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Full Paper

Ion Selective Electrodes for Stability-Indicating Determination of Gemifloxacin Mesylate

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Abstract- Three novel polyvinyl chloride (PVC) membrane sensors for the determination of gemifoxacin mesylate are described and characterized. The sensors are based on the use of the ion association complexes of gemifoxacin cation with ammonium reineckate counter anions as ion exchange sites in the PVC matrix. The membranes incorporate ion association complexes of gemifoxacin with dibutylsebathete (sensor 1), dioctylphthalate (sensor 2), nitrophenyl octyl ether (sensor 3). The performance characteristics of these sensors were evaluated according to IUPAC recommendations, which reveal a fast, stable and linear response for gemifoxacin over the concentration range of 10^{-5}-10^{-2} M for the three sensors with cationic slopes of -14.30, -20.44 and -22.37 mV per concentration decade for the three sensors, respectively. The proposed sensors displayed useful analytical characteristics for the determination of gemifoxacin mesylate in bulk powder, pharmaceutical formulation, and biological fluids (plasma) and in the presence of its acid degradation products and thus could be used for stability-indicating studies. Validation of the method shows suitability of the proposed sensors for use in the quality control assessment of gemifoxacin mesylate. The developed method was found to be simple, accurate and precise when compared with a manufacturer HPLC method.

Keywords- Gemifoxacin Mesylate, Ion Selective Electrodes, PVC Membranes, Ammonium Reineckate

1. INTRODUCTION

Gemifoxacin mesylate (GM), is a new synthetic third generation fluorinated quinolone antibacterial used in the treatment of severe systematic infections as bronchitis pneumonia as it has a broad spectrum activity against many pathogenic gram -ve and +ve bacteria including many of the so called atypical respiratory pathogens [1]. It overcomes the microbial resistance against common classes of antibiotics which is increasingly important global problem [2] as it is a significant phenomenon in terms of its clinical and economic impact. Patients who were infected with resistant organisms had longer hospitalizations than those infected with susceptible bacteria. In addition, increased costs were associated with infection caused by resistant species and increased mortality, despite the fact that patients received appropriate antimicrobial therapy [3].

GM is (±)7 [3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1, 4- dihydro-4oxo-1, 8-naphthyridine-3-carboxylic acid was prepared in 1995 and 1997 [4]. Its chemical structure was shown in Fig.1. The great bactericidal activity of GM is due to the presence of 4-oxo-3-carboxylic acid [5].

