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RESEARCH ARTICLE

New in situ modified PVC membrane electrodes for potentiometric determination of gentamicin sulfate in its pharmaceutical formulation and biological fluids

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Abstract

The construction and performance characteristics of five new, simple, rapid, selective and sensitive in situ modified poly vinyl chloride (IPVC) membrane electrodes are described for determination of gentamicin sulfate (GNS) in raw material, pharmaceutical formulations and biological fluids (plasma and urine) using dibutylphthalate (DBP) as plasticizer. Sensor 1(S1) was fabricated using cation exchanger sodium tetraphenylborate (NaTPB), sensor 2 (S2) used phosphotungstic acid (PTA), sensor 3 (S3) used Phosphomolybdic acid (PMA), sensor 4 (S4) used ammonium reineckate (RN) and sensor 5 (S5) used a mixture of RN + NaTPB. The electrodes under investigation exhibit suitable response to GNS in the concentration range from 1 $x10^{-6}$ to 1 $x10^{-2}$ M for S1, S2, S3 and S5 and from 1 $x10^{-5}$ to 1 $x10^{-2}$ M for S4. The electrodes have values of 30.5±0.8, 28.7±0.6, 29.9±0.7, 25.8±0.5 and 31.0±0.9 mV $decade^{-1}$ with detection limit values of 8.9 x 10^{-7}, 9.4 x 10^{-7}, 9.1 $x10^{-7}$, 9.7 x 10^{-6} and 8.5 x 10^{-7} M GNS using S1, S2, S3, S4 and S5, respectively. The electrode response is independent of pH in the range of 3-9 for S1, S2, S3 and S5 and in the range of 3-7 for S4. All electrodes shows fast response time which is very short (≤ 12 s), and were used over a period of 60 days with good reproducibility. The selectivity coefficients indicated good selectivity for GNS drug over a large number of interfering materials (inorganic cations, amino acids and sugars). The proposed electrodes were successfully applied to the determination of GNS in raw material, pharmaceutical formulations and biological fluids. The results were validated by comparison with the official method.

1. Introduction

Gentamicin sulfate, isolated in 1958 and reported in 1963 by Weinstein et al., is an aminoglycoside based broad spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria [1]. Gentamicin was originally isolated from the microorganism Micromonospora purpurea in the research laboratories of Schering-Plough Corporation, and four aminoglycosides have been identified in the USP-NF 2013 (cUSP) as C1, C1a, C2a and C2 [2,3] with three structures were listed in the compendium [Fig-1]. Gentamicin and its related substances are highly polar compounds with high water solubility, very low solubility in most organic solvents and all of them have a weak UV chromophore. Drug products containing gentamicin sulfate are used to treatment various infections where Cream and ointment dosage forms are used in the treatment of skin infections and burns complicated by Pseudomonemia, the ophthalmic solution is commonly used to treat eye infections while an injectable solution including 10- and 40-mg/mL gentamicin sulfate is used for serious systemic and genitou-rinary tract infection [4].

Several methods have been reported for the determination of GNS, these include HPLC with UV detection or fluorescence detection [5–18], HPLC using Refractive Index (RI) [19], mass spectrometry (MS) detection [20–

24], Evaporative Light Scattering Detection (ELSD) [25,26], and Electrochemical Detection (ECD) [27–29]. However, these methods need expensive instruments as well as laborious and time-consuming extraction procedures.

Electrochemical methods have proved to be very sensitive for the determination of organic molecules, including drugs and related substances in pharmaceutical dosage forms and biological fluids. For instance, potentiometric ion-selective electrodes (ISEs) have been extensively used in pharmaceutical analysis because of their simplicity, low-cost, fast response time, wide pH range of application, applicability to turbid and colored solutions, high selectivity and easy interfacing with automated and computerized systems.

The present work describes the use of NaTPB, PTA, PMA, RN and a mixture of NaTPB+RN as ion exchangers, for the development of new in situ modified PVC membrane electrodes for determination of GNS in pure form, pharmaceutical preparations and biological fluids. Performance characteristics of novel electrodes reveal low detection limit, high sensitivity, good selectivity, widen the pH range, widen the concentration range, fast response, long life span and application for accurate determination of GNS in pure form, pharmaceutical preparations and biological fluids.

2. Experimental:

2.1. Materials and reagents

All chemicals were of analytical grade. Double distilled water was used throughout all experiments. Pure gentamicin sulfate (GNS) was provided by Sigma for pharmaceutical industry, Egypt. Dioctylphthalate (DOP), dibutylphthalate (DBP) and dioctylsebacate (DOS) were supplied from Merck (Germany) and tricresylphosphate (TCP) was supplied from Aldrich (USA). Glucose, lactose, maltose, fructose, glycine, urea, β-aniline, calcium chloride, cobalt chloride, ferric chloride, lithium chloride, cupper chloride, magnesium chloride, ammonium chloride, potassium chloride and sodium chloride were supplied from Merck (Germany). Sodium tetraphenylborate (NaTPB), ammonium reineckate (RN) and phosphotungstic acid (PTA) were purchased from Fluka (Switzerland). Phosphomolybdic acid (PMA) was purchased from Aldrich (USA). Poly (vinyl chloride) (PVC) of high molecular mass and tetrahydrofuran (THF) were obtained from Aldrich (USA). The GNS pharmaceutical preparation was Epigent (gentamicin sulfate, 80 mg/ 2ml ampoules, EIPICO Egy. Int. Pharm. Ind., Egypt).

