PREPARATION OF A NEW MOLECULARLY IMPRINTED POLYMERS AND ITS USE IN THE SELECTIVE EXTRACTION FOR DETERMINATION BROMHEXINE HYDROCHLORIDE AT PHARMACEUTICALS

A. R. Mahdi [(1)] Y. K. Al-Bayati [(2)] S. T. Ameen [(1)]
Researcher Assist. Prof. Prof.
Department of Chemistry, College of Science, University of Tikrit, IRAQ
Department of Chemistry, College of Science, University of Baghdad, IRAQ
Email: yahyaalbayti@yahoo.com

ABSTRACT

This study was aimed four electrodes were synthesized based on molecularly imprinted polymers (MIPs). Two MIPs were prepared by using bromhexine hydrochloride (BHH) as the template, acryl amide (AA) and methyl methacrylate (MMA) as monomers as well as ethylene glycol dimethacrylate (EGDMA) and penta erythritol triacrylate (PETA) as cross linkers respectively and benzoyl peroxide as initiator. The same composition was used in preparation of non-imprinted polymers (NIPs), but without the template (Bromhexine hydrochloride). To prepare the membranes, different plasticizers were used in PVC matrix such as: Di butyl sebacate (DBS), acetophenone (AP), di-octyl phthalate (DOPH) and tri-2-ethyl hexyl phosphate (TEHP). The characteristics studied are the slop, detection limit, life time and linearity range of BHH–MIPs electrodes. Results obtained of selectivity measurements on interfering cations (Al^{+3}, Ca^{+2}, K^{+}) and some pharmaceutical additives such as methylparaben, propylparaben, trisodium citrate show that no interfering with drug bromhexine hydrochloride. The preparation electrodes have been shown good response including testing pharmaceutical analysis.

Keywords: Molecularly imprinted electrodes, bromhexine hydrochloride, potentiometric method, (MMA), (AA) monomers, different plasticizers.

مجلة العلوم الزراعية العراقية -2019 :50(3):886-900 مهدي وآخرون

تحضير بوليمرات طبعة جزيئية جديدة واستخدامها في الاستخلاص الانتقائي لتقدير برومهيكسين هيدروكلوريد في المستحضرات الصيدلانية

عدنان رضا مهدي[(1)] يحيى كمال البياتي[(2)] سهام توفيق أمين[(1)]
باحث أستاذ مساعد أستاذ
قسم الكيمياء – كلية العلوم / جامعة تكريت / العراق ، قسم الكيمياء – كلية العلوم / جامعة بغداد / العراق

المستخلص

هدفت هذه الدراسة إلى تصنيع أربعة أقطاب بناءً على بوليميرات طبعة جزيئيَّة (MIPs). تم تحضير اثنين من MIPs باستخدام برومهيكسين هيدروكلوريد (BHH) كقالب، أكريل أميد (AA) وميثيل ميثاكريلات (MMA) كمونومرات وكذلك إيثيلين كلايكول ثنائي ميثاكريلات (EGDMA) وبنتا إريثريتول تراي أكريليت (PETA) كمواد تشابك وبنزويل بيروكسيد كبادئ. واستخدمت نفس التركيبة في تحضير البوليمرات غير المطبوعة (NIPs) ، ولكن بدون القالب (Bromhexine hydrochloride). لتحضير الأغشية، تم استخدام مواد ملدنة مختلفة مع بولي فاينيل كلورايد كساند مثل: ثنائي بيوتيل سبكيت (DBS) ، الأسيتوفينون (AP) ، ثنائي أوكتيل الفثالات (DOPH) وثلاثي إيثيل هكسيل الفوسفات (TEHP). الخصائص التي شملتها الدراسة هي الميل ، حد الكشف ، العمر الزمني ومدى الخطية لأقطاب MIPs––BHH. أظهرت النتائج التي تم الحصول عليها من قياسات الانتقائية على بعض الكاتيونات المسببة للتداخل (K^{+}, Ca^{+2}, Al^{+3}) وبعض المضافات الصيدلانية مثل الميثيل بارابين والبروبيل بارابين وثلاثي سيترات الصوديوم أنه لا يوجد تداخل مع دواء برومهيكسين هيدروكلوريد. وقد أظهرت ألاقطاب المحضرة استجابة جيدة تضمنت اختبار التحليل الصيدلاني.

الكلمات المفتاحية: أقطاب الطبعة الجزيئية، برومهيكسين هيدروكلورايد، طريقة جهدية، مونمرات (MMA) و (AA)، ملدنات مختلفة

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INTRODUCTION

Bromhexine hydrochloride [N-(2-Amino-3,5-dibromobenzyl)-N-methylcyclohexanamine hydrochloride]. Bromhexine hydrochloride is a white or almost white, crystalline powder, very slightly soluble in water, slightly soluble in alcohol and in methylene chloride [17]. Bromhexine hydrochloride is a mucolytic agent that reduces sputum thickness, and works by breaking down mucus so it is easier to cough out. It helps patients breathe deeply, and is used to treat abnormal mucus secretions, including influenza, respiratory tract infections, and the common cold. In an over-the-counter form, bromhexine hydrochloride is used to treat cheat congestions and coughs [6] . Bromhexine hydrochloride's side effects include rash, diarrhea, vomiting, nausea, and pain in the upper stomach. This medication should not be used by pregnant or breastfeeding women. This medication should be avoided in patients with gastric ulcers due to the increased risk of adverse events[22]. The chemical formula of bromhexine hydrochloride is $C_{14}H_{20}Br_2N_2.HCl$, (M.Wt.:412.6 $g.mol^{-1}$) and its structural formula show in Fig. 1.

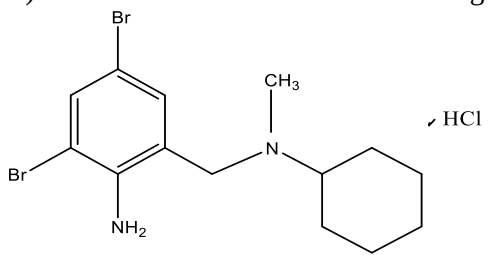

Fig. 1. Structure of Bromhexine hydrochloride

According to review, bromhexine hydrochloride was determination by several methods such as these recent methods that employed reverse phase-high performance liquid chromatography[16, 19] , spectrophotometry[14], potentiometric-flow injection[11] , electrochemiluminescence system[12], TLC densitometric[18]. Several techniques used for determination drugs and pharmaceuticals. The selective electrodes technique one of these techniques that used for determination bromhexine hydrochloride because this technique has many characteristics like fast response time, easy used, rapid, low cost and selectivity. The potentiometric sensors techniques that is based on PVC membranes electrodes widely available used for analysis of drugs and ionic species[9,21,7]. Molecularly imprinted polymers (MIPs) were used the bromhexine hydrochloride as the template while the monomers which used the acryl amide (AA) and methyl methacrylate (MMA) as monomers as well as ethylene glycol dimethacrylate (EGDMA) and penta erythritol triacrylate (PETA) as cross linkers respectively and benzoyl peroxide as initiator to achieved the polymerization process. There are a variety of ion selective electrode determined drugs that depended on MIPs as re cognition membranes like ibuprofen [1], warfarin [4], phenytoin [3] and metronidazole benzoate [2]. In this study used different plasticizers to the construction of membranes electrodes based on BHH-MIPs such as di butyl sebacate (DBS), acetophenone (AP), di-octyl phthalate (DOPH) and tri-2-ethyl hexyl phosphate (TEHP).

