Potentiometric Transducers for the Selective Recognition of Risperidone Based on Molecularly Imprinted Polymer

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Abstract

Graphite Coated Electrodes (GCE) based on molecularly imprinted polymers were fabricated for the selective potentiometric determination of Risperidone (Ris). The molecularly imprinted (MIP) and nonimprinted (NIP) polymers were synthesized by bulk polymerization using (Ris.) as a template, acrylic acid (AA) and acrylamide (AAm) as monomers, ethylene glycol dimethacrylate (EGDMA) as a cross-linker and benzoyl peroxide (BPO) as an initiator. The imprinted membranes and the non-imprinted membranes were prepared using dioctyl phthalate (DOP) and Dibutylphthalate (DBP) as plasticizers in PVC matrix. The membranes were coated on graphite electrodes. The MIP electrodes using (AA) and (AAm) showed a near nernstian and nernstian response with slopes of 55.2±0.1 and 59.0±0.2 mV/decade, correlation coefficient (r^2) 0.9997 and 0.9999, a linear response for a concentration range of (1.0×10^{-6} - 1.0×10^{-2}) M and (5.0×10^{-7} to 1.0×10^{-2}) M respectively. The response time of the prepared electrodes was less than 30 seconds. The electrode responses were stable in a pH range (4-8). The electrodes exhibited good selectivity over a wide range of interference. The most effective electrode (Ris-MIP GCE) was used to determine the concentration of (Ris.) in some pharmaceutical formulations. The electrodes could be successfully used within (7and13) weeks respectively without any drift.

Keywords: Ion-selective electrodes, Molecularly imprinted polymer, PVC membrane coated graphite electrode, Risperidone.

متحسسات جهدية للتعيين الإنتقائي للرسبيريدون المعتمد على بوليمرات الطبعة الجزيئية

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الخلاصة

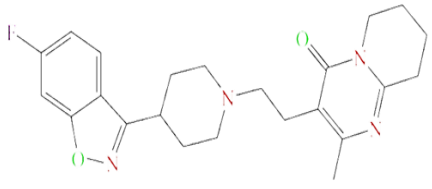
تم تحضير أقطاب كرافيت لبوليمرات طبعت بعقار (الرسبيريدون) للتعيين الأنتقائي لهذا العقار.حيث حضرت بوليمرات الطبعة الجزيئية والبوليمرات غير المطبوعة بطريقة بلمرة المجمل بإستعمال (الرسبيريدون) كقالب (طبعة)، حامض الاكريليك والأكريل أمايد كمونيمرات فعالة ،أثيلين كلايكول ثنائي مثيل أكريلت كرابط تقاطعي وبيروكسيد البنزويل كبادئ. حضرت أغشية الطبعة الجزيئية والأغشية غير المطبوعة ببعثرة دقائق بوليمر الطبعة الجزيئية والبوليمر غير المطبوع في فثالات ثنائي الأوكتيل وفثالات ثنائي البيوتيل كمذيبات ملدنة في متعدد كلوريد الفاينيل. هذه الأغشية المحضرة طليت بها اقطاب الكرافيت لتقدير عقار (الرسبيريدون).

أظهرت أقطاب الطبعة الجزيئية للرسبيريدون المعتمدة عل حامض الأكريليك و والأكريل أمايد كمونيمرات فعالة إنحدارات قرب نيرنستية ونيرنستية مقدارها mV/decade (59.0 – 55.2) بمعاملات ارتباط (٠,٩٩٩٧ و٠,٩٩٩٩) مع مدى تراكيز خطية تتراوح بين M (1.0×10^{-2}–1.0×10^{-6}) و M(5.0×10^{-2}–1.0×10^{-7}) وحدود تحسس مقدارها M(3.3×10^{-7} و2.0×10^{-7}) على التوالي. كما كان زمن استجابة الأقطاب المحضرة أقل من ٣٠ ثانية.وأظهرت الأقطاب أستجابة مستقرة في مدى دالة حامضية تراوحت (٤ – ٨) وإنتقائية جيدة بوجود العديد من الأصناف المتداخلة. إستعمل القطب الأكثر تأثيرا (Ris-MIP GCE) في تعيين تركيز الرسبيريدون في بعض المستحضرات الدوائية.أمكن إستعمال الأقطاب لفترة (٧و١٣) أسبوع على التوالي دون حدوث إنحراف في الجهد.

الكلمات المفتاحية : أقطاب إنتقائية آيونية ،بوليمرات الطبعة الجزيئية ، أقطاب كرافيت مطلية بأغشية متعدد كلوريد الفاينيل ، الرسبيريدون.

Introduction

Risperidone (Ris.) belongs as a chemical structure to an important antipsychotic medication chemical group called "benzioxazoles"[(1,2)] that is mainly used to treat schizophrenia , schizoaffective disorder, the mixed and manic states of bipolar disorder, and irritability in people with autism. (Ris.) undergoes hepatic metabolism and renal excretion. [(2)] It is a second-generation atypical antipsychotic agents (SGAs) which are broad-spectrum antagonists of dopamine, alpha noradrenergic, serotonin and histamine receptors. The structural formula of (Ris.) is shown in Scheme (1).



Scheme (1): The Structural Formula of Risperidone.

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The mechanism by which atypical drugs achieve this highly selective approach to treatment is the antagonism of a specific serotonin receptor, 5-HT_{2A} .This receptor can be found on the axon terminal of neurons that produce dopamine.When serotonin activates those receptors, the release of dopamine decreases. By building into the typical antipsychotics a mechanism to block the 5-HT_{2A} receptors from serotonin, dopamine release is increased [3, 4] .

(Ris.) was determined by using other techniques; spectrophotometric[5], high performance liquid chromatography(HPLC)[6] and solid phase extraction (SPE) [7] .