In the analysis of biological fluids, human urine and plasma were used; plasma was obtained from VESRA, Cairo, Egypt.

2.2. Apparatus

Laboratory potential measurements were performed using HANNA pH-mV meter model 211. Silver-silver chloride double - junction reference electrode (Metrohm 6.0726.100) in conjugation with different drug ion selective electrode was used. Digital multimeter connected to a portable PC and Brand digital burette was used for the measurement of the drug under investigation.

2.3. Procedures

2.3.1. Construction of membrane electrodes

The matrix membranes of optimum composition for S1, S2, S3, S4 and S5 were prepared by dissolving the required amounts of PVC, plasticizer and varying ratios of ion pairing agent (1.5-8%) in 5 ml THF. The mixture of every membrane was poured in a glass petri dish (5 cm diameter). The petri dishes were covered with filter papers and left to stand overnight to allow solvent evaporation at room temperature. A master membrane with a thickness of 0.1 mm is obtained. From each master membrane, a disk (about 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 GNS and 10^{-1} M sodium chloride 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 internal reference electrode. The sensors were conditioned by soaking each sensor into 10^{-2} M aqueous GNS solutions for 24 h and storing in the same solution when not in use.

2.3.2 Sensors calibration

The calibration of the electrodes under investigation was established by immersing the working electrodes in conjunction with Ag/AgCl reference electrode in 50 mL beakers containing known aliquots of $1x\ 10^{-7}$- $1x\ 10^{-2}$ M GNS standard solution. The potential reading was plotted against the negative logarithmic value of GNS concentrations. The established calibration graph was used for subsequent determination of unknown GNS concentrations.

2.3.3. Effect of pH

The effect of pH on the response of the investigated electrodes was studied using 10^{-2}, 10^{-3} and 10^{-4} M GNS solutions over the pH range of 1–12. This is done by immersing the electrodes in the drug solution. The pH was gradually increased or decreased by addition of very small volumes of diluted NaOH or HCl solutions, respectively. The potential obtained at each pH was recorded.

2.3.4. Effect of the foreign compounds on the electrode selectivity

The potentiometric selectivity coefficients ($K^{pot}_{A,B}$) were determined according to IUPAC guidelines using the separate solutions (SSM) and matched potential (MPM) methods [30,31].

2.3.5. Analytical application

2.3.5.1 Application to pure form and pharmaceutical formulation

The proposed electrodes were found to be useful in the potentiometric determination of GNS in pure solution and in pharmaceutical preparation by using the standard addition and potentiometric titration methods.

In the standard addition method, known increments of GNS standard solution were added to constant volume of samples of different concentrations. The voltage was first measured in the pure sample, then the standard was added and the solutions were mixed well; a second reading was taken. From the change in potential readings for each increment the concentration of the unknown sample was calculated using the following equation [32]:

$$C_x = \{C_s[V_s)/(V_x+V_s)]\}x\{10^{n(\Delta E/s)} - [(V_x)/(V_s+V_x)]\}^{-1} \quad (1)$$

Where C_x and V_x are the concentration and the volume of unknown, respectively. C_s and V_s are the concentration and the volume of the standard, respectively. S the slope of the calibration graph and ΔE is the change in millivolt due to the addition of the standard.

In the potentiometric titration aliquots of the drug solution containing different weights from drug mg transferred to a 25 mL beaker. A standard solution of NaTPB, PTA and PMA were used as a titrant and the titration is monitored using proposed electrodes as indicator electrodes conjugated with Ag/AgCl as the reference electrode. The potential values were plotted against the volume of the titrant added and the end points were determined from the S- shaped curves using the first derivative plots.

2.3.5.2. Application to plasma samples

One milliliter of each 10^{-2}, 10^{-3} and 10^{-4} M standard drug solution were added separately into two 20-mL stoppered shaking tubes each containing 9 mL of plasma and the tubes were shaken for 1 min. The electrodes were immersed in conjunction with the reference electrode in these solutions and washed with distilled water between measurements. The emf for each solution measured by the proposed electrodes, and the concentration of GNS was determined from the corresponding calibration curve.

2.3.5.3. Application to urine samples

For urine analysis, different quantities of the drug and 5 mL urine were transferred to a 100 mL volumetric flask, left for 5 min, completed to the mark with distilled water and a small volume of 0.01 M HCl or NaOH was added to give solutions of pH 6. These solutions were subjected to the standard addition methods for the drug determinations.

3. Results and discussion

3.1 Electrode composition

The electro-analytical performance and electrode potential of an ISE is dependent upon the selective extraction of the target ion which creates the electrochemical phase boundary potential due to thermodynamic equilibria at the sample/electrode interface. Using of a suitable ion pairing agent in the electrode matrix followed by soaking in the drug solution may lead to the formation of an ion exchanger at the electrode surface by adsorption of a counter ion (analyte) from the solution during the measurement itself and can be extracted by the plasticizer in to the electrode bulk. Using of a suitable ion pairing agent in the electrode matrix has the advantage of reducing the time required for the electrode preparation where there is no need for ion pair (IP) preparation as well as expansion of the application of ion selective electrodes (ISEs) for the determination of drugs that cannot be precipitated as suitable IPs.

