Pharmaceutica Analytica Acta

Babanejad M, Pharm Anal Acta 2016, 7:7
DOI: 10.4172/2153-2435.1000499

Research Article Open Access

# Potentiometric Determination of Clonazepam Using Carbon Paste Electrode Based on Molecular Imprinted Polymer (MIP) in Solution and in a Biological Fluid Model

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**Received date:** May 14, 2016; **Accepted date:** Jul 21, 2016; **Published date:** Jul 25, 2016



## Abstract

A potentiometric method is reported for clonazepam determination in biological fluid models. A simple, rapid and sensitive method for the determination of clonazepam in biological fluid models and pharmaceutical preparations using modified carbon paste electrodes is developed. The clonazepam selective MIP was synthesized from methacrylic acid as the functional monomer and ethylene glycol dimethacrylate as the cross-linker in methanol solution using clonazepam as the template molecule and 2, 2-azobis isobutyronitrile as the initiator. A non-imprinted polymer (NIP) was prepared by the same procedure, but in the absence of template molecule then incorporated in the carbon paste electrodes (CPEs). The effect of template ratio to monomer on the sensor performance was investigated and important role for this ratio was shown. The MIP–CP electrode showed high recognition ability in comparison to NIP-CPE. Some parameters affecting the sensor response were optimized and then the calibration curve was plotted. After optimization, the carbon past electrode constructed with a MIP exhibited a Nernstian response 29.66 ± 1.0 mVdecade−1 in a wide concentration range, from $1.0\times10^{-7}$ to $1.0\times10^{-1}$ M, with a low detection limit of $7.3\times10^{-7}$ M and the electrode showed a response time of less than 15 s. The optimum pH values for quantitative uptake of drug were 6 and it was determined by measuring the drug content in the supernatant liquid. Finally, the proposed electrode was successfully used for potentiometric determination of clonazepam in biological fluid models and pharmaceutical samples.

**Keywords:** Clonazepam; Molecularly imprinted polymer; Carbon paste electrode; Potentiometric; Tablets

## Introduction

Clonazepam, 5-(2-chlorphenyl)-1, 3-dihydro-7-nitro-2H-1, 4-benzodiazepin-2-one (Figure 1) as a benzodiazepine derivative, is an anticonvulsant agent primarily used in the treatment of epilepsy for both adults and children [1-4]. It is used based on daily administration of oral doses to prevent seizures or intravenous infusions in status epileptics [5] and neonatal convulsions [6], hence it is necessary and important for determination of the concentration of clonazepam in the plasma of epileptic patients in order to control its dosage [7-9]. In general, the recommended therapeutic concentration is in the range of 10 to 50 ng/mL at predose sampling [10-12]. Moreover, a risk of increased seizure frequency is present, when the concentration of clonazepam in plasma exceeds 120 ng/mL [13].

Hence, it is necessary to develop a specific and rapid method for the determination of clonazepam in biological fluids. Several methods were already published involving spectrophotometry [14-18], gas chromatography [19-23] with or without derivatization and liquid chromatography (LC) [24-29], spectrofluorimetry, differential pulse polarography and non-aqueous titration [30-40], HPLC with UV spectrophotometric and fluorescence detection systems [41-46]. Most of the LC methods suffer from either extensive sample preparation involving two extractions, time-consuming for sample preparation, long-time analysis or expensive equipment, and therefore they are not suitable for routine works. However, each of the above methods has shortcomings such as lack of selectivity, expensive instruments for their operation or being time consuming.

The methodology of molecular imprinting, first introduced by Anderson, has also been exploited, and has significant advantages. The molecular imprinting technique is a powerful and novel method for producing high selective synthetic receptors with molecular recognition sites designed for a particular analyte. This technique is used for a wide range of target molecules. MLP production process is based on the chemical polymerization of a functional monomer and cross-linking in the present of template. After the removal of target molecule, MIPs are designed according to fit in active site of the Drug. Molecular imprinting is a quite remarkable method to identify the drugs in biological fluids using designing artificial antibody-like binding sites. Due to the presence MMA as functional monomer with carboxyl functional group, binding interactions clonazepam to MIP were attributed to the hydrogen bonds and the electrostatic force.

