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Development of Electrochemical Sensor Based on Magnetic Molecularly Imprinted Polymer Implanted onto a Graphite-Epoxy Composite Electrode for Sulfonamides Recognition

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Abstract

In this work we developed a sensitive electrochemical magneto sensor based on modified magnetic molecularly imprinted polymer (MMIP) and magneto graphite-epoxy composite electrode (m-GEC) for sulfanilamide (SN) recognition. The sulfanilamide based magnetic molecularly imprinted polymer (MMIP) was first synthesized and then the surface feature of the core-shell MMIPs modified graphite-epoxy composite (mag-MIP/GEC) was characterized by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The response behavior of the mag-MIP/GEC electrode, known to affect was studied and optimized by various factors. The developed mag-MIP/GEC sensor shows high recognition ability and affinity for the template molecule with good sensitivity and stability. Under optimum experimental conditions, satisfactory linearity was observed between the current response of the redox probe and SN concentrations which range from 1.0×10^{-8} to 1.0×10^{-7} M, shown the detection limit of 3.0×10^{-9} M. The developed method was effectively applied to analyze SN in milk sample with satisfactory recoveries of 93.5 to 102.2%, representing an excellent characteristic of the mag-MIP/GEC as a sensor towards the recognition of SN in real samples.

Keywords: Sulfonamides; Magnetic nanoparticles; Electro-chemical analysis; Molecularly imprinted polymer; Graphite epoxy electrode; Differential pulse voltammetry

Introduction

Since the mid-twentieth century, there has been widespread application of sulfonamides in both animals and humans medicines [1,2]. These substances have been used for their antimicrobial properties [3]. In the history of medicine, it has long been accepted that antibiotic use potentially contributes to resistance to substances with antimicrobial properties [4]. Sulfonamides have been found to cause an array of health effects such as skin conditions as well as being one of the substances that are cancer causing [2]. It interestingly appears that with the growing demand for good health in animals and humans alike, sulfonamides are gaining access to the environment through many ways. These include ways such as waste from drug manufacturing firms being disposed in the environment. As much as twenty thousand tons of such waste comprising of sulfonamides is estimated to be released to the environment globally [1]. It is therefore imperative that measures be put in place to regulate the amount of these substances being released into the environment as a way of promoting safety of food as well as of the environment. Recently, Dmitrienko et al. delved into a research that was aimed at looking into latest trends towards the detection of sulfonamides [1]. The process of screening to find residues of sulfonamides is a rather long one requiring expert workers [1] and complex equipment e.g., capillary electrophoresis [5], liquid chromatography [6,7] and mass spectrometry [8]. It is also important to note that other upcoming applications for the same results are being used [9,10]. These new technologies like bio-sensing are being explored because results prove they are more reliable due to their high nature of sensitivity [11]. It is worth considering that even the most preferred methods of analysis could sometimes fail or be surpassed by earlier methods thought to work less efficiently [1]. The voltammetry behavior and quantification of sulfa drugs have been carried out in bulk form, pharmaceutical formulation and serum onto the electrode of mercury [12]. It is however known that one disadvantage of using mercury as an electrode is that mercury is toxic [13]. In most experiments, carbon comes in handy as a substrate that is cheap used also as an electrode in electro-analysis. Carbon improves transfer of electron kinetics both within electrolyte and electrode uses [14]. A variety of agents used for adjustment were applied by either dispersion inside a matrix that conducts [15] or by coating it on the surface of a solid electrode. An interesting alternative tool for construction of electrochemical sensors include carbon-epoxy composite, which have strong conduction power. These composites has the capacities to integrate various materials which in turn improve the sensitivity and selectivity as compare to other conventional composites [16,17]. The electrochemical behavior of graphite-epoxy carbon composites has been found to represent highest sensitivity when compared to graphite epoxy-nylon and glassy carbon electrodes [18]. The high sensitivity of this composite as compare to a widely used glassy carbon electrochemical sensor is due to the microelectrode array an d porous nature of the carbon epoxy materials [19]. The sensitivity reduction of the graphite-epoxy nylon transducer mainly due to diffusion barrier of the nylon membrane towards the electro active species [18].

The sulfonamides that had the capability to reduce were quantified [20-22] by the use of composite material that was modified with Fe_3O_4 nanoparticles (MNPs) and Molecularly Imprinted Polymer (MIP). As a

