Potentiometric Determination of L-Dopa, Carbidopa, Methyldopa and Aspartame Using a New Trinitrobenzenesulfonate Selective Electrode

S.S. Badawy, Y.M. Issa* and *A.S. Tag-Eldin*

Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt

Received: October 5, 1995
Final version: December 5, 1995

Abstract
A selective PVC membrane electrode based on the ion-pair of cetylpyridinium (CP^+) and 2,4,6-trinitrobenzenesulfonate ($TNBS^-$) as the electroactive species was developed. The electrode exhibits a rapid and Nernstian response to $TNBS^-$ from 5.0×10^{-5} to 1.0×10^{-2} M at 25 ± 0.1°C. The response is unaffected by the change of pH over the range 2–12. Selectivity coefficients for a larger number of organic and inorganic anions and sugars are given. The electrode has been successfully applied to the determination of L-dopa, carbidopa, methyldopa and aspartame in pure solutions and in their pharmaceutical formulations with a precision and accuracy of 0.9–1.3% without interference from excipients.

Keywords: Trinitrobenzenesulfonic acid, L-Dopa, Carbidopa, Methyldopa, Aspartame, Ion selective electrodes, Potentiometry

1. Introduction

The use of 2,4,6-trinitrobenzenesulfonic acid (TNBS) as a reagent for determining the concentration of amino acids, amines, peptides and proteins has been widely accepted [1–5] since its introduction in 1960 by Satake et al. [6]. TNBS is water soluble, relatively stable and reacts with amino groups under comparatively mild conditions, as shown in Scheme 1.

The sensitivity of the system depends strongly on the pH value, and the reaction goes to completion at pH > 10 which gives the potentiometric methods using selective electrode sensors a great advantage for monitoring the reaction than the spectrophotometric methods since TNBS forms a yellow product with hydroxide ion which absorb strongly at the same wavelength as TNBS [1].

Levodopa, [59-92-7], [(-)-3-(3,4-dihydroxyphenyl)-L-alanine] (Scheme 2), and carbidopa, [28860-95-9], [(-)-L-α-methyldopa hydrazine] (Scheme 3) are used together to treat the symptoms of Parkinson's disease. Levodopa acts to replenish dopamine in the brain, while carbidopa ensures that enough levodopa gets to the brain where it is needed. Methyldopa,[555-30-6],[L-α-methyldopa] (Scheme 4) is an effective antihypertensive agent.

Aspartame, [22839-47-0], [L-α-aspartyl L-phenylalanine methyl ester] (Scheme 5), is a very promising sweetener which is used as an aid in weight control programs and for diabetics.

Several methods have been reported for the determination of L-dopa, carbidopa, methyldopa and aspartame, including spectrophotometry [7–9], high performance liquid chromatography (HPLC) [10–12], potentiometry using an enzyme electrode [13], titrimetry [14, 15] and voltammetry [16, 17]. Nevertheless, most of these methods involve several manipulation steps before the final result of the analysis is obtained. This is in contrast to the potentiometric methods of analysis using ion-selective electrodes, with their inherent advantages, i.e., high selectivity, sensitivity, freedom from optical interferences, economy and applicability to samples of different natures.

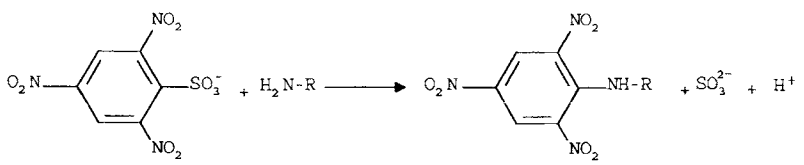
In the present work, a $TNBS^-$-electrode was prepared, based on incorporation of the cetylpyridinium-trinitrobenzenesulfonate (CP-TNBS) ion-pair in a polyvinyl chloride (PVC) membrane plasticized with dibutylphthalate (DBP). The electrode was successfully used for the determination of L-dopa, carbidopa, methyldopa and aspartame in pure solutions and in pharmaceutical preparations based on monitoring their reaction with TNBS with no special sample pretreatment, reagent requirements or use of sophisticated equipment.

