Construction and performance characterization of screen printed and carbon paste ion selective electrodes for potentiometric determination of naphazoline hydrochloride in pharmaceutical preparations†

Eman Y. Z. Frag,* Gehad G. Mohamed, F. A. Nour El-Dien and Marwa E. Mohamed

Received 22nd May 2010, Accepted 10th September 2010
DOI: 10.1039/c0an00343c

This paper describes the development of screen-printed (SPE) and carbon paste (CPE) sensors for the rapid and sensitive quantification of naphazoline hydrochloride (NPZ) in pharmaceutical formulations. This work compares the electroactivity of conventional carbon paste and screen-printed carbon paste electrodes towards potentiometric titration of NPZ. The repeatability and accuracy of measurements performed in the analysis of these pharmaceutical matrices using new screen printed sensors were evaluated. 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 Nernstain responses in the concentration range from 1×10^{-6} to 1×10^{-2} mol L^{-1} with slopes of 57.5 ± 1.3 and 55.9 ± 1.6 mV per decade for SPE and CPE, respectively, and was found to be very precise and usable within the pH range 3–8. These sensors exhibited a fast response time (about 3 s for both SPE and CPE, respectively), a low detection limit (3.5×10^{-6} and 1.5×10^{-6} M for SPE and CPE, respectively), a long lifetime (3 and 2 months for SPE and CPE, respectively) and good stability. The selectivity of the electrode toward a large number of inorganic cations, sugars and amino acids was tested. It was applied to potentiometric determination of NPZ in pure state and pharmaceutical preparation under batch conditions. The percentage recovery values for the assay of NPZ in tablets (relative standard deviations $\leq 0.3\%$ for $n = 4$) were compared well with those obtained by the official method.

1. Introduction

Naphazoline hydrochloride (NPZ) (Fig. 1) has the IUPAC name 2-(1-naphthylmethyl)-2-imidazoline monohydrochloride of molecular weight 246.74g mol^{-1} and molecular formula $C_{14}H_{14}N_2HCl$.[1] It acts as an ocular vasoconstrictor where it constricts the vascular system of the conjunctiva. It is presumed that this effect is due to the direct stimulation action of the drug upon the alpha adrenergic receptors in the arterioles of the conjunctiva resulting in decreasing the conjunctival congestion. Naphazoline belongs to the imidazoline class of sympathomimetics.

A number of studies were described for the determination of NPZ in both pure and pharmaceutical samples including micellar electrokinetic chromatography,[2,3] high-performance liquid chromatographic (HPLC),[4] spectrophotometric[5–10] and potentiometric methods.[11] Most of these methods involve time-consuming procedures and use of sophisticated instruments.

Ion-selective membrane electrodes are now widely used for the direct potentiometric determination of ion activities or ion concentrations in different samples.[12–17] Particularly, the feasibility, their use in continuous as well as *in situ* applications impose a strong competition on the currently established methods like official chromatographic methods. Under a variety of membrane types, solvent polymeric membranes have proved to be especially suited for clinical analysis since they can easily be manufactured in different sizes and shapes and are less affected by the response of biological substrates such as protein, enzyme, antibody,…[18–20] Their advantages are simple design, low cost, adequate selectivity, low detection limit, high accuracy, wide concentration range and applicability to coloured and turbid solutions.[21] The membranes were made from liquid and plasticized polyvinylchloride (PVC) and are based on a water-insoluble ion-pair complex acting as ion-exchanger.[22,23] Some problems have been encountered with the membranes, *e.g.* the requirement of extensive pre-conditioning treatment, care in

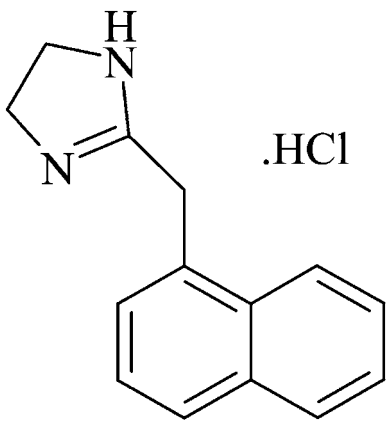

Fig. 1 Structure of naphazoline hydrochloride.

Chemistry Department, Faculty of Science, Cairo University, Gamaa Str., 12613, Giza, Egypt. E-mail: e_uossry@yahoo.com

† Electronic supplementary information (ESI) available: Schematic diagrams. See DOI: 10.1039/c0an00343c

storage, and sensitivity to some hydrophobic counter ions and a relative short lifetime.