result, MIP have the potential of individually identifying and binding molecules via non-covalent bonds between the host and guest molecules including hydrogen bonding, Van der Waals forces, electrostatic forces, metal ion coordination and hydrophobic interactions [23]. Molecularly Imprinted Polymers have successfully ben used in chromatography, sensors and solid phase extractions [24-34]. There are opportunities to try and use them in innovative techniques of sensing when combined both sensing and imprinting technologies [35]. These polymers have been likened to a certain degree, to what are known as biomimetic receptors. This is because of their power to the recognition mechanist and high selectivity to the target molecule [36]. It is also widely accepted that these polymers are way more stable than recognition elements that are biological so that a sensor that has been improved with a molecularly imprinted polymer becomes an attractive approach to chemical sensing. The use of MIP in sensors has advantages that sample preparation can be simplified and possibility of impurities from the matrix is eliminated [37-41]. Thus, the electrochemical sensors based on MIPs, combines the intrinsic properties of MIPs with specific electrochemical reactions to bring about a more elaborate feedback of the sensor and make them useful in determination of the target molecule in real samples [42]. On the other hand, bio-sensing technology brings in together elements of bio-recognition combined with a transducing structure that brings up a particular indicator when an analyte has been found. The indicator can be in form of a mechanical, optical or electrochemical signal. Elements of bio-recognition may vary in choice, with many applications being with oligonucleotides, antibodies, enzymes or other biomolecules. Molecularly Imprinted Polymers are also being considered for use as elements of bio-recognition [43,44] especially when the molecules in question are minute to the extent that they are not easy to get [11]. They are mainly artificial polymers obtained by copolymerization of functional monomer and cross-linker with the presence of molecule whose size and use is harmonized to the analyte being targeted [45]. It is worth noting that traditionally prepared molecularly imprinted polymers exhibit a number of drawbacks when being applied for instance, mixed dispersal of binding sites, low mass transfer and poor site approach to the target analyte [46]. The surface molecular imprinting method comes in handy when looking for a way to counter these drawbacks of which the most impressive is the surface imprinting method. When this method has been employed, the polymers get better performing sites for recognition and become quicker in the process of transfer of mass [47]. Surface imprinting is attained by imprinting molecules on the surface of a solid support [48]. One of the commonly used nanoparticles in imprinting methods to form core-shell structural MIP for solid support is $Fe_3O_4@SiO_2$ because it is chemically stable, compatible and reactive with other agents and the characteristics of the magnetic component [49,50]. So by keeping in mind the characteristics of $Fe_3O_4@SiO_2$ we used magnetic nanoparticles modified MIP fabricated graphite-epoxy composite mag-MIP/GEC electrode for determination of sulfanilamide (SN), which to the best of our knowledge has not been reported.

In this paper, we reported a novel selective and sensitive electrochemical sensor based on MNPs modified with MIP fabricated onto m-GEC electrode for recognition of sulfonamides. A sulfonamide based magnetic molecularly imprinted polymer (MMIP), that bears with it recognition sites for SN based on core-shell MNPs, was produced, characterized by VSM and SEM and then was immobilized onto the surface of m-GEC. The prepared electrochemical mag-MIP/GEC sensor was characterized using cyclic voltammetry and differential pulse voltammetry. The experimental standards affecting the performance of imprinted sensor were examined and optimized. Consequently, this developed sensor was effectively applied for the recognition of target molecule in milk sample.

Experimental

Materials and reagents

All the chemicals used in the experiments were of HPLC or otherwise analytical grade and were used without any other action. Chemicals for buffer preparation as well as supporting electrolytes, like HCl, H_2SO_4, H_3PO_4, NaH_2PO_4, Na_2HPO_4, H_3BO_3, CH_3COOH, and NaOH were graded reagent (Sigma-Aldrich). SN, ammonium hydroxide, iron (II) chloride tetrahydrate, iron (III) chloride hexahydrate, tetraethoxysilane (TEOS), EGDMA, APTES, sulfamerazine (SM) and norofloxacin (NF) were purchased from Sigma-Aldrich. Acetonitrile (ACN), ethanol, methanol (MeOH), acetic acid and toluene were purchased from Panreac-Quimica. Britton-Robinson buffer solution (CH_3COOH 0.04 mol L^{-1} and H_3PO_4 0.04 mol L^{-1}, H_3BO_3 0.04 mol L^{-1}) with pH 3 was used as the rebinding solution to SN. All the solutions were prepared with ultrapure water.

Apparatus

Magnetic properties of the prepared samples were carried out at a room tempreture of 333 K with the help of a vibrating sample magnetometer (VSM, Riken BHV-55 Japan). The electrochemical calibration by CV and DPV was done using a potentiostate Autolab PGSTAT30 held on to a micro-computer which does the functions of registering and storing the information got from using Nova 2.0 as a control software. The pH of the solutions was measured using a model 538, WTW pH-meter (Austria) with combination to working and reference electrodes. An orthodox electrochemical cell containing three electrodes, reference-electrode Ag/AgCl (KCl 3.0 mol L^{-1}) platinum wire being auxiliary electrode and magneto graphite-epoxy composite electrode as a working electrode were put into use. In the convective transport, a magnetic stirrer was applied. All the experiments were carried out at room temperature. In addition, solutions involved were de-aerated with nitrogen of great purity ten minutes prior to every electrochemical test.

