Fabrication and evaluation of potentiometric sensors of an anticancer drug (Gemcitabine)

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ABSTRACT

Accurate, rapid and inexpensive determination of gemcitabine, an anticancer drug, is of high interest. This manuscript describes the use of potentiometric sensors as a basis for this work given their known attractive characteristics that meet our needs. Potentiometric sensors were comprised of carbon paste S1, coated wire S2 and PVC membrane S3 of gemcitabine (an anticancer drug) were fabricated, studied and evaluated. The calibration plots for these electrodes showed a Nernstian slope of 58.4±0.3, 59.5±0.3 and 58.3±0.3 mV per decade with the limit of detection: 6.50×10⁻⁵, 7.20×10⁻⁵ and 4.60×10⁻⁵ for sensors S 1, S 2 and S 3, respectively. The electrodes have a short and stable response time of ~5 seconds and good reproducibility in a pH range of 2.5-9.5. The present sensors show distinct selectivity toward the drug ion in comparison to several inorganic ions, sugars, amino acids and some common drug excipients. Gemcitabine was determined successfully in ampoules and urine using these sensors by the calibration curve method.

1. Introduction

The chemical structure of Gemcitabine (GEM) is given in Figure 1. Chemical name of its hydrochloride salt is 4-amino-1-[(2R, 4R, 5R)-3, 3-difluoro-4-hydroxy-5-(hydroxymethyl)-oxolan-2-yl]pyrimidin-2-one mono hydrochloride. Gemcitabine is a chemotherapy medication administered by intravenous infusion to treat human malignancies [1] and a number of types of cancer including advanced and metastatic pancreatic [2-4], breast cancer [5], ovarian cancer [6], non-small cell lung cancer [7] and bladder cancer [8,9].

Several analytical techniques have been used to evaluate gemcitabine in pharmaceutical products including HPLC [10,11], HPLC-MS [12], LC-MS/MS [13,14], UV spectrophotometric methods [15-17] and some electrochemical methods [18-21]. However, these techniques are generally complicated, time-consuming, expensive, and require relatively complex processes of specimen treatment. Ion selective electrode (ISE) techniques have been widely used for the analysis of pharmaceuticals as they are highly sensitive, simple, easily miniaturized, inexpensive, and require relatively short analysis time [22].

Figure 1. Chemical structure of gemcitabine hydrochloride.
showed that an increased concentration of the primary ion in the inner solution leads to its extraction from them together with its counter ions forming ion fluxes from the membrane to the sample [23,24]. This process changes the ion activity at the phase boundary thus significantly worsening the detection limit. One strategy to counteract this behavior is elimination of the inner solution by using a solid inner contact. In solid-contact SC-ISEs, the sensing membrane is sandwiched between the sample solution and a SC-ISEs. In recent years, therefore, research has intensified to develop solid contact electrodes with low LODs [25]. CPEs are also known to overcome most of the problems encountered in liquid membrane electrodes where transport into or out of the inner filling solution incur changes in the membrane response [26]. In addition, they have favorable characteristics such as miniaturized size, lower detection limit, simple design, flexible use and low cost. Moreover, reducing the amount of work by providing fresh paste in electrode renewal paid for in improving the analytical results. These favorable characteristics attracted attention and paved the way for their expanding use in a variety of sensing and detection applications [27]. To shed light on these concepts we have designed three new CP, CW, and PVC electrodes for GEM analysis and made a comparative study on their detection limits, concentration ranges and the effect of the internal solution on the results. To the best of our knowledge, no CP, CW or PVC electrodes for the detection of GEM have been reported to date.

