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Membrane electrodes for the determination of glutathione

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Abstract

Four glutathione (GSH)-selective electrodes were developed with different techniques and in different polymeric matrices. Precipitation-based technique with bathophenanthroline–ferrous as cationic exchanger in polyvinyl chloride (PVC) matrix was used for sensor 1 fabrication. β-Cyclodextrin (β-CD)-based technique with either tetrakis(4-chlorophenyl)borate (T*p*ClPB) or bathophenanthroline–ferrous as fixed anionic and cationic sites in PVC matrix was used for fabrication of sensors 2 and 3, respectively.

β-CD-based technique with T*p*ClPB as fixed anionic site in polyurethane (Tecoflex) matrix was used for sensor 4 fabrication. Linear responses of 1×10^{-5} to 1×10^{-4} M and 1×10^{-6} to 1×10^{-3} M with slopes of 37.5 and 32.0 mV/decade within pH 7–8 were obtained by using electrodes 1 and 3, respectively. On the other hand, linear responses of 1×10^{-5} to 1×10^{-2} and 1×10^{-5} to 1×10^{-3} M with slopes of 47.9 and 54.3 mV/decade within pH 5–6 were obtained by using electrodes 2 and 4, respectively. The percentage recoveries for determination of GSH by the four proposed GSH-selective electrodes were 100 ± 1, 100.5 ± 0.7, 100 ± 1 and 99.0 ± 0.8% for sensors 1, 2, 3 and 4, respectively. Determination of GSH in capsules by the proposed electrodes revealed their applicability for determination of GSH in its pharmaceutical formulations. Also, they were used to determine GSH selectively in presence of its oxidized form (GSSG). Sensor 4 was successfully applied for determination of glutathione in plasma with average recovery of 100.4 ± 1.11%. The proposed method was compared with a reported one. No significant difference for both accuracy and precision was observed.

Keywords: Glutathione; Potentiometry; β-Cyclodextrin; Poly(vinyl chloride); Bathophenanthroline; Tetrakis(*p*-chlorophenyl)borate; Tecoflex; Pharmaceutical formulations; Plasma

1. Introduction

Glutathione is found in most living cells in the reduced (GSH) and oxidized (GSSG) forms at concentration levels depending upon the activity of glutathione reductase enzyme, which appears to be as ubiquitous in nature as GSH [1]. Determination of these substances is essential in many clinical diagnoses. Erythrocytes of normal individuals contain 0.6–0.65 mg of GSH, 30–80 μg of GSSG and 6.3–39 mIU of glutathione reductase/cm^3, and higher concentrations (∼3.1 mg of GSH, 255 μg GSSG and 4–100 IU of enzyme/gm) are commonly found in various biological tissues [1,2]. The level of GSH increases in myeloproliferative disorders and sharply decreases in malnutrition, renal and hepatic failure, diabetes, mental diseases as well as with deficiencies of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and Vitamin B_2 [3].

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Glutathione (GSH) is a nonprotein tripeptide composed of the amino acids, glutamate, cysteine and glycine [4]. It is among the most powerful antioxidants present in biological systems, and has been called the "master antioxidant"; it regulates the actions of lesser antioxidants such as Vitamin C and E [5].

GSH has been determined by several analytical methods, most of them determine GSH in biological fluids. These include titrimetry [6,7], spectrophotometry [8–10], spectrofluorimetry [11,12], HPLC [13–16], capillary zone electrophoresis [17–20] and voltammetry [21–23]. Potentiometric flow injection analysis (FIA) method has also been described [24]. Determination of GSH and GSH reductase with silver sulfide membrane electrode has also been developed [25].

Modern ion selective electrodes based on material transport across a specific membrane are now widely used in the determination of trace amounts of analytes. The material transport includes both neutral and charged complex species, or simple ions.

The high selectivity of these electrodes imparts a great advantage over other techniques [26]. Analytes in colored, turbid and viscous samples can be determined accurately. They show rapid response to changes in the concentration.

Furthermore, they may be used for measurement over a wide concentration range. Ion selective electrodes (ISEs) are generally tolerant of small changes in pH. A further advantage is that they are relatively simple and cheep to develop, set up and run. Moreover, the chemical design of the electrodes has been developed to give superior selectivity and response [27].

In the present work, both anionic and cationic properties of GSH have been fully studied with both cationic and anionic exchangers, respectively. The partially dissociated molecule of GSH is stable to form negatively and positively charged complexes in the membrane phase. Thus, precipitation-based technique of GSH with bathophenanthroline–iron(II) to form an ion association complex was used in the preparation of sensor 1. In addition, β-CD-based technique was used in the preparation of sensors 2, 3 and 4.