Fe_3O_4 NPs synthesis

$FeCl_2.4H_2O$ weighing 8 mmol and $FeCl_3.6H_2O$ weighing 16 mmol were dissolved in 80 ml of deaerated highly purified H_2O that was in a three neck flask and vigorously stirred at 800 rounds per minute under nitrogen. As temperatures were raised to 80°C, 10 ml of ammonium hydroxide was introduced drop by drop and the reaction was refluxed for thirty minutes. Separation of the black end-product was achieved by placing the vessel on a permanent magnet and the supernatant was decanted. The unreacted chemicals were removed away with by washing the precipitate 3 times using highly purified H_2O. Fe_3O_4 as the black product was dried in a vacuum.

$Fe_3O_4@SiO_2$ NPs synthesis

300 mg of super-paramagnetic NPs were dissolved in 50 ml of 2-propanol and 4 ml of highly purified H_2O by sonication for fifteen minutes. This was then accompanied by adding 5 ml of ammonium hydroxide and 2 ml of TEOS in a sequence. A resulting mixture was then reacted for 12 h at room temperature while ensuring it was being stirred continuously. The product that resulted was then collected
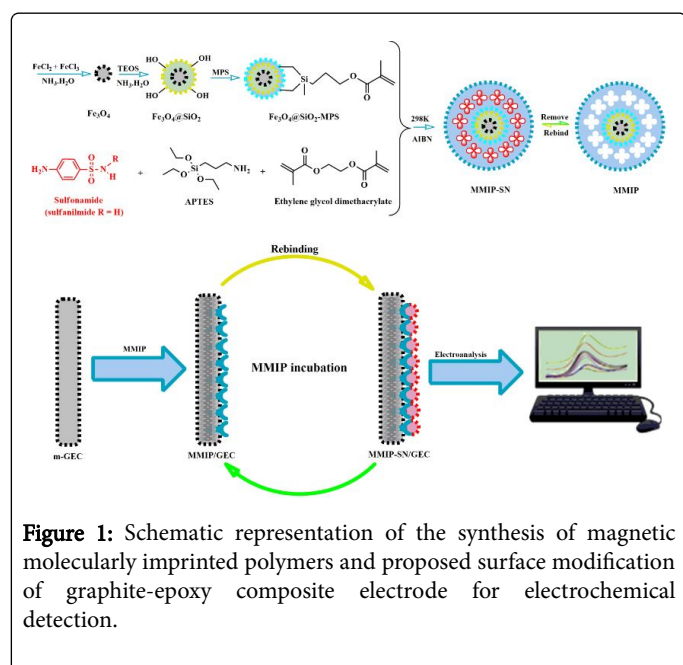
through an external magnetic field, rinsed 3 times with highly purified H_2O and finally dried in a vacuum.

Synthesis of $Fe_3O_4@SiO_2$-MPS NPs

$Fe_3O_4@SiO_2$, measured at 200 mg was dissolved in 50 ml of methanol by sonication in a total of 10 min. The resulting mixture was vigorously stirred at 500-550 rpm. 3 ml of MPS was also introduced drop by drop. A 24 hr reaction of the resulting mixture was performed in room temperature where stirring was done continuously. The product that ensued was then collected under an external magnetic field, thereafter rinsed in methanol for a total of 3 times and finally dried in a vacuum.

Synthesis of MMIP

Schematic diagram for methodology is shown in Figure 1. Non-covalent imprinting methodology was followed for the preparation of MMIP. In a 30 mL ethanol mixture containing the 0.6 mmol template molecule SN and 2 mL functional monomer, APTES was stirred for 30 min followed by the addition of 4 mL EGDMA (cross-liker) to the mixture (ultrasonic vibration for about 15 min) and stirred for 1 hr. 250 mg of $Fe_3O_4@SiO_2$ NPs and 1 mL of 0.01 M HCl solution were added to the mixture and stirred for 15 h at room temperature. Finally, the MMIP were collected by external magnet and dried for 15 h at 60C. The MMIPs obtained after polymerization were washed by soxhlet extraction with a 9:1 (v/v) MeOH and acetic acid solution until the template molecule was fully removed (observed by UV-Vis spectrophotometry at 258 nm after each 8 hr wash). The same procedure was followed for the synthesis of magnetic non-imprinted polymers (MNIPs) for comparison with the MMIPs but without the involvement of the template SN.

Figure 1: Schematic representation of the synthesis of magnetic molecularly imprinted polymers and proposed surface modification of graphite-epoxy composite electrode for electrochemical detection.

Construction and fabrication of mag-MIP/GEC sensor

The construction of m-GEC electrode was carried out by following the method used previously [51]. For this purpose in the weight ratio of 1:4 (w/w), graphite powder and epoxy resin were hand-mixed. The

resulting conductive paste was placed in a body of the PVC cylindrical sleeve (Id 6 mm) with an electrical contact at a depth of 3 mm. A small magnet (Id 3 mm) was placed in the center of this electrode after adding a thin layer of the GEC paste to avoid direct contact between the magnet and the electrical connector. After that, the electrode was completely filled with the soft paste body and packed well. Dispersion of the 3 mg mL^{-1} MMIP nanoparticles was performed in a solution of water:ethanol solution under sonication for 30 min. Preparation of the MMIP improved electrode was achieved by immersion of the m-GEC electrode in the above MMIP solution for 10 seconds and dried at room temperature for analysis. The developed electrode was washed after each analysis by immersion in methanol for 5 min to remove the analyte adsorbed.