2. Experimental

2.1. Reagents

All reagents used were analytical grade. Distilled water was used throughout experiments. Gencitabine hydrochloride was purchased from Mylan USA, NEON India and TEVA Israel. Graphite powder (G, 99.9%, <45 μm), poly vinyl chloride (PVC) of high relative molecular weight and tetrahydrofuran (THF), as well as the plasticizers, diocetyl phthalate (DOP), dibutyl phthalate (DBP), tris(2-ethylhexyl)phosphate (DOPh), diocetyl sebacate (DOS), and paraffin oil as well as metal salts were purchased from Sigma-Aldrich. Phosphotungstic acid (PTA) H3[PW12O40], sodium tetraphenylborate (STPB) Na[C24H20B], the salts of the following cations were prepared: Na+, K+, Mg2+ and Ca2+ were obtained from Sigma- Aldrich. In addition, the sample [23,24]. This process changes the ion activity at the

2.2. Equipment

Potentiometric automatic titrator AT-400 was used for potential measurements, pH measured were made on a digital pH meter (TOA Electronics HM-60V). CHNS Elemental Analyzer (VARIO EL III Germany) was used for analysis of GEM-PT ion pair, and saturated calomel electrode from Sigma-Aldrich were used for potential measurements in cell assemblies: Hg, HgCl2(s), KCl (sat) as assemblies: Hg, Hg2Cl2(s), KCl (sat)

2.3. Preparation of ion-exchanger complex

The ion-exchangers, GEM-PT were made by mixing 10 mL of 0.01 M of GEM and 10 mL of 0.0033 M of PTA, according to a previously reported method [20]. Instant reactions took place that produced sparingly soluble material that precipitated from the reaction mixture. These were collected, washed, dried and characterized by CHNS Elemental Analyzer that gave the following results: Anal. calcld. for C27H36N9F6O52W12P: C, 88.4; H, 0.99; N, 3.44. Found: C, 8.89; H, 0.98; N, 3.46%.

2.4. Preparation of carbon paste electrode

The desired amounts of charcoal, the selected plasticizer and the ion-pair making a total of 1.0 g were intimately mixed in a petri dish to make a homogenous paste which was packed at the end of a small syringe cut at one end. Electrical contact was secured by a stainless-steel screw which was moved down to squeeze the paste against a smooth paper to ensure smoothness of the surface. The electrode was repeatedly used for potential determination until its deterioration indicating saturation of the surface layer which is skimmed off to replenish the electrode activity.

2.5. Preparation of PVC membrane electrode

PVC-membranes were prepared as previously described elsewhere [29,30]. The membranes were prepared by dissolving optimized amounts of PVC, different plasticizers and ion-exchangers in 10 mL of tetrahydrofuran. The mixture was shaken vigorously and the clear solution was poured into a glass dish 7 cm in diameter. The solvent was allowed to evaporate overnight leaving a homogeneous flexible and transparent membrane. Small disks (10 mm) were punched from the cast films and mounted on home-made electrode bodies. The electrodes were filled with the internal filling solution (0.01 M KCl and 0.001 GEM) and preconditioned by soaking for 30 min in 0.001 M GEM solution.

2.6. Preparation of Coated-Wire Electrodes

The coated-wire electrodes (CWEs) were prepared according to a previously reported method [30]. Certain amounts of PVC, the ion-exchanger and the selected plasticizer were dissolved in about 10 mL of THF. A silver, copper, and graphite wires about 1 mm diameter and 50 mm length were first polished on a cloth pad and washed with acetone. One end of the wire was then coated by repeated dipping into the membrane solution in THF. A membrane was formed on the wire surface and was allowed to dry overnight. The prepared electrodes were finally conditioned by soaking for 30 min in 0.001 M of GEM solution.

2.7. Construction of calibration curves

Solutions having concentrations 2.0×10−7-1.0×1.0−2 M were made and used to investigate performance of the electrodes with continuous stirring by measuring the potential and plotting as a logarithmic function of GEM ion activities. 

