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Development and Validation of Potentiometric Ion Selective Screen Printed Electrode for Sensitive Determination of Linagliptin in Pure Form and in Pharmaceutical Formulation; Application to Dissolution Testing

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Abstract- A novel screen printed graphite ion-selective electrode (SPE) was prepared and characterized for determination of linagliptin (LNG) in pure form and in pharmaceutical formulation. The electrode is based on LNG-tetraphenyl borate (LNG-TPB) as ion exchanger and tricresyl phosphate (TCP) as a plasticizer. Several parameters such as the type of solvent mediator, amount of the ion-exchanger and working pH range were studied for optimal potentiometric response. Under optimal experimental conditions, the sensor exhibited a Nernstian slope of 60.10±1.02 mV/decade (n=3) over the concentration range from 1.00×10^{-5} to 1.00×10^{-2} M (r =0.9999) with LOD of 4.50×10^{-6} M. The electrode can be used safely over a wide pH range from 4.0 to 8.0. The electrode showed a high selectivity for LNG against common interfering substances. Fast potentiometric response was obtained within 5 seconds and remained stable for at least 60 S. The electrode was applied to the analysis of LNG in pure form and in pharmaceutical dosage form with high accuracy (98.00-102.00%) and precision (%RSD ≤2%). Our electrode has the advantages of being inexpensive, simple, sensitive, portable and disposable.

Keywords- Screen printed; Potentiometric response; Solid-state electrode; Ion selective; Dipeptidyl peptidase-4 Inhibitor

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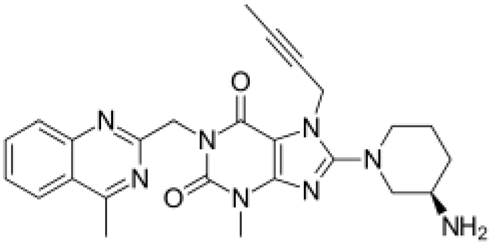
1. INTRODUCTION

Linagliptin (LNG) is chemically known as 1H-purine-2,6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butyn-1-yl)-3,7-dihydro-3-methyl-1-[(4-methyl-2-quinazolinyl)methyl]- [1] (Figure 1). It is a DPP-4 (dipeptidyl peptidase-4) inhibitor used for treatment of type-II diabetes. LNG acts by blocking the action of DPP-4 enzyme that destroys the glucagon-like peptide-1 hormone (GLP-1) which helps increase insulin secretions and inhibits the release of glucagon resulting in decreasing the glucose level in the circulation [2,3].



Molecular Formula: $C_{25}H_{28}N_8O_2$
Molecular Weight: 472.54 g/mol

Figure 1. Chemical structure of LNG [1]

Several analytical methods including chromatography [4-14], spectrophotometry [15-20] and spectrofluorimetry [21] were developed for the determination of LNG.

The chromatographic methods are expensive, time and solvent consuming and use expensive sophisticated equipment. On the other hand, the spectrophotometric methods are highly dependent on the selection of the wavelength region which crucially affects the precision and accuracy; besides, they exhibited a narrow linear range with high detection limits and use tedious lengthy calculations. Thus, a great interest has been focusing on the development of an easy, simple and cost-effective analytical method that enables LNG analysis in a short time.

Ion selective electrodes (ISEs) are potentiometric sensors by which the activity of a particular ion dissolved in a solution is converted into an electrical potential that can be measured by a voltammeter. Potentiometric ISE represents a cost-effective approach for measuring ionic compounds in turbid and colored solutions. However, this advantage is compromised by the large volumes of the sample. To overcome this problem, disposable screen printed electrodes with small sizes were produced via screen printing technology [22-26].

In this study we present a new disposable screen printed electrodes (SPEs) which can be used for determination of LNG in pharmaceutical dosage form. The potentiometric response was characterized according to IUPAC recommendations [27]. The potentiometric performance of the electrode was optimized by accurate selection of the ion exchanger and plasticizer. The electrode was applied for the assay and dissolution testing of LNG.

2. EXPERIMENTAL SECTION

2.1. Materials and Reagents

Poly vinyl chloride (PVC) of high molecular weight, dibutylphthalate (DBP) and dioctylphthalate (DOP) were obtained from Sigma-Aldrich, St. Louis, USA. Sodium tetra phenyl borate (Na-TPB) and graphite powder (particle size <50μm) were purchased from Merck, Darmstadt, Germany. Tricresylphosphate (TCP) was purchased from Fluka, Switzerland. Phosphomolybdic acid (PMA) was purchased from S.D Fine-Chem. limited, Mumbai, India. Hydrochloric acid was obtained from Honey Well Specialty Chemicals, Germany. Sodium chloride, potassium chloride, calcium chloride, potassium di-hydrogen orthophosphate, sodium di-hydrogen orthophosphate and sodium hydroxide pellets were purchased from Adwic Chemical Co., Cairo, Egypt. Amino acids Alanine, Lucien, Lysine, Glycine, Valine, Proline, Methionine and Glutamine were obtained from Sigma-Aldrich, Germany. Lactose monohydrate was obtained from Amoun Co., Egypt. All solutions were prepared using double distilled water.

2.1.1. Raw Materials

LNG (Batch No. LG0040616 (PVA)) manufactured by Honour Lab Limited, Hetero Infrastructure Ltd. SEZ, Nakkapalli, India was obtained from The Arab Company for Gelatin and Pharmaceutical Products, Egypt. Its purity was certified to be 99.40%**.**

2.1.2. Dosage Forms

Trajenta® tablets (Batch No. AA1846A, 5 mg LNG/tablet), manufactured by Boehringer Ingelheim, Germany, were purchased from a local pharmacy.

2.2. Apparatus

A JENWAY 3510 pH/mV meter (Staffordshire, England) with combined glass electrode was used for potential measurements. All potential measurements were carried out against Ag/AgCl (3M KCl) reference electrode. The potentiometric cell can be represented as follows: Ag/AgCl / 3M KCl // Sample / SPE. The pH measurement was performed using a Cyberscan 500 digital pH/mV meter (Eutech Instruments, Thermo Scientific, USA). A four-digit Scientech SA-210 balance (USA) was used for weighing the chemicals. Imaging the electrode surface was carried out using a JSM-6700F scanning electron microscope, Japan Electro Company, Japan.

Dissolution testing was carried out using USP II dissolution device (Hanson Research, SR8 Plus; USA), model: 0405-057.