The influence of the plasticizer type and its quantity on the characteristics of the studied sensors was investigated by using four plasticizers with different polarities including: DBP, DOP, TCP and DOS. Different plasticizer/PVC (w/w) ratios were studied. The 1:1 plasticizer/PVC ratio produced maximum sensitivity for all of the plasticizers. The electrodes containing DBP showed better potentiometric responses.

In order to determine the suitable content of ion pairing agents (NaTPB, PTA, PMA, RN and a mixture of NaTPB + RN), several electrodes of a varying nature and ratio of ion pairing agent were prepared from the systematic investigation of each electrode composition. Experimental trials proved that a certain percentage of each ion pairing agents or mixture of them was optimum, indicated by the Nernstian behavior of the electrodes. However, further increase of the ion pairing agents over this percentage resulted in a diminished response slope of the electrode, most probably due to some inhomogenities and possible saturation of the membrane [33]. The results which given in Table 1 show that the optimum ion pairing agent percentage is 4% NaTPB, 5% PTA, 3% PMA, 4% RN and (3.5% RN+ 0.5% NaTPB) that gave the highest slope values of 30.5±0.8, 28.7±0.6, 29.9±0.7, 25.8±0.5 and 31.0±0.9 mV decade^{-1} for S1, S2, S3, S4 and S5, respectively.

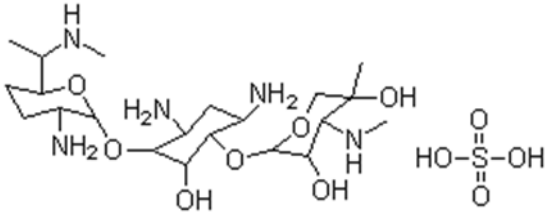

Fig. 1- Structure of gentamicin sulfate

Table 1: Effect of the content of ion pairing agents on the performance of the proposed electrodes.

Electrode	Composition			Concentration range (M)	Slope (mV decade^{-1})	r^2
	IP agent %	PVC %	plasticizer %			
S1 (NaTPB)	1.5	49.25	49.25	10^{-6}- 10^{-2}	22.5±0.8	0.969
	3.0	48.5	48.5	10^{-6}- 10^{-2}	26.2±0.7	0.968
	4.0	**48.0**	**48.0**	**10^{-6}- 10^{-2}**	**30.5±0.8**	**0.999**
	5.0	47.5	47.5	10^{-6}- 10^{-2}	25.9±1.0	0.988
	6.5	46.75	46.75	10^{-6}- 10^{-2}	24.1±0.6	0.991
	8.0	46.0	46.0	10^{-6}- 10^{-2}	19.7±0.5	0.996
S2 (PTA)	1.5	49.25	49.25	10^{-6}- 10^{-2}	18.2±0.4	0.998
	3.0	48.5	48.5	10^{-6}- 10^{-2}	21.1±0.7	0.998
	4.0	48.0	48.0	10^{-6}- 10^{-2}	24.5±0.5	0.992
	5.0	**47.5**	**47.5**	**10^{-6}- 10^{-2}**	**28.7±0.6**	**0.999**
	6.5	46.75	46.75	10^{-6}- 10^{-2}	23.6±0.6	0.992
	8.0	46.0	46.0	10^{-6}- 10^{-2}	20.1±1.0	0.986
S3 (PMA)	1.0	49.5	49.5	10^{-6}- 10^{-2}	23.5±0.6	0.979
	2.0	49.0	49.0	10^{-6}- 10^{-2}	27.0±0.8	0.988
	3.0	**48.5**	**48.5**	**10^{-6}- 10^{-2}**	**29.9±0.7**	**0.998**
	4.0	48.0	48.0	10^{-6}- 10^{-2}	27.8±1.1	0.988
	5.0	47.5	47.5	10^{-6}- 10^{-2}	25.5±0.9	0.991
	6.0	47.0	47.0	10^{-6}- 10^{-2}	22.2±0.5	0.993
S4 (RN)	1.5	49.25	49.25	10^{-5}- 10^{-2}	20.8±0.7	0.985
	3.0	48.5	48.5	10^{-5}- 10^{-2}	23.5±0.6	0.991
	4.0	**48.0**	**48.0**	**10^{-5}- 10^{-2}**	**25.8±0.5**	**0.996**
	5.0	47.5	47.5	10^{-5}- 10^{-2}	19.9±0.8	0.992
	6.5	46.75	46.75	10^{-5}- 10^{-2}	17.6±0.4	0.990
	8.0	46.0	46.0	10^{-5}- 10^{-2}	15.2±0.9	0.993
S5 (RN+ NaTPB)	(3.9+0.1)	48.0	48.0	10^{-7}- 10^{-2}	26.8±0.5	0.992
	(3.7+0.3)	48.0	48.0	10^{-7}- 10^{-2}	28.4±0.8	0.987
	(3.5+0.5)	**48.0**	**48.0**	**10^{-7}- 10^{-2}**	**31.0±0.9**	**0.999**
	(3.3+0.7)	48.0	48.0	10^{-7}- 10^{-2}	28.1±0.3	0.994