2.8. Effect of interfering ions

The selectivity of the electrodes was explored by the separate solution method (SSM) and the modified separate solution method (MSSM). Basically, SSM measures the potential of a prospected interferent and the drug one at a time to obtain the net effect of each species [31,32]. The following equation,

\[
\log K_{D}^\text{pot} = \frac{E_J - E_D}{S} + (1 - \frac{Z_J}{Z_D}) \log a_D
\]

where EJ and ED are the measured potentials of the interfering ion and GEM, respectively and S is the slope of the calibration
graph. $z_S$ and $z_J$ are the charge of GEM and interfering species respectively, and $E_D$ is the activity of the GEM. In addition, MSSM involves construction of the calibration curve of every selected ion as well as that of the drug ion separately. By extrapolation to 0, it is possible to get the selectivity coefficient from this [33,34].

$$\log K_{D,J}^{pot} = \frac{E_J - E_D}{S_D}$$

This approach eliminates any effect caused by the ionic charge.

### 2.9. Effect of temperature on the electrode potential

To study the thermal stability of the electrodes, calibration curves were constructed at different test solution-temperatures at 15, 25, 35, and 45 °C. The slope, linear concentration range and limit of detection of the electrodes were determined at each temperature.

### 2.10. Effect of $pH$ on the electrode potential

The effect of $pH$ of the test solution on the potential values of the electrode system in solutions of different concentrations (1.0×10⁻⁴ and 1.0×10⁻⁵ M) of GEM. Aliquots of the GEM were transferred to 100 mL titration cell and the tested ISE in conjunction with the SCE, and a combined glass electrode were immersed in the same solution. The $pH$ of the solution was varied over the range of 1.0-11.0 by addition of very small volumes of (0.1 or 1.0 M) HCl and/or NaOH solution. The $mV$-readings were plotted against the $pH$-values for the different concentrations.

### 2.11. Direct potentiometric method

In the calibration curve method, different amounts of GEM were added to 50 mL of water comprising a concentration range from 1.0×10⁻⁵ to 1.0×10⁻³ M and the measured potential was recorded using the present electrodes. Data were plotted as potential versus logarithm of the GEM activity and the resulting curve was used for subsequent determination of unknown surfactant concentration.

### 2.12. Potentiometric titration

Potentiometric titration of 10 mL of 1.0×10⁻² M GEM solution were transferred to a 25 mL beaker, and titrated with a standard solution of PTA and TPB using the prepared GEM electrodes as indicator electrodes. The end points were determined from the S-shaped curve.

### 2.13. Analysis of GEM in injection solution vials

An equivalent amount of 1.0×10⁻⁴ to 1.0×10⁻⁶ M GEM were prepared from the stock solution (1000 mg GEM in 25 mL) presented in different preparations. The measured potential of each solution was used to calculate the concentration of the solution from the calibration plot constructed early.

### 2.14. Determination of GEM in spiked human urine samples

The concentration of GEM-spiked urine samples obtained by mixing 5.0 mL of urine down to 0.25 mL of urine and diluting with distilled water to 25.0 mL to make 1.0×10⁻⁵ M, 1.0×10⁻⁴ M and 1.0×10⁻³ M GEM solutions that were measured by the calibration curve method. Each analysis was repeated three times and the standard deviation of the results was evaluated.

### 3. Result and discussion

Designing and development of new electrodes to measure various chemical species such as GEM is a prospering area of research. It is rewarding to get new fabricated electrodes with competitive properties. One has to utilize the properties of the composite materials as efficiently as possible to achieve this goal. With these points in mind, we have intimately worked in the design and characterization of these electrodes: CP, CW, and PVC electrodes of GEM then compared their properties in light of these considerations.

#### 3.1. Composition of the electrodes

It is well known that the performance characteristics of IESs based on ion-exchanger depend to a large extent on the nature of these ion-exchanger and their lipophilicities [30], the type of solvent mediator [35] and any additives used [36]. Therefore, the influences of membrane composition, nature and amount of solvent mediator as well as the amount of additives, such as sodium tetraphenylborate, on the potential response of the proposed sensors were tested and the obtained results are given in Table 1.

