RESEARCH ARTICLE

Molecular Imprinted Polymer Based Impedimetric Sensor for Trace Level Determination of Digoxin in Biological and Pharmaceutical Samples

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Abstract: Background: Molecularly imprinted polymers (MIPs) are used as artificial receptors in biosensors for the detection of a wide range of analytes from small drug molecules to large molecular weight biomolecules. Quantification of Digoxin (DIG) in human serum and pharmaceutical samples has very high importance due to its low margin of safety. In this report, a highly sensitive molecularly imprinted polymer for DIG is prepared and the detection of analyte was conducted using electrochemical impedance spectroscopy.

Methods: Bulk polymerization method was chosen for the preparation of MIP because of its better preservation of the pockets formed during the polymerization. DIG imprinted polymer was prepared by thermal polymerization of methacrylic acid (MAA) cross-linked with ethylene glycol dimethacrylate (EGDMA) in the presence of DIG. The molecular interactions via hydrogen bonding between template and monomer in MIP and non-imprinted polymer (NIP) were confirmed using Fourier transform infrared (FTIR) spectroscopy. Morphological studies were performed before and after extraction of the template using scanning electron microscopy (SEM). MIP modified carbon paste electrode (MIPCPE) was fabricated by mixing optimized quantities of graphite, the polymer, and eicosane. The change in charge transfer resistance (Rct) at the electrode-electrolyte (MIPCPE-electrolyte) interface before and after addition of DIG solution was studied using electrochemical impedimetric sensing of DIG.

Results: The surface morphology of the MIPCPE shows better porosity in comparison to non-imprinted polymer CPE (NIPCPE) and MIPCPE before extraction of the template. In contrast to NIPCE, the fabricated MIPCPE shows a significant increase in Rct after addition of DIG. The MIPCPE based impedimetric sensor is able to detect DIG over a wide range of concentration from $1.0 \times 10^{-9}$ M to $0.5 \times 10^{-7}$ M with a detection limit $6.95 \times 10^{-11}$ M. The selectivity coefficients obtained for the MIPCPE and NIPCPE reveal specific recognition MIPCPE sensor towards DIG. The recovery rates of DIG from the spiked blood serum and pharmaceutical samples are in the range of sensor 89-101%.

Conclusion: In the present work, the capability of MIPs as molecular recognition elements is proved by reporting a selective and sensitive impedimetric sensor for DIG. The selectivity coefficients (K) of MIPCPE and NIPCPE obtained for the DIG and interferents show better selectivity of the sensor towards DIG in the presence of interferents. The proposed sensor also shows satisfactory stability, reproducibility, and repeatability for DIG. The proposed sensor was successfully applied for the quantitative estimation of DIG in serum and pharmaceutical samples with appreciable recoveries.

Keywords: Digoxin, Molecularly imprinted polymer, Free-radical polymerization, Scanning electron microscope, Electrochemical impedance spectroscopy, Carbon paste electrode.

1. INTRODUCTION

DIG is a glycosidic steroidal toxin prescribed for the treatment of cardiac failures, cardiac arrhythmias, atrial fibrillation and atrial flutter [1]. When DIG concentration in plasma is higher than the therapeutic range, it can be harmful and may cause heart block, fatal arrhythmias, and disturbances in the central nervous system like malaise, delirium, confusion, and visual disturbance [2, 3]. As a result, therapeutic range of plasma concentrations of DIG has fallen to 0.5 – 0.9 ng mL$^{-1}$ in the treatment of heart failure [4-6]. The most common techniques by which DIG was estimated are enzyme-linked chemiluminescent immunosorbent assay [7], radioimmunoassay (RIA) [8], enzyme-multiplied immunoassay technique (EMIT) [9], fluorescence polarisation immunoassay [10], high performance liquid chromatography (HPLC) with RIA [11], liquid chromatography with mass spectrometry (LC-MS) [12], HPLC [13], liquid chromatog-
raphy with immunochemical detection (LC-ICD) [14], and liquid chromatography with solid phase extraction [15]. Some of the above reported conventional methods are relatively slow and are replaced by RIA which is a more practical method for estimating DIG. Though RIA is sensitive it has radionuclides with some cross reaction with metabolites of DIG and endogenous DIG analogues [16-18]. HPLC masks such problems but is less sensitive to determine trace amount of DIG [19].