	(3.0+1.0)	48.0	48.0	10^{-7}- 10^{-2}	27.3±0.6	0.996
	(2.5+1.5)	48.0	48.0	10^{-7}- 10^{-2}	26.6±0.2	0.990

3.2. Electro-analytical performance characteristics of the electrodes

3.2.1. Calibration plots

The results presented in Table 2 and Fig. 2 showed that the prepared sensors can be successfully applied for the potentiometric determination of the drug under investigation with linear response in the concentration range of 10^{-6} to 10^{-2} M with slope values of 30.5±0.8, 28.7±0.6, 29.9±0.7 and 31.0±0.9 mV decade^{-1} for S1, S2, S3 and S5, respectively, and of 10^{-5} to 10^{-2} M with slope value 25.8±0.5 mV decade^{-1} for S4.

Table 2: Response characterization of the proposed electrodes.

Parameters	**S1 (NaTPB)**	**S2 (PTA)**	**S3 (PMA)**	**S4 (RN)**	**S5 (RN + NaTPB)**
Slope (mV/decade)	30.5±0.8	28.7±0.6	29.9±0.7	25.8±0.5	31.0±0.9
Correlation coefficient (r^2)	0.999	0.996	0.997	0.994	0.998
Detection limit (M)	$8.9x10^{-7}$	$9.4x10^{-7}$	$9.1x10^{-7}$	$9.72x10^{-6}$	$8.5x10^{-7}$
Response time	≤ 12 s	≤ 12 s	≤ 12 s	≤ 12 s	≤ 12 s
Working pH range	3–9	3–9	3–9	3–7	3–9
Life time (days)	62	63	61	31	64
Concentration range (M)	10^{-6} – 10^{-2}	10^{-6} – 10^{-2}	10^{-6} – 10^{-2}	10^{-5} – 10^{-2}	10^{-6} – 10^{-2}
SD	0.13	0.31	0.34	0.42	0.26
RSD (%)	0.45	1.08	1.14	1.63	0.85
Isothermal coefficient (mV/^{0}C)	0.47	0.55	0.61	0.75	0.52

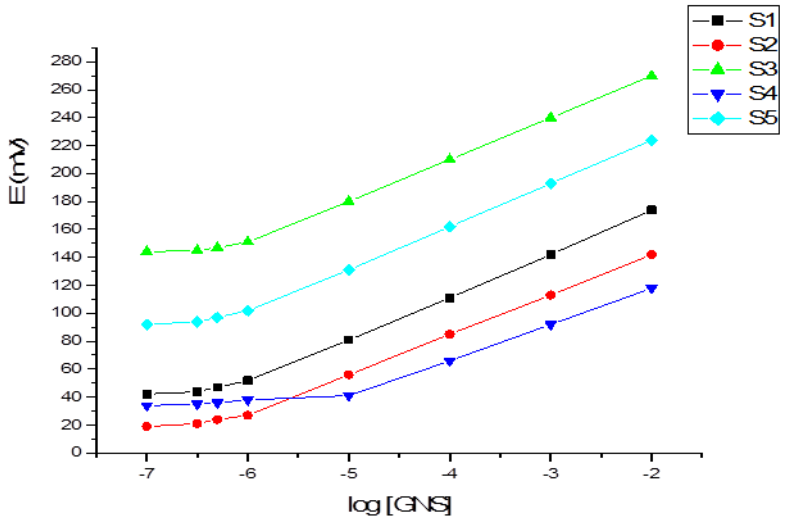

Fig. 2: Calibration graphs for S1, S2, S3, S4 and S5 at optimum membrane composition.

3.2.2. Effect of pH

To investigate pH effect on the electrode potential, the potential readings were recorded for three different concentrations (10^{-4}, 10^{-3} or 10^{-2} M) of GNS at different pH values (pH 1–12). The potential changes as a function of

pH are shown in Fig. 3. This figure indicates that the electrodes response was independent of the pH within the range from 3 to 9 for S1, S2, S3 and S5 and from 3 to 7 for S4. At lower pH values the increase in mV readings can be due to the interference of hydronium ion, while at higher pH values the decrease in the mV readings may be due to the deprotonation of the drug molecules.

