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# Application of Ion Selective Electrode for Determination of Lamivudine in Pure Form, Drug Product, Plasma and in presence of its Related Impurities

**Kareem M. Younes***

*Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, El-Kasr El-Aini Street, ET-11562 Cairo, Egypt*

* Corresponding Author, Tel.:+2.02.01141731229; Fax: +2.02.23628246
E-Mail: kareemchem99@yahoo.com

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**Abstract**- The field of Electro-chemical sensors development is now highly grown for the determination of traces amounts of drugs**.** A novel polyvinylchloride membrane sensor for the determination of lamivudine is prepared and characterized. The sensor is based on the use of the ion association complex of lamivudine anion with iron(II)-phenanthroline counter cation as an ion exchange site in the polyvinylchloride matrix. The performance characteristics of this sensor was evaluated according to IUPAC recommendations, which reveal a fast, stable and linear response for lamivudine over the concentration range of $10^{-6}$ to $10^{-2}$ mol $L^{-1}$ for the sensor with anionic slope of 27.3 mV per concentration decade for the sensor. The direct potentiometric determination of lamivudine using the proposed sensor gave recovery of 99.57±0.62% for the sensor. The proposed sensor displayed useful analytical characteristics for the determination of lamivudine in bulk powder, pharmaceutical formulations, and biological fluids (plasma) and in the presence of some related impurities, namely cytosine, uracil and salicylic acid. Validation of the method shows suitability of the proposed sensor for the use in quality control assessment of lamivudine. The developed method was found to be simple, accurate and precise when compared with an official high performance liquid chromatographic method. Also the method allows direct determination of lamivudine without pretreatment or separation steps in turbid or colored solutions in presence of some related impurities.

**Keywords-** Lamivudine, Ion selective electrode, PVC membrane, Phenanthroline, Iron(II), Sensor, Related impurities

## 1. INTRODUCTION

Lamivudine (LAM), 2′-deoxy-3′-thiacytidine [1], Fig. 1, is a cytosine analog with potent activity against human immunodeficiency (HIV) and hepatitis B viruses (HBV) through inhibition of reversed transcriptase activity. LAM is used in the treatment of HBV infections and it has strongly recommended for the treatment of HIV infections in combination with other antiviral drugs [2]. Moreover, lamivudine is active against zidovudine-resistant HIV [3] The US Department of Health and Human Services' current guideline for the treatment of established HIV infection strongly recommends LAM in combination with another nucleoside reverse transcriptase inhibitor and either a protease inhibitor or efavirenz [4]. The usual dosage of LAM is 150 mg twice daily or 300 mg once daily in combination with other antiretroviral agents [5]. WHO mentioned some related impurities of LAM such as cytosine, uracil and salicylic acid [6].

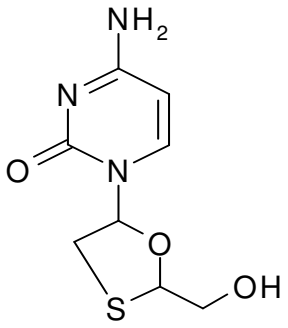


**Fig. 1.** Chemical structure of Lamivudine

Several analytical methods have been developed for the determination of LAM either individually or in combination with other anti-retroviral drugs in the dosage forms and in biological fluids. Examples of these methods are spectrophotometric [7-18], high performance liquid chromatographic (HPLC) [19-27], liquid chromatographic mass spectrometric (LC-MS) [28-33] and capillary electophoretic methods [34-44].

In the last three decades, being commercially and not expensive, ion selective electrodes have become an item of general equipment of analytical work. This result happens because ion selective electrodes have rapid, simple, lower cost and give accurate measurements of ionic species.

The key to constructing such an electrode is to produce a sensitive and selective membrane that responds to a particular drug. Such membrane is usually prepared by incorporating an appropriate ion-exchanger and solvent mediator into a polyvinyl chloride (PVC) matrix.