Furthermore, among various application investigated for MIPs, electrochemistry is one of favorable techniques for determination of templates, because potential abilities of MIPs including superior stability, low cost, and ease of preparation make them interesting in electrochemistry. In addition, MIPs may replace with natural receptors as the detection components of selective electrodes owing to their long-term stability, chemical inertness and insolubility in water [47-49] In summary, MIPs are made by synthesizing highly cross linked polymers in the presence of imprint molecules (the template). After

removal of the template, the polymer can be used as a selective binding medium for the print molecules or other structurally related compounds. MIPs that have complementary sites (i.e., specific recognition sites for the template molecules) can be acquired with various configurations that are adapted for the structure of target molecules.

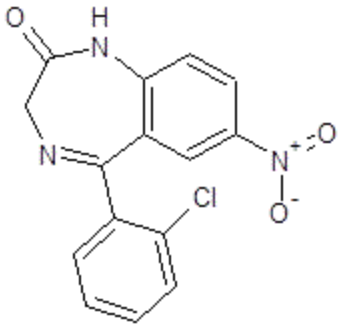

**Figure 1:** Structure of clonazepam as the investigated drug in this study.

The potentiometric sensor that has already been prepared by MIP is used for determination template. The ion-selective electrode technique has the advantage of selectivity, simplicity and rapidity. Nowadays, conventional potentiometric carbon paste electrodes (CPEs) are highly selective, highly sensitive, and of low detection limit. Moreover, compared to membrane electrodes, carbon paste electrodes as ion selective electrodes have gained considerable attention mainly due to their advantages such as renewability, stable response, low-ohmic resistance as well as no need for internal solution. The CPEs are one of the convenient conductive matrices to prepare chemically electrodes, by simply mixing the graphite/binder paste.

They are inexpensive and possess many advantages, such as low background current, easy fabrication and rapid renewal [50-53]. The aim of this work is to develop new simple, low cost, sensitive ion-selective electrodes for determining trace amounts of clonazepam in pharmaceutical preparations as well as in biological fluids. In the present work, a simple potentiometric chemically carbon paste electrode based on molecularly imprinted polymers for the determination of clonazepam is presented.

This scheme allows the sensitive, simple and inexpensive detection of the analyte-receptor binding without using additional reagents or instruments. These electrodes were found to give accurate results for the determination of clonazepam in biological and pharmaceuticals samples.

## Materials

### Reagents

Clonazepam was provided from Marham Daroo Pharmaceutical Co. (Tehran, Iran), methacrylic acid (MAA) was distilled to use in order to remove the stabilizers, and ethylene glycol dimethacrylate (EGDMA) and 2, 2-azobisisobutyronitrile (AIBN) obtained from Merck (Darmstadt, Germany), graphite fine powder extra pure was purchased from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany). Since clonazepam generally had a low solubility in water, a stock solution of clonazepam was prepared by dissolving appropriate amount in analytical pure grade methanol. Then, it was diluted with distilled water (water–methanol 1:1). Daily diluted solutions were prepared from the stock solution. The phosphate buffer solution, 0.01 M, with a pH value of 6 was prepared in de-ionized water and used.

### MIP and NIP preparation with precipitation polymerization

The schematic exhibition for the preparation of the clonazepam imprinted polymer and the removal of clonazepam from it conformed to reference [42] that are shown in (Figure 2).