MATERIALS AND METHODS

Preparation of MIP and NIP

For preparation first bromhexine hydrochloride molecularly imprinted polymer (BHH-MIP1), 0.485 mmol (0.20 g) from bromhexine hydrochloride then mixed with 9.980 mmol (1.00 g) methyl methacrylate as the monomer, after that added 11.296 mmol (3.37 g) penta erythritol triacrylate to the solution as the cross linker, followed that added 0.099 mmol (0.02 g) benzoyl peroxide as the initiator. All these materials were dissolved in 5±mL methanol (CH_3OH). While the second bromhexine hydrochloride molecularly imprinted polymer (BHH-MIP2) were achieved by mixed 0.227 mmol (0.09g) from bromhexine hydrochloride as the template, 1.500 mmol (0.10g) acryl amide as the monomer, 15.00 mmol (2.97g) ethylene glycol dimethacrylate as the cross linker and 0.099mmol (0.02 g) benzoyl peroxide as the initiator which dissolved in 5±mL of methanol (CH_3OH). For obtained a homogeneous solution, the mixture was stirred for 5 minutes. N_2 passes for 30 minutes on the mixture to remove oxygen from solution. After that the solution was placed in a water bath at 60˚C. when the reaction completes the molecularly imprinted polymer became hardened, after the polymerization process the polymer was draying and crashed to obtain a polymer particle. These particles were sonicated in CH_3OH / CH_3COOH (40:4 v/v) to remove the template from MIP. The particles size of BHH-MIP1 and BHH-MIP2 were between 43 – 60 µm and 75-125µm respectively. The

preparation of non-molecularly imprinted polymers using the same substances and conditions that formed BHH-MIP1 and BHH-MIP2 but without the bromhexine hydrochloride (template). The same composition was used in preparation of non-imprinted polymers (NIPs), but without the template (Bromhexine hydrochloride).

Instruments

Ion analyzer used in this work (WTW model, Germany), a pH meter (WTW model pH 720, Germany), and a saturated calomel electrode (Gallenkamp, USA). The electrode BHH-MIP used was construction in the laboratory and all potentiometric measurements was made at room temperature. The bromhexine hydrochloride-MIP electrode combined with Ag-AgCl electrode and the reference electrode was 0.1 M internal solution of bromhexine hydrochloride. The PVC tube (1-4 cm long) was flattened and polished by putting it on a glass plate and soaking with THF. The membrane was cut similar to the external diameter of the PVC tubing and pasted on the polished end. The other direction of the PVC tubing was then linked to the electrode body. To make the electrodes more sensitive were by soaking in 0.1 M bromhexine hydrochloride solution for at least (2-3 hours) before the use of the electrodes.

Materials and chemicals

1. Standard bromhexine hydrochloride obtained from industries of pharmaceuticals (IRAQ-SDI -Samara). Solvodin 20 tablets 8 mg from (SDI -Iraq), Bisolvon 20 tablets 8 mg from (Boehringer Tngelheim- Germany), Mucolyte 20 tablets 8 mg from (Julphar-U.A.E.) were purchased from local pharmacies
2. Plasticizers, di butyl sebacate (DBS) (97.0% purity), acetophenone (AP) (99.9% purity), di-octyl phthalate (DOP) (99.5% purity), Tri-2-ethyl hexyl phosphate (TEHP) (97.0% purity), were purchased from Sigma Aldrich. Other chemicals and reagents materials were obtained from Fluka, BDH and Sigma Aldrich

Preparing of standard solutions

1.For preparing standard solution of 0.1 M bromhexine hydrochloride by dissolving 2.063 g of standard bromhexine hydrochloride in methanol and completed to 50 mL in the volumetric flask. The other solutions were prepared in 25 mL at the ranged from 10^{-6} – 10^{-1} M in the same procedure

2. The stock standard solution of 1×10^{-3} M, 1×10^{-4} M, phospho molybdic acid was prepared by dissolving 0.225g, 0.022g respectively in distilled water and completed to 100 mL.

3. All interfering cations (Al^{+3}, Ca^{+2}, K^{+}) and some pharmaceutical additives such as methylparaben, propylparaben, trisodium citrate 0.1 M stock solution prepared at ranged from 10^{-6} - 10^{-1} M which present the interfering ions were prepared and diluted to 100 mL.

Synthesis of membrane molecularly imprinted polymers electrode

Bromhexine hydrochloride membrane was immobilized into the PVC tube as portrayed by Thomas and Moody[15] · BHH-MIP of 0.036g was mixed with different of plasticizers 0.4g used in this work such as: DBS (electrode A1), AP (electrode A2), DOPH (electrode B1) and TEHP (electrode B2). Then added 0.17g of PVC powder was scattered on 7± mL of tetra hydro furan with stirring until a clear viscous solution was acquired. Then the solutions mixed with stirring until the mixture became homogeneous. The mixture was casted into a glass ring 30-35 mm diameter and unwind on a glass plate and a ribbon of filter was placed on top of the glass. The solvent was then allowed to evaporate according to room temperature more than 24-48 hours at least. The thickness of the membrane obtained was different of membrane to other's, the thickness was about 0.4 -0.7 mm. That size of membrane was adequate to prepare electrodes.

Scanning electron microscope SEM

In scanning electron microscopy, a fine beam of electrons scans the membrane surface. This causes several kinds of interactions generating different signals, also used in image formation. The SEM can be used to get an idea about the size, geometry, and distribution of pore surface of the membranes. SEM analysis showed the highly ordered and regular pore structure of the molecular imprinted polymer surface and the cross-section. Several papers showed that the molecular imprinted membranes recognized the template molecule effectively and transported it with good efficiency due to porous structures of the molecular imprinted

polymer. The ordered porous and cross section on surface shows the sites of interaction, and MIP showed the highest transport rate towered the template molecule. The morphology of MIP before and after washing showed by electron microscope in Figure (2a, 2b) and Figure (3a, 3b). Micro emulsion polymerization gives very small particles size around (600-850) nm for methyl methacrylate (MMA) polymer and (400-1,000) nm for acryl amide (AA) polymer can be distinguished in the related image.

