

New Plastic Membrane Sensors for Selective Determination of Pyridine as a Hazardous Pollutant: Validation and Applications to Flow Injection Analysis

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A new validated potentiometric method was described for static and hydrodynamic monitoring of pyridinium ions (Py^+) as an organic pollutant. The method implied the development of a potentiometric sensors responsive to Py^+ based on the use of pyridinium tetraphenyl borate [Py^+][TPB⁻] ion-association complex and/or synthesized β -cyclodextrine (β -CD) polymer as an electro-active material, in a poly(vinyl chloride) matrix membrane plasticized with dioctyl phthalate (DOP). Optimization of the performance characteristics of the sensors was described. Under static mode of operation, the sensors revealed a near Nernstian response over a wide Py^+ concentration range 5.0×10^{-6} and 5.0×10^{-5} to 1.0×10^{-2} mol L⁻¹ with a detection limit of 3.1×10^{-6} and 3.5×10^{-5} mol L⁻¹, respectively. In flow injection potentiometry, excellent reproducibility (RSD % \pm 0.9%), fast response, high sensitivity with a near- Nernstian 67.2 ± 0.8 and 72.8 ± 1.1 mV decade⁻¹, linear range 1.0×10^{-4} - 1.0×10^{-2} mol L⁻¹, detection limit 6.0×10^{-5} and 1.0×10^{-4} mol L⁻¹, high sampling rate (35-40 and 30-35 sample h⁻¹) and stable baseline was observed in the presence of 0.01 mol L⁻¹ acetate solution, pH 3.0 as a carrier for ([Py^+][TPB⁻] and (β -CD) polymer membrane based sensors, respectively. Validation of the assay method is tested by measuring the lower detection limit, range, linearity, trueness, accuracy, precision, selectivity and ruggedness (robustness). The results reveal good potentiometric performance of the proposed sensor for determination of Py^+ in spiked water and soil samples.

Keywords: Potentiometric sensors; PVC membranes; Pyridine monitoring; Flow injection analysis (FIA).

1. INTRODUCTION

Pyridine is an organic, colorless, volatile liquid of disagreeable odor, produced from coal tar or by chemical synthesis [1]. It is widely used as a solvent and intermediate the production of piperidine,

agro-chemicals, drugs, dyestuffs and paints, rubber products, polycarbonate resins and textile water repellents, as well as in laboratories [2]. Trace quantities of pyridine and structurally related pyridine bases may be present in surface water as a result of discharge from coking operations and petroleum processing [3]. It is also present in crude tar distillate along with benzene; xylene, and toluene, etc. [4, 5]. Pyridine is an amphiphilic molecule exhibiting high solubility in water, so it can be considered as a teratogenic compound rated as a priority pollutant by the United States Environmental Protection Agency (USEPA) [6]. The typical concentration of pyridine in wastewater produced by a pharmaceutical plant is reported in the range of 20–300 mg L⁻¹ [7]. Disposal of industrial wastewater to adjoining surface water bodies and/or on to land may pose risk of groundwater aquifers contamination, leading to serious health hazards. The USEPA [8] has prescribed a regulatory level of 5 mg L⁻¹ for pyridine in wastewater for disposal. Therefore, the levels of pyridine in industrial wastewater need to be carefully monitored for their effective control.

Several methods are available in literature for the determination of pyridine in various matrices using spectrophotometry [9-11], kinetic spectrophotometry [12], high-performance liquid chromatography (HPLC) [13], and gas chromatography (GC) [14-16]. Although they provide specific responses, the overall procedure is time-consuming and too expensive for routine analytical measurements. Thus, in view of the increasing manufacture and use of pyridine and subsequent enforcement of the stringent regulations for the control of wastewater disposal containing toxic chemicals, it becomes a priority to develop new, simple, rapid, reliable cost-effective methods for the determination of pyridine in different matrices including water and wastewater. Alternative and advantageous methods should rely on expeditious and efficient procedures providing highly specific and sensitive measurements. Ion-selective sensor's utility and simplicity have replaced for long other wet analytical methods, because they offer high precision and rapidity, low cost of analysis, enhanced selectivity and sensitivity over a wide range of concentrations [17,18].

In This work, new pyridine sensors incorporating ion association of pyridinium tetraphenyl borate [Py⁺][TPB⁻] complex and (β-CD) polymer as electroactive materials dispersed in PVC matrix plasticized with dioctyl phthalate (DOP) as solvent mediator, were introduced. These sensors exhibited significantly high sensitivity, long term stability, and reasonable selectivity for Py⁺ ions over many common organic and inorganic cations. They were also successfully used for accurate determination of Py⁺ contents of spiked water and wastewater samples. Tubular detector was used in a flow injection manifold for continuous assay of pyridinium ions.

2. EXPERIMENTAL

2.1. Equipment

All potentiometric measurements were made at 25±0.1°C with an Orion pH/mV meter (model SA 720) and Py⁺ ion-PVC membrane sensors in conjunction with an Orion Ag/AgCl double junction reference electrode (model 90-02) with 10% (w/v) KNO₃ in the outer compartment. A combination Ross glass pH electrode (Orion 81-02) was used for all pH measurements.

The cell assembly was of the type: KCl (0.1 mol L^{-1})/sample test solution //sensor membrane // internal filling solution/AgCl/Ag internal reference electrode.