MIPs are biomimetic sensing material which provides molecular recognition for different types of biologically active molecules because of physical robustness, mechanical and chemical stability of polymeric matrix. MIPs exhibit properties of selective recognition properties and stable physical characteristics [20, 21]. The so called biomimetics also find applications in many areas such as liquid chromatography [22], capillary electrochromatography [23], drug delivery [24], solid-phase extraction [25], cancer biomarkers and sensing devices [26]. MIPs have been widely used for the determination of various biologically active moieties and also used for sensor application as a possible alternative for an unstable natural receptor. MIP is synthesized by cross linking the template - functional monomer complex by a cross linking agent having covalent or non-covalent interactions. Subsequent elution of the template creates specific cavities or molecular memories in the polymer matrix. These molecular recognition sites provide selective binding of a specific molecule as the sites are complementary in size, shape and functional groups [27, 28]. A fluorosensor has been reported based on molecularly imprinted polymer (MIP) [29]. Recently, an immunosensor has been fabricated for DIG using core shell gold nanoparticle–antibody conjugate [30].

Electrochemical impedance spectroscopy (EIS) is a powerful technique to study the interfacial phenomenon that takes place at the electrode-electrolyte and binding kinetics of molecules [31]. EIS has also been applied for the analysis of frequency-dependent changes in tissue impedance [32]. EIS provides the information regarding Faradaic impedance which is termed as the resistance of electrode surface against the electron transfer (Rc) or charge transfer resistance [33]. EIS is a simple, cheap and sensitive method having numerous applications in the fields of corrosion, battery, fuel cell development, sensors and physical electrochemistry [34-36]. DIG has been detected by various techniques namely HPLC, LC-MS and also electrochemically detected by CV [12, 13, 37]. During the past decade, electrochemical sensors have been receiving tremendous scientific interest due to their significant role in the development of diagnostic tools and point-of-care devices [38, 39]. There are few reports on the electrochemical detection of DIG. In 1985, Kenneth et al. reported enzyme immunoassay based amperometric detection of DIG [40]. Later, labelless impedimetric anti-body based with pg ml⁻¹ detection and antigen labelled Fe₃O₄-Au-NPs were also reported [41]. As the most recent ones, aptamer based Ag-NPs decorated graphene oxide and fluorine doped tin oxide surface can also be found in the literature for voltammetric detection of DIG [42, 43]. However, no research work was reported on an impedimetric determination of DIG using molecular imprinting method.

The present work focuses on fabrication and evaluation of molecularly imprinted polymer based on the impedimetric sensor for DIG. DIG imprinted polymer was prepared by thermal polymerization of methacrylic acid (MAA) cross-linked with ethyleneglycol dimethacrylate (EGDMA) in the presence of DIG. The morphology of obtained porous structured polymer was characterized by electron microscopy. The hydrogen bonding interactions between the hydroxyl groups of DIG and carbonyl groups of MAA was confirmed using FT-IR spectroscopy. It is observed that there is a significant increase in the impedance of MIP-CPE after the addition of DIG where as there is negligible increase in impedance in the case of NIP. It shows an evidence for the formation of sensitive cavities in MIP-CPE during the polymerization. The successful application of sensor has been shown from the recovery of DIG from the spiked human blood sera and pharmaceutical samples.

2. EXPERIMENTAL PROCEDURES

2.1. Reagents and Apparatus

Digoxin (DIG) was purchased from Fluka. Methacrylic acid (MAA), Ethylene glycol dimethacrylate (EGDMA), azobisisobutyronitrile (AIBN), eicosane, graphite powder (particle size <20 micron), ascorbic acid and cholesterol were purchased from Sigma Aldrich. Dipotassium monohydrogen phosphate, mono potassium hydrogen phosphate, potassium chloride, urea, glucose, lactose, and tryptophan were purchased from Merck. Potassium ferrocyanide K₃[Fe(CN)₆] and potassium ferricyanide K₃[Fe(CN)₅] were purchased from Fischer Scientific. Lanoxin tablet bought from a general medical store.