The present study originates from the fact that GSH possess primary amino group and two carboxylic groups that can react with anionic and cationic exchangers, either inside or outside the membrane phase.

2. Experimental

2.1. Instruments

- Jenway digital ion analyzer model 3330 (UK) with Ag/AgCl double junction reference electrode no. 924017-LO3-Q11C.
- Bandelin Sonorox, Rx 510 S, magnetic stirrer (Hungarian).
- pH glass electrode Jenway (UK) no. 924005-BO3-Q11C.

2.2. Materials

2.2.1. Reference sample

Glutathione pure sample was kindly supplied by ADWIA, 10th of Ramadan City, Egypt, batch no. 0330921 and assayed for its purity according to a compendial method [6] to contain $99.69 \pm 0.64\%$.

2.2.2. Pharmaceutical formulations

- Livit capsules (50 mg) manufactured by the European Egyptian Co., batch no. 226101.
- Hepanox capsules (50 mg) packed by Technopharm Egypt Co., under license for: Tishcon Corporation U.S.A, batch no. 00135 BN.
- Hipamax capsules (50 mg) manufactured by Farco Co., under license for: Mercyler U.S.A, batch no. 146.

2.3. Reagents

All chemicals and reagents used throughout this work were of analytical grade (water used was bi-distilled):

- Bathophenanthroline (4,7-diphenyl-1,10-phenanthroline); Sigma, GmbH, Germany.
- Dioctyl phthalate (DOP); Sigma.
- 2-Nitrophenyl octyl ether (*o*NPOE); Aldrich.
- 2-Nitrophenyl phenyl ether (*o*NPPE); Aldrich.
- Poly(vinyl chloride) (PVC) of high molecular weight; Fluka Chemie GmbH, Germany.
- Tetrakis(4-chlorophenyl)borate (T*p*ClPB) freshly prepared, saturated aqueous solution; Aldrich.
- Sodium hydroxide, 1 M aqueous solution; Prolabo.
- Hydrochloric acid, 1 M aqueous solution; Prolabo.
- Ammonia solution, 10% aqueous solution; Prolabo.
- Iodine aqueous solution, 0.05 M aqueous solution; Prolabo.
- Borate buffer, prepared by mixing 50 ml of 0.1 M boric acid in 43.7 ml of 0.1 M sodium hydroxide.
- Ferrous ammonium sulphate; Prolabo.
- Potassium chloride, 1×10^{-2} M aqueous solution; Prolabo.
- β-Cyclodextrin (β-CD), Fluka Chemie GmbH, Germany.
- Polyurethane (Tecoflex); Fluka Chemie GmbH, Germany.
- Tetrahydrofuran (THF); BDH.
- Diethyl ether (DEE); BDH.
- Riboflavin, BN: UQ30422026; thiamine hydrochloride, BN: UQ20708332; zinc sulphate, BN: 2/2004; ascorbic acid, BN: TL00307493; nicotinamide, BN: 010591N; pyridoxine, BN: H09AS66; Vitamin A, BN: 1191; silymarine, BN: S/30042; Vitamin E, BN: EC2002/215C; were kindly supplied from El-Kahira Pharmaceutical and Chemical Industries Company with certificates of their purity.
- Bathophenanthroline–iron(II) complex, prepared by dissolving 100 mg of bathophenanthroline in 20 ml of 10^{-2} M iron(II) ammonium sulphate, followed by drops of ethanol and water to keep a clear solution.

2.4. Standard solutions

2.4.1. Glutathione stock solution (1×10^{-1} M)

It was freshly prepared daily by transferring 3.07 g of GSH into 100 ml volumetric flask and dissolving in distilled water and tightly closed.

2.4.2. Glutathione working solutions (1×10^{-7} to 1×10^{-2} M)

They were freshly prepared by suitable dilution from their stock solution using distilled water and kept in well-closed tight containers to avoid air oxidation.

2.4.3. Oxidized glutathione (GSSG) solution (1×10^{-2} M)

It was prepared by transferring accurately weighed amount of 614.7 mg of GSH in a conical flask and dissolving in 10 ml water. One millilitre of starch solution was added, and the solution was titrated with 0.05 M iodine solution until the first blue color appeared. The solution was then transferred quantitatively into 100 ml volumetric flask and completed to volume with deionized water.

2.5. Procedures

2.5.1. Precipitation-based technique for the preparation of PVC-membrane sensor (sensor 1)

A 5 ml aliquot of 10^{-1} M aqueous GSH solution was treated with two drops of 10% ammonia solution, mixed with 5 ml of bathophenanthroline–iron(II) solution, and shaken for 5 min.

The resultant precipitate formed was filtered using Whatmann no. 42 paper, washed with cold water, dried at room temperature (about 20 °C) and grounded to fine powder.

