Scientific paper

Miniaturized Membrane Sensors for Potentiometric Determination of Metoprolol Tartrate and Hydrochlorothiazide

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Abstract

Four microsized graphite and platinum wire poly(vinyl chloride) matrix membrane electrodes responsive to some drugs affecting cardiovascular system, Metoprolol tartrate (MT) and Hydrochlorothiazide (HZ) were developed, described and characterized. These sensors were constructed by using (2-Hydroxypropyl)-β-cyclodextrin (2HP β-CD) as an ionophore which has a significant influence on increasing both membrane sensitivity and selectivity. The four sensors were fabricated in a polymeric matrix of carboxylated polyvinyl chloride (PVC–COOH) and dioctylphthalate (DOP) as a plasticizer, based on the interaction between the drugs and the dissociated COOH groups in the PVC–COOH. Fast and stable Nernstian responses of 1.0×10^{-6}–1.0×10^{-2} M for MT (sensors 1 and 2) and of 1.0×10^{-7}–1.0×10^{-3} M for HZ (sensors 3 and 4) over pH range 3.0–9.0 and 3.0–7.0 for the MT and HZ sensors respectively were obtained. Nernstian slopes of 56.2, 54.6, 19.0 and 20.8 mV/decade for electrodes 1–4 respectively were observed. The proposed method displayed useful analytical characteristics for the determination of MT and HZ in their pure powder forms with average recoveries of 99.11 ± 0.357, 99.21 ± 0.389, 100.08 ± 0.459 and 100.28 ± 0.438% for sensors 1–4 respectively. The lower limit of detection (LOD) were 5.5×10^{-6}, 4.5×10^{-6}, 4.8×10^{-8} and 5.0×10^{-8} M for sensors 1–4 respectively indicated high sensitivity. The four sensors displayed a good stability over a period of 6 weeks. The selectivity coefficients of the developed sensors indicated excellent selectivity.

Results obtained by the four electrodes revealed the performance characteristics of these electrodes which evaluated according to IUPAC recommendations. The method was successively applied for the determination of MT and HZ in presence of each other, in presence of Salamide (SA), the main degradation product of HZ, in their pharmaceutical formulations and in human plasma samples. Statistical comparison between the results obtained by this method and those obtained by the official methods of the drugs was done and no significant difference was found.

Keywords: Metoprolol tartrate, Hydrochlorothiazide, microsized electrodes, cyclodextrin, human plasma

1. Introduction

Metoprolol tartrate (MT), (Fig. 1a) is a selective adrenergic antagonist that is devoid of intrinsic sympathomimetic activity[1]. It is used in the management of hypertension, angina pectoris, cardiac arrhythmia, myocardial infarction.[2] While *Hydrochlorothiazide (HZ)*, (Fig. 1b) is a thiazide diuretic that used in the treatment of hypertension, either alone or with other antihypertensives. It is also used to treat oedema associated with heart failure, with renal and hepatic disorders and with acute glomerulonephritis.[1,2] MT and HZ are co-formulated together in commercial tablets for their anti hypertensive effect.

Determination of MT is described in British Pharmacopeia (BP)[3] by non-aqueous titration method. While HZ is described in United State Pharmacopeia (USP)[4] by HPLC method. MT and HZ are also determined by several methods involving spectrophotometric methods,[5–14] HPLC,[15–24] electrophoresis[25–28] and potentiometric methods.[29,30]

The scientific novelty of the present work is that the method used is simple, rapid, selective and less expensive and less time consuming compared with other published spectrophotometric and HPLC methods.

The focus of the present study is to develop and validate potentiometric method for the determinations of

MT and HZ in presence of each others, in the presence of Salamide (SA) the main degradation product of HZ,[31] and the applicability of the method for the determination of MT and HZ in their dosage form and in human plasma sample.

Microelectrodes have been widely used in the recent years. Their small physical size allows exploration of microscopic domains, such as biological systems. Their fast response time, due to diffusion layer, allows rapid scan rates to be used.[32]

Metallic and graphite-based conductors of many geometric shapes can be used, such as wire, disc and cylinders.[33–35] These electrodes behave as two interface devices, membrane/ electrolyte interface and membrane/metal interface.[36] Thus, the membrane potential is due to the electric potential difference between the two interfaces.

