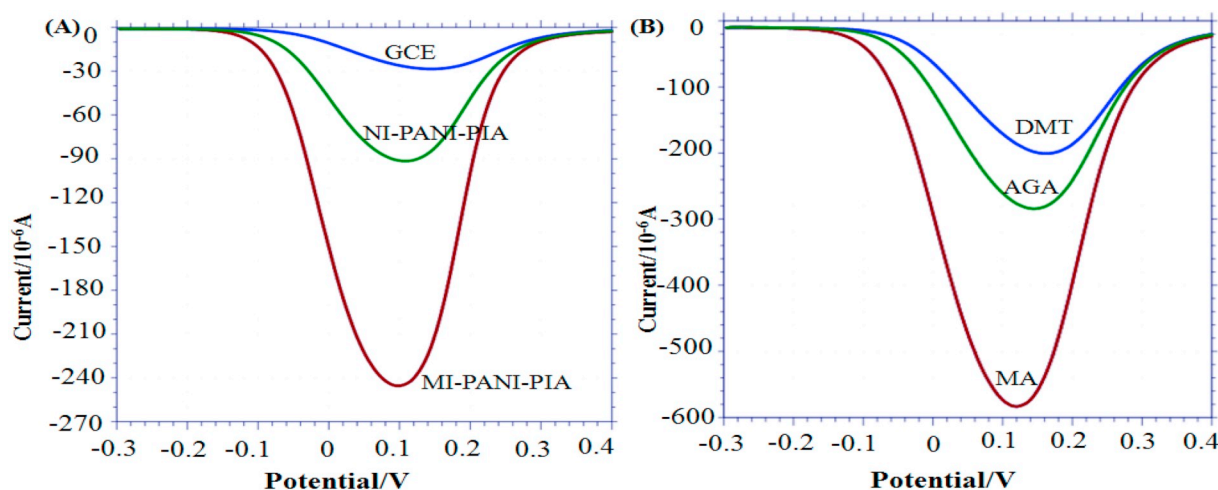
not significant. The current result confirms that the proposed sensor is selective to the target molecule.

### 3.7. Reproducibility, reusability and stability

The fabrication reproducibility of MI-PANI-PIA/GCE was evaluated by preparing three electrodes under the same conditions and used for the detection of 0.01 mM MA in ABS with pH 7.0 by SWV (Fig. 8A) and the relative standard deviation (RSD) of the current responses were found to be 4.3% for MA, confirming that the developed fabrication method was highly repeatable with good precision.

Measurement reproducibility was also tested for the proposed sensor by measuring the same quantity of MA five times using the same electrode. The RSD of the measurements was 3.2% which is  $< 5$  showing that there is good agreement among the individual measurements.

The reusability is the important parameter of the MIP sensors. The MI-PANI-PIA/GCE was subjected to several washing and incubation processes to test its possible number of reuses. After measuring the current responses, the possible number of uses and the percentage of responses maintained relative the first response were studied. It was observed that the electrodes retained about 89.6% of its initial current



**Fig. 7.** Specificity and selectivity tests of different sensors evaluated by SWV between  $-0.3$  V to  $+0.4$  V, pulse amplitude 25 mV and frequency of 20 Hz.