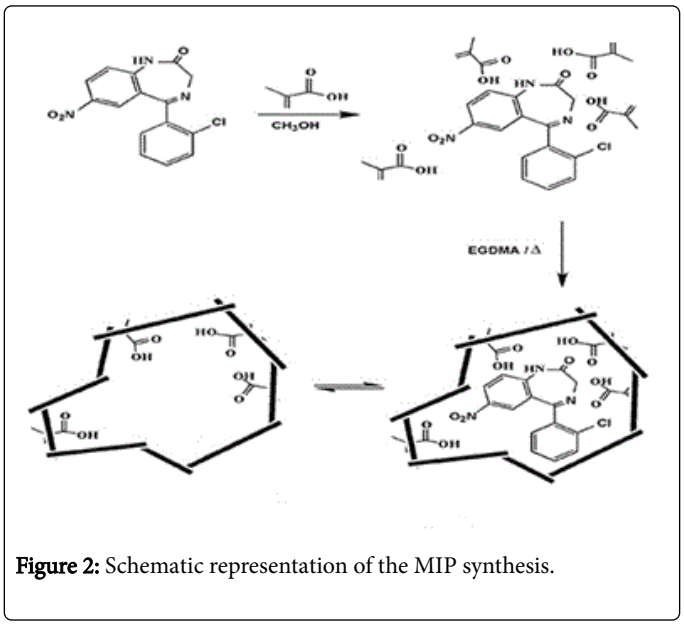


**Figure 2:** Schematic representation of the MIP synthesis.

The molecular imprinted polymer particles for clonazepam were prepared by taking (0.06 mL, 0.68 mmol) of methacrylic acid, (0.6 mL, 3.18 mmol) of ethylene glycol dimethacrylate, (0.05 g, 0.17 mmol) of clonazepam in 30 mL methanol whereas the mixture were placed in a glass tube and was uniformLy dispersed by sonication for 5 min and the initiator (AIBN) (12 mg, 0.08 mmol) were then added to the above solution and then it was purged with N2 for 15 min to remove oxygen ,the glass tube was sealed under this atmosphere. Then the solution was placed in a paraffin bath at 60°C to begin reaction. The reaction was allowed to proceed for 24 h under these conditions.

The product polymer, after drying in air overnight, was yellow and possessed a soft structure, a sieve with 200 meshes was employed to select particles with certain narrow range of size and washed with acetone and methanol before the template removal.

The template and un-polymerized monomers were removed by Soxhlet extraction with 150 mL methanol by refluxing for 20 h. A control polymer (NIP) was also synthesized following exactly the same procedure, including washing but when polymerization was carried out in the absence of clonazepam. Non-imprinted polymer was used to determine the presence of any nonspecific binding of the target molecule.

## Apparatus

Electrochemical data were obtained with a two-electrode system. The differently prepared MIP or NIP involved sensors were used as a

working electrode and the Ag/AgCl electrode was used as reference electrode. Potentiometric measurements were carried out with a digital millivolt meter (Hung Chang model 4510). The pH measurements were made on pH meter (Metrohom model 827) at room temperature (25.0 ± 1.0°C).

The performance of the electrodes was investigated by measuring the electromotive forces (emfs) of clonazepam solutions in a concentration range of $10^{-7}$-$10^{-1}$ M. Each solution was stirred and the potential reading was recorded when it stabilized, and plotted as a logarithmic function of clonazepam concentration that are calculated according to the Nernst equation. UV–Vis absorption spectra were recorded using the double-beam in-time spectrophotometer (Cary-100 conc UV-Visible- varian) and IR spectra were taken on a (Termo Nicolet- Nexus 870 FT-IR) instrument.

### FTIR experiments and studies

FTIR spectra were collected under room temperature/humidity control after background correction. The number of scans was 30 for both sample and background. X-axis was wave number, ranging from 500 to 4000 $cm^{-1}$, and Y-axis was % transmittance. Resolution was set to 4.000. The FTIR spectra of all the un-leached and leached MIPs and NIP recognized the absence of clonazepam on its surface. They showed similar characteristic peaks, indicating similarity in backbone structures of the different polymers.

They were also displayed two peaks at about 1731 $cm^{-1}$ and 1159 $cm^{-1}$, proportionate with –C=O or –C–O stretches, respectively. These bonds are in all spectra that use from one of the kind of cross linker, EGDMA. There are no present bands in the area of 2850–3000 $cm^{-1}$ indicating the absence of C–H groups in imprinted polymeric because the most of monomers were suitably polymerized.

There is also an additional –O–H stretch band and –O-H vibration at 3566 $cm^{-1}$ and 1457$cm^{-1}$ in the leached MIP, respectively. As a result of the hydrogen bonding in un-leached polymer, these bonds shifted (about 5 $cm^{-1}$) in corresponding un-leached MIP. Therefore the spectra confirmed the presence of carboxylic groups in the polymer.

### Thermogravimetric analysis

Figure 3 exhibits the plots of the leached NIP and leached MIP particles. Regarding leached MIP particles, TGA displayed a decomposition state that is between 100 and 200°C (~5% weight loss), assigned to the free monomer and cross-linker, and since no drug is in the polymers, TGA did not revealed the clonazepam of decomposition as the melting point of clonazepam is about 237°C [43].

