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Potentiometric and Fluorimetric Methods for the Determination of Terazosin HCl in Drug Substance and Dosage Forms

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Determination of terazosin hydrochloride dihydrate (TRZ) in drug substance and tablets was studied by potentiometeric and fluorimetric techniques. potentiometeric method has focused on the fabrication of two carbon paste ion selective electrodes for determination of the drug under investigation using potentiometric titration with phosphomolybdic acid (PMA), and Phosphotungestic acid (PTA). The influence of the electrode composition, conditioning time of the electrode and pH of the test solution, on the electrode performance were investigated. The drug electrode showed Nernstian response in the concentration range from 1×10^{-6}-1×10^{-2} mol L^{-1}, 2×10^{-6}-1×10^{-2} mol L^{-1} with slope of 58.4±0.35, and 57.3±0.23 mV $decade^{-1}$ using PMA, and PTA respectively. It was found to be very precise and usable within the pH range 2-6. These sensors exhibited a low detection limit (8×10^{-7}, $6x10^{-7}$ mol L^{-1}). The fluorimetric methods are based on: (1) measurement of the native fluorescence of the drug in water at 750 nm after excitation at 330 nm. (2) sensitizing the native fluorescence by formation a binary complex of drug with aqueous uranyl acetate (0.1% w/v) at the same E_x / E_m. under the described condition. the proposed methods were applicable over the concentration range of (10 -1000 and 0.5-12 ng mL^{-1}) with good correlation ((r^2=0.9982 and 0.9987), limit of detection of (3.47 and 0.198 ng/mL) and a lower limit of quantification of (10.5 and 0.6 ng mL^{-1}) for method (1) and (2), respectively. The described methods were successfully applied for the determination of TRZ in its commercial tablets without interference from common excipients.

Keywords: Terazosin HCl; carbon paste electrode; potentiometric and fluorimetric determination; Nernstian response.

1. INTRODUCTION

Terazosin HCl has the IUPAC name of 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-[(tetrahydro-2furanyl) carbonyl] piperazine hydrochloride dihydrate. Figure. (1). Its molecular formula is $C_{19}H_{25}N_5O_4$, HCl, H_2O. Terazosin HCl (TRZ.HCl) is an $alpha_1$-adrenoceptor blocker with actions

similar to those of prazosin, but a longer duration of action. It is used in the management of hypertension and in benign prostatic hyperplasia to relieve symptoms of urinary obstruction.

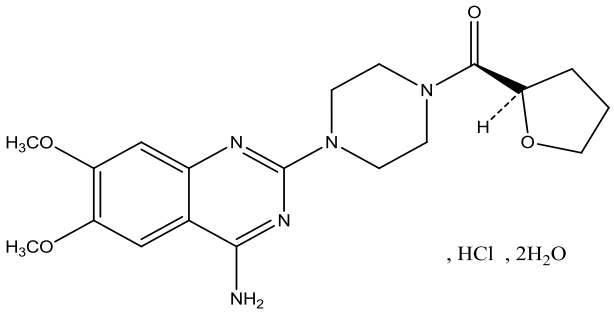

Figure 1. Structural formula of terazosin HCl

An acid-base titrimetric method is recommended for the determination of TRZ.HCl as drug substance in British pharmacopoeia [1], but its formulation is not officially up till now in any pharmacopoeia. Owing to the therapeutic importance of TRZ.HCl, various analytical procedures have been established for its quantitative determination in drug substance, drug products and human plasma.

These procedures include high performance liquid chromatography (HPLC) with fluorescence detection [2–4], with mass spectrometric or UV detection [5,6], X-ray fluorescence spectrometry based on the formation of ion-pair associates with zinc thiocyanate [7], spectrophotometric [8-13] chemometric and capillary zone electrophoresis methods [14,15],.potentiometric [16] voltammetric technique[17] and fluorimetry [18].

Potentiometric carbon paste electrode, native fluorescence and sensitizing the native fluorescence by formation of a binary complex of the drug with uranyl acetate were not reported in these articles.

So, simple potentiometric and fluorescence methods were developed and validated for the quantitative determination of terazosin HCl in drug substance and its dosage form.

Uranyl acetate is a yellow free flowing, crystalline, water soluble uranium compound. It has been used as a chromogenic agent for the spectrophotometric determination of different drugs through complex formation [19,20]. It is also used as a fluorescent indicator for TLC chromatographic determination of complexing agents [21]. It has quenching effect on the fluorescence intensity by the formation of binary complexes [22]. The sensitization effect of uranyl acetate on the native fluorescence through the formation of binary complex was studied.

2. EXPERIMENTAL

2.1. Reagents and Materials

All chemicals and reagents used were of analytical reagent grade. Bidistilled water was used throughout all experiments. The raw material terazosin hydrochloride (TRZ.HCl) was kindly provided by Abbott/Kahira, Egypt Company for Pharmaceutical Industry. Its purity was found to be

99.80%±0.19% according to the official titrimetric method [1]. Itrin tablets B.N. 19503 labeled to contain 5 mg of terazosin hydrochloride, manufactured by Abbott Pharmaceutical Industries, and Prostasin tablets B.N. P4691212, labeled to contain 5 mg of terazosin hydrochloride, manufactured by (Pharco). Uranium acetate reagent (BDH chemicals) 0.1% w/v was freshly prepared in water. *o*-Nitrophenyloctylether (*o*-NPOE) was supplied from Fluka while dioctylphthalate (DOP), and dibutylphthalate (DBP) were supplied from BDH. Tricresylphosphate (TCP), polyvinylchloride (PVC relative high molecular weight) and graphite powder (synthetic 1–2 μm) were supplied from Aldrich. Phosphomolybdic acid (PMA); $H_3[PMo_{12}O_{40}]$, Phosphotungestic acid, (PTA), $H_3[PW_{12}O_{40}.xH_2O]$,.were applied as a titrant for the drug under investigation and purchased from Aldrich chemical company.

2.2. Drug substance

Stock solutions of terazosin, 1×10^{-2} mol L^{-1}, were prepared by dissolving accurately weighed amount of pure solid in double distilled water for potentiometric method, stored in dark bottles and kept in the refrigerator for no more than 3 days, solutions of low concentrations were prepared daily by appropriate dilution.

TRZ.HCl drug substance in 50 mL bidistilled water and diluting to mark with bidistilled Stock solution of TRZ.HCl drug substance was prepared into 100.0 mL measuring flask by dissolving 459.92 mg in water.