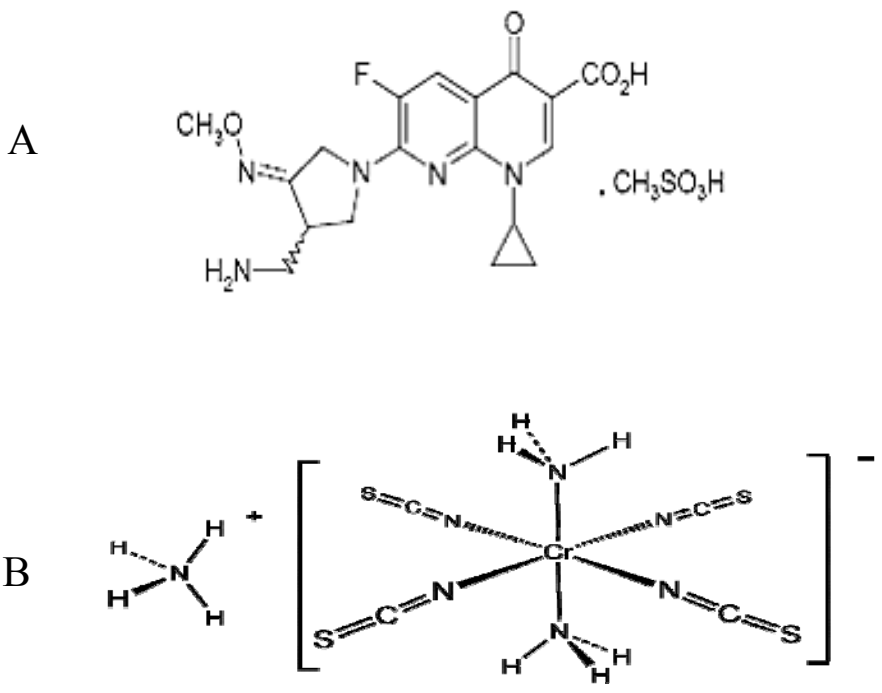

Fig. 1. Chemical structure of gemifloxacin mesylate (A) and ammonium reineckate (B)

It is recently being approved by the US Food Drug Administration for the treatment of upper respiratory tract infections [6]. GM determination is not yet described in any pharmacopoeias. The literature survey reveals that few analytical methods have been reported for determination of GM in bulk powder, pharmaceutical formulation and in biological

samples which include HPLC [7-13], TLC [13], spectrophotometry [13-15] and capillary electrophoresis [16-19] methods.

From these procedures, the HPLC techniques [7,13], TLC [13], spectrophotometry [13] and capillary electrophoresis [19] were recommended as stability-indicating assays. There is no stability-indicating electrochemical method reported for the estimation of gemifoxacin mesylate in its pharmaceutical formulations. Therefore, attempts were made in this study to develop a fast, sensitive, selective and stability-indicating method for its determination.

The rapid growth in the analytical chemistry techniques is necessary to match the development of a wide variety of science and technology approaches. 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, and low 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 a membrane is usually prepared by incorporating an appropriate Ion-exchanger and solvent mediator into a poly vinyl chloride (PVC) membrane matrix. The present work originates from the fact that GM behave as cation. This fact suggests the use of anionic type of ion exchangers, forming water insoluble ion association complexes. Ammonium reineckate as an anionic exchanger (Fig.1) was used for construction of water insoluble ion-association complex with GM. The high lipophilicity and remarkable stability of this complex suggested its selective use as electroactive materials in PVC matrix membrane sensors for the determination of GM, without interference in laboratory prepared mixtures, pharmaceutical formulations and in plasma.

2. EXPERIMENTAL

2.1. Instrument

Potentiometric measurements were made at 25±1 with a Jenway (Model 3510) pH/mV meter. A single junction calomel reference electrode (Model HI 5412) was used in conjunction with the drug sensor and a pH combined glass electrode (WPA, model CD 740). Another equipment was a sonication bath (Bandelin, model RK 510-S), a magnetic stirrer and a silver wire (3 mm diameter) immersed in the internal solutions were also applied.

2.2. Reagents

All chemicals were of analytical grade and bidistilled water was used. Tetrahydrofuran (THF) 99% (Lab Scan), high molecular weight (10,000) polyvinyl chloride (PVC) powder (Aldrich), nitrophenyl octyl ether (*o*-NPOE) and dioctylphthalate (DOP) were obtained from

Aldrich, dibutylsebathete and ammonium reineckate (R) were obtained from Sigma. Phosphate buffer pH 4 was prepared [20]. Plasma was supplied by VACSERA (Giza, Egypt).

2.3. Samples

2.3.1. Pure sample

GM was kindly supplied by Hikma Pharma, Cairo, Egypt. Its purity was found to be 99.94 ±1.27 according to the HPLC manufacturer procedure.

2.3.2. Pharmaceutical dosage form

Factive tablets (Hikma Pharma, Cairo, Egypt), batch no. 003. Each tablet claimed to contain GM 426.39 mg equivalent to 320 mg gemifloxacin.

2.4. Prepared solutions

2.4.1. Stock standard solutions

GM stock solution (10^{-2} M) in either water or phosphate buffer pH 4 was prepared by transferring 0.4855 g of GM into two separate 100 ml measuring flasks. Fifty millilitres of either water or phosphate buffer pH 4 were added, shacked or few minutes and completed to volume with the same solvent.