All of the materials decomposed before obtaining the temperature of 400°C. Figure 3 shows that leached MIP and NIP particle have similar decomposition mould up to 400°C and in this temperature degradation of polymeric matrix occurs completely.

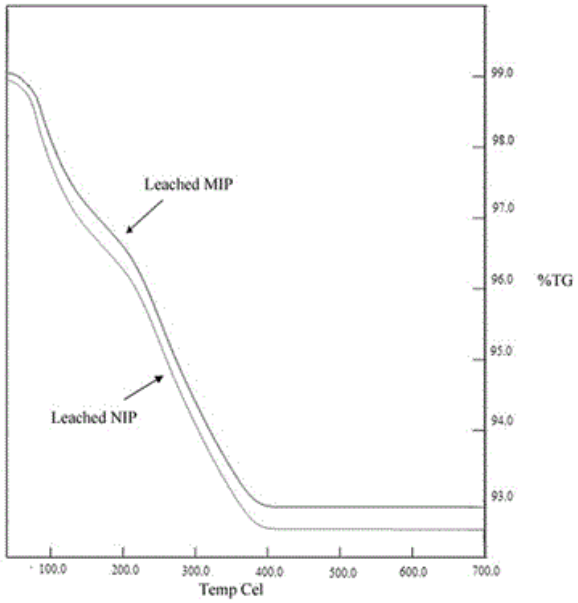


**Figure 3:** TGA plots of the leached NIP and leached MIP particles.

## Procedures

### Preparation of synthetic serum samples and extraction procedure

For the preparation of standard solutions, 250 µL of $1.0\times10^{-4}$ M clonazepam standard solution was transferred in to a 25 mL flask and then it diluted with synthetic serum [41] and vortexed for 2 min. Then, the solution was adjusted to a pH of 6 by the addition of a concentrated sodium hydroxide solution. For the determination of clonazepam, 2 mL dichloromethane was added to 1 mL of synthetic serum samples and vortexed for 4 min. The mixture was centrifuged at 6000 rpm for 3 min to separate the aqueous and organic layers. After removal of the organic layer the extraction was repeated two more times on the residual aqueous layer. The dichloromethane layers were pooled and dried at 40°C under a gentle stream of nitrogen. After drying, the samples were reconstituted with 15 mL of acetate buffer. Then, the analysis was conducted, as indicated in the general analytical procedure. The calibration curve for synthetic serum samples contained clonazepam in the range of $1.0\times10^{-6}$ to $1.0\times10^{-4}$ M was prepared using buffer solution.

### Determination of clonazepam in tablets

Potentiometric analysis was conducted on oral dosage forms of pharmaceutical preparations, with a labeled amount of 1000 mg clonazepam per tablet. Ten tablets were weighed, finely powdered, mixed for 20 min and a suitable amount of powder equivalent to the weight of one tablet was transferred to a 100 mL volumetric flask. This powder was dissolved in suitable volume water and methanol (60:40% v/v) and acetate buffer was added to flask after sonication for 15 min and filtered. A 1.0 mL aliquot of the clear supernatant was diluted with acetate buffer of pH 6 in 10 mL volumetric flask.

### Preparation of the MIP-modified carbon paste electrodes

The modified carbon paste electrode was prepared by thorough mixing analytical grade graphite powder, paraffin oil and, MIP (or NIP) 5, 10, 12, and 15% (w/w %) ratio. each mixture was mixed by mortar for at least 10 min to become homogeneous. After the mixture homogenization, the obtained paste was packed into one end of a glass tube to avoid the possible air gaps in which electrical contact was made with a copper rod without blank that was inserted into the opposite end and runs through the center glass tube. The electrode surface was polished using soft paper to produce reproducible working surface. Electrochemical behavior of clonazepam at the surface of each electrode was studied using potentiometric technique. Best results were obtained at 64:21:15 (w/w %) ratio of graphite powder, paraffin oil and MIP (or NIP). This optimized electrode composition was then used for the potentiometric determination of clonazepam. Modified electrode surfaces were conditioned by submerging into $1.0\times10^{-3}$ M clonazepam solution at the pH value of 6 for 24 h. The electrodes were rinsed with deionized water.

### Potentiometric measurements

All electromotive forces (EMF) measurements were carried out with the following cell assembly.

Ag/AgCl (s) ||sample solution| carbon paste electrode.