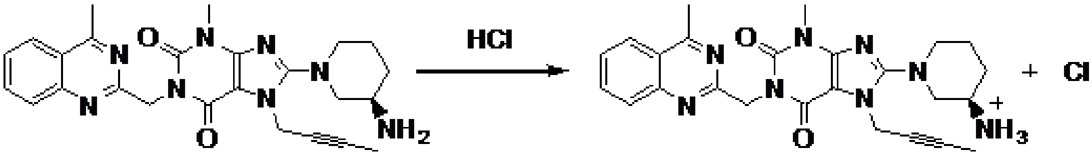
Reference HPLC experiment [14] was conducted using an Agilent-1260 series HPLC device. The separation was performed using Agilent C18 column (150 mm×4.6, 5μm) maintained at ambient temperature. The mobile phase consists of acetonitrile: phosphate buffer (pH 4.6) (80:20 v/v). The flow rate was adjusted 1ml/min and the detection was carried out at 299 nm.

2.3. Procedures

2.3.1. Preparation of Solutions

a-Standard Stock solution

LNG stock solution (1.0×10^{-2} M) was prepared into 100-mL volumetric flask by dissolving an accurately weighed 472.54 mg of LNG reference material in 0.10% HCl. That results in the formation of a charged species (Scheme 1) which gives the potentiometric response.



Scheme 1. Formation of a charged species of LNG

b-Sample Stock Solution of LNG

Thirty tablets of Trajenta® tablets were finely ground in a mortar. An amount of the powder equivalent to 118.0 mg LNG was accurately weighed and transferred into 25-mL volumetric flask. The powder was dissolved in 15 mL 0.1% HCl and sonicated for 15 min; afterwards, the flask was completed to volume using 0.1% HCl.

2.3.2. Preparation of LNG-TPB Ion-Exchanger

The ion-exchanger (electro-active species) was prepared by adding 25 mL of 0.01 M of Na-TPB solution to 25 mL of 0.01 M of LNG solution in 0.1% HCl. The solution was stirred for 10 min. and filtered. The precipitate (LNG-TPB) was washed several times with copious amount of double distilled water and left to dry overnight at room temperature before the preparation of the electrodes. The composition of the ion-exchanger was found to be 1:1 (LNG: TPB), as confirmed by elemental analysis data (Table1). The elemental analysis was performed at the Micro-analytical Center, Cairo University.

Table 1. Elemental analysis of the ion pair (IP) LNG-TPB

	LNG-TPB (1:1)	
Element	**Calculated%**	**Found%**
C	72.25	74.17
H	5.90	5.93
N	13.87	14.13

2.3.3. Preparation of LNG-Based Carbon Paste Electrodes

LNG-based carbon paste electrode CPE1 was prepared by hand mixing of LNG-TPB (2.0% w/w) with graphite powder and TCP as pasting liquid [ratio of graphite powder to pasting liquid was 1.2:1.0 (w/w)] in a mortar. The electrode was filled with the paste by pressing the tip (at its wide opening) against the paste several times. The electrode surface was polished on a filter paper and used directly for potentiometric measurements without preconditioning. The same procedure was followed for preparation of other carbon paste electrode using LNG-phosphomolybdic acid (LNG-PMA), in presence of different pasting liquids including dibutylphthalate (DBP) and dioctylphthalate (DOP).

2.3.4. Preparation of the Screen Printed Electrodes (SPEs)

LNG SPEs were prepared by a simple manual screen printing procedure. Array of seven carbon electrodes were prepared by printing carbon ink on a PVC flexible plastic sheet (≈ 200 µm thick) using a polystyrene mesh and a squeegee, The PVC sheet is preferred over other substrates as it has good mechanical properties and high flexibility that enable easy use, handling and cutting at home using an office scissor. The electrodes were cured at 60 °C for 2 h in a vacuum oven. The sensing PVC membrane cocktail SPE1 (2% electro-active species, 65.0% TCP and 33% PVC in 1:1 cyclohexanone: acetone, unless otherwise stated) was printed upon the conductive carbon array and left to dry over night at room temperature. The electrodes were covered with an insulating tape leaving a rectangular area (3x3 mm) at the both terminals as a sensing part (sensing PVC membrane) and conductive part for connection to the instrument (Figure 2). Connecting the electrode with the instrument was carried out using an alligator clip.

2.4. Construction of Calibration Graph

The SPEs were conditioned in 1.0×10^{-2} M of LNG solution for 30 min before use. All calibration graphs were constructed by successive addition of small aliquots of LNG solution (1.0×10^{-2} M) to 50 mL phosphate buffer pH 6.8 to cover the concentration range from 1.0×10^{-6} to 1.0×10^{-3} M. The membrane sensors, in conjunction with the Ag/AgCl reference electrode, were immersed in the above test solutions and the potential readings were recorded. Potential of higher concentrations (up to 1.0×10^{-2} M) was recorded by dipping the electrode in this solution separately and recording the corresponding mV reading. All potential measurements were recorded under constant stirring at ambient temperature. The calibration graphs were constructed by plotting the electrode potential (mV) *vs.* -log Conc. (M).

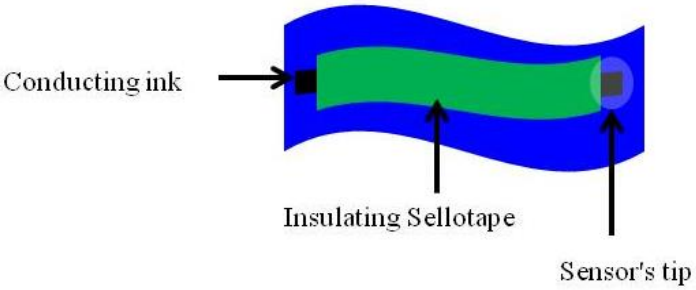


Figure 2. Shows the screen printed sensor covered with Sellotape to define the sensing area and cover the conductive carbon track

2.5. Selectivity

The potentiometric selectivity coefficients log $K_{drug,j}^{Pot.}$ of the proposed sensor towards various inorganic cations and amino acids was studied by the separate solution method [27]. The selectivity coefficients were calculated by applying Nickolsky- Eisenman equation (1).

$$\log K_{drug,j}^{pot} = \frac{(E_j - E_{drug})}{2.303RT / Z_{drug}F} + (1 - \frac{Z_{drug}}{Z_j}) \log a \qquad \text{Eq. (1)}$$

2.6. Potentiometric Titration Method

Aliquots from LNG standard stock solution (1.0×10^{-2} mole L^{-1}) were diluted each to 50 mL with double distilled water to prepare 4.0×10^{-4} M and 8.0×10^{-4} M. each solution was titrated against 1.0×10^{-2} M Na-TPB solution in presence of the proposed LNG-SPE as indicator electrode and the change of the cell potential upon addition of Na-TPB was recorded. The volume of titrant at equivalence point was obtained using the first derivative method.