Screen-printing technique seems to be one of the most promising approaches allowing simple, rapid and inexpensive biosensors production.[24,25] The biosensors based on screen-printed electrodes have been extensively used for detections of biomolecules, pesticides, antigens and anions.[26] Electrochemical biosensors based on screen-printed electrodes are in tune with the requirements of *in situ* screening devices, since all the equipment needed for the electrochemical analysis is portable. They have all the major performance characteristics of biosensors, among them the minimum sample preparation, the simplicity of the apparatus, the obtaining of fast results, moreover they are cost effective, small and becoming miniaturized with new technologies.[27]

In this paper, we performed a screen-printed and carbon paste sensors for rapid and sensitive quantification of NPZ in both pure and pharmaceutical preparations. The present work aims to fabricate new screen-printed carbon paste electrodes (SPEs) as a potentiometric NPZ sensor using home made printing carbon ink as well as comparing the performance of such electrodes with those of the CPEs and conventional PVC (polyvinyl chloride) membrane electrodes. The proposed method shows adequate sensitivity, low cost, versatility, simplicity and effectiveness. Our aim was to develop a new method based on ion selective electrodes (SPE and CPE) able to analyze pharmaceuticals formulations, avoiding or minimizing the number of steps needed to assess the concentration of the NPZ.

2. Experimental

2.1. Reagents

All chemicals and reagents used were of analytical reagent grade. Bi-distilled water was used throughout all experiments. Naphazoline hydrochloride (NPZ) provided by Misr Company for Pharmaceutical Industry and its Pharmaceutical preparation Neozoline (Eye/Nasal drops) was produced by Amoun Pharmaceutical Company, El-Obour City, Cairo, Egypt (each 100 mL contains 50 mg naphazoline hydrochloride). o-Nitrophenyloctylether (*o*-NPOE) was supplied from Fluka, while dioctylphthalate (DOP), dibutylphthalate (DBP) and dioctylsebacate (DOS) were purchased from BDH. Tricresylphosphate (TCP), polyvinylchloride (PVC relative high molecular weight) and graphite powder (synthetic 1–2 μm) were supplied from Aldrich. The polyacetate sheet (Fuji Medical X-ray Film) is supplied from Fuji Flim Co, Tokyo, Japan. The silver wire has a purity of 99.9% and it is supplied from Zhejiang Leyin Alloy Co., Ltd., China.

Potassium tetraphenylborate (KTPB) and ammonium reineckate (RN) $[NH_4(Cr(NH_3)_2(SCN)_4)\cdot H_2O]$ were supplied from Fluka. Phosphotungstic acid (PTA); $H_3[PW_{12}O_{40}]$, and phosphomolybdic acid (PMA); $H_3[PMo_{12}O_{40}]$, were purchased from BDH.

2.2. Solutions

10^{-2} mol L^{-1} NPZ solution was prepared by dissolving the accurate weighed amount in a definite volume of bi-distilled water to get the required concentration. KTPB solution (10^{-2} M) was prepared by dissolving an accurate weighed amount in warm water, adjusted to pH 9 by adding sodium hydroxide and completed to the desired volume with bi-distilled water. The resulting solution was standardized potentiometrically against standard (10^{-2} M) thallium(I) acetate solution.[28]

2.3. Apparatus

Laboratory potential measurements were performed using 716 DMS Titrino Metrohm connected with 728 Metrohm stirrers. This Titrino had a combined electrode, which was more convenient to be used, equipped with silver–silver chloride double-junction reference electrode (Metrohm 6.0222.100) in conjugation with different ion selective electrode. Digital multimeter connected to a portable PC and Brand digital burette was used for the measurement of the drug under investigation.

2.4. Electrode preparation

2.4.1. Screen-printed electrodes. A polyacetate sheet (sheet for X-ray cleaned with concentrated HNO_3 and washed several time with water and then cleaned with commercial acetone) was used as a substrate which was not affected by the curing temperature or the ink solvent and easily cut by scissors. The graphite ink was prepared by mixing 1.8 g o-NPOE, 5 g 8% PVC solution and 3 g carbon powder. The well mixed graphite ink was poured onto the mesh and forced into the mesh with the aid of a 6 inch squeegee (Sericol SE–C52 medium hardness) held at angle of approximately 60 °C and mesh was held away from the polyacetate sheet. The squeegee was then pulled back across the template; this ensured the electrode templates were fully load with the ink. The wooden frame was pushed down onto the polyacetate sheet and the squeegee drawn across the template in a single swift action, which forced the ink through the mesh and onto the epoxy acetate sheet (Suppl. Fig. 1†). The stencil frame was then released, revealing the electrodes printed onto the polyacetate sheet.[29,30] The electrodes were cured at 60 °C for 2 h and then cut out from the substrate. After finishing the printing process, the stencil was cleaned with commercial acetone solution in order to remove the excess of ink within the template.