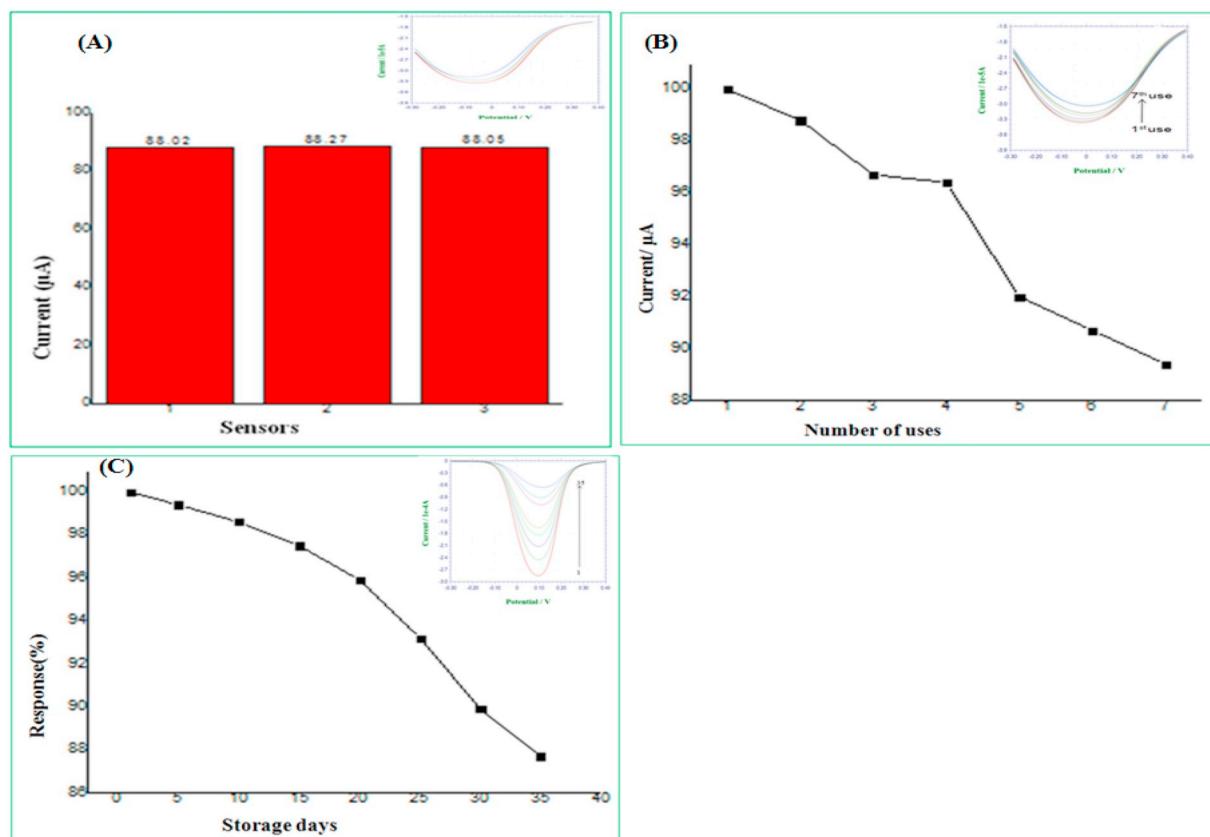


Fig. 8. Reproducibility(A), reusability(B) and stability(C) tests of the fabricated sensor evaluated by SWV between  $-0.3$  V to  $+0.4$  V, pulse amplitude 25 mV and frequency of 20 Hz after incubating in 0.01 mM MA and then transferred to 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  containing 0.1 M KCl.

response after seven cycles (Fig. 8B) with RSD of 4.02% which indicates the fabricated sensor has good measurement capabilities for repeatedly done measurements.

The stability of MIPs and the binding sites created in/on the polymer films are the most important factors that directly affect the selectivity and sensitivity of the sensor during operational storage [14,45]. The developed sensor retained its current response for a storage of 35 days in acetate buffer solution (5 mM, pH 7) when stored at  $-4$  °C. From the curve of storage stability (Fig. 8C), it is clear that the sensor can maintain its performance ( $> 90\%$  (RSD  $\leq 4.67\%$ ) for 7 cycles up to 30 days storage. However, over 30 days it started to decrease significantly which might be due to either the damage of the binding sites by repeated washing or the loose of the films from the electrode's surface.

### 3.8. Comparison with the literature reports

It is important to compare the developed method with the previously reported methods to get an insight about the developed method (Table 1).

It was noticed that MI-PANI-PIA/GCE sensor shows encouraging analytical performance in terms of wide linear range and low detection limit over other modified electrodes confirming that the proposed sensor is suitable for the detection of MA. The SWV measurements were done in triplicate. The results show good recovery with relative standard deviation (RSD  $\leq 4.56\%$ ) from 95.87–101.50% indicating that the sensor is suitable to be applied for real sample analysis (Table 2).

### 3.9. Application of the sensor to real samples analysis

The practical application of the MI-PANI-PIA/GCE was evaluated by the analysis of the milk real samples. 1.0 g powder infant formula or

Table 1  
Comparison of different MIP based sensors for the detection of MA.

Method	LR	LOD	Reference
MIP-Electrochemical	50 $\mu\text{M}$ -10 mM	6 $\mu\text{M}$	[46]
MIP-Optical sensor	0.625–0.8 $\mu\text{M}$	155 nM	[47]
MIP-Electrochemical sensor	100 $\mu\text{M}$ –50 $\mu\text{M}$	3 nM	[48]
MIP-Electrochemical sensor	1 $\mu\text{M}$ -10 mM	88.6 $\mu\text{M}$	[49]
MIP-Electrochemical sensor	100 nM-10 mM	1.75 pM	[50]
MIP Electrochemical sensor	0.4 to 9.2 $\mu\text{M}$	0.11 $\mu\text{M}$	[51]
MIP-Optical sensor	10 nM – 10 mM	5.1 pM	[52]
MIP-Optical sensor	5–50 $\mu\text{M}$	12 $\mu\text{M}$	[53]
MIP-Electrochemical sensor	0.25–100 nM	127 pM	Present work

Table 2  
Recovery studies infant formula milk samples spiked with different amounts of MA.

Sample	Added ( $\mu\text{g}$ )	Found ( $\mu\text{g}$ )	Recovery (%)	RSD (%) (n = 3)
Infant formula	100	97.6	97.60	4.56
	200	199.9	99.95	3.42
	300	304.5	101.50	3.74
Raw milk	100	95.87	95.87	5.10
	200	199.1	99.55	4.81
	300	303.27	101.09	4.32

1.0 mL of raw liquid milk were dissolved in acetonitrile, heated in ultrasonic bath for 10 min and centrifuged at 10,000 rpm for 10 min, then filtered through 0.45  $\mu\text{m}$  filter membrane to obtain the supernatant [34].



#### 4. Conclusions

This work describes the development of new sensitive MI-PANI-PIA copolymer film based electrochemical sensor for the detection of MA based on electropolymerization technique. The sensing layer was the first materials of its kind for MA sensing application and simple preparation procedure resulted in an MI-PANI-PIA film with a broad linear range, high sensitivity, selectivity, and reproducibility after careful optimization of important parameters. The sensitivity of the sensor may attribute to the presence of multiple binding sites from PANI and PIA respectively through synergistic effects. This indicates that polymer blends are the promising materials for sensor development. Moreover, the developed sensor was applied for the analysis of complex milk matrices evidencing the applicability of the sensor for the detection of MA in real samples. The fabricated MI-PANI-PIA based sensor is simple to fabricate, easy to operate, sensitive and selective towards the target analyte. Finally, the sensing layer is considered as the potential material for sensor fabrication for other analytes also.

#### Availability of data and materials

All data supporting our findings is contained within the manuscript.

#### Authors' contributions

SMR and SK designed and performed the study as well as prepared the draft manuscript. TRS and OEF are scientific advisors and edited the paper. SS analyzed the results, PCR provided all the facilities to perform the experimental work and edited the article.

#### Consent for publication

Not applicable.

#### Declaration of Competing Interest

The authors declare that they have no competing interests to publish the article.

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