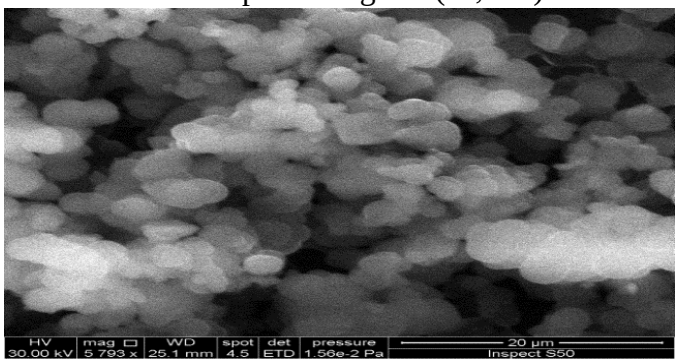

Fig. 2a. SEM for the MIP1 before washing

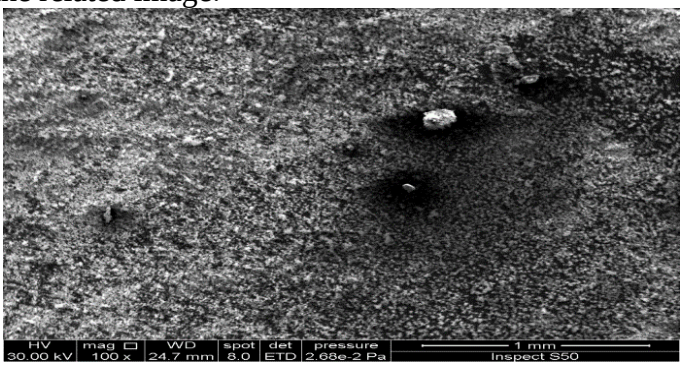

Fig.2b. shows the SEM for MIP1after washing

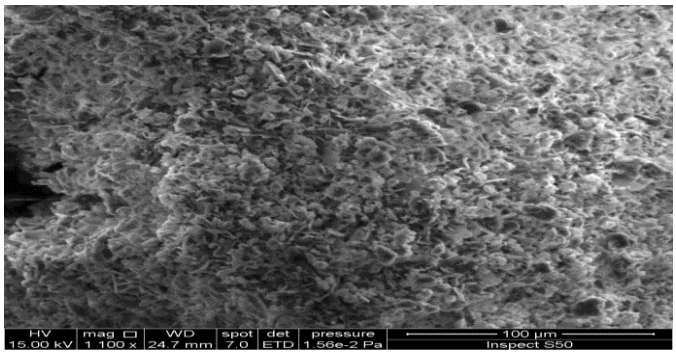

Fig. 3a. SEM for the MIP2 before washing

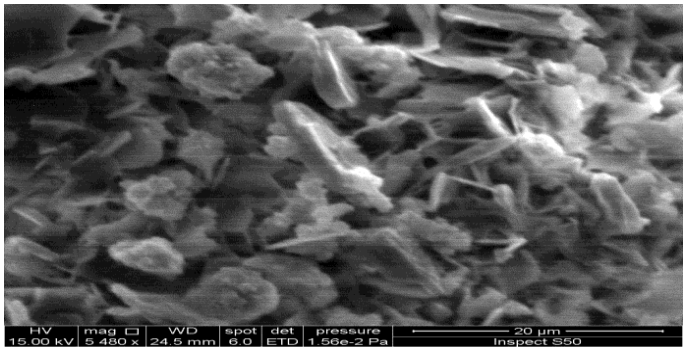

Fig.3b. shows the SEM for MIP2 after washing

The morphology of the prepared membrane using the BHH-MIP1 before washing is shown in figure 2a and after washing is shown in figure 2b. Figure 2a (before washing) reveals that the particles of the complex are formed in a regular spherical shape with an average of about 200 µm in diameter. In other hand, figure 2b (after washing) shows that the formed particles look like a colloidal particle growing in a solution, this might take place due to a presence of excess of DFS that form ionic atmosphere surrounding the complex and create the formation of electric double layers.

Construction of ion-selective electrodes

The building of the electrode body and the immobilization were achieved as portrayed by Mahajan et al [(13)]. Bromhexine hydrochloride solution (0.1) M was filled in the glass tube as an internal solution. Preferred immersing the membrane in standard solution of (0.1) M of bromhexine hydrochloride for at least (2-3) hours before measurements which represents stipulations of membrane electrode

Preparation of pharmaceutical samples

To obtained the powder of tablets pharmaceutical samples using pestle and mortar to grinding the tablets then taken a suitable weight for preparation in 50 mL of solutions .Used appropriate amount of methanol (CH_3OH) for dissolved pharmaceutical samples and complete to 50 mL in the volumetric flask by methanol as well as using the magnetic stirrer for more than 30 minutes .After that filtered the solution by using 0.07µm cellulose filter paper for preparing and obtained the concentrations of 1 $x10^{-3}$ M and 1 $\times10^{-4}$ M bromhexine hydrochloride.

RESULTS AND DISCUSSION

Ion-selective electrodes (ISE) is one of the sensors that considered most common which used voltage through measurements. Used this measurement in the laboratory tests, industry,

process control, physiological measurements, and environmental monitoring. [5] Electrodes membranes that responded to the concentration analysis using a chemical reaction to generate ions that can be monitored with ion selective electrode [20]. These membrane electrodes included two main categories are ions selective electrodes which be sensitive to ionic species and molecular selective electrodes that applied to determination of molecular analytes [8,15]. The principle working of ion-selective electrodes consist of two different types of electrical conductivity which are in metals the electric current is carried by electrons while in Liquids the electric current is carried by ions [13]. The measurement of conductivity for each electrochemical process can be achieved in one of this type galvanic cell, electrolysis and electrical analysis. This type of cells must be contact with the solution on both sides of the cell membrane also there is some ISE arrangements with wire connection to one side of the membrane. Traditional composition of the cell is:

Outer ref. / Test solution / membrane/ internal ref.

Or

Outer ref. / Test solution / ion-selective electrode

The current which passed through the electrolytical cell must be equals zero depending on this condition the cell is designed according to the basic rule of designing of electrolytic cells.

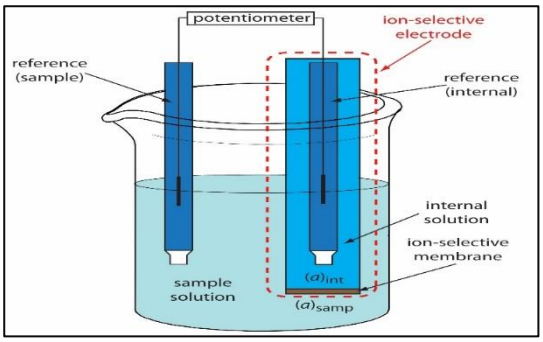

Fig. 4. Schematic diagram showing a typical potentiometric cell with an ion-selective electrode

Two MIPs have been prepared by using the bromhexine hydrochloride as the template, acryl amide (AA) and methyl methacrylate (MMA) as monomers as well as ethylene glycol dimethacrylate (EGDMA) and penta erythritol triacrylate (PETA) as cross linkers respectively and benzoyl peroxide as initiator. A plasticizer is an important component in an ISE membrane. Compatibility with the polymer and other membrane constituents provides a homogeneous environment for membrane when the plasticizers using as a solvent for the membrane practical use of ISE membrane should be avoided leaching of the plasticizer, otherwise it would affect the electrode performance over time. Four electrodes have been construct based on PVC matrix. These plasticizers such as: di butyl sebacate (DBS), acetophenone (AP), di-octyl phthalate (DOP), Tri-2- ethyl hexyl phosphate (TEHP). The characteristics was studied for all electrodes based on BHH-MIP1 (A1, A2 membranes) and BHH-MIP2 (B1, B2 membranes) which included linearity range, correlation coefficients, detection limit (M) and life time (day) respectively. The results obtained showed in the table 1 and figure 5.