2.7. Analytical Applications

2.7.1. Standard Addition Method

The standard addition method was applied for the potentiometric determination of LNG in the drug substance and pharmaceutical dosage form. In this method, known small volumes of standard LNG solution (0.01 M) is added to 50 mL of the sample solution (the sample is dissolved in phosphate buffer pH 6.8). The change in mV readings was recorded after each addition and used to calculate the concentration of the drug in the sample solution using equation (2) [22-24]:

$$C_x = C_s \left(\frac{V_s}{V_x + V_s}\right) \left(10^{n\,(\Delta E/s)} - \frac{V_x}{V_x + V_s}\right)^{-1} \qquad \text{Eq. (2)}$$

where, C_x is the concentration to be determined, V_x is the volume of the original sample solution, V_s and C_s are the volume and concentration of standard solution added to the sample to be analyzed, respectively, ΔE is the change in potential after addition of a certain volume of the standard solution and S is the slope of the calibration graph**.**

2.7.2. Dissolution Monitoring

The dissolution profile of LNG was investigated with Potentiometric and HPLC methods. For LNG dosage form, 10 tablets of Trajenta® (5 mg/tablet) were dissolved in 500 mL of (dissolution medium) phosphate buffer of pH 6.8 in the dissolution vessels maintained at 37^0C $\pm 0.5^0$C using USP II dissolution apparatus (Hanson Research, SR8 plus; USA) with paddle rotation rate at 50 rpm. For HPLC measurements samples (5 mL) were withdrawn from the dissolution vessels every 5 min. and replaced with 5 mL of fresh dissolution medium added to the vessels. The samples were filtered through syringe filter 0.45 µm then analyzed using HPLC method [14]. For potentiometric measurements the proposed screen printed electrode (SPE) together with the reference electrode were dipped directly in the dissolution medium then stirring the content of the vessel at a rate of 50 rpm and the potential in mV was recorded each 5 min. Percentage dissolution was calculated and then dissolution curves were plotted against time in minutes.

3. RESULTS AND DISCUSSION

3.1. Surface Morphology of the SPE

A typical scanning electron microscope (SEM) cross section view of the fabricated sensor is shown in Figure 3. Figure 3 is an SEM cross section view of the proposed electrode.

The picture shows three layers with distinct morphology (top, middle and bottom layers). The upper layer is the ion selective membrane (ISM), the middle layer is the conductive carbon track and the bottom layer is the PVC flexible substrate. The ISM layer has a thickness of ≤60 µm and is well adhered to the underneath conductive carbon track with no gaps at the interface. The conductive carbon layer has a thickness of ≤90 µm; the picture shows that the graphite flacks are well connected by the PVC binder. It is obvious that this layer is well sealed to the PVC substrate. The bottom layer is the PVC substrate and has a thickness about 300 µm.

3.2. Effect of Composition of Sensor

Construction of (ISEs) with optimal potentiometric response is enforced by several factors including the type of ion exchanger and the type of plasticizer. The ion exchanger is one of the most crucial components of ion selective electrodes it must be practically insoluble in water, hydrophobic, stable over a wide pH range and should exist in the ion selective membrane at an intermediate concentration that can provide a maximum exchange current density at the membrane/solution interface. The plasticizer is another important component; it must be nonvolatile, capable of dissolving the ion exchanger and be immiscible with water. Herein, two types of LNG ion exchangers, namely LNG-TPB and LNG-PMA, were prepared and used for preparation of LNG ion selective electrodes. Three types of plasticizers (TCP, DBP and DOP) were investigated for the preparation of LNG selective electrode with optimal potentiometric response.

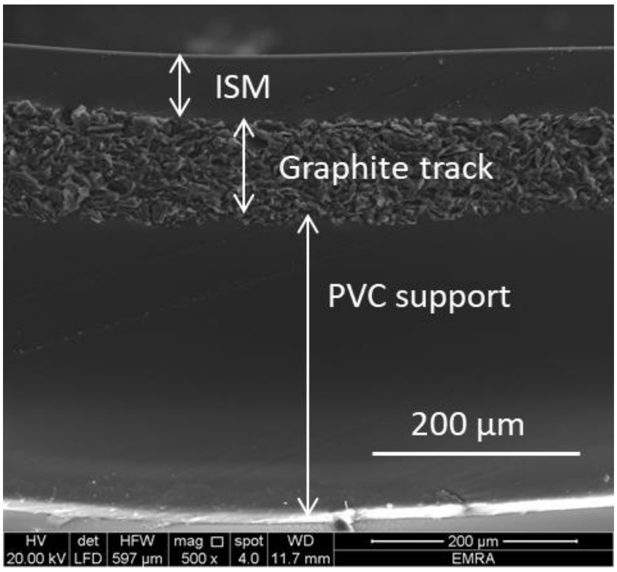


Figure 3. SEM cross section view of the printed LNG ion selective electrode

Calibration graphs for each membrane composition were constructed in phosphate buffer pH 6.8 (Figure 4) and the slope, linear range and limit of detection were calculated for each electrode as shown in table 2. The best performance was obtained with SPE1 (2% LNG-TPB, 65% TCP and 33% PVC) showed a Nernstian response of 60.1 mV/decade over the concentration range from 1.0×10^{-5} to 1.0×10^{-2} M. Also we have noticed, by varying the percentage of the ion exchanger with keeping the percentage of (PVC: Plasticizer) equal to (1:2) as in the SPE2 and SPE3 exhibit a Nernstian slopes (59.2 and 58.3 mV/decade) over the concentration range from 1.0×10^{-5} to 1.0×10^{-2} M this is confirmed that any small changes in the amount of LNG-TPB (2 ± 1%) in the membrane are not affecting the potentiometric characteristics of the sensor indicating the robustness of the prepared SPE but further increase in the percentage of LNG-TPB (SPE4) resulted in a sub- Nernstian slope 53.2 mV/decade a diminished response slope of the sensor is due to homogeneities and possible saturation of the membrane [28]. Other ion exchanger LNG-PMA (2% LNG-PM, 65% TCP and 33% PVC) was tested (SPE5) showed a sub- Nernstian response of 52.25 mV/decade over the concentration range from1.0×10^{-5} to 1.0×10^{-2} M (Table 2) the sub-Nernstian response would be due to leaching the LNG-PMA from the membrane when the sensor is brought in contact with the solution. Furthermore, the nature of plasticizers influences the di-electric constant of the membrane and consequently the mobility of the ion exchanger so the effect of the plasticizer on the potentiometric response of LNG-TPB based electrode was studied using DBP and DOP plasticizers. The slope was dropped to (50.13 and 44.21 mV/decade) for SPE6 and SPE7; respectively (Table 2). This would be due to the limited solubility of the ion exchanger (LNG-TPB) in both DBP and DOP or low distribution of the ion exchanger (LNG-TPB) in these solvent mediators. It seems that TCP provides more appropriate conditions for incorporation of the highly lipophile LNG+ ion into the membrane.

