



Original scientific paper

## New potentiometric electrode based on ion pair complex for determination of tropicamide in pure and pharmaceutical formulations

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### Abstract

Construction and general performance of a novel modified carbon paste electrode (MCPE) for determination of tropicamide (TPC) in pure form and pharmaceutical formulations have been examined. Tropicamide-tetraphenylborate (TPC-TPB) ion pair has been prepared and used as electroactive material. The best MCPE electrode was composed of 7 % ion-pair, 46.5 % dioctylphthalat and 46.5 % graphite powder. The electrode shows stable potentiometric response for TPC in the concentration range 0.3–221.0  $\mu\text{M}$  at 25 °C and pH range of 2.0–8.0. The electrode exhibits near Nernstian slope of  $59.71 \pm 0.30$  mV/decade and lower limit of detection of 0.09  $\mu\text{M}$  with fast response time (less than 15 s). The selectivity of the electrode (TPC-TPB) was investigated with respect to some organic and inorganic cations. The MCPE was designed to have better mechanical resistance. The proposed method was successfully applied for determination of TPC in eye drop formulation.

### Keywords

Tropicamide; Sodium tetraphenylborate; Ion pair complex; Carbon paste electrode; Pharmaceutical formulations

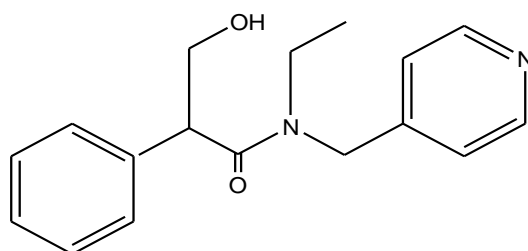
### Introduction

Tropicamide (TPC), (R,S)-N-ethyl-3-hydroxy-2-phenyl-N-(pyrid-4-yl-methyl) propionamide (Fig. 1), is an antimuscarinic agent with short duration of mydriatic and cycloplegic effect. TPC is used for refractive examinations and preservative for optimal eye tolerance and activity [1]. The action of TPC is more rapid in onset and wears off more rapidly than most other mydriatics and its use is generally much the same as those described for other mydriatics [2,3].

Several methods have been reported for determination of TPC including spectrophotometry [4–8], high performance liquid chromatography (HPLC) [9–12] and gas chromatography–mass spectrometry (GC–MS) [13]. In the United States Pharmacopoeia (USP) [14] and British Pharmacopoeia (BP) [15] the non-aqueous titration method for determination of TPC in raw materials and the extractive spectrophotometric method for pharmaceutical preparations have been described, respectively.

In the literature survey, no potentiometric method has been reported yet for determination of TPC. Therefore, the objective of the present study was to develop an accurate and validated potentiometric method for determination of TPC in raw material and pharmaceutical dosage forms, that can also be used in quality control laboratories.

Since their innovation by Adams [16], carbon paste electrodes (CPEs) have widely been used in many fields such as voltammetry, amperometry and potentiometry [17]. In comparison with ion-selective electrodes based on polymeric membranes, chemically modified carbon paste electrodes (CMCPEs) possess some advantages. In addition to lower limits of detection with respect to electrodes with an internal reference solution, CMCPEs are ease for preparation and regeneration, have stable response and very low ohmic resistance [18].



**Figure 1.** Chemical structure of tropicamide.

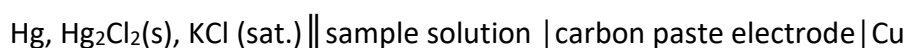
## Experimental

### Reagents and chemicals

All chemicals used were of analytical grade. Double distilled water was used throughout all experiments. Working reference standard of tropicamide (TPC  $C_{17}H_{20}N_2O_2$ , 284.35 g/mole) was obtained from Kunshan Chemical and Pharmaceutical Co., Ltd (India). Its purity was found to be 99.5 % according to the compendial testing method. Pharmaceutical preparation Mydriamed eye drop was provided by Medico Company for Pharmaceutical Industries (Homs, Syria). Sodium tetrphenylborate (Na–TPB) was from Merck, while dibutyl phthalate (DBP), dioctyl phthalate (DOP) and paraffin oil (p.Oil) were from BDH. Graphite powder was obtained from Aldrich.

