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## Potentiometric determination of methylene blue in pharmaceutical preparation using simple PVC electrode

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### ABSTRACT

The construction and performance characteristics of methylene blue (MB) PVC electrodes are described. Different methods for electrode fabrication (modified with the ion-pair, ion pairing agent or soaking the plain electrode in the ion-pair suspension) are described. Matrix compositions were optimized on the basis of effects of type and content of the modifier as well as influence of the plasticizers. The fabricated electrodes worked satisfactorily in the concentration range from  $7 \times 10^{-8}$  to  $10^{-3}$  M with Nernstian cationic slopes depends on the method of electrode fabrication. The MB-TPB modified electrode showed the best performance (slope  $58.3 \pm 2.0$  mV decade<sup>-1</sup>) compared with the plain electrodes or modified with NaTPB and fast response time of about 10 sec and adequate lifetime (2 months). The developed electrodes have been successfully applied for the potentiometric determination of MB in pure solution and in pharmaceutical preparations under batch conditions as well as end point indicator electrode for the potentiometric titration of MB. The solubility product of different MB ion-pair and the formation constant of the precipitation reaction leading to the ion-pair formation were determined conductometrically.

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### KEYWORDS

Methylene blue;  
PVC electrode;  
Potentiometry;  
Pharmaceutical preparations;  
Solubility products.

### INTRODUCTION

Methylene blue, (3,7-bis(dimethylamino)phenothiazin-5-ium chloride trihydrate) was an antimethemoglobinemic agent and an antidote to cyanide<sup>[1]</sup>. Various methods had been used for its determination, e.g. complexometry<sup>[2]</sup>, spectrophotometry<sup>[3,4]</sup>, ion-exchange chromatography<sup>[5]</sup>, thin layer and high performance liquid chromatography<sup>[6]</sup>, fast atom bombardment and tandem mass spectrometry<sup>[7]</sup>, coulometric titration<sup>[8]</sup>, reductimetric titration<sup>[9]</sup>, polarography<sup>[10-12]</sup> and poten-

tiometric titration<sup>[13-15]</sup>.

The widespread dosefication and/or adulteration of commercially available pharmaceutical preparations demand reliable methods for drug quality control that is preferably selective, rapid and can be undertaken with simple equipment. Nevertheless, most of aforementioned methods involve several manipulation steps before the final result of the analysis or require expensive apparatus. In contrast to other analytical techniques, electrochemical techniques such as potentiometric measurements have analytical and economic advantages

including simple instrumentation, fast response, application to turbid or colored solutions, high sensitivity, wide linear dynamic ranges, low cost, nondestructive measurement of the analyte sample as well as possible interfacing with flow injection analysis systems (FIA)<sup>[16]</sup>. Although ISEs had found very wide applications for drug quality control<sup>[17-19]</sup>, only a PVC electrode based on MB-silicotungstate (SLT) ion association as electroactive material was described<sup>[20]</sup>. The proposed electrode showed a useful working range of  $10^{-6}$ - $10^{-3}$  M with a detection limit of  $7.65 \times 10^{-7}$  M and dynamic response time about 1 min.

The present study is concerned with preparation, characterization and application of simple potentiometric sensors for rapid determination of MB in pure and dosage forms. Electrodes were fabricated in plain and modified forms and then subjected to a series of tests to elect sensor possessing the most favorable analytical characteristics. The developed sensors were also applied in the potentiometric determination of MB using standard addition and potentiometric titration.

## EXPERIMENTAL

### Apparatus

All potential measurements were performed using a 4310 Jenway digital pH meter with PC interface, equipped with silver-silver chloride double junction reference electrode in conjugation with the sensing drug ISE. A combined pH glass electrode was used for all pH measurements. Conductance was measured using 4310 Jenway Conductivity meter. Manual potentiometric titrations were performed using a Brand Digital Burette.

### Reagents

All reagents were of the analytical grade and double distilled water was used throughout the experiments. Methylene blue ((MB, 3, 7-Bis (dimethylamino)-phenothiazin-5-ium chloride,  $C_{16}H_{18}ClN_3S$ , Fluka) was used without further purification. *o*-Nitrophenyloctylether (*o*-NPOE) from Sigma was used for preparation of the sensors. Other types of plasticizers, namely dibutylphthalate (DBP), dioctylphthalate (DOP), dioctylsebacate (DOS) and tricresylphosphate (TCP) and Tri (2-ethylhexyl) trimellitate (TOTM, S) were purchased from BDH, Sigma, AVOCADO and Fluka,

respectively.