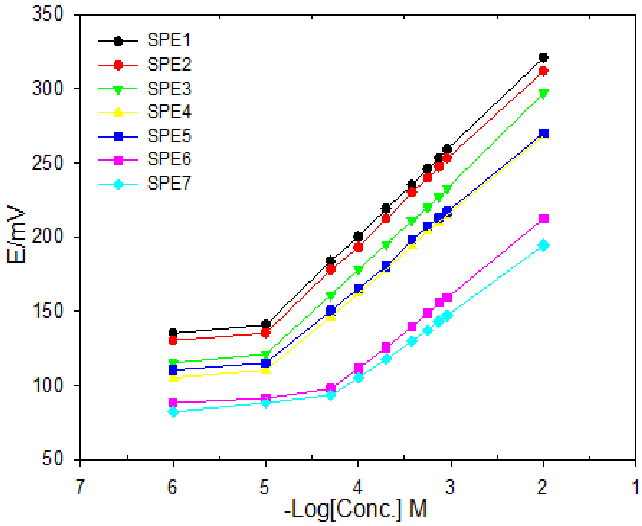


Figure 4. Calibration graphs of LNG screen printed electrodes with different compositions in phosphate buffer pH 6.8

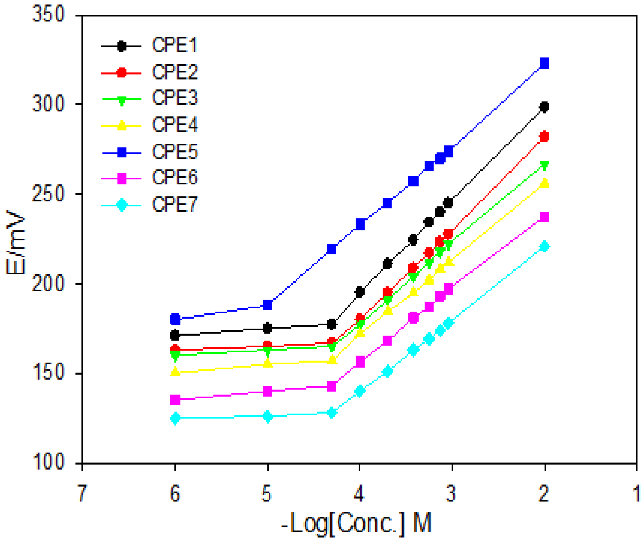


Figure 5. Calibration graphs for LNG carbon paste electrodes prepared using different types of ion pair, different amount of ion pair and different solvent mediators

Carbon paste electrodes (CPEs) have also been prepared (Figure 5) and their potentiometric characteristics were compared side-by-side with the screen printed electrodes (SPEs). The Nernstian behavior, linear range and detection limits are summarized in table 3. Some of the carbon paste electrodes exhibited a sub-Nernstian response (≤52.35 mV/decade.) and others electrodes exhibited a non-Nernstian response ≤44.57 mV/decade. Therefore, all subsequent studies including pH, dynamic response time, selectivity and analytical applications were carried out with screen printed ISEs (SPE1) which not only provides higher values of a Nernstian slope but also more stable potential readings and lower limit of detection.

Table 2. Effect of membrane composition on the potentiometric response of LNG SPEs in phosphate buffer pH 6.8

Sensor	**Composition (% w/w)**			**Slope mV/decade**	**Linear range (M)**	**Limit of detection (M)**	**Correlation coefficient (r)**
	Ion pair (%)	**Plasticizers (%)**	**PVC (%)**				
SPE1	2.0% TPB	65% TCP	33%	60.10	1.00×10^{-5} to 1.00×10^{-2}	4.50×10^{-6}	0.9999
SPE2	1.0% TPB	66% TCP	33%	59.22	1.00×10^{-5} to 1.00×10^{-2}	4.50×10^{-6}	0.9995
SPE3	3.0% TPB	65% TCP	32%	58.30	1.00×10^{-5} to 1.00×10^{-2}	5.01×10^{-6}	0.9993
SPE4	4.0% TPB	64% TCP	32%	53.22	1.00×10^{-5} to 1.00×10^{-2}	6.31×10^{-6}	0.9995
SPE5	2.0% PMA	65% TCP	33%	52.25	1.00×10^{-5} to 1.00×10^{-2}	6.31×10^{-6}	0.9992
SPE6	2.0% TPB	65% DBP	33%	50.13	5.00×10^{-5} to 1.00×10^{-2}	1.25×10^{-5}	0.9994
SPE7	2.0% TPB	65% DOP	33%	44.21	5.00×10^{-5} to 1.00×10^{-2}	1.58×10^{-5}	0.9991

Table 3. Potentiometric performance parameters of LNG carbon paste electrodes

Paste No.	**Composition (% w/w)**			**Slope (mV/decade)**	**Linear range (M)**	**Limit of detection (M)**	**Correlation coefficient (r)**
	Ion pair (%)	**plasticizer (%)**	**graphite (%)**				
1	2.0%TPB	44%TCP	54%	52.35	5.00×10^{-5} to 1.00×10^{-2}	2.00×10^{-5}	0.9996
2	1.0%TPB	44.5%TCP	54.5%	50.20	5.00×10^{-5} to 1.00×10^{-2}	2.51×10^{-5}	0.9992
3	3.0%TPB	44%TCP	53%	47.11	5.00×10^{-5} to 1.00×10^{-2}	2.51×10^{-5}	0.9995
4	4.0%TPB	43%TCP	53%	45.12	5.00×10^{-5} to 1.00×10^{-2}	2.00×10^{-5}	0.9991
5	2.0%PMA	44%TCP	54%	44.57	1.00×10^{-5} to 1.00×10^{-2}	6.31×10^{-6}	0.9993
6	2.0%0TPB	44% DBP	54%	41.23	5.00×10^{-5} to 1.00×10^{-2}	2.82×10^{-5}	0.9991
7	2.0%TPB	44% DOP	54%	40.38	5.00×10^{-5} to 1.00×10^{-2}	3.16×10^{-5}	0.9995

3.3. Effect of Buffer

The membrane response was checked by distilled water and Phosphate buffer (Figure 6). The best results in terms of slope, linear range and potential stability were obtained in phosphate buffer pH 6.8. Hence, phosphate buffer pH 6.8 was selected for all subsequent studies. The potentiometric results in buffer and double distilled water are summarized in table 4.