Stock standard solutions for two flourimetric methods were prepared by dissolving TRZ.HCl 0.1 mg mL^{-1} in water. The working standard solutions within linearity range were prepared using water.

2.3. Drug products (tablets)

(a) Ten tablets were accurately weighed and finely powdered. The required amount from the tablets powder of each pharmaceutical product (Itrin®, and Prostasine, 5 mg/tablet) were weighed, and then dissolved in 50 mL of bidistilled water by sonication for 10 min. The solution mixture was shacked in a mechanical shaker and the mixture was filtered then transferred accurately to 50 mL measuring flask, completed to the mark with bidistilled water shacked and finally determined by the proposed sensors.

For potentiometric method: An Aliquot of analyte solution (a) containing 20 mg of drug was pipette into a 100-mL beaker, and the solution was diluted to 50 mL with bidistilled water. The solution was titrated with $3.3x10^{-3}$ mol L^{-1} PMA solution using the proposed electrode (TRZ-PMA). The volume of the titrant at the end point was obtained using the differential method.

For fluorimetric method: An Aliquot of analyte solution (a) containing 10 mg of drug was pipette into a 100-mL beaker, and the solution was diluted to 100 mL with bidistilled water. The

procedure was completed as mentioned under general procedure. The nominal content of the tablets was determined either from the calibration curve or using the corresponding regression equation

2.4. Apparatus

The potentiometric measurements were carried out using Orion 940 pH meter, which was more convenient to be used.

Saturated Calomel electrode (SCE) was used as reference electrode.

All measurements were carried out at 25 ºC with a cell of the following type: TRZ-CMCPE/ test solution/ SCE.

Shimadzu RF -1501 spectofluorimeter, equipped with Xenon arc lamp, using quartz cell (1 x1 x 4.5 cm).

2.5. Preparation of ion-association complexes

The ion-association complexes Trazosin-phosphotungestate (TRZ-PTA), and Trazosin-phosphomolybdate (TRZ-PMA), were prepared by mixing stoichiometric amounts of 100 ml of 10^{-2} mol L^{-1} (TRZ.HCl) solution to 100 ml of $3.3x10^{-3}$ mol L^{-1} solution each of PTA, and PMA. The precipitates were filtered and washed thoroughly with deionized water for several times. Then precipitates were dried at room temperature for at least 48 h. and ground to fine powders. The composition of the solid ion-exchanger was confirmed by elemental analysis and found to be 3:1 TRZ-PMA or TRZ-PTA. The C, H and N percentages are 22.2, 2.4, 6.6% and 16.5, 1.7, 5.0%, the corresponding calculated ones for (TRZ-PMA) ion associate are 22.09, 2.43, and 6.7%, respectively and the corresponding calculated ones for (TRZ-PTA) ion associate are 16.49, 1.8 and 5.06%, respectively.

2.6. Preparation of carbon paste electrodes (CPEs)

The sensing carbon paste electrodes (TRZ.PMA and TRZ.PTA) were prepared by mixing accurately 500 mg mixtures of TRZ.PMA or TRZ.PTA (1-7 % w/w) with spectroscopic graphite powder (Aldrich, 1-2 micron) and *o*-Nitrophenyloctylether (*o*-NPOE) as pasting liquid [ratio graphite powder to pasting liquid was 1:1 (w/w)] in an agate mortar until it was uniformly wetted. The mixture was used for filling the electrode body, the electrode surface was polished using a filter paper to obtain a reproducible working surface and used directly for potentiometeric measurements without preconditioning.

2.7. Calibration graph for potentiometeric method

The performance of the electrode was investigated by measuring its potential in prepared Trazocin solutions of a concentration range of 10^{-7} to 10^{-2} mol L^{-1} by serial dilution. Each solution was

stirred and the potential was recorded using carbon paste electrode and saturated calomel electrode when it became stable. The potential readings of the stirred solutions were measured at 25±1 ºC and plotted versus the negative logarithmic of the drug concentration, pTRZ (-log [TRZ.Cl]). The constructed calibration graphs were used for subsequent measurements of unknown TRZ.HCl test solutions.

2.6. Measurement of selectivity

Potentiometric selectivity coefficient $\mathbf{K}_{i,j}^{Pot}$ for different inorganic and organic cations was evaluated using the separate solution method (SSM) [23] and matched potential method (MPM) [24]. In the SSM the EMF value (E_i and E_j) of the electrode in pure solution of each of the primary and the interfering ion, of equal concentration, are used for calculating the selectivity coefficient. The selectivity coefficient $\mathbf{K}_{i,j}^{Pot}$ is calculated using Nickolsky-Eisenman equation.

$$\log K_{i,J}^{Pot} = \frac{(E_j - E_i)}{2.303RT/Z_iF} + \left(1 - \frac{Z_i}{Z_J}\right)\log a \qquad a_i = a_J$$

In the matched potential method the concentration of terazocin hydrochloride solution was increased from $a_i = 1\times10^{-6}$ mol L^{-1} (reference solution) to $a_i' = 1\times10^{-3}$ mol L^{-1}, and the change in potential (ΔE) was recorded. Then, small amounts of a solution of an interfering ion of concentration a_j (from 1×10^{-2} to 1×10^{-3} mol L^{-1}) was added to a new 1×10^{-6} mol L^{-1} reference terazocin hydrochloride solution until the same potential change (ΔE) is achieved. The selectivity coefficient was calculated using the following equation:

$$K_{i,J}^{Pot} = \frac{\Delta a_i}{a_j}$$

With

$$\Delta a_i = a_i' - a_i$$

2.7. Fluorimetric determination of TRZ.HCl

2.7.1. Native fluorescence method

The native fluorescence study of TRZ.HCl: Aliquots containing different amounts of the analyses, between 0.1-10 µg mL^{-1}(100-10000 ng mL^{-1}) were pipette into 10 mL calibrated flasks, and diluted to the mark with water. The fluorescence emission was measured at 750 nm using excitation wavelength of 330 nm, against a blank solution.

2.7.2. Uranyl acetate method

For sensitizing method : Aliquots corresponded to 5-120 ng TRZ.HCl were transferred from stock solution to a series of 10-mL volumetric flasks, followed by adding 2 mL 0.1% w/v of uranyl

acetate and completed to the mark with water. The fluorescence intensity of solution was measured at 750 nm with excitation at 330 nm against a blank prepared similarly.