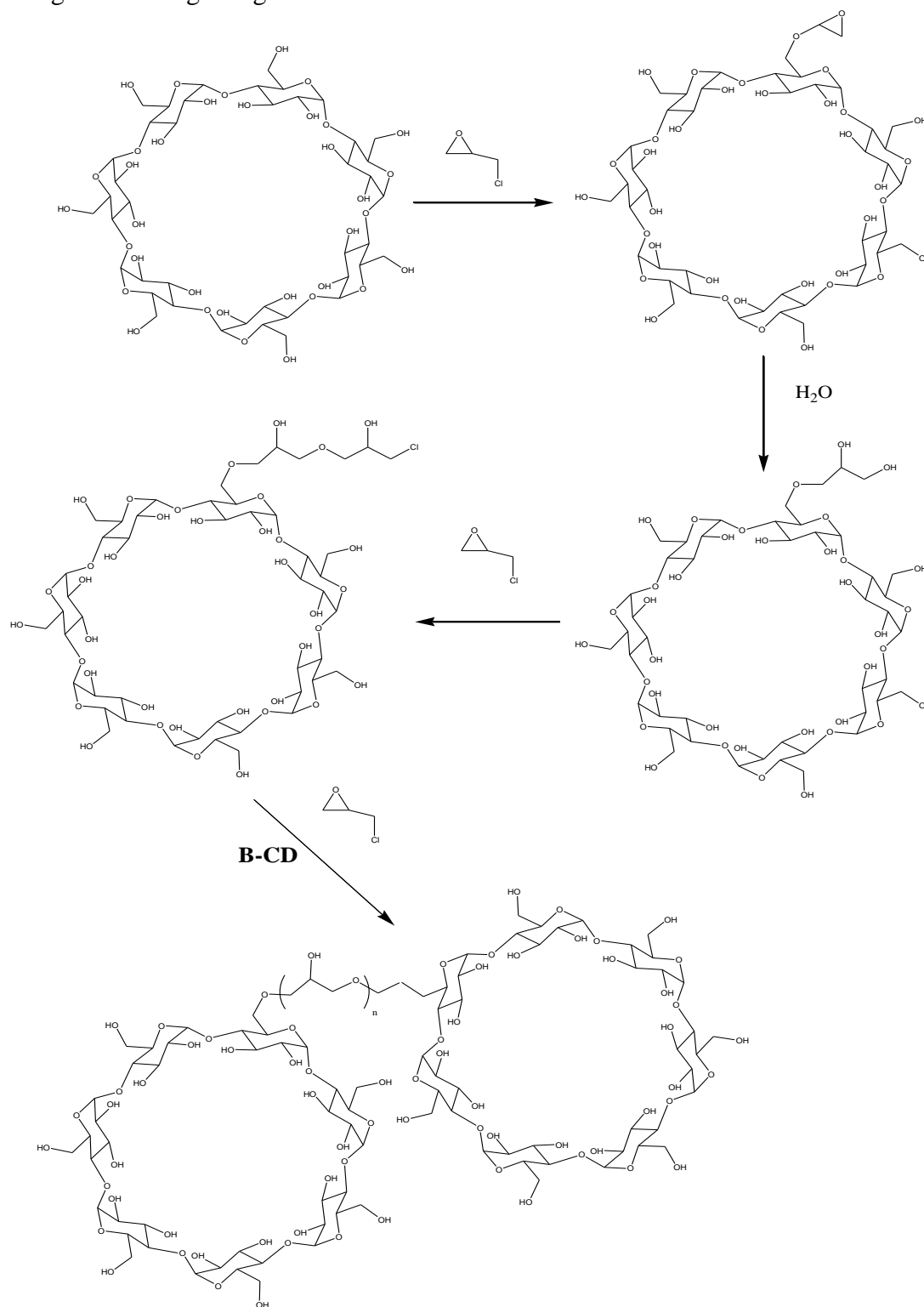


Figure 1. Schematic illustration of reaction between β -CD and EPI

The FIA system represented in Fig. 1 consisted of an Ismatech MS-REGLO pump and an Omnifit injection valve (Omnifit, Cambridge, UK) with sample of $200\ \mu\text{L}$ volume. The potential

measurements were obtained with a high resolution data logger [Pico Technology limited] (model ADC-16). The flow Tygon tubes were obtained from (AIKEM) (P/N A00349 and P/N A000355), the pump tubes were red/red 0.71 MJD and blue/blue 0.065N JD. The distance between the injection valve and the detector was 40 cm. The end of the tube was placed in a Petri dish where a double junction Ag/AgCl reference electrode was placed downstream from the indicator sensor just before the solution went to waste.

2.2. Materials

All chemicals used were of analytical reagent grade and were used without further purification unless otherwise stated. Twice distilled water was used throughout all experiments. Dioctyl phthalate (DOP), β -cyclodextrine (β -CD), epichlorohydrin (EPI), potassium *p*-chloro tetraphenyl borate (*p*Cl-TPB) and poly (vinyl chloride) (PVC) were obtained from Sigma (St. Louis, MO). Pyridine and tetrahydrofuran (THF) were purchased from Aldrich Chemical Company Inc. A 0.01 mol L⁻¹ stock Py⁺ solution was prepared by dissolving 0.31 mL in 100 mL acetate solution (0.01 mol L⁻¹) of pH 3.

2.3. β -CD polymer preparation

A typical procedure for preparing β -CD polymer was described as follows [19]. β -CD (5 g, 0.44 mmol) was mixed with 8 ml NaOH (50%, by mass) solution and mechanically stirred for 20 min till β -CD was dissolved completely. Then, 15 ml EPI was added in dropwise as the mixture was heated gently up to 65 °C. The reaction mixture was polymerized at 65 °C under vigorous stirring (200 r·min⁻¹). After stirring for about 1-2 h, precipitate could be observed, and the viscosity of the solution was also increased. The solution was mixed with 100 ml acetone, and the insoluble polymers were poured into water. The resultant product was filtrated, and further washed with acetone in a Soxhlet extractor for 24 h. After drying in vacuum oven at 80 °C for 12 h, the polymer product was crushed and granulated to 160-250 μ m in diameter. A schematic illustration of reaction between β -CD and EPI is shown in Fig. 1.