### Apparatus

All potentiometric measurements were made at  $25 \pm 1$  °C using a Sanwal potentiometer (pH meter, Ion meter model of DT9201A) with combined pH electrode (model 250A, Orion, USA) for pH measurements. Saturated calomel electrode (SCE) was used as the reference electrode. The electrochemical cell is represented as follows:



### Standard solution

Standard stock solution of  $1.0 \times 10^{-2}$  M TPC was prepared daily by dissolving the appropriate amount of drug in double distilled water. Standard working solutions  $1.0 \times 10^{-3}$  and  $1.0 \times 10^{-4}$  M were freshly prepared by suitable dilutions of the stock solution with double distilled water.

### Calibration graph

Different amounts of  $1.0 \times 10^{-4}$  or  $1.0 \times 10^{-3}$  M TPC were added to 50 mL of double distilled water to achieve the concentration range from 0.3 to 221.0  $\mu$ M. The measured potential was recorded. The calibration graph was constructed by plotting the potential value versus  $pC_{\text{TPC}}$  ( $-\log$  concentration of TPC). The amount of drug was obtained from the regression equation.

### Analysis of eye drop

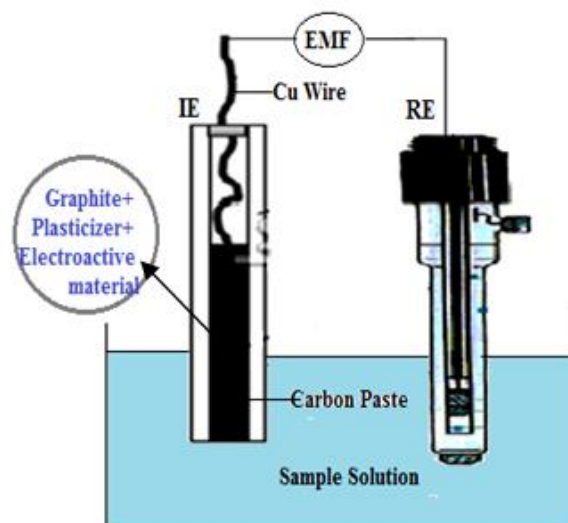
0.1 mL of the commercial eye drop was transferred into a 100 mL volumetric flask and diluted with double distilled water. The general procedure was then followed in the concentration range mentioned above.

### Preparation of tropicamide-tetraphenylborate (TPC-TPB) ion-pair

Sensing element used in the carbon paste electrode is the ion-pair compound made by interaction of TPC and Na-TPB. It was prepared by mixing 20 mL of 0.01 M acidic solution of TPC with 20 mL aqueous solution of 0.01 M Na-TPB. The resulting solution was then filtered, the precipitate washed with double distilled water and dried at room temperature [19].

### Modified carbon paste electrodes (MCPEs)

MCPEs were prepared by thoroughly mixing various amounts of ion pairing agents with carbon powder and plasticizer in the mortar, until homogenization of this mixture was achieved. The resulting paste was then packed firmly into the hole of the electrode body. Electrical contact to the carbon paste was made with a copper wire. Fresh surface was obtained by applying manual pressure to the carbon paste and polished on a filter paper to a shiny surface (Fig.2).



**Figure 2.** Schematic illustration of electrochemical cell with MCPE as ~~an~~ indicator electrode (IE) and SCE as reference electrode (RE).

### Selectivity of the electrode

Potentiometric selectivity coefficient was evaluated using the matched potential method (MPM) [20]. According to the MPM, the activity of the analyte was increased from  $\alpha_A = 9.90 \times 10^{-5}$  M (reference solution) to  $\alpha'_A = 1.01 \times 10^{-4}$  M and the corresponding change in potential ( $\Delta E$ ) is measured. Then,  $1.00 \times 10^{-1}$  M solution of an interfering ion was added to a new  $9.90 \times 10^{-5}$  M analyte reference solution, until the same  $\Delta E$  is recorded, determining thus the concentration of the added amount,  $\alpha_B$ . The selectivity coefficient  $K_{A,B}^{\text{MPM}}$  for each interfering ion was calculated using the following equation:

$$K_{A,B}^{MPM} = \frac{\alpha_A' - \alpha_A}{\alpha_B}$$

### Results and discussion

#### Calibration graph and effect of ion pair (IP) percentage on electrode potential

It is known that sensitivity and linearity of an electrode depend significantly on the amount of the ion-pair (IP) in the carbon paste composition. Thus, influence of the TPC-TPB IP percentage in the carbon paste composition was investigated first. Preliminary experiment showed that carbon paste electrode without IP modifier has no response towards the analyte. For this purpose, ten electrodes were prepared containing the IP modifier percentage from 1 to 10 % and the results are summarized in Fig. 3 and Table 1.

Increase-of IP percentage in the paste is found to increase the electrode response and stability of potentiometric readings, as well as slopes (S) of linear parts of calibration graphs defined by equation  $E = f(pC_{TPC})$ , reaching  $S = 59.714 \text{ mV dec}^{-1}$  at 7 % TPC-TPB (Fig. 4). At percentages above 7 %, a decrease of slope and reduction of linearity range where  $E = f(pC_{TPC})$  of the MCPE electrode are observed due to the kinetics of IP within the paste.

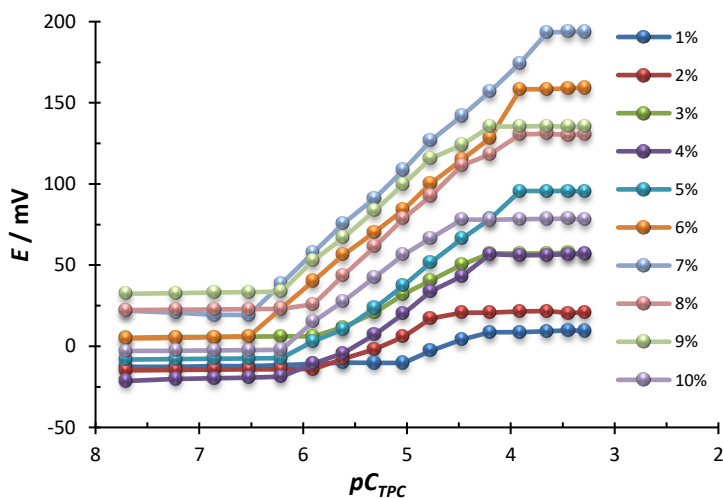


Figure 3. Effect of IP percentage in the MCPE on the calibration graph of TPC

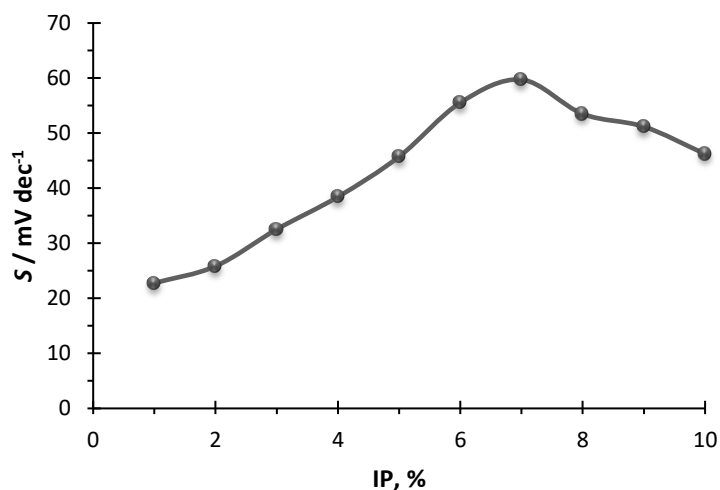


Figure 4. Effect of IP percentage in MCPE on the slope (S) of the linear,  $E = f(pC_{TPC})$ , range of the calibration graph of TPC.

