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# New electrochemical methods for the determination of Prasugrel using drug selective membranes

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***ABSTRACT:*** *Prasugrel ion selective electrodes were constructed from PVC matrix containing Prasugrel HCl and [ ammonium Reineckate (PRS-RNK )] or [ sodium Tungstate (PRS-TUN) as the sensing elements in presence of di-n-butyl phthalate (DBP) as the plasticizing solvent mediator . the best PVC membrane electrodes were made of 4% ion pair for both (PRS-RNK) and (PRS-TUN) , 48% PVC , 48% DBP . the two electrodes showed a fast , stable and Nernstian response . the electrodes exhibit linear response over concentration range $10^{-6}$-$10^{-2}$mol /l with Nernstian slope 59.08 or 55.09 for sensor 1 and sensor 2 respectively with detection limit (LOD ) 0.035µM-0.09µM and quantification limit (LOQ) 0.108 µM-0.3µM for sensor 1 and 2 respectively. the both electrodes were effective at* ***pH*** *range between 1-4 . the electrodes showed very good selectivity for Prasugrel in presence of different inorganic cations and pharmacological related compounds . the electrodes exhibited a fast dynamic response of (15±2sec / 20±2 sec) for a period of 8 days/2days without significant change in electrode parameters , the methods are precise as shown by mean recoveries range of (98.8-101 %)– (97-102 %) with mean relative standard deviation less than 3.94 and 4.9% for sensor 1 and 2respectively . Prasugrel is determined successfully in pure and pharmaceutical preparations by this described methods .*

***Keywords:*** *Ion selective electrodes , Prasugrel, Nernstian slope, potentiometric determination , ammonium Reineckate– sodium tungstate .*

## 1. Introduction

Prasugrel chemically is 5-[2-cyclopropyl -1-(2-fluro-phenyl ) 2-oxoethyl ]-4,5,6,7-tetra hydro thieno [3,2-c] pyridine 2-yl acetate [1] . Prasugrel is a platelet inhibitor belonging to thieno pyridine class developed by Daiichi Sankyo Co. and produced by Ube and currently marketed in the US in codperation with Eli Lilly [2,3] .on July 10, 2009 the US FDI approved the use of Prasugrel for the reduction of thrombotic cardiovascular events [4] . it acts as ADP receptor antagonist , which makes it different is its safety profile and pharmacokinetic properties . although Clopidogrel is a widely prescribed agent it has some limitation , because of its disadvantages. researchers developed a more effective agents such as the novel Prasugrel . literature survey revealed very few methods for the estimation of Prasugrel hydrochloride such as LS-MS [5] , HPTLC [6], UV –VIS spectrophotometric method [7].

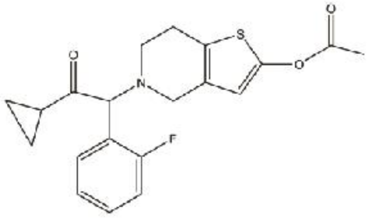

**Fig .1 :chemical structure of PRS**

Ion selective electrode have many advantages relation to other analytical techniques, being accurate , fast , economic , simple and sensitive also have extremely wide range of application [8].

The aim of this work was to develop a sensitive , selective and validated a ISE$_s$ for the determination of Prasugrel hydrochloride in bulk and pharmaceutical formulations .

### 1.1. Research Highlights

The proposed methods depend on the reaction of Prasugrel with ammonium Reineckate or sodium Tungstate to form ion pairs complexes (PRS-RNK ) or (PRS-TUN) that are useful in the potentiometric determination of PRS in pure form and in pharmaceutical dosage forms with average recovery between 98.8 –101% and between 97.00–102.0 % for AB with RSD less than 3.94% and 4.9% respectively.

## 2. Materials and Methods

### 2.1. Apparatus :

The electrochemical measurements were made with ion check 10 **pH/mv** meter – Radiometer analytical S.A., France , with Prasugrel –reineckate salt/sodium tungstate – PVC- DBP plasticizer membrane electrode in conjunction with double junction Ag/Ag cl electrode as external reference electrode as external reference electrode ( lab. assembly ) , containing 1M KCl in the outer compartment . crison –Glp 21/Eu **pH** meter was used for **pH** adjustment for all measurements .ultrasonic processor model powersonic 405 was used to sonicate the sample solutions . all potentiometric measurements were made at 25±1°C with constant stirring using hot-plate magnetic stirrer MS 300 BANTE, China. All weights were taken by analytical balance (BP 221S SARTORIUS, Germany) with accuracy ±0.1mg. Conductivity meter (inoLab-cond 720, Germany) used for bi-distill water quality.