3. RESULTS AND DISCUSSION

3.1. Potentiometeric method using Carbon paste sensors

3.1.1. Composition, response behavior and characteristics of the Carbon paste electrode

Terazosin-phosphomolybdate (TRZ-PMA) or Terazosin-phosphotungestate (TRZ-PTA) as ion-sensors was found to be highly sensitive to TRZ^+ with respect to several other cations. Therefore, the performance of the CMCPE containing this ion-exchanger in aqueous solutions was studied in details. It is well known that the selectivity, linear dynamic range and sensitivity obtained for a given CMCPE depend significantly on the paste composition [25], the nature of the solvent mediator [26,27] and additives used [28].

The amount of ion-exchanger in the paste affects the response of the electrode, so three paste compositions were prepared by varying the percentage of TRZ-PMA or TRZ-PTA. The results in Table (1) show that the electrodes containing 3, 5 and 7% have slopes of 56.17, 58.40 and 54.22 mV/decade, respectively using TRZ-PMA and 53.20, 57.30 and 52.76 mV/decade, respectively using TRZ-PTA. The three electrodes of each sensor have the same linear range but the slope of the paste containing 5% of TRZ-PMA or TRZ-PTA has wide linear range and lower detection limit and life time up to 4 days Figure (2).

Table 1. Composition, slope, linear ranges, and detection limits of calibration curves for terazosin chemically modified carbon paste electrode at 25±1 ºC

Paste No.	Composition % w/w			Slope mV/ decade	Linear range (mol L^{-1})	LOD (mol L^{-1})
	sensor	Graphite	Plasticizer (*o*-NPOE)			
1	(TRZ-PMA) 3	48.5	48.5	56.17,	1×10^{-6}-1×10^{-2}	8×10^{-7}
2	5*	47.5	47.5	58.40	1×10^{-6}-1×10^{-2}	8×10^{-7}
3	7	46.5	46.5	54.22	1×10^{-6}-1×10^{-2}	8×10^{-7}
4	(TRZ-PTA) 3	48.5	48.5	53.20,	2×10^{-6}-1×10^{-2}	6×10^{-7}
5	5*	47.5	47.5	57.30	2×10^{-6}-1×10^{-2}	6×10^{-7}
6	7	48.5	48.5	52.76	2×10^{-6}-1×10^{-2}	6×10^{-7}

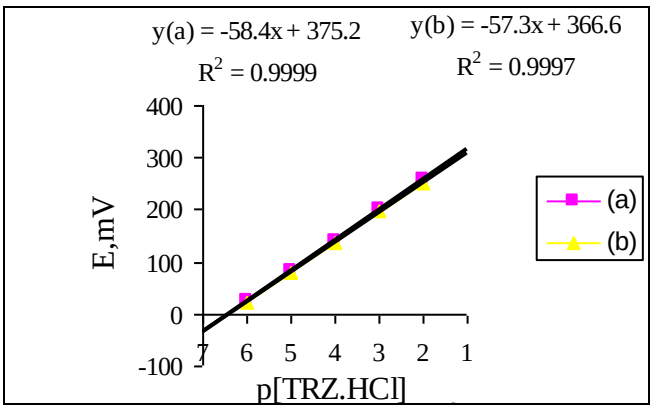

Figure 2. Calibration curves for TRZ.HCl using a) TRZ-PMA b) TRZ-PTA CMCP electrodes

3.1.2. Effect of soaking time

Freshly prepared CMCPE electrode must be soaked to activate the surface of the membrane to form an infinitesimally thin gel layer at which ion exchange occurs. This preconditioning process requires different times depending on diffusion and equilibration at the electrode-test solution interface; a fast establishment of equilibrium is certainly a condition for a fast potential response [29]. Thus, the performance characteristics of the TRZ.HCl ion-selective electrode were investigated as a function of soaking time. For this purpose the CMCP electrode was soaked in TRZ-PMA ion-pair and the titration curves were plotted from which the total potential changes are recorded after 0, 15, 30, 60, 120 min. and 12 and 24 h. The optimum soaking time was found to be 0 min, where the highest total potential change, potential break at the end point are obtained, and the slope of the calibration graph are decreased with increasing soaking time, at 25 °C. This is may be attributed to the memory effect of the electrode due to contamination. Soaking for longer than 24 h is not recommended to avoid leaching of, although very little, the electro-active species into the bathing solution. The CMCP electrode should be stored in a refrigerator while not in use. The end point, potential change and total potential changes are shown in Table (2).

Table 2. Effect of soaking time on the CMCPE performance in the potentiometric titration of 3 mL of 10^{-2} mol L^{-1} TRZ.HCl with $3.3x10^{-3}$ mol L^{-1} PMA

Time of soaking	Total potential change , mV	Potential break at the end point, mV	ΔE/ΔV mV/mL
Without	280	196	570
15 min	241	166	498
30 min	230	150	450
60 min.	238	145	435
120 min.	233	138	414
12 h	227	124	372
24 h	223	103	309

3.1.3. Effect of pH

The effect of pH on the performance of the potentiometric titration of the drug with PMA and PTA were evaluated in concentrations of 1.0×10^{-2} and 1.0×10^{-4} mol L^{-1} of TRZ.HCl at different pH values (1-10) by addition of small volumes of HCl and/or NaOH solution (0.1−1 mol L^{-1} of each) to the solution medium using CMCPE. The potential change at each pH value was reported. It is obvious that, within the pH range from 2.0 to 6.0 the electrode potential is practically independent on pH and in this range the CMCPE can be safely used for TRZ.HCl determination Figure (3). The decrease in mV readings at pH < 2 may be due to interference of hydronium ion. At higher pH values (pH > 6.0), free-base precipitates in the test solution and consequently, the concentration of unprotonated species gradually increased [30]. As a result, lower e.m.f. readings were recorded.