2.4.2. Working standard solutions

GM working solutions (10^{-6}-10^{-3} M) were prepared by suitable dilution from its stock solution using either water or phosphate buffer pH 4.

2.4.3. Laboratory-prepared mixtures

2.5 ml GM from its stock solution (10^{-2} M) was transferred accurately to a series of 25 ml measuring flasks. Aliquots from GM acid degradate (10^{-2} M) solution were added to prepare mixtures containing 10-80% degradation product.

2.5. Procedures

2.5.1. Precipitation-based technique for the preparation of PVC- membrane sensor (sensors 1–3)

Ten milliliters of 10^{-2} M GM aqueous solution was mixed with 10 ml of a saturated aqueous solution of ammonium reineckate. The resulting precipitate was filtered, washed with cold water, allowed to dry at room temperature and grounded to fine powder.

In three glass Petri dishes (5 cm diameter), 10 mg of the previously prepared ion association complex were mixed thoroughly with 0.35 ml of either dibutylsebathete (sensor 1) or dioctylphthalate (sensor 2) or nitrophenyl octyl ether (sensor 3) then add 0.19 g of poly vinyl chloride (PVC). These mixtures were dissolved in 5ml tetrahydrofuran (THF). The

dishes were covered with a filter paper and left to stand overnight to allow slow evaporation of the solvent at room temperature forming master membrane with 0.1mm thickness [21].

Sensors were assembled using a disk of an appropriate diameter (about 8.0 mm) were cut from the previously prepared master membranes and cemented to the flat end of PVC tubing with THF. The electrodes were then filled with an internal solution of equal volumes of 10^{-2} M GM and 10^{-2} M KCl. Ag/AgCl coated wire (3mm diameter) was employed as an internal reference electrode. The sensors were conditioned by soaking for 24 h in a solution of 10^{-2} M of drug and stored in the same solution when not in use.

2.5.2. Sensors calibration

The conditioned sensors were calibrated by separately transferring 50 mL aliquots of solutions (10^{-6} to 10^{-2} M) of GM into a series of 100-mL beakers. The membrane sensors, in conjunction with single junction calomel reference electrode, were immersed in the above test solutions and allowed to equilibrate while stirring. The potential was recorded after stabilizing to ±1 mV, and the electromotive force was plotted as a function of the negative logarithm of GM concentration.

2.5.3. Effect of pH

The effect of pH on the potential values of the three electrode systems was studied over pH range of 1–12 at 1-pH interval by immersing electrodes in 10^{-3} and 10^{-4} M GM solutions. The pH was gradually increased or decreased by adding aliquots of diluted sodium hydroxide or hydrochloric acid solutions, respectively. The potential obtained at each pH was recorded.

2.5.4. Sensors selectivity

The potentiometric selectivity coefficients ($K^{pot}_{GM,\ I}$) of the proposed sensors towards different substances were determined by a separate solution method using the following equation [21]:

$$-\log (K^{pot}_{A.B}) = \frac{E_1 - E_2}{2.303\ RT/Z_AF} + \left[1 - \frac{Z_A}{Z_B}\right] \log a_A$$

where $K^{pot}_{A.B}$ is the potentiometric selectivity coefficient, E1 is the potential measured in 10^{-3} M GM solution, E_2 is the potential measured in 10^{-3} M interferent solution, Z_A and Z_B are the charges of GM and interfering ion, respectively, aA is the activity of the drug and $2.303RT/Z_AF$ represents the slope of the investigated sensors (mV/concentration decade).