Ion pairing agents namely, sodium tetraphenylborate (NaTPB, Fluka), reineckate ammonium salt (RAS, Fluka), phosphotungstic acid (PTA, BDH) and phosphomolybdic acid (PMA, Fluka) were used for precipitation of different MB ion pairs.

### Standard solutions

Sodium tetraphenylborate (NaTPB) solution (ca.0.01M) was prepared by dissolving a weighed amount of the substance in worm water, then adjusted to pH 9 by adding sodium hydroxide solution and completed to the desired volume with water. The resulting solution was standardized potentiometrically against standard 0.01M thallium (I) nitrate solution<sup>[21]</sup>.

### Electrode construction

#### Ion pair preparation

Different MB ion-pairs were prepared by drop wise addition of 0.01 M of NaTPB, PTA, RAS or MPA to 50 ml of 0.01 M MB solution with continuous stirring. The resulting precipitates were filtered, washed several times with distilled water and then dried at 50°C. The chemical compositions of the different IPs were confirmed by (C, H, N and S) elemental analysis at Microanalytical Lab., National Research Center using Elementar (Vario El, Germany).

#### Conductometric determination of the stoichiometric ratios

Series of the dye solutions ( $10^{-4}$ - $10^{-2}$ M) were placed in the titration vessel followed by addition of  $10^{-2}$ M NaTPB, RAS or  $10^{-3}$ M PMA, PTA solutions and the conductance was measured after each addition (0.2mL). The corrected conductivity was then plotted against the volume added of titrant where the end point was determined to predict the stoichiometric ratio<sup>[22-24]</sup>.

#### Conductometric determination of the solubility products

The conductivities of MB, NaTPB, RAS, PMA and PTA solutions of different concentrations ( $10^{-4}$ - $10^{-2}$  M) were measured at 25°C. The specific conductivities (K), corrected for the effect of dilution were calculated and used to obtain the equivalent conductivities ( $\lambda$ ) of the solutions.

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$\lambda = 1000 \text{ K/C}$

Straight line plots of  $\lambda$  versus  $C^{1/2}$ , were constructed and the equivalent conductance values at infinitive dilution ( $\lambda_{\text{oMB}}$ ,  $\lambda_{\text{oTPB}}$ ,  $\lambda_{\text{oRN}}$ ,  $\lambda_{\text{oPMA}}$  and  $\lambda_{\text{oPTA}}$ ) can be evaluated. The equivalent conductance values of the IPs ( $\lambda_{\text{oIP}}$ ) were calculated from Kohlrausch's law:

$$\lambda_{\text{oIP}} = n \lambda_{\text{oMB}} + \lambda_{\text{o ion pairing agent}}$$

where  $n$  is the stoichiometric ratio.

The solubility ( $S$ ) and solubility products ( $K_{\text{sp}}$ ) of a particular ion associates were calculated using the following equations:

$$S = K_s \times 1000 / \lambda_{\text{oIP}}$$

$$K_{\text{sp}} = S^2 \text{ for } 1:1 \text{ ion associate}$$

$$K_{\text{sp}} = 27S^4 \text{ for } 1:3 \text{ ion associate}$$

where  $K_s$ , is the specific conductivity of a saturated solution of IP measured at 25°C.

### Sensor construction

Matrices compositions composed of 20 mg of MB-TPB or 40 mg of NaTPB were mixed with 240 mg o-NPOE, 6 mL THF and 240 mg PVC for electrode fabricated by different methods were described elsewhere<sup>[25]</sup>. The internal filling solution ( $10^{-3} \text{ M MB}$  and  $10^{-2} \text{ M KCl}$ ) and Ag/AgCl internal reference electrode were used. The fabricated sensors were conditioned for 24h in  $10^{-3} \text{ M MB}$  before use and soaked in the same solution. Plain electrode was prepared in the same manner using the plain PVC membrane and presoaked in freshly prepared MB-IPs suspension for 24h.

### Analytical procedure

#### Calibration of sensors

Sensors were calibrated by transferring 25 ml aliquots of  $10^{-8}$ - $10^{-3} \text{ M MB}$  solutions into a 50 mL double jacket thermostated glass cell at 25°C followed by immersing the sensor in conjugation with Ag/AgCl double junction reference electrode in the solution. The potential readings were recorded after stabilization and plotted against drug concentration in logarithmic scale ( $-\log [\text{MB}]$ ). The sensors performances were evaluated according to IUPAC recommendation<sup>[26]</sup>.