**Table 1.** Potentiometric data for MCPE with different IP percentage obtained from linear equations.

Composition, %			S / mV dec <sup>-1</sup>	Intercept	Linear range, $\mu\text{M}$	r
Graphite	Plasticizer	IP				
49.5	49.5	1	22.77	105.17	9.27-64.70	0.9873
49.0	49.0	2	25.83	136.41	1.25-33.40	0.9810
48.5	48.5	3	32.53	190.24	1.25-64.70	0.9942
48.0	48.0	4	38.43	215.31	0.62-64.70	0.9868
47.5	47.5	5	45.73	268.98	0.62-122.00	0.9906
47.0	47.0	6	55.53	366.42	0.62-64.70	0.9912
46.5	46.5	7	59.71	409.40	0.30-221.00	0.9995
46.0	46.0	8	53.49	344.94	1.25-64.70	0.9904
45.5	45.5	9	51.17	354.48	0.62-64.70	0.9922
45.0	45.0	10	46.22	286.89	0.62-33.40	0.9971

### Response characteristics and statistical data

The characteristics performance of the best electrode (shadowed in blue in Table 1) was determined and the results are summarized in Table 2. The best proposed MCPE electrode shows nearly Nernstian response over the concentration range 0.3-221.0  $\mu\text{M}$ .

**Table 2.** Response characteristics of the best MCPE-TPC electrode.

Parameter	TPC-TPB electrode
IP, %	7
Regression equation	$E = -59.7\text{pC}_{\text{TPC}} + 409.40$
Correlation coefficient (r)	0.9995
Linear range, $\mu\text{M}$	0.3–221.0
LOD, $\mu\text{M}$	0.09
LOQ, $\mu\text{M}$	0.300
Response time, sec	$\leq 15$
Life time, day	70
Working pH range	2.0–8.0

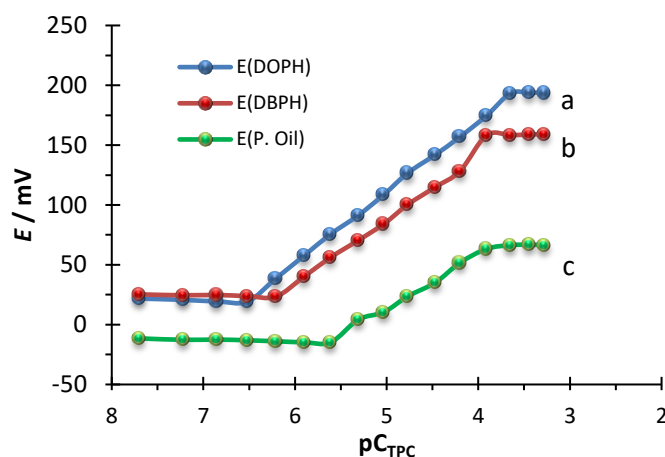
The slope of calibration graph for the best MCPE is 59.7 mV/decade for TPC concentrations in the range of 0.3–221.0  $\mu\text{M}$ , with standard deviation of  $\pm 0.3$  mV after five replicate measurements. Limit of detection (LOD) was calculated from the intersection of two extrapolated segments of the calibration graph [21]. LOD and limit of quantification (LOQ) were 0.09 and 0.3  $\mu\text{M}$ , respectively.

### Effect of plasticizer on the potential response

In this study, three plasticizers, di-octylphthalate (DOP), di-butylphthalate (DBP) and paraffin oil (p. Oil) were used to examine possible optimization of the paste. Contents of examined plasticizers were 46.5, 46.0 and 45.0 w %, contents of graphite powder were 46.5, 46.0 and 45.0 w % and contents of electroactive compound (TPC–TPB IP) were 7, 8 and 10 w %, respectively. The sum of percentages of all three components was always adjusted to 100 %. The results obtained showed that response performances of prepared pastes are rather different, depending on the kind of plasticizer, proportion of the plasticizer towards graphite and amount of electroactive compound (Table 3). Typical potential responses of electrodes constructed with three plasticizers are given in Fig.5. As shown in Fig. 5, the DOP-graphite electrodes were superior to DBP-graphite and p.Oil-graphite electrodes in both the response slope and linear concentration range. So, DOP was selected as the plasticizer of the carbon paste. The best paste composition of the DOP-graphite electrode was 46.5 % graphite, 46.5 % DOP and 7 % IP.