2.4.2. Preparation of carbon paste electrode (CPE). The CPE electrode was prepared by intimate mixing accurately weight (500 mg) of highly pure graphite powder and plasticizer (0.2 ml of DOP, TCP, DBP, DOS or *o*-NPOE). This matrix was thoroughly mixed in the mortar and the resulting past was used to fill the electrode body (Suppl. Fig. 2†). A fresh surface was obtained by gently pushing the stainless-steel screw forward and polishing the new carbon-paste surface with filter paper to obtain a shiny new surface.[29,30]

2.4.3. Preparation of coated wire and coated graphite electrodes. For the preparation of silver CWEs, the silver wire 1.5 mm diameter was sealed into the end of PVC tube. The wire was polished, carefully cleaned in strong ammonia solution, rinsed, carefully dipped in 50% nitric acid for 1 min and rinsed with distilled water. The wire was cathodized[21,22] against a silver anode at 5 mA cm^{-2} in 0.1 M HCl for 30 s, the bubbles formed near the electrode were allowed to disperse from the wire and the

electrode was washed and left to dry. The electrode was immersed in the cocktail consisting of (240 mg *o*-NPOE + 240 mg PVC + 6 ml THF) 20 times. After each immersion in the cocktail, the solvent was evaporated using an air gun.[29]

Consequently, a commercially available pencil of fine Japanese type was used as a graphite-based material for the ion-sensitive electrode. The rod was immersed in chloroform for 10 min, formed to the required size and then ignited in a colourless flame for 1 min. After cooling, the rod was mounted into a Teflon tube. The open end of the tube was then connected to a slight vacuum and the other end containing carbon rod was immersed into the cocktail (240 mg *o*-NPOE + 240 mg PVC + 6 ml THF) 20 times and after each immersion, the solvent was evaporated using an air gun. The electrode was kept dry at room temperature for 24 h and preconditioned by soaking in a drug/ion pair suspended solution for 24 h.[29]

2.5. Sample analysis

The potentiometric titration of NPZ solution: aliquots of the drug solution containing 7.4 mg were transferred into a 25 ml beaker and titrated with a standard solution of KTPB (10^{-2} mol L^{-1}) using the different fabricated electrodes (SPE, CPE, PVC, CWE and CGE) as indicator electrodes. The end points were determined from the S-shaped curves using the first derivative plots. In the calibration graph method, the indicator electrodes (SPE and CPE) plasticized with o-NPOE was immersed in conjunction with the double junction Ag/AgCl reference electrode in solutions of NPZ in the range of 10^{-2}–10^{-7} M. The electrodes (SPE and CPE) were allowed to equilibrate whilst stirring and recording the e.m.f. readings within ±1 mV. The sensors were washed between measurements with water. The e.m.f. was plotted as a function of the logarithm of the NPZ concentration.

3. Results and discussion

The isolated solid [NPZ]:[TPB] ion pair has a white colour and is characterized using elemental analysis which indicates the formation of 1 : 1 [NPZ]:[TPB] ion pair with calculated: % C = 86.20, %H = 6.62, % N = 5.29 and found: %C = 86.48, %H = 6.14 and % N = 5.38.

3.1. Optimization of the printing ink composition and printing process

The first part of the present work is oriented to the optimization of the printing inks formulations, where the performance of the printed electrodes is tested in the potentiometric titration of NPZ with KTPB.

3.2. Effect of soaking

Freshly prepared SPE electrodes must be soaked in NPZ–TPB ion pair suspended solution in order to activate the surface of the carbon paste layer 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;[11] thus, the performance characteristics of the NPZ ion-selective SPEs was investigated as a function of soaking time. For this purpose the SPE was soaked in aqueous suspension of NPZ ion pair and the titration curves were plotted from which the total potential changes are recorded after 0, 15, 30, 60, 90, 120 and 180 min and 12 and 24 h (Fig. 2). The optimum soaking time was found to be 15 min, when the slope of the calibration curves was 57.5 mV per decade, at 25 °C. Soaking for a longer time i.e. more than 15 min is not recommended to avoid leaching, although very little of the electroactive species does leach into the bathing solutions.[11,31] The SPE should be kept dry in an opaque closed vessel and stored in a refrigerator while not in use.

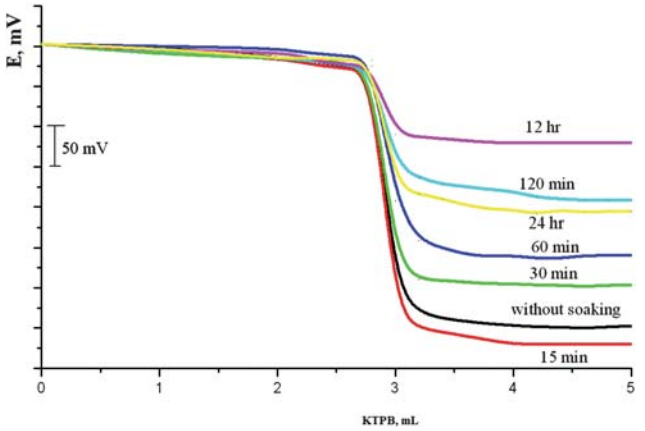

Fig. 2 Effect of soaking time on the SPEs performance in the potentiometric titration of 3 mL of 10^{-2} M NPZ with 10^{-2} M KTPB.