Molecular imprinting is a process in which functional and cross-linking monomers are copolymerized in the presence of the target analyte (the imprint molecule), which acts as a molecular template. [8, 9] The functional monomer initially interacts with the imprint molecule to form a multi-action spot (complex). [10] After polymerization, their functional groups are held in position by the highly cross-linked polymeric structure [8, 9]. Additionally, the steric arrangement of these interactions around a given substrate (molecular template) is a crucial aspect necessary for the creation of binding pockets providing complementary size, shape, and functionality for preferentially facilitating selective recognition along with a high affinity toward the target. Thus, the recognition process in MIPs may be described in analogy with mechanisms established for enzyme – substrate-complexes such as the lock-and-key [11] .

The synthesis of MIPs usually involves a parallel process involving synthesis of a control non-imprinted polymer (NIP) under conditions identical to those of the MIP except that the template is absent. In principle, the NIP is entirely analogous to the MIP except that any binding sites within its porous structure are non-selective. The NIP can therefore be used as a benchmark for assessing the selectivity of the MIP [12] .

Experimental

Apparatus

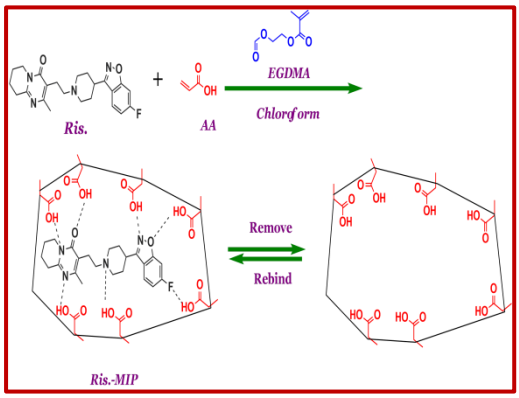
The FTIR spectra of MIPs and NIPs were obtained using FTIR-8400S spectrometer (Shimadzu, Japan). A Shimadzu 1800 UV-Visible, double beam spectrophotometer (Kyoto–Japan) with 1 cm path length quartz cell. All potentiometric measurements were made at room temperature with a pH/mV meter Philips pW9421, England. The potentiometric measurements were recorded using the fabricated Ris-MIP membrane coated on a homemade graphite electrode in conjugation with a reference saturated calomel electrode (SCE). The pH values were recorded using WTW GmbH , inolab pH 7110 Germany pH meter.

Reagents

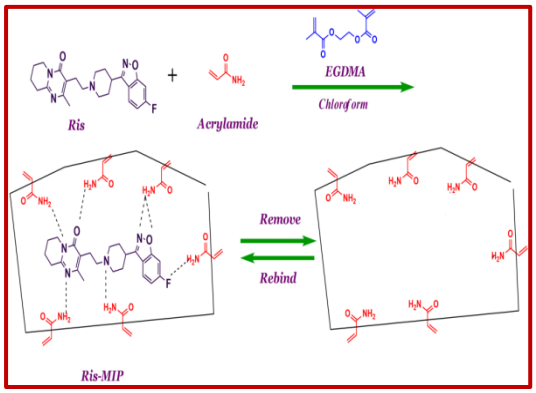
The entire chemical used were reagent grade with highest purity and used as received without further purification. Acrylic acid (AA) (99.0% Himedia Ltd), Acrylamide (AAm) (99.0%, Fluka), benzoyl peroxide (BPO) (75%,Acros Organics), Ethylene glycol di-methacrylate (EGDMA) (95%, BDH), dioctyl phthalate (DOP) (99%, BDH), tetrahydrofuran (THF) (99.0%, Riedel-dehaen). High-molecular-weight polyvinyl chloride (PVC) (99.5%, BDH). For pH control, sodium hydroxide (0.1 M) and hydrochloric acid (0.1 M) were used. Pharmaceutical grade risperidone powder received in pure form (99.99 %) were provided from (Arab Pharmaceutical Manufacturing Company, Jordan) as a gift from the University of Science and Technology Amman, Jordan. Pharmaceutical grade Domperidone and Cephalexin powders received in pure form (99.99 %) were provided as a gift from State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI). Respal caplets (2mg), Joswe medical (Jordan) and Rispond tablets (4mg), India were purchased from local markets.

Synthesis of Ris-MIPs

MIPs for (Ris.) were prepared using a bulk polymerization procedure. 3.6542 mmol of the template (Ris) was dissolved in 5 mL of chloroform in a thick walled glass tube. 14.6168 mmol of functional monomer acidic (AA) or basic (AAm), 73.084 mmol of cross-linker (EGDMA), and 0.32 mmol of initiator (BPO) were then added to the above solution respectively. The mixture was degassed by purging nitrogen for 10 minutes and shacked in ultrasonic water bath. The reaction flask was then removed, sealed and heated at 70°C in a water bath for 15 min. to allow initiation of the reaction. Orange colored polymers with a rigid structure were formed. Schemes (2) and (3) illustrate the synthesis of MIPs for (Ris.) based on (AA) and (AAm) as acidic and basic monomers.



Scheme (2): Ris-MIP synthesis, using acrylic acid (AA) as an acidic functional monomer.



Scheme (3): Ris-MIP synthesis, using acrylamide (AAm) as a basic functional monomer.

Non-reacted species (excessive reagents or template) were removed from the polymers by consecutive washout of the particles with chloroform then diethyl ether and were dried at room temperature overnight. The polymers were then crushed grounded using mortar and pestle and sieved to particles size 150µm (using 100 mesh sieve).

The template (Ris.) was removed by repeated washing the MIPs successively with 100 mL portions of 30% (v/v) acetic acid / water solution. The elimination of (Ris.) from the MIPs was confirmed by measuring the absorbance of the washout solution at 274 nm. After the polymer was completely dried at ambient temperature, it was used as an active material in the selective sensor.

A control non-imprinted polymer (NIP) was prepared according to the same procedure, but excluding the target molecule, (Ris.) [13, 14] .