Electrochemical measurements

Cyclic Voltammetry and Differential Pulse Voltammetry technique was employed to perform electrochemical measurements. Characterization of the MMIP modified graphite-epoxy composite electrode (mag-MIP/GEC) was ensured by CV (concentrations of SN: 50 μM of SN in PBS (pH 6.4) on the mag-MIP/GEC electrode at different scan rated 10-200 mV/s) and DPV (concentrations of SN: 0.01, 0.05, 0.1, 0.5, 1, 30, 50, 70 μM. Accumulation time=10 min; Measurement conditions: B-R buffer of pH 3.0, scan rate=80 mVs^{-1}). The cyclic voltammetry measurements were conducted by applying a potential from -0.2 V to +1.6 V for purposes of studying electrochemical performance of SN in a PBS. All electro-analytical studies were conducted in 0.1 M PBS (pH 6.4) and B-R buffer solution. Before analyzing each set of electrochemical, the electrode was activated during each potential cycle ranging from -1.0 V to 1.0 V at scan rates of 100 mV/s. Finally, the template molecule SN was removed by immersion of the prepared mag-MIP/GEC electrode in the methanol solution for 2 minutes.

Preparation of milk sample

Milk for the experiment was got from a local store and be treated in accordance to the following procedure. 5 g of milk, weighed accurately at a precision of 0.1 mg was put in a polypropylene centrifuge tube with a capacity of 50 ml. Extraction solvent measuring 40 ml was then added. The mixture was shaken for a total of 10 min after which it was centrifuged for ten minutes at a rate of 4000 rpm. A 0.22 μm filter membrane was used to filter out the supernatant. The resulting filtrate was then diluted by an extraction solvent to achieve 50 ml. An appropriate amount of SN solution was used to spike the milk.

Results and Discussion

Characterization of the magnetic nanoparticles

At this point, magnetic properties of Fe_3O_4, $Fe_3O_4@SiO_2$, $Fe_3O_4@SiO_2$-MPS and MMIP nanoparticles were examined by use of magnetic hysteresis loops. The magnetic saturation values for Fe_3O_4, $Fe_3O_4@SiO_2$ $Fe_3O_4@SiO_2$-MPS and MMIP are 62.4 emu g^{-1}, 28.36 emu g^{-1}, 17.85 emu g^{-1} and 5.32 emu g^{-1} respectively are shown in Figure 2. It ensued in the findings that stability of magnetic nano-composite on the surface of the electrode is dependent on 4 main factors; these include washing and magnetic force, that might may end up in the detachment of the magnetic nano-composite and also magnetic interaction and physical adsorption that works to stimulate its own adhesion. Since saturation magnetization of the MMIP (5.32 emu g^{-1})

is enough for the stability of magnetic interaction, so the magnetic interaction causes the MMIP nanoparticle to hold on relentlessly on the surface of the electrode [52].

It is also worthwhile noting that diameters of extra steady physical characteristics of Fe_3O_4, $Fe_3O_4@SiO_2$ as well as MMIP by SEM as shown in Figure 3, increased gradually which shows that surface nanoparticles were modified accordingly as the other groups were introduced. The increased surface of MMIP as compare to conventional MIP will provide a better surface for the target molecule to adsorb the analyte.
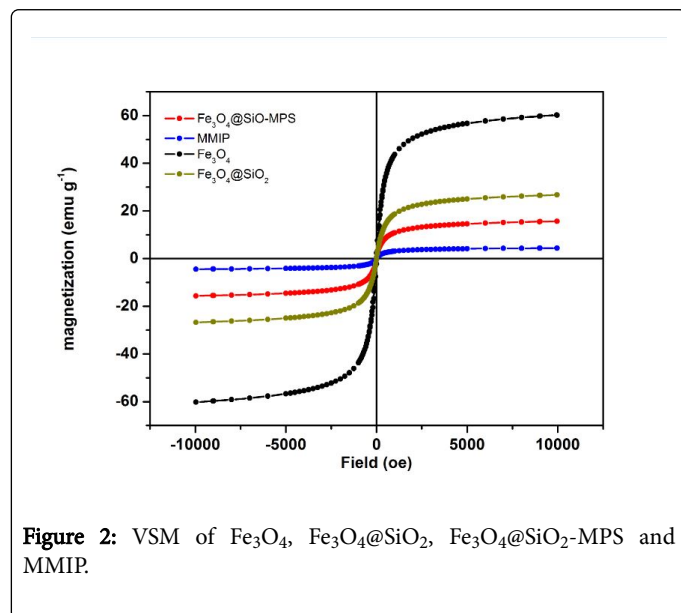