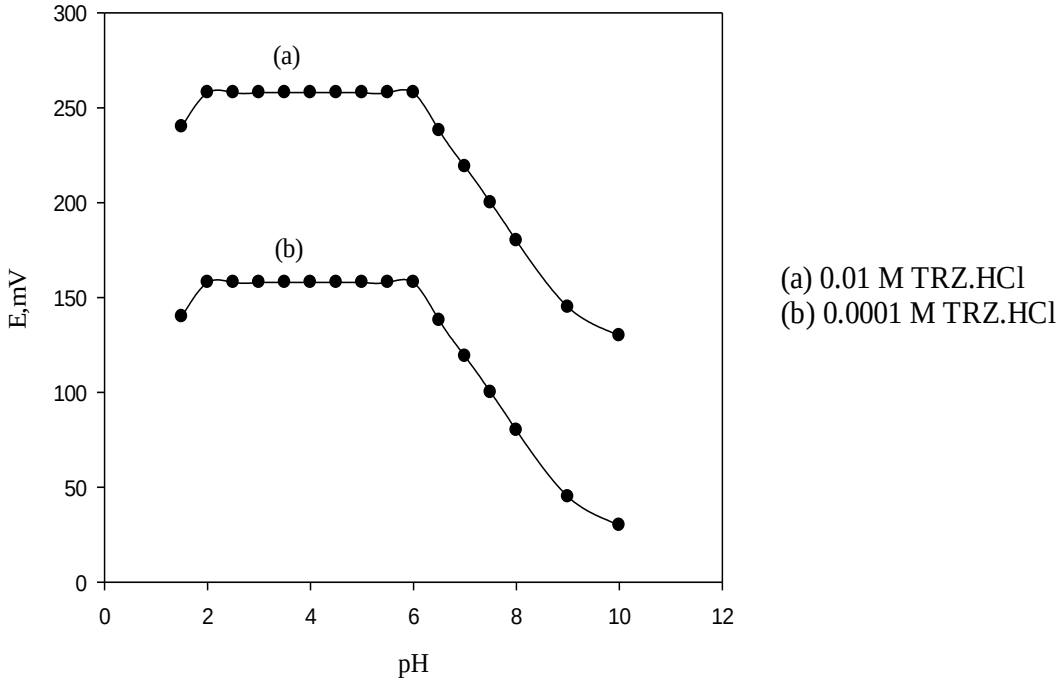

Figure 3. Effect of pH at different TRZ.HCl concentrations using TRZ-PMA CMCP electrode

3.1.4. Effect of temperature

To study the effect of temperature, the electrode potential of 10^{-2}–10^{-7} mol L^{-1} TRZ.HCL solutions were measured at different temperature intervals of 10, 20, 30, 40, 50 and 60 °C and the calibration graphs were constructed. For the determination of the isothermal coefficient (dE^o/dt) of the CPE electrode, the standard electrode potentials (E^o) against the normal hydrogen electrode at the different temperatures were obtained from calibration graphs as the intercepts at p[TRZ.HCl] = 0 (after subtracting the values of the standard electrode potential of the calomel electrode at these temperatures) and were plotted versus (t−25), where t was the temperature of the test solution in °C Figure (4). A straight-line plot is obtained according to Antropov's equation (4) [31, 32].

$E° = E°(25) + (dE°/dt)\ (t-25)$ (4)

Where $E^o(25)$ is the standard electrode potential at 25 °C, the slope of the straight-line obtained represents the isothermal coefficient of the electrode (0.7895 V/°C). The value of the obtained isothermal coefficient of the electrode indicates that the TRZ-PMA electrode has a fairly high thermal stability within the investigated temperature range. The investigated electrode was found to be usable up to 50 °C without noticeable deviation from the Nernstian behaviour.

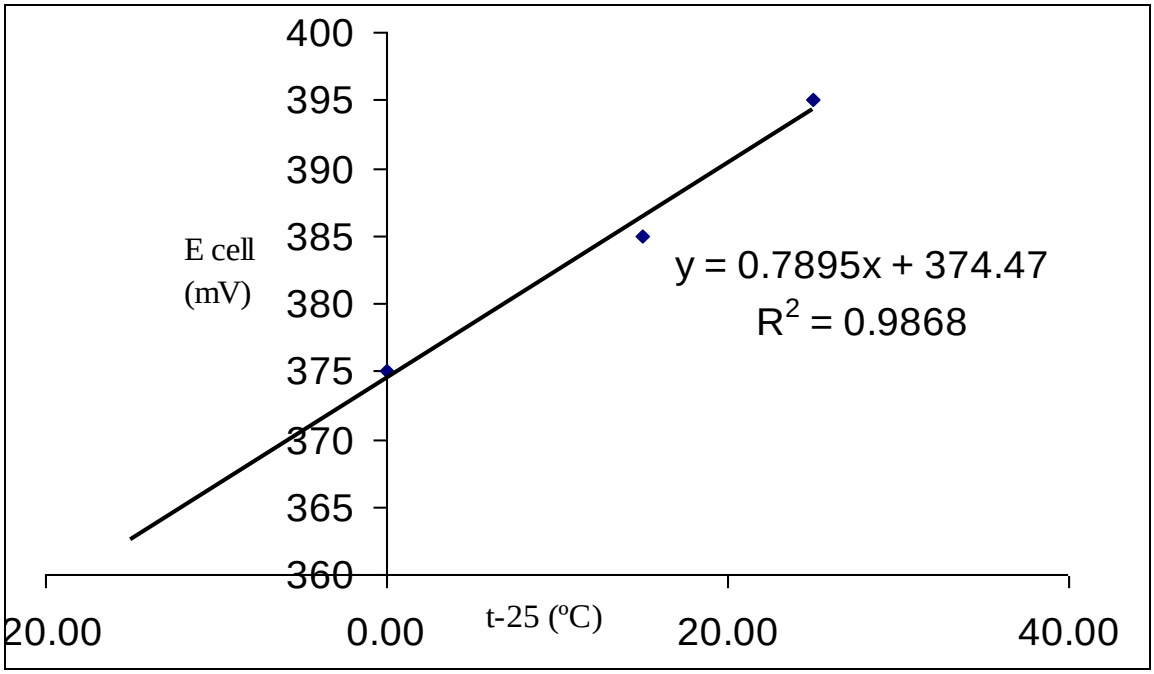

Figure 4. Varriation of the cell emf with the temperature for the CMCP electrode

3.1.5 Response time

The dynamic response time [33] of the electrode was tested by measuring the time required to achieve a steady state potential (within ±1 mV) after successive immersion any one of the two electrodes in a series of TRZ^+ solutions, each having a 10-fold increase in concentration from to 1.0×10^{-6} to 1.0×10^{-2} mol L^{-1}. The electrodes yielded steady potentials within 8-10 s. The potential reading stays constant, to within ±1 mV, for at least 2 minute

3.1.6. Selectivity of the electrode

The influence of some inorganic cations, sugars and glycine on the electrode was investigated. The selectivity coefficients were determined by the modified separate solution method using the rearranged Nicolsky equation (5) [34-36]:

$\text{Log } K_{D,B}^{pot} = ((E_1-E_2)/S) + (1 + (z_1/z_2))\ \log a$ (5)

Where, E_1 is the potential measured in 1×10^{-3} mol L^{-1} TRZ.HCl (D), E_2 the potential measured in 1×10^{-3} mol L^{-1} of the interfering compound (B), z_1 and z_2 are the charges of the TRZ.HCl (D) and interfering species (B), respectively and S is slope of the electrode calibration plot.