The objective of this work is to develop a sensitive ion selective electrode for the determination of LAM based on the fact that LAM has an alcoholic hydroxyl group that can act as an anion in basic medium, which suggests the use of ion exchangers of the cationic type like iron(II)-phenanthroline, forming water insoluble ion association complex.

The high lipophilicity and remarkable stability of this complex suggested its selective use as an electro-active material in PVC matrix membrane sensor for the determination of LAM. This sensor showed no interference either from its related impurities or from Co-administered drugs, such as ribavirin and zidovudine.

## 2. EXPERIMENTAL

### 2.1. Apparatus

Potentiometric measurements were made at 25±1ºC with a Hanna (Model 211) pH/mV meter. A single junction calomel reference electrode (Model HI 5412) was used in conjunction with the drug sensor. A WPA pH combined glass electrode (Model CD 740) was used for pH measurements. Bandelin Sonorex, RK 510 S, magnetic stirrer and a silver wire (3 mm diameter) immersed in the internal solution was also applied.

### 2.2. Chemicals and Reagents

All chemicals used were of analytical grade and bi-distilled water was used. Tetrahydrofuran (THF) 99% (Lab Scan), high molecular weight (10.000) polyvinyl chloride (PVC) powder were obtained from Aldrich (Steinheium, Switzerland). Dibutylsebathete (DBS), ferrous sulfate, phenanthroline, cytosine and uracil were obtained from Sigma (St. Louis, USA). Salicylic acid was supplied from El Nasr Chemical Company (Cairo, Egypt). Phosphate buffer pH 8 was prepared [45]. Ammonia solution 10% (v/v), 0.1 M NaOH and 0.1 M HCl solutions were freshly prepared.

### 2.3. Samples

#### *2.3.1. Pure sample*

LAM was kindly supplied by Eva-pharm Pharmaceutical Company (Cairo, Egypt). The purity was found to be 99.91±0.47 according to the official HPLC method [46].

#### *2.3.2. Pharmaceutical dosage form*

Lamidine® and Zeffix™ tablets (batch No. 701050 and 070053 labeled to contain 150 and 100 mg lamivudine expressed as base per tablet, respectively) are provided from EVA and Glaxo Smith Kline (GSK) Pharmaceutical Co. (Cairo, Egypt).

*2.3.3. Plasma*

Fresh human plasma was obtained from VACSERA (Giza, Egypt). It was kept frozen until use after gentle thawing.

**2.4. Standard Solutions**

*2.4.1. LAM working standard solutions*

LAM working solutions ($1\times10^{-6}$ to $1\times10^{-2}$ mol $L^{-1}$) were prepared by serial dilutions from LAM stock solution ($1\times10^{-2}$ mol $L^{-1}$) using phosphate buffer pH 8 as a solvent.

*2.4.2. Iron(II)–phenanthroline solution*

It is prepared by dissolving 100 mg of phenanthroline in 20 mL of $10^{-2}$ mol $L^{-1}$ iron(II) sulfate, followed by drops of ethanol and water to keep a clear solution.

*2.4.3. Laboratory-prepared mixtures*

2.5 mL LAM from its stock solution ($10^{-2}$ mol $L^{-1}$) was transferred accurately to a series of 25 mL measuring flasks. Different Aliquots from each impurity solution ($10^{-2}$ mol $L^{-1}$) were added to prepare different mixtures of LAM and each impurity up to 80%.

**2.5. Procedures**

*2.5.1. Precipitation-based technique for the preparation of the sensor*

A 5 mL aliquot of $10^{-2}$ mol $L^{-1}$ aqueous LAM solution was treated with two drops of 10% ammonia solution, mixed with 5 ml of phenanthroline-iron(II) solution and shaken well for 5 minutes. The resultant precipitate formed was filtered using Whatman No. 42 paper, washed with cold water, dried at room temperature and grinded to a fine powder.