2.4. Potentiometric assessment of Pyridine

2.4.1. Pyridinium-tetraphenyl borate ion associate complex

Pyridinium-tetraphenyl borate ion associate complex [Py⁺][TPB⁻] was prepared by mixing 25 mL of 10⁻¹ mol L⁻¹ aqueous pyridinium solution with 25 mL of 10⁻¹ mol L⁻¹ tetraphenyl borate (TPB). The mixture was shaken well; the precipitate was filtered off through G4 sintered glass crucible, washed with de-ionized bi-distilled water, dried at room temperature and grounded to a fine powder. Elemental analysis of the complex gave C 88.32, H 5.61, N 3.52% for the Pyridinium-tetraphenyl borate ion-pair complex [(C₅H₆N) [C₂₄H₂₀B], C 88.55, H 5.34%, N 3.52%).

2.4.2. Pyridine-PVC membrane sensors

The plastic membranes were prepared by mixing 10 mg portion of either $[\text{Py}^+][\text{TPB}^-]$ and/ or ($\beta\text{-CD}$) polymer with 190 mg of PVC, 350 mg of the plasticizer DOP and 4 mL THF in a glass Petri dish (5 cm diameter) covered with a filter paper and left to stand overnight to allow evaporation of the solvent at room temperature. Semi-transparent master PVC membranes of approximately 0.1 mm thick were obtained. A disc (8 mm diameter) was cut using a cork borer and glued to polyethylene tube (3 cm x 8 mm i.d.) which was clipped onto the end of the electrode glass body. The electrode was filled with the internal reference solution consisted of an equal volume of $1 \times 10^{-2} \text{ mol L}^{-1}$ of Py^+ and KCl solutions. An Ag/AgCl internal reference wire electrode (1 mm diameter) was immersed in the internal solutions. Pyridine sensors were finally preconditioned by soaking in $1 \times 10^{-2} \text{ mol L}^{-1}$ pyridinium ions for one day before use and were stored in the same solution when not in use.

The emf of the sensors were measured in buffer solutions obtained by transferring 0.5 mL aliquots of $1.0 \times 10^{-2} - 1.0 \times 10^{-6} \text{ mol L}^{-1}$ Py^+ aqueous solutions to 50 mL beaker containing 10.0 mL of 0.01 mol L^{-1} acetate solution of pH 3. Potential readings were recorded after stabilization to $\pm 0.2 \text{ mV}$ and emf was plotted as a function of logarithm Py^+ concentration. Calibration graphs were used for subsequent determination of unknown Py^+ concentrations. General working characteristics of the pyridine selective electrodes were evaluated after calibration procedures carried out.

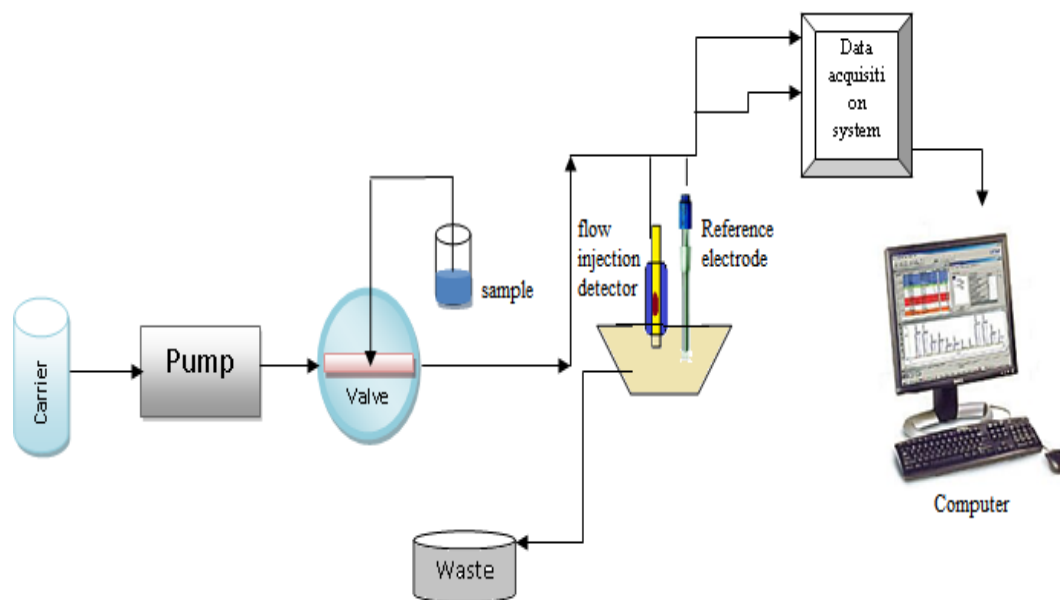


Figure 2. FIA manifold for the evaluation of pyridinium. A 0.01 mol L^{-1} carrier acetate buffer solution pH 3.0; loop sample $200 \mu\text{L}$; and flow rate 3.5 mL min^{-1}

For continuous measurements (FIA), the flow cell used for detection of pyridine was designed with a constant geometry to accommodate small sensor size to avoid large dispersion of the sample in the cell and to give high response with short recovery time. With short tubing (10 cm) between the injector and detector, and using an injection volume of $200 \mu\text{L}$, the dispersion in the system was kept

to minimum (Fig. 2). A flow stream of 0.01 mol L⁻¹ acetate solution of pH 3.0 carrier solution were allowed to pass through the flow-cell at a flow rate 3.5 mL min⁻¹. Successive 200 µL aliquots of the standard pyridine and unknown test sample solutions were injected into the flowing stream. The corresponding potential change was measured and recorded versus time. A typical calibration plot was made and used to determine the concentration of pyridine in the unknown samples.