2. Experimental

2.1. Reagents and Materials

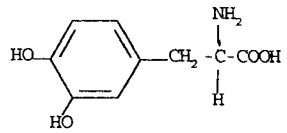
All reagents used were chemically pure. Double distilled water was used throughout all experiments. 2,4,6-Trinitrobenzenesulfonic acid trihydrate (Eastman Kodak, Rochester, New York, USA), cetylpyridinium bromide (CPBr) (Fluka), dibutylphthalate (Fluka), PVC of relatively high molecular weight (Aldrich), L-dopa (Merck), carbidopa (Merck), methyldopa (Merck) and aspartame (Fluka) were used. The pharmaceutical preparations Sinemet tablets 25/250 (carbidopa/L-dopa, Merck & Co. Inc., Whitehouse Station, NJ, USA), Aldomet tablets (methyldopa, Merck & Co. Inc., Rahway, NJ, USA) and Diet Sweet tablets (aspartame, Amirya Pharmaceutical Industries Co., Alexandria, Egypt) were obtained from local drug stores.

Stock sodium trinitrobenzenesulfonate solution, 0.1000 M was prepared by dissolving an accurately weighed 3.4721 g of TNBS acid form in 70 mL of water. The solution was neutralized with 1 M NaOH to pH of about 7, then transferred

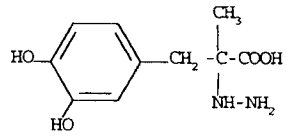


Scheme 1.

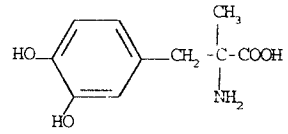
  1040-0397/96/1111-1060 $ 10.00+.25/0



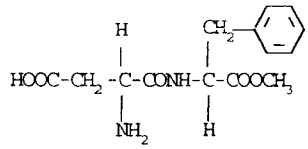
Scheme 2. Levodopa.



Scheme 3. Carbidopa.



Scheme 4. Methyldopa.



Scheme 5. Aspartame.

to a 100 mL calibrated flask and diluted to the mark. More dilute solutions were prepared by appropriate dilutions. All TNBS solutions were kept in dark brown bottles.

0.01 M solutions of L-dopa, carbidopa and methyldopa were prepared daily by dissolving the appropriate amount of the drug in 5 mL of 0.1 M hydrochloric acid and diluting with water to 100 mL. Aspartame solution was prepared daily by dissolving the appropriate amount of aspartame in double distilled water.

2.2. Sample Preparation

The contents of not less than 20 tablets were weighed and finely powdered. An accurately weighed 0.24426 g of the powder containing appropriate amount of Sinemet (25/250) or 0.2924 g Aldomet was dissolved in 5 mL 0.1 M HCl and diluted with water to the mark of a 100 mL calibrated flask. For Diet-Sweet, 1.2922 g was dissolved in 100 mL water in a calibrated flask.

2.3. Preparation of CP-TNBS Ion-Pair

The ion-pair was prepared by mixing 50 mL 0.1 M ($TNBS^-$) and 50 mL 0.1 M (CPBr) solutions. The resulting yellow precipitate was filtered, washed with deionized water and dried at room temperature. The composition of the ion-pair was confirmed by elemental and thermogravimetric analysis to be 1:1 (CP:TNBS). The obtained values were in agreement with those required for the tentative formula $C_{27}H_{40}N_4SO_9 \cdot H_2O$, the found values for %C, %H, %N and %S were 52.82, 7.44, 9.27 and 5.10 while the calculated values were 52.76, 6.84, 9.12 and 5.21, respectively.

Table 1. Composition of the membrane and slope of the calibration graphs at 25 ± 0.1°C (3 h soaking in 10^{-3} M $TNBS^-$).

Membrane	*Composition % (w./w.)*			*Slope [mV/decade]*	*S [a] (%)*
	Ion-Pair	*DBP*	*PVC*		
I	5	47.5	47.5	51.5	0.42
II	10	45.0	45.0	53.0	1.50
III	15	42.5	42.5	56.5	0.82
IV	20	40.0	40.0	48.5	0.62
V	25	37.5	37.5	37.0	1.32

[a] Relative standard deviation (five preparations).

2.4. Membrane Composition

Five membrane compositions were tried (Table 1). The electrode bodies were filled with a solution containing 0.1 M NaCl and 10^{-3} M $TNBS^-$ and preconditioned by soaking in 10^{-3} M $TNBS^-$ solution.

2.5. Apparatus

Potentiometric and pH measurements were carried out using a SEIBOLD G-103 digital pH/mV meter. A Techne circulator thermostat Model C-100, was used to control the temperature of the test solution. A saturated calomel electrode (SCE) was used as the external reference while a Ag/AgCl electrode as the internal reference. The electrochemical system is thus represented as follows: Ag/AgCl|inner solution|membrane|test solution||KCl salt bridge||SCE.

2.6. Construction of the Calibration Graphs

Suitable increments of standard ($TNBS^-$) solution were added to 50 mL of 10^{-6} M TNBS solution at the appropriate pH value (2–12) to cover the concentration range 10^{-6}–5×10^{-2} M. In this solution, the sensor and the reference electrodes were immersed and the emf was recorded, at 25 ± 0.1°C, after each addition. The electrode potentials, Eelec., were calculated from the emf values and plotted versus $pTNBS^-$.