Figure 2: VSM of Fe_3O_4, $Fe_3O_4@SiO_2$, $Fe_3O_4@SiO_2$-MPS and MMIP.
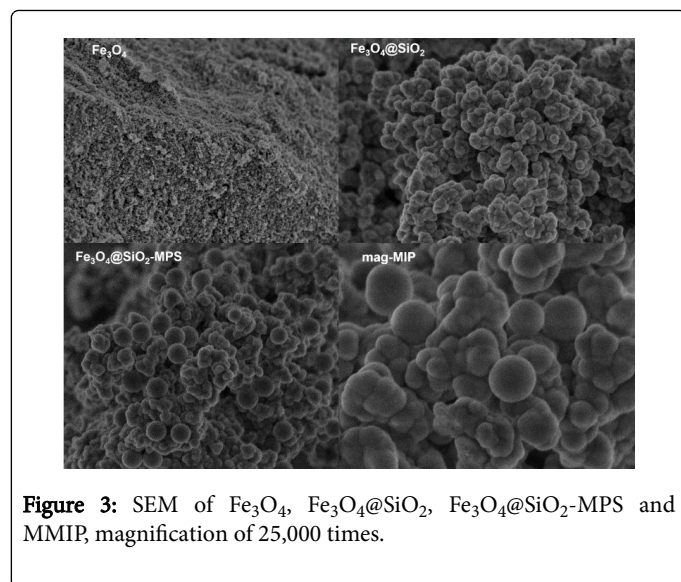
Figure 3: SEM of Fe_3O_4, $Fe_3O_4@SiO_2$, $Fe_3O_4@SiO_2$-MPS and MMIP, magnification of 25,000 times.

Electrochemical behavior and optimization of the experimental conditions for SN

Cyclic voltammetric behavior of sulfanilamide: Experiments of cyclic voltammetry of 100 μM SN were performed by the use of electrodes. Cathode waves with irreversible condition bearing potentials of reduction peak at -0.65 V might be seen in m-GEC and

mag-MIP/GEC surfaces. It emerges that reduction peak current (i_p) at m-GEC is much lower than at mag-MIP/GEC as shown in Figure 4. This signifies that the MMIP nanoparticles are enriched more efficiently onto the surfaces of the m-GEC. The rather weak peak current signal that is observed on the mag-NIP/GEC is because of the non-specific adsorption of the template molecule SN. The mag-MIP/GEC electrode however shows no current signal once the electrochemical measurements were done in solutions that are free of SN. It is known that sulfonamides are oxidized in electrochemical reactions mostly in NH_2^- group while being reduced in SO_2^{2-} group [53]. Reduction however has been observed to depend of R group (Figure 1) characteristics. R has however little known effects in the oxidation potential of sulfonamides [54]. Other previous investigations [7,55,56] have mentioned in the electrochemical behavior of sulfonamides. The same studies put forward the possibility of a permanent reaction that depends on a reaction of two-electron pH leading to oxidation in aqueous medium. This means that the reduction reaction depends on the chemical structure of the sulfonamide which is opposite in the oxidation reaction. This electrochemical phenomenon has been instrumental in distinguishing between distinct sulfonamides mixed together [57]. These compounds are electrochemically active and have been studied by voltammetry using various electrodes [22,55,58].

After the influence of the possible scan rate (v) on SN (50 μM of SN on the mag-MIP/GEC electrode at different scan rate (mV/s)) oxidation peak current at the mag-MIP/GEC was also examined and it was observed that peak current i_p gradually increased as the scan rate increased as can be seen in Figure 5. After logarithmic calculation between peak current i_p (μA) and scan rates v in rang 10-200 mV s^{-1}, a linear regression equation (R^2=0.985) was obtained which depicts that the SN oxidation on mag-MIP/GEC electrode is diffusion controlled process and the slop value (0.47) is near to the reported theoretical values for this process [59,60].
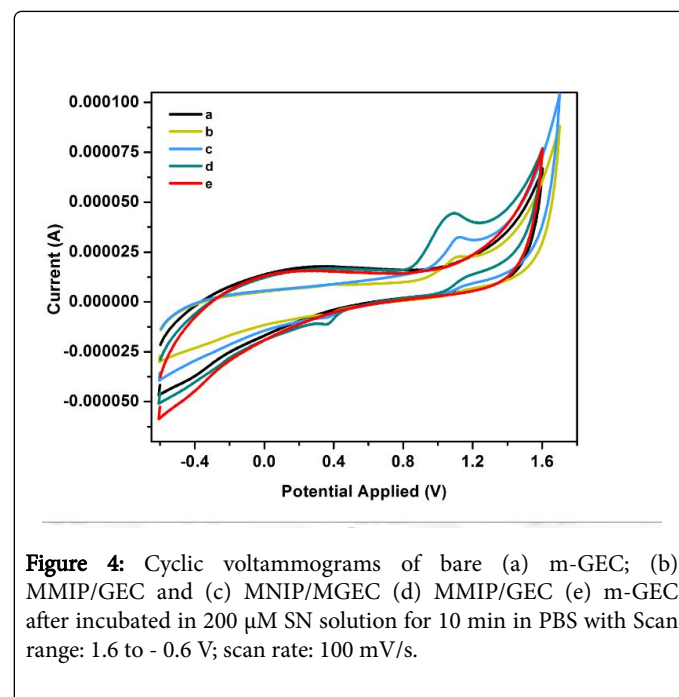