#### 3.1.1. Optimization of the amount of the ion-pair complex in the paste

The ion-pair is a form of the analyte normally a lipophilic salt and liable to dissociate in the paste phase. It is practically insoluble in the aqueous phase and incorporated in the ion-selective electrode to make it responsive to changes in the concentration of the analyte and developing a corresponding potential difference useful for determination of unknown amounts of the analyte. The ion exchanger incorporated in each electrode presented here was an ion-association complex of the GEM cation with phosphotungstic acid $H_3PW_{12}O_{40}$. This ion exchanger, with high molecular weight anions: 2880 g/mol, has high lipophilicities and stabilities. This ion-exchanger was used as electroactive materials in CP, CW, and PVC electrodes. The ion-pair was prepared by stoichiometric reactions of aqueous solutions of their precursors in 3:1 molar ratio and characterized by CHNS-Elemental Analysis. Electrodes comprising variable amounts of GEM-PT as an ion pair were tested in exploration of the response that is closest to Nernstian behavior towards choosing the most appropriate material and using that as a starting point of building up and characterization of the electrode that provides the best response. Fortunately, electrodes numbered 7, 15 and 24, for CP, CW, and PVC electrodes in Table 1 comprising 2.0, 1.0, and 1.0 % of GEM-PT respectively gave good results and that provided flexibility in devising new electrodes for determination of GEM. Figure 2 shows the measured potential vs. GEM for the present electrodes comprising GEM-PT that clearly indicate excellent behavior. As anticipated, electrodes containing zero ion pair gave poor response, a shortcoming that made reluctance on its further characterization. In addition, electrodes comprising increasing amounts of the ion pairs (>2%) showed inferior response: larger detection limits and smaller slopes likely due to partial dissociation and back diffusion of the drug ion in the paste resulting in a smaller buildup of charge separation across the paste surface.

As the initial step in working towards spotting the best composition of the intended electrode, mixtures comprising selected graphite and plasticizers in ratios of 0.80-1.20 were tested to find the required ratio of the components of the electrode for this purpose. The electrodes comprising a mixture with g/p = 1.00 as shown in Table 1 produced the most appropriate response with the lowest detection limit and the closest Nernstian slope.
These electrodes are workable, however, electrodes comprising pastes with larger ratios are brittle and those with smaller ratios are butter-like and not workable.

3.1.2. The influence of anionic additives

Additives such as lipophilic anions reduce ohmic resistance and improve response behavior and selectivity in cation-selective electrodes. In addition, they enhance the selectivity of the membrane electrode in cases where the extraction capability of the ion-exchanger is poor. Furthermore, the lipophilic additive may catalyze the exchange kinetics at the sample-electrode interface [37]. Comparison of the data for CP, CW, and PVC electrodes in Table 1 revealed that the sensitivity of the sensor increased and the slope of the calibration curve increased from 46.6 to 58.4 mV/decade with the addition of 0.2 wt% of STPB in CP, 56.2 to 59.5 mV/decade with the addition of 0.2 wt% of STPB in CW, and 54.7 to 58.3 mV/decade with the addition of 0.1 wt% of STPB in PVC electrode. Clearly, this additive contributed significantly to the dielectric constant of the membrane in addition to the effect of the plasticizer.

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3.1.3. Solvent mediators (plasticizers) effect

The solvent mediator, in particular, has a dual function: it acts as a liquefying agent, making the membrane material workable, that is enabling homogenous solubilization and modifying the distribution constant of the ion-exchanger used and sustaining these characteristics on continued use. The proportion of solvent mediator must be optimized in order to minimize the electrical asymmetry of the membrane in order to keep the sensor as clean as possible and to stop leaching to the aqueous phase [38]. For a plasticizer to be adequate for use in sensors, it should gather certain properties and characteristics such as having high lipophilicity, high molecular weight, low tendency for exudation from the membrane matrix, low vapor pressure and high capacity to dissolve the substrate and other additives present in the membrane [39].