Table 1. Characteristics of the bromhexine hydrochloride-MIP electrode based on different functional monomers and plasticizers

Membrane composition	BHH-MIP1 + DBS (A1)	BHHMIP1 +ACPH (A2)	BHH-MIP2 +DOPH (B1)	BHH-MIP2 +TEHP (B2)
Slop (mV/decade)	-18.02	-20.45	-19.47	-17.00
Linearity range (M)	5×10^{-5}-1×10^{-1}	5×10^{-5}-1×10^{-1}	5×10^{-5}-1×10^{-1}	5×10^{-5}-1×10^{-1}
Correlation coefficient	0.9998	0.9998	0.9932	0.9995
Detection limit (M)	2.6×10^{-5}	1.8×10^{-5}	9.2×10^{-6}	9×10^{-6}
Life time (day)	14	9	19	8

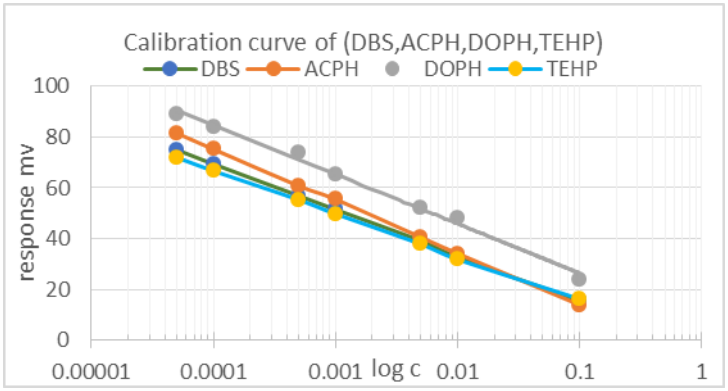

Fig. 5. Calibration curve for BHH-MIP1 and BHH-MIP2 membranes electrodes

Effect of pH on electrodes response

The study of pH effected on BHH-HCl membranes electrodes by prepared various concentrations of BHH-HCl (1×10^{-2}, 1×10^{-3} and 1×10^{-4}) M. To measurement the selective pH at ranged (1-11) by using the hydrochloric acid (0.1 M ,1 M) and/or sodium hydroxide (0.1 M ,1 M) for pH studies. The results obtained by adding appropriate volume of HCl/NaOH as shows in the Table (2) and Fig. (6,7). The change in potentials at differential pH values my be due to the composition of electrodes. This composition also effects on response and life time for electrodes.

Table 2. Working pH range for Bromhexine Hydrochloride Selective electrode

Number and composition of MIPs	Membranes	Membrane composition	pH range		
			1×10^{-2}M	1×10^{-3}M	1×10^{-4}M
MIP1	A1	BHH-MIP1 +DBS	3.5-8.5	3.0-8.5	3.0-8.5
BHH+MMA+PEHP	A2	BHH-MIP1 +ACPH	3.5-8.0	4.0-8.5	3.5-8.5
MIP2	B1	BHH-MIP2+DOPH	2.5-9.5	3.5-9.0	3.0-9.5
BHH+ AA +EGDMA	B2	BHH-MIP2 +TEHP	3.5-8.0	4.0-7.5	4.0-7.5

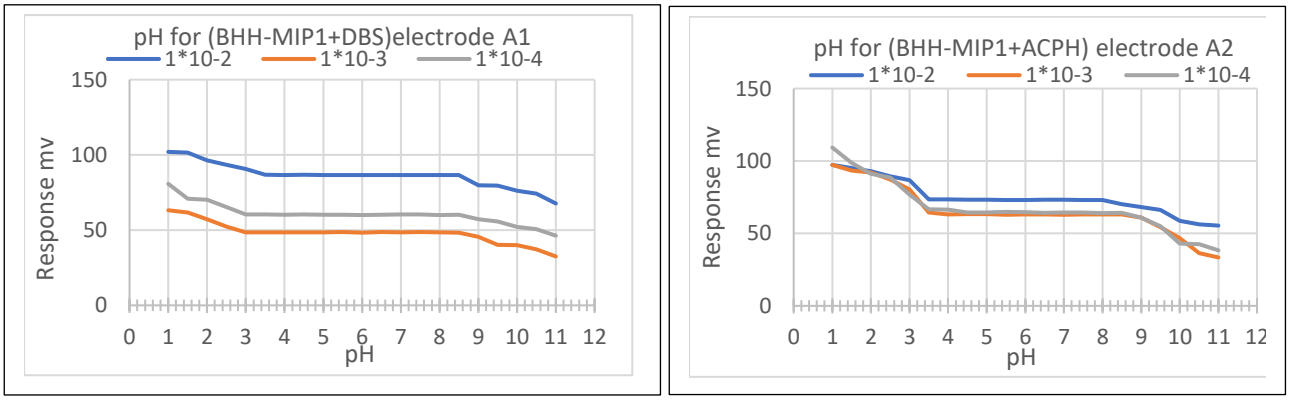

Fig. 6. Effect of pH on the Bromhexine Hydrochloride {BHH-MIP1 + DBS (A1) and BHH-MIP1 +ACPH (A2)} electrodes at concentration 1×10^{-2}, 1×10^{-3} and 1×10^{-4}M

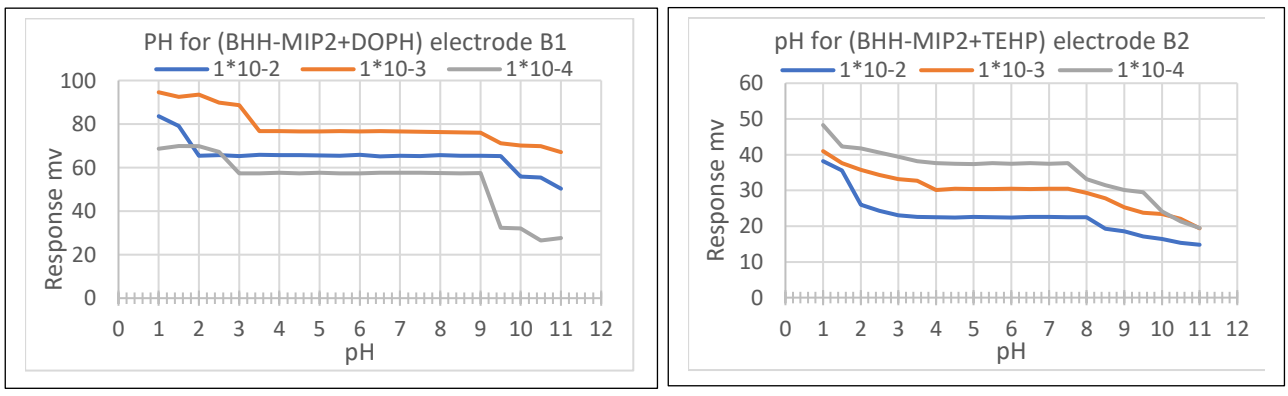

Fig.7. Effect of pH on the Bromhexine Hydrochloride {BHH-MIP2 + DOPH (B1) and BHH-MIP2 +TEHP (B2)} electrodes at concentration 1×10^{-2}, 1×10^{-3} and 1×10^{-4}M.

Interference studies

For calculated the selectivity coefficient measurement was used the separate solution method. Used the separate equation for these measurements according to the equation below.

Log K pot= [(EB − EA)/ (2.303RT/z F)] + (1 –zA/zB) log Aa

EA, EB; zA, zB; and aA, represents the potentials, charge numbers, and activities for the primary A and interfering B ions, respectively at aA = aB, R, the ideal gas constant (3.314 joule $mole^{-1}$ K^{-1}), K^{pot}, selectivity coefficient. The results obtained for selectivity coefficients of primary ion and interfering ions like cations and some pharmaceutical additives used in this work. The selectivity coefficients depend on charges of both primary ion and interfering ions also depends on concentration as well as the composition of electrodes. All values for selectivity coefficients were listed in the table 3, 4, 5, 6 and figures (8, 9).