### 2.2. Reagent and Materials:

Analytical grade Prasugrel hydrochloride ( Glenmarak Generics Ltd- INDIA) , high molecular weight PVC (SABC ,KSA ) , di –n- butyl phthalate (DBP) 99% , tetrahydroforan 97% , HCL , NaOH , KCL (GR grade, MERCK, Germany) were used ,Bi-distilled water (conductivity≤10 μS/cm), sodium tungstate $Na_2\ Wo_4$ , ammonium REINECKATE $NH_4[Cr(SCN)_4(NH_3)_2].H_2O$ ($NH_4$RNK), (BDH Laboratory, England) .

### 2.3. Standard Drug Solutions

Stock standard (0.01 M) Prasugrel HCL ($M_W$=409.902 g.mol$^{-1}$) was prepared by dissolving accurate weight in bi – distilled water using ultra sonic bath to assist the dissolution of the drug substance . this solution was found to be stable for 24 hrs if kept at room temperature . working solution ranging 0.1-1000μM were prepared by serial dilution of the stock solution . Britton –Robinson universal buffer 0.2 M was used [9] .

### 2.4. Ion Selective Electrodes

### 2.4.1. Preparation of Ion-Pairs

The ion pairs were prepared by mixing 50 ml of 1 m M Prasugrel solution to 50 ml of 1mM ammonium reineckate, or 50 ml of 1m M PRS to 50 ml of 1m M TUN. the precipitates Prasugrel – ammonium Reineckate ( faint pink powder ) and Prasugrel – sodium tungstate (off- white powder) were filtered , washed thoroughly with distilled water until the conductivity of the washed water is close to the conductivity of bi –distilled water and dried at room temperature for 24 hrs . ion pairs were stored in will – closed amber class bottles . the molecular ratios of the both complexes were found to be 1:1.

### 2.4.2. Membranes Composition:

The membranes were prepared by dissolving equal weights of matrix PVC and the plasticizer (DBP) and the suitable weight of ion pair . the matrix is dissolved by minimum volume of THF . The solutions were poured into a petri –dish , covered with a filter paper and the solvent was allowed to evaporate slowly at room temperature . leaving the casted ion selective membranes that represent the electro- active part of ion selective electrodes .

### 2.4.3. Construction of ISE :

A punched circular membrane was attached to poly-ethylene tube in an electrode configuration and attached to the end of glass tube . this electrode was filled with internal solution consisting of 1 m M Prasugrel in 1 M KCl . Ag/AgCl wire electrode was used as an internal reference electrode [10,11] . then this electrode attached to double junction Ag/Agcl electrode as external reference electrode , containing 1 M KCl in the outer compartment . the circuit closed by attaching the cell to pH/mv meter [12-14].

### 2.4.4. Electrode Calibration :

25 ml aliquots of 0.1-10000(**100000**) µM Prasugrel standard solutions were transferred into 50 ml beaker and the sensor in conjunction with Ag/AgCl reference electrode was immersed in solution . all potentiometric measurements were performed using the cell:

Ag/AgCl – kCl (1M)+PRS (1m M)**ll**PRS –RNK or PRS-TUN –DBP –PVC membrane**ll**test solution**ll**Ag/AgCl –KCl (1M)

The measured potential was plotted against the minus logarithm of Prasugrel concentration. The electrodes were washed with bi –distilled water and dried with tissue paper between measurements.

### 2.4.5. Effect of Ion Pair Percentage on Electrode Potential :

We have prepared a set of membranes containing a different percentage of ion pair ranging between 2-8 % , then we studied the effect of this percentage on electrode potential and we selected the percentage that showed the closet slope to Nernstian slope.

### 2.4.6. Effect of pH:

The effect of pH on the potential response was studied using 10 and 1 m M Prasugrel solutions. The pH of the solutions were adjusted between 1-6 using suitable amounts of 1M KOH or HCl solution. The potential readings corresponding to different pH value were recorded . on the other hand , the study was repeated using Britton – Robinson universal buffer . The pH of the buffer solutions was adjusted between 1-6, and a specific volume of it was added to the drug solution.