3.3. Effect of plasticizer type

The role of plasticizer may be considered analogous to that of the organic solvent in liquid membrane electrodes where it influences both the selectivity and sensitivity of the electrode. When the SPEs are used to monitor the potentiometric titration based on ion pair formation, the magnitude of both the potential break and sharpness at the inflexion point of the titration curve is predetermined by the plasticizer polarity (dielecterical constants, ε) as a result of higher extractability of the ion pair into the plasticizer.[32] The influence of the plasticizer choice on the electrode performances has been studied as the SPE plasticized with *o*-NPOE is compared with those plasticized with DBP, DOP, DOS, or TCP (Fig. 3 and Table 1). From the all tested plasticizers, *o*-NPOE shows the highest total potential change and the highest potential break at the end point which may be attributed to the high dielecterical constant of *o*-NPOE and the high extractability of the formed NPZ ion pair into the electrode matrix compared with other tested plasticizers (ε values are 24, 3.88, 5.2, 4.7 and 17.6, for *o*-NPOE, DOS, DOP, DBP and TCP, respectively). Due to the high extractability of the formed ion pair in the electrode matrix, no electrode preconditioning is needed before applying in the potentiometric titration and excellent titration curves can be achieved from the second titration process, while electrodes fabricated using other plasticizers need either to operate the titration process at least 5–7 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.

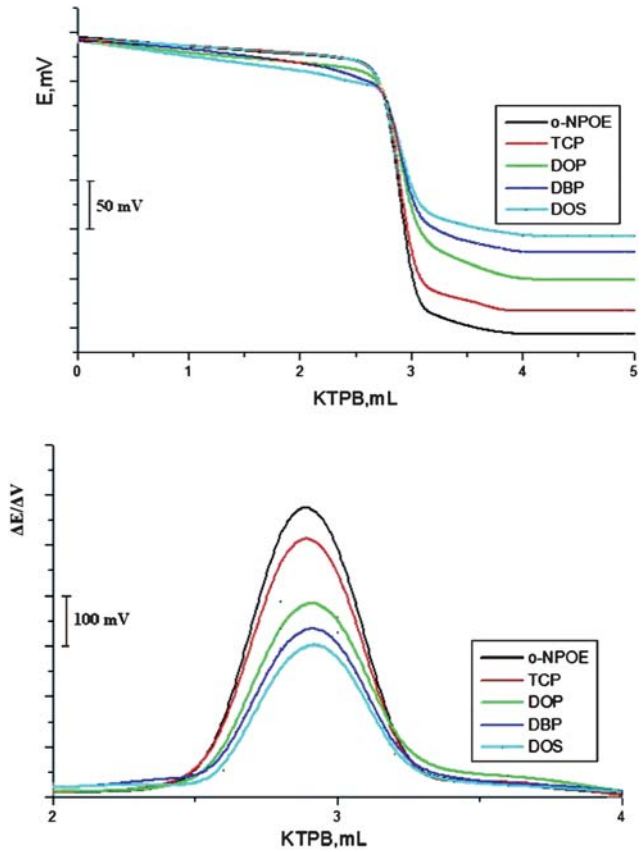

Fig. 3 The effect of plasticizer type on the SPCPEs performance in the potentiometric titration of 3 mL of 10^{-2} M NPZ with 10^{-2} M KTPB.

Table 1 Effect of the plasticizer type on the SPEs performance in the potentiometer titration of 3 mL of 10^{-2} M NPZ with 10^{-2} M KTPB

Plasticizer	Total potential change, mV	Potential break at the end point, mV	ΔE/ΔV
o-NPOE	302	220	617
TCP	276	193	546
DOP	243	139	407
DBP	215	127	357
DOS	198	115	327

Different plasticizers will exhibit different characteristics in both the ease with which they form the plasticized material and in the resulting mechanical and physical properties of the flexible product. According to the Lubricating Theory of plasticization,[33] the plasticizer molecules diffuse into the polymer and weaken the polymer-polymer interactions (van der Waals' forces). The plasticizer molecules act as shields to reduce polymer-polymer interactive forces and prevent the formation of a rigid network. This lowers the PVC Tg and allows the polymer chains to move rapidly past each other, resulting in increased flexibility, softness, and elongation.

The mechanistic explanation of plasticization considers the interactions of the plasticizer with the PVC resin macromolecules. It assumes that the plasticizer molecules are not permanently bound to the PVC resin molecules but are free to self-associate and to associate with the polymer molecules at certain sites such as amorphous sites. As these interactions are weak, there is a dynamic exchange process whereby, as one plasticizer molecule becomes attached at a site or center, it is readily dislodged and replaced by another. Different plasticizers yield different plasticization effects because of the differences in the strengths of the plasticizer-polymer and plasticizer-plasticizer interactions. At low plasticizer levels, the plasticizer-PVC interactions are the dominant interactions, while at high plasticizer concentrations plasticizer-plasticizer interactions can become more significant. The polar portion of the molecule must be able to bind reversibly with the PVC polymer, thus softening the PVC, while the non-polar portion of the molecule allows the PVC interaction to be controlled so it is not so powerful a solvator as to destroy the PVC crystallinity. Plasticizers have a strong affinity for PVC polymers, but do not undergo a chemical reaction that causes bonding, or grafting, to the polymer.