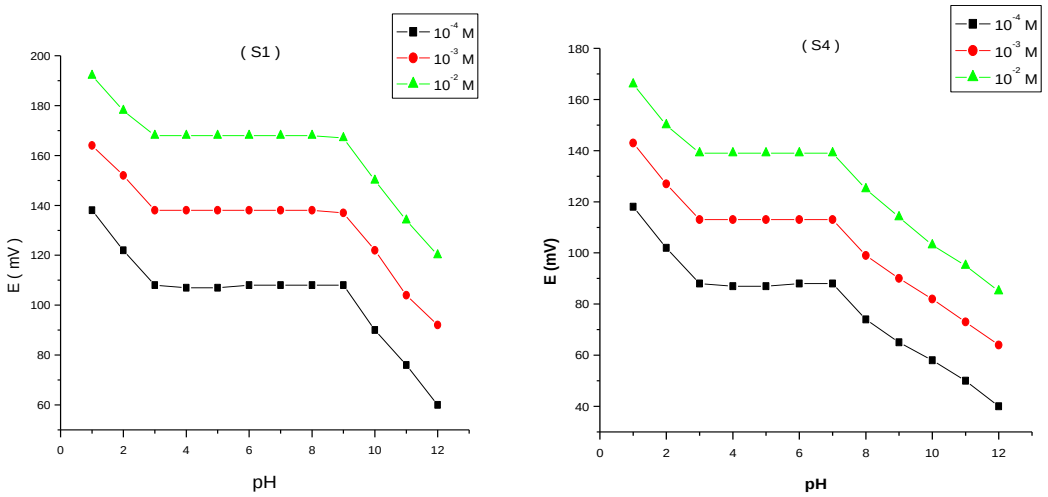

Fig. 3: Effect of pH at different GNS concentrations on emf values using S1 and S4.

3.2.3. Effect of temperature

In order to determine the isothermal coefficient of the electrodes (dE^o/dt), the standard electrode potentials (E^o) were determined from the calibration graphs (the intercepts at p[GNS]=0) of proposed electrodes carried out at different temperatures (10, 20, 30, 40, 50 and 60 ^oC) and plotted versus (t−25), where (t) is the temperature of the experiment in degree centigrade and according to the following equation [34]:

$$E° = E°_{(25)} + (dE°/dT)\,(t - 25) \qquad (2)$$

A straight line is obtained and the slope of this line represents the isothermal coefficients of the electrodes which were found to be 0.47, 0.55, 0.61, 0.75 and 0.52 mV/^{o}C for S1, S2, S3, S4 and S5, respectively. The obtained values of the isothermal coefficient reveal that the electrodes under investigation had high thermal stability within the used range of temperature. The investigated electrodes can be used up to 60 ^{o}C without noticeable deviation from the Nernstian behavior.

3.2.4. Interference studies

Potentiometric selectivity coefficient refers to the ability of the ISE to differentiate a particular (primary) ion from others (interfering ions). The potentiometric selectivity coefficient ($K^{Pot}_{A,B}$) was determined according to IUPAC recommendations using the SSM and MPM [30, 31]. In the MPM the selectivity coefficient is defined as the activity ratio of the primary ion and interfering ion which gives the same potential change in a reference solution. This method does not require Nernstian responses to the activity (concentration) of either primary or interfering ions. It is a suitable method for determination of selectivity coefficients of the neutral compounds.

As shown in Table 3 the results obtained show no significant interference and this reflects a very high selectivity of the electrodes under investigation toward GNS. The results also indicate that there was no serious interference from glucose, sucrose, maltose, fructose and lactose due to the difference in polarity and lipophilic nature of their molecules relative to GNS^{2+} cation. The inorganic cations did not interfere due to the differences in their ionic size and hence their mobilities and permeabilities as compared to those of GNS^{2+} cation.

Table 3: Selectivity coefficients for the proposed electrodes.

Interfering ion	$K^{pot}_{A,B}$									
	S1		S2		S3		S4		S5	
	SSM	MPM	SSM	MPM	SSM	MPM	SSM	MPM	SSM	MPM
Maltose	----	6.43×10^{-5}	----	3.21×10^{-5}	----	2.16×10^{-5}	----	1.46×10^{-6}	----	4.37×10^{-5}
Lactose	----	5.65×10^{-5}	----	3.92×10^{-5}	----	1.99×10^{-5}	----	1.53×10^{-6}	----	3.19×10^{-5}

Fructose	----	$3.96x10^{-5}$	----	$2.53x10^{-5}$	----	$2.66x10^{-5}$	----	$8.07x10^{-6}$	----	$3.55x10^{-5}$
Glucose	----	$7.13x10^{-5}$	----	$3.95x10^{-5}$	----	$3.11x10^{-5}$	----	$3.39x10^{-6}$	----	$4.51x10^{-6}$
Urea	----	$1.94x10^{-5}$	----	$2.95x10^{-5}$	----	$2.13x10^{-5}$	----	$6.65x10^{-6}$	----	$3.17x10^{-5}$
β-aniline	----	$7.62x10^{-5}$	----	$4.98x10^{-5}$	----	$4.58x10^{-5}$	----	$4.43x10^{-6}$	----	$6.14x10^{-5}$
Glycine	----	$4.88x10^{-5}$	----	$2.40x10^{-5}$	----	$1.86x10^{-5}$	----	$2.06x10^{-5}$	----	$3.48x10^{-5}$
K^+	$3.98x10^{-6}$	----	$2.29x10^{-6}$	----	$4.90x10^{-6}$	----	$8.61x10^{-4}$	----	$8.31x10^{-6}$	----
Li^+	$2.63x10^{-6}$	----	$1.58x10^{-5}$	----	$7.59x10^{-6}$	----	$8.98x10^{-4}$	----	$9.14x10^{-6}$	----
Co^{2+}	$1.61x10^{-5}$	----	$8.51x10^{-6}$	----	$5.62x10^{-5}$	----	$9.51x10^{-4}$	----	$3.42x10^{-5}$	----
Mg^{2+}	$2.34x10^{-5}$	----	$1.29x10^{-5}$	----	$3.63x10^{-5}$	----	$9.28x10^{-4}$	----	$4.22x10^{-5}$	----
Fe^{3+}	$2.45x10^{-4}$	----	$2.24x10^{-4}$	----	$3.31x10^{-4}$	----	$9.38x10^{-3}$	----	$2.99x10^{-4}$	----
Cu^{2+}	$4.37x10^{-5}$	----	$1.82x10^{-5}$	----	$5.75x10^{-5}$	----	$1.33x10^{-3}$	----	$1.66x10^{-5}$	----
Ca^{2+}	$3.68x10^{-5}$	----	$2.57x10^{-5}$	----	$4.37x10^{-4}$	----	$9.46x10^{-4}$	----	$2.15x10^{-5}$	----
NH_4^+	$5.89x10^{-6}$	----	$4.68x10^{-6}$	----	$7.41x10^{-6}$	----	$8.89x10^{-4}$	----	$8.21x10^{-6}$	----