### 2.4.7. Selectivity:

The response of the electrode was examined in the presence of some inorganic compounds by matched potential method [15]. In this method , the potentiometric selectivity coefficient is defined as the activity ratio of primary and interfering ion that give the same potential change under the same conditions.

$$K=(\grave{a}-a)\ /\ a_{B}$$

At first , a known activity ($\grave{a}_{prs}$) of the primary ion solution is added into a reference solution that contains a fixed activity ($\grave{a}_{prs}$) of primary ions , and the corresponding potential change (ΔE) is recorded . next , a solution of an interfering ion is added to the reference solution until the same potential change (ΔE) recorded again [16].

### Determination of Prasugrel in Pharmaceutical Dosage Forms:

Local manufacturing formulations, Targol ( film coated tablets , universal pharmaceutical industries ) and Prassuclear ( Ibn al Haytham ) containing 5 or 10 mg of PRS.HCl . Were used for the analysis of PRS by direct potentiometric determination.

Ten tablets of Targol and Prassuclear were finely powdered and accurate weight equivalent to one tablet , dissolved using the same condition used to dissolve the drug substance . The solution was filtered through a common filter paper and transferred to 250 ml volumetric flask and diluted to the mark with bi–distilled water. The final solution was analyzed as described under electrode calibration.

## 5. Results and Discussions

### 5.1. Calibration Graphs and the Best Electrode:

The analytical range of a potentiometric sensor was the linear part of the calibration graphs, it was ($10^{-2}$-$10^{-6}$ M ) pc =( 2-6) (for two sensors). Increasing IP percentage in the membrane was found to increase the electrode response and increasing the slope of the linear area reaching -59.08 m.v.decade$^{-1}$ for sensor 1 and -55.09m.v.decade$^{-1}$ for sensor 2 at 4% ion pair ratio, but at percentage higher than this we found a decrease in electrode response and slope, that due to the kinetic of the IP inside the membrane.

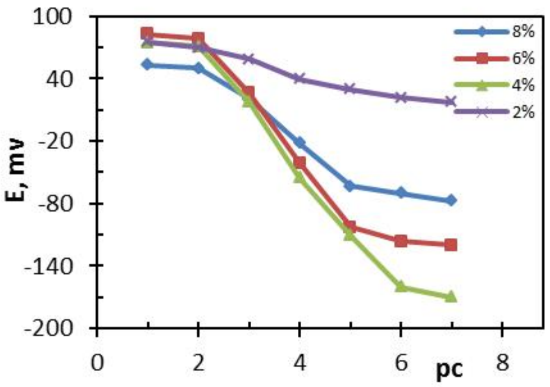

**Fig. 2: Effect of IP content on PRS calibration curve.**

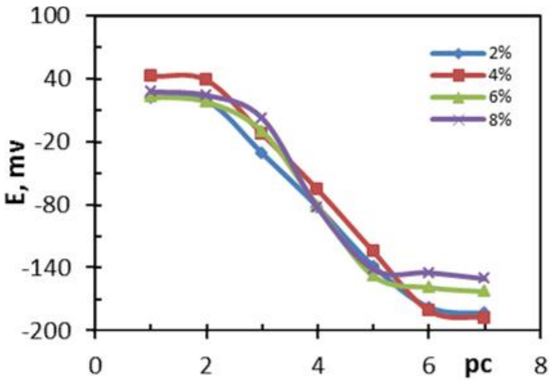

**Fig. 3: Effect of IP content on PRS-TUN calibration curve**

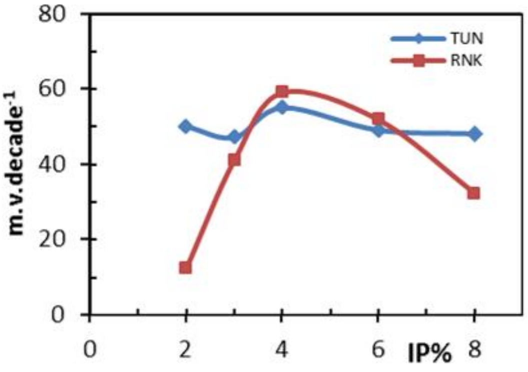

**Fig. 4: Effect of IP percentage in the ion selective membrane on the slope of the liner area for equation curve: E = f(pCprs)**