2.7. Selectivity of the Electrode

The selectivity coefficients, $K^{pot}_{TNBS^-,J^{z-}}$, were evaluated by the separate solution method (method I) [18] and mixed solution method (method II) [19].

2.8. Potentiometric Determination of $TNBS^-$

The standard addition method [18] was used, in which small increments of 0.1 M $TNBS^-$ solution were added to 50 mL samples of various concentrations at the appropriate pH value (2–12). The change in emf was recorded after each addition and used to calculate the concentration of the $TNBS^-$ sample solution.

2.9. Potentiometric Titration of $TNBS^-$

An aliquot of $TNBS^-$ solution containing 3.7–52.0 mg was transferred into a 100 mL double wall titration cell, and the solution was diluted to 50 mL with distilled water. The resulting solution was titrated with 10^{-2} M CPBr solution at 25 ± 0.1°C.

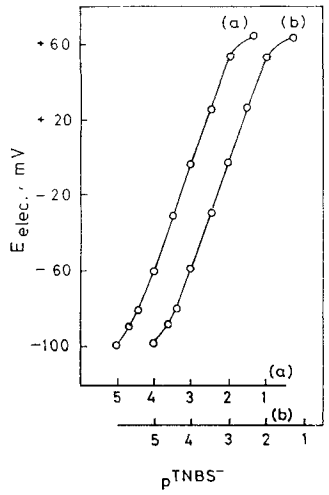


Fig. 1. Calibration curves for the membrane composition containing 15% of the ion-pair CP-TNBS at pH 7 (a) and pH 11 (b).

2.10. Determination of L-Dopa, Carbidopa, Methyldopa and Aspartame

The stoichiometry of the reaction of TNBS with the studied drugs was confirmed, using a $TNBS^-$-electrode, as follows:

(i) The potential (E_i) of a mixture containing 10.00 mL phosphate buffer solution pH 11, 8.00 mL of 3.00×10^{-3} M $TNBS^-$ solution, and 2.00 mL distilled water was recorded.
(ii) The potentials (E_f) of mixtures containing 10.00 mL phosphate buffer solution pH 11, 8.00 mL of 3.00×10^{-3} M TNBS solution, and 2.00 mL of drug solutions of different concentrations (1.0×10^{-2}–1.0×10^{-3} M) were recorded. From E_i and E_f the amount of $TNBS^-$ in millimoles which reacts with the drug can be calculated, according to the equation,

$$10^{-\Delta E/S} = \frac{[TNBS]_f}{[TNBS]_i} = K \qquad (1)$$

Where:
S is the slope value, $\Delta E = E_f - E_i$, $[TNBS]_i$ and $[TNBS]_f$ are the initial and final concentrations of $TNBS^-$ in the mixture, respectively.

$$\text{mmole } TNBS^- = V \cdot [TNBS]_i(1 - K) \qquad (2)$$

Where: V is the final volume of the measured solution (20 mL).

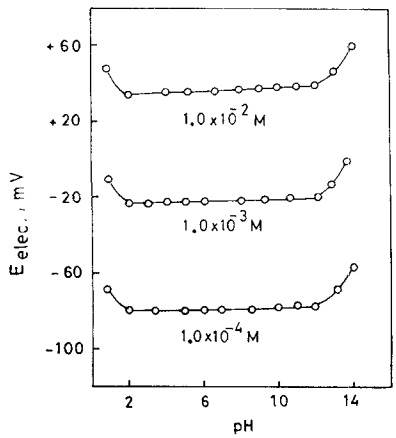


Fig. 2. Effect of pH on the potential of CP-TNBS ion-pair membrane electrode.

Table 2. Selectivity coefficient for CP-TNBS electrode.

Interferant	$K^{Pot}_{TNBS^-,J^{z-}}$ [a]	*Interferant*	$K^{Pot}_{TNBS^-,J^{z-}}$ [a]
Cl^-	1.7×10^{-3}	SO_4^{2-}	6.1×10^{-4}
Br^-	1.9×10^{-3}	$S_2O_8^{2-}$	6.0×10^{-4}
I^-	1.4×10^{-3}	CO_3^{2-}	7.4×10^{-4}
ClO_3^-	1.9×10^{-3}	$C_2O_4^{2-}$	5.1×10^{-4}
BrO_3^-	1.8×10^{-3}	HPO_4^{2-}	4.0×10^{-4}
IO_3^-	2.1×10^{-3}	PO_4^{3-}	2.3×10^{-4}
IO_4^-	6.8×10^{-2}	Borate	5.8×10^{-4}
NO_2^-	1.8×10^{-3}	Citrate	9.2×10^{-4}
NO_3^-	3.7×10^{-3}	Tartrate	2.5×10^{-4}
HCO_3^-	2.3×10^{-3}	Glucose	1.3×10^{-3}
CH_3COO	1.9×10^{-3}	Maltose	1.4×10^{-3}
C_6H_5COO	2.4×10^{-3}	Lactose	9.2×10^{-4}
$H_2PO_4^-$	1.2×10^{-3}	Picric acid	1.6