3.2.5. Response time

The response time is an important factor which characterizes ISEs. It can be defined as the time taken by the electrodes to reach steady potential values of 90% of the final equilibrium values, after successive immersions in a series of solutions, each having a 10- fold concentration difference [35, 36]. The dynamic response time of the sensors under study was determined for the concentration range from $1.0x10^{-6}$ to $1.0x10^{-2}$ M. Fig. 4 illustrates a representative plot of the potential changes versus time for electrodes under study. The electrodes yielded steady potential within ≤ 12 s this is most probably due to the fast exchange kinetics of association-dissociation of GNS ion with the ionophores at the solution-membrane interface.

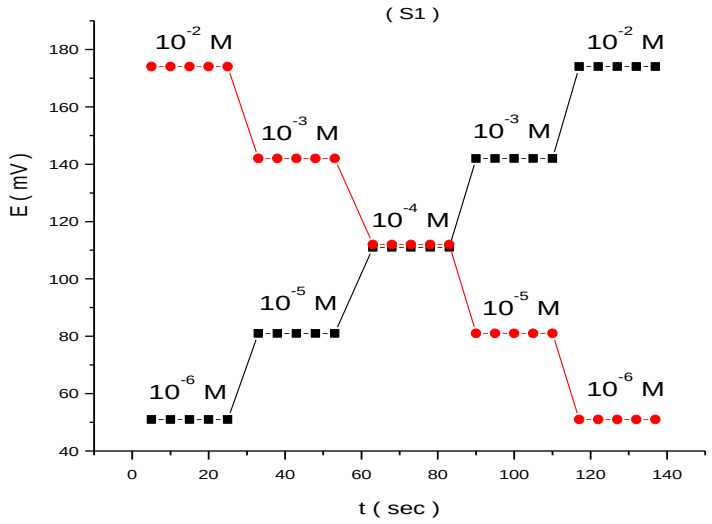

Fig. 4: Dynamic response time of S1 for step changes in concentrations of GNS from low to high and vice versa.

3.2.6. Life time

The life time of the investigated sensors was studied by periodically constructing the calibration graphs under optimum conditions in the concentration range of 10^{-6} - 10^{-2} M of GNS on different days. For sensors under investigation it was found that S1, S2, S3, S4 and S5 have a life time of 62, 63, 61, 31 and 64 days, respectively. After preparation of the proposed electrodes, they were kept at 4 ^{o}C and directly used for potentiometric measurements.

3.2.7. Analytical applications

3.2.7.1. Potentiometric determination of GNS in pharmaceutical dosage form

The optimized sensors under investigation have been successfully used for the potentiometric determination of GNS (pure form and pharmaceutical form) by using the standard addition and potentiometric titration methods, and the results are summarized in Table 4. In order to estimate the quality of the results, recovery

values were also determined and are presented in the same table. Corresponding titration curves for S1 and differential curves for S5 are shown in Fig. 5 and Fig. 6, respectively, The obtained results in Table 4 using the proposed sensors were in good agreement with those obtained using the official method [37].

Table 4: Determination of GNS in pure form and pharmaceutical formulation using the proposed sensors