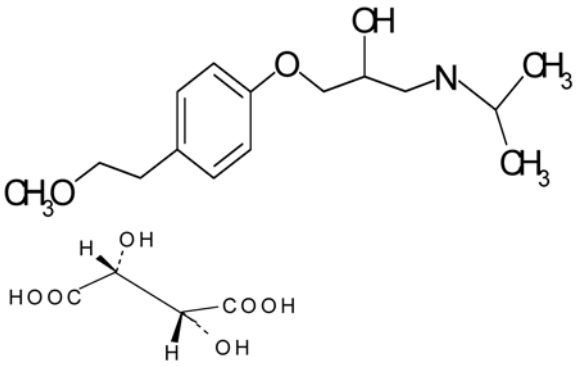


Fig. 1a. Metoprolol tartrate

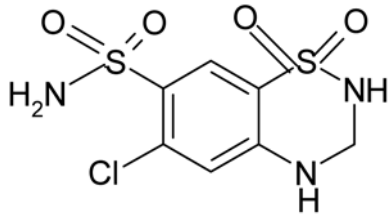


Fig. 1b. Hydrochlorothiazide

Membranes are prepared from polymer, ionophore and plasticizer. The polymer provides an inert solid support structure in which the rest of components are embedded. The ionophore can be viewed as molecular receptor, because its chemical structure provides well defined inclusion cavities with a specific receptor function.[37] The ionophores can form stable host–guest inclusion complexes or nanostructure supramolecular assemblies in their hydrophobic cavity, showing high molecular selectivity and enantioselectivity.[38] The plasticizer affects the lipophilicity of polymer membrane. It also alters the distribution coefficients (K) of different species thus affecting the performance characteristics of electrode.[39]

Analytes in colored, turbid and viscous samples can be determined accurately. They show rapid response to change in the concentration. Furthermore, they may be used for measurement over a wide concentration range. Ion selective electrodes are generally tolerant to small changes in pH. A further advantage is that they are relatively simple and not expensive to develop, set up and run. Moreover, the chemical design of the electrodes has been developed to give superior selectivity and response.

The present work described the use of functionalised cyclodextrin derivatives as neutral ionophore for the development of novel sensors for the determination of MT and HZ. Linear responses of MT and HZ within the concentration ranges of 1×10^{-6}–1×10^{-2} M for sensors 1 and 2 and of 1×10^{-7}–1×10^{-3} M for sensors 3 and 4 were obtained. Nernstian slopes of 56.2, 54.6, 19.0 and 20.8 mV/decade over the pH range of 3.0–9.0 and 3.0–7.0 for the MT and HZ sensors respectively were observed. The selectivity coefficients of the developed sensors indicated excellent selectivity for MT and HZ. The LOD of the proposed sensors were 5.5×10^{-6}, 4.5×10^{-6}, 4.8×10^{-8} and 5.0×10^{-8} M revealed high sensitivity. The proposed electrodes are successfully applied for the determination of MT and HZ in their pure powder forms with average recoveries of 99.11 ± 0.357, 99.21 ± 0.389, 100.08 ± 0.459 and 100.28 ± 0.438% for sensors 1–4 respectively.

2. Experimental

2. 1. Apparatus

– Jenway digital ion analyzer model 3330 (UK) with Ag/AgCl double junction reference electrode no. Z113107-1EAPW (Aldrich Chemical Co.).
– pH glass electrode Jenway (Jenway, UK) no. 924005-BO3-Q11C.
– Magnetic stirrer, Bandelin Sonorox, Rx510S (Budapest, Hungarian).

2. 2. Reference Samples

Metoprolol tartrate (MT) and Hydrochlorothiazide (HZ)-Pure samples were kindly supplied by CID pharmaceuticals CO., batch numbers C0028 and C0976; respectively, (Giza–Egypt). MT and HZ percentages purity were 99.42 ± 0.421% and 100.56 ± 0.420%; respectively, according to the official methods.[3,4]

2. 3. Market Samples

Lopressor HCT (100:50) and *Lopressor HCT (100:25)* tablets-Batch numbers K0016 and K0095A respectively. Each tablet is claimed to contain 100 mg of MT and 50 mg or 25 mg of HZ. Manufactured by: Novartis pharma, (USA).

2. 4. Reagents

All chemicals and solvents used were of analytical grade (water was used doubly distilled). Polyvinyl chloride carboxylated (PVC–COOH), (Fluka chemie GmbH Germany). Dioctylphthalate (DOP), Dibutylsebacate (DBS), 2-nitrophenylphenylether (oNPPE) and 2-nitrophenyloctylether (oNPOE), (Sigma/Aldrich, St.Louis, MO), (2-Hydroxypropyl)-β-cyclodextrin (2HP β-CD), (Fluka Chemie GmbH, Germany). Tetrahydrofuran (THF) – (BDH, limited Poole, England). Sodium hydroxide,1.0 M and Hydrochloric acid, 1.0 M aqueous solution, (Prolabo, VWR International, West Chester, PA). Phosphate buffer pH 7.0 and acetate buffer pH 3.0.[40] Salamide (Degradation product of HZ), (Sigma/Aldrich, St.Louis, MO).

2. 5. Standard Solutions

MT and HZ stock solutions (1.0×10^{-2} M and 1.0×10^{-3} M)

MT stock solution was prepared by transferring 0.684 gm of MT in 100.0 ml phosphate buffer pH 7.0. While HZ stock solution was prepared by transferring 0.0297 gm in 100.0 ml acetate buffer pH 3.0.

MT (1.0×10^{-6} to 1.0×10^{-2} M) and HZ (1.0×10^{-7} to 1.0×10^{-3} M) working solutions

They were prepared by proper dilution from their stock solutions using the corresponding buffers.