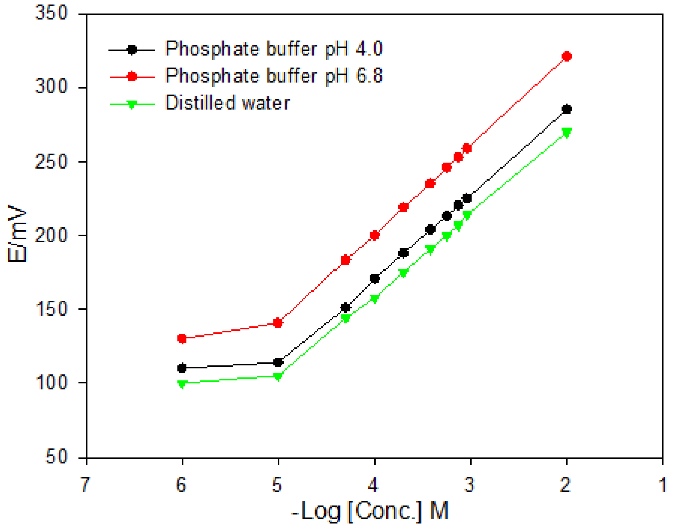


Figure 6. Calibration graphs for LNG SPEs in phosphate buffer at different pH values and in bi-distilled water

Table 4. **P**erformance parameters for LNG SPE at different pH values of phosphate buffer

Buffer type	Slope (mV/decade)	Linear range (M)	Limit of detection (M)	Correlation coefficient (r)
No buffer (water)	55.10	1.00×10^{-5} to 1.00×10^{-2}	6.31×10^{-6}	0.9995
KH_2PO_4 buffer pH 6.80	60.10	1.00×10^{-5} to 1.00×10^{-2}	4.50×10^{-6}	0.9999
NaH_2PO_4 buffer pH 4.00	57.27	1.00×10^{-5} to 1.00×10^{-2}	5.01×10^{-6}	0.9997

3.4. Effect of pH

Effect of pH on the potential of LNG electrode was tested by monitoring the potential of LNG electrode in LNG solutions with concentrations of 1.0×10^{-2} and 1.0×10^{-3} M. The pH of the solution was changed by adding appropriate amounts of HCl and NaOH (0.1 M each). The effect of pH of the electrode response is shown in figure 7. It is clear that the potential is not affected by changing the pH of the solution from 4 to 8 so the potentiometric measurements should be performed within this range. The slight change in the potential of the electrode, at pH lower than 4, is most likely due to interference from H^+ ion. The dramatic increase in the potential values at pH higher than 8 is due to the formation of LNG base; interference from

OH^- is also a possible. In order to avoid the interference from H^+, all measurements were carried out in a buffer solution adjusted at pH 6.8.

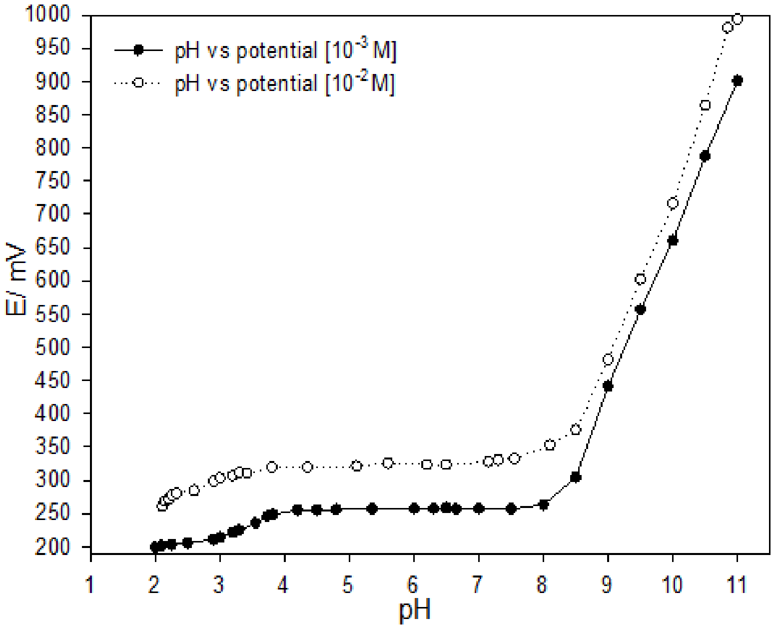


Figure 7. Effect of different pH values on the potential response of 10^{-2} M and 10^{-3} M of LNG solutions

3.5. Dynamic Response Time and Hysteresis

The dynamic response time of potentiometric ion-selective electrodes is one of the characteristics that should be studied; it is the time needed by the electrode to reach a limiting potential value (steady state potential) after successive immersing the electrode in a sample solution of the analyte each having a 10-fold increase in concentration. The hysteresis, On the other hand, results from electrode memory and it appears as a shift in the potential reading when the electrode is frequently subjected to solutions of different concentrations of the target analyte (the measurements were performed in the sequence of high-to-low sample concentration). It affects the reproducibility of the electrode. The response time and hysteresis were studied according to the IUPAC recommendations [27]. The potentiometric dynamic response time of the proposed electrode was evaluated by monitoring the electrode potential for 10 min. at different analyte concentrations from 1.0×10^{-4} to 1.0×10^{-2} M (Figure 8 (A)). The electrode showed a fast and stable potential response in 15 sec. The potential reading stays constant and the change was within ±1.0 mV, for at least 5 minute.