Figure 4: Cyclic voltammograms of bare (a) m-GEC; (b) MMIP/GEC and (c) MNIP/MGEC (d) MMIP/GEC (e) m-GEC after incubated in 200 μM SN solution for 10 min in PBS with Scan range: 1.6 to - 0.6 V; scan rate: 100 mV/s.
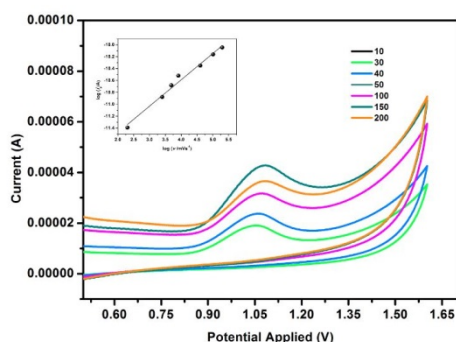
Figure 5: Cyclic voltamograms data of 50 μM of SN in PBS (6.4) on the mag-MIP/GEC electrode at different scan rated: from inner to outer are: (a) 10, (b) 30, (c) 40, (d) 50, (e) 100, (f) 150 and (g) 200 mV/s. Insert shows the logarithmic relationship between scan rate and the reduction peak current.

Influence of MMIP amount: The MMIP amount applied in order to modify the m-GEC is a very important factor while considering extracting SN on the sensor's surface. The interrelationship existing between the amount of MMIP on the m-GEC and the peak current was investigated by CV. This was done with varying the amount of MIP and the volume of the MMIP suspension remained at a constant (1 mL). It was noted that peak current increased at a significant level along with concentration of MMIP amount from a low of 2 to 3 mg mL^{-1} as shown in Figure 6. This indicated that MMIP has the effect of increasing the active area as well as the accumulation efficiency of m-GEC. While an ongoing increase in the concentration of MMIP amount to a high of 4 mg mL^{-1}, the peak current was noted to decline gradually. The reason behind this could be that conductivity of the surface of the electrode was decreasing due to large amount of MIP used to modify the surface of m-GEC.
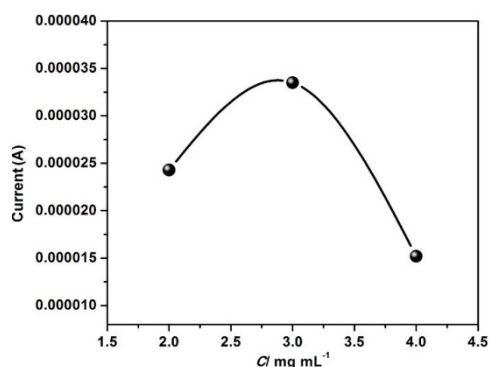

Figure 6: Effects of the amounts of MMIP suspension on the reduction peak current of 150 μM SN. Accumulation time=10 min; scan rate=100 mV/s.

The effect of pH: An study of how pH value affects the peak current i_p, a concentration of 100 μM SN at the mag-MIP/GEC was done by CV at 0.04 M B-R with the scan rate of 100 mV s^{-1} and ranging in pH