2. 6. Procedures

2. 6. 1. Preparation of Electroactive Coating Membrane: (PVC–COOH /2-HP b-CD / DOP)

In a glass petri dish (5 cm diameter), portion of 0.9 gm PVC–COOH was thoroughly mixed with 0.4 gm DOP and 0.3 gm 2-HP β-CD, then dissolved in 15 ml THF. The petri dish was covered with filter paper and left to stand for 1 h to allow slow solvent evaporation. A thick homogeneous solution was produced which used for preparation of sensors (1–4).

2. 6. 1. 1. Preparation of Graphite Substrate Based Sensors

Two rods of spectrographic graphite (5 mm in diameter and 15 mm long) were inserted in a polyethylene sleeve, and about 3 mm of the other end of the protruded rods served as a measuring surface. These ends of the rods were washed with acetone, dried in air for 3 hours, and dipped rapidly into the previously prepared PVC–COOH / 2-HP β-CD / DOP solution. The solvent was allowed to evaporate in air after each dipping, and the dipping process was repeated 6–8 times to produce uniform membranes on the surface of the graphite rods. Drops of mercury were added in the polyethylene sleeve to ensure electrical contact with the connection cable. The coated graphite rods were conditioned by soaking in a 1.0×10^{-2} M MT solution in case of sensor 1 and in 1.0×10^{-3} M HZ solution in case of sensor 3 for 4 hours for both sensors, and stored in the same solutions when not in use.

2. 6. 1. 2. Preparation of Platinum Substrate Based Sensors

The cover of insulated platinum wires was removed for a length of about one cm at both ends. One end of each wire was immersed in the previously prepared PVC–COOH / 2-HP β-CD / DOP solution and then allowed to air dry for 10 min., forming thin membranes around each wire end. The resultant coated wires membrane sensors were conditioned in 1.0×10^{-2} M MT solution in case of sensor 2 and in 1.0×10^{-3} M HZ solution in case of sensor 4 for 3 hours for both sensors and were stored in the same solutions when not in use.

2. 6. 2. Direct Potentiometric Determination of the Drugs in their Pure Samples

Electrodes 1 and 2 in conjunction with the double junction Ag/AgCl reference electrode were immersed in the solutions of MT in concentration range 1.0×10^{-6} – 1.0×10^{-2} M. While electrodes 3 and 4 in conjunction with the double junction Ag/AgCl reference electrode were immersed in the solutions of HZ in concentration range 1.0×10^{-7} – 1.0×10^{-3} M. The four electrodes were allowed to equilibrate while stirring and the emfs were recorded. The membrane sensors were washed with the corresponding buffers between measurements. Calibration graphs were plotted relating the recorded potentials vs. p drug concentrations. These calibration graphs or the computed regression equations were used for subsequent measurements of unknown concentrations of MT and HZ.

2. 6. 3. Identification of the Slope, Response Time and Operative Life of the Studied Electrodes

The electrochemical performance of the four proposed sensors was evaluated according to the IUPAC recommendations data.[41]

The dynamic response times for the electrodes in the discussion to reach values ±1.0 mV of the final equilibrium potential after increasing the drug concentration 10 folds were measured.

2. 6. 4. Effect of pH

The effect of pH on the potential values of the four electrodes was studied over pH range of 2.0 to 11.0 by adding drops of 1.0 M HCl and 1.0 M NaOH, the potentials obtained at each value were recorded.

2. 6. 5. Effect of Temperature

The effect of the temperature was studied, the potential response displayed by the investigated electrodes as a function of temperature in the range of 20°–40°C was monitored. The potentials obtained at each temperature were recorded.

2. 6. 6. Effect of Interfering Compounds on the Electrodes Selectivity

The potential response of the four studied sensors in the presence of each other and in presence of a number of related substances was studied, and the potentiometric selectivity coefficient, was used to evaluate the extent to which a foreign ion would interfere with the response of the electrodes to its primary ion. The selectivity coefficients were calculated by the separate solutions method (SSM)[42], where potentials were measured for 10^{-3} M drug solution and then for 10^{-3}M interferent solution, separately, then potentiometric selectivity coefficients were calculated using the following equation:

Log $K^{pot}_{A;B} = E_B / S - E_A + 1 / Z_B - Z_A \log a_A$ Where $K^{pot}_{A;B}$ is the potentiometric selectivity coefficient, S is the slope of the calibration plot, E_A and E_B are the potentials measured in 10^{-3} M solution of primary ion and interfering ion, respectively, a_A is the activity of MT or HZ, and Z_A and Z_B are the charges on MT or HZ and the interfering ion, respectively.

2. 6. 7. Analysis of Laboratory Prepared Mixtures

In a series of 25 ml volumetric flasks, different volumes of MT (1.0×10^{-3} M) and HZ (1.0×10^{-3} M) and SA (1.0×10^{-3} M) were accurately transferred and diluted to the mark with acetate buffer pH 3.0 to prepares mixtures containing different ratios of the two drugs and the degradation product of HZ.