Elemental analysis of the formed complex confirmed the formation of drug:ion exchanger in a ratio of 1:1.

In a glass Petri dish (5 cm diameter), 10 mg of GSH-ion exchanger was thoroughly mixed with 0.35 ml of DOP and 0.19 g of PVC. The mixture was dissolved in 5 ml of THF. The Petri dish was covered with a filter paper and left to stand overnight to allow solvent evaporation at room temperature. A master membrane with a thickness of 0.1 mm was obtained.

From the master membrane, a disk (≃8 mm diameter) was cut using a cork borer and pasted using THF, to an interchangeable PVC tip that was clipped into the end of the electrode glass body. Equal volumes of 10^{-2} M GSH and 10^{-2} M KCl were mixed and this solution was used as an internal reference solution. Ag/AgCl wire (1 mm diameter) was immersed in the internal reference solution as an internal reference electrode.

2.5.2. β-CD-based technique for the preparation of PVC-membrane sensors (sensors 2 and 3)

β-CD, 0.04 g, was mixed with 0.4 g *o*NPOE and with 0.01 g of either T*p*ClPB or bathophenanthroline–iron(II) for the preparation of sensors 2 and 3, respectively.

PVC, 0.18 g, previously dissolved in 6 ml THF was added and procedure was completed as under Section 2.5.1 starting from "The Petri dish was covered with a filter paper and left to stand overnight . . .".

2.5.3. β-CD-based technique for the preparation of Tecoflex-membrane sensor (sensor 4)

The same procedure was carried out as under Section 2.5.2 using T*p*ClPB as ion exchanger and Tecoflex instead of PVC as polymer.

2.5.4. Direct potentiometric determination of glutathione in its pure sample

The sensors were conditioned by soaking in 10^{-2} M aqueous GSH solution for 24, 2, 2 h and for 15 min for sensors 1, 2, 3 and 4, respectively. Storage was in the same solution when not in use. The conditioned sensors were calibrated by separately transferring 50 ml aliquots of solutions covering the concentration range of (1×10^{-7} to 1×10^{-2} M) GSH, into a series of 100 ml beakers. The electrode system was immersed in each solution, with constant stirring in conjunction with a Jenway reference electrode.

The sensors were stored in distilled water between measurements. The electrode potential was plotted versus each negative logarithmic concentration of GSH. The calibration plots obtained were used for subsequent measurements of unknown samples.

2.5.5. Direct potentiometric determination of glutathione in its pharmaceutical formulations

Capsules were softened using hot water, and then cut with a sharp blade. The contents of five capsules were evacuated using a small spoon in a 250 ml volumetric flask using 100 ml water. The spoon and softened capsules were washed with water into the flask and the mixture was stirred for 15 min, and the volume was then completed to the mark with water. The solution was filtered and a suitable volume equivalent to 30.7 mg of GSH was transferred into a 100 ml volumetric flask and diluted to volume with distilled water to prepare 1×10^{-3} M aqueous solution of GSH. Suitable dilutions were performed using distilled water to obtain serial of 10^{-5} to 10^{-4} M GSH. Procedure was then completed as under Section 2.5.4.

2.5.6. Direct potentiometric determination of glutathione in plasma

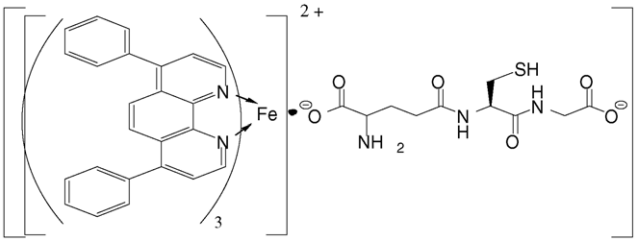
The pH of plasma was adjusted to 7 using borate buffer. A volume of 5 ml plasma was placed in a stoppered shaking tube. Then 1.0, 0.1 and 0.01 ml of 10^{-1} M GSH standard stock solutions followed by 20 ml DEE were separately added. Prepared solutions were allowed to centrifugate for 5 min, and the organic layer was rejected. The extraction with 20 ml DEE was repeated, and traces of DEE were then evaporated by a flow of nitrogen. The resultant aqueous phase was transferred into 100 ml volumetric

flask, and the volume was completed to the mark with distilled water. Procedure was then completed as under Section 2.5.4.

3. Results and discussion

Selective membranes in ion selective electrodes (ISEs) have shown both ion exchange and perm-selectivity of the sensor ions [28].