[a] Mean of four determinations

Sample	Electrode type	taken (mg)	Standard addition		Potentiometric titration		Official Method	
			Recovery (%)±SD	RSD(%)[a]	Recovery (%)±SD	RSD (%)[a]	Recovery (%)±SD	RSD (%)[a]
Pure Form	S1	1.73	98.35±0.21	0.216	99.15±0.86	0.879	98.0±0.34 97.30±0.43 98.72±0.28	0.362 0.450 0.302
		5.18	98.75±0.24	0.245	99.25±0.77	0.788		
		8.64	99.20±0.19	0.198	99.60±0.69	0.714		
	S2	1.73	96.60±0.31	0.321	97.30±0.99	1.123		
		5.18	97.25±0.54	0.584	97.85±0.96	0.986		
		8.64	98.10±0.35	0.383	98.40±0.69	0.725		
	S3	1.73	97.35±0.42	0.447	97.94±0.62	0.665		
		5.18	97.70±0.33	0.362	98.90±0.45	0.514		
		8.64	98.52±0.29	0.308	99.12±0.56	0.598		
	S4	1.73	95.95±0.92	0.979	96.25±0.98	1.214		
		5.18	96.55±0.89	0.925	96.65±0.85	0.997		
		8.64	96.90±0.85	0.874	96.98±0.96	1.089		
	S5	1.73	98.45±0.27	0.281	98.95±0.64	0.698		
		5.18	99.15±0.30	0.318	99.54±0.47	0.508		
		8.64	99.50±0.40	0.429	99.80±0.39	0.419		
Epigent(80 mg/2ml)	S1	1.73	98.96±0.34	0.363	98.88±0.34	0.368		
		5.18	98.64±0.29	0.312	99.24±0.40	0.426		
		8.64	99.75±0.26	0.284	99.80±0.29	0.316		
	S2	1.73	96.75±0.45	0.466	96.95±0.55	0.594		
		5.18	97.12±0.61	0.624	97.82±0.84	0.882		
		8.64	98.33±0.51	0.537	99.22±0.66	0.714		
	S3	1.73	97.52±0.39	0.415	97.88±0.45	0.489		
		5.18	97.87±0.40	0.419	98.50±0.38	0.407		
		8.64	98.96±0.56	0.584	99.15±0.51	0.543		
	S4	1.73	95.90±0.82	0.845	96.10±0.99	1.320		
		5.18	96.75±0.91	0.922	96.80±0.97	1.184		
		8.64	97.30±0.75	0.779	97.50±0.88	0.946		
	S5	1.73	98.50±0.35	0.372	98.90±0.52	0.612		
		5.18	99.45±0.30	0.315	99.56±0.46	0.499		
		8.64	99.30±0.40	0.418	99.80±0.60	0.711		

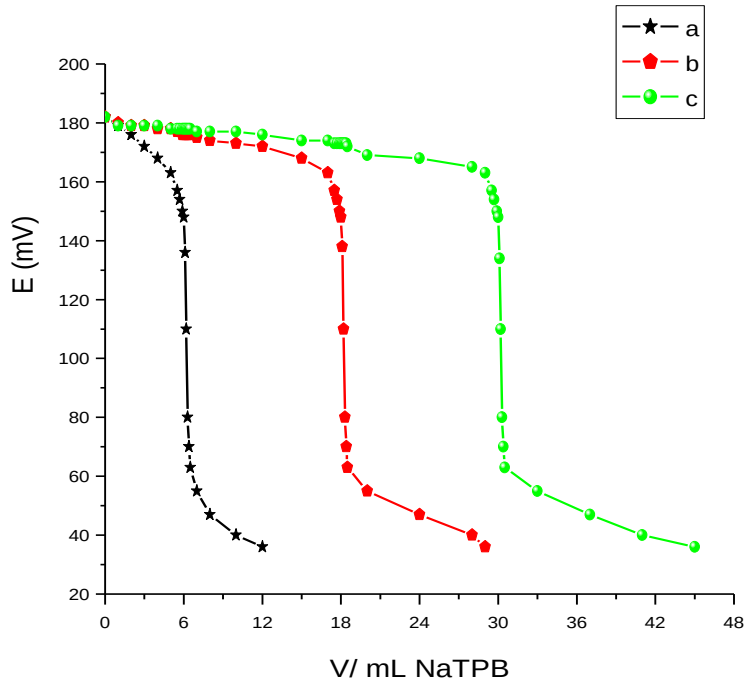

Fig. 5: Potentiometric titration curves of (a) 3, (b) 9 and (c) 15 mL of 10^{-2} M GNS using S1 electrode and 10^{-2} M NaTPB as titrant.

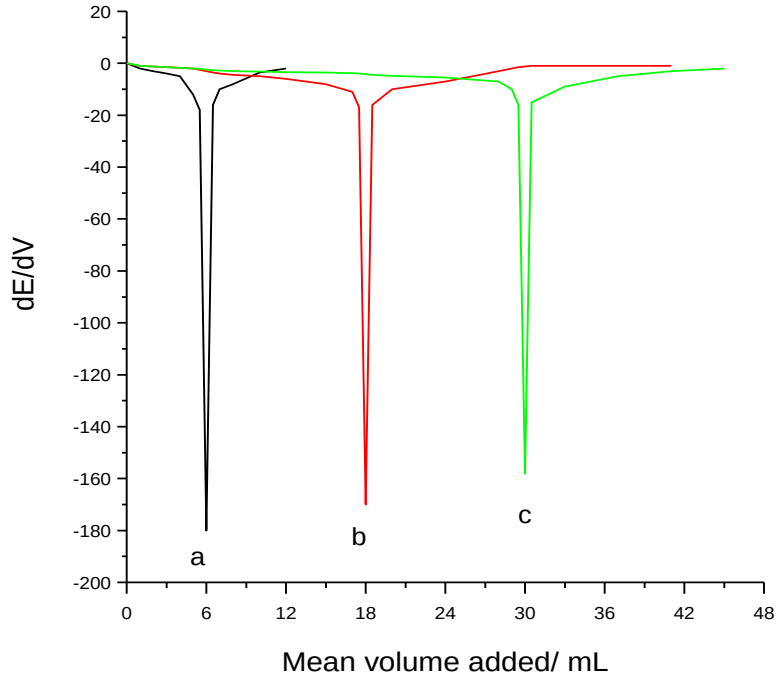

Fig. 6: Differential curves for potentiometric determination of (a) 3, (b) 9 and (c) 15 mL 10^{-2} M GNS using S5 electrode and 10^{-2} M NaTPB as titrant.