Construction of the MIP membrane graphite electrode

The sensing PVC membrane was prepared by mixing 0.17 g of high molecular weight PVC, 0.4 g of plasticizer either DOP or DBP and 0.02 g of the MIP or NIP particles. After homogenization, 2-3 mL of THF was added and stirred. The graphite coated electrode (GCE) was constructed by dipping a graphite rod (3 mm diameter and 10 cm long) in the above solution and allowing it to dry in air. The procedure was repeated until the coating thickness is about 1.0 (±0.1) mm. [13, 15]

EMF measurements

The performance of the electrode was investigated by measuring the e.m.f. using the following cell:

$Hg_{(l)}$ | $Hg_2Cl_{2(s)}$, KCl(sat'd) || Test solution | MIP membrane | GE

Preparation of 1.0×10^{-2} M (4.105 mg/mL) Ris.'s stock solution

A standard stock solution of (Ris.) was prepared by dissolving accurately weighed 0.4105 g of pure drug in 10 mL of 0.1 M HCl and the volume was completed up to the mark in 100 mL volumetric flask with distilled water. Working solutions were freshly prepared by subsequent dilutions.

Potentiometric measurements of the proposed sensors

All potentiometric measurements were carried out at room temperature (25±2˚C) in stirred solutions. Aliquots from standard drug solution 1.0×10^{-2}M were diluted to obtain a series of solutions with concentrations ranged (5.0×10^{-9}-1.0×10^{-2}) M in 50mL volumetric flasks. 30mL of each solution was transferred to a beaker and the e.m.f. values were recorded using (Ris.) electrode in conjunction with a reference SCE after stabilization to ±0.2 mV and the calibration curve was plotted. The calibration plot was used for measuring unknown concentrations under the same conditions. [16]

Binding experiments

Binding experiments were carried out by placing 0.02 g of Ris-MIP in contact with 10.0mL (Ris.) solutions ranged (0.005–0.32) mM .The mixtures were shacked for 2h at (25˚C±2) and the solid phase was separated by centrifugation (٦٠٠٠ rpm) for 40 minutes.

The concentrations of free drugs in the supernatant were detected by UV-VIS spectrophotometry at 238 nm. The amount of drugs bound to the polymer was calculated by subtracting the concentration of free drug from the initial drug concentration. The data obtained were used for a Scatchard analysis. [16, 14]

Determination of (Ris.) in pharmaceutical formulations

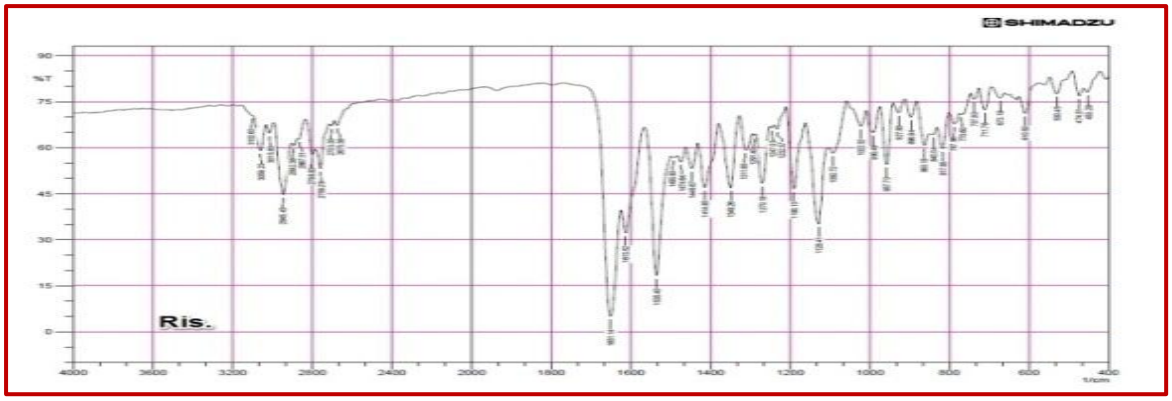
MIP electrode for (Ris.) based on (AAm) as basic monomer was applied for the direct and standard addition determinations of (Ris.) in two pharmaceutical formulations; Respal® (2mg) caplets and Rispond (4mg) tablets. Ten tablets were finely ground, homogenized, then 0.2546 gm of Respal®,

0.3572gm of Respond were weighed accurately and separately, dissolved in 12mL of 0.1NHCl. The mixture was sonicated for 20min to aid dissolution and left to stand for (1 h). The solution was filtered by using Whatman filter paper No.41 to avoid any suspended or un-dissolved material before use. The resulting filtrate was transferred into a 25 ml volumetric flask and the volume was completed to the mark with distilled water to get 1.0×10^{-3}M. Working solutions were freshly prepared by subsequent dilutions with distilled water, and analyzed by the recommended procedure.

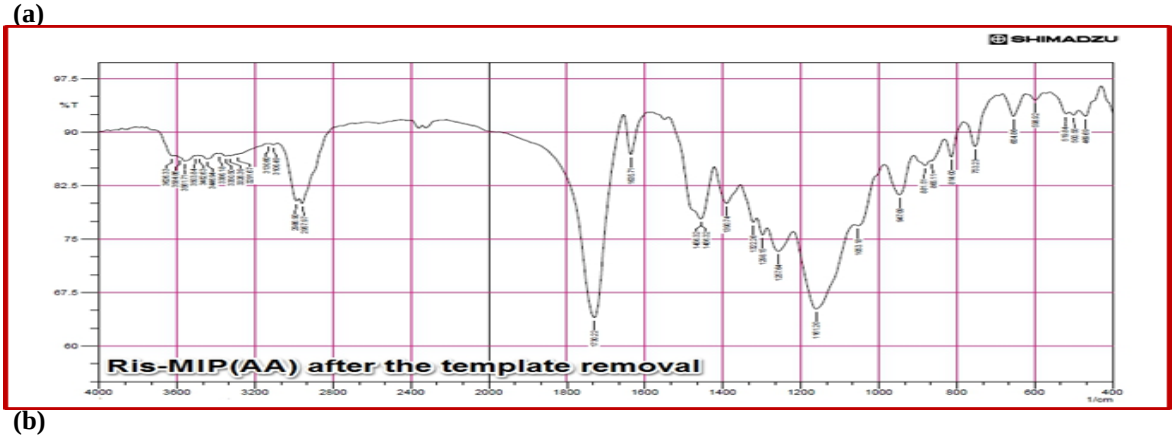
Results and Discussion

FTIR of acidic and basic MIPs of (Ris.)