The present study originates from the fact that GSH can behave both as anion in basic medium or cation in acid medium as the dissociation constants (pK_a) was found to be pK_1' (–COOH) 2.12, pK_2' (–COOH) 3.53, pK_3' ($-NH_2$) 8.66 and pK_4' (–SH) 9.12 [4]. This fact suggests the use of ion exchangers of both the anionic and cationic types. It has been found that bathophenanthroline–iron(II) and T*p*ClPB were the optimum anionic and cationic exchangers for the studied drug, respectively, for their low solubility product and suitable grain size. The suggested structural formulae of ion association complexes of GSH with both bathophenanthroline–iron(II) and T*p*ClPB are:



GSH was found to form 1:1 ion association complexes with each of bathophenanthroline–iron(II) and T*p*ClPB as proved by elemental analysis, as divalent anion and monovalent cation with the two ion exchangers, respectively.

3.1. Sensors fabrication

The four proposed sensors were prepared and electrochemically GSH behaviour evaluated as prospective sensors for GSH according to the IUPAC guidelines [29] (Table 1).

It has been reported that PVC matrix is a regular support and reproducible trap for ion association complexes in ISEs. Nevertheless, its use creates a need for plasticization and places a constraint on the choice of mediator [30].

In the present study, DOP or *o*NPOE have been used in the fabrication of sensor 1 and other proposed sensors, respectively. They plasticized the membrane and adjusted both permittivity of the final organic membranes and mobility of the ion exchanger sites. The potentiometric response of sensor 1 was not influenced by the polarity of the plasticizer; sensors prepared with *o*NPPE gave the same responses as the original ones. In the other β-CD-sensors (2, 3 and 4), the use of plasticizer with high dielectric constant such as *o*NPOE and *o*NPPE, facilitated the inclusion of organic molecules by competitive inclusion, they gave the optimal results. The membranes constituents were dissolved in THF that was slowly evaporated at room temperature leading to membrane formation.

Cyclodextrins are optically active oligosaccharides that form inclusion compounds in the aqueous and in solid state with organic molecules. They were previously applied as sensor ionophores to potentiometric ISEs for the determination of protonated amines [31] and chiral molecules incorporating aryl rings [32]. β-CD-based sensors showed accurate results in both response and selectivity.

The electrochemical cell of the suggested membrane electrodes for the determination of glutathione can be illustrated

Table 1
General working characteristics of the four investigated glutathione-selective electrodes

Parameter	Value			
	Electrode 1	Electrode 2	Electrode 3	Electrode 4
Slope (mV/decade)[a]	37.5	47.9	32.0	54.3
Response time (s)	5	60	60	10
Working pH range	7–8	5–6	7–8	5–6
Concentration range (M)	1×10^{-5} to 1×10^{-4}	1×10^{-5} to 1×10^{-2}	1×10^{-6} to 1×10^{-3}	1×10^{-5} to 1×10^{-3}
Stability (days)	15	7	14	7
Average recovery (%)	100	100.5	100	99.0
Precision (%)	1	0.7	1	0.8
Correlation coefficient	0.979	0.997	0.995	0.988
Intercept (mV)	156.8	162.7	99.3	244.4
Robustness[b]	98.11	99.84	99.13	99.75
Ruggedness[c]	99.43	98.13	98.86	98.91

[a] Results of five determinations.

[b] Average recovery percent of determining 10^{-5}, 10^{-4} and 10^{-3} M solutions of GSH for the four studied electrodes preparing the membrane using *o*NPPE as plasticizer instead of DOP.

[c] Average recovery percent of determining 10^{-5}, 10^{-4} and 10^{-3} M solutions of GSH for the four studied electrodes using Jenway 3310 digital ion analyzer instead of 3330 model.

diagrammatically as follows:

Sensor 1:

Double junction Ag/AgCl reference electrode	Test solution	Glutathione association complex incorporated in PVC matrix	Internal reference solution: 10^{-2} M KCl + 10^{-2} M glutathione in 1:1 ratio	Ag/AgCl internal reference wire

Sensors 2 & 3:

Double junction Ag/AgCl reference electrode	Test solution	β-CD + cationic or anionic additive incorporated in PVC matrix	Internal reference solution: 10^{-2} M KCl + 10^{-2} M glutathione in 1:1 ratio	Ag/AgCl internal reference wire

Sensor 4:

Double junction Ag/AgCl reference electrode	Test solution	β-CD + anionic additive incorporated in Tecoflex membrane	Internal reference solution: 10^{-2} M KCl + 10^{-2} M glutathione in 1:1 ratio	Ag/AgCl internal reference wire

3.2. Sensors calibration and response time

The potential displayed by the four proposed electrodes for constructive measurements of standard drug solution in the same day and from day-to-day did not vary by more than ±1 mV. Calibration slopes did not change by more than ±1 mV/decade concentration over a period of 15, 7, 14 and 7 days for sensors 1, 2, 3 and 4, respectively. The required time for the electrodes to reach values within ±1 mV of the final equilibrium potential after increasing the drug concentration 10-folds was found to be 5, 60, 60 and 10 s for electrodes 1, 2, 3 and 4, respectively.