2.5.5. Determination of GM in the presence of its acid degradate

A degraded sample of GM was prepared as follows: 100.00 mg amount of intact GF was refluxed for 7 hours with 50 ml 2 N HCl. The resulting solution was tested for complete degradation by the thin layer chromatography technique using chloroform: methanol: toluene: diethylamine: water (33.6:33.6:16.8:10.8:6, by volume) as a mobile phase and detecting the

spots at 254 nm. The solution was cooled, neutralized using 2 N NaOH and transferred into 100 ml volumetric flask. The volume was completed to the mark by distilled water. Aliquots of standard drug solution (10^{-3} M) were mixed with its degraded sample (10^{-3} M) in different ratios. The membrane sensor was immersed in conjunction with the single junction calomel reference electrode in the different laboratory prepared mixtures. The membrane sensor was washed with water between measurements. The e.m.f. produced for each mixture was measured by the three proposed electrodes then the concentration of GM was determined from the corresponding regression equation.

2.5.6. Determination of GM in pharmaceutical preparation

A portion of Factive ® tablets powder equivalent to 0.0485 g GM was transferred into 50 mL volumetric flasks and filled to the mark with phosphate buffer solution pH 4. The concentration of the prepared samples was 10^{-3} M. The potentiometric measurements were performed using the proposed sensors in conjunction with single junction calomel reference electrode, and the potential readings were compared to the calibration plots.

2.5.7. Determination of GM in plasma

4.5 ml of plasma were placed into 2 stoppard shaking tube, then 0.5 ml of 10^{-2} and 10^{-3} M GM were added separately and shacked. The membrane sensor was immersed in conjunction with the single junction calomel reference electrode in these solutions. The membrane sensor was washed with water between measurements. The e.m.f. produced for each solution was measured by the three proposed electrodes then the concentration of GM was determined from the corresponding regression equations.

3. RESULTS AND DISCUSSION

The development and application of ion-selective electrodes (ISEs) continue to be of interest for pharmaceutical analysis because these sensors offer the advantages of simple design and operation, fast response, reasonable selectivity, low detection limit, high accuracy, wide concentration range applicability to colored and turbid solutions, and possible interfacing with automated and computerized systems [22].

GM reacted with ammonium reineckate to form stable 1:3 water insoluble ion association complex, with low solubility product and suitable grain size precipitate.

This ratio was confirmed by the Nernstian response of the suggested sensors which was about 20 mV; the typical value for trivalent drugs [21]. Deviations from the ideal Nernstian slop (20 mv) stems from the fact that the electrode responds to the activity of the drug rather than its concentration.

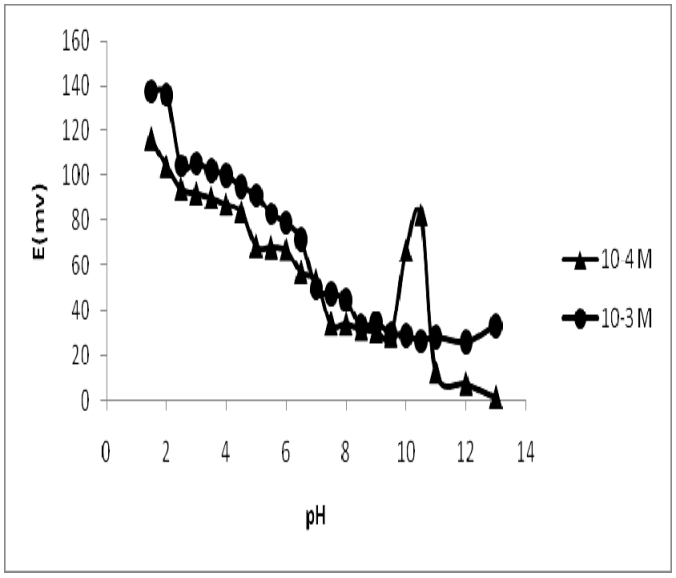

Fig. 2. Effect of pH on the response of sensor 1

The introduction of high molecular PVC, as regular support matrix and traps for the sensed ions, creates a need for a plasticizer [23]. In the present investigation, dibutylsebathete and dioctylphthalate were chosen from diesters of dicarboxylic acids and nitrophenyl octyl ether was chosen as an example of the nitroaromatic group. They gave responses similar to each other with no noise in the same calibration range from 10^{-5}-10^{-2} M. With PVC, they plasticize the membrane, dissolve the ion association complex, and adjust both permittivity 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.