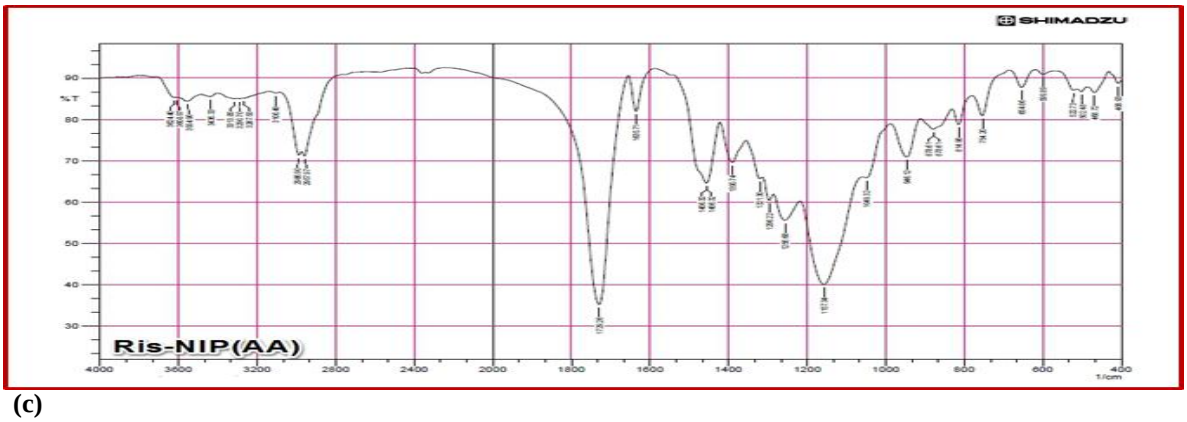
The FTIR spectra of (Ris.), control non-imprinted and molecularly imprinted polymer based on (AA) as a functional monomer prepared using radical bulk polymerization are shown in Figure (1). Both polymers have similar IR spectra indicating the similarity in the backbone structure. In the IR spectra, the absorptions due to sp^2-CH str.(3228)cm^{-1}, sp^3-CH str. 2peaks(2985,2956) cm^{-1}, C=O str.(1732) cm^{-1}, C=C str.residual(1668) cm^{-1}, sp^3-CH scissoring (1456) cm^{-1}, sp^3-CH rocking (1390) cm^{-1}, O–C str.(1257) cm^{-1}, C–O acryl str.(1159) cm^{-1}, C–O alkoxy str.(1047) cm^{-1},sp^2-CH ben. Out of plane (960) cm^{-1} were observed.



(a)



(b)



(c)

Figure (1): FTIR of (a): (Ris.) (b): Ris-MIP(AA) after the removal of (Ris.) (c) : Ris-NIP(AA).

In addition to backbone similarity concluding, those absorbances attributed to the previous groups for the molecularly imprinted polymer are relatively stronger than for non-imprinted polymer. From this comparison it was found that presence of imprint molecule (Ris.) causes incorporation of ethylene glycol dimethacrylate in the preparation of polymer to be increased. [17] On the other hand the FTIR spectra of (Ris.), control non-imprinted and molecularly imprinted polymer based on (AAm) as a functional monomer prepared using radical bulk polymerization are shown in Figure (2). Both polymers have similar IR spectra indicating the similarity in the backbone structure. In the IR spectra, the absorptions due to -NH2 str. 2peaks (3435, 3370)) cm^{-1}, sp^2-CH str.(3200)) cm^{-1}, sp^3-CH str.(2957)) cm^{-1}, Ester C=O str.(1726)) cm^{-1}, Amide C=O str.(1690)) cm^{-1}, C=C str. Residual(1645)) cm^{-1}, NH ben.(1535)) cm^{-1}, sp^3-CH scissoring(1456)) cm^{-1}, sp^3-CH rocking(1382)cm^{-1}, O–C str.(1295)) cm^{-1}, C-N str.(1261)) cm^{-1}, C–O acryl str.(1159)) cm^{-1}, C–O alkoxy str.(1051)) cm^{-1}, sp^2-CH ben.Out-of-plane(948)) cm^{-1} were observed. In addition to backbone similarity concluding, those absorbances attributed to the previous groups for the molecularly imprinted polymer are relatively stronger than for non-imprinted polymer. From this comparison it was found that presence of imprint molecule (Ris.) causes incorporation of ethylene glycol dimethacrylate in the preparation of polymer to be increased [17].