The prepared carbon paste electrode was connected to the pH/mV meter as an indicator electrode and the Ag/AgCl as a reference electrode was connected to the reference terminal of the meter. For all measurements the two electrodes were immersed in a 50 mL beaker containing standard solution was stirred using a magnetic stirrer. The pHs of the test solutions were adjusted to about 6.0 by the addition of 0.01 M acetate buffer.

The electrode was allowed to equilibrate until a steady state response was achieved (up to 15 s) and clonazepam concentration range from $1.0\times10^{-1}$ to $1\times10^{-7}$ M was obtained. The potential of the carbon paste electrode against the Ag/AgCl reference electrode was recorded after different clonazepam concentration addition, and then plotted as a logarithmic function of clonazepam concentration.

## Results and Discussions

### The ratio of template to monomer

In our investigations, we planned to study the ratio of template to monomer of the MIP electrode for clonazepam determination. The ratio of template to monomer was important in the sensor performance because the template–monomer functional ratio of MIP enhance specific affinity of polymer and the number of MIPs recognition sites available for selective rebinding of clonazepam. Hence, we prepared five different MIP with molar ratio of the template to the monomer MMA of 1:2,1:4,1:5,1:6 and used in the experiment. The optimum ratio of functional monomer to template for the selective rebinding of clonazepam was 1:4 Table 1. It had highest recovery of 96% ± 3.1 and the best specific affinity; unlike corresponding NIPs was low at 27% ± 1.8.

Therefore, MIPs synthesized by the optimal molar ratio 1:4:20 template: monomer: cross-linker and was used for further studies.

| Concentration of clonazepam (M) | Average of potential; E(mV) | Time of record (day) | Precision (R.S.D, %) |
|---|---|---|---|
| $1\times10^{-5}$ | 82.41 | 1 | 0.01 (n=6) |
| $1\times10^{-5}$ | 82.31 | 16 | 0.10 (n=6) |

**Table 1:** Precision and reproducibility of the MIP sensor for analysis clonazepam.

### CPEs response characteristics

In preliminary experiments, the concentration of target molecule by MIP-carbon paste electrode was determined with high selectivity. CPEs generated stable potentials in the solution with clonazepam ions after conditioning in a $1.0\times10^{-4}$ M clonazepam solution. CPEs specified remarkable selectivity for clonazepam comparison with the non-imprinted based CPEs (Figure 4). Potentiometric response of the carbon paste electrodes investigated through changing proportion of the graphite powder, the MIP and the paraffin oil. The comparison of the carbon paste electrodes and their potentiometric in Table 2.

| Clonazepam added (mg/mL) | Mean found (mg/mL) | Accuracy (%) | Number of experiment (n) |
|---|---|---|---|
| $9.8\times10^{-4}$ | $1.23\times10^{-5}$ | 98.73 | 6 |
| $5.0\times10^{-2}$ | $5.65\times10^{-3}$ | 88.7 | 6 |

**Table 2:** Accuracy of the results obtained with the modified CPE for analysis clonazepam.

They were measured in the acetate buffer solution (pH=6). Clonazepam ion concentration in all of the studied CPEs was in the range of $1.0\times10^{-7}$ to $1.0\times10^{-1}$ M. The response of the made of electrodes was evaluated based on the IUPAC recommendations [44]. The slopes, linear ranges and LODs of the resulting calibration curve for clonazepam ion-selective CPEs are indicated in Table 3 according to the result in prepared solution with buffer at the pH value of 6.0 obtained.

According to Table 3, the modified CPE with percentage ratio of 64% graphite powder, 15% MIP and 21% paraffin oil has the optimal membrane ingredient composition in comparison with other electrodes. It showed a Nernstian slop of 29.66 ± 1.0 mV decade−1, intercept 229.82 and correlation coefficient of 0.99 in a clonazepam concentration range between $1.0\times10^{-6}$ to $1.0\times10^{-1}$ M.

The carbon paste electrode with non-imprinted polymer presented a nonlinear potentiometric response with a low slope (6.68 mVdecade −1) that is comparable with modified CPE applied in the studies.

LOD for modified CPE calculated $7.3\times10^{-7}$ M that this value as the clonazepam ion concentration was obtained via extrapolating the linear regions of the calibration graphs to baseline potential (Table 3).