Table 3. Potentiometric selectivity coefficient of PTA-TRZ CMCPE plasticized with *o*-NPOE.

Interfering ions (B)	$K^{pot}_{D,B}$
PTA-TRZ CPE	
Glucose	$3.10\text{x}10^{-6}$
Lactose	$4.61\text{x}10^{-6}$
Fructose	$4.59\text{x}10^{-5}$
Maltose	$7.98\text{x}10^{-6}$
Starch	$3.32\text{x}10^{-5}$
Sucrose	$4.67\text{x}10^{-6}$
Glycine	$1.51\text{x}10^{-4}$
Co^{2+}	$2.56\text{x}10^{-4}$
Ni^{2+}	$2.18\text{x}10^{-3}$
Ca^{2+}	$4.09\text{x}10^{-2}$
NH^{4+}	$1.72\text{x}10^{-4}$
Zn^{2+}	$1.56\text{x}10^{-3}$
Na^{+}	$3.90\text{x}10^{-5}$
Cd^{2+}	$6.22\text{x}10^{-5}$
Al^{3+}	$8.12\ \text{x}10^{-5}$

The results obtained are summarized in Table (3). The mechanism of selectivity is mainly based on the stereo-specificity and electrostatic environment, and is dependent on how much matching is present between the location of the lipophilic sites in the two competing species in the bathing solution side and those present in the receptor of the ion exchanger. A reasonable selectivity toward TRZ.HCl in the presence of many nitrogenous compounds such as amines, glycine, and some inorganic cations was observed. The results showed no serious interference by a number of pharmaceutical excipients and active ingredients commonly used in the drug formulations (e.g. glucose, lactose, maltose, fructose, starch and sucrose) at concentration as high as a 10–100-fold molar excess over TRZ.HCl.

3.1.7. Effect of plasticizer

It is well known that the sensitivity and selectivity obtained for a given ion-selective electrode is greatly influenced by the polarity of the electrode matrix, which is defined by the dielectric constant of the electrode plasticizer [37, 38]. The influence of the plasticizer on the TRZ-PMA CMCP electrode performance was studied using five plasticizers having different dielectric constants, namely, *o*-NPOE, DOP, DBP and TCP. Electrode plasticized with *o*-NPOE shows the highest total potential change (280 mV) and the highest potential break at the end point ($\Delta E/\Delta V$ = 570 mV/mL) [39].

No electrode preconditioning is needed before applying in the potentiometric titration and excellent titration curves can be achieved, while electrode fabricated using other plasticizers need either to operate the titration process at least 5 times or to soak the electrode in the aqueous solution of the ion pair for 15 min before using these electrodes in the titration process.

3.1.8. Effect of concentration of TRZ.HCl

The effect of concentration of TRZ.HCl on the performance of the potentiometric titration of TRZ.HCl is investigated by addition of different volumes (1, 3, 5 and 10 mL) of 10^{-2} mol L^{-1} TRZ.HCl drug to the titration medium which prove that the reaction between the drug and PTA or PMA occurs with ratio of 3:1 (i.e.3 $[TRZ]^{+}[PTA]^{-}$ and 3 $[TRZ]^{+}$:$[PMA]^{-}$).

3.1.9. Effect of titrant

The effect of titrant on the performance of the potentiometric titration of TRZ.HCl is investigated as phosphomolybdic acid (PMA) is replaced by ammonium reineckate (RN), phosphotungstic acid (PTA). TRZ.HCl reacts with PMA and PTA in the molar ratio of 3:1 while with RN the ratios are 1:1. The highest total potential change is obtained using PMA Table (4).

Table 4. Potentiometric titration of 3 mL of $1x10^{-2}$ mol L^{-1} TRZ.HCl with different titrants using CMCPE: a) 3.3×10^{-3} mol L^{-1} PMA, b) 3.3×10^{-3} mol L^{-1} PTA, and c) 1×10^{-2} mol L^{-1} RN.

Titrants	Total potential change, mV	Potential break at the end point, mV	ΔE/ΔV mV/mL
PMA	280	196	570
PTA	269	187	555
RN	157	98	289

3.1.10. Life time

Life time, the period in which the electrode functions properly, was measured by plotting the titration curve Figures (5) periodically of TRZ.HCl with PMA standard solution on different days and calculating the total potential change, potential break at the end point and end point investigated the lifetime of the electrodes Table (5). It is clear from the figure that there is a change in the potential break at the end point by 15.8 % after 60 days for *o*-NPOE. A new surface for measurement can be achieved daily by simply squeezing out a small amount of the paste and polishing the electrode surface on a smooth filter paper till a shiny surface is obtained.