[a] Mean value of methods (I) and (II).

Equation 1 can be modified to

$$[TNBS]_i 10^{-\Delta E/S} = [TNBS]_i - [Drug] \qquad (3)$$

From Equation 3 the concentrations of the studied drug can be determined.

3. Results and Discussion

3.1. Study of the Electrode Characteristics

The results showed that the electrode made by membrane III (Table 1) with 15% CP-TNBS ion-pair exhibits the best Nernstian behavior (response time $\leq$ 10 s and a linear calibration plot with a slope of 56.5 mV/decade in the range of 5.0×10^{-5} to 1.0×10^{-2} M $TNBS^-$ at 25 ± 0.1°C after 3 h of soaking in 10^{-3} M $TNBS^-$ solution). Representative curves are shown in Figure 1.

3.2. Effect of Soaking

Calibration graphs were constructed after the electrode was soaked continuously in 10^{-3} M $TNBS^-$ for 1, 3, 5, 10 and 24 h, and 2, 3, 7, 15, 25, 30 and 42 days. The calibration graph slope decreased slightly to 52.0 mV/decade after 30 days and continued to decrease reaching 48.0 mV/decade after 42 days.

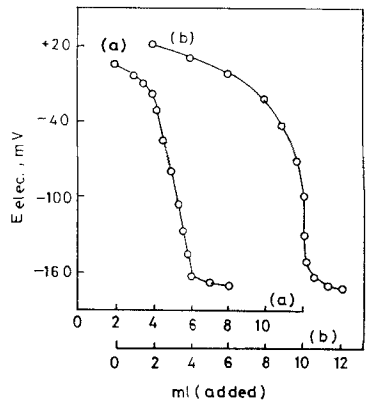


Fig. 3. Potentiometric titrations of 10 mL of 5×10^{-3} M $TNBS^-$ (a) and 10 mL of 1.0×10^{-2} M $TNBS^-$ (b).

Table 3. Standard addition and potentiometric titration methods for determination of $TNBS^-$ using CP-TNBS electrode.

Standard addition			*Potentiometric titration*		
Taken [mg]	*Mean recovery [%]*	*RSD [a] [%]*	*Taken [mg]*	*Mean recovery [%]*	*RSD [a] [%]*
0.9	100.4	0.28	3.7	101.0	0.16
4.6	101.0	0.14	18.5	99.8	0.23
9.2	100.2	0.12	37.0	100.6	0.13
20.0	100.3	0.15	52.0	100.7	0.18

[a] Relative standard deviation (five determinations).

3.3. Effect of pH

The effect of pH of the test solution (1.0×10^{-4}, 1.0×10^{-3}, and 1.0×10^{-2} M $TNBS^-$) on the electrode potential was investigated by following the variation of potential readings with change in pH by using the CP-TNBS electrode. The pH was varied by the addition of very small volumes of 3 M H_2SO_4 and/or 3 M NaOH. The results are given in Figure 2. From the plot obtained it is clear that the potential response is practically independent of pH between 2 and 12. The increase in mV reading of the electrode below pH 2, may be due to penetration of H^+ into the membrane surface, while the increase in mV reading at pH more than 12 is probably due to changes in liquid-junction potentials or the formation of an SO_3^{2-} complex of TNBS after its reaction with the OH^- group [1].

3.4. Selectivity of the Electrode

The selectivity of the ion-pair based membrane electrodes depends on the selectivity of the ion-exchange process at the membrane–test solution interface and the mobilities of the respective ions in the membrane. The data presented showed that the CP-TNBS electrode is highly selective for the TNBS anion (Table 2). The organic and inorganic anions did not interfere due to the differences in their mobilities and permeabilities as compared to the TNBS anions. It was found that the periodate ion and picric acid interfere with the CP-TNBS electrode. In the case of sugars, the high selectivity is mainly attributed to the difference in polarity and the lipophilic nature of their molecules relative to the TNBS anion or CP cation.