The memory of the electrode was studied by exposing the electrode to LNG solutions of 1.0×10^{-4} and 1.0×10^{-3} M (Figure 8 (B)). The electrode showed a satisfactory reproducibility indicating a negligible memory effect when it is exposed to different concentrations of LNG.

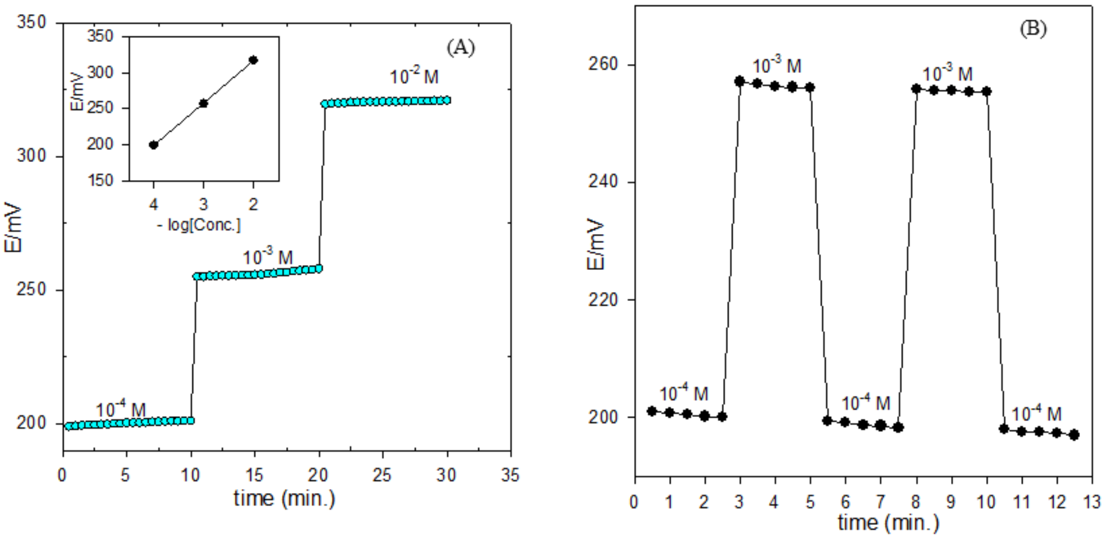


Figure 8. (A) Response time of the proposed SPE at different concentrations and (B) Hysteresis of the proposed SPE for several high to low cycles

3.6. Method validation

The proposed method was validated according to the recommendation of ICH Q2 (R1) guidelines [29]. Validation parameters include linearity and range, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and selectivity.

3.6.1. Linearity and Range

A linear relationship was obtained by plotting the potential of the proposed SPE against –log concentration of LNG over the concentration range from 1.0×10^{-5} to 1.0×10^{-2} M with LOD of 4.50×10^{-6} M and LOQ of 1.0×10^{-5} M.

The regression equation was computed and found to be:

$$Y= -60.10X+441.10 \qquad (r = 0.9999)$$

where Y is the potential in mV, X is -log LNG molar concentration, and r is the correlation coefficient.

The high values of the correlation coefficients (r) indicate good linearity of the calibration graphs while small values of standard deviation (SD) and relative standard deviation (RSD), indicate that the points are closer to the linear regression line over the specified concentration range. The calibration data of the proposed method are presented in Table 5.

3.6.2. Detection Limit

The limit of detection (LOD) for the proposed SPE was calculated according to IUPAC recommendations [27]. The LOD is the concentration at the intersection of the two segments of the calibration plot (Figure 9). The LOD was found to be 4.50×10^{-6} M as presented in Table 5.

Table 5. Calibration and Performance data of the proposed method for determination of LNG in pure form

Parameters	**LNG**
Concentration range (M)	1. 0×10^{-5} to 1.0×10^{-2}
Intercept (mV)	441.10
Slope (mV/decade)	60.10
Correlation coefficient (r)	0.9999
% Recovery	98.00 - 102.00
Mean recovery ± S.D	100.30 ± 1.61
%RSD	1.61
% Er	0.54
LOD (M)	4.50×10^{-6}
LOQ (M)	1.00×10^{-5}

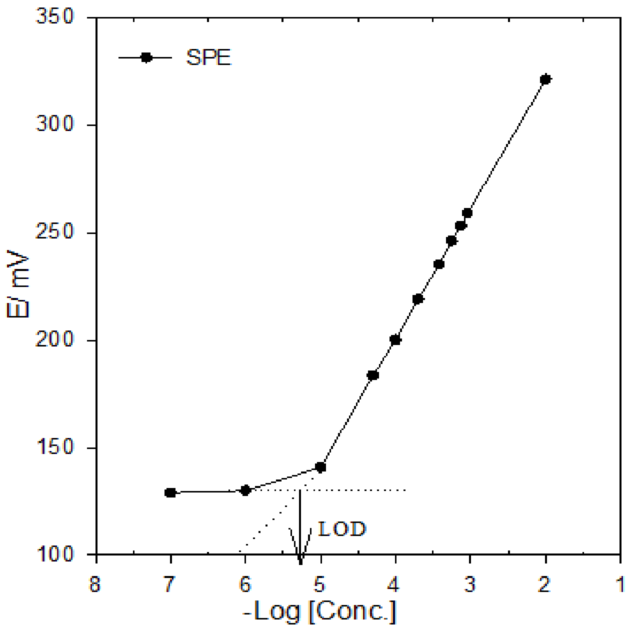


Figure 9. Determination of the detection limit of the proposed SPE; the intersection of the two segments of the calibration graph shows the detection limit of the electrode

3.6.3. Precision and Accuracy

The precision of the proposed method was assessed by the analysis of 1.0×10^{-4}, 2.00×10^{-4} and 3.80×10^{-4} M LNG within the same day to evaluate repeatability (intraday precision) and over three days to evaluate intermediate precision (inter day precision) (Table 6).The accuracy was found to be within ± 1.5% and the relative standard deviation (%RSD) is ≤ 2%, indicating high accuracy and precision of the proposed method.