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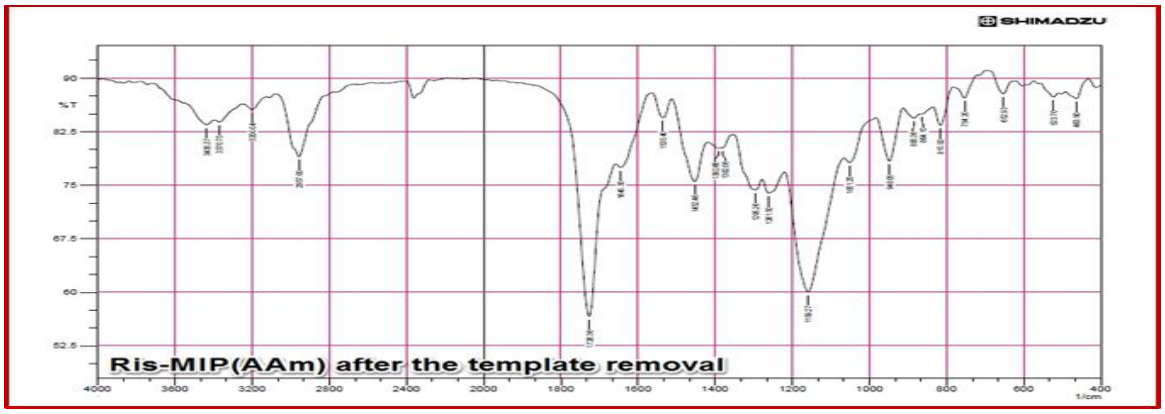
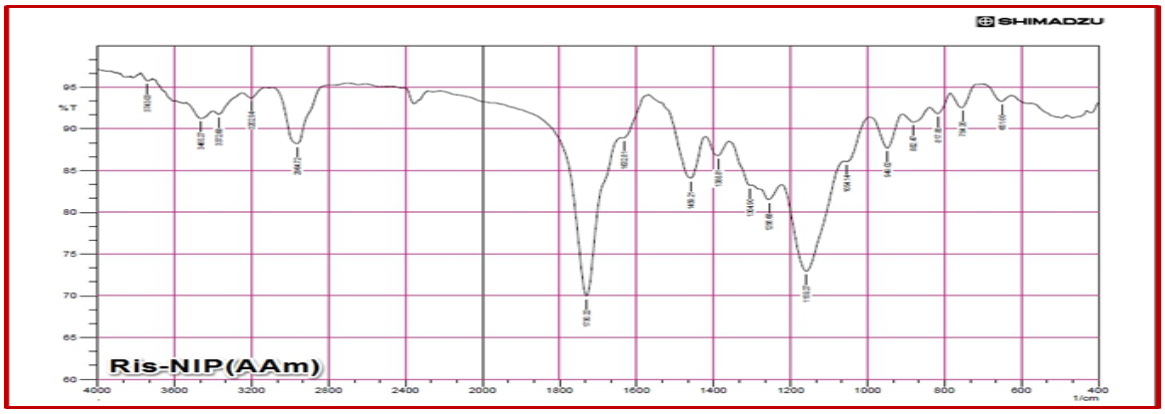


(a)



(b)

Figure (2): FTIR of (a): Ris -MIP(AAm) after the removal of (Ris.), (b) : Ris -NIP(AAm).

Electrodes performance

Characterization of the primary analytical features [according to IUPAC recommendations] of graphite electrodes based on (Ris.) MIP or NIP particles incorporated in PVC membrane and plasticized with DOP was investigated [18].The results are shown in Table (1). Ris – MIP sensors using AA and AAm as monomers displayed slopes of 55.2 ± 0.1 and 59.0±0.2 m V decade $^{-1}$ respectively . The sensors showed linear responses in the range of (1.0×10^{-6} – 1.0×10^{-2}) M and (5.0×10^{-7} – $1.0\times^{-2}$) M of (Ris) , with detection limits of 3.3×10^{-7} M ,respectively as shown in figures (3,4) .

The life time of electrodes were estimated by their periodical recalibration and calculations of slope and limit of detection. A drift in the performance of electrodes I and III was noticed after 7 and 13 weeks respectively which indicate the end of electrodes lives.

Two membrane compositions were investigated by using two types of plasticizers (DOP and DBP) as shown in Figure (5).The results shown in table (1) for electrodes (III and V) indicate that membrane plasticized with (DOP) displayed better performance than that plasticized with (DBP). The (DOP) appeared to be more compatible with the Ris-MIP as it produces a homogenous and clear membrane having better slope and longer life time than (DBP).

Table (1): The characteristics of the (Ris.) GCE based on MIPs and NIPs using different functional monomers and plasticizers.

Type of Plasticizer	**(DOP) as Plasticizer**				**(DBP) as Plasticizer**
Electrode no.	**I**	**II**	**III**	**IV**	**V**
Type of Electrode	Ris-MIP (AA)	Ris-NIP (AA)	Ris-MIP (AAm)	Ris-NIP (AAm)	Ris-MIP (AAm)
Slope	55.2±0.1	36.855	59.0±0.2	26.8	47.9±0.9
Correlation Coefficient (r^2)	0.9997	0.9975	0.9999	0.9796	0.9988
Linear range(M)	(1.0×10^{-6} - 1.0×10^{-2})	(٥.٠×١٠$^{-٥}$ - 1.0×10^{-2})	(5.0×10^{-7} - 1.0×10^{-2})	(5.0×10^{-7} - 1.0×10^{-2})	(5.0×10^{-7} - 1.0×10^{-2})
LOD(M)	3.3×10^{-7}	٣.٠×١٠$^{-٦}$	2.0×10^{-7}	4.0×10^{-7}	3×10^{-7}
Response time (s)	15-20	15-20	15-20	15-20	30
Life time(week)	7	-	13	-	3
RSD%	0.1329	-	0.2989	-	1.9951
RE%	-0.0767	-	-0.1717	-	-2.2513

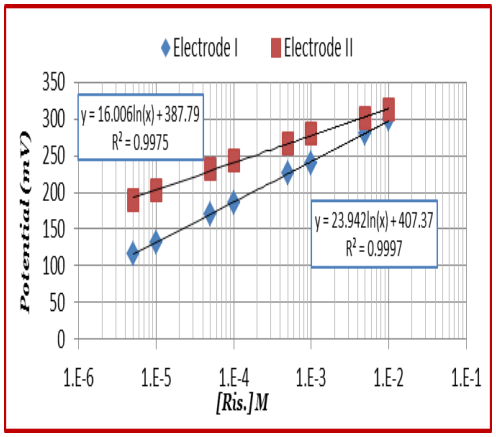


Figure (3): Calibration curve of Ris-MIP (I) and Ris-NIP (II) GCEs based on (AA) as a functional monomers and DOP as plasticizer.