To spot a suitable plasticizer for constructing this electrode, we tested four plasticizers, with a range of characteristics, namely: the values of dielectric constants, lipophilicity and molecular weight respectively are in parentheses, DBP (ε = 6.4, \( \log P_{LTC} = 4.5, \text{M.Wt} = 278 \)), DOP (ε = 5.1, \( \log P_{LTC} = 7.1, \text{M.Wt} = 390 \)), DOS (ε = 4.2, \( \log P_{LTC} = 10.1 \)), and DOPh (ε = 4.8, \( \log P_{LTC} = 10.2, \text{M.Wt} = 435 \)). DOPh as solvent mediators produced the best results for CP, CW, and PVC electrodes, as shown in Table 1. It is not clear why these mediators were the best among those used, but one can say that the outcome of their properties was the most effective on the electrode response. It is likely that increasing the lipophilicity of the ion-exchanger increases its solubility in the membrane and the electrode potential as well. However, the higher the molecular weight of the plasticizer the less soluble the ion-exchanger in the membrane. We are in front of a situation where two opposing factors are in effect and what we see is the outcome of both of these factors that produce similar effect on utilizing these plasticizers.

Among the different compositions studied, the electrode containing 2.0 wt% GEM-PT, 48.9 wt% graphite, 48.9 wt% DOPh, and 0.2 wt% STPB for CPE, 1.0 wt% GEM-PT, 49.4 wt% PVC, 49.4 wt% DOPh, and 0.2 wt% STPB for CW electrode, and 1.0 wt% GEM-PT, 49.4 wt% PVC, 49.4 wt% DOPh, and 0.2 wt% STPB for PVC electrode exhibited the best compromised characteristics: the slope closest to Nerstian value and the corresponding detection limit.

Therefore, these compositions were used to study various operation parameters of the electrodes. The electrochemical performance characteristics of these electrodes were systematically evaluated according to the International Union of Pure and Applied Chemistry (IUPAC) recommendations [40]. The potentiometric responses of the electrodes were examined in the concentration range from 2.0×10^{-7} M to 1.0×10^{-2} M GEM solutions. The calibration plots for these electrodes, represented in Figure 2, show linearity over the concentration range 6.80×10^{-5} - 1.00×10^{-2} M, 7.50×10^{-5} - 1.00×10^{-2} M, and 4.80×10^{-5} - 1.00×10^{-2} M and the limits of detection were 6.50×10^{-5}, 7.20×10^{-5}, and 4.60×10^{-5} M for CP, CW, and PVC electrodes, respectively. Comparison of the slopes, linear ranges and detection limits of the electrodes is given in Table 1. The results revealed that characteristics of solid contact electrode surpass the PVC electrode due to elimination of the internal solution.

3.2. Effect of electrode bed

To investigate the effect of the bed nature on the efficiency of coated wire electrodes, the optimized coating mixture was used for preparation of electrodes with different conductive beds, namely silver, copper, and graphite. After conditioning, each electrode was examined in the concentration range from 2.0×10^{-7} to 1.0×10^{-2} M GEM solution. Examining the results compiled in Table 1, one can notice that all wires give inferior response towards GEM as compared to that of silver wire. Ag wire-coated electrode has a slope 59.5 mV/decade and a detection limit of 7.50×10^{-5} M. This is attributed to low resistivity (1.62 μΩ·m) of silver [30]. Therefore, silver wire was used as the inner solid contact for the electrodes in this study.

3.3. Effect of diverse ions

As its name indicates an ion-selective electrode is designed such that it selectively senses and measures the amount of a specified ion in a test solution, so its selectivity is crucial for proper function to the intended goal. Selectivity of an ion-selective electrode quantitatively depends on the equilibria at the sample-electrode interface. Logically, they also depend on the structure and composition of the ion-pair. Other ions present in solution affect the response of the electrode according the simplified Nicolsky-Eisenman equation [41].