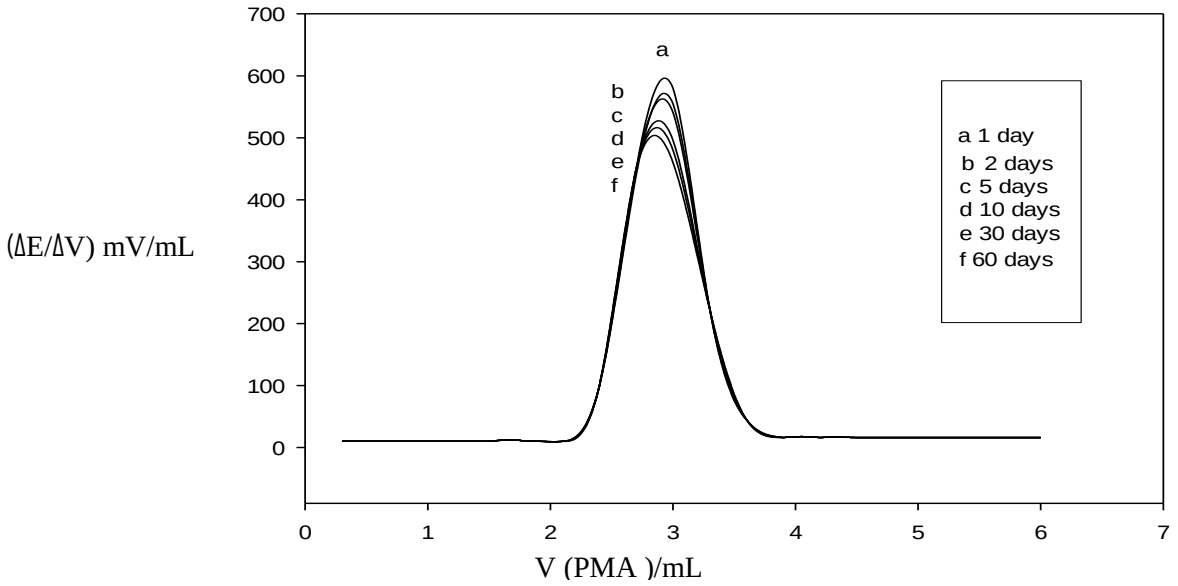

Figure 5. Life time of the carbon paste electrode performance in the potentiometric titration of 3 mL of 10^{-2} mol L^{-1} TRZ.HCl with $3.3x10^{-3}$ mol L^{-1} PMA using *o*-NPOE.

Table 5. Life time of the carbon paste electrode in the potentiometric titration of 3 mL of 10^{-2} mol L^{-1} TRZ.HCl with $3.3x10^{-3}$ mol L^{-1} PMA using *o*-NPOE.

Time (day)	End point (mL)	Recovery %	Total potential change, mV	Potential break at the end point, mV	ΔE/ΔV (mV/mL)
1	2.98	99.33	280	196	570
2	2.98	99.33	280	196	567
3	2.97	99.00	276	190	560
5	2.97	99.00	274	186	555
8	2.97	99.00	274	186	550
9	2.96	98.66	273	184	546
10	2.96	98.66	272	182	541
11	2.95	98.33	272	182	538
17	2.95	98.33	270	180	534
18	2.94	98.00	270	180	528
22	2.94	98.00	270	180	521
25	2.93	97.66	268	175	516
30	2.93	97.66	268	175	511
33	2.93	97.66	267	170	506
38	2.93	97.66	265	168	502
45	2.92	97.33	261	166	496
51	2.92	97.33	261	166	492
59	2.91	97.00	256	163	487
60	2.90	96.66	250	157	480

3.2. Flourimertric Method

3.2.1. Fluorescence spectral properties of TRZ. HCl

TRZ.HCl is freely soluble in water; the solution exhibited an intense native fluorescence in different media, such as water, 0.1 M HCl, 0.1 M NaOH, and methanol. The highest fluorescence intensity was obtained in water; at λ emission 750 nm upon using 330 nm as λ excitation.

Uranyl acetate solution has high fluorescence intensity [40] and when added to the studied drug its fluorescence intensity increases. This behavior has been observed due to formation of binary complex between TRZ. HCl and uranyl acetate, which confers structural rigidity and enhancing fluorescence, Figure (6).

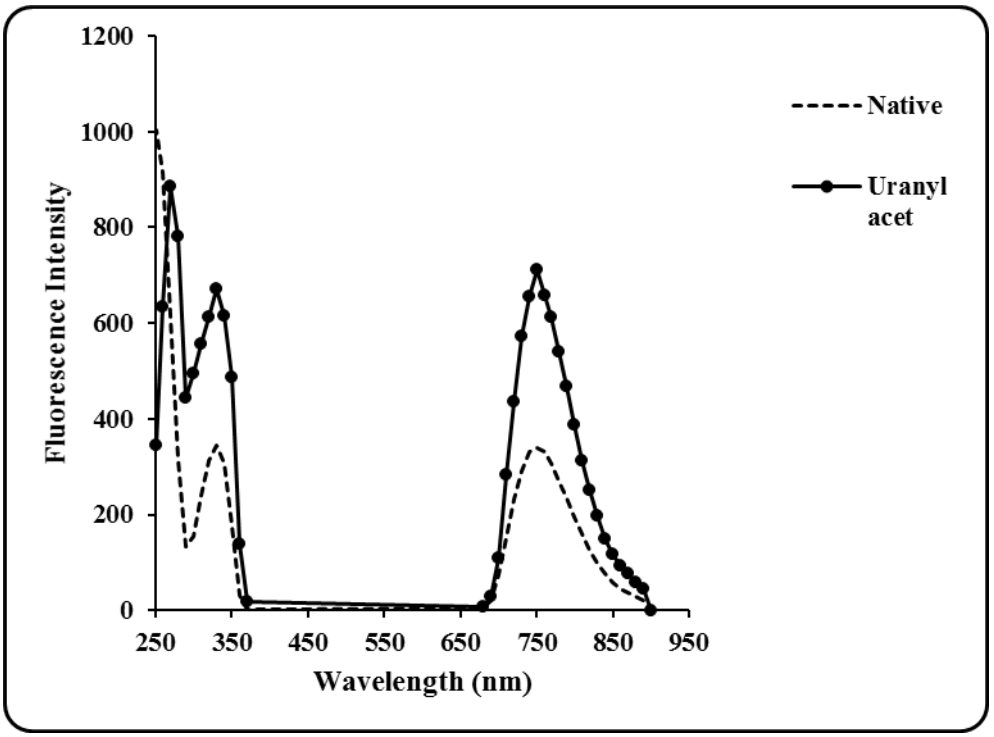

Figure 6. Excitation and Emission spectra of TRZ. solution (0.4 μg mL^{-1}), Excitation and Emission spectra after reaction with uranyl acetate (0.1% w/v) and TRZ.(4 ng mL^{-1}).

Optimum conditions for binary complex formation were studied. Different concentrations of aqueous uranyl acetate ranging from (0.025-0.3 % w/v) were tried and the most suitable volume was also used Figure (7). It was found that 2 ml of 0.1% w/v aqueous solution was adequate for best results. The fluorescence signal was linearly related to the concentration in the range (10-1000 ng ml^{-1}) for native and (0.5-12 ng ml^{-1}) for complex with mean percentage recoveries 100.15 ± 0.226 and 100.41 ± 0.234, respectively. The binary complex is formed immediately and remains stable at least 6 h. as shown in (Table 6).