2.4. Analytical applications

Groundwater samples were analyzed by spiking with known amounts of Py⁺. The pH values of these ground water samples (ca. 50 mL) were adjusted to pH 3.0 with 0.01 mol L⁻¹ acetate solution. The samples were analyzed via standard addition method using pyridine membrane based sensors as mentioned above.

For testing the method applicability in soil samples, constant weights (~ 2.0 g) of different soil samples were transferred to 15 mL polypropylene sample tubes. Different aliquots of pyridine and 10 mL portion of 0.01 mol L⁻¹ acetate solution pH 3.0 thoroughly mixed with the soil sample. A sonication period for 5 min was allowed to ensure convenient extraction of the analyte. Analytical measurements were conducted over this solution after completing the flask to 25 mL with buffer. For batch assessment, the sensor and reference electrode were immersed in the solution, and the potential readings were recorded after reaching the equilibrium response (10–20 s). The concentration of Py⁺ was calculated using a calibration graph.

For hydrodynamic measurements (FIA), a flow stream of 1.0x10⁻² mol L⁻¹ acetate solution of pH 3.0 carrier solution was allowed to pass through the flow-cell at a flow rate 3.5 mL min⁻¹. Successive 200µL aliquots of the standard Py⁺ and unknown test sample solutions were injected into the flowing stream. The corresponding potential change was measured and recorded vs. time. A typical calibration plot was made and used to determine the concentration of the unknown samples.

3. RESULTS AND DISCUSSION

3.1. Performance characteristics of the sensors

Pyridine-tetraphenylborate [Py⁺][TPB⁻] and synthesized β-cyclodextrine (β-CD) polymer membrane based sensors were prepared and examined for potentiometric pyridinium determination with the composition 2:34:64 wt% of the ion pair complex (or ionophore), PVC and plasticizer, respectively [20]. The sensors based on [Py⁺][TPB⁻] exhibit a potentiometric response towards Py⁺ ions with near Nernstian slope of 54.9± 1.1 (r²=0.9978) and 43.3±1.2 (0.999) mV decade⁻¹ with a detection limit 3.1x10⁻⁶ and 3.5x10⁻⁵ mol L⁻¹ for [Py⁺][TPB⁻] and (β-CD) polymer membrane based sensors, respectively (Fig. 3).

Addition of TPB⁻ (30 mmol% relative to the ionophore) to membrane plasticized with DOP and incorporating (β-CD) polymer improve the response towards pyridinium ions with a slope 59.8±0.5 mV (r²=0.9989) decade⁻¹ and detection limit 5.0x10⁻⁶ mol L⁻¹. All potentiometric response

characteristics of the sensors are shown in Table 1. From the results, it can be concluded that, the response of sensors based on (β -CD) polymer is based on a neutral carrier mechanism, in which the ionophore exhibits strong affinity towards Py^+ to create positively charged complexes in the membrane phase. To stabilize the presence of such complexes in the membrane, lipophilic anionic sites must be present. For sensors based on $[\text{Py}^+][\text{TPB}^-]$, their response is mainly based on an ion exchanger mechanism, in which addition of anionic sites has no significant improvement in detection limit of the sensor.

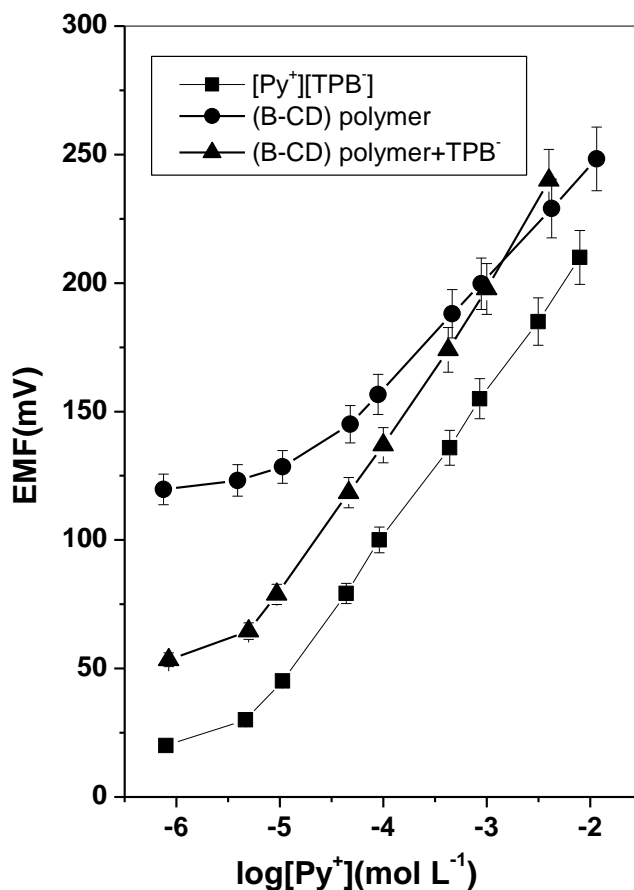


Figure 3. Potentiometric response of pyridinium PVC membrane sensors under static mode of operation.