Table 3. Selectivity coefficients for (BHH –MIP1 +DBS) electrode at different concentrations of bromhexine hydrochloride

Interfering ions	Concentrations of Bromhexine. HCl (M): Concentration of interference ions (M)													
	10^{-1}		10^{-2}		5×10^{-3}		1×10^{-3}		5×10^{-4}		1×10^{-4}		5×10^{-5}	
	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$
K^+	18.3	0.6393	33.8	0.6024	42.5	0.3712	53.8	0.5759	62.4	0.3589	71.3	0.5824	65.5	0.6162
Ca^{+2}	19.2	0.1896	37.1	0.4864	39.8	0.5824	50.3	0.7378	57.6	0.5263	72.3	0.6519	71.9	0.3840
Al^{+3}	17.3	0.1377	33.6	0.5088	49.0	0.1458	59.7	0.2313	62.4	0.2708	70.8	0.4975	80.6	0.1017
T. S. C	19.2	0.0727	34.6	0.1075	44.8	0.1746	56.2	0.3280	65.3	0.2739	72.3	0.2589	71.5	0.0950
M. P.	19.5	0.5555	38.7	0.3840	44.2	0.4017	58.6	0.4062	62.3	0.3712	75.9	0.3431	75.3	0.3589
P. P.	23.6	0.3375	49.1	0.1075	46.8	0.1746	54.2	0.3280	60.8	0.2739	70.6	0.2589	74.3	0.0950

Table 4. Selectivity coefficients for (BHH –MIP1 +ACPH) electrode at different concentrations of bromhexine hydrochloride

Interfering ions	Concentrations of Bromhexine. HCl (M): Concentration of interference ions (M)													
	10^{-1}		10^{-2}		5×10^{-3}		1×10^{-3}		5×10^{-4}		1×10^{-4}		5×10^{-5}	
	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$
K^+	21.6	0.2211	33.8	0.4598	45.7	0.3431	67.2	0.4249	73.9	0.5505	79.6	0.4249	82.3	0.2589
Ca^{+2}	24.3	0.1374	43.8	0.01746	52.3	0.0097	62.8	0.0040	64.4	0.0120	86.2	0.0006	87.1	0.0017
Al^{+3}	44.6	0.0476	47.2	0.0082	49.0	0.0073	58.9	0.0023	67.3	0.0020	77.3	0.0005	80.6	0.0001
T. S. C	49.2	0.1096	54.8	0.0161	59.8	0.0122	67.6	0.0023	72.3	0.0021	79.3	0.0012	84.6	0.0006
M. P.	47.9	0.1595	52.6	0.2067	59	0.1806	65.3	0.1362	69.8	0.1347	74.1	0.1458	82.3	0.1393
P. P.	47.2	0.5146	54.3	0.2997	60.2	0.2801	69.2	0.1560	72.0	0.2531	76.0	0.2678	79.3	0.2801

Table 5. Selectivity coefficients for (BHH –MIP2 +DOPH) electrode at different concentrations of bromhexine hydrochloride

Interfering ions	Concentrations of Bromhexine. HCl (M): Concentration of interference ions (M)													
	10^{-1}		10^{-2}		5×10^{-3}		1×10^{-3}		5×10^{-4}		1×10^{-4}		5×10^{-5}	
	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$
K^+	35.7	0.0204	40.3	0.0592	49.3	0.3431	67.2	0.4249	73.9	0.5505	79.6	0.4249	92.3	0.2589
Ca^{+2}	75.3	0.0334	79.9	0.0066	84.6	0.0050	88.1	0.0021	92.8	0.0014	100.6	0.0002	102.7	0.0003
Al^{+3}	64.7	0.0068	69.7	0.0012	78.6	0.0004	84.6	0.0001	98.7	0.0002	104.3	0.0006	112.3	0.0001
T. S. C	46.1	0.0150	53.6	0.0059	68.9	0.0009	72.4	0.0004	88.3	0.0004	97.4	0.0001	105.6	5×10^{-6}
M. P.	75.0	0.0298	79.3	0.0235	84.3	0.0143	88.2	0.0111	91.3	0.0131	97.6	0.0148	105.7	0.0104
P. P.	42.0	0.0235	49.7	0.0513	58.2	0.0961	64.7	0.1247	78.9	0.0445	88.3	0.0715	98.7	0.0415

Table 6. Selectivity coefficients for (BHH –MIP2 +TEHP) electrode at different concentrations of bromhexine hydrochloride

Interfering ions	Concentrations of Bromhexine. HCl (M): Concentration of interference ions (M)													
	10^{-1}		10^{-2}		5×10^{-3}		1×10^{-3}		5×10^{-4}		1×10^{-4}		5×10^{-5}	
	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$	E_B (mv)	$K_{A,B}$
K^+	62.3	0.0388	68.7	0.0367	72.5	0.0559	76.2	0.0733	79.5	0.0649	83.4	0.0530	87.9	0.0817
Ca^{+2}	49.8	0.0450	58.6	0.0303	69.2	0.0051	74.3	0.0041	75.1	0.0034	81.8	0.0010	92.5	0.0004
Al^{+3}	71.7	0.0014	82.3	0.0003	88.9	0.0001	110.3	7×10^{-6}	112.8	8×10^{-6}	121.8	1.3×10^{-6}	131.2	3.5×10^{-7}
T. S. C	96.3	3.3×10^{-5}	97.9	1.5×10^{-5}	88.9	9.0×10^{-5}	92.4	6.8×10^{-5}	102.8	2.4×10^{-5}	124.6	6.6×10^{-7}	130.8	3.5×10^{-7}
M. P.	44.6	0.2446	49.3	0.2195	55.9	0.1586	58.6	0.1943	60.9	0.2480	65.3	0.2618	74.8	0.2225
P. P.	44.7	0.2917	50.2	0.4879	58.8	0.2917	59.9	0.4879	64.8	0.5013	70.1	0.6142	76.8	0.4379

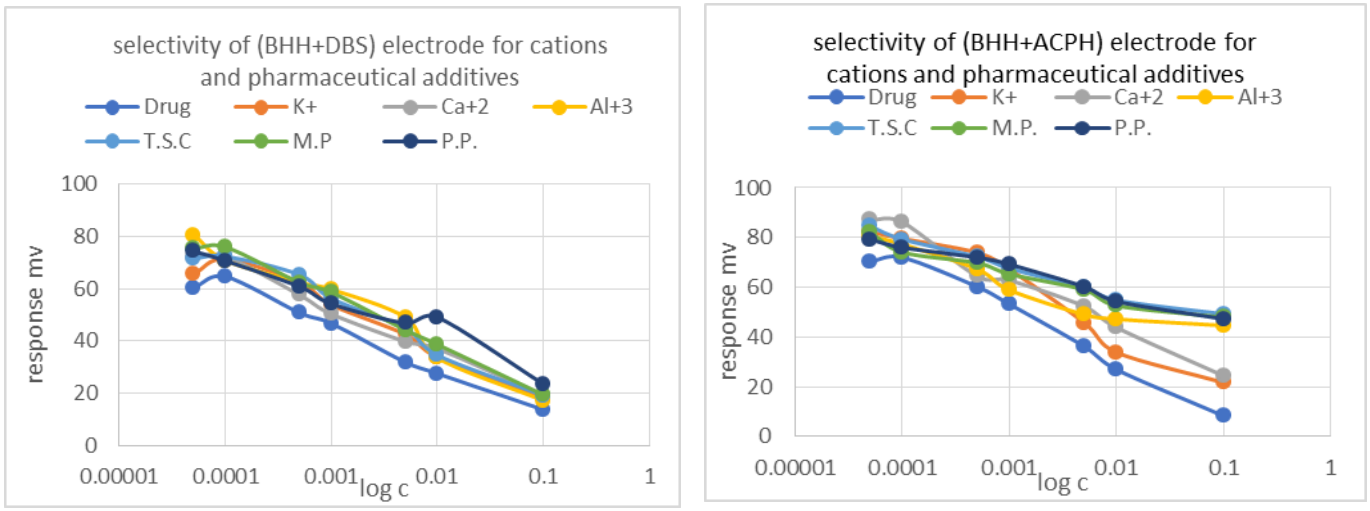

Fig. 8. Selectivity of (BHH-MIP 1 + DBS and BHH-MIP 1 + ACPH) electrodes with cations (K^+, Ca^{+2}, Al^{+3}) and some pharmaceutical additives (T.S.C, M.P, P.P) via Separation Solution Method