3.4. Effect of pH

The effect of pH on the performance of the potentiometric titration of the drug with KTPB was evaluated in concentrations of 1.0×10^{-2} and 1.0×10^{-6} M of NPZ at different pH values (2–12) by addition of small volumes of HCl and/or NaOH solution (0.1 – 1 M of each) to the titration medium using SPE and CPE. The total potential change and the potential break at the end point at each pH value were calculated. It is obvious that, within the pH range from 3.0 to 8.0 the electrode potential is practically independent of pH, and in this range the electrodes (SPCPE and CPE) can be safely used for NPZ determination (Fig. 4 and 5). The decrease in mV readings at pH < 3 may be due to interference of hydronium ion. At higher pH values (pH > 8.0), free-base precipitates in the test solution and consequently, the concentration of unprotonated species gradually increased. As a result, lower e.m.f. readings were recorded. Therefore, pH 3 is recommended for subsequent studies.

3.5. Response time

For analytical applications, the response time of a fabricated sensor is of critical importance. The average time required for the electrode to reach a steady potential response within ±1 mV of

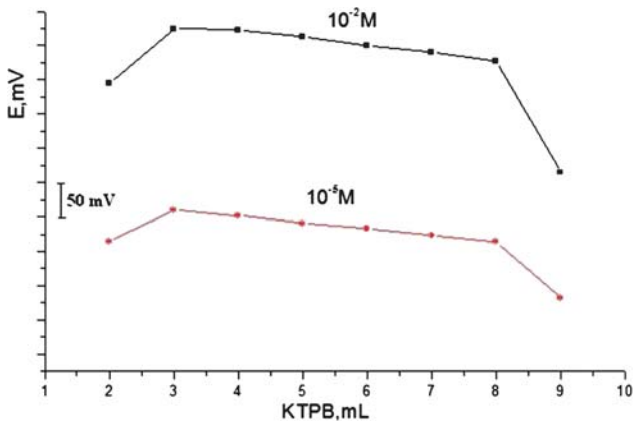

Fig. 4 Effect of pH (2–9) using SPCPE.

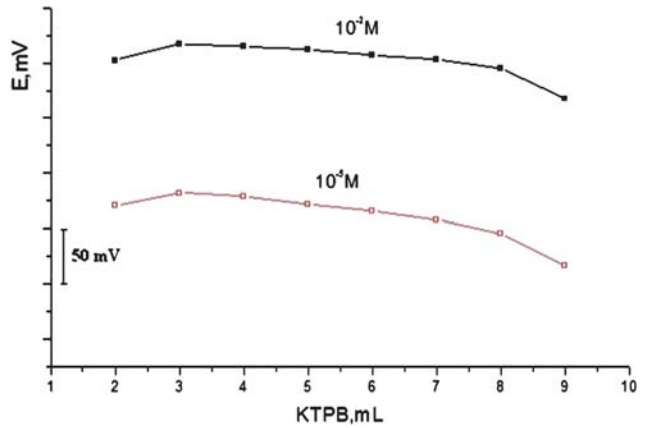

Fig. 5 Effect of pH (2–9) using CPE.

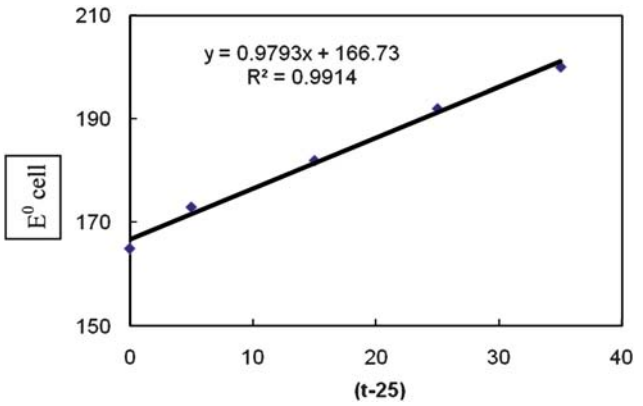

Fig. 6 Variation of the cell e.m.f. with the temperature for the NPZ electrode.

the final equilibrium value after successive immersion of a series of NPZ solutions, each having a 10-fold difference in concentration, is investigated.[34] The electrode response time of SPE and CPE is found to be 3 s which is much shorter than any previously mentioned drug electrode[35] and the equilibrium potentials essentially remained constant for over 10 min. This fast and stable potential reading is reflected on the time needed for complete titration process as it is only about 3–5 min.

3.6. Life time

All the printed sensors have high mechanical durability and good adherent to the PVC substrate. For the determination of the storage stability, sensors fabricated in the same production cycle have been used. Every week a new electrode is used in the potentiometric titration of NPZ with KTPB under optimum conditions on different days and every time the measurement of the total potential change, potential break at the end point and end point are recorded using CPE and SPEs using *o*-NPOE, DOS, DOP, DBP and TCP as plasticizers. It was found that the proposed SPEs showed longer shelf-life and operational time when compared with CPE tested within the present study. Although the electrode is a disposable device, longer stability tests are also investigated and the electrodes are successfully used for at least 50 consecutive titration processes (in duration of 3 months) without a significant change in the potential break at the end point.