Electrochemical measurements were performed with the help of three electrode system. Electrochemical impedance spectra (EIS) were recorded using Parstat 4000 potentiostat (Princeton Applied Research Ametek). CPE, Ag/AgCl electrode (Princeton applied research) and Pt wire were used as working, reference and counter electrodes respectively. Fourier transform infrared (FTIR) spectra of the polymeric particles were recorded using Shimadzu IR- Affinity instrument. The sample for FTIR analysis was prepared by mixing particles with KBr and mixture was ground and placed in the holder for recording the FTIR spectra. All FT-IR measurements were recorded as an average of three scans from 4000-500 cm⁻¹ with 4 cm⁻¹ resolution. Absorption spectra of the samples were analyzed by Shimadzu model UV-1800 spectrophotometer equipped with quartz cell of 1cm path length. The surface morphology was studied using MIRA3 TISCAN scanning electron microscope. The pH measurements were done using Toshniwal pH meter.

2.2. Preparation of Molecularly Imprinted Polymer for DIG

The preparation of DIG MIP was adopted from the previous report [30]. Preparation of MIP for DIG was carried out using thermal polymerization of MAA (5 mmol) as monomer and template DIG (0.5 mmol). MAA and DIG were dissolved in dimethyl sulfoxide (DMSO):acetoniitrile (ACN) [1:4] as a solvent. The mixture was kept in the ice bath for 10 minutes followed by the addition of 10 mmol cross linker (EGDMA) and 0.15 mmol initiator (AIBN) to
initiate the polymerization. The contents were sonicated for 5 minutes followed by nitrogen purging for 10 minutes to maintain an inert atmosphere. The resultant mixture was heated for 24 h at (60 °C). The monolith formed was washed, crushed and pulverized in pestle and mortar to micro particle size and sieved by wire mesh sieve. The control or non-imprinted polymer (NIP) was prepared using same experimental condition without the addition of template molecule.

The template (DIG) was extracted by washing the MIP in methanol and acetic acid (9:1 v/v) mixture by several times. The washing eluents of MIP obtained during the extraction were analyzed using UV-VIS spectroscopy. Complete elution of template was confirmed from the disappearance of the absorption peak 220 nm in UV spectra. Further, excess acetic acid was removed by washing the particles with 1 mM Na2CO3 followed by water and dried at 50 °C under vacuum. The particles were then used for further studies.

2.3. Fabrication of MIP and NIP Modified CPE

The carbon paste electrode (CPE) was fabricated by mixing graphite powder, MIP/NIP particles and n-eicosane (in molten state at 60 °C) in suitable weight percentages. The MIP and NIP modified CPE were fabricated with the addition of optimized quantity of MIP (or NIP), graphite powder and n-eicosane. The mixture was homogenized in a granite mortar and pestle for 10 min so as to form carbon paste. The paste was stuffed into a Teflon tube (3 mm i.d., 5 mm o.d.) at one end and a thin copper (1 mm Dia) wire inserted through the opposite end to establish electrical contact. The surface of the electrode is polished by placing its surface exactly vertical to the surface of a sand paper and slowly moved in a circular path to obtain a reproducible working surface. Polishing of the electrode surface was carried until the surface had a glossy shiny appearance. The optimized composition of the MIPCPE consists of MIP, eicosane and graphite powder with weight percentages of 25, 5 and 70 respectively was used for further electrochemical measurement.

2.4. Impedance Measurements

Electrochemical characterisation of MIPCPE and NIPCPE was performed using CV and EIS. The imprinted sensor was characterized by CV in presence of Fe(CN)63-/Fe(CN)64- containing 0.1 M KCl prepared in phosphate buffer solution (PBS) adjusted to pH 7.0. The potential range from -0.2 V to 1 V was applied to record CV with a scan rate of 100 mVs⁻¹. EIS was performed for electrochemical characterization of MIPCPE, NIPCPE and bare CPE (unmodified). All EIS measurements were carried out in room temperature at half-wave peak potential (0.210 V) of the 10 ml redox mixture consists of 5 mM Fe²⁺/Fe³⁺ system and 0.1 M KCl prepared in 0.1 M PBS (pH 7.0). The AC frequency was scanned ranging from 100 kHz to 0.01 Hz with excitation amplitude of 10 mV to record EIS.