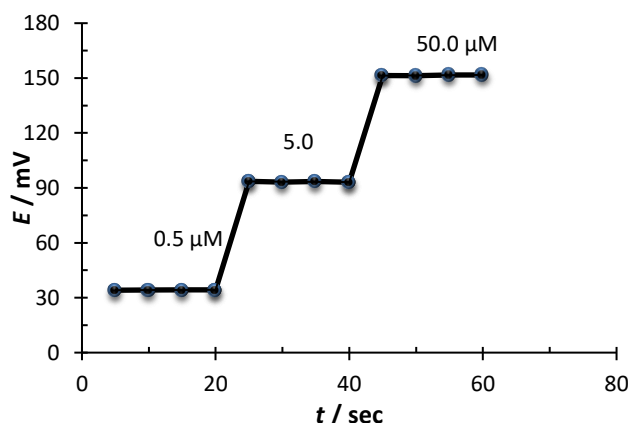
**Table 3.** General characteristic of different plasticizers of MCPE-TPC electrode.

Composition of the plasticizer, %	IP, %	S / mV dec <sup>-1</sup>	Linear range, $\mu\text{M}$	LOD, $\mu\text{M}$	Response time, s
DOP (46.5)	7	59.71	0.30 – 221.00	0.09	15
DBP (46.0)	8	52.18	0.62 – 64.70	0.31	20
p. Oil (45.0)	10	44.88	2.48 – 122.00	0.89	30

**Figure 5.** Optimization of plasticizers with CPE compositions: **(a)** DOP 46.5 %, graphite 46.5 %, IP 7.0 %, **(b)** DBP 46.0%, Graphite 46.0 %, IP 8.0 %), **(c)** p. Oil 45.0 %, graphite 45.0, IP 10.0 %.

#### Dynamic response time

Dynamic response time is the required time for the electrode to achieve values within  $\pm 1$  mV of the final equilibrium potential after successive immersions in the sample solutions [22]. Its calculation involved the variation and the recording of the TPC concentration in a series of solutions from 0.5 to 50.0  $\mu\text{M}$ . The electrode was able to quickly reach its equilibrium response in the whole concentration range. This time for the MCPE was about 15 seconds in the concentrated solutions  $\leq 221.00$   $\mu\text{M}$  (Fig 6).

**Figure 6.** Response time of MCPE for different concentrations of TPC.

#### Selectivity of the electrode

Influence of various basic substances on the response of MCPE-TPC electrode was investigated by measuring the potentiometric interference from different kinds of sugars, inorganic cations and certain alkaloids. Selectivity coefficients were evaluated by the matched potential method (MPM). Table 4 showed that the proposed MCPE is highly selective towards TPC. The electrode showed no response to a number of potentially interfering ions usually used in the manufacturing of pharmaceutical preparations, such as starch and lactose. The inorganic cations did not interfere due to differences in their mobilities and permittivities as compared with TPC cation.

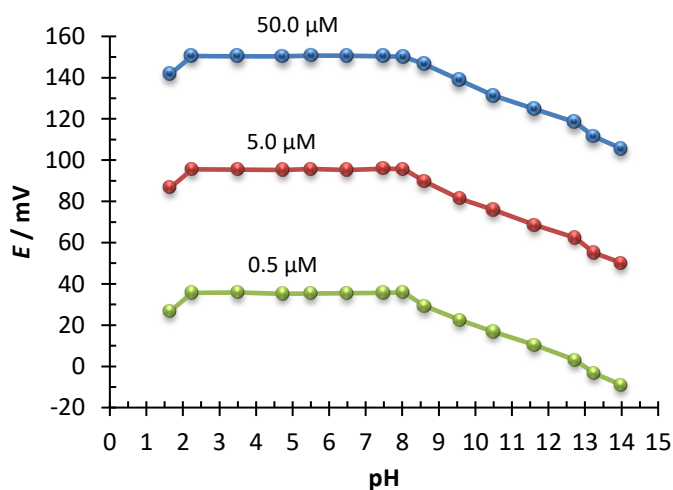
**Table 4.** Selectivity coefficients of some interfering ions