from 2.0 to 10.0. It was observed that i_p increases as pH value increases from 2.0 to 3.0, and then decreases as the pH value increases from 3.0 to 10.0 as shown in Figure 7. The pH 3.0 has been chosen for further SN study as it shows the smaller peak width and higher current peak potential. Protons being directly involved in SN oxidation as the increase of pH from 2.0 to 10 proven from oxidation peak potential shifted negatively with linear regression equation (R=0.996) as the pH values raise. According to Nernst equation which is mostly applicable for reverse reactions, the SN irreversible redox process can be employed to predict the transfer of protons and electron involved. The slope value (0.049) obtained in SN oxidation is near to the slope value of Nernst equation (0.0592), which shows the equal number of protons and electron are involved in electrochemical reaction. The formula E_{pa}-$E_{pa/2}$=47.7 mV/α n [59], has been used to calculate the numbers of protons and electrons involved in the reaction of SN (E_{Pa} is peak potential, $E_{Pa/2}$ is peak potential at half height, α is electronic constant coefficient and n is the number of electrons). After applying the formula given, it was observed that one electron was involved in the SN oxidation reaction. The structure of SN as shown in Figure 1 is similar to p-aminobenzensulfonic acid [61] and p-aminobenzoic acid [62], which predicts that combination of free radicals forming hydrabenzene sulfonamides, may cause the possible oxidation of SN in amino group. Effect of accumulation potential and incubation time: The stage of buildup is often a reliable while still maintaining not to be a difficult way of improving the MIP sensor's sensitivity. Peak current at 100 μM SN for mag-MIP/GEC investigated by CV was seen to increase as shown in Figure 8 rapidly with up to 10 min incubation time and thereafter remaining at an unchanged state. This may be due to saturation of binding sites at the surface of the constructed MMIP electrode. So keeping in mind the effect of incubation time, a 10 min incubation time was set for extraction of SN under open circuit conditions.
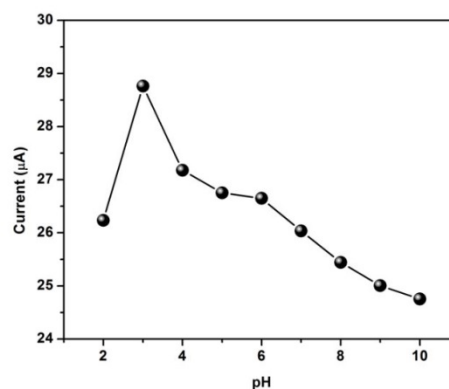

Figure 7: Influence of pH on the reduction peak current and potential of SN at the modified electrode.
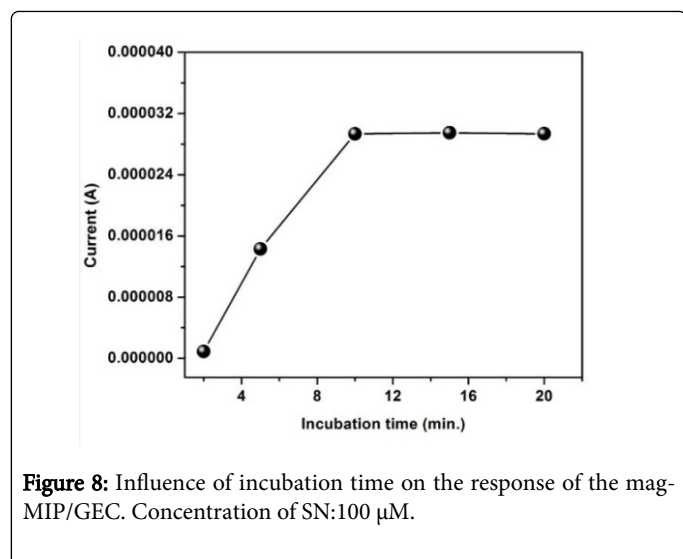
Figure 8: Influence of incubation time on the response of the mag-MIP/GEC. Concentration of SN:100 µM.

Performance of the imprinted sensor

Calibration curve and detection limit: N determination that was done in different concentrations was achieved through the application of DPV under the ideal analytical circumstances. Procedures of determination were found to be the sharp as well as the well-illustrated reduction peak current increased linearly as the concentration of SN increased as shown in Figure 9. A linear relationship as shown in inset graph of Figure 9, after the logarithmic calculation was achieved from the calibration graph as the SN concentration ranges 0.01-70 µM with the linear regression equation as i_p (µA)=-0.6846+0.212c (µM) (R=0.9772). The estimated limit of detection (LOD) and limit of quantification (LOQ) were to be 0.003 and 0.01 µM respectively. This electrochemical sensor showed lower LOD and linear range of the developed electrochemical mag-MIP/GEC sensors were better or similar to the sensors when compared with the reported ones as shown in Table 1, which demonstrate the sensitivity of the prepared sensor for SN recognition in real sample [63-66].

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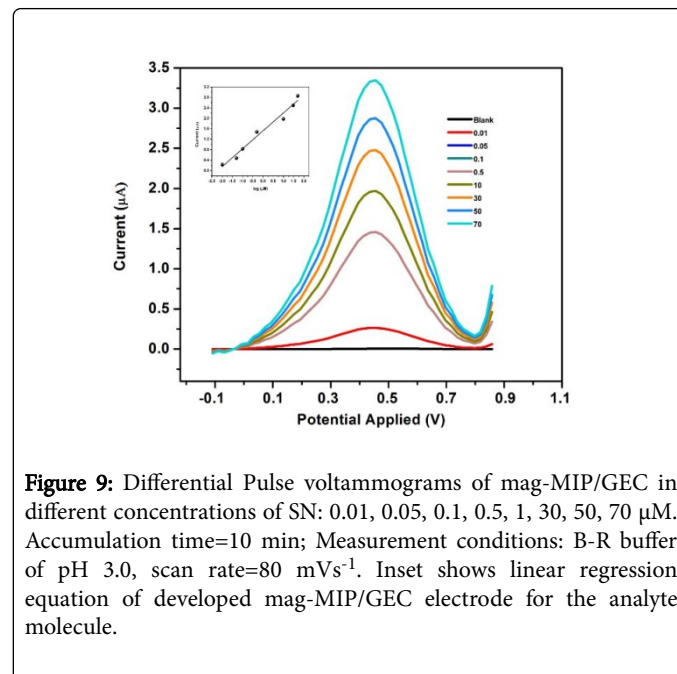