2. 6. 8. Application of the Proposed Method for Simultaneous Determination of MT and HZ in Lopressor HCT Tablets

Ten tablets of the drug formulations were weighed accurately and finely powdered in a small dish. An amount of powder equivalent to 68.4 mg of MT from each of the two formulations was accurately transferred to a 100-ml volumetric flask and diluted to the mark with phosphate buffer pH 7.0 to prepare 1.0×10^{-3} M solution of MT. Another amount of powder equivalent to 29.7 mg of HZ from each of the two formulations was accurately transferred to a 100-ml volumetric flask and diluted to the mark with acetate buffer pH 3.0 to prepare 1.0×10^{-3} M solution of HZ. The potentials readings produced by immersing the prepared electrodes in conjunction with the double junction Ag/AgCl reference electrode in the prepared solutions were recorded and compared with the calibration graphs.

2. 6 .9. Direct Potentiometric Determination of MT and HZ in Spiked Human Plasma Sample

Aliquots equivalent to 4.5 ml of human plasma were placed into four stoppered shaking tubes, and then 0.5 ml of 1.0×10^{-4} and 1.0×10^{-5} M MT and 0.5 ml of 1.0×10^{-5} and 1.0×10^{-6} HZ were added separately and shacked. The membrane sensors were immersed in conjunction with the Ag/AgCl reference electrode in these solutions. The membrane sensors were washed with the corresponding buffers between measurements. The potentials readings produced by immersing the prepared electrodes in conjunction with the double junction Ag/AgCl reference electrode in the prepared solutions were recorded and compared with the calibration plots. No adverse effect on the responses of the electrodes was observed when the drugs were spiked with the human plasma samples without prior removal of the protein.

3. Results and Discussion

A variety of electrode materials of different shapes and sizes have been reported, but the most commonly used electrode materials are platinum,[43] gold and carbon fiber.[44]

The replacement of the classical internal filling solution in potentiometric sensors is important because that design offers a number of advantages such as simplicity, lower cost, better mechanical flexibility and possibility of miniaturization.[43]

3. 1. Sensors Fabrication

– The microsized graphite rods and platinum wires were coated with the previously prepared thin films of PVC–COOH / 2-HP β-CD / DOP for sensors (1–4) and used as potentiometric sensors for MT and HZ.
– Upon soaking graphite rod and platinum wire for sensors 1 and 2 respectively in 1.0×10^{-2} M MT test solution, homogenous electroactive layers from PVC–COOH / 2-HP β-CD / DOP / MT were formed which induce a potentiometric response for MT.
– While upon soaking graphite rod and platinum wire for sensors 3 and 4 respectively in 1.0×10^{-3} M HZ test solution, homogenous electroactive layers from PVC–COOH / 2-HP β-CD / DOP / HZ were formed which induce a potentiometric response for HZ.

3. 1. 1. Plasticizer

In the present investigation different plasticizers were tried, DOP was found to be the optimum available me-

diator for the investigated membrane sensors. It adjusted the membrane permittivity to give the highest possible selectivity and sensitivity. The use of other placticizers such as oNPPE and oNPOE failed in dissolving the electroactive coating complex and gave noisy responses.

3. 1. 2. Cyclodextrin

The possibility of using functionalized lipophilic CD derivatives (2-HP β-CD) as the sensor ionophore in the preparation of MT and HZ selective electrodes (sensors 1–4) with PVC–COOH to immobilize the sensors and to attain the formation of highly stable complex was evaluated. 2-HP β-CD has better aqueous solubility (up to 0.7 M) than β-CD which is poorly soluble in water (0.02 M). Therefore, using 2-HP β-CD as an ionophore provides high stability of the complex between that molecule and the cationic drug present in solution, thus the membrane selectivity and sensitivity were substantially enlarged (Tables 1,2).

3. 1. 3. PVC–COOH

It is characterized by its partial dissociation and high adhesion properties. Sensors (1–4) were simply fabricated without the need of ion association complex. They were only preconditioned by soaking in corresponding drug solution for 4 hours where acid-base interactions take place between the dissociated COO^- group of the PVC and amino group of the drugs in the test solution until chemical equilibrium is attained.

PVC–COOH also acts as a regular support matrix, as trap for the ion and as polymeric matrix to immobilize the sensor and to attain the formation of highly stable complex.

3. 2. Sensors Calibration and Response Time

Electrochemical performance characteristics of the proposed sensors were systematically evaluated according to IUPAC standards.[41]

Table 1 shows the results obtained over a period of six weeks for the four investigated sensors. Typical potential profiles are shown in Fig. 2. The four sensors displayed a good stability over a period of 6 weeks.

The required time for the sensors to reach the values of the final equilibrium potential after increasing drug concentration 10-folds was found to be 10 and 20 seconds, for sensors 1,3 and 2,4; respectively. The slopes of the calibration plots were 56.2 and 54.6 mV/concentration decade for sensor 1 and 2, 19.0 and 20.8 mV/concentration decade for sensor 3 and 4, respectively, the typical value of monovalent and trivalent substances as MT behaves as monovalent and HZ as a trivalent cation via their amino groups. Deviation from the ideal Nernstian slope (60 mV/decade and 20 mV/decade) for sensors 1,2 and 3,4, stems from the fact that the electrodes respond to the activities of the drug rather than its concentration.