2.5. Real Sample Analysis

The fabricated MIPCPE was used to determine the concentration of DIG in pharmaceutical and human blood sera samples. The sera samples of DIG administered patients were collected from the pathology lab of Government Medical College, Nagpur. The serum samples were stored at (-4 °C) before use. Different concentrations were prepared using standard addition method by spiking DIG into the drug free serum. Pharmaceutical sample solution of Lanoxin (1 mM) was prepared by dissolving proper weight of powdered tablets (sufficient weight of tablets) in PBS (pH 7.0). Different dilutions were made by mixing aliquots of the sample solution and phosphate buffer for spiking and calculating the % recovery.

3. RESULTS AND DISCUSSION

3.1. FT-IR Spectra of MIP and NIP

The recorded FTIR spectra of MIP particles (before and after elution of DIG) and NIP are shown in Fig. (1A). It provides information regarding the molecular interactions such as hydrogen bonding between surface functional groups of MAA and DIG. The peaks at 3439 cm⁻¹, 2953 cm⁻¹, and 1722 cm⁻¹ are the characteristic peaks for O—H, C—H and carboxyl group as observed in the FTIR of DIG Fig. (1B). It is reported in the literature that MAA shows characteristic peaks at 3300 cm⁻¹, 2815 cm⁻¹, 1689 cm⁻¹ and 1631 cm⁻¹ for stretching vibration of O—H, C—H, carboxyl and C=C groups respectively [44]. Fig. (1A) shows the spectra of MIP and NIP having back bone of same functional groups with some changes in the frequency of O—H and carboxyl groups. The IR spectra of MIP before extraction shows characteristic peaks at 3542 cm⁻¹ and 1728 cm⁻¹ respective to O—H and carboxyl stretching which represents red shift in frequency of these groups due to the formation of hydrogen bonding between MAA and DIG. However, after extraction of DIG from the polymer, a blue shift was observed at 3624 cm⁻¹ and 1730 cm⁻¹ for free O—H and carboxyl groups due to the removal of hydrogen bonding as seen in Fig. (1B). Also FTIR of NIP Fig. (1C) shows the characteristic peaks at 1725 cm⁻¹ (carboxyl group of carboxylic acid (MAA) and peaks at 3499 cm⁻¹ corresponds to free hydroxyl group of MAA, confirming the absence of DIG template in NIP.

3.2. Morphological Studies by SEM

The surface morphologies of MIP before (A) and after extraction of the template (B) and NIP particles (C) formed during bulk polymerization were studied using scanning electron microscope (SEM) (Fig. 2). SEM micrographs show a significant difference in the morphologies of MIP and NIP. The surface of MIP (with DIG) was smooth and homogeneous throughout the sample. In the case of MIP (without DIG) rough and globular surface with greater porosity due to the cavities formed during the elution of the template was observed and the particle size was relatively smaller. The NIP had similar morphology that of MIP with DIG because of the same functional monomer and crosslinker used.

3.3. Cyclic Voltammetry

CV was performed to characterize the MIPCPE in the presence of mixture consisting 5 mM [Fe(CN)6]³⁻ and 5 mM [Fe(CN)6]⁴⁻ as a redox probe (Fig. 3). Well defined reversible redox peaks of ferro-ferri system with the peak potential difference (ΔEₚ = Eₚ-Eₚc) of 619 mV were observed at bare CPE as a consequence of diffusion limited processes. The redox peak current was successively decreased due to the
incorporation of MIP particles with non-conducting nature in the MIPCE. Compared to NIPCE, MIPCE shows increment in the redox peak current due to its porosity formed during extraction of DIG (see Sec. 2.2 for experimental details) from the polymeric network. A pair of clear redox-reaction peaks (530 μA) was observed at MIPCE (after extraction of DIG) with the ΔE_{p} of 550 mV, where as NIPCE shows negligible current values. Fast electron transfer kinetics at MIPCE was found to be due to DIG imprinted specific pores which provide free channels to diffusion of the ions. The absence of these channels in MIPCE before extraction of template results in a sluggish redox reaction. When the MIPCE incubated in DIG, some of the cavities were blocked by DIG which interrupted ease of redox couple ions diffusion that leads to producing low current values (433 μA).