**TABLE 1: The least squares equations data obtained from the liner equation**

| | **PRS-RNK** | | | | **PRS-TUN** | | | |
|---|---|---|---|---|---|---|---|---|
| **IP %** | 2 | 4 | 6 | 8 | 2 | 4 | 6 | 8 |
| **S , mv** | -12.42 | -59.08 | -51.88 | -32.37 | -50.11 | -55.09 | -49.13 | -48.17 |
| **b , mv** | 93.86 | 188.86 | 176.28 | 112.5 | 118.56 | 151.42 | 120.64 | 124.8 |
| **$R^2$** | 0.9778 | 0.9959 | 0.9763 | 0.9648 | 0.9975 | 0.9993 | 0.9569 | 0.9289 |

### 5.2. Effect of pH on potential:

The results showed that the potential remained constant despite the pH change in the range between 1-4, and the same results when we used a Britton –Robinson universal buffer in both PRS- RNK and PRS –TUN sensors , that indicate the applicability of this electrode in the pH specified range .

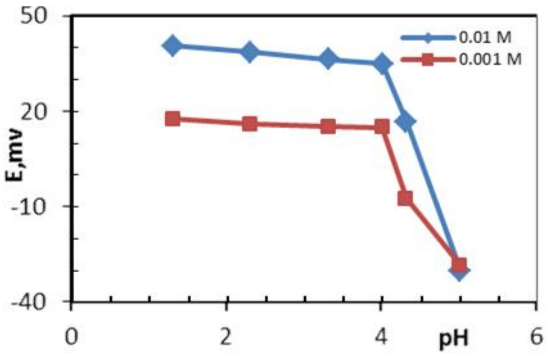

**Fig. 5: Effect of pH on the potential response of the PRS-RNK sensors using 10 mM PRS solution, 1 mM PRS solution, in BRITTON-ROBINSON universal buffers solution**

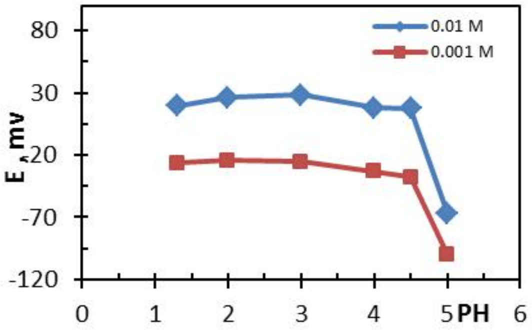

**Fig. 6: Effect of pH on the potential response of the PRS-TUN sensors using 10 mM PRS solution, 1 mM PRS solution, in BRITTON-ROBINSON universal buffers solution**

### 5.3. Selectivity of the Electrodes:

the results of the potentiometric selectivity coefficients of the prepared electrodes showed high selectivity toward Prasugrel in presence of different inorganic cations and some pharmacologically related compounds.

**TABLE 2: Selectivity coefficient of some interfering ions by PRS-ISEs**

| Interfering, B | KMFX,B | |
|---|---|---|
| | PRS-RNK | PRS-TUN |
| Potassium chloride | $1.92*10^{-3}$ | $1.5*10^{-3}$ |
| Calcium chloride | $3.5*10^{-3}$ | $9.4*10^{-3}$ |
| Ammonium chloride | $3.7*10^{-3}$ | $7.5*10^{-3}$ |
| Sodium chloride | $5.1*10^{-3}$ | $3.2*10^{-3}$ |
| Dextrose | $9.7*10^{-3}$ | $17*10^{-3}$ |
| Microcrystalline cellulose | $1.89*10^{-3}$ | $19*10^{-3}$ |

### 5.4. Life Time Study :

Prasugrel electrodes life time were estimated with calibration curves periodical test of a standard solutions ($10^{-2}$-$10^{-7}$ M ) and calculation of its response slope . The calibration curves were plotted after optimum soaking time of 6 hrs in 1 m M PRS solution. The slopes were -59.08 m.v.decade$^{-1}$ for sensor 1 and -55.09 m.v.decade$^{-1}$ for sensor 2 at 25 °C. The electrodes were continuously soaked in 1 m M solution of PRS for about 10 days (we changed the inner solution every day because of Prasugrel sensitivity to degradation in water solutions). The calibration plot slopes decrease slightly to be -52.3 after 8 days for sensor 1 and -50.6 after 2 days for sensor 2 . These indicate that soaking sensors in drug solution for a long time has a negative effect on the response of membranes.