Interfering ion	Log $K_{MPM}$	Interfering ion	Log $K_{MPM}$
Na <sup>+</sup>	-3.51	Ba <sup>2+</sup>	-2.51
K <sup>+</sup>	-3.38	Fructose	-
Ca <sup>2+</sup>	-2.97	Glucose	-
Mg <sup>2+</sup>	-3.60	Sucrose	-
NH <sub>4</sub> <sup>+</sup>	-2.43	Maltose	-
Mn <sup>2+</sup>	-2.71	Lactose	-
Zn <sup>2+</sup>	-2.43	Starch	-

### Effect of pH

To examine the effect of pH on the MCPE-TPC electrode response, the potential was measured at specific concentration of the TPC solution (50.0, 5.0 and 0.5 μM) having pH value of 1.0 up to 14.0 (concentrated NaOH or HCl solutions were employed for pH adjustments). The results showed that the potential remained constant despite pH change in the range of 2.0-8.0, indicating applicability of this electrode in the specified pH range (Fig. 7).

Relatively prominent fluctuations in the potential vs. pH behavior took place below and above the formerly stated pH limits. In detail, the fluctuations above the pH value of 8.0 might be justified by removing the positive charge on the drug molecule. Fluctuations below the pH value of 2.0 may be due to interference of hydronium ion.



**Figure 7.** The pH effect on potential response of the MCPE-TPC electrode.

### Effect of temperature

To investigate thermal stability of the electrode, the effect of temperature of TPC solution on the MCPE was studied at different temperature values (20-70 °C). The MCPE exhibited good Nernstian behavior in the temperature range of (20-60 °C). The standard cell potentials ( $E^{\circ}_{\text{cell}}$ ) were determined at different temperatures and used to determine the isothermal temperature coefficient ( $dE^{\circ}/dt$ ) of the cell with the aid of the following equation [23]:

$$E^{\circ}_{\text{cell}} = E^{\circ}_{\text{cell},25^{\circ}\text{C}} + (dE^{\circ}/dt)_{\text{cell}} t$$

Plotting  $t$  vs.  $E^{\circ}_{\text{cell},25^{\circ}\text{C}}$  produced the straight line (Fig. 8). The slope of this line was taken as the isothermal coefficient of the cell which was found to be  $-1.96 \times 10^{-3}$  V/°C. The values of isothermal coefficient of the electrode revealed relatively high thermal stability within the investigated temperature range.

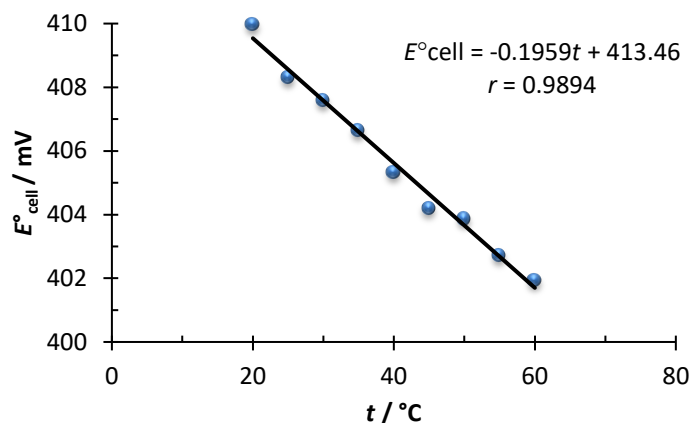


Figure 8. Variation of the  $E^{\circ}_{cell}$  with temperature for the MCPE-TPC electrode.

Potentiometric titration

Representative titration curve for determination of the investigated drug is shown in Fig.9. This MCPE electrode can be used successfully as indicator electrode in potentiometric titrations of the investigated drug. It also indicates that 1:1 [TPC]:[TPB] is formed as seen from the curve.