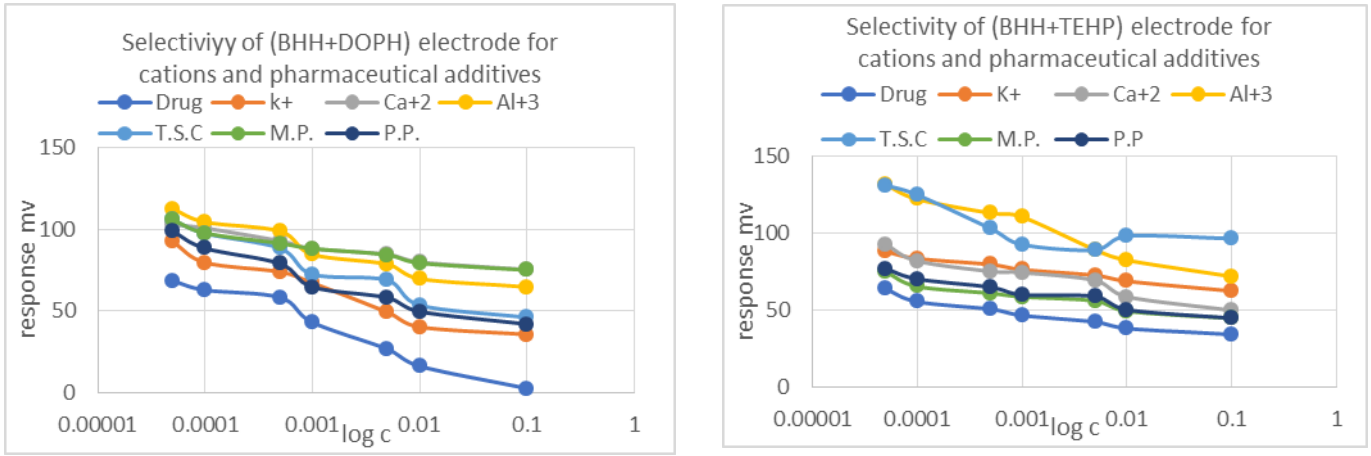

Fig. 9. Selectivity of (BHH-MIP 2 + DOPH and BHH-MIP 2 + TEHP) electrodes with cations (K^+, Ca^{+2}, Al^{+3}) and some pharmaceutical additives (T.S.C, M.P, P.P) via Separation Solution Method

Calculation by Multiple Standard Addition Method (MSA)

The concentrations used for applied in this method (1×10^{-3} & 1×10^{-4}) for two solutions of bromhexine hydrochloride for plotting the antilog E/S (Y-axis) against volume of standard bromhexine hydrochloride (X-axis). Fig. (10, 11, 12 and 13) represents the results of bromhexine hydrochloride concentrations

calculated via the electrodes based on BHH- MIP1+ DBS, BHH-MIP2+TEHP

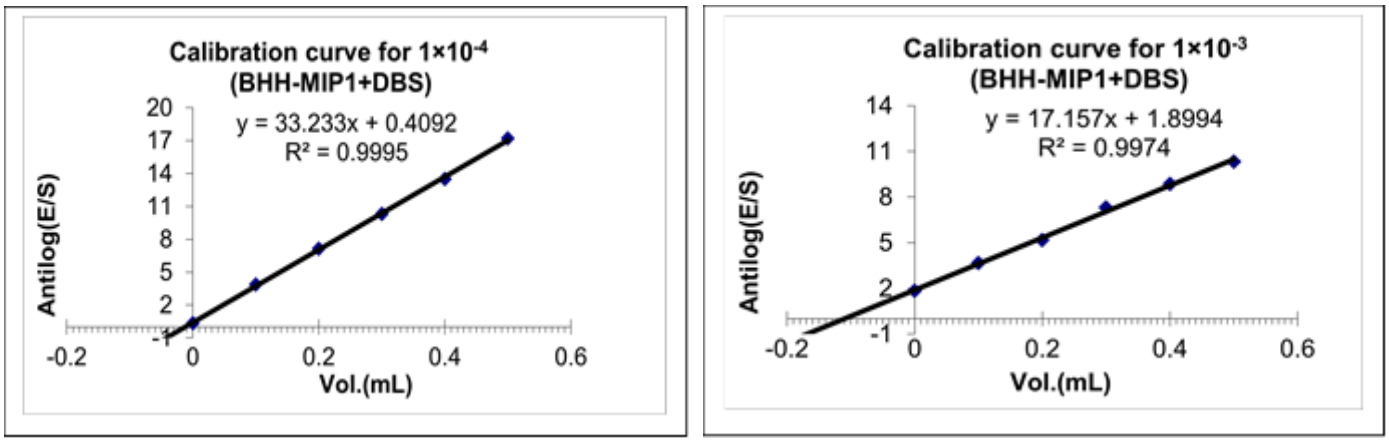

Fig.10. Antilog (E / S) against the volume of the added standard for the determination of bromhexine hydrochloride solution (1×10^{-3} and 1×10^{-4}) by MSA using (BHH–MIP1 + DBS) electrode

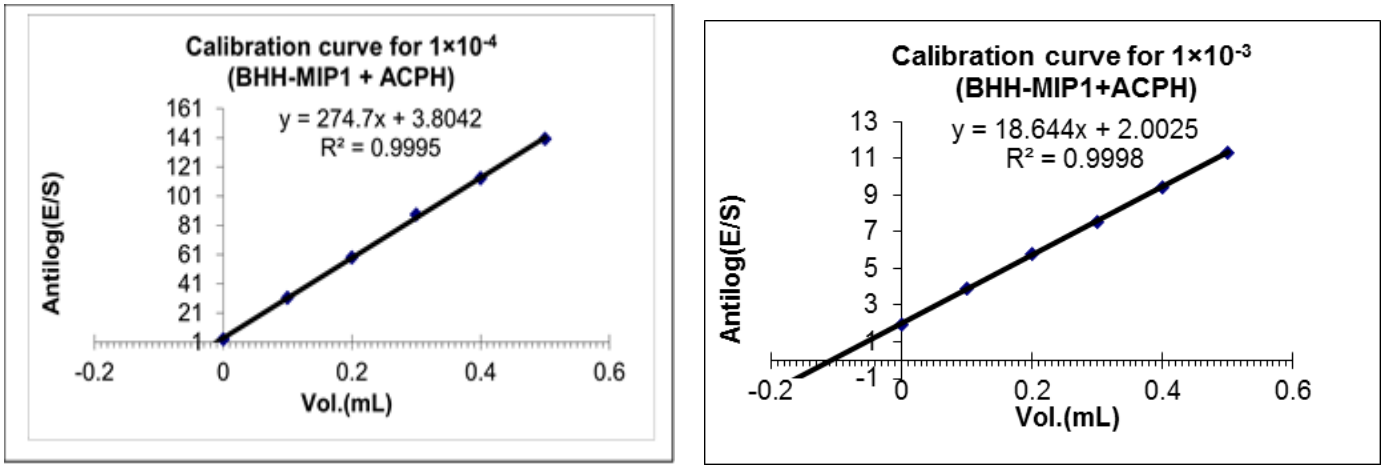

Fig.11. Antilog (E / S) against the volume of the added standard for the determination of bromhexine hydrochloride solution (1×10^{-3} and 1×10^{-4}) by MSA using (BHH–MIP1 + ACPH) electrode