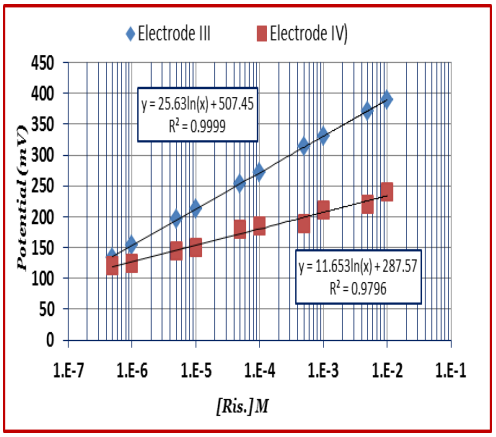
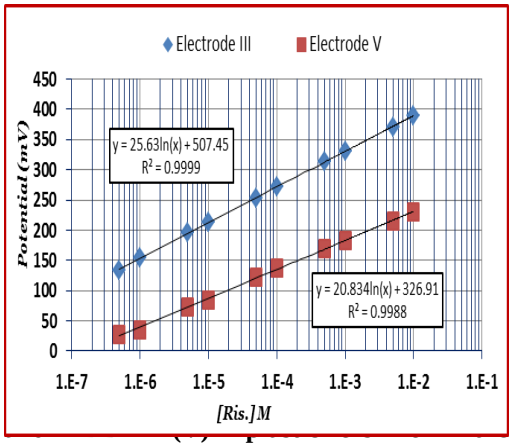


Figure (4): Calibration curve of Ris-MIP (III) and Ris-NIP (IV) GCEs based on (AAm) as a functional monomers and DOP as plasticizer.



characteristics of (Ris.) GCEs based on MIP using (AAm) monomer.

Effect of pH

The effect of pH on the potential readings of the electrodes (I and III) was studied by immersing a combined glass electrode, (Ris.) GCE and a saturated calomel reference electrode in 50 ml beakers containing 25 ml aliquots of 1.0×10-3 and 1.0×10-4 M of (Ris.) aqueous solutions. The pH of each solution was adjusted by 0.1N sodium hydroxide or hydrochloric acid solutions. The potential reading at each pH value ranging 1-10 was recorded. A plot of pH values versus the potential of the electrode is illustrated in figure (6). The electrode potential did not affect by the pH change in the range (4 – 8) which considered as the working pH range of the proposed sensor.At pH lower than 4 the electrode potential decreased due to the formation of di-protonated form of (Ris.) where nitrogen atom of the pyrimidine ring is the preferred site for the second protonation (19). At pH value higher than 8, the decreased electrode potential might attribute to the deprotonation form of (Ris.) which lead to the precipitation of the drug in the test solution.

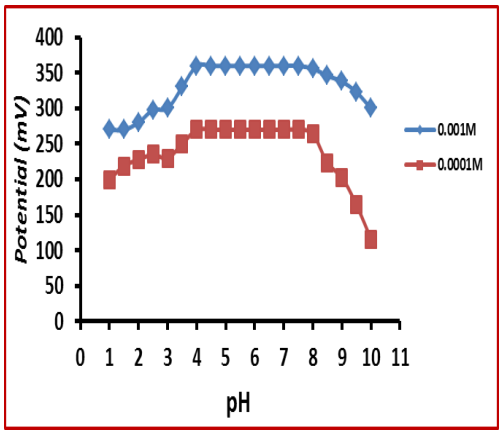


Figure (6): pH effect on potential response of Ris-MIP(AAm) electrode.

The selectivity study

The influence of the interfering ions on the response behavior of ion selective membrane electrode is usually described in terms of selectivity coefficients. The selectivity coefficients for (Ris.) ($K_{Ris.,J}^{pot}$) over a wide range of interfering molecules, mono-valente and di-valent ions were determined by the separate solutions method (SSM) using electrode (III) and listed in table (2).The selectivity coefficients calculated for (Ris.) over each interference at $a_A = a_B$ in a series of concentrations ranged (1.0×10^{-7}-1.0×10^{-2}) M for each of the two separate solutions ,i.e. the (Ris.) solutions and the interference solutions, were below the unity which indicates that these species do not interfere in the determination of (Ris.) .

The values of the selectivity coefficients were calculated according to the following equation [20]:

$$logK_{IJ}^{pot} = \frac{(E_J - E_I)z_I F}{2.303RT} + (1 - z_I/z_J)\, loga_I \quad (1)$$

Plots of selectivity of electrode (III) to interfering molecules and ions are shown in figure (7)

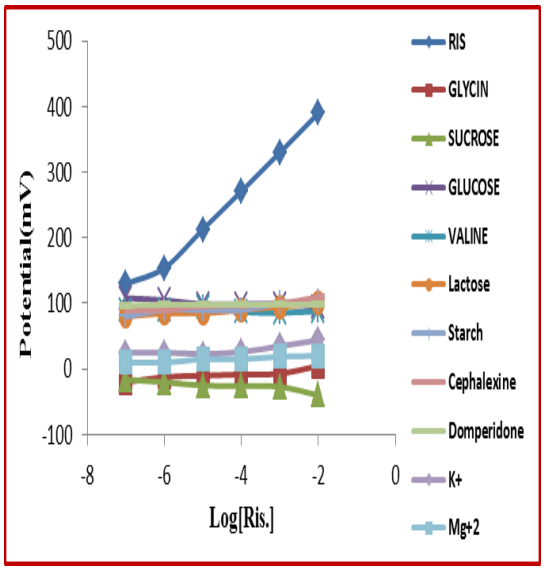


Figure (7): Selectivity of the electrode (III) to interfering species by separate solution methods (SSM).

Table (2): Selectivity coefficient values of some interfering species calculated by separate solution method using electrodes (III).