3.2. Method Validation

The method development and establishment phase defines the chemical assay. The fundamental parameters for an analytical method validation are accuracy, precision, selectivity, sensitivity, reproducibility, and stability. Measurements for each analyte in any matrix should be validated. In addition, the stability of the analyte in spiked samples should be determined. Typical method development and establishment for an analytical method include determination of (1) Linearity and detection limit, (2) accuracy, precision, recovery, robustness, (3) selectivity, and (4) stability of analyte in spiked samples. In the present work, six batches (6 replicates each) of Pyridinium cation

(Py⁺) were used for assessment of the linearity, limit of detection, accuracy (trueness), precision, selectivity, and method robustness.

3.2.1. Method Linearity and Detection Limit

The linear range of the calibration plot is 1.0×10^{-2} – 5.0×10^{-6} mol L⁻¹ (0.31 mg mL⁻¹– 0.155 μg mL⁻¹) and 1.0×10^{-2} – 8.0×10^{-6} (0.31 mg mL⁻¹– 0.248 μg mL⁻¹) for [Py⁺][TPB⁻] and ((β-CD) polymer /TPB) membrane based sensors, respectively. Least square analysis of the data gives Equations 1 and 2:

$$E(\text{mV}) = (54.9 \pm 1.1) \log [\text{Py}^+] + (281.5 \pm 0.7) \quad (1)$$

$$E(\text{mV}) = (59.8 \pm 0.8) \log [\text{Py}^+] + (229.8 \pm 0.4) \quad (2)$$

The lower detection limit (LOD) was calculated according to IUPAC guidelines and found to be 3.1×10^{-6} mol L⁻¹ (0.096 μg mL⁻¹) and 5.0×10^{-6} mol L⁻¹ (0.15 μg mL) Py⁺ ions for [Py⁺][TPB⁻] and ((β-CD) polymer/TPB) membrane based sensors, respectively. It is defined as the Py⁺ concentration corresponding to the intersection of the extrapolated linear segment of the calibration graph [21]. The lower limit of quantitation (LLQ) was ~ 0.155 and 0.248 μg mL⁻¹. In order to know if the investigated sensor exhibits any fixed or proportional bias, a simple linear regression for the observed concentrations against expected values (4 points) was performed. The slopes of the regression lines were near to those of the ideal value of unity ($r^2=0.9996$). The potential variability of the intercepts was very small indicating that there is no systematic difference between the determined and expected concentrations within the investigated range using the present method.

3.2.2. Method Accuracy, Precision and Robustness

Table 1. Potentiometric response characteristics of pyridine membrane based sensors

Parameter	[Py ⁺][TPB ⁻]	(β-CD) polymer	((β-CD) polymer + (30 mmol % TPB ⁻))
Slope (mVdecade ⁻¹)	54.9±1.1	43.3±1.2	59.8±0.5
Correlation coefficient, r (n=5)	0.9978	0.9990	0.9989
Linear range (mol L ⁻¹)	5.0×10^{-6} 1.0×10^{-2}	5.0×10^{-5} 1.0×10^{-2}	8.0×10^{-6} 1.0×10^{-2}
Detection limit (mol L ⁻¹)	3.1×10^{-6}	3.5×10^{-5}	5.0×10^{-6}
Working range (pH)	2.0 - 4.0	3.0 - 4.0	3.0 - 4.0
Response time (s)	< 10	< 20	< 15
Standard deviation σ _v (mV)	1.2	1.5	0.8
Repeatability, C _v w (%)	1.2	1.3	1.1
Accuracy (%)	99.2	98.8	99.3

The accuracy (trueness) and precision (relative standard deviation, RSD) of the batch and flow injection procedures were calculated according to Equations 3 and 4 [22, 23], respectively:

$$\text{Accuracy \%} = (x / \mu) \times 100 \quad (3)$$

$$\text{Precision \%} = (SD / x) \times 100 \quad (4)$$

Where: x , μ and SD are the average measured concentration found, reference-value and standard deviation, respectively Table 1.

The robustness of the method was evaluated by testing the influence of pH variation and measuring time on the accuracy of the results. The effect of pH on the potentiometric response of $[\text{Py}^+][\text{TPB}^-]$ and $(\beta\text{-CD})$ polymer based membrane sensors was examined with standard 1.0×10^{-4} and $1.0 \times 10^{-3} \text{ mol L}^{-1} \text{ Py}^+$ solutions over a pH range of 2–8. The pH of the solution was adjusted with either hydrochloric acid and/or lithium hydroxide solutions. The results indicate that the variation of solution pH over the range 2.0–4.0 and 3.0–4.0 has no significant effect on the sensor response of $[\text{Py}^+][\text{TPB}^-]$ and $(\beta\text{-CD})$ polymer based membrane sensors, respectively (Fig. 4).