Selectivity coefficients values range from zero, that indicates no interference, to greater than unity, when the electrode responds to the interfering ion is greater than that to the primary ion. Eisenman equation for calculation of selectivity coefficients holds for ions of the same charge but gives erroneous results for ions with different charges. Therefore, one must adopt an alternative approach to measure selectivity of ions with different charge such as the separate solution method described by which was employed to assess the selectivity of other species likely present in preparations of this drug [33]. The electrodes were tested in presence of substances administered with GEM as cancer treatment protocols, organic ions and some electrolytes as well as excipients commonly encountered in pharmaceutical preparations. Measurements listed in Table 2 shows that the present sensors display high selectivity for GEM over common drugs and other species as a consequence of the similarity in composition of the electrode constituent’s and the drug ion in the analyte and amounting to better compatibility and enhanced response on one hand but the interferents marginally affect the electrodes for diminished similarity between them on the other.

Organic cations and electrolytes do not interfere as they are normally small in size with high charge to size ratio. In contrary, GEM is more bulk and thus the differences in ionic size, permeability and mobility of the GEM over these cations supports the response of the electrode. Notably, the MSSM produced better results than the SSM for the first gives what is considered unbiased and thermodynamic selectivity coefficients.

In the results collected in Table 2 for SSM method, some compounds such as sugars, amino acids and some drugs were tested for interference but showed no effect on the measured potential. No data was listed in the relevant positions. This is reasonable as these materials are not ionic and consequently do not interact with ionic materials present in solution.

3.4. The effect of pH on the response of the electrodes

The influence of the pH of the solution on the response of the proposed electrodes was studied for from 1.0×10^{-3} and 1.0×10^{-5} M GEM ion in the pH range of 2.0-11.0. The pH was adjusted with 0.1 mol/L solutions of hydrochloric acid or sodium hydroxide. It can be seen from Figure 3 that the variation in potential is acceptable in the pH range 3.1-9.5 for CPE, from 2.5 to 9.5 for CWE, and from 3.2 to 9.0 for PVC electrode.

Nevertheless, CPE at pH < 3.1, CWE at pH < 2.5, and PVC electrode at pH < 3.2 showed a nonlinear response with slight increase in the potential adjusted with 0.1 mol/L. This is likely due to the effect of the increase in the hydronium ion concentration on the electrode behavior.
with PT− ions which are anions of the polyprotic acid (that contain a proton or more). Thus, the equilibrium is disrupted and shifted to the right with the effect of gradual decrease of the ion-exchanger and a decrease in the concentration of the active ingredient of the sensor. This explanation is similar to the ion-exchanger and that proposed recently [42].

### 3.5. Response time

Response time is the time elapsed between the addition of a given amount of the analyte and the time when a stable potential response is attained by the electrode as over several 10-fold concentration increments. It is considered an important characteristic of the electrode for short response time is an attractive property. Response time is normally measured over concentration increments from 1.0×10−4 M to 1.0×10−2 M and was 5-7 seconds which is notably short and indicates a good quality of the present electrodes. It most likely stems from fast exchange kinetics between drug and the ion-pair at the electrode surface.

At high pH the OH ions penetrate the membrane and react with PT− ions which are anions of the polyprotic acid (that contain a proton or more). Thus, the equilibrium is disrupted and shifted to the right with the effect of gradual decrease of the ion-exchanger and a decrease in the concentration of the active ingredient of the sensor. This explanation is similar to that proposed recently [42].

### 3.6. Effect of temperature

To examine its reversibility, the electrode potential was measured alternately in solutions containing 1.0×10−4 M and 1.0×10−5 M as shown in Figure 4 which clearly shows that equilibrium is reached in a notably short time (5-7 s). However, there is a slight decrease of the measured potential with time apparently due to memory effect and partial saturation of the surface of the electrode.