3.7. Effect of temperature of the test solution

Calibration graphs (electrode potential (E_{elec}) *versus* p[NPZ]) were constructed at different test solution temperatures (25, 30, 40, 50, 60 and 65 °C) using SPE. For the determination of the isothermal coefficient (dE^0/dt) of the electrode, the standard electrode potentials (E^0) against the normal hydrogen electrode at the different temperatures were obtained from calibration graphs as the intercepts at p[NPZ] = 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 (Fig. 6). A straight-line plot is obtained according to Antropov's equation:[36]

$$E^0 = E^0_{(25)} + (dE^0/dt)(t\text{-}25)$$

where $E^0_{(25)}$ is the standard electrode potential at 25 °C, the slope of the straight-line obtained represents the isothermal coefficient of the electrode (0.001 V $°C^{-1}$). The value of the obtained isothermal coefficient of the electrode indicates that the SPE electrode has a fairly high thermal stability within the investigated temperature range. The investigated SPE was found to be usable up to 60 °C without noticeable deviation from the Nearnestian behaviour.

3.8. Selectivity of the SPE and CPE

The selectivity coefficient (log $K^{pot}_{D,B}$) of the SPE and CPE was determined employing separate solution method (SSM) with the rearranged Nicolsky equation:[37–39]

$$\log K^{pot}_{D,B} = ((E_1\text{-}E_2)/S) + (1 + (Z_1/Z_2))\log a$$

where, E_1 is the potential measured in 1×10^{-3} mol L^{-1} NPZ (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 NPZ (D) and interfering species (B), respectively, and S is slope of the electrode

Table 2 Potentiometric selectivity coefficient of o-NPOE plasticized SPE and CPE

	$K^{pot}_{D,B}$	
Interfering ions (B)	SPE	CPE
Glucose	1.13×10^{-6}	8.13×10^{-7}
Lactose	8.19×10^{-7}	7.18×10^{-7}
Fractose	1.43×10^{-6}	7.80×10^{-7}
Maltose	8.52×10^{-7}	1.18×10^{-6}
Starch	8.19×10^{-7}	8.13×10^{-7}
Sucrose	6.97×10^{-7}	7.79×10^{-7}
Glycine	1.49×10^{-6}	1.23×10^{-6}
p-Aminophenol	1.80×10^{-6}	4.60×10^{-6}
Ascorbic acid	3.19×10^{-6}	2.82×10^{-6}
Ca^{2+}	3.71×10^{-5}	3.44×10^{-5}
NH_4^+	1.17×10^{-6}	1.28×10^{-6}
K^+	6.70×10^{-7}	2.11×10^{-6}
Na^+	1.08×10^{-6}	6.08×10^{-7}
Cd^{2+}	7.04×10^{-5}	7.25×10^{-5}

calibration plot. The results obtained are summarized in Table 2. A reasonable selectivity toward NPZ in the presence of many nitrogenous compounds such as amines, amino acid, and some inorganic cations was observed. The results showed no serious interference by a number of pharmaceutical excipients, diluents 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 NPZ.

3.9. Between day assays

For the developed drug electrode (SPE), four different titration runs of 3 mL of 10^{-2} mol L^{-1} NPZ with 10^{-2} mol L^{-1} KTPB standard solution are performed on 4 different days, in order to evaluate the reproducibility of the results obtained. Table 3 gives a statistical summary of each of the titration series using the SPEs, including the means of the end point volumes and the potential break at the end point.

Table 3 Between-day precision of the potentiometric titration of 3 mL of 10^{-2} M NPZ with 10^{-2} M KTPB using SPE

Analytical method	End point			ΔE/ΔV			Total potential change		
	mL	SD[a]	RSD[a]	mV mL^{-1}	SD[a]	RSD[a]	mV	SD[a]	RSD[a]
SPE	2.97	0.005	0.19	619	10.90	1.76	215.0	4.27	1.99

[a] Average of four determinations

Table 4 Potentiometric titration of 3 mL of 10^{-2} M NPZ with different titrants (a) 1×10^{-2} M KTPB, (b) 1×10^{-2} M RN, (c) 3.3×10^{-3} M PTA, (d) 3.3×10^{-3} M PMA, using SPE

Titrants	Total potential change, mV	Potential break at the end point, mV	ΔE/ΔV
KTPB	355	220	617
RN	217	92	361
PTA	193	87	308
PMA	158	84	264