Table 6. Repeatability data of the proposed ion-selective electrode method for the determination of LNG in pure form

Parameters	LNG		
	Intra-day precision (Repeatability)		
Conc. (M)	1.00×10^{-4}	2.00×10^{-4}	3.80×10^{-4}
%Recovery	100.00 101.00 98.00	102.00 99.80 102.00	99.51 100.60 100.33
%Average recovery	99.80	101.27	100.15
%RSD	1.59	1.25	0.57
	Inter-day precision (Intermediate precision)		
Conc. (M)	1.00×10^{-4}	2.00×10^{-4}	3.80×10^{-4}
%Recovery	100.20 97.80 100.20	102.11 102.37 100.00	100.76 99.23 100.76
%Average recovery	99.40	101.49	100.25
%RSD	1.39	1.28	0.88

3.6.4. Statistical Analysis

The results obtained by the proposed and the reference methods were statistically compared using the student's t-test and variance ratio F-test [30]. The results showed no significant difference between the performance of the two methods regarding the accuracy and precision (Table 7)

3.6.5. Selectivity and Selectivity Coefficients

Direct application of the proposed electrode for determination of LNG in pharmaceutical formulation without sample pretreatment or extraction requires a former knowledge about the selectivity of the electrode towards inactive ingredients that exist in the formulation. The selectivity coefficient is defined as the ability of the ISEs to discriminate between the target analyte (I) and the interferant (j). Herein, the selectivity coefficients for common cations such as Na^+ K^+ and Ca^{2+}; Lactose; and amino acids such as Methionine, Alanine, Valine, Proline, Glutamine, Glycine, lysine, Lucien were studied by the separate solution method [27]. Furthermore, calibration graphs were individually constructed for LNG and each of the interfering compounds. As shown in figure 10, no response towards the interfering cations, whereas, a Nernstian response was achieved for the analyte ion; so that the proposed method can be used for determination of LNG in presence of these foreign compounds without fear of interference. Commonly, acceptable selectivity coefficient log $K_{i,j}^{Pot.}$ for a given electrodes is ≥ 0.1. The values of log $K_{i,j}^{Pot.}$ for our electrode are summarized in table 8. The values are in the range from 2.66 to 4.30 indicating a high selectivity of the proposed sensor towards LNG over the studied interfering compounds.

Table 7. Statistical analysis of the results for determination of LNG in pure form by the proposed method, compared with a reference method [14]

Parameters	**LNG**			
	Conc. taken (M)	**Conc. found (M)**	**%recovery proposed method**	**%recovery ref. method** [14]
	1.00×10^{-4}	1.025×10^{-4}	102.50	100.64
	1.00×10^{-4}	1.005×10^{-4}	100.50	102.10
	1.00×10^{-4}	1.002×10^{-4}	100.25	100.14
	1.00×10^{-4}	0.972×10^{-4}	97.25	101.80
	1.00×10^{-4}	0.982×10^{-4}	98.25	99.71
% Average recovery			99.75	100.88
No. of experiments			5	5
Variance			4.22	1.08
F-ratio			3.92 (6.39)*	
Students T-test			1.10 (2.45)*	

*Values between parentheses are the tabulated F and t values respectively at 95% confidence level [30].

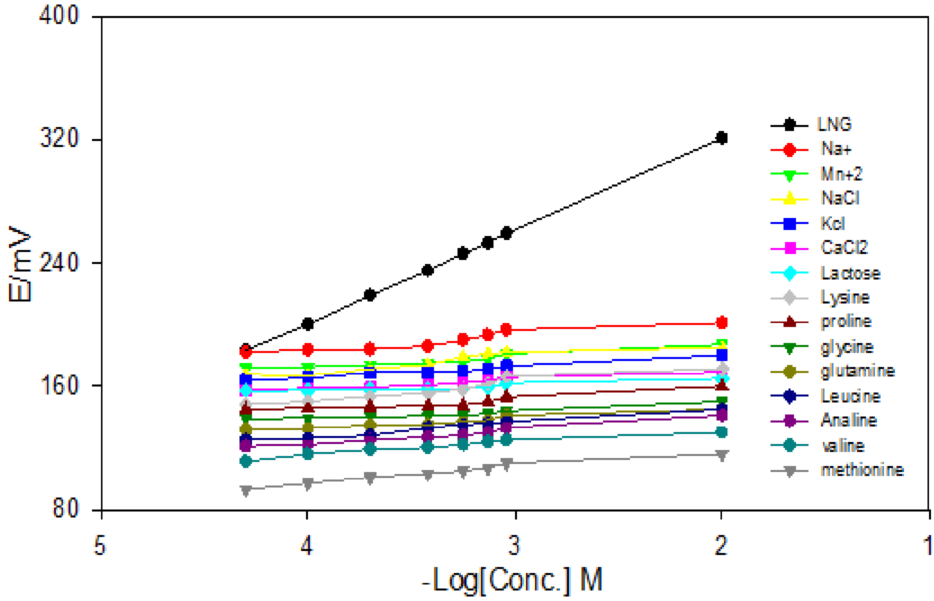


Figure 10. Calibration graphs for LNG and possible interfering substances

Table 8. Selectivity coefficient values $-\log K^{Pot.}_{i,j}$ for SPE using the separate solution method

Interferant	log $K^{Pot.}_{i,j}$ −
Na^+	2.66
Mn^{2+}	3.86
NaCl	3.16
KCl	3.33
$CaCl_2$	4.00
Lactose	3.78
Lysine	4.00
Leucine	4.16
Glutamine	4.11
Glycine	4.08
Analine	4.20
Proline	4.05
Valine	4.25
Methionine	4.30

3.7. Analytical applications

3.7.1. Potentiometric Titration of LNG

LNG was determined successfully using the potentiometric titration method. Figure 11 shows the potentiometric titration curves of 2 mL and 4 mL of 1.0×10^{-2} M LNG standard solution using SPE1 against 1.0×10^{-2} M sodium tetra phenyl borate used as a titrant. The end point is detected by first derivative method. The accuracy and precision results are presented in table 9. The obtained results indicate a high accuracy and precision of the proposed method.