The slopes of the calibration plots were 37.5, 47.9, 32.0 and 54.3 mV/concentration decade for GSH–bathophenanthroline–iron(II) (electrode 1), β-CD–T*p*ClPB–PVC (electrode 2), β-CD–bathophenanthroline–iron(II)–PVC (electrode 3) and the β-CD–T*p*ClPB–Tecoflex (electrode 4), respectively (Fig. 1). Slopes values of electrodes 1 and 3 were about 30 mV; the typical value of divalent substances as GSH behaves as divalent anion via its two carboxylic groups. An increase of GSH concentration in the measured outer solution will cause suppression of dissociation of the formed complex and decrease of the positive charge on the membrane, as shown in Fig. 1. On the other hand, slope values of electrodes 2 and 4 were about 60; the typical value of monovalent substances as GSH behaves as monovalent cation via its primary amino groups. Fig. 1 shows a decrease in negative potential as the concentration increases due to the decrease in negative charge on the membrane.

Deviation from the ideal Nernstian slope (30 mV) for sensors 1 and 3, and (60 mV) for sensors 2 and 4, stems from the fact that the electrode responds to the activities of drug anion and cation rather than its concentration. The investigated electrodes exhibit fast response time (5–60 s) and fair stability (7–15 days). The response characteristics of the four electrodes are summarized in Table 1.

The proposed method was compared with a reported one [6], no significant difference was observed.

3.3. Sensors pH and temperature

For quantitative measurements with ISEs, studies were carried out to reach the optimum experimental conditions.

The effect of pH on the response of the four investigated electrodes using 1×10^{-4} and 1×10^{-3} M solutions of GSH for electrodes 1 and 3, respectively, and 1×10^{-5} and 1×10^{-4} M solutions of GSH for electrodes 2 and 4, respectively, was studied. It was apparent from the potential–pH profile that the responses are fairly constant over the pH range 7–8, i.e., in this pH range GSH is completely ionized, dissociated and sensed. Above pH 8 variable electrode response was observed, this may be explained by a variable increase of

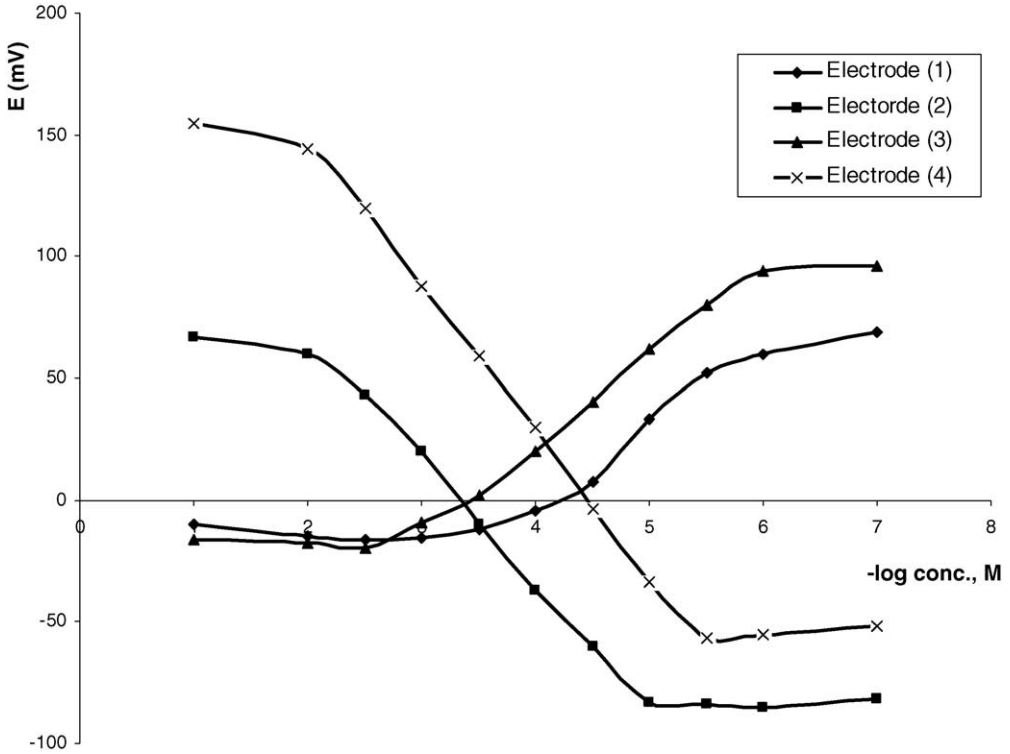


Fig. 1. Profile of the potential in mV vs. −log concentration in M using the four GSH-selective electrodes: GSH-bathophenanthroline–iron(II) (electrode 1); β-CD-bathophenanthroline–iron(II)–PVC (electrode 3) at pH 7–8 and β-CD–T*p*ClPB–PVC (electrode 2); β-CD–T*p*ClPB–Tecoflex (electrode 4) at pH 5–6.