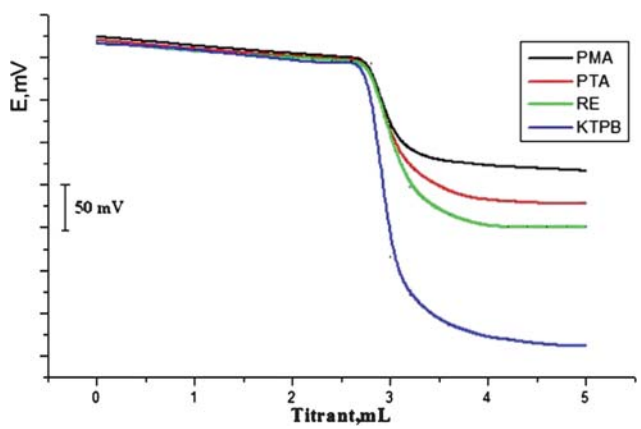

Fig. 7 Potentiometric titration of 3 mL of 10^{-2} M NPZ with different titrants using SPCPEs.

Table 5 Potentiometric determination of 2.03×10^{-3} M NPZ drug in pharmaceutical formulation against 2.03×10^{-3} M KTPB using SPE and CPE plasticized with o-NPOE and TCP

Sample	Taken/mg mL^{-1}	SPE			CPE			British pharmacopoeia	
		Found/mg mL^{-1}	Recovery (%)	RSD[a]	Found/mg mL^{-1}	Recovery (%)	RSD[a]	Found/μg mL^{-1}	Recovery (%)
Neozoline		1.48	99.0	0.10	1.46	97.30	0.3	2.95	98.40
(a) using o-NPOE	1.5								0.22
(b) using TCP		1.47	98.30	0.27	1.46	97.20	0.35	2.95	98.40
									0.22

[a] Average of four determinations.

3.10. Effect of titrant

The effect of titrant on the performance of the potentiometric titration of NPZ is investigated using SPE, as KTPB is replaced by ammonium reineckate (RN), phosphotungstic acid (PTA) and phosphomolybdic acid (PMA). NPZ reacts with PTA and PMA in the molar ratio of 3 : 1 and with KTPB and RN the ratios are 1 : 1. The highest total potential change is obtained using KTPB as a titrant with good reproducibility compared with other titrants, (Table 4 and Fig. 7).

3.11. Analytical applications

Specificity is the ability of the method to measure the analyte response in the presence of all the potential interference. The response of the analyte with excipients, were compared with the response of pure NPZ. It was found that assay results were changed. Therefore, the excipients interfere with the quantization of NPZ as such in synthetic as commercial eye drop samples. Therefore, aliquot of eye drop sample equivalent to 1.5 mg is evaporated in a water path till dryness. The residue is washed two times with the least amount of methylene chloride where it will be dissolved while NPZ will not. Then, the remaining solid residues were dissolved in 3 mL bi-distilled water and the titration against KTPB is carried out. The procedure was repeated several times to optimize the amount of methylene chloride needed for complete removal of the interfering.

This SPE and CPE, plasticized with o-NPOE and TCP, can be successfully used for analysis of NPZ in pharmaceutical preparations. The determinations were made on a type sample, *i.e.* in eye droplets. As the conventional method for determination of NPZ (titration in non-aqueous solvents) was difficult and time-consuming as well as using expensive solvents, but this method (potentiometric determination) is easy, fast and inexpensive (Table 5). One of the important applications of these drug-selective electrodes would be the study and investigation of NPZ.

Conclusions

The proposed NPZ selective electrodes were successfully applied for fast and accurate determination of NPZ.HCl in pharmaceutical preparations. The results of the present study indicate that the electrochemical method can be used to study the interaction between NPZ with KTPB. Newly developed NPZ-IESs based on SPE and CPE were constructed and used for determining NPZ in pure and in pharmaceutical preparations. Developed ISEs have shown good performance characteristics with time stability up to three and two months for SPE and CPE, respectively. Elimination of potential drift by stable internal reference electrode and possibility of using these electrodes in routine analysis where symmetrical ISEs are difficult to operate, give these electrodes an advantage for applications in NPZ determinations and its quality control. Detection limits are far less than pharmacopoeia methods. Developed methods have shown to be simple, accurate and precise. Although, HPLC method has lower detection limit, simplicity and low cost give the developed methods superiority over the numerous HPLC methods previously published.[40]

Acknowledgements

The financial support of the Faculty of Science, Cairo University, is gratefully acknowledged.