Interfering Species	$K^{pot}_{Ris.,J}$ at $a_A = a_B$					
	1.0×10^{-7} M	**1.0×10^{-6} M**	**1.0×10^{-5} M**	**1.0×10^{-4} M**	**1.0×10^{-3} M**	**1.0×10^{-2} M**
K^+	2.8×10^{-3}	1.5×10^{-3}	1.6×10^{-4}	1.8×10^{-5}	1.9×10^{-6}	2.9×10^{-7}
Mg^{+2}	2.9×10^{-6}	3.6×10^{-6}	1.4×10^{-6}	4.6×10^{-7}	2.5×10^{-7}	5.4×10^{-8}
Tartaric acid	7.6×10^{-3}	2.9×10^{-3}	2.6×10^{-4}	5.6×10^{-5}	1.8×10^{-5}	1.7×10^{-6}
Glycine	2.8×10^{-3}	1.5×10^{-3}	1.6×10^{-4}	1.8×10^{-5}	1.9×10^{-6}	2.9×10^{-7}
Valine	2.1×10^{-1}	8.2×10^{-2}	1.0×10^{-2}	7.6×10^{-4}	7.3×10^{-5}	7.6×10^{-6}
Glucose	4.2×10^{-1}	1.5×10^{-1}	1.2×10^{-2}	1.2×10^{-3}	1.2×10^{-4}	1.0×10^{-5}
Sucrose	3.6×10^{-3}	1.1×10^{-3}	9.2×10^{-5}	9.3×10^{-6}	8.9×10^{-7}	5.2×10^{-8}
Lactose	1.4×10^{-1}	6.8×10^{-2}	6.8×10^{-3}	8.6×10^{-4}	1.5×10^{-4}	1.2×10^{-5}
Starch	1.4×10^{-1}	8.2×10^{-2}	8.2×10^{-3}	8.6×10^{-4}	1.9×10^{-4}	1.4×10^{-5}
Cephalexin	2.1×10^{-1}	8.2×10^{-2}	1.0×10^{-2}	1.1×10^{-3}	1.9×10^{-4}	1.8×10^{-5}
Domperidone	2.7×10^{-1}	11×10^{-1}	1.1×10^{-2}	1.2×10^{-3}	1.7×10^{-4}	1.1×10^{-5}

Validation of the proposed method

The proposed sensor no.(III) was successfully applied for the determination of (Ris.) in pure solutions. Four solutions of (Ris.) with different concentrations were prepared and analyzed with triplicate measurements. The validation of the method was achieved by the accuracy and precision test of the proposed sensor (III) expressed in terms of percent relative error and percent relative standard deviation for inter-day (repeated over 3 days) and intra-day (repeated independently three times a day) measurements of the prepared solutions. The results are shown in table (3). The recoveries of (Ris.) for each concentration were also calculated. The results have shown good accuracy and precision.

Table (3): Validation of the proposed method for the determination of (Ris.) in pure form

	Taken [Ris.] Mole /L	***Found [Ris.] Mole /L**	**RE %**	**RSD %**	**Recovery %**
Intra-day	1.0000×10^{-6}	1.0200×10^{-6}	2.0000	0.6792	102.0000
	1.0000×10^{-5}	1.0400×10^{-5}	4.0000	1.9208	104.0000
	1.0000×10^{-4}	9.9800×10^{-5}	-0.2000	2.2672	99.8000
	1.0000×10^{-3}	9.9700×10^{-4}	-0.3000	2.2682	99.7000
	Taken [Ris.] Mole /L	**Found [Ris.] Mole /L**	**RE %**	**RSD %**	**Recovery %**
Inter-day	1.0×10^{-6}	1.0500×10^{-6}	-1.0000	2.2300	105.0000
	1.0×10^{-5}	1.0400×10^{-5}	2.0000	2.3046	104.0000
	1.0×10^{-4}	9.7200×10^{-5}	-2.8000	2.2404	97.2000
	1.0×10^{-3}	1.0100×10^{-3}	1.0000	2.2389	101.0000

***Average of three determinations**

Determination of (Ris.) in pharmaceutical formulations

The direct, the standard addition (S.A) and multiple standard addition (M.S.A) methods were applied for the determination of (Ris.) in two types of pharmaceutical formulations; Rispal[@] 2mg caplets and Rispond 4mg tablets. The results obtained using graphite coated electrode no.(III) are listed in Table (4).

Table (4): Analysis of (Ris.) in some pharmaceutical formulations by the direct and the standard addition methods using electrode (III).

Sample	Pot. Methods	Taken [Ris.] M	Found [Ris.] M	Weight found mg/ dosage	*RSD%	Rec %
Respal 2mg	**Direct**	1.0000×10^{-4}	0.9400×10^{-4}	1.8800	2.2451	94.0000
		1.0000×10^{-5}	0.9400×10^{-5}	1.8800	5.2477	94.0000
		1.0000×10^{-6}	0.9880×10^{-6}	1.9760	0.1168	98.8000
	SAM	1.0000×10^{-4}	1.0409×10^{-4}	2.0818	0.7433	104.0900
		1.0000×10^{-5}	1.0137×10^{-5}	2.0274	1.1628	101.3700
		1.0000×10^{-6}	1.0904×10^{-6}	2.1808	0.4336	109.0400
	MSA	1.0000×10^{-4}	1.0010×10^{-4}	2.0020	0.3539	100.1000
		1.0000×10^{-5}	1.0009×10^{-5}	2.0018	0.4965	100.0900
		1.0000×10^{-6}	0.9917×10^{-6}	1.9834	0.3920	99.1700
Rispond 4mg	**Direct**	1.0000×10^{-4}	0.9120×10^{-4}	3.6480	0.1064	91.2000
		1.0000×10^{-5}	9.9900×10^{-5}	3.996	1.8148	99.9000
		1.0000×10^{-6}	0.9910×10^{-6}	3.9640	0.8159	99.1000
	SAM	1.0000×10^{-4}	1.0129×10^{-4}	4.0516	1.2912	101.2900
		1.0000×10^{-5}	0.9889×10^{-5}	3.9556	1.1724	98.8900
		1.0000×10^{-6}	0.9678×10^{-6}	3.8712	0.4419	96.7800
	MSA	1.0000×10^{-4}	0.9905×10^{-4}	3.9620	0.1426	99.0500
		1.0000×10^{-5}	0.9904×10^{-5}	3.9616	0.0761	99.0400
		1.0000×10^{-6}	1.0115×10^{-6}	4.0460	0.0653	101.1500

***Average of three determinations**

Multiple standard addition (M.S.A) method has exhibited better results over the direct method. The method showed RSD% ranged between (0.0653-0.4965) and Rec% ranged between (99.0400 - 101.1500). The reported percent recoveries of the drugs in Table 4 were calculated using the values reported by the manufacturer which was obtained using the recommended assay by the British Pharmacopoeia.