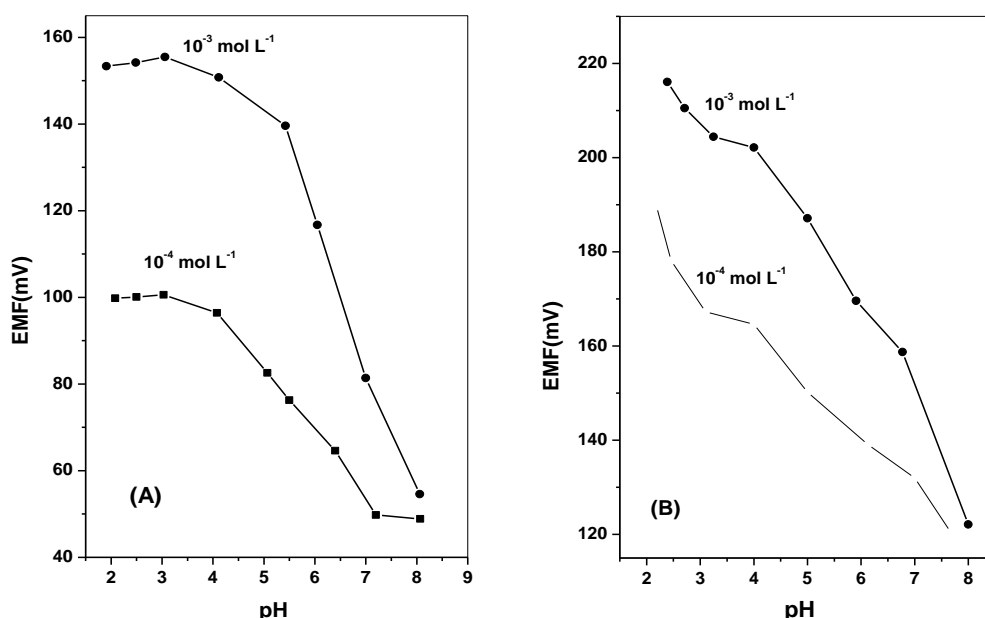


Figure 4. Effect of pH for on the response of pyridinium membrane sensors .

Since the pK_a of pyridine is 5.25, 2 and 3 pH units below the pK_a resulted in 99 and 99.9 % ionization (protonation) of pyridine, respectively. The potential of the sensor considerably declined with negative drift at higher pH values probably due to progressive formation of the free pyridine base. At $\text{pH} < 2$, the sensor response was severely influenced by H_3O^+ .

The response time of an ISE is an important parameter that must be considered if the sensor is going to have any type of practical utility. For conventional membrane-based ISEs, the potentiometric response is due to the phase-boundary potential that results at the sample–membrane interface when activity changes occur at the surface layer at the sample/membrane interface [24]. The optimum equilibration time for the membrane sensor in $1 \times 10^{-3} \text{ mol L}^{-1} \text{ Py}^+$ solution was found to be 12 h for the sensor based on $[\text{Py}^+][\text{TPB}^-]$ and 24 h for the sensor based on $(\beta\text{-CD})$ polymer, respectively, upon reaching the stage of stable and reproducible potential. The time required to achieve a steady potential response ($\pm 3 \text{ mV}$) using the proposed sensors in $1.0 \times 10^{-3} \text{ mol L}^{-1} \text{ Py}^+$ solutions with a rapid 10-fold increase in concentration were $< 10 \text{ s}$ for $[\text{Py}^+][\text{TPB}^-]$ and $< 20 \text{ s}$ for $(\beta\text{-CD})$ polymer membrane

based sensors, respectively. After several calibrations for each sensor, low potential drift, long-term stability and negligible change in sensors response were observed. When not in use, the sensors were stored and conditioned in 10^{-3} mol L⁻¹Py⁺. For all sensors examined, the detection limits, response times, linear range and calibration slopes were reproducible within $\pm 2.5\%$ of their original values over a period of at least 9 weeks.

3.2.3. Selectivity

Potentiometric selectivity factors of the sensors were evaluated by applying the matched potential method (MPM) [21]. In this method, the activity of Py⁺ was increased from $a_A = 1.0 \times 10^{-5}$ mol L⁻¹ (reference solution) to $a'_A = 5.0 \times 10^{-5}$ mol L⁻¹, and the change in potential (ΔE) corresponding to this increase was measured. Next, a solution of an interfering ion of concentration a_B in the range 1.0×10^{-1} – 1.0×10^{-2} mol L⁻¹ is added to a new 1.0×10^{-5} mol L⁻¹ (reference solution) until the same potential change (ΔE) was recorded. The selectivity factor, $K^{MPM}_{Py,B}$ for each interferent was calculated using the following equation:

$$K^{MPM}_{Py,B} = (a'_A - a_A) / a_B$$

Table 2 summarizes the potentiometric selectivity characteristics of membranes containing, [Py⁺][TPB⁻] and ((β -CD) polymer) with and without lipophilic anionic additive (TPB⁻). The selectivity order for [Py⁺][TPB⁻] membrane based sensor was: Py⁺ ~ cetylpyridinium > quinine > caffeine > phenylalanine > serine > histidine > glutamine > cysteine > glycine > urea > NH₄⁺ > Na⁺ > K⁺ > Ca²⁺. The selectivity patterns of ((β -CD) polymer) membrane with and without a lipophilic anionic additive (TPB⁻) in the membrane sensor was investigated. The selectivity order of ((β -CD) polymer) membrane based sensor is: Py⁺ > Na⁺ > K⁺ > quinine > phenylalanine > serine > caffeine > histidine > cetylpyridinium > glutamine > cysteine > glycine > urea > NH₄⁺ > Ca²⁺.