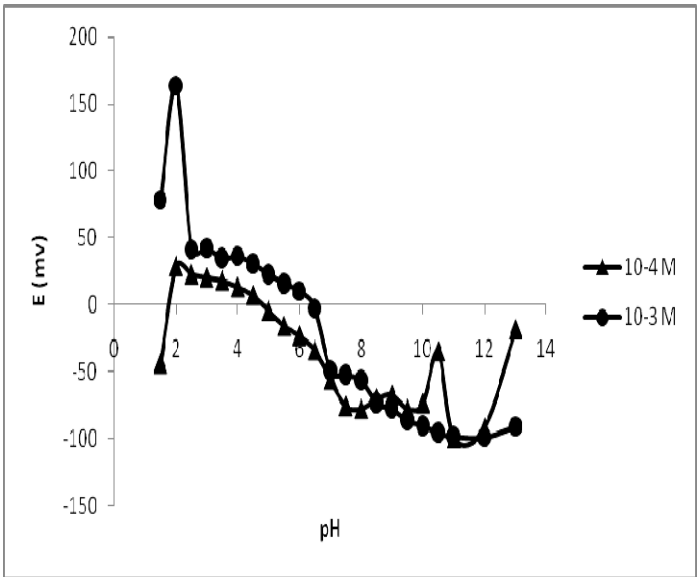

Fig. 3. Effect of pH on the response of sensor 2

The response time of the electrodes were tested for concentrations of the drug from 10^{-6}–10^{-2} M. The measurements was characterized by a fast stable response within 20-30 seconds for concentrations less than 10^{-4} M and 10-20 seconds for concentrations more than 10^{-4} M.

The effect of pH on the electrode potential was investigated and it was found that the electrodes gave a stable potential over a pH range from 2-5 for all sensors, Fig. (2-4). Above and below this pH range, the potentials displayed by the electrodes were noisy.

The potentiometric response of the three studied electrodes at the optimum pH was linear with constant slopes over a drug concentration range 10^{-5}–10^{-2} M for all sensors, Fig. 5.

The accuracy of the proposed membrane sensors for the quantification of blind samples of GM was assessed by using the three sensors. The results showed average recoveries of 99.40±1.02, 100.05±1.09 and 99.93±1.13, respectively.

Table 1. Electrochemical response characteristics of the three investigated GM sensors

Parameter	Sensor 1	Sensor 2	Sensor 3
Slope (mV/decade)[a]	14.30	20.44	22.37
Intercept (mV)	77.00	122.10	176.52
LOD (mol L^{-1})[b]	1.1×10^{-6}	2.8×10^{-6}	3.4×10^{-6}
Response time (s)	20	20	20
Working pH range	2–5	2–5	2–5
Concentration Range (mol L^{-1})	10^{-5} to 10^{-2}	10^{-5} to 10^{-2}	10^{-5} to 10^{-2}
Stability (days)	35	40	40
Average recovery (%) ±S.D.[a]	99.40±1.02	100.05±1.09	99.93±1.13
Correlation coefficient	0.9999	0.9995	0.9995

[a] Average of five determinations

[b] Limit of detection (measured by interception of the extrapolated arms of Fig. 5)