In a glass Petri dish (5 cm diameter), 10.0 mg of LAM-ion exchanger was thoroughly mixed with 0.35 gm of dibutylsebathete (DBS) and 0.19 gm of polyvinyl chloride (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.

*2.5.2. Preparation of the electrode assemblies*

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 glass electrode body. Equal volumes of $10^{-2}$ mol $L^{-1}$ LAM and $10^{-2}$ mol $L^{-1}$ potassium 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 an internal reference electrode.

The electrode was conditioned by soaking for 24 h in a solution of $10^{-2}$ mol $L^{-1}$ of drug and stored in the same solution when not in use.

*2.5.3. Electrode Calibration*

The electrode was conditioned by soaking in $10^{-2}$ mol $L^{-1}$ LAM solution for 24 h. Storage was in the same solution when not in use. The conditioned electrode was immersed in conjunction with the single junction calomel reference electrode in solutions of LAM in the range of $10^{-6}$-$10^{-2}$ mol $L^{-1}$. They were allowed to equilibrate whilst stirring and recording the e.m.f readings within ±1 mV. The membrane sensor was washed between measurements with water. The mV-concentration profiles were plotted. The regression equation for the linear part of the curve was computed and used for subsequent determination of unknown concentrations of LAM.

*2.5.4. Determination of LAM in its pharmaceutical dosage form (tablets)*

The content of ten lamidine® or zeffix™ tablets was mixed. An amount of this powder equivalent to 22.9 mg LAM was accurately transferred separately to a 50-ml volumetric flask and the volume was completed to the mark with phosphate buffer of pH 8 to prepare $10^{-3}$ mol $L^{-1}$ of LAM. The e.m.f. produced by immersing the prepared electrode in conjunction with calomel reference electrode in the prepared solution was determined then the concentration of LAM was calculated from the regression equation of the corresponding electrode.

*2.5.5. Determination of LAM in spiked plasma samples*

4.5 mL plasma was placed into two stoppered shaking tubes then 0.5 ml of $10^{-3}$ and $10^{-4}$ mol $L^{-1}$ LAM were added separately and shaken. The membrane sensor was immersed in conjunction with calomel reference electrode in spiked plasma solutions. The e.m.f. produced for each solution was measured by the proposed electrode then the concentration of LAM was determined from the corresponding regression equation.

*2.5.6. Determination of LAM in the presence of its related impurities*

Aliquots of standard drug solution ($10^{-3}$ mol $L^{-1}$) were mixed with its related impurities each one is ($10^{-3}$ mol $L^{-1}$) in different ratios. The emf values of these laboratory-prepared mixtures were recorded and results were compared with the calibration plot.

## 3. RESULTS AND DISCUSSION

Preparation of the proposed sensor originates from the fact that LAM behaves as an anion in basic medium; this fact suggests the use of cationic exchangers**.** The most common cationic exchanger that react with anionic drugs is, iron(II)–phenanthroline. It forms an insoluble ion association complex with suitable grain size with LAM. The ratio of LAM to the ion exchanger in the formed complex was found to be 2:1 as proven by the obtained Nernstian slope (about 30mV/decade). The cationic exchangers were incorporated with a suitable solvent mediator in poly (vinyl chloride) matrix to produce plastic membranes which were used for constructing the electrodes. The complexes were formed in situ by soaking the prepared membranes in $1\times10^{-2}$ mol $L^{-1}$ LAM solution. The LAM extraction into the membrane sensors was a result of the ion-pair tendency to exchange with LAM cation. LAM (containing alcoholic OH group) was ionized in alkaline medium and then reacted with this ion exchanger to form stable 2:1 water insoluble ion association complex (Fig. 2).