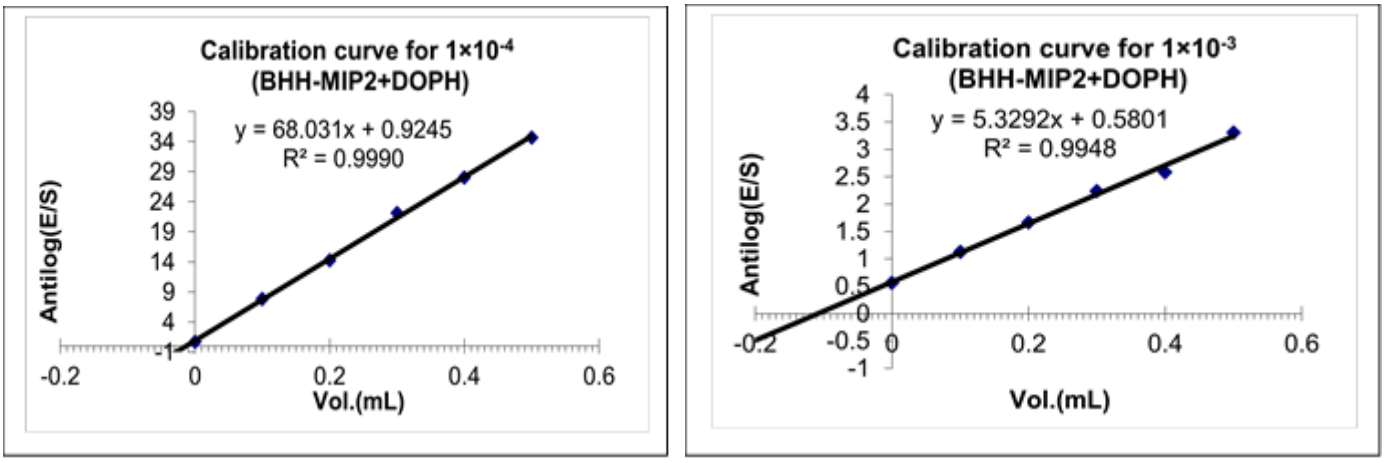

Fig.12. Antilog (E / S) against the volume of the added standard for the determination of bromhexine hydrochloride solution (1×10^{-3} and 1×10^{-4}) by MSA using (BHH–MIP2 +DOPH) electrode

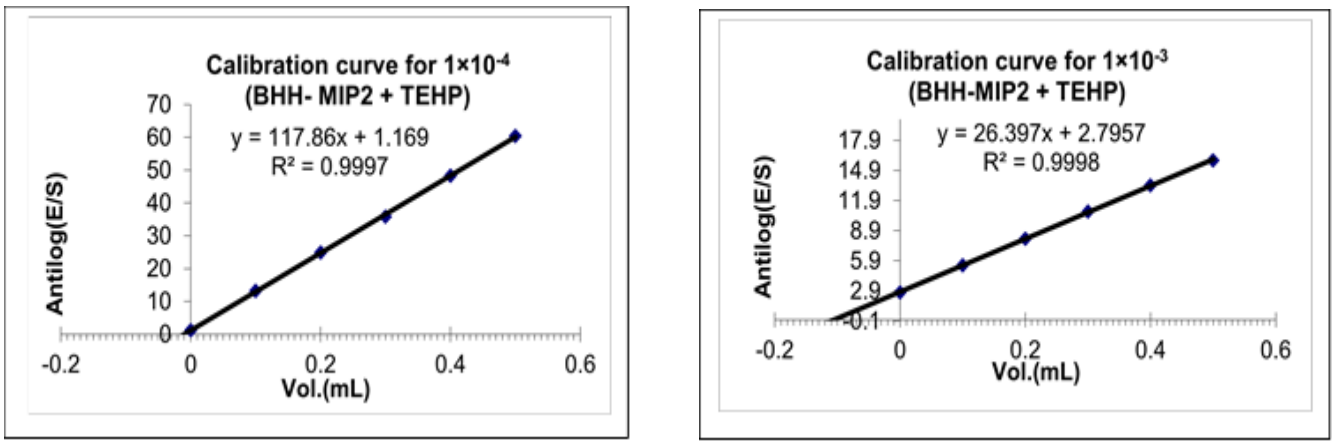

Fig.13. Antilog (E / S) against the volume of the added standard for the determination of bromhexine hydrochloride solution (1×10^{-3} and 1×10^{-4}) by MSA using (BHH–MIP2 +TEHP) electrode

Titration methods (Titrimetry)

In this method the measurement is depended changes that to be a large shift in the electrode response for the detection of the end point of titration. The process has been achieved by used volumetric analysis of concentrations (1×10^{-3} and 1×10^{-4}) M of bromhexine hydrochloride versus solutions (1×10^{-3} and 1×10^{-4}) M of concentrations (PMA). The results for parameters RSD%, RC% and RE% for all electrodes are listed in the table (7).

Table 7. Bromhexine hydrochloride sample analyses by using titration method for BHH electrodes

Electrode No.	Concentration (M)	
	Sample	Measured using PMA as titrant
BHH – MIP_1 + DBS (1)	1×10^{-3}	1.0485×10^{-3}
	RSD%	2.10
	RC%	104.85
	RE%	4.85
	1×10^{-4}	1.0413×10^{-4}
	RSD%	3.336
	RC%	104.13
	RE%	4.13
BHH – MIP_1 + ACPH (2)	1×10^{-3}	1.00384×10^{-3}
	RSD%	2.77
	RC%	103.73
	RE%	3.73
	1×10^{-4}	1.0456×10^{-4}
	RSD%	2.156
	RC%	104.56
	RE%	4.56

Electrode No.	Concentration (M)	
	Sample	Measured using PMA as titrant
BHH – MIP2 + DOPH (1)	1×10^{-3}	1.0411×10^{-3}
	RSD%	2.63
	RC%	104.11
	RE%	4.11
	1×10^{-4}	0.9609×10^{-4}
	RSD%	2.24
	RC%	96.09
	RE%	-3.91
BHH – MIP2 + TEHP (2)	1×10^{-3}	1.019×10^{-3}
	RSD%	2.64
	RC%	101.9
	RE%	1.9
	1×10^{-4}	0.993×10^{-4}
	RSD%	3.92
	RC%	99.3
	RE%	-0.7

Applications of pharmaceuticals

Ion selective electrodes that based on molecularly imprinted polymers were used for determination of bromhexine hydrochloride in pharmaceuticals. This ISEs measurements including: standard addition, direct, Gran plot and multiple standard addition method. Preparation solutions of bromhexine hydrochloride at concentrations 1×10^{-3} and 1×10^{-4} M. The RE%, RC% and RSD% were calculated of bromhexine hydrochloride in pharmaceuticals. The results obtained represented in the table (9, 10, 11, and 12).