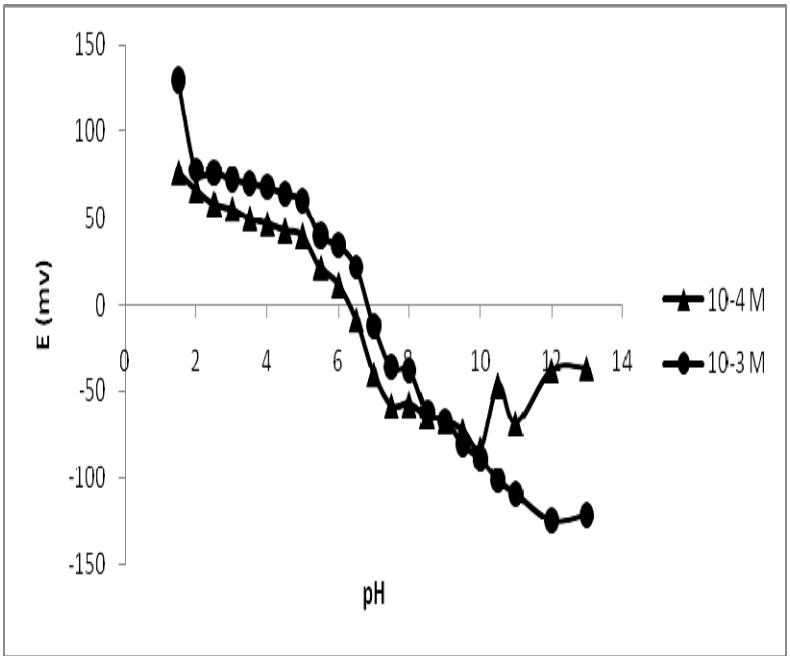

Fig. 4. Effect of pH on the response of sensor 3

Table 2. Potentiometric selectivity coefficients ($K^{pot}_{GM, I}$) of the three proposed sensors using the separate solutions method (SSM) [21]

Interfering substance	**Selectivity coefficient**		
	Sensor 1	**Sensor 2**	**Sensor 3**
Degradates	0.79×10^{-3}	0.75×10^{-3}	0.53×10^{-3}
Na^+	0.86×10^{-3}	0.48×10^{-3}	0.24×10^{-3}
K^+	0.94×10^{-3}	0.67×10^{-3}	0.34×10^{-3}
NH_4^+	0.96×10^{-3}	0.66×10^{-3}	0.43×10^{-3}
Ca^{2+}	1.01×10^{-3}	0.69×10^{-3}	0.58×10^{-3}
Mg^{2+}	1.01×10^{-3}	0.73×10^{-3}	0.57×10^{-3}
Cellulose	0.99×10^{-3}	0.68×10^{-3}	0.49×10^{-3}
Copovidone	1.05×10^{-3}	0.78×10^{-3}	0.51×10^{-3}

Table 3. Determination of GM in Factive tablet by the proposed electrodes

Dosage form	(%) Recovery S.D.[a] of GM±		
	Sensor 1	Sensor 2	Sensor 3
Factive tablet (batch no. 003)	97.67 ±0.66	98.44 ±0.82	98.13 ±0.31

[a] Average of five determinations

Table 2 shows the potentiometric selectivity coefficients of the proposed sensors in the presence of degradates and some other inorganic cations (K^+, Na^+, NH_4^+, Mg^{++}, and Ca^{++}) that are usually found in biological fluids.