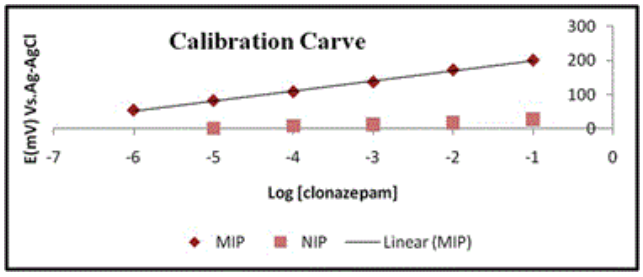


**Figure 4:** Potentiometric response of carbon paste electrodes, based on the MIP as modifier (upper) slope=29.663 ± 1.5 mV decade−1 n=6, and without modifier (lower), slope=6.688 ± 2mVdecade−1, n=5, in pH value 6.0.

The optimum time for increase conductive the electrode, in the clonazepam solution $1.0\times10^{-3}$ M, was 24 h. After this time period, the electrode in contact with the clonazepam solution supplied stable potentials.

## Accuracy

The accuracy of the results obtained with the described method for analysis of clonazepam was verified by calculating percentage recovery of a known clonazepam concentration solution ($9.8\times10^{-4}$ M). Percentage recovery obtained by applying the calibration curve method, was 98.73% (n=6) and was compared with the recovery obtained using HPLC method [40]. The results are shown in Table 4.

| Electrode | Composition (%) | | | | Slope | Linear range | LOD (M) |
|---|---|---|---|---|---|---|---|
| | MIP | Graphite powder | Paraffin oil | NIP | (mV decade-1) | | |
| CPE1 | 0 | 64 | 21 | 0 | 6.688 | - | $1.50\times10^{-7}$ |
| CPE2 | 5 | 71 | 24 | 0 | 10.214 | $1.0\times10^{-5}$-$1.0\times10^{-1}$ | $1.90\times10^{-7}$ |
| CPE3 | 7 | 70 | 23 | 0 | 12.046 | $1.0\times10^{-6}$-$1.0\times10^{-1}$ | $1.13\times10^{-6}$ |
| CPE4 | 10 | 68 | 22 | 0 | 11.791 | $1.0\times10^{-6}$-$1.0\times10^{-1}$ | $3.07\times10^{-7}$ |
| CPE5 | 15 | 64 | 21 | 0 | 29.663 | $1.0\times10^{-6}$-$1.0\times10^{-1}$ | $7.30\times10^{-7}$ |

**Table 3:** Structure of the carbon paste electrodes and comparison of their potentiometric response characteristics, pH=6.

| MIP | MMA (mmol) | Clonazepam (mmol) | EGDMA (mmol) | AIBN (mmol) | Extraction (%)[a] |
|---|---|---|---|---|---|
| | | | | | Mean ( ± SD) |
| MIP1 | 0.342 | 0.171 | 3.181 | 0.082 | 67( ± 2.4) |
| MIP2 | 0.684 | 0.171 | 3.181 | 0.082 | 96( ± 3.1) |
| MIP3 | 0.855 | 0.171 | 3.181 | 0.082 | 84( ± 2.8) |
| MIP4 | 1.026 | 0.171 | 3.181 | 0.082 | 75( ± 2.5) |
| NIP1 | 0.342 | 0 | 3.181 | 0.082 | 34( ± 2.1) |
| NIP2 | 0.684 | 0 | 3.181 | 0.082 | 27(± 1.8) |
| NIP3 | 0.855 | 0 | 3.181 | 0.082 | 25( ± 1.7) |
| NIP4 | 1.026 | 0 | 3.181 | 0.082 | 39( ± 2.3) |

**Table 4:** Comparisons of adsorption clonazepam by MIP using measurement of clonazepam amount extracted from clonazepam standard solution (5 mL, 100/µgL) with 30 mg of various composition polymers applied as sorbents at pH 6.0. [a]Average of tree determinations.

## Precision and reproducibility

The reproducibility of the sensor was evaluated with five repeated potentiometric measurements of the $1.0\times10^{-5}$ M clonazepam solution. The precision of the used procedure of relative standard deviation in one day was 0.01% and during sixteen days was 0.1% as presented in Table 2. The analysis of clonazepam in spike samples for 16 days showed the standard deviation of both slopes of 0.0023 and the intercept of 82.402, indicating adequate precision of the proposed method (data are shown in Table 3).