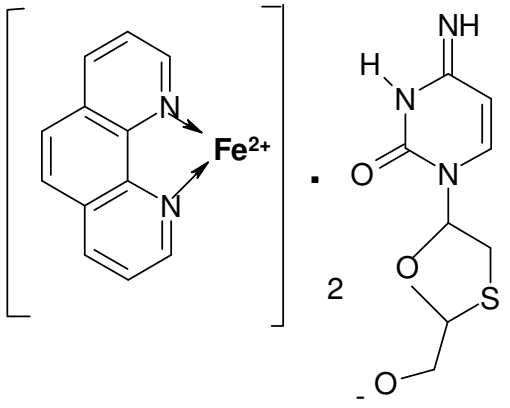


**Fig. 2.** The suggested Chemical structure of LAM association complex with iron(II)-phenanthroline

Based on the IUPAC recommendations [47] the response characteristics of the designed electrode were assessed. Table 1 displays the results obtained over a period of two months for two different assemblies of the sensor. The calibration plot was presented in Fig. 3. The slope of the calibration plot was 27.3 mV/concentration decade for the suggested sensor. The deviation from the ideal Nernstian slope (30 mV/decade), is due to the fact that the electrode responds to activities of the drug rather than the concentration. The suggested electrode displayed constant potential readings for day to day measurements, and the calibration slope did not change by more than ±2 mV/decade over a period of 6 weeks. The detection limit of the sensor was estimated according to the IUPAC definition [47].

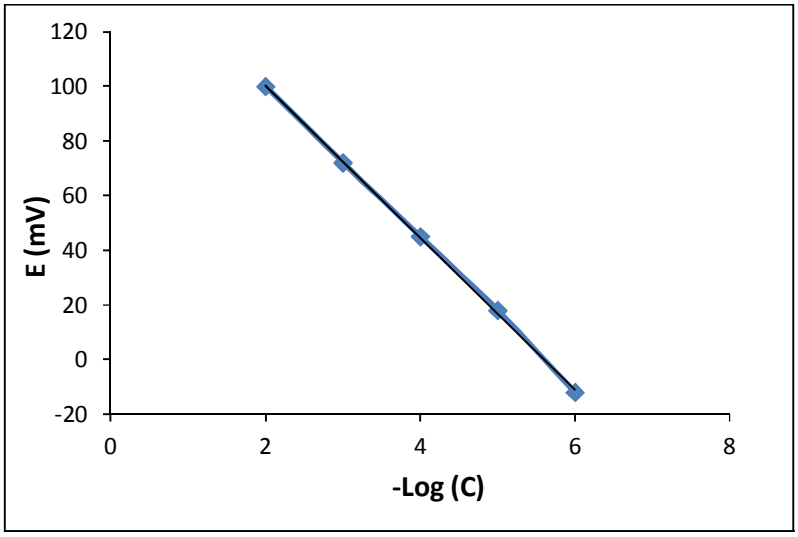


**Fig. 3.** Profile of the potential in mV to – log concentration in mol $L^{-1}$ obtained with sensor

The introduction of high molecular weight PVC, as regular support matrix and traps for the sensed ions, creates a need for a plasticizer [48]. In the present investigation, dibutylsebathete (DBS) was chosen from diesters of di-carboxylic acids. With PVC, it plasticize the membrane, dissolves the ion association complex and adjusts both permeability of the final organic membrane and mobility of the ion exchange sites. Such adjustments influence the partition coefficient of the studied drug with subsequent effect on electrode selectivity.

A fast response time was recorded by increasing LAM concentration by up to 10-fold. The required time for the sensor to reach values within ±1 mV of the final equilibrium potential was 10-15 s. The optimum equilibration time for the electrode, after soaking in $1.0\times10^{-2}$ mol $L^{-1}$ LAM, was 12 hours. After this time period, the electrode generated stable potentials in contact with the LAM solution. On soaking for a longer time the slopes decreased gradually and this may be attributed to the gradual leaching of the electroactive species into the bathing solution [49]. Therefore, when not in use for a long time, the electrodes should be kept dry.