### 5.5. Response Characteristics and Statistical Data:

The result summarized in tablet 3. The sensors show nearly Nernestain response in the range between 10-100000 µM , PC (2-6) .

**TABLE 3: Response characteristics of PRS-sensors**

| Parameter | PRS-RNK | PRS - TUN |
|---|---|---|
| **IP%** | 4% | 4% |
| **Slope, mV.decade$^{-1}$** | -59.08 | -55.09 |
| **Intercept, mV.decade-1** | 188.86 | 151.42 |
| **Correlation coefficient (R2)** | 0.9959 | 0.9993 |
| **Linear range, µM** | 10-100000 | 10-100000 |
| **LOD, µM** | 0.035 | 0.09 |
| **LOQ, µM** | 0.108 | 0.3 |
| **Response time for 1 mM, sec** | 15 ± 2 | 20± 2 |
| **Life time, day** | 8 | 2 |
| **Working pH range** | 1-4 | 1-4 |

### 5.6. Quantification of Prasugrel HCl:

The developed sensors found to be effective in the potentiometric determination of Prasugrel in both pure form and pharmaceutical preparations.

**TABLE 4: Direct determinations of PRS in bulk solution using proposed sensors**

| Taken $C_{PRS.HCl}$ | | PRS-RNK | | | PRS-TUN | | |
|---|---|---|---|---|---|---|---|
| **(µg/mL)** | **mol/L** | **% R** | **SD** | **RSD%** | **R%** | **SD** | **RSD%** |
| 4099 | $1x10^{-2}$ | 101 | 53.8 | 1.29 | 102.7 | 59.5 | 1.5 |
| 409.9 | $1x10^{-3}$ | 100.26 | 9.62 | 2.34 | 97.5 | 7.14 | 1.7 |
| 40.9 | $1x10^{-4}$ | 98.8 | 1.07 | 2.64 | 98.2 | 1.49 | 3.5 |
| 4.09 | $1x10^{-5}$ | 99.53 | 0.12 | 2.94 | 102.3 | 0.13 | 3.2 |
| 0.409 | $1x10^{-6}$ | 99.04 | 0.016 | 3.94 | 101.2 | 0.02 | 4.9 |

*Average of five replicates

The results obtained were compared with a reference RP- HPLC method [17]. As shown in table 5. Statistical analysis of the result obtained by the proposed and comparison methods using STUDENT's t-test and variance ratio F-test, showed no significant difference between them regarding accuracy and precision, respectively [18].

**TABLE 5: Determinations of PRS in pharmaceutical preparations using proposed sensors**

| Ion Pair | X ± SD, mg$^{a}$ | R% | t-value$^{b}$ | F-value$^{c}$ |
|---|---|---|---|---|
| **Targol 10 mg** | | | | |
| PRS –RNK | 10.025 ±0.49 | 100.25 | 0.87 | 1.12 |
| PRS-TUN | 10.19 ± 0.71 | 101.9 | 1.09 | 1.8 |
| **Targol 5 mg** | | | | |
| PRS- RNK | 5.015 ± 0.14 | 100.3 | 1.25 | 3.68 |
| PRS – TUN | 5.24 ±0.41 | 104 | 1.62 | 2.28 |
| **Prassuclear** | | | | |
| PRS – RNK | 10.18 ±0.78 | 101.8 | 0.56 | 1.87 |
| PRS – TUN | 10 ± 0.63 | 100 | 0.06 | 1.21 |

[a] Average of five replicates.
[b] Tabulated t-value at 95% confidence level is 2.776.
[c] Tabulated F-value at 95% confidence level is 6.39.

## 6. Conclusion

New constructed sensors were developed for Prasugrel determination over a wide range of concentration in pH range between 1-4. The electrodes showed a very good selectivity to Prasugrel in the presence of various common related compounds. Those electrodes can be used as alternative analytical method for the determination of Prasugrel in bulk powder and pharmaceutical preparations.

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