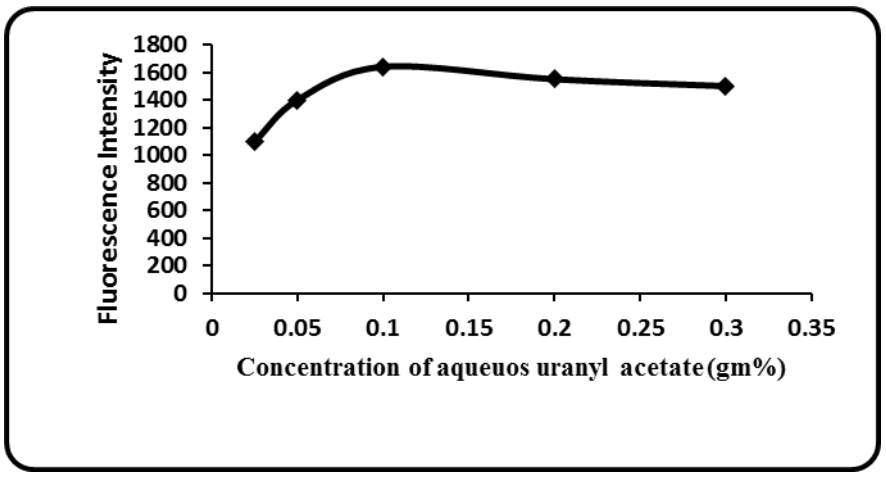

Figure 7. Effect of concentration of aqueous uranyl acetate on the sensitization fluorescence after reaction with TRZ. (10 ng ml^{-1}).

Table 6. Quantitative parameters and statistical data of the regression equations for the fluorimetric analysis of terazocine HCl in their drug substances using native fluorescing and sanitization by using uranyl acetate

Parameters	Terazosin HCl	
	Native method	Uranyl acetate method
$\lambda_{Ex/Em}$	330 / 750	330 / 750
Linearity range (ng ml^{-1})	10 -1000	0.5 – 12
Regression equation [a]	F = 0.843 C – 0.196	F = 166.3C – 30.72
Slop	0.8339	166.313
SD of slope	0.0000412	0.00182
S E of slope	0.014491	2.44933
Confidence limit of slope [b]	0.7967 – 0.87122	160.017 – 172.609
Intercept	- 0.1960	- 30.7232
SD of intercept	0.0000433	0.003967
S E of intercept	7.43457	16.7726
Confidence limit of intercept [b]	- 19.307 – 18.915	-73.8387 - 12.3923
Correlation coefficient (r)	0.9985	0.9987
SE of (r)	13.2159	26.4369
Accuracy (Mean [c] ± RSD %)	100.15 ± 0.226	100.41 ± 0.234
Precision Repeatability	100.2 0 ± 0.216 100.10 ± 0.271	100.07 ± 0.152 100.06 ± 0.096
LOD (ng ml^{-1})	3.47	0.198
LOQ (ng ml^{-1})	10.5	0.6

[a] F = Fluorescence intensity, C = Concentration ng ml^{-1}
[b] mean of six different experiments.
[c] 95% confidence limit.

3.2.2. Determination of Fluorescence Quantum Yield of TRZ.HCl and uranyl acetate:

Diluted solution of quinine sulfate dissolved in 0.05 mol L^{-1} sulfuric acid with fluorescence quantum yield of 0.55 was used as reference reagent, and an equation shown was used to calculate the fluorescence quantum yield of TRZ. [41].

$$Yu = Ys (Fu / Fs) (As / Au). \quad (6)$$

Yu and Ys referred to the fluorescence quantum yield of TRZ. and quinine sulfate, respectively; Fu and Fs represented the integral fluorescence intensity of TRZ. and quinine sulfate, respectively; Au and As referred to the absorbance of TRZ. and quinine sulfate at the excited wavelength, respectively. The concentration was selected so that the absorbance was less than 0.05 to minimize error arising from inner effect [42]. The fluorescence quantum yield found to be 0.42 of the TRZ.HCl with uranyl acetate complex.

3.2.3. Method validation

Validation of the proposed methods was assessed according to USP guidelines [43] for linearity and range, accuracy, precision, detection limit, quantitation limit and robustness.

3.2.3.1. Linearity and range

The fluorescence concentration plot for the studied drug was linear over the range of 10 -1000 $ngml^{-1}$for native method and 0.5 – 12 ng mL^{-1} for uranyl acetate method, Linear regression analysis of the data gave the equations listed in Table (6).

The proposed methods were evaluated by studying the accuracy as percent relative error and precision as percent relative standard deviation, the results are abridged in Table 6.

3.2.3.2. Accuracy

As a part of determining accuracy of the proposed methods, different levels of drug concentrations were prepared from independent stock solution and analysed (N=6). Accuracy was assessed as the standard deviation and percentage relative standard deviation studies were found to be satisfactory Table 6. To give additional support to accuracy of the developed assay method, standard addition method was done. The percent recovery of the added pure drug was calculated as,

$$\% \text{ Recovery} = [(C_y – C_u) / C_a] \times 100 \quad (7)$$

Where C_y is the total drug concentration measured after standard addition. C_u is the drug concentration in the formulation. C_a is the drug concentration added to the formulation Table (7).

Table 7. Results of standard addition method

Method	Concentration of drug in formulation (µg mL^{-1})	Concentration of pure drug added (µg mL^{-1})	% level of pure drug added	Total concentration of drug found (µg mL^{-1})	% Analytical recovery ± SD
Native method	5	0.02	2	5.01	99.8±0.01
	5	0.2	20	5.21	100.2±0.15
	5	0.8	80	5.82	100.4±0.12
Uranyl acetate method	5	0.004	0.4	5.003	99.9±0.03
	5	0.008	0.8	5.009	100.2±0.08
	5	0.012	1.2	5.03	100.4±0.01

Each value is a result of three separated determination

3.2.3.3. Precision

Repeatability was determined by using different levels of drug concentration (same concentration levels taken in accuracy study), prepared from independent stock solution and analysed (N=6) Table 1. Inter-day and intra- day variation and instrument variation were taken to determine intermediate precision of the proposed methods (N=6). The % relative standard deviation of the predicated concentrations from the regression equation was taken as precision Table (6).