Table 8. Determination of Bromhexine hydrochloride Samples by Ion Selective electrodes (ISEs) techniques based on PVC membranes.

Electrode No.	Sample	Concentrations (M) Measurements using potentiometric methods		
		Direct	SAM	MSA
BHH - MIP_1+ DBS (1)	1×10^{-3}	0.9663×10^{-3}	0.9975×10^{-3}	1.0089×10^{-3}
	*RSD%	3.33	4.84	----------
	REC%	96.63	98.86	100.89
	RE%	-3.37	-1.14	0.89
	1×10^{-4}	0.9666×10^{-4}	0.9710×10^{-4}	0.9905×10^{-4}
	*RSD%	2.55	3.78	----------
	REC%	96.66	97.11	99.05
	RE%	-3.34	-2.89	-0.95
Electrode No.	Sample	Concentrations (M) Measurements using potentiometric methods		
		Direct	SAM	MSA
BHH - MIP_1+ ACPH (2)	1×10^{-3}	0.9768×10^{-3}	0.986×10^{-3}	0.982×10^{-3}
	*RSD%	2.25	1.15	----------
	RC%	97.68	98.67	98.20
	RE%	-2.32	-1.33	-1.77
	1×10^{-4}	1.0238×10^{-4}	0.985×10^{-4}	0.998×10^{-4}
	*RSD%	2.82	1.13	----------
	RC%	102.38	98.57	99.84
	RE%	2.38	-1.43	-0.16
Electrode No.	Sample	Concentrations (M) Measurements using potentiometric methods		
		Direct	SAM	MSA
BHH – MIP2+ DOPH (1)	1×10^{-3}	1.0563×10^{-3}	1.0157×10^{-3}	0.9888×10^{-3}
	*RSD%	0.59	3.36	----------
	REC%	105.63	101.57	98.88
	RE%	5.63	1.57	-1.12
	1×10^{-4}	1.0336×10^{-4}	1.0195×10^{-4}	1.0166×10^{-4}
	*RSD%	0.92	4.16	----------
	REC%	103.36	101.95	101.66
	RE%	3.36	1.95	1.66
Electrode No.	Sample	Concentrations (M) Measurements using potentiometric methods		
		Direct	SAM	MSA
BHH – MIP2+ TEHP	1×10^{-3}	0.9714×10^{-3}	0.968×10^{-3}	0.982×10^{-3}
	*RSD%	3.71	0.02	----------
	RC%	97.14	96.80	98.20
	RE%	-2.86	-3.2	-1.77
	1×10^{-4}	0.9810×10^{-4}	0.957×10^{-4}	0.988×10^{-4}
	*RSD%	1.58	0.036	----------
	RC%	98.10	95.7	98.80
	RE%	-1.90	-4.3	-1.20

Table 9. Sample Analysis of Pharmaceuticals Bromhexine Hydrochloride by using ISE

Pharmaceutical	Samarra (Iraq)			
	Direct method	SAM	MSA	Titration method
Concentration prepared	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}
*Found	0.9624×10^{-3}	1.0121×10^{-3}	1.0056×10^{-3}	1.0494×10^{-3}
REC%	96.24	101.21	100.56	104.94
RE%	-3.76	1.21	0.56	4.94
*RSD%	4.09	4.61	-------	3.67
F experimental	7.86	2.41	-------	9.00
F theoretical		19.2		
Pharmaceutical	Samarra (Iraq)			
	Direct method	SAM	MSA	Titration method
Concentration prepared	1×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-4}
*Found	0.9631×10^{-4}	1.0156×10^{-4}	1.0041×10^{-4}	1.0287×10^{-4}
REC%	96.31	101.56	100.41	102.87
RE%	-3.69	1.56	0.41	2.87
*RSD%	4.90	3.68	-------	4.36
F experimental	5.70	3.24	-------	5.06
F theoretical		19.2		

Table 10. Sample Analysis of Pharmaceuticals Bromhexine Hydrochloride by using ISE.

Pharmaceutical	Samarra (Iraq)			
	Direct method	SAM	MSA	Titration method
Concentration prepared	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}
*Found	0.9769×10^{-3}	0.9779×10^{-3}	1.0145×10^{-3}	1.0339×10^{-3}
REC%	97.69	97.80	101.45	103.39
RE%	-2.31	-2.20	1.45	3.39
*RSD%	3.20	2.94	-------	2.68
F experimental	12.0	3.37	-------	12.4
F theoretical		19.2		
Pharmaceutical	Samarra (Iraq)			
	Direct method	SAM	MSA	Titration method
Concentration prepared	1×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-4}
*Found	0.9715×10^{-4}	0.9896×10^{-4}	0.9938×10^{-4}	1.0341×10^{-4}
REC%	97.15	98.96	99.38	103.41
RE%	-2.85	-1.04	-0.62	3.41
*RSD%	3.00	1.51	-------	1.13
F experimental	11.7	8.1	-------	18
F theoretical		19.2		

Table 11. Sample Analysis of Pharmaceuticals Bromhexine hydrochloride by using ISE

Pharmaceutical	Samarra (Iraq)			
	Direct method	SAM	MSA	Titration method
Concentration prepared	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}
*Found	0.9686×10^{-3}	1.0227×10^{-3}	0.9784×10^{-3}	0.9617×10^{-3}
REC%	96.55	102.27	97.84	96.12
RE%	-3.45	2.27	-2.16	-3.88
*RSD%	0.99	4.30	-------	2.66
F experimental	3.2	16.2	-------	13.5
F theoretical		19.2		

Pharmaceutical	Samarra (Iraq)			
	Direct method	SAM	MSA	Titration method
Concentration prepared	1×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-4}
*Found	0.9573×10^{-4}	0.9707×10^{-4}	0.9863×10^{-4}	1.044×10^{-4}
REC%	95.73	97.08	98.63	104.40
RE%	-4.27	-2.92	-1.37	4.40
*RSD%	0.32	1.49	-------	2.22
F experimental	16.2	13.6	-------	18.4
F theoretical		19.2		

Table 12. Sample Analysis of Pharmaceuticals Bromhexine Hydrochloride by using ISE

Pharmaceutical	Samarra (Iraq)			
	Direct method	SAM	MSA	Titration method
Concentration prepared	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}
*Found	0.979×10^{-3}	0.991×10^{-3}	0.994×10^{-3}	1.011×10^{-3}
RC%	97.99	99.16	99.47	101.16
RE%	-2.01	-0.84	-0.53	1.16
*RSD%	2.84	0.88	-------	3.71
F experimental	8.6	13.7	-------	4.6
F theoretical		19.2		

Pharmaceutical	Samarra (Iraq)			
	Direct method	SAM	MSA	Titration method
Concentration prepared	1×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-4}
*Found	0.972×10^{-4}	1.022×10^{-4}	1.010×10^{-4}	1.025×10^{-4}
RC%	97.22	102.26	101.02	102.56
RE%	-2.78	2.26	1.02	2.56
*RSD%	2.32	2.04	-------	1.61
F experimental	13.6	5.9	-------	3.2
F theoretical		19.2		

Conclusion

Bromhexine hydrochloride membranes selective electrodes can be constructed by mixing with different plasticizers. These plasticizers DBS, ACPH, DOPH and TEHP were used to prepared bromhexine

hydrochloride membranes electrodes based on PVC. The results obtained for all electrodes were excellent as well as applied on standard and pharmaceutical solutions. The aim of construction electrodes for used in determination bromhexine hydrochloride in pharmaceuticals analysis

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