3.4. EIS of MIPCE, NIPCE and Bare CPE

EIS studies were performed for the characterization of MIPCE, NIPCE and bare CPE. It is an efficient method to analyze the interfacial electron transfer kinetics occurring at an electrode-electrolyte interface. In a typical Nyquist plot (real part of Z' vs. imaginary part of Z'), a semicircle part represents a diffusion-limited process i.e., the oxidation and reduction reaction of ferro/ferri redox probe at the electrode-electrolyte interface is limited by a finite reaction rate. The corresponding charge transfer resistance (R_{ct}) of the reaction can be determined from its diameter. On the other hand, a straight line represents the diffusion controlled process and termed as Warburg impedance [45]. Fig. (4) shows Nyquist plots of bare CPE, NIPCE, MIPCE before extraction and MIPCE after extraction of template (without incubation and with incubation of template) molecule with the relative impedance of 3.4, 11.6, 7.2, 4 and 5.1 kΩ respectively. The R_{ct} was obtained by fitting the EIS plot into Randles equivalent circuit shown in the inset of Fig. (4). The circuit contains important terms such as R_{ct}, R_{s}, C_{d} and W corresponding to charge transfer resistance, solution resistance, double layer capacitance and Warburg impedance [46]. Bare CPE shows the least R_{ct} as a result of conducting behavior of graphite carbon paste. However, incorporation of insulating MIP particles in CPE would lead to the increment in R_{ct}. The non-conducting smooth surface of NIPCE as seen in the SEM images would lead to slow electron transfer of redox system corresponds to highest R_{ct} value (11.6 kΩ). Also MIPCE shows high R_{ct} compared to Bare CPE but relatively less R_{ct} compared to NIPCE due to the porosity of MIP incorporated.
MIPCPE after extraction of DIG (see Sec. 2.2 for experimental details) leaves DIG specific cavities that result into an easy electron transfer of ions and shows less \( R_{ct} \) (4 k\( \Omega \)). It is interesting to observe the increase in \( R_{ct} \) (5.1 k\( \Omega \)) after incubation of MIPCPE into DIG solution due to binding of DIG into specific cavities which hinders electron transfer kinetics. The \( R_{ct} \) of MIPCPE before template extraction shows high value (7.4 k\( \Omega \)) due to the absence of DIG cavities. These results confirm the formation of MIP with specific cavities to DIG while the absence of the same in the case of the NIP.

3.5. Optimisation of Conditions

The optimization of various conditions such as composition of carbon paste, pH of supporting electrolyte and incubation time were essential for achieving desired sensitivity to the CPE sensor. The optimized parameters increase the performance ability of the electrode. All these parameters were optimized by analyzing impedance changes (\( R_{ct} \) changes or \( \Delta R \)). The effect was measured by varying one component while keeping the other constant.

The pH of the redox probe solution influences binding kinetics of the template on the MIPCPE, which eventually changes the rate of electron transfer and hence ultimately effects change in \( R_{ct} \). A series of test solutions of PBS [Fe(CN)\(_6\)]\(^{3-}/4^-\) with pH values from 4.0 to 10.0 was investi-
attract DIG due to lack of the specific cavities and hence the obtained $R_{ct}$ was constant after addition of the template.

### 3.7. Impedimetric Detection of DIG

Electrochemical impedance spectroscopy (EIS) was used for the detection of DIG using MIPCPE. Fig. (7A) shows the Nyquist plots (the real vs. imaginary parts of the impedance) obtained for MIPCPE (under optimized conditions) after gradual increase in concentration of DIG from $1.0 \times 10^{-9}$ M to $0.5 \times 10^{-7}$ M. It is evident from the figure that $R_{ct}$ of the Nyquist plots increases with increasing concentration of DIG which results from the blocking of molecularly imprinted cavities present at the electrode surface. The increase in $R_{ct}$ is attributed to diffusion limiting electron transfer process of redox system. A calibration curve was plotted between $R_{ct}$ and concentration of DIG from $1 \times 10^{-9}$ M to $0.5 \times 10^{-7}$ M.