3.2.3.4. limit Of Detection (LOD) and Limit Of Quantitation (LOQ)

The LOD and LOQ of the terazocin HCl by proposed methods were determined by using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ /S, respectively, where S is slope of the calibration curve and σ is standard deviation of y-intercept of regression equation Table (6).

3.3. Analytical application

The proposed electrodes were proved useful for the assay of terazosin in the drug substance and pharmaceutical product by potentiometric titration using phosphomolybdic acid as a titrant. The end point was determined by the first derivative method. The accuracy and precision were tested at concentration 20 mg/50 mL, five samples (20 mg/50 mL) were used. The mean recovery obtained was within ± 2% for the drug substance and Itrin®, Prostasine tablets, respectively. The relative standard deviation was ≤2.0%, indicate reasonable repeatability and reproducibility of the proposed methods. The accuracy and precision of the assay of terazocin hydrochloride in the drug substance and in Itrin®, Prostasine tablets using the proposed electrodes are summarized in Table (8).

The application of fluorimetric determination of TRZ.HCl in the drug substance and pharmaceutical products gives good results as shown in Tables (9,10).

3.4. Statistical treatment of the results

Table 8. Accuracy and precision for quantification of TRZ.HCl in the drug substance**,** Itrin® and Prostasin tablets Using TRZ-PMA CMCPE plasticized with *o*-NPOE.

Sample	Proposed		Official		% Recovery		SD* (RSD*, %)	SD** (RSD**, %) [Drug]
	[Drug] mg		[Drug] mg		Ion selective method	Official method[1,16]		
	Taken	Found	Taken	Found				
Drug substance	20	19.89	20	19.65	99.45	98.25	0.031 (0.16)	0.056 (0.28)
Itrin® tablets (5 mg TRZ per tab.)	20	19.86	20	19.65	99.3	99.950	0.010 (0.050)	0.06 (0.061)
Prostasin tablets (5 mg TRZ per tab.)	20	19.71	20	19.65	98.55	99.950	0.010 (0.050)	0.032 (0.162)

* Average of four determinations.

** Average of four determinations.

Table 9. Analysis Terazocine HCl in its drug substances using native fluorescing method and sanitization by using uranyl acetate method, and potentiometric method compared with the offecial titrimetric method.

Parameters	Fluorometric method		
	Native method	Uranyl acetate method	Reference method[1] BP
Mean Recovery %	100.09	100.42	100.05
±RDS [a] %	0.268	0.169	0.129
Variance	0.072	0.029	0.016
SE	0.0346	0.069	0.053
t-test	0.63	4.25	(2.228)[b]
F-test	4.3	1.72	(5.1)[b]
	Potentiometric method		
	TRZ-PMA	TRZ-PTA	Reference method[1] BP
Mean Recovery %	99.35	98.95	99.2
±RDS [a] %	0.16	0.279	0.129
Variance	0.025	0.076	0.016
SE	0.065	0.113	0.053
t-test	1.87	3.125	(2.228)[b]
F-test	1.5	4.50	(5.1)[b]

[a] Average of six different experiments.

[b] Theoretical values.

Table 10. Analysis of terazosin HCl in their drug products by the proposed (native fluorescing and formation of uranyl acetate complex), and potentiometric method methods and compared with the reported method.

Parameters	Itrein 5 mg			Prostasin 5 mg		
	Fluorimetric method		Reported HPLC Method[6]	Fluorimetric method		Reported HPLC Method[6]
	Native method	Uranyl acetate method		Native method	Uranyl acetate method	
Mean*	100.2	100.08	100.1	100.5	100	99.36
± RDS %	0.224	0.237	0.225	1.11	0.48	0.61
Variance	0.050	0.056	0.051	1.23	0.23	0.372
SE	0.091	0.096	0.092	0.453	0.272	0.262
t- test	0.833	0.15	(2.228)[a]	2.1	1.91	(2.228)[a]
F-test	1.02	1.09	(5.1)[a]	3.3	1.61	(5.1)[a]
	Potentiometric method using TRZ-PMA		Reported method[16]	Potentiometric method TRZ-PMA		Reported method[16]
Mean*	99.3		99.550	98.55		98.95
±RDS%	0.061		0.050	0.162		0.100
Variance	0.0036		0.0025	0.025		0.0096
SE	0.025		0.020	0.065		0.020
t- test	0.78		(2.228)[a]	5.88		(2.228)[a]
F-test	1.44		(5.1)[a]	2.6		(5.1)[a]

The calculated F values [44] were less than the tabulated F values where υ1 =6 and υ2 = 6 for batch condition at 95% confidence level. The t-test [48] was also done at 99.9% confidence level and the results are shown in Tables (9, 10). The applied method does not exhibit significant difference in comparison with the reference method [1, 6,16] which reflects the accuracy and precision of the proposed methods.

4. CONCLUSION

The proposed chemically modified carbon paste electrode based on terazosin phosphomolybdate or phosphotungestate as electroactive ion-exchangers might be a useful alternative analytical tool for the determination of TRZ^+ in different samples. Table (11) compares the slope, response time, detection limit, and life time of the proposed terazosin electrodes with those terazosin -

selective electrodes reported in the literature. We can observe that, our electrodes show a wider linear range, lower detection limit, and faster response time than those reported in the literature [16]. The electrodes were used successfully for the determination of TRZ^+ content in pure solution and in pharmaceutical formulations.

The two spectrofluorimetric methods proved to be successful, providing a rapid, simple and low cost for the determination of TRZ.HCl in pure solutions and in pharmaceutical preparations.

Table 11. General performance characteristics of the previously developed terazocin electrodes

Ion-recognition+ plasticizer + supporting material	Slope mV decade^{-1}	Detection limit mol L^{-1}	Linear range (mol L$^{-1)}$	Response time (s	Reference
7%TRZ-TPB/30% PVC/63% DBP	59.5	of 7.9×10^{-6}	1×10^{-5}-1×10^{-2}	≤ 15	[16]
5%TRZ-PMA/ 47.5%C/47.5%NPOE	58.4 ± 0.35	8×10^{-7}	1×10^{-6}-1×10^{-2}	< 8-10	This work
5%TRZ-PTA/ 47.5%C/47.5%NPOE	57.3± 0.23	6×10^{-7}	2×10^{-6}-1×10^{-2}	<8-10	This work

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