### Table 2. Selectivity coefficients of various suggested interferent on S1, S2, S3

| Interfering species          | S1     | S2     | S3     |
|------------------------------|--------|--------|--------|
| Na+                          | -3.02  | -4.36  | -5.08  |
| K+                           | -3.08  | -4.47  | -5.26  |
| Mg2+                         | -1.86  | -2.47  | -2.48  |
| Ca2+                         | -2.03  | -2.38  | -2.46  |
| Glucose                      | -4.53  | -4.49  | -4.54  |
| Glucose                     | -4.19  | -4.42  | -4.89  |
| Fructose                     | -4.19  | -4.42  | -4.89  |
| Sucrose                      | -4.19  | -4.42  | -4.89  |
| Maltose                      | -4.17  | -4.64  | -4.27  |
| Lactose                      | -4.16  | -4.78  | -3.95  |
| Dextrose                     | -4.21  | -4.58  | -3.87  |
| Glycine                      | -4.24  | -4.74  | -4.31  |
| Histidine                    | -3.79  | -4.42  | -3.37  |
| Glutamic acid                | -3.70  | -4.32  | -3.70  |
| Aspartic acid                | -3.47  | -4.42  | -3.62  |
| Chloropheniramine maleate    | -4.37  | -4.59  | -3.99  |
| Acetaminophen (Paracetamol)  | -4.46  | -4.27  | -3.95  |
| Ibuprofen                    | -4.64  | -4.59  | -4.01  |
| Tramadol                     | -4.02  | -4.64  | -3.90  |
| Metoclopramide               | -3.85  | -3.93  | -3.95  |
| Levocetirazine.HCl           | -2.18  | -2.38  | -2.72  |
| Diclofenac potassium         | -3.22  | -5.00  | -2.93  |
| Lidocaine HCl                | -2.33  | -3.05  | -3.15  |
| Batidine HCl                 | -2.39  | -3.15  | -3.42  |
| Tramadole HCl                | -2.36  | -3.65  | -3.35  |
| 5-Fluroakukan                | -4.27  | -3.85  | -3.20  |
| Oxaalplatin                  | -4.32  | -4.32  | -4.27  |
| Doxorubicin HCl              | -2.60  | -2.98  | -3.00  |
| Dacarbazine HCl              | -2.55  | -2.60  | -3.10  |
| Cytrabine HCl                | -2.33  | -2.73  | -2.91  |

To study the thermal stability of the electrodes, calibration graphs were constructed at different test solution temperatures of the test solution covering the range 15-45 °C. The slope, response time, concentration range and the detection limit were obtained from the calibration plot corresponding to each temperature. It is noted that raising temperature from 15 to 25 °C slightly improved the response but further increase to 45 °C slightly deteriorate the response. The results indicate that no appreciable change in the calibration characteristics of the electrodes was observed in the temperature range 15-45 °C.
3.7. Stability, repeatability, surface-renewal and reproducibility of the electrodes

The stability of the response of each electrode was tested by measuring the potential in a 1.0×10⁻⁵ M GEM solution at 5 min intervals for two hours. The relative standard deviation of the measurements, for n = 6 was 1.52% for CPE, 1.50 for CWE, and 1.43 for PVC electrodes, indicates dependable stability of the electrode. The repeatability of the potential measurements for each electrode was examined subsequentially in 1.0×10⁻⁵ M GEM solution immediately after measuring the first set of solutions, for n = 6 was 1.52% for CPE, 1.50 for CWE, and 1.43 for PVC electrodes, indicates dependable stability of the electrodes. To sum up, the present sensors were utilized to determine GEM in pharmaceutical preparations using the standard additions, calibration curve and potentiometric titration methods.