3. 3. Effect of pH and Temperature

Different factors affecting the response of the electrodes (emfs) were studied to reach the optimum experimental conditions. The effect of pH on the response of the four investigated electrodes was studied (Fig. 3, 4). It is apparent from the potential-pH profile that the responses are fairly constant over pH range 3.0–9.0 and 3.0–7.0 for sensors (1, 2) and (3, 4); respectively, ie., in these pH MT and HZ are completely ionized, dissociated and sensed.

Above pH 9.0 for electrodes (1, 2) and above pH 7.0 for electrodes (3, 4), the potentials show sharp decrease due to the formation of non protonated amino group of MT and HZ. While below pH 3.0, the response of electro-

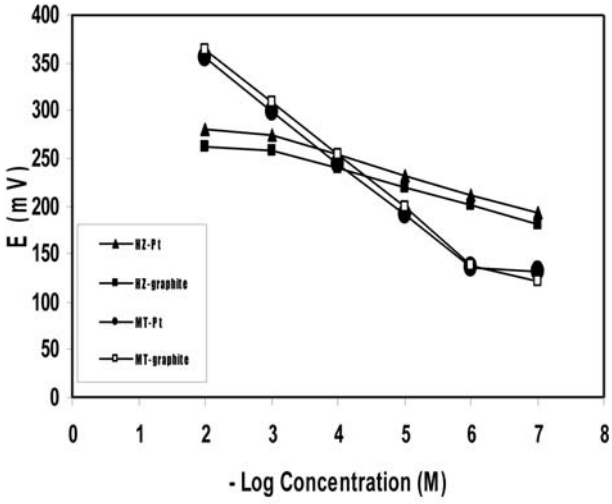


Fig. 2: Profile of the potential in mV/- Log concentration in (M) of MT and HZ using graphite rod (sensors 1,3) and Pt. wire (sensors 2,4)

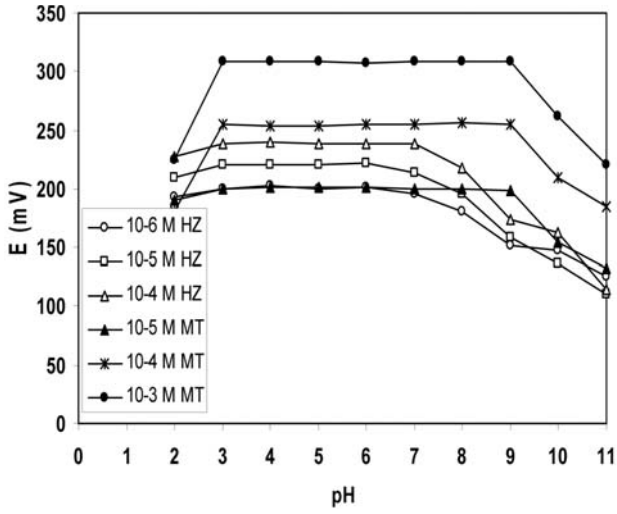


Fig. 3: Effect of pH on the response of MT-graphite and HZ-graphite electrodes (sensors 1,3)

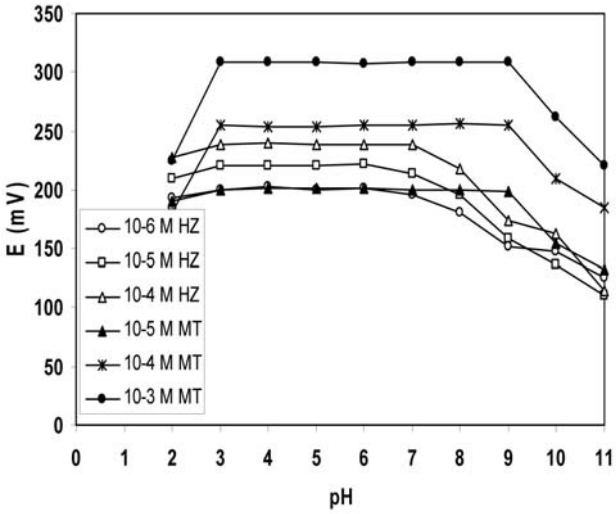


Fig. 4: Effect of pH on the response of MT-pt and HZ-Pt electrodes (sensors 2,4)

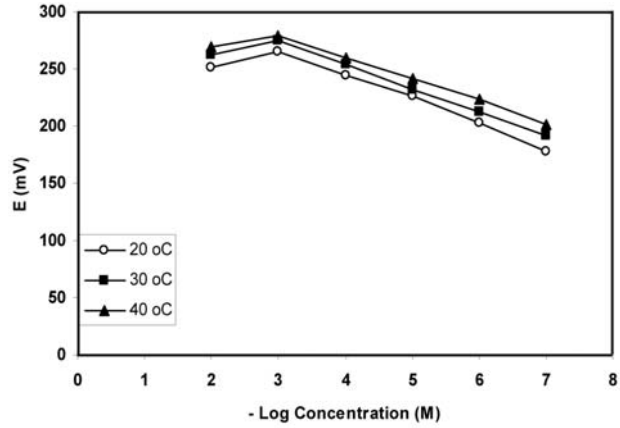


Fig. 5: Effect of Temp on the response of MT-graphite electrode (sensor 1)