Binding characteristics

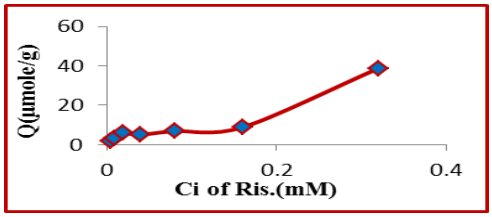
To understand the way how small molecules interact with adsorbent surface, the mode of binding and site distributions in the interaction, adsorption isotherms are an important tool obtained by plotting of the equilibrium concentrations of bound MIP versus free drug. In liquid-phase applications of MIPs, a molecule in solution interacts with binding sites in a solid adsorbent, and after equilibrium the free drug concentration becomes constant and is easily quantified to plot the corresponding adsorption isotherm. [21]

This was determined by plotting the binding capacity (Q) against the free (Ris.). Q was calculated according to the following equation: [22]

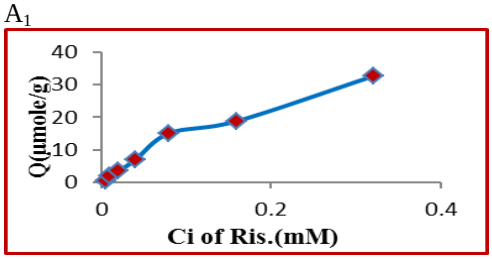
$$Q = \frac{(C_i - C_f)V_s \times 1000}{mass_{MIP}} \qquad (2)$$

Where Q is the binding capacity of MIPs (µmol/g), C_i is the initial (Ris.) concentration (mM), C_{free} is the final (Ris) concentration (mM), V_s is the volume of test solution (L) $mass_{MIP}$ is the mass of the dried MIP (g).

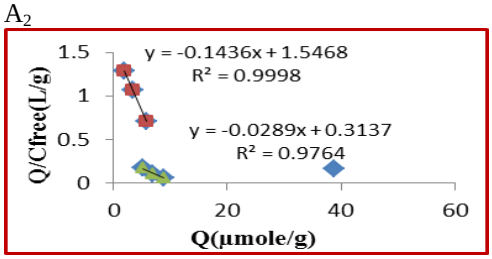
The adsorption isotherms obtained after shaking varying concentrations of (Ris.) with the synthesized particles for 2 hours in a thermostatic water bath shaker at 25°C were plotted in Figs. (8).



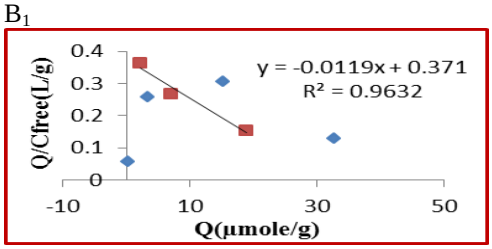
A_1



A_2



B_1



B_2

Figure (8): A_1,A_2,B_1,B_2 are binding isotherm and Scatchard plots of Ris- MIP(AA) and Ris-MIP(AAm) respectively.

In general, adsorption data showed that the binding capacity of Ris-MIPs increased with increasing initial concentrations of (Ris.), leading to saturation at higher concentrations. The binding parameters of the binding Ris-MIPs were calculated by Scatchard analysis, with the following equation:

$$\frac{Q}{C_{free}} = \frac{Q_{max} - Q}{K_d} \qquad (3)$$

Where C_{free} is the free analytical concentration at equilibrium (mM), Q_{max} is the maximum apparent binding capacity. Kd is the dissociation constant at binding site.

The equilibrium dissociation constant was calculated from the slopes and the apparent maximum number of binding sites from the y-intercepts in the linear plot of Q/C_i vs. Q as shown in Fig. (8).The Scatchard plot for MIP based on (AA) as a monomer was not linear in all (Ris.) concentration ranges, suggesting that the binding sites in the MIP were not uniform. The plot shows two distinct sections that can be regarded as straight lines, revealing two types of distributing binding sites in the MIP. The equilibrium dissociation constant $Kd_{1(AA)}$ and the apparent maximum amount $Q_{max1(AA)}$ for the higher affinity binding sites were calculated to be 34.6021 µM and 10.8547 µmol/g respectively for the dry polymer. Using the same treatment, $Kd_{2(AA)}$ and $Q_{max2(AA)}$ for the lower affinity binding sites were calculated to be 6.9638 µM and 10.7716 µmol/g respectively. In contrast, when (AAm) was used as a monomer, the Scatchard plot was linear in all concentration ranges, suggesting that the binding sites were homogeneous and of one type. The equilibrium dissociation constant $Kd_{(AAm)}$ and the apparent maximum amount $Q_{max(AAm)}$ for the higher affinity binding sites were calculated to be 84.0336 µM and 31.1765 µmol/g respectively for the dry polymer [12] .

According to the equilibrium dissociation constants (Kd) of the two acidic and basic Ris -MIPs, it was noticed that basic Ris-MIP has homogenous binding site distributions which lead to better results in its application than acidic Ris-MIP which exhibited high (Kd) value as well as heterogeneous binding sites distributions. [11, 22]

Conclusions

The molecular imprinting technique was employed to produce (Ris.) host – tailored sensors incorporated in PVC matrix and coated on a graphite electrode for potentiometric transduction. The electrodes were successfully used for the determination of (Ris.) in their pure form and pharmaceutical formulations. The MIP based electrodes using (AAm) showed a higher affinity for the templates (Ris.) than (AA) based electrodes in the sense of their Nernsation slopes. The electrodes exhibit a short response time good stability, sensitivity, selectivity and life time more than two months. The rebinding procedure confirms the performance of (Ris.) MIP GCE based on (AAm) as basic monomer.

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