## Effect of pH

The effect of solution pH was investigated using MIP sensor response. It was examined in the range of pH 2-7 by recording potentiometric measurement. MIP sensor response depends on partial clonazepam deprotonation in the sample solutions and binding interaction of clonazepam to MIP was attributed to the hydrogen bonds and the electrostatic force.

The adjustment of pH parameter in measurement of samples by applied electrode is important. However, in the range of 3-7.5, changing the pH did not affect the conductivity of the carbon paste electrode. The binding interactions in pH values higher than 7.5 increases and in values lower than 3.5 decreases rapidly.

## Interference studies

Interference determined by the matched potential method (MPM), according to this manner, a solution of primary ion of specified concentration was added to $1\times10^{-6}$ M as reference solution and it changed to corresponding changes in potential (ΔE) were measured and second experiment a solution of an interfering ion of activity or

concentration in the defined was added to a new $1.0\times10^{-6}$ M as reference solution until the same potential change (ΔE) was recorded.

The selectivity factor was calculated for interferences. Using primary ion activity (concentration) to the interfering ion activity ratio according by the following equation.

In addition, the selectivity coefficients of interfering species for the clonazepam ion-selective electrode to at the constant pH value of 6.0 assayed by the fixed interference method (FIM). In this manner, the CPE and the reference electrode was placed in container within 20.0 mL of $1.0\times10^{-4}$ M interference ion solution and different volumes of 0.0001, 0.001 or 0.01 M of clonazepam solution were added to it by micro syringe.

The solution was stirred by magnetic stirrer and after each addition, the results were recorded and this measurement was repeated 5 times. The pH values of total solutions were about 6.0. The MPM and FIM selectivity coefficients for the clonazepam carbon paste electrode at the constant pH value of 6.0 are presented in Table 5.

| Sample | Claimed value (mg/ tablet) | Amount added (mg) | Found (mg) |
|---|---|---|---|
| Clonazepam | 1 | - | 0.97(n=5) |
| | 1 | 10 | 9.23(n=5) |

**Table 5:** Analytical results for clonazepam tablet and claimed value in tablet formulations by proposed sensor at pH~6.0.

As a result, both on the template molecular structure and the interactions between the target molecule and the imprinted polymer are important in MIP molecular recognition.

### Response time of the electrode

Dynamic response time is dynamic in important factor that calculated through to reach a potential response within ± 1mV of the final equilibrium value by electrode after successive immersion of clonazepam solutions, each having a 10-fold difference in concentration, the measurements of potential versus time were showed from lower ($1\times10^{-6}$ M) to higher ($1\times10^{-2}$) concentration Figure 5.

In all the concentration, time required for reaching electrode to equilibrium was less than 15 second as is shown in Figure 5.

## Analytical Application

### Analysis of clonazepam tablets

To investigate the feasibility of the proposed potentiometric procedure for the clonazepam determination in tablets, the MIP-clonazepam sensor was applied in solution made from clonazepam tablets, and pH adjusted by acetate buffer (pH=6). Data obtained using calibration curve procedure and the resulting data were compared with the labeled amounts in clonazepam tablets (Table 6).

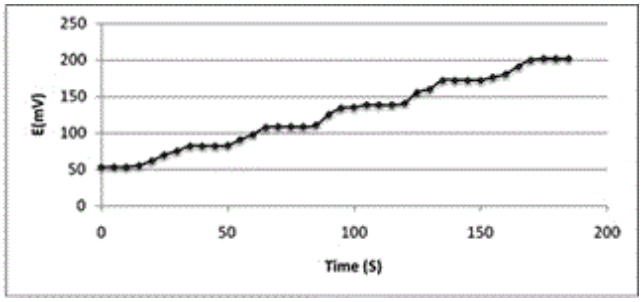


**Figure 5:** Dynamic response of the modified carbon paste electrode for analysis of clonazepam.

| Interfering agent, B | | |
|---|---|---|
| Diazepam | 2.1 | 1.9 |
| Aspirin | 4.2 | 4.1 |

**Table 6:** Potentiometric selectivity coefficient value ,.

### Clonazepam assay in synthetic serum

Application of the present electrode was successfully used to evaluation of clonazepam in spiked synthetic serum [43]. The results of the recovery and their study are present in Table 7. The recoveries were in the range of 97-109% for the spiked synthetic serum. As a result, the suggested method was sensitive and precise.