Figure 9: Differential Pulse voltammograms of mag-MIP/GEC in different concentrations of SN: 0.01, 0.05, 0.1, 0.5, 1, 30, 50, 70 µM. Accumulation time=10 min; Measurement conditions: B-R buffer of pH 3.0, scan rate=80 mVs^{-1}. Inset shows linear regression equation of developed mag-MIP/GEC electrode for the analyte molecule.

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Electrode	Method	Detection range (M)	LOD (M)	Ref.
Ppy-MIP/PGE	DPV	5.8×10^{-6} to 4.8×10^{-5}	2.0×10^{-8}	[52]
Overoxidized Polypyrrole Electrodes	DPV	2.5×10^{-5} to 1.0×10^{-3}	1.53×10^{-6}	[53]
MIP/GO/GCE	SWV	5.8×10^{-8} to 5.8×10^{-6}	-	[54]
GCE	SWV	3×10^{-6} to 2.5×10^{-4}	6.4×10^{-7}	[55]
mag-MIP/GEC electrode	DPV	1.0×10^{-8} to 7.0×10^{-7}	3.0×10^{-9}	This work

Table 1: Comparison of the pretreated mag-MIP/GEC electrode with other reported electrodes for SN detection.

Selectivity of the imprinted sensor: Analogs of the template SN, sulfamerazine (SM) and norofloxacin (NF) and that were structurally similar were employed in order to assess the imprinted sensor's selectivity. They were applied to study the interfering on determination of SN with an equimolar concentration (100 µM) with the comparison of the selectivity co-efficient K, where K is define as $K=i_{int}/i_{SN}$ (i_{int} and i_{SN} are the corresponding response of the sensor for interferent and SN respectively) and the K values obtained are 0.053 for SM and 0.042 for NF. The results demonstrate that the cavities in the imprinted polymer for determination of the target molecules had been created and the imprinted sensor showed excellent selectivity for the template SN recognition.

Reproducibility and stability of mag-MIP/GEC: For the purposes of studying the prepared mag-MIP/GEC sensor's reproducibility, a 100 µM SN solution had to be measured in applying 4 mag-MIP modified electrodes that had been independently prepared under similar conditions. As such, an ensuing relative standard deviation (RSD) value was 4.3%. The same electrode was also studied for the repeatability purposes with the SN concentration of 100 µM. Template extraction were applied to regenerate the used mag-MIP/GEC. With the experiments of determination, pre-concentration, rinsing and regeneration the RSD value for the 4 replicates was 4.1%. The change in peak current of 100 µM SN was measured every day for the purpose of evaluating the stability of the electrode. The prepared mag-MIP/GEC sensor retains its 93.3% initial response when stored at room temperature for 30 days, which demonstrate the reproducibility and stability of the sensor for SN recognition.

Application in real sample analysis: SN concentration in milk was determined in order to investigate the viability in the current electrochemical mag-MIP/GEC sensor that can be applied in real sample situations. The standard addition method was used to test the sensor's recovery performance and the results are shown in Table 2. It was found that the recoveries ranged from 93.5-102.2% with good RSD (%) values. These results demonstrate that this imprinted sensor that has been modified with mag-MIP could possibly be effective and reliable in a process for the determining SN in food samples.

Sample	Amount added	Amount found	Recovery
	(µM)	(µM)	(%)
Milk	0	ND	-
	0.5	0.47	93.5
	2	1.96	98
	5	5.11	102.2
ND= not detected			

Table 2: Determination result of SN in real sample (n=3).

Conclusions

This study has shown a sensitive electrochemical sensing procedure for application in recognition of SN, by magnetic nanoparticles modified with molecularly imprinted polymer fabricated onto m-GEC electrode. The mag-MIP synthesized was characterized by SEM and VSM. The sensor prepared presents an excellent discovery capability due to high levels of sensitivity as well as being stable. The developed mag-MIP/GEC sensor shows high recognition ability and affinity for the template molecule with good sensitivity and stability with satisfactory linearity between the current response of the redox probe and SN concentrations which ranges from 1.0×10^{-8} to 1.0×10^{-7} M and the detection limit of 3.0×10^{-9} M was observed. Likewise, the outcome got from the determination of SN shows the possibility of applying this same procedure in analyzing real samples and demonstrated excellent results for application of SN in milk samples and shown good recoveries of 93.5-102.2%. The idea of the construction of mag-MIP/GEC senor was novel, low-cost and easy to handle, which demonstrated that MMIP based sensors can be an active tool for recognition purposes in real samples.

Conflicts of Interest

Authors declare no conflicts of interest.

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