Fig. (7B) is linearly fitted to the following equation; $R_{ct} = 7.95 + 0.08 \times [\text{DIG}]$ with $R^2$ value of 0.985. The detection limit has been determined according to the following Eq.

Detection limit = $(3.3 \times \text{S. D.})/m$

Where S. D. refers to the standard deviation of the blank and $m$ is the slope of the calibration curve. The detection limit is found to be $6.95 \times 10^{-11}$.

Moreover, Relative change in $R_{ct}$ was taken as the basis for calculating relative sensitivity of the impedimetric sensor MIPCPE [47]. The relative sensitivity was defined as follows

Relative sensitivity = $\frac{[R_{ct}(\text{DIG}) - R_{ct}(\text{Blank})]}{R_{ct}(\text{DIG})} \times 100$

Where, $R_{ct}(\text{DIG})$ and $R_{ct}(\text{Blank})$ corresponds to 0 and 20 nM of DIG in the electrolytic solution. Relative sensitivity of MIPCPE was found to be 33.33 $\Omega \text{ nM}^{-1}$.

### 3.8. Selectivity Studies

In order to verify the selective recognition ability of the proposed impedimetric sensor for the detection of DIG, the impedance spectra of MIPCPE and NIPCPE were recorded in the presence of the most common interference such as tryptophan, urea, glucose, cholesterol, lactose, ascorbic acid, cholesterol, estradiol, and tetracycline. The selectivity of the fabricated sensor MIPCPE and NIPCPE was investigated by comparing selectivity coefficient ($K$) obtained using equivalent concentration of DIG and interferents (20 nM). $K$ is the relative coefficient and termed as $K = R_{ct}(C_I)/R_{ct}(C_D)$, where $R_{ct}(C_I)$ is charge transfer resistance corresponding to interferent and $R_{ct}(C_D)$ for DIG. The obtained selectivity coefficient values for MIPCPE and NIPCPE are given in Table 1. If $K$ value is closer to 0.10, the selectivity was considered as good. Increase in $K$ values from 0.10 would be considered as unacceptable selectivity [48, 49]. To evaluate the specificity of MIPCPE, selectivity coefficient of NIPCPE was also analyzed for various interferent same as MIPCPE. From results in Table 1, it is clear that surface of MIPCPE exclusively selective to DIG in comparison with NIPCPE.

![Fig. (6).](image1) 

**Fig. (6).** (A) Impedance spectra of NIPCPE, (B) of MIPCPE performed in 5 mM 5mM [Fe(CN)$_6$]$^{3-}$/[Fe(CN)$_6$]$^{4-}$ containing 0.1 M KCl prepared in PBS after incubation in different concentration of DIG. (Excitation potential: 0.21 V; frequency range of 100 kHz to 100 mHz and AC amplitude 5mV).

![Fig. (7).](image2) 

**Fig. (7).** (A) Overlay of Nyquist plots obtained for MIPCPE on the binding of DIG of various concentrations incubation for 10 minutes; (a) blank, (b) $1 \times 10^{-9}$, (c) $5 \times 10^{-9}$, (d) $10 \times 10^{-9}$, (e) $20 \times 10^{-9}$, (f) $30 \times 10^{-9}$, (g) $40 \times 10^{-9}$ M and (h) $50 \times 10^{-9}$ M (B) the corresponding calibration plot showing the linear relationship between the change in $R_{ct}$ and concentration of DIG.
3.9. Reproducibility, Repeatability and Stability

The reproducibility of the developed sensor was checked by the EIS analysis of seven MIPCPE sensors under identical experimental conditions. Relative change in $R_{ct}$ was obtained by using each of the MIPCPE sensors in 20 nM DIG. Relative standard deviation (RSD) was found to be 2.17%, which shows good reproducibility of the sensor. Repeatability of the fabricated sensor was studied with the use of same MIPCPE which were used for calibration and same impedimetric experiments were repeated in an optimized identical condition after some interval of 1 hour. The RSD of repeatability was found to be 2.9% favors good repeatability of MIPCPE. Also, stability is one of the important factors to evaluate the performance of sensor. The MIPCPE was stored at room temperature and its impedance response was measured in 5 mM redox probe containing 20 nM DIG after every 5 days of earlier experiments. The MIPCPE gives a consistent impedimetric response with recovery percentages of 95 up to 40 days and then sensor lead to deviate from its actual response.