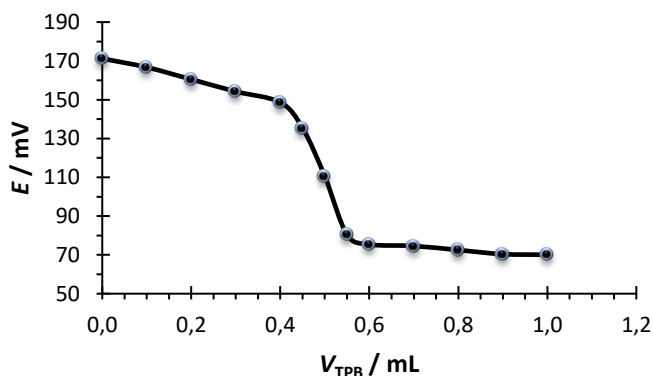


Figure 9. Typical potentiometric titration of 50 mL of  $1 \times 10^{-4} M$  TPC with  $1 \times 10^{-2} M$  TPB using MCPE

Life-time study

The MCPE electrode life time was estimated with the calibration graph, periodical tests with standard solutions (0.30-221.0  $\mu M$ ) and calculation of its response slope. For this estimation, the electrode was employed extensively (1 hour per day) for 80 days. As it can be seen from Table 5, in the case of MCPE this time is 70 days which shows the long-term stability of this kind of electrode. In MCPEs the surface of the electrode is renewable and can be used for longer time.

Table 5. Life time of MCPE electrode

Time, day	S / mV dec <sup>-1</sup>	Linear range, $\mu M$	Time, day	S / mV dec <sup>-1</sup>	Linear range, $\mu M$
1 / 24	59.71	0.30-221.00	45	59.65	0.30-122.00
1	59.35	0.30-221.00	50	60.02	0.62-122.00
2	59.50	0.30-221.00	55	59.22	0.62-122.00
5	59.96	0.30-221.00	60	59.11	0.62-221.00
10	59.60	0.30-221.00	65	59.09	0.30-122.00
15	59.41	0.30-221.00	70	58.65	1.25-122.00
20	59.75	0.30-221.00	75	58.31	1.25-122.00
25	59.18	0.30-221.00	80	57.76	2.48-64.70
30	59.15	0.30-221.00			
35	59.31	0.30-221.00			
40	59.55	0.30-221.00			

### Accuracy and precision

The precision and accuracy of the method were also evaluated. The standard deviation, relative standard deviation and recovery of different TPC amounts were determined and recorded in Table 6. The accuracy of the method is indicated by excellent recovery (99.80-101.40 %) and precision is supported by low standard deviation.

**Table 6.** Accuracy and precision for determination of TPC in pure form by the proposed method.

Taken	C <sub>TPC</sub> , μM		RSD, %	Recovery, %
	Found ± SD*			
0.5	0.499±0.01		2.00	99.80
5.0	5.07±0.08		1.58	101.40
50.0	50.10±0.58		1.16	100.20
100.0	99.80±0.82		0.82	99.80

\*Average of five replicates

### Analytical application

The application of the proposed method using calibration graph gives good results as shown in Table 7. The results were compared with the official method [14] and shown that the MCPE has good efficiency as regard of sensitivity, index of retrieving and repetition.

**Table 7.** Determination of TPC in eye drop by here proposed and official methods.

Formulation	Label claim	Recovery, % ± SD <sup>a</sup>	
		Proposed method	Official method [14]
Mydriamed	0.5 % 5 mg/mL	99.60±0.10	99.80±0.09
		$t^b=0.45$ $F^c=1.23$	$t^b=0.25$
	1.0 % 10 mg/mL	100.90±0.11	100.60±0.10
		$t^b=1.83$ $F^c=1.21$	$t^b=1.34$

<sup>a</sup> Average of five replicates; <sup>b</sup> Tabulated  $t$ -value at 95 % confidence level is 2.776. <sup>c</sup> Tabulated  $F$ -value at 95 % confidence level is 6.26

### Conclusion

The proposed chemically modified carbon paste electrode (MCPE) demonstrated advanced performance with fast response time and long stability, and shows high sensitivity, reasonable selectivity, and applicability over wide concentration range without sample pretreatment. The proposed procedure can be used for the routine analysis of TPC in bulk and eye drop. The sample recoveries from all samples were in good agreement with their respective label claims.

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