References

1 British Pharmacopoeia, Stationary Office London, 1517, 2008.
2 J. M. L. Gallego and J. P. Arroyo, *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.*, 2003, **784**, 39.
3 J. M. L. Gallego and J. P. Arroyo, *J. Sep. Sci.*, 2003, **26**, 947.
4 C. Petr, Š. Dalibor and S. Petr, *Talanta*, 2006, **70**, 408.
5 E. Souri, M. Amanlou, H. Farsam and A. Afshari, *Chem. Pharm. Bull.*, 2006, **54**, 119.
6 H. Goicoechea, M. Collado, M. Satuf and A. Olivieri, *Anal. Bioanal. Chem.*, 2003, **376**, 460.
7 G. J. M. Lemus and A. J. Perez, *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.*, 2003, **784**, 39.
8 J. Charles and M. Bertucat, *Anal. Lett.*, 1999, **32**, 373.
9 S. O. Hman, *Drug Dev. Ind. Pharm.*, 1987, **13**, 1257.
10 S. Belal, M. A. Elsayed, M. E. Abdel-Hamid and H. Abdine, *J. Pharm. Sci.*, 2006, **70**, 127.
11 S. M. Ghoreishi, M. Behpour and M. Nabi, *Sens. Actuators, B*, 2006, **113**, 963.
12 N. Alizadeh and R. Mehdipour, *J. Pharm. Biomed. Anal.*, 2002, **30**, 725.
13 N. Alizadeh, S. Ershad, H. Naeimi, H. Sharghi and M. Shamsipour, *Fresenius J. Anal. Chem.*, 1999, **365**, 511.
14 M. F. Mousavi, S. Sahari, N. Alizadeh and M. Shamsipour, *Anal. Chim. Acta*, 2000, **414**, 189.
15 N. Alizadeh and M. Mahmodian, *Electroanalysis*, 2000, **12**, 509.
16 A. R. Fakhari, M. R. Ganjali and M. Shamsipur, *Anal. Chem.*, 1997, **69**, 3693.
17 M. R. Ganjali, A. Moghimi and M. Shamsipur, *Anal. Chem.*, 1998, **70**, 5259.
18 D. Ammann, W. E. Morf, P. Anker, P. C. Meier, E. Pretsch and W. Simon, *Ion Sel. Elec. Rev.*, 1983, **5**, 3.
19 C. Meier, D. Amman, W. E. Morf, W. Simon, in: J. Koryta (Ed.), *Applications of Electrochemical Devices in Medical and Biological*, John Wiley and Sons, Chichester, New York, Brisbane, Toronto, (1980) 13.
20 D. R. Thomas, *Anal. Chim. Acta*, 1986, **180**, 289.
21 S. S. M. Hassan, W. Mahmoud and A. Othman, *Talanta*, 1997, **44**, 1087.
22 V. Cossofret and R. P. Buck, *Analyst*, 1984, **109**, 1321.
23 N. Takisawa, D. G. Hall, E. Win-Jones and P. Brown, *J. Chem. Soc. Faraday Trans.*, 1988, **1**(84), 3059.
24 Noelia A. Martinez, Germ'an A. Messina, Franco A. Bertolino, Eloy Salinas and Julio Raba, *Sens. Actuators, B*, 2008, **133**, 256.
25 S. Ledru, N. Ruille and M. Boujtita, *Biosens. Bioelectron.*, 2006, **21**, 1591.
26 M. Albareda-Sirvent, A. Merkoci and S. Alegret, *Sens. Actuators, B*, 2000, **69**, 153.
27 G. Bagni, D. Osella, E. Sturchio and M. Mascini, *Anal. Chim. Acta*, 2006, **573**, 81.
28 K. Vytras, Potentiometric titrations based on ion-pair formation, *Ion Sel. Elec. Rev.*, 1985, **7**, 77.
29 E. Khaled, G. G. Mohamed and T. Awad, *Sens. Actuators, B*, 2008, **135**, 74.
30 Gehad G. Mohamed, Tamer Awad Ali, M. F. El-Shahat, A. M. Al-Sabagh, M. A. Migahed and E. Khaled, *Anal. Chim. Acta*, 2010, **673**, 79.
31 S. M. Ghoreishi, M. Behpour and M. Nabi, *Sens. Actuators, B*, 2006, **113**, 963.
32 K. Vytras, J. Jezkova, V. Dalabka and J. Kalous, *Scientific papers of the University of Pardubice, Series A*, 1997, **3**, 307.
33 C. E. Wilkes, C. A. Daniels and J. W. Summers, *PVC Handbook, ISBN 3-446-22714-8*, 2005.
34 R. P. Buck and E. Lindner, *Pure Appl. Chem.*, 1994, **66**, 2527.

35 (*a*) R. Puac, M. Bosnar, M. Bilic and Z. Grabaric, *Sens. Actuators, B*, 2005, **106**, 221; (*b*) R. Puac, M. Bosnar, M. Bilic and B. S. Grabaric, *Electroanalysis*, 2004, **16**, 843.
36 L. I. Antropov, *Theoretical Electrochemistry*, Mir Publisher, Moscow, (1977).
37 T. S. Ma, S. S. M. Hassan, *Organic Analysis Using Ion Selective Electrodes*, Academic Press, London, (1982).
38 S. S. M. Hassan, W. H. Mahmoud and A. M. Othman, *Talanta*, 1997, **44**, 1087(and references cited therein).
39 A. M. Othman, N. M. H. Rizk and M. S. El-Shahawi, *Anal. Chim. Acta*, 2004, **515**, 303.
40 K. Chiba, H. Hidekazu, T. Chiba, Y. Kato and T. Hirano, *J. Chromatogr. B*, 1995, **668**, 77.