3.10. Analytical Application

To demonstrate the analytical applicability of the fabricated impedimetric sensor, the MIPCPE was evaluated for the determination of DIG in human blood serum sample and DIG tablets containing 0.25 mg DIG. As the quantity of DIG in the samples is below the detection limit of the sensor, the recovery tests were performed by spiking DIG in the samples of three different concentrations. The percentage of recovery of exogenously added DIG in diluted serum sample was calculated using data obtained from the calibration curve. The percentages of recoveries of DIG obtained using MIPCE are given in Table 2. As can be seen from the values, the sensor shows the percentage of recoveries in the range of 89-101% for both the samples. The results prove the capability of the MIPCPE sensor for the determination of DIG of a nanomolar concentration range in blood sera and pharmaceutical samples.

CONCLUSION

The present work represents the fabrication of an impedimetric sensor (MIPCPE) based on a molecular imprinted technique for selective determination of DIG. The MIPCPE was developed using DIG MIP formed by bulk polymerization of MAA and DIG. The MIP provides highly selective recognition sites for DIG in the CPE. Various experimental parameters such as pH, MIP composition and incubation time were studied and then electrochemical measurements (CV and EIS) were performed under optimised condition.

| Table 1. The selectivity coefficients (K) values for DIG in presence of equivalent concentration of interferents (20 nM) at MIP CPE and NIPCPE. |
|-----------------|-----------------|-------------------|
| Interferents    | MIPCPE          | NIPCPE            |
| DIG             | 1               | .......           |
| Glucose         | 0.032           | 0.019             |
| Lactose         | 0.062           | 0.023             |
| Tryptophan      | 0.085           | 0.022             |
| Ascorbic acid   | 0.097           | 0.024             |
| Urea            | 0.057           | 0.025             |
| Cholesterol     | 0.102           | 0.020             |
| Estradiol       | 0.098           | 0.021             |
| Tetracycline    | 0.089           | 0.026             |

<p>| Table 2. Determination of the content of DIG in blood serum and Lanoxin tablets. |
|-----------------|-----------------|-----------------|</p>
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<th>S. No</th>
<th>Sample</th>
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<th>Recovery (%R)</th>
<th>Average Recovery (%)</th>
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<td>99.5</td>
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<tr>
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The determination of DIG in human blood serum sample and DIG tablets containing 0.25 mg DIG. As the quantity of DIG in the samples is below the detection limit of the sensor, the recovery tests were performed by spiking DIG in the samples of three different concentrations. The percentage of recovery of exogenously added DIG in diluted serum sample was calculated using data obtained from the calibration curve. The percentages of recoveries of DIG obtained using MIPCE are given in Table 2. As can be seen from the values, the sensor shows the percentage of recoveries in the range of 89-101% for both the samples. The results prove the capability of the MIPCPE sensor for the determination of DIG of a nanomolar concentration range in blood sera and pharmaceutical samples.
FT-IR spectra and SEM images show the molecular interaction and surface morphology of MIP particles. EIS was employed for the impedimetric determination of DIG using MIPCPE. The NIPCPE shows negligible response toward DIG whereas MIPCPE selectively sensed DIG with the linearity of $1 \times 10^{-6}$ to $0.5 \times 10^{-3}$ M. The lower detection limit was found to be $6.95 \times 10^{-11}$ nM with $R^2$ value of 0.985. The sensitivity of impedimetric sensor was found to be $33.33 \Omega \text{nM}^{-1}$. The proposed MIPCPE shows better response to DIG in comparison to the most common interferents in serum and structure analogues of DIG. The proposed sensor shows stable and reproducible results for the sensing of DIG. MIPCPE was applied for the determination of DIG in sera samples and pharmaceutical samples with good recovery rates.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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