| Amount added | Amount found (M) | Recovery (%) |
|---|---|---|
| $1.0\times10^{-6}$ | $1.09\times10^{-6}$ | 109% |
| $1.0\times10^{-5}$ | $1.04\times10^{-5}$ | 104% |
| $1.0\times10^{-4}$ | $9.72\times10^{-5}$ | 97.20% |
| $1.0\times10^{-3}$ | $9.7\times10^{-4}$ | 97.70% |

**Table 7:** Application of the optimized sensor to the clonazepam concentration measurements in synthetic serum samples.

### Determination of binding capacities of MIPs

Binding capacities of the imprinted and non-imprinted monolith polymers were investigated by adding 20.0 mg of MIP washed particles in 10.0 mL standard solutions of clonazepam prepared with methanol and water (60:40% v/v) in the range of 0.03-4.0 mM. The mixtures were centrifuged at 4500 rpm for 30 min at room temperature and the absorbance was measured against a blank solution prepared with the same amount of methanol, but no clonazepam by spectrophotometer at 309 nm. This method was applied for NIP and standard solution to compare their absorbance. Thus clonazepam concentrations were measured by UV spectrophotometry at 309 nm against clonazepam standard solution (Figure 6).

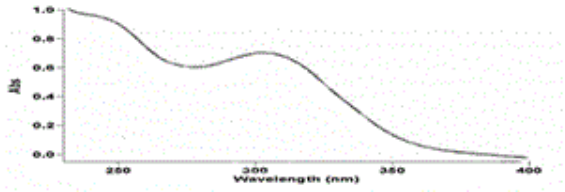


**Figure 6 (a):** Template (Drug) Spectra-Control Group.

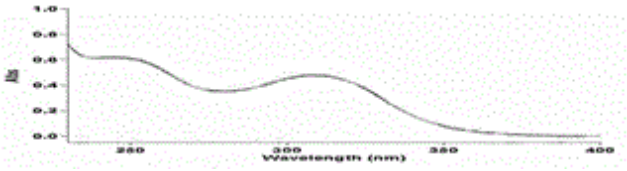


**Figure 6 (b):** The spectra of the template after separation from the loaded MIP in desorption step.

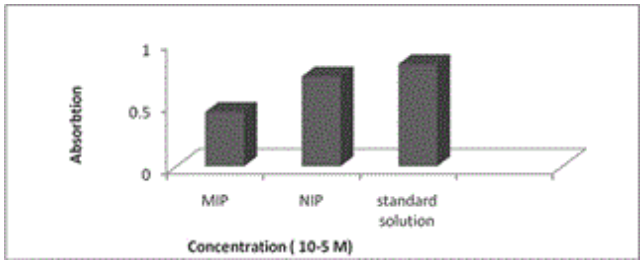


**Figure 6 (c):** Comparison to absorbance at 309 nm, between the solutions of the MIP, NIP and standard solution.

Figure 6-(A),(B),(C): UV-Vis absorption spectra of NIP, MIP and comparison to their absorbance.

## Conclusion

In this work, we synthesized MIP for clonazepam and investigated the effect of MIP composition. According to data of this study, the potentiometric method applied is an attractive alternative for clonazepam assessment. The proper attention to the polymer structure and the surface adsorption on MIPs can be effective in appropriately designing and preparing MIPs. In experiments conducted, the MIP shows higher up taking capability to clonazepam, in comparison to NIP. A sensitive electrochemical sensor was developed for clonazepam determination in tablet and similar biological fluid using molecularly imprinted polymer. The described procedure can be easily created at low cost and has shown good repeatability, stability, selectivity and accuracy. After optimization of the effective parameters of the MIP sensor, the applied method in this article was used for clonazepam determination in samples successfully. The proposed low cost chemical sensor could find application in the measurement of clonazepam level in clinical samples as well as in pharmaceutical industry.

## Acknowledgement

This work was supported by Drug Design and Bioinformatics Unit, Biotechnology Research Center (BRC), Pasteur Institute Iran and Islamic Azad University Tehran, Iran. The authors thank Marham Daroo Co, for their gift of clonazepam used in this study.

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