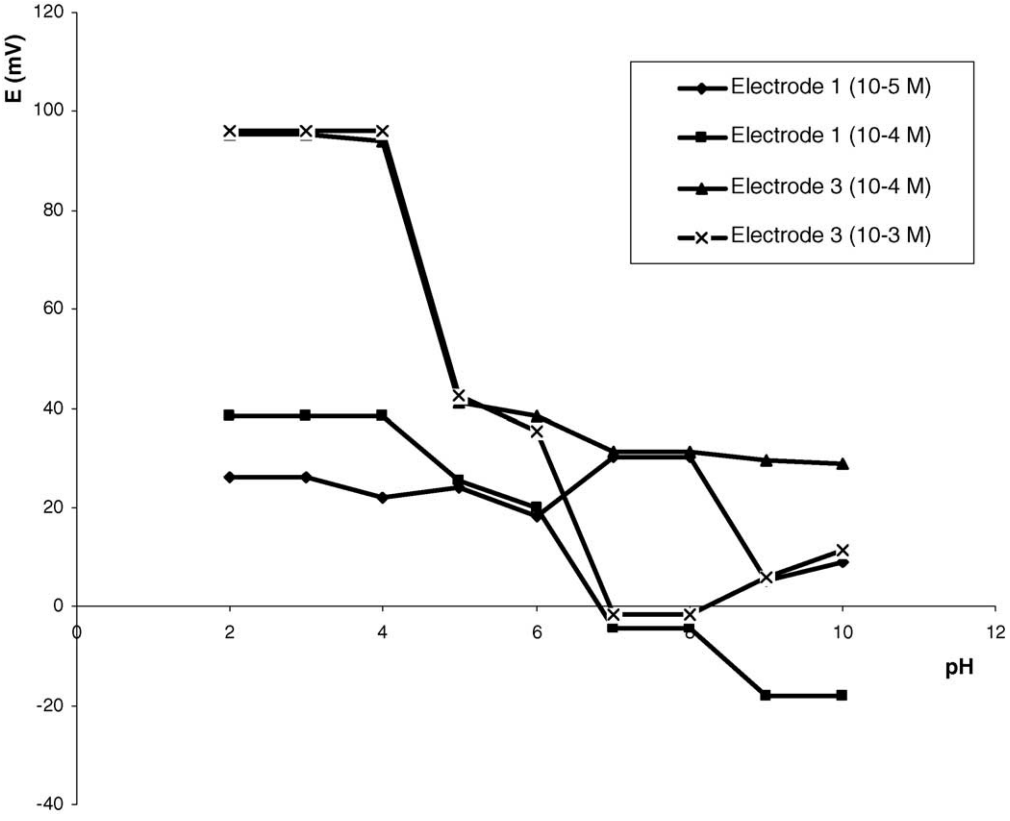


Fig. 2. Effect of pH on the responses of sensors 1 and 3.