To evaluate the precision of measurements, three concentrations within the linear concentration range ($10^{-4}$, $10^{-3}$ and $10^{-2}$, mol $L^{-1}$ solutions) of LAM were chosen. Three solutions of each concentration were prepared and analyzed in triplicate (repeatability assay). This assay was repeated on three different days (reproducibility assay) (Table 1). As for the robustness, the method demonstrated efficient stability when the plasticizer was changed. Also, the wide range of pH (7-9) made the method robust. To study the method's ruggedness, $10^{-3}$ mol $L^{-1}$ solution of LAM was analyzed by the suggested electrode using Jenway 3505

digital ion analyzer instead of Hanna 211 Model. Results proved the ruggedness of the method upon changing the instrument Table 1.

**Table 1.** Electrochemical response characteristics and method validation for the investigated LAM sensor

| **Parameter** | **LAM sensor** |
|---|---|
| Slope (mV/ decade) | -27.3 |
| Intercept (mV) | 153.4 |
| Correlation coefficient | 0.9998 |
| LOD (mol $L^{-1}$) | $3.9\times10^{-7}$ |
| Response Time (s) | 20-30 |
| Working pH Range | 4-6 |
| Concentration Range (mol $L^{-1}$) | $10^{-6}$-$10^{-2}$ |
| Life Span (days) | 30 |
| Accuracy (mean ± S.D.)* | 99.57 ± 0.62 |
| Repeatability** | 99.26 ± 0.39 |
| Intermediate Precision*** | 99.14 ± 0.49 |
| Ruggedness**** | RSD is 1.21% |

* Average of five determinations.

** The intra-day variability of LAM concentrations of $10^{-2}$, $10^{-3}$ and $10^{-4}$ mol $L^{-1}$ (n =3).

*** The inter-day variability of LAM concentration of $10^{-2}$, $10^{-3}$ and $10^{-4}$ mol $L^{-1}$ (n =3).

****Relative standard deviation % of the potential produced by $10^{-3}$ mol $L^{-1}$ solution using Jenway 3505 digital ion analyzer instead of Hanna 211.

### 3.1. Effect of pH on the electrode response

The potential response of the suggested electrode was found sensitive to pH changes. Fig. 4 shows a typical pH response curve for the prepared sensor over a pH range of 2-11, where the pH was varied by addition of diluted sodium hydroxide or hydrochloric acid solutions. The sensor responses were hardly affected by the pH change from 7 up to 9, i.e., in this pH range LAM is completely ionized, dissociated and sensed, so all measurements were carried out at pH 8.

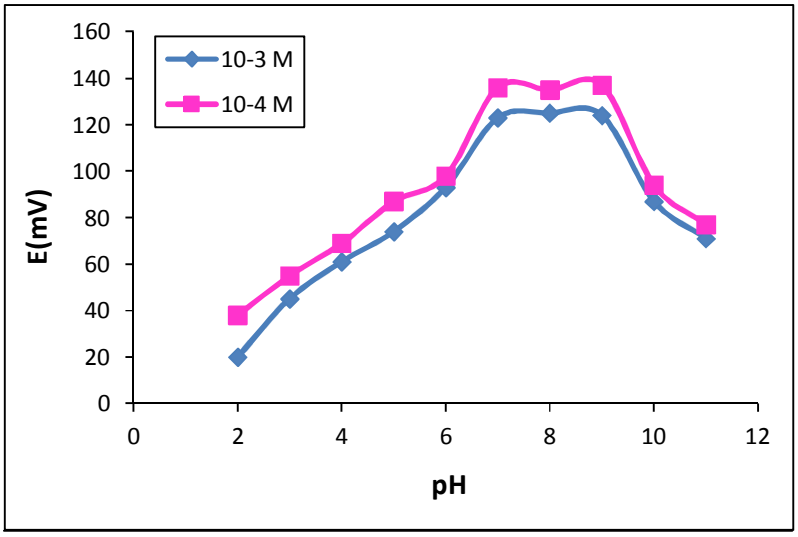


**Fig. 4.** Effect of pH on the response of LAM sensor

### 3.2. Effect of temperature on the electrode response

The potential response displayed by the investigated electrode was affected by temperature. Fig. 5 shows the typical response for the prepared sensor over temperature range of 25º-40º C. The sensor response increased by raising temperature up to 40º C, but on construction a calibration plot at different temperature, the same slope value was obtained.