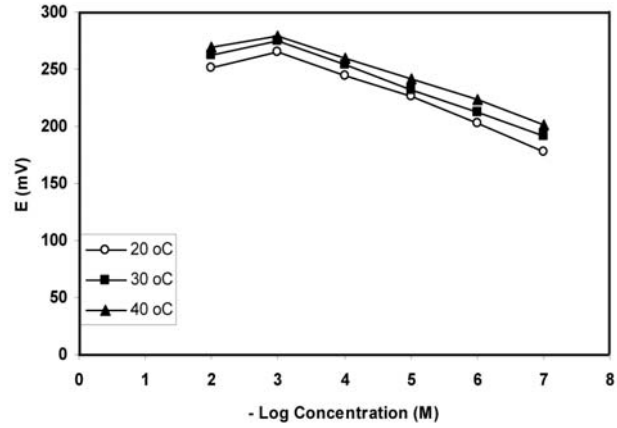


Fig. 6: Effect of Temp on the response of HZ-Pt electrode (sensor 4)

des (1–4) decrease with the increase of analyte acidity; as at such high acidity the dissociation of the carboxyl groups of PVC–COOH is limited, also the membrane may extract H^+, furthermore, the neutralization of OH^- groups of 2HP β-CD takes place and the inclusion function of the ionosphere is decreased leading to weak responses.

So MT was dissolved in phosphate buffer pH 7.0 and HZ was dissolved in acetate buffer pH 3.0 (as high concentrations of HZ did not dissolve in phosphate buffer pH 7.0).

Upon studying the effect of temperature the suggested sensors exhibit slight gradual increase in their potentials as the temperature increases in the range of 20–40 °C, however the calibration graphs obtained at different

Table (1): Response Characteristics of the investigated selective electrodes and the validation parameters of the response and the regression equations

Parameter	**Value**			
	Sensor 1	**Sensor 2**	**Sensor 3**	**Sensor 4**
Validation of regression equations				
Slope (mV/decade)[a]	56.2	54.6	19	20.8
Intercept (mV)[a]	477.2	463.0	314.6	337
Correlation coefficient (r)	0.9997	0.9999	0.9997	0.9996
Validation of responses				
Concentration range (M)	$1 \times 10^{-6} - 1 \times 10^{-2}$	$1 \times 10^{-7} - 1 \times 10^{-3}$		
Response time (sec)	10	20	10	20
Working pH range	3–9	3–9	3–7	3–7
LOD (M)[b]	5.5×10^{-6}	4.5×10^{-6}	4.8×10^{-8}	5.0×10^{-8}
Stability (weeks)	6	6	6	6
Accuracy (mean ±S.D.)	99.11 ± 0.354	99.21 ± 0.386	100.08 ± 0.459	100.28 ± 0.439
Relative standard deviation (precision %)	0.357%	0.389%	0.459%	0.438%
Repeatability [c] (%)	0.278	0.413	0.520	0.464
Intermediate precision [d] (%)	0.634	0.440	0.619	0.407

[a] Results of five determinations, [b] The lower detection limit (LOD) defined as drug concentration obtained at the intersection of the extrapolated high concentration (linear segment) with the low concentration (zero slope segment) of the calibration plot. [c] n = 3 × 3 [d] n = 3 × 3

temperatures were parallel, slope and response time did not significantly vary with variation of temperature indicating reasonable thermal stability of the suggested sensors up to 40 °C, (Fig. 5, 6).

3. 4. Sensors Selectivity

The effect of interfering substances upon the performance of the sensors was studied by separate solutions method.[42]

To evaluate precision and accuracy, three concentrations within the linear range (1.0×10^{-5}, 1.0×10^{-4} and 1.0×10^{-3} M solutions of MT and HZ) were chosen. Three solutions of each concentration were prepared and analyzed in triplicate (repeatability assay). This assay was repeated on three different days (intermediate precision assay), (Table 1).

The performance of the four sensors in the presence of tablets excipients, organic and inorganic related substance were assessed by measuring and comparing the potentiometric selectivity coefficients. The results revealed that the proposed membrane sensors displayed high selectivity, and no significant interference was observed from interfering species, (Table 2).

MT is fairly stable and does not undergo any degradation even under drastic conditions. While HZ undergoes alkaline degradation, (Fig 7) to produce 4-amino-6-chlorobenzene-1, 3-disulphonamide (SA), which is also one of HZ process impurities.[3,12,31]

Table 3 shows the results obtained upon analysis of synthetic mixtures containing different ratios of MT, HZ and SA. The results showed that sensors 1 and 2 can be successfully used for selective determination of MT in presence HZ and SA, while sensors 3 and 4 can be successfully used for selective determination of HZ in presence of MT and up to 60% SA.

Table 4 shows the results obtained for the determination of MT and HZ in Lopressor HCT tablets that proves the applicability of the method without prior treatment or separation, using the four sensors for the determination of MT and HZ, as demonstrated by the accurate and precise percentage recovery. Table 5 shows the results obtained for the determination of MT and HZ in spiked human plasma.