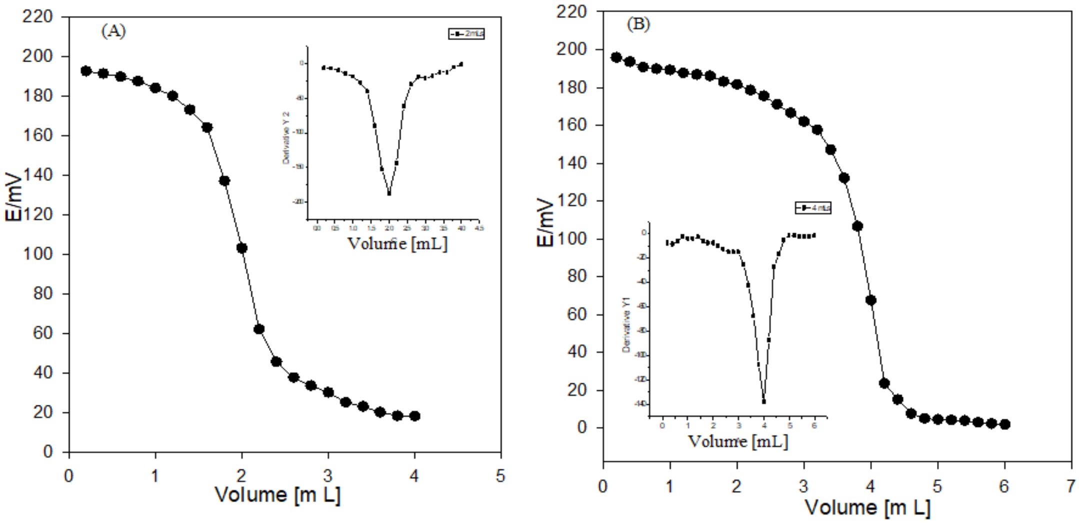


Figure 11. Potentiometric titration curves of (A) 2 and (B) 4 mL of 1.0×10^{-2} M LNG standard solution using SPE1 and 1.0×10^{-2} M Na-TPB as titrant; the insets are its first order derivative

Table 9. Potentiometric results for determination of LNG in standard solutions

Sample No.	Conc. Taken (mg/50mL)	Conc. Found (mg/50mL)	%Recovery	Mean Recovery(%) ± SD	% RSD
1	9.45	9.53	100.85	100.83± 0.29	0.28
		9.55	101.10		
		9.50	100.53		
2	18.90	18.89	99.95	100.50± 0.88	0.87
		18.91	100.03		
		19.18	101.51		

3.7.2. Determination of LNG in Pharmaceutical Preparation

The proposed SPE was successfully applied for the accurate determination of LNG in pharmaceutical dosage form using the standard addition technique. The obtained results (Table 10) indicate that the sensor gives high recovery values ranging from 98.0 to 102 %. The %RSD values were found to be < 2 % reflecting the precision of the proposed electrochemical method.

Table 10. Determination of LNG in tablet dosage form by applying the standard addition method for the proposed electrode

Sample	Taken conc. (mg/50mL)	Found conc. (mg/50mL)	Mean Recovery(%) ± SD•	% RSD*
Trajenta® tablets	2.36	2.39	101.29± 1.12	1.11
	4.72	4.70	99.51± 1.83	1.84
	7.09	7.06	99.57± 1.97	1.98
	9.45	9.28	98.15± 1.48	1.51

• **%**Recovery ± SD (Standard deviation) of 6 replicate measurements
*Relative standard deviation for 6 replicate measurements

3.7.3. Dissolution Monitoring of Trajenta® Tablets

Dissolution monitoring is always needed for bio weaver study which provided in-vitro drug release information for quality control proposes to predict in-vivo drug release profiles which processed the dissolution in the vivo study (bioequivalence), drug dissolution testing is sufficient in many cases to establish safety and efficacy of drug product without in-vivo tests. LNG exhibiting high solubility and low permeability; both tablets exhibit an immediate release profile with a very fast release in the dissolution medium. Monitoring dissolution via HPLC was performed; samples were withdrawn from the dissolution medium at five minutes time intervals, filtered, then injected and chromatographed using reference HPLC method [14]. Monitoring the dissolution for LNG was performed via the proposed method; the screen printed electrodes and reference electrodes are dipped into the dissolution medium and the potential in mV was recorded at five time intervals, then these readings were converted into % dissolution and plotted against time in minutes. The drug dissolution profile curves are obtained by plotting the percentage of the dissolved drug versus the dissolution time. (Figure 12)

HPLC method was relatively time consuming due to multiple sampling and purification steps, the advantages of the proposed method in studying the dissolution profiles of drug are that it saves the analysis time because it permits the online determination of the amount of drug dissolved by time and it does not need filtration and demonstrates a very low limit of detection which allows for the accurate online determination of very low concentrations of the dissolved drug**.**

The studied drug complied with the acceptance dissolution criterion stated by the USP which indicates that the drug dissolution profiles of fast-release drug products reach 85-100% dissolution within 30-45 min.; it was found that reaching 100% dissolution was achieved by LNG after 30 min.

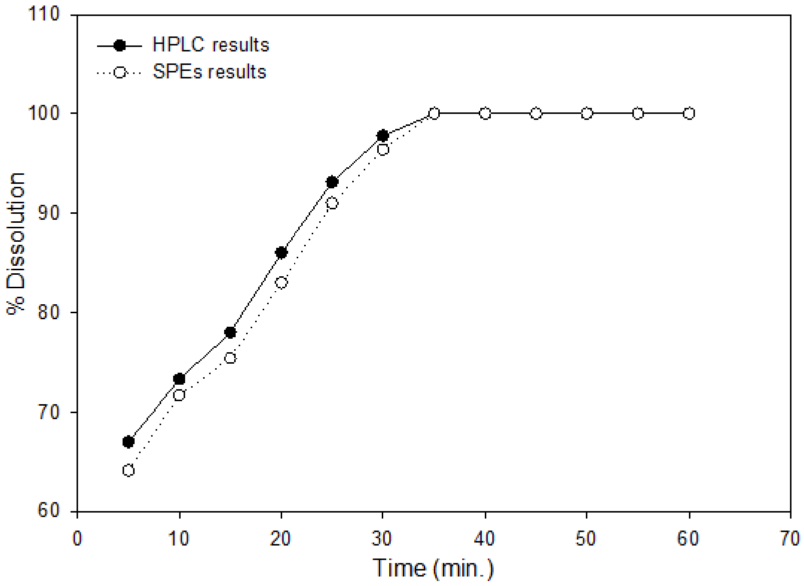


Figure 12. Dissolution profile for LNG in Trajenta® (5 mg LNG/tab) obtained by HPLC method and the proposed Potentiometric method

4. CONCLUSION

A home-made screen printed ion selective electrode was fabricated and characterized for the determination of LNG. The electrode is simple, inexpensive, portable, disposable and showed satisfactory sensitivity and selectivity It can be considered as an attractive alternative to numerous complicated methods used for the determination of the studied drug. The proposed method was successfully applied for the determination of LNG in pure form and in pharmaceutical formulations with high accuracy and precision; the results are in good agreement with those obtained by a reported method.

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