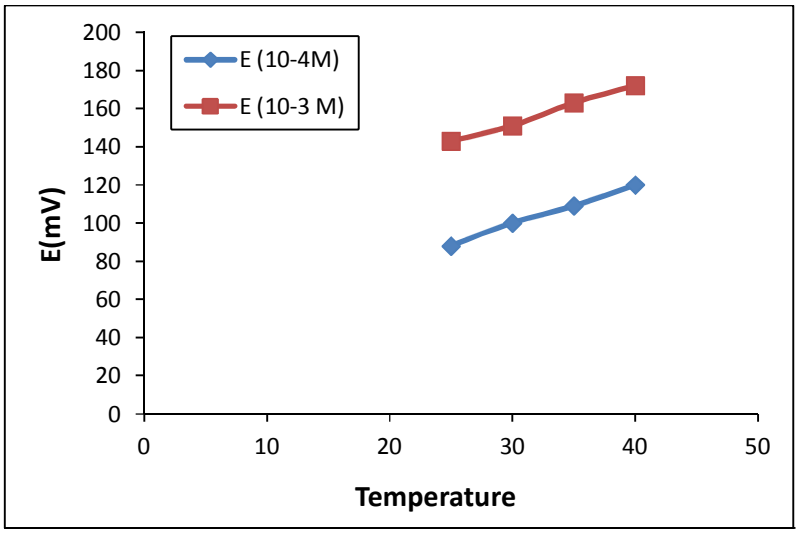


**Fig. 5.** Effect of temperature on the response of LAM sensor

### 3.3. Sensors selectivity

The selectivity of an ion-pair based membrane electrode depends on the physico-chemical characteristics of the ion-exchange process at the membrane. Table 2 shows the potentiometric selectivity coefficients of the proposed sensor in the presence of a number of pharmaceutical additives commonly used in tablets and other co-administered drugs. The selectivity coefficients were determined by the separate solution method and calculated from the rearranged Nicolsky Eisenman equation (Eq. 1) [47]:

$$\mathrm{Log}K_{A,B}\,Pot = \frac{(E_B - E_A)}{S} + \left(1 - \frac{Z_A}{Z_B}\right) Loga_A \qquad (1)$$

Where $E_B$ and $E_A$ are the potential readings recorded after exposing the electrode to the same concentration of the studied drug and the interferent, respectively. $K^{pot}_{A,B}$ is the potentiometric selectivity coefficient, S is the slope of calibration plot, $a_A$ the activity of LAM and $Z_A$ and $Z_B$ are the charges on LAM and the interfering ion, respectively. As it was obvious from Table 2, none of the tested interfering species had a significant influence on the potentiometric response of the electrode towards LAM.

**Table 2.** Potentiometric selectivity coefficient ($K^{pot}_{A,B}$) for the proposed sensor using the separate solution method (SSM)

| **Interference**** | **Selectivity coefficient*** |
|---|---|
| | **LAM-PH** |
| Lactose | $3.1\times10^{-3}$ |
| Urea | $2.5\times10^{-3}$ |
| L-phenylalanine | $4.6\times10^{-3}$ |
| $Na^+$ | $1.68\times10^{-3}$ |
| $K^+$ | $2.23\times10^{-3}$ |
| $Mg^{+2}$ | $1.84\times10^{-3}$ |
| $NH_4^+$ | $3.5\times10^{-3}$ |
| Ribavirin*** | $2.44\times10^{-3}$ |
| Zidovudine*** | $2.63\times10^{-3}$ |

* Average of three determinations
** All interferences are in the form of $1\times10^{-3}$ mol $L^{-1}$ solution
*** Co-administered drugs

### 3.4. Potentiometric determination of LAM in dosage form (tablets)

As none of the commonly used tablets additives show significant interference with the determination of LAM, the new proposed sensor was successfully applied for LAM determination in tablets as shown in Table 3. Results obtained prove the applicability of the method as demonstrated by the accurate and precise recovery percentages.