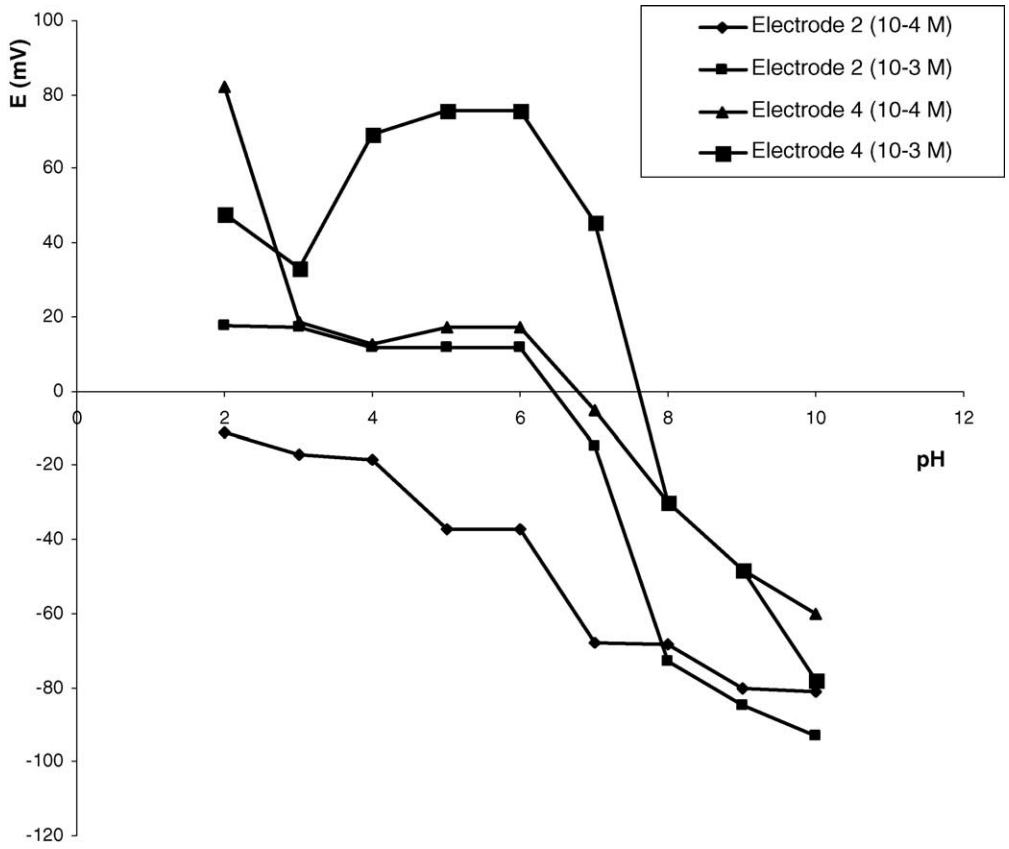


Fig. 3. Effect of pH on the responses of sensors 2 and 4.

dissociation of the formed ion association complex. While below pH 7, the electrode response decreased with the increase of analyte acidity; at such high acidity the dissociation of the carboxyl groups of GSH in solution are limited, also the membrane may extract H^+ [33], leading to noisy responses (Fig. 2).

While, for electrodes 2 and 4, above pH 6, the potential showed a sharp decrease due to the formation of non-protonated primary amino group of GSH.

Below pH 5, the electrode response increased with the increase of analyte acidity; the membrane may extract H^+ [33], leading to noisy responses (Fig. 3).

Upon studying the effect of temperature, the four suggested electrodes exhibit slight increase in their potentials as the temperature rises in the range of 20–35 °C, however, the calibration graphs obtained at different temperatures were parallel. The limit of detection, slope and response time did not significantly vary with variation of temperature, indicat-

Table 2
Potentiometric selectivity coefficients (average of five measurements) ($-\log K^{\text{Pot.}}_{\text{glutathione, interferent}}$) of the four proposed sensors by separate selectivity method (SSM) [29]

Interferent[a]	Sensor 1	Sensor 2	Sensor 3	Sensor 4
Riboflavin	2.2×10^{-4}	1.0×10^{-4}	1.9×10^{-4}	1.8×10^{-4}
Thiamine hydrochloride	1.8×10^{-4}	1.3×10^{-4}	2.1×10^{-4}	1.6×10^{-4}
Zinc sulphate	2.2×10^{-4}	1.3×10^{-4}	2.0×10^{-4}	2.1×10^{-4}
Ascorbic acid	2.1×10^{-4}	9.6×10^{-3}	2.2×10^{-4}	1.9×10^{-4}
Nicotinamide	1.1×10^{-4}	5.4×10^{-3}	2.2×10^{-4}	1.8×10^{-4}
Pyridoxine	8.6×10^{-3}	1.5×10^{-4}	1.9×10^{-4}	1.1×10^{-4}
Oxidized glutathione	1.7×10^{-4}	9.5×10^{-3}	1.3×10^{-4}	1.8×10^{-4}
Glycine	0.3×10^{-3}	0.4×10^{-3}	1.7×10^{-3}	1.9×10^{-3}
Cysteine	5.2×10^{-2}	6.4×10^{-2}	8.1×10^{-2}	3.1×10^{-2}
1-Glutamate	3.0×10^{-3}	8.1×10^{-3}	7.2×10^{-2}	8.1×10^{-3}
Glutamyl cysteine	1.6×10^{-2}	4.1×10^{-2}	2.4×10^{-2}	4.1×10^{-2}
5-Oxoproline	1.3×10^{-3}	1.3×10^{-3}	4.8×10^{-3}	3.5×10^{-3}
Cyste amine	6.2×10^{-2}	4.6×10^{-2}	8.4×10^{-2}	3.3×10^{-2}

[a] Aqueous solutions of 1×10^{-4} M were used.