Addition of TPB⁻ to the membrane (30 mmol% relative to the ionophore) was found to improve the selectivity behavior such that: Py⁺ > K⁺ > Na⁺ > quinine > cetylpyridinium > caffeine > phenylalanine > serine > histidine > glutamine > cysteine > glycine > urea > NH₄⁺ > Ca²⁺. The selectivity differences between membranes containing the neutral carrier only and those containing neutral carrier plus TPB⁻ (30 mmol% relative to the ionophore) could be due to direct interaction between the complexed cations and the counter-anion sites in the membrane. For membrane sensors containing [Py⁺][TPB⁻], the response mechanism for pyridinium cation is based on the ion-exchange properties in the polymer matrix. The electrostatic interaction plays the dominate role for the cation transfer across the organic/water interface. The hydration energy of the analyte cations is overcome by the electrostatic affinity. The selectivity sequence is, however, determined by the order of the hydration energy or by the hydrophilicity of the tested cations. It is well established that the selectivity of neutral cation-selective carrier-based liquid-polymeric membrane sensors can be optimized by the addition of lipophilic anionic additives in the membrane. These additive sites reduce membrane resistance, minimize interference by anions at high sample activities, increase the availability of the

free carrier for cations complexing, and improve the membrane selectivity. The optimum concentration of such lipophilic additives in the membrane phase depends in part on the charge of the primary ion and its complexation stoichiometry with the carrier relative to that of the interfering ion [25].

Table 2. Selectivity coefficient values for Py^+ selective sensors as calculated by matched potential method (MPM)

Interferent	$\text{Log } K_{\text{Cet, J}}^{\text{pot}}$		
	$[\text{Py}^+][\text{TPB}^-]$	(β -CD) polymer	(β -CD) polymer + (30 mmol % TPB $^-$)
Py^+	0	0	0
Cetylpyridinium	-0.54	-2.82	-1.90
Quinine	-1.13	-1.6	-1.72
Caffeine	-1.32	-2.32	-1.92
Phenylalanine	-1.51	-1.75	-2.01
Serine	-1.80	-2.12	-2.30
Histidine	-1.82	-2.62	-2.60
Glutamine	-1.92	-2.92	-2.91
Cystiene	-2.02	-3.10	-3.10
Glycine	-2.3	-3.11	-3.20
Urea	-2.7	-3.32	-3.28
NH_4^+	-3.0	-3.42	-3.41
Na^+	-3.8	-0.6	-0.7
K^+	-3.9	-0.8	-0.65
Ca^{2+}	-4.01	-3.83	-3.91

3.3. Pyridinium Assay

3.3.1. Determination of Py^+ in spiked water and soil samples

Potentiometric determination of Py^+ in spiked water and soil samples under static and hydrodynamic mode of operations was carried out. The results showed average recoveries (accuracy) of 97.3 ± 0.7 and $97.8 \pm 0.8\%$ and $95.8 \pm 0.7\%$ and 96.1 ± 0.9 in water and soil samples using batch and FIA techniques, respectively (Table 3). These data were compared with results obtained by the method described [26]. This confirms the applicability of the method for accurate routine analysis of Py^+ in spiked samples. The sensors can be used for up to 4 weeks before noticeable drift is detected, probably due to contamination of the PVC membrane with the matrix.

An *F*-test showed no significant difference at 95% confidence level between the means and variances of the results. The calculated *F*-values ($n=10$) of the results obtained by the proposed sensor

and different potentiometric techniques (Table 3) for spiked samples were less than 2.019, compared with the theoretical tabulated value ($F=3.18$).

Table 3. Determination of Pyridinium ions in spiked water and soil samples using $[\text{Py}^+][\text{TPB}^-]$ based membrane sensor.

Matrix	Spiked concentration ($\mu\text{g mL}^{-1}$)	Recovery found * (%)		
		Static	FIA	Spectrophotometry [26]
Water	2.0	96.2±1.1	92.7±0.8	96.4±1.3
	5.0	96.2±0.9	96.2±1.3	97.2±0.1
	10.0	97.8±0.6	96.2±0.3	98.7±0.3
	20.0	99.1±0.5	98.2±0.7	99.2±0.2
Soil	2.0	95.2±1.2	92.3±1.2	97.7±0.4
	5.0	96.8±0.6	95.2±0.7	97.0±0.3
	10.0	98.1±0.4	98.4±0.4	98.4±0.2
	20.0	98.2±1.2	98.2±1.1	98.7±1.2

*Average of 5 determinations

3.3.2. Continuous monitoring of Py^+

Flow injection analysis (FIA) is a simple, rapid, and versatile technique that is now firmly established, with widespread applications in quantitative chemical analysis. ISEs in flow injection potentiometry (FIP) have several advantages compared to steady-state measurements. They include, fast sample through-put, high precision, small sample volumes, economical use of reagents, correction of electrode drift by the measurement of peak heights and ease of computer automation [27–32]. The flow cell used for Py^+ assessment was designed with a constant geometry and a minimum “dead” volume in order to accommodate small sensor size. This setup avoids large sample dispersion and results in high response with short recovery time. A tubular-type detector incorporating $[\text{Py}^+][\text{TPB}^-]$ and ((β -CD) polymer) based membrane sensor was prepared and used under hydrodynamic mode of operation for continuous monitoring of Py^+ .

Important variables of a single-line flow setup are confined to sampling volumes and flow-rates. The effect of varying sample loop volume from 50 to 500 μL for Py^+ solution ranging from 10^{-5} to 10^{-2} mol L^{-1} on the potentiometric response (slope in mV decade^{-1}) at pH 3.0 was initially evaluated. The potentiometric response increased with the increase of sample volumes from 50 to 200 μL and was maintained constant in sample volume higher than 200 μL . Therefore, a sample volume of 200 μL was selected for further experiments.

The effect of carrier buffer flow rate was examined over a range of flow rates from 1.5 to 5 mL min^{-1} for Py^+ solutions ranging from 10^{-6} to 10^{-2} mol L^{-1} . The potentiometric response (slope in mV decade^{-1}) was recorded against the flow rate. The optimal flow rate was chosen to be 3.5 mL min^{-1} . In flow rates lower than 3.0 mL min^{-1} , the tubular detector showed a slight memory effect, long washing

times, and low analytical frequency. At flow rates higher than 4.0 mL min^{-1} , the detector response decreases and the peak width become narrow because high flow rates decrease the residence time of the sample. The recommended optimal flow rate was chosen to be 3.5 mL min^{-1} .