**Table 3.** Determination of LAM in its dosage forms by the proposed electrode

| **Dosage form Batch No.** | **Recovery% ± S.D.* of LAM** |
|---|---|
| Lamidine® (701050) | 99.58±0.68 |
| Zeffix™ (070053) | 100.32±0.59 |

* Average of three determinations.

### 3.5. Potentiometric determination of LAM in the presence of its related impurities

Table 4 shows the results obtained upon analysis of synthetic mixtures containing different ratios of intact drug and its related impurities, namely cytosine, uracil and salicylic acid. From the presented results it was obvious that the proposed sensor could be used for selective determination of intact drug in the presence of up to 80% of each impurity.

**Table 4.** Determination of LAM in laboratory prepared mixtures containing different ratios of LAM and its related impurities by the proposed electrode

| **Ratio(%)** | **Drug Recovery % ± S.D*** | | |
|---|---|---|---|
| **Drug : impurity** | **Cytosine** | **Uracil** | **Salicylic acid** |
| 80 : 20 | 96.88 ± 0.95 | 97.67 ± 0.44 | 98.72 ± 0.39 |
| 70 : 30 | 97.93 ± 0.39 | 98.23 ± 0.51 | 98.22 ± 0.46 |
| 50 : 50 | 98.55 ± 0.64 | 97.88 ± 0.34 | 97.91 ± 0.57 |
| 30 : 70 | 96.33 ± 0.52 | 96.92 ± 0.57 | 97.36 ± 0.61 |
| 20 : 80 | 95.21 ± 0.68 | 97.13 ± 0.31 | 97.21 ± 0.36 |

*Average of three determinations

### 3.6. Potentiometric determination of LAM in human plasma

On application to the biological fluids, plasma electrolytes did not show any interference. It has been found that electrode gave stable results as revealed by high precision and accuracy of recoveries of the spiked plasma samples, Table 5.

**Table 5.** Determination of LAM in spiked human plasma by the proposed sensor

| **Concentration (mol $L^{-1}$)** | **Recovery % ± S.D.* of LAM** |
|---|---|
| $1\times10^{-5}$ | 99.16 ± 0.39 |
| $1\times10^{-6}$ | 98.49 ± 0.76 |

* Average of three determinations

Statistical evaluation of the results of analysis of pure LAM by the proposed electrode and the official method [46] showed that there is no significant difference between the proposed method and the official one in terms of accuracy and precision, Table 6.

**Table 6.** Statistical analysis of the results obtained by applying the proposed sensor and the official one for the analysis of LAM in pure form

| **Item** | **LAM sensor** | **Official method [46]**** |
|---|---|---|
| Mean±S.D. | 99.57±0.62 | 99.91±0.47 |
| N | 6 | 6 |
| Variance | 0.38 | 0.22 |
| T test (2.23)* | 1.07 | …… |
| F test (5.05)* | 1.73 | …… |

*The values between parentheses are the corresponding theoretical values of t and F at 95% Confidence level.
** Official HPLC method

## 4. CONCLUSION

The use of the proposed sensor offers the advantages of fast response, elimination of drug pretreatment or separation steps, low detection limits and direct determination of drug in turbid or colored solutions. The proposed sensor can be used for determination of LAM in presence of its related impurities. The method is sensitive, simple and precise so that it can be used for routine analysis of the LAM in quality control laboratories.

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