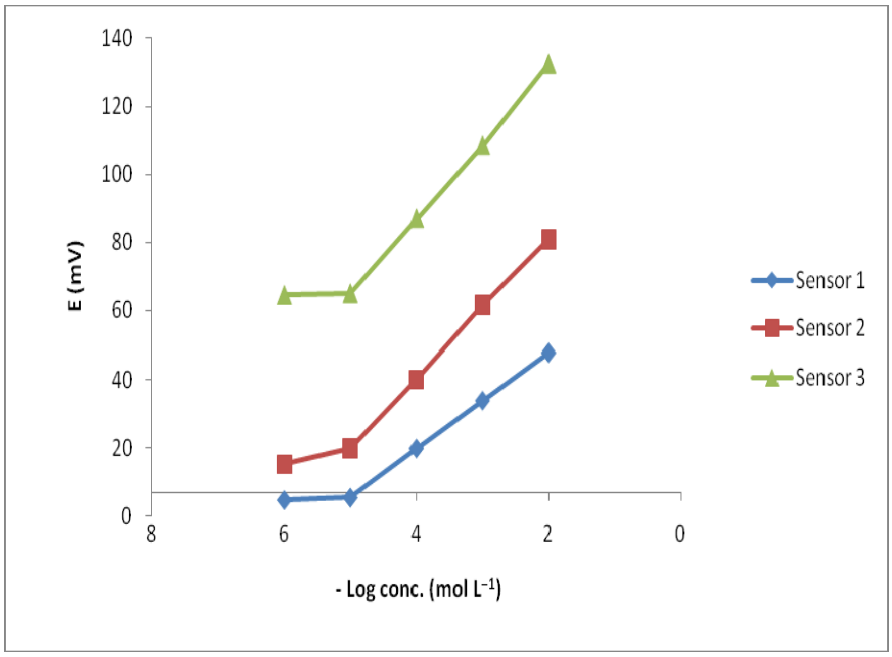

Fig. 5. Profile of the potential in mV *vs.* −log concentrations of GM in mol L^{-1} obtained with sensors 1, 2 and 3

Table 4. Determination of GM in laboratory prepared mixtures containing different ratios of GM and its acidic degradation products by the proposed sensors

Ratio (%) drug:degradate	Drug recovery (%) ±S.D.[a]		
	Sensor 1	Sensor 2	Sensor 3
100:0	100.02±0.33	100.05±1.09	99.93±1.13
90:10	94.34±0.31	103.61±0.93	96.88±0.95
70:30	94.55±0.21	103.57±0.89	95.31±0.52
50:50	96.55±0.49	101.78±0.92	97.93±0.39
30:70	103.95±0.73	101.25±0.65	96.67±0.93

[a] Average of three determinations

Pharmaceutical additives such as Cellulose and Copovidone did not show any interference. Thus, analysis was carried out without prior treatment or extraction. The three sensors were successfully used for the determination of GM in Factive tablets, Table 3.

Table 4 shows the results obtained upon analysis of synthetic mixtures containing different ratios of intact drug and degraded sample varying from 100:0 to 30:70, intact GM: degradates, respectively. The results show that the sensors can be successfully used for selective determination of intact drug in the presence of up to 70% of its degradates. Thus, these sensors are recommended for use in stability-indicating methods.

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

Table 5. Determination of GM in spiked human plasma by the proposed sensors

Concentration [M]	Recovery (%) ± S.D.[a]		
	Sensor 1	Sensor 2	Sensor 3
1×10^{-3}	102.83±1.13	103.96±1.51	99.91±1.40
1×10^{-4}	102.79±1.75	96.19±1.12	101.99±1.86

[a] Average of three determinations

Table 6. Statistical analysis of the results obtained by the proposed method and the reported method [13] for the analysis of GM in pure powder form

Item	Sensor 1	Sensor 2	Sensor 3	Reported method [13]
Mean	99.40	100.05	99.93	99.41
R.S.D.	1.02	1.09	1.13	1.74
N	5	5	5	6
Variance	1.040	1.188	1.277	3.00
T test (1.833)[a]	0.011	0.713	0.575	--------
F test (6.26)[a]	2.885	2.525	2.347	--------

[a]The values between parenthesis are the corresponding theoretical values of t and F at the 95 % confidence level

Statistical evaluation of the results of analysis of pure GM by the proposed electrodes and the reported method [13] showed that there is no significant difference between the proposed and reported method in terms of accuracy and precision, Table 6.

4. CONCLUSION

As a conclusion, the use of the proposed sensors offers the advantages of fast response, elimination of drug pretreatment or separation steps, low detection limit and direct determination of drugs in turbid and colored solutions. They can therefore be used for routine analysis of the GM in quality control laboratories.

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