Table 3
Determination of glutathione in pharmaceutical formulations (three formulations were freshly prepared and obtained from the manufacturing firms) by the suggested potentiometric procedures and assessing the accuracy of the results by standard additions

Pharmaceutical preparation	Sensor 1		Sensor 2		Sensor 3		Sensor 4	
	Found (%)	Recovery of standard added (%)[a]	Found (%)	Recovery of standard added (%)[a]	Found (%)	Recovery of standard added (%)[a]	Found (%)	Recovery of standard added (%)[a]
Livit capsules (50 mg) batch no. 226101	100 ± 2	99.8 ± 0.8	98.3 ± 0.9	100.3 ± 0.8	100 ± 2	100 ± 2	100.2 ± 0.5	100.0 ± 0.4
Hepanox capsules (50 mg) batch no. 00135 BN	101 ± 2	100 ± 2	99 ± 2	99.6 ± 0.7	100 ± 2	101 ± 2	100.5 ± 0.9	99.7 ± 0.5
Hipamax capsules (50 mg) batch no. 146	101 ± 2	100 ± 1	100.1 ± 0.4	98.9 ± 0.8	100 ± 2	100.9 ± 0.9	99.8 ± 0.7	98.3 ± 0.4

[a] Average of six determinations.

ing reasonable thermal stability of both PVC and Tecoflex membranes up to 35 °C.

3.4. Sensors selectivity

The effect of interfering substances upon the performance of the sensors was studied by separate solution method using the following equation [29]:

$$-\log\left(K^{\text{Pot.}}_{\text{primary ion, interferent}}\right) = \left[\frac{(E_{\text{GSH}} - E_{\text{M}})}{(2.303\,RT/Z_{\text{GSH}}F)}\right] + \left[1 + \frac{Z_{\text{GSH}}}{Z_{\text{M}}}\right]\log[\text{GSH}]$$

where E_{GSH} is the potential measured in 10^{-4} M GSH solution, E_{M} the potential measured in 10^{-4} M interferent solution, Z_{GSH} and Z_{M} are the charges of GSH and interfering ion, respectively, and $2.303RT/Z_{\text{GSH}}F$ represents the slope of the investigated sensor (mV/concentration decade).

Table 2 shows the selectivity of the proposed sensors in the presence of its oxidized form (GSSG), and in the presence of the related substances that always present with GSH in the dosage formulations.

Being insoluble in aqueous solution, Vitamin A, Vitamin E and silymarine show no interference with the measured potential of GSH.

Table 3 shows the results obtained for the determination of GSH in pharmaceutical formulations that proves the applicability of the method, as demonstrated by the accurate and precise percentage recovery. Placebo experiments contain all additives in the same ratio as that used in capsules were investigated.

Gelatin, glycerin, methyl- and propyl parabens, and lecithin that are normally used in soft gelatin capsule formulations did not show any interference. Thus, analysis was carried out without prior treatment or extraction.

The proposed GSH-selective sensors have shown excellent selectivity.

On application to biological fluids, it has been found that the sensors incorporated in PVC polymer showed a drop in slope accompanied by noise responses in comparison to the original slope and responses of the aqueous solution. This may be attributed to the fact that any protein adherence to the PVC matrix leads to its fouling.

On the other hand, Tecoflex gave stable results in both slopes and mV readings as revealed by high precision and accuracy of recovery results of spiked plasma samples (Table 4).

Table 4
Determination of glutathione in spiked human using β-CD–T*p*ClPB–Tecoflex (electrode 4)

Added (μg/ml)	Recovery (%) ± S.D.[a]
310	100.7 ± 0.9
31	101 ± 2
3.1	99.3 ± 0.9

[a] Mean of three separate determinations; S.D., standard deviation.

Deprotonation step was found essential to avoid any interference from cellular thiols (CSH) and protein thiols (PSH).

As for robustness, determining 10^{-5}, 10^{-4} and 10^{-3} M solutions of GSH for the four studied electrodes preparing the membrane using *o*NPPE as plasticizer instead of DOP was studied; the methods demonstrated efficient stability. To study the methods, ruggedness, 10^{-5}, 10^{-4} and 10^{-3} M solutions of GSH were analyzed by the four studied electrodes using Jenway 3310 digital ion analyzer instead of 3330 Model; proved stability of the methods upon change of the instrument.

4. Conclusion

The potentiometric responses of the four GSH-selective electrodes at the optimum pH range and temperature are linear over a drug concentration range of 3.07–30.73, 3.07–3073.30, 0.31–307.33 and 3.07–307.33 $\mu g\,ml^{-1}$ for electrodes 1, 2, 3 and 4, respectively (Figs. 1 and 2). Electrodes 1 and 3 gave more stability than 2 and 4 (about double the life time is attained). Electrodes 1 and 4 were faster than 2 and 3, thus the response times were more or less instantaneous (5 and 10 min for electrodes 1 and 4, respectively), while those of 2 and 3 were about 1 min. Precisions of sensors 2 and 4 were higher than 1 and 3 (less than one). Only electrode 4 gave successful application in plasma.

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