3.5. Analytical Applications

3.5.1. Determination of $TNBS^-$ (Method I)

The electrode proved to be useful for the determination of $TNBS^-$ by the standard addition method and by potentiometric titration in pure solution. Representative titration curves are shown in Figure 3. Collective results are given in Table 3. The results revealed that the proposed method satisfies a high degree of accuracy, a mean recovery of 99.8 to 101%, and excellent precision as indicated by the small values of the relative standard deviation.

Table 4. Determination of L-Dopa, carbidopa, methyldopa and aspartame in pharmaceutical formulations.

Sample	*Amount [mg]*		*RSD [a]* [%]
	Labeled by the manufacturer	*Found by the present method*	
Sinemet tablets	275	274.18	1.20
Aldomet tablets	250	252.00	1.22
Diet-Sweet tablets	20	20.10	0.92

[a] Relative standard deviation (five determinations).

3.5.2. Determination of L-Dopa, Carbidopa, Methyldopa and Aspartame (Method II)

It was found that TNBS reacts with the investigated drugs in the ratio of 1 : 1. The proposed method was applied successfully to the determination of L-dopa (0.394–3.994 mg), carbidopa (0.453–4.525 mg), methyldopa (0.422–4.220 mg) and aspartame (0.589–5.886 mg) in pure solutions with mean recoveries of 99.6–100.5% and RSD % values < 1. The results of the analysis of some commercial formulations given in Table 4 indicate the high accuracy and precision of the present work as compared to those previously reported [12, 20, 21] depending on more or less complicated instrumentation or time-consuming pretreatment steps, while the combination of sensitivity, selectivity and simplicity of ion-selective electrode potentiometry makes it an excellent and versatile technique.

4. Conclusion

A new $TNBS^-$-selective PVC membrane electrode based on a CP-TNBS ion-pair is constructed and applied in the potentiometric determination and titration of $TNBS^-$. The electrode proved to be successful providing a rapid, simple and a low cost potentiometric method for the determination of L-dopa, carbidopa, methyldopa and aspartame in pure solutions and pharmaceutical formulations.

5. References

[1] R. Fields, *Biochem. J.* **1974**, *124*, 581.
[2] K. Satake, T. Take, Matsuo, K. Tazaki, Y. Hiraga, *J Biochem.* **1966**, *60*, 12.
[3] F. Edwards-Levy, M.C. Andry, M.C. Levy, *Int. J. Pharm.* **1993**, *96*, 85.
[4] Z. Jonusiene, G. Dienys, J. Stepanovicius, *Zh. Anal. Khim.* **1988**, *43*, 536.
[5] K.A. Holm, *Analyst* **1980**, *105*, 18.
[6] K. Satake, T. Okuyama, M. Ohashi, T. Shinoda, *J. Biochem.* **1960**, *47*, 654.
[7] J. de Araujo Noberga, O.F. Fatibello, I. da Cruz Vieira, *Analyst* **1994**, *119*, 2101.
[8] Z. Hu, Z. Lou, X. Qian, *Zhongguo Yiyao Gongye Zazhi* **1993**, *24*, 313. (*Analyt. Abst.*, **1994**, *56*, 6G 168).
[9] P.B. Issopoulos, *Pharm. Acta. Helv.* **1989**, *64*, 82.
[10] G. Verzella, G. Bagnasco, A. Mangia, *J. Chromatogr.* **1985**, *349*, 83.

[11] S. Ting, *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 169.
[12] J.B. Kafil, B.S. Dhingra, *J. Chromatogr.* **1994**, *667*, 175.
[13] G.G. Guilbault, G.J. Lubrano, J.M. Kauffmann, G.J. Patriarche, *Anal. Chim. Acta* **1988**, *206*, 369.
[14] D. Amin, *Analyst* **1986**, *111*, 255.
[15] W.I. Mohamed, F.B. Salem, *Anal. Lett.* **1984**, *17*, 191.
[16] P.P. Di, F. Yin, H.M. Mao, *Fenxi Huaxue* **1992**, *20*, 1416. (Analyt. Abst., **1993**, *55*, 9 G237).
[17] E. Bishop, W. Hussein, *Analyst* **1984**, *109*, 627.
[18] S.S. Badawy, A.F. Shoukry, Y.M. Issa, *Analyst* **1986**, *111*, 1363.
[19] G.J. Moody, J.D.R. Thomas, *Talanta* **1972**, *19*, 623.
[20] P.B. Issopoulos, P.T. Economou, *Indian Drugs* **1992**, *29*, 355.
[21] F. Garcia Sanchez, A. Aguilar Gallardo, *Anal. Chim. Acta* **1992**, *270*, 45.