The results obtained were also compared with those obtained by using official methods 3,4 for MT and HZ. No significant difference in results was found, (Table 6).

Table 1 shows all the validation parameters of the proposed method including linearity, range, accuracy and precision.

4. Conclusion

The described sensors are sufficiently simple and selective for the quantitative determination of MT and HZ in pure form, in presence of each others, in presence of up to 60% of HZ main degradation product, in spiked human plasma sample and pharmaceutical formulations. The graphite rode gave lower response time as in sensors 1 and 3 in comparison with sensors 2 and 4. While the use of platinum wire increased the membrane selectivity as in sensors 2 and 4 in comparison with sensors 1 and 3. The use of the proposed sensors offers advantages of fast response and elimination of drug pretreatment or separation steps. They can therefore, be used for routine analysis of MT and HZ in quality control laboratories.

Table 2: Potentiometric selectivity coefficients ($K^{pot}_{primary\ ion,\ interferent}$) of the four proposed sensors

Interfernts [a]	Sensor (1)	Sensor (2)	Sensor (3)	Sensor (4)
glucose	2.05×10^{-4}	7.49×10^{-5}	3.64×10^{-4}	6.22×10^{-5}
lactose	2.56×10^{-4}	8.01×10^{-5}	3.97×10^{-4}	7.82×10^{-5}
starch	1.65×10^{-4}	9.56×10^{-5}	3.58×10^{-4}	8.13×10^{-5}
talc	1.73×10^{-4}	9.38×10^{-5}	2.86×10^{-4}	9.90×10^{-5}
NaCl	4.90×10^{-4}	2.13×10^{-4}	4.15×10^{-4}	1.92×10^{-4}
KCl	4.50×10^{-4}	2.34×10^{-4}	4.39×10^{-4}	2.05×10^{-4}
NH_4Cl	6.11×10^{-4}	3.21×10^{-4}	5.72×10^{-4}	2.88×10^{-4}
$CaCl_2$	7.61×10^{-4}	5.07×10^{-4}	5.99×10^{-4}	3.37×10^{-4}
Urea	1.17×10^{-3}	8.35×10^{-4}	2.78×10^{-3}	8.83×10^{-4}
Salamide	1.85×10^{-3}	9.94×10^{-4}	3.61×10^{-3}	1.02×10^{-3}
MT	–	–	6.23×10^{-3}	1.19×10^{-3}
HZ	5.39×10^{-3}	1.25×10^{-3}	–	–

[a] Average of 3 determinations. All interferents are in the form of 1×10^{-3} M solution.

Table 3: Determination of intact MT and HZ in laboratory prepared mixtures by the proposed potentiometric method

Ratio	Concentration (M)			Recovery %			
				MT		HZ	
MT:HZ:SA	MT	HZ	SA	Sensor (1)	Sensor (2)	Sensor (3)	Sensor (4)
4:1:0.2	4.0×10^{-4}	1.0×10^{-4}	2.0×10^{-5}	100.94	98.98	99.12	98.10
3:1:0.3	3.0×10^{-4}	1.0×10^{-4}	3.0×10^{-5}	101.05	101.13	99.82	98.89
2:1:0.4	2.0×10^{-4}	1.0×10^{-4}	4.0×10^{-5}	100.77	99.65	100.19	99.57
1:2:1	1.0×10^{-4}	2.0×10^{-4}	1.0×10^{-4}	101.59	99.70	100.23	98.07
1:1:0.6	1.0×10^{-4}	1.0×10^{-4}	6.0×10^{-5}	100.18	100.67	98.83	98.55
		Mean		100.90	100.02	99.64	98.63
		R.S.D.%		0.504	0.862	0.636	0.631

Table 4: Determination of MT and HZ in pharmaceutical formulations by the suggested potentiometric procedure

Sensors	Pharmaceutical formulations	Recovery [a] % ± R.S.D.%
Sensor 1	Lopressor HCT tablets (100:50 mg) B.N. K0016	100.89 ± 0.130
	Lopressor HCT tablets (100:25 mg) B.N. K0095A	99.76 ± 0.529
Sensor 2	Lopressor HCT tablets (100:50 mg) B.N. K0016	101.35 ± 0.228
	Lopressor HCT tablets (100:25 mg)B.N. K0095A	102.00 ± 0.767
Sensor 3	Lopressor HCT tablets (100:50 mg) B.N. K0016	98.44 ± 0640
	Lopressor HCT tablets (100:25 mg) B.N. K0095A	99.11 ± 0.309
Sensor 4	Lopressor HCT tablets (100:50 mg) B.N. K0016	100.48 ± 0.337
	Lopressor HCT tablets (100:25 mg) B.N. K0095A	100.85 ± 0.192

[a] Average of three determinations.