3.2.7.2. Potentiometric determination of GNS in urine and plasma

In urine samples the standard addition technique was applied to overcome the matrix effects in these samples. Also the results, in table 5, obtained for the determination of GNS in spiked human plasma show that a wide concentration range of the drug can be determined by the investigated sensors with high precision and accuracy. The response times of the proposed sensors are instant (within 15s), so the sensors are rapidly transferred back and forth between the biological samples and the bi-distilled water between measurements to protect the sensing component from adhering to the surface of some matrix components. It is concluded that the proposed sensors can be successfully applied to in vitro studies and for clinical use. This confirms that the sensitivity and limit of quantification (LOQ) are adequate for determination of GNS in pharmacokinetic studies.

Table 5: Determination of GNS in biological fluids (plasma and urine).

[a] Mean of three determinations

Electrode type	Spiked human plasma			Spiked urine		
	Taken (mg)	Recovery (%)±SD	RSD(%)[a]	Taken (mg)	Recovery (%)±SD	RSD (%)[a]
S1	0.576	98.13±0.30	0.219	0.576	98.20±0.36	0.299
	2.880	98.31±0.22	0.235	2.880	98.25±0.27	0.251
	5.760	98.43±0.24	0.202	5.760	98.23±0.32	0.276
S2	0.576	96.52±0.31	0.321	0.576	96.74±0.40	0.310
	2.880	97.15±0.57	0.434	2.880	97.46±0.37	0.301
	5.760	97.55±0.29	0.323	5.760	98.10±0.36	0.297
S3	0.576	97.08±0.35	0.347	0.576	97.54±0.52	0.398
	2.880	97.36±0.30	0.322	2.880	97.90±0.44	0.312
	5.760	97.96±0.31	0.330	5.760	98.15±0.48	0.342
S4	0.576	95.84±0.90	0.963	0.576	96.15±0.97	1.203
	2.880	96.30±0.88	0.915	2.880	96.54±0.80	0.876
	5.760	96.43±0.81	0.854	5.760	96.68±0.86	0.910
S5	0.576	97.45±0.42	0.311	0.576	97.93±0.54	0.418
	2.880	97.61±0.38	0.319	2.880	98.24±0.47	0.338
	5.760	97.75±0.40	0.403	5.760	98.31±0.50	0.392

3.3. Method validation

3.3.1. Accuracy

Accuracy is an important requirement of electroanalytical methods. It can be defined as the closeness between the true or accepted reference value and the obtained value [38]. The accuracy of the proposed method using the proposed electrodes was investigated by the determination of GNS in spiked samples prepared from serial concentrations of GNS reference standards. The results summarized in Table 4 and Table 5 show high accuracy of the proposed method, as indicated by the percentage recovery values.

3.3.2. Linearity

Under the optimal experimental conditions, linear relationships exist between the electrode potential/mV and the log [GNS]. The regression data, correlation coefficients (r^2) and other statistical parameter are listed in Table 2.

3.3.3. Detection limit

LOD is the lowest quantity of the investigated compound in a sample that can be detected, but not necessarily quantified with an acceptable uncertainly. LOD of an electroanalytical method is an important factor if quantitative measurements are to be made at concentrations close to it. Especially, LOD is necessary for the trace analysis of drug active components in pharmaceuticals and/or biological samples [38]. The values of LOD that are presented in Table 2 indicate that the sensors under investigation are highly sensitive, selective and can be applied in determination of small amounts of GNS.

3.3.4. Specificity

The specificity of the method was examined by observing any interference caused by the common excipients of the pharmaceutical formulation. It was found that these compounds did not interfere with the results of the proposed method as shown in Table 3.

3.3.5. Precision

Precision is a measure of how close results are to one another. Precision is also expressed as the closeness of agreement between independent test results obtained under stipulated conditions. Precision is usually expressed as standard or relative standard deviations of the replicate analysis [38]. Hence the precision of the proposed potentiometric method using the sensors under investigation was measured as percentage relative standard deviation (RSD %) as shown in Table 4 and Table 5.

4. Conclusion

The present work involves the preparation of novel PVC membrane electrodes with in situ mode of modification. The described sensors are sufficiently selective for the quantitative determination of GNS in pure form, pharmaceutical dosage form, human urine and plasma. The present electrodes show high sensitivity, reasonable selectivity, fast static response, long-term stability and applicability over a wide pH range with no sample pretreatment. The presented methods for the determination of GNS with the prescribed electrodes are advantageous over the previously described procedures being faster than many other techniques (response time of 10s). The suggested methods are characterized by low LOD values amounting to 10^{-7} M using electrodes; thus they are more sensitive than many spectrophotometric and chromatographic methods. These electrodes were used satisfactory for analyses of biological fluids without any interference from the matrix.

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