Under these conditions, a linear relationship was obtained between the FIA signals and Py^+ concentrations over the range $10^{-6} - 10^{-2} \text{ mol L}^{-1}$. The slopes of the calibration plot were 67.2 ± 0.8 and $72.8 \pm 1.1 \text{ mV decade}^{-1}$ with detection limits 6.0×10^{-5} and $1.0 \times 10^{-4} \text{ mol L}^{-1}$ for $[\text{Py}^+][\text{TPB}^-]$ and $(\beta\text{-CD}) \text{ polymer} / \text{TPB}^-$ membrane based sensors, respectively (Fig. 5). The relative standard deviations of the FIA signals for the sensors were $\sim 1\text{-}2\%$ for $10^{-6} - 10^{-2} \text{ mol L}^{-1} \text{ Py}^+$.

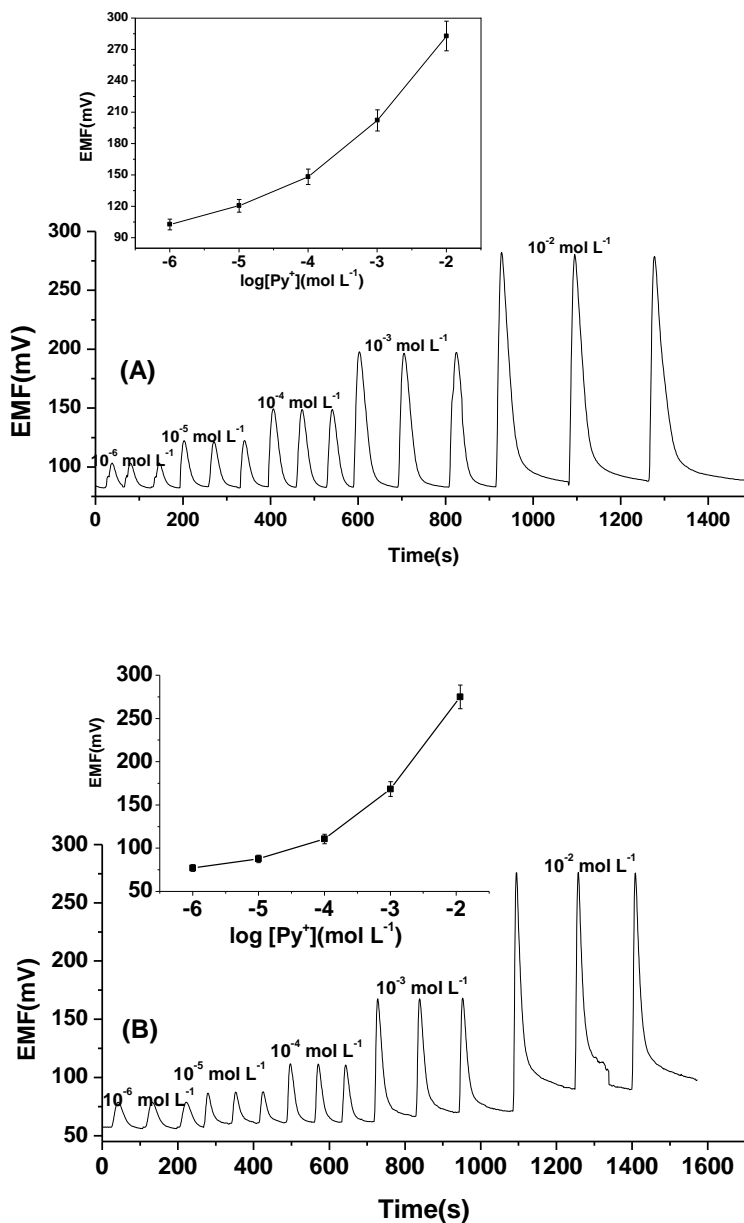


Figure 5. Typical (FIA) peaks produced by injection of $200 \mu\text{L}$ aqueous solutions of standard Py^+ into a stream of $10^{-2} \text{ mol L}^{-1}$ acetate buffer pH 3.0 flowing at 3.5 mL min^{-1} using: (A) $[\text{Py}^+][\text{TPB}^-]$ and (B) $(\beta\text{-CD}) \text{ polymer} / \text{TPB}^-$ membrane based sensors.

4. CONCLUSIONS

A Py^+ potentiometric sensors based on the use of ion association complex of (Py^+) cation with tetraphenyl borate (TPB) anion, and ((β -CD) polymer) as a neutral carrier, exhibited excellent potentiometric performances such as quick response, a wide range of working pH, high sensitivity, long-term stability, good selectivity and self feasibility. The use of these sensors as detectors for the continuous monitoring of Py^+ offered an advantage of simple design, ease of construction and possible application in the routine control of pyridinium ions samples. Optimization and full validation of the assay method enable accurate, precise and rapid measurements of as low as $3.1 \times 10^{-6} \text{ mol L}^{-1}$ ($0.096 \mu\text{g mL}^{-1}$) and $5.0 \times 10^{-6} \text{ mol L}^{-1}$ ($0.15 \mu\text{g mL}^{-1}$) Py^+ ions in different samples for [Py^+][TPB $^-$] and ((β -CD) polymer)/TPB) membrane based sensors, respectively. No pretreatment or prior separation steps are used. Application to pyridine evaluation in water and soil matrices revealed good results. The results are favorably compared with data obtained using the standard method [26].

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