Table 5: Determination of MT and HZ drugs in spiked human plasma by the proposed electrodes

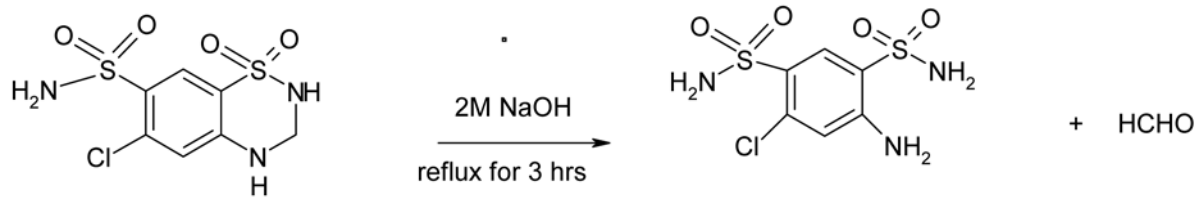
Concentration of MT (M)	Recovery % ± R.S.D. Sensor 1	Sensor 2	Concentration of HZ (M)	Recovery % ± R.S.D. Sensor 3	Sensor 4
10^{-5}	100.23 ± 0.148 %	99.19 ± 0.654 %	10^{-6}	100.44 ± 0.650 %	99.22 ± 0.554 %
10^{-6}	99.44 ± 0.651 %	99.87 ± 0.980 %	10^{-7}	100.70 ± 0.109%	99.87 ± 0.642 %

[a] Average of three determinations.

Table 6: Statistical analysis between the results obtained for the determination of MT and HZ drugs in pure samples by the proposed method and those obtained by the official[3, 4] methods.

Item	Sensor 1	Sensor 2	Official method[a(3)] for MT	Sensor 3	Sensor 4	Official method[b(4)] for HZ
Mean	99.11	99.21	99.45	100.08	100.28	100.34
S.D	0.354	0.386	0.473	0.459	0.439	0.501
R.S.D %	0.357	0.389	0.475	0.459	0.438	0.499
Variance	0.125	0.149	0.223	0.211	0.193	0.251
n	5	5	6	5	5	6
F test	1.784 (6.26)[c]	1.496 (6.26)[c]		1.189 (6.26)[c]	1.300 (6.26)[c]	
Student's t test	1.325 (2.262)[c]	0.909 (2.262)[c]		0.890 (2.262)[c]	0.209 (2.262)[c]	

[a(3)] Non-aqueous titration using 0.1 M perchloric acid, determining the end point potentiometrically. [b(4)] HPLC method using 0.1 M monobasic sodium phosphate and acetonitrile (9:1 v/v), adjust with phosphoric acid to a pH 3.0 ± 0.1 as a mobile phase [c] Figures between parenthesis are the corresponding tabulated values (P = 0.05)



HZ SA (M.wt = 267.7)

Fig 7: Degradation pathway of HZ

6. References

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Povzetek

Razvili, opisali in okarakterizirali smo štiri mikroelektrode iz grafita ali platinaste žice z membrano iz polivinilklorida, ki zaznavajo nekatere učinkovine za kardiovaskularni sistem: metoprolol tartrat (MT) in hidroklorotiazid (HZ). Senzorje smo izdelali z uporabo (2-hidroksipropil)- β-ciklodekstrina (2HP β-CD) kot ionofora, kar znatno poveča občutljivost in selektivnost membrane. Senzorje smo izdelali v polimernem nosilcu karboksiliranega polivinilklorida (PVC–COOH) in dioktil ftalata (DOP) kot plastifikatorja na osnovi interakcije med učinkovinami ter disociiranimi COOH skupinami v PVC–COOH. Dobili smo hiter in stabilen Nernstov odziv med $1{,}0 \times 10^{-6}$–$1{,}0 \times 10^{-2}$ M v pH območju 3,0–9,0 za MT (senzorja 1 in 2) ter $1{,}0 \times 10^{-7}$–$1{,}0 \times 10^{-3}$ M v pH območju 3,0–7,0 za HZ (senzorja 3 in 4). Opazili smo Nernstove naklone 56,2, 54,6, 19,0 in 20,8 mV/dekado za elektrode 1–4. Predlagana metoda se je pokazala kot uporabna za določitev MT in HZ v čisti praškasti obliki s povprečnim izkoristkom 99,1 ± 0,4, 99,2 ± 0,4, 100,1 ± 0,5 in 100,3 ± 0,4 % za senzorje 1–4. Meje zaznave so bile pri senzorjih 1–4 $5{,}5 \times 10^{-6}$, $4{,}5 \times 10^{-6}$, $4{,}8 \times 10^{-8}$ in $5{,}0 \times 10^{-8}$ M, kar kaže na visoko obč utljivost. Vsi štirje senzorji so bili stabilni preko obdobja 6 tednov. Selektivnostni koeficienti kažejo na odlično selektivnost. Delovne karakteristike vseh štirih senzorjev smo evaluirali po IUPACovih smernicah. Metodo smo uspešno uporabili za določ itev MT in HZ v farmacevtskih pripravkih ter v vzorcih humane plazme v prisotnosti drug drugega ter v prisotnosti salamida (SA), ki je glavni razgradni produkt HZ. Naredili smo statistično primerjavo med rezultati, dobljenimi s to metodo in z uradnimi metodami. Ni bilo signifikantnih razlik.