![Figure 4. Dynamic response of the S₁, S₂ and S₃ for 1.0×10⁻⁵ to 1.0×10⁻² M.](image)

3.8. Analytical applications

The designed sensors were utilized to determine GEM in pharmaceutical preparations, obtaining the standard additions, calibration curve and potentiometric titration methods.

3.8.1. The potentiometric titration method

As potentiometric titration is a valuable analytical technique where there is a remarkable change in the concentrations of the reactants and a big shift in the electrode potential over addition of a small measured amount of titrant. In accordance, the present electrodes were successfully applied as an indicator in potentiometric titration of 10.0-mL samples of 0.001M GEM with a 0.001 M solution of PTA & STPB one at a time.

A representative plot using 0.001 M solution of GEM, Figure 5, shows a steep potential jump at the end point indicating completeness of the titration. The added titrant instantly combines with the drug, forming an ion-pair complex, causing gradual depletion in solution and in the corresponding measured potential. To sum up, the present electrodes can be dependently used as indicators to determine the amount of the drug in solutions.
3.8.2. Determination of drug ions in urine and pharmaceutical preparations

Potentiometric measurements with ion-selective electrodes is still one of the most promising analytical tools for drug determination [44]. Therefore, it is important to check the applicability of the present electrodes in determination of GEM in biological samples such as urine and pharmaceutical preparations. The present electrodes were utilized to determine the drug GEM in various ampoules and urine samples. The daily dose is 2000 mg of GEM out of which about 10% (≈ 200 mg) is excreted into the urine [45], making an estimated concentration of 3.33×10⁻⁴ M considering a total volume of urine to be 2L daily and the molar mass of the drug to be 299.66 g/mol. The above-mentioned concentration lies the linear concentration range covered by the present electrodes. Experimentally, it was found that spiking 0.25 mL samples of the urine resulted in about 99% recovery and using larger samples lowered percent recovery due to matrix effects of urine samples. Assessment of these findings, collected in Table 3, indicate accuracy, reproducibility and dependability that are paid for by the fabrication of these electrodes for determination of the drug in urine samples.

GEM ampoule samples from different sources were tested by the calibration curve method. The results were collected and the data, listed in Table 3, indicate that these electrodes can be used dependently in the intended analysis of GEM ampoule drug samples.

3.8.3. Statistical treatment of results

The collected results utilizing the present electrodes were assessed by comparison with those reported spectrophotometrically [46]. The precision was checked using F-test and the accuracy by applying the t-test on the results as shown in Table 3. The calculated F and t-values show no significant difference in precision and accuracy of the results obtained by using the present electrodes with a confidence level above 95%.

4. Comparison with other electrodes

The performance characteristics of some reported methods are better than for the proposed electrodes especially HPLC methods which have detection limits down to 3.67×10⁻⁹ in HPLC-MS. Other methods have higher detection limits larger than this value. However, the present electrodes cover the range normally found in natural samples such as pharmaceutical preparations and urine. In addition, these electrodes provide accurate, fast and dependable means of analysis for this important drug. Moreover, they are compact, cheap tools that merit for the intended analysis that was experimentally proven.

5. Conclusions

Three kinds of potentiometric (CP, CW, and PVC) electrodes were constructed for determination of gemcitabine and a comparison was made between them. The sensors show favorable performance characteristics with short response times (≤5s), low detection limits of 6.90×10⁻⁵, 7.20×10⁻⁵ M, and 4.60×10⁻⁵ M over the concentration range from 6.30×10⁻⁵-1.00×10⁻² M, 6.70×10⁻⁵-1.00×10⁻² M, and 4.40×10⁻⁵-1.00×10⁻² M for CP, CW, and PVC electrodes respectively. Clearly, the coated wire electrode shows a lower detection limit due to its diminished current flux. The sensors were effectively used for determination of GEM in pharmaceutical preparations.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.

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Ethical approval: All ethical guidelines have been adhered.

Sample availability: Samples of the compounds are available from the author.

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