#### Electrode response time

The dynamic response time of the electrode was tested by measuring the time required to achieve a steady

state potential (within  $\pm 1 \text{ mV}$ ) after successive immersion of the electrode in a series of dye solutions, each having a 10-fold excess in concentration from  $10^{-6}$  to  $10^{-3} \text{ M}$ .

### Effect of pH

The influence of pH on the response of PVC was checked by recording the potential readings of the cell for solutions containing  $10^{-2} \text{ M}$  of each dye at different pH values (pH 3–11). Variation of pH value was done by adding very small volumes of HCl and/or NaOH solution (0.1-1M of each) to the drug solution.

### Potentiometric determination of MB in pharmaceutical preparations

MB was potentiometrically determined in pure solution and pharmaceutical preparations using the developed electrodes with potentiometric titration methods, aliquots of the sample solutions were titrated against standard NaTPB solution. The titration process was monitored using MB sensor in conjugation with Ag/AgCl reference electrode where the emf values were plotted against the ml added from the titrant to estimate the end point.

## RESULTS AND DISCUSSION

### Preliminary IP identification studies

MB is a tertiary amine cation which forms water insoluble ion-pair complexes with the oppositely charged anions such as TPB, RAS, PTA or PMA. The resultant IPs can be used as ion exchangers for MB potentiometric sensors. From this point of view, different types of MB-IPs were prepared and their stoichiometric ratios were estimated from elemental analysis and conductometric titration data. The elemental analysis data (TABLE 1) revealed that MB forms 1:1 IPs with both TPB and RAS. Complexes of MB with PTA and PMA showed ratio 1:3.

The stoichiometric ratios of the IPs formed can be estimated from the conductometric titration curve, obtained by plotting the change in conductance versus volume of titrant added (Figure 1). By addition of the titrant to MB solution, the system showed a regular rise in conductance up to the equivalence point where a sudden change in the conductance observed. Intersect of the two straight lines determine the stoichiometric

TABLE 1: Characterization of different MB-IPs

PI	MW <sub>cal</sub>	C%		H%		N%		S%		Tentative formula	K <sub>sp</sub>
		Calc.	F	C	F	C	F	C	F		
MB-TPB	638.3	75.2	75.0	5.95	5.92	6.57	6.42	5.01	4.95	[C <sub>16</sub> H <sub>18</sub> ClN <sub>3</sub> S][C <sub>24</sub> H <sub>20</sub> B]	1.96×10 <sup>-8</sup>
MB-RN	637.5	37.7	38.0	3.76	3.65	19.8	19.5	25.9	25.0	[C <sub>16</sub> H <sub>18</sub> ClN <sub>3</sub> S][C <sub>4</sub> H <sub>10</sub> CrN <sub>7</sub> S <sub>4</sub> ]	4.86×10 <sup>-9</sup>
MB-PMA	2780.5	20.7	20.9	0.64	0.58	4.52	4.35	3.40	3.20	[C <sub>16</sub> H <sub>18</sub> ClN <sub>3</sub> S] <sub>3</sub> [PMO <sub>12</sub> O <sub>40</sub> ]	1.35×10 <sup>-15</sup>
MB-PTA	3825.5	15.0	15.0	0.47	0.35	3.28	3.08	2.50	2.42	[C <sub>16</sub> H <sub>18</sub> ClN <sub>3</sub> S] <sub>3</sub> [PW <sub>12</sub> O <sub>40</sub> ]	6.35×10 <sup>-18</sup>

ratio of the complexes formed. The obtained results sustained the elemental analysis data for the complex formation ratios.

Solubility product of the IPs is important since its reciprocal is approximately equal to its formation constant, which in turns is tightly related to the degree of

hydrophobicity of the ion exchanger and its solubility in the electrode matrix. The solubility products of the ion associates were determined conductimetrically, and found to be 1.96×10<sup>-8</sup>, 4.86×10<sup>-9</sup>, 1.35×10<sup>-15</sup>, and 6.35×10<sup>-18</sup>, for MB-TPB, MB-RN, MB-PMA, and MB-PTA, respectively.



Figure 1: Conductometric titration of MB with different ion pairing agents

### Optimization of the electrode performance

For quantitative and qualitative composition optimization of the developed PVC sensors, an election scheme was followed. Both unmodified (plain) and modified electrodes (either with the MB-IPs, or the ion pairing agents) electrodes were prepared and tested for nature and content of modifier, type of plasticizer,

pH effect, response time and applications.

### Electrodes modified with MB- ion pairs

The most traditional way for the preparation of cationic dye sensors is the incorporation of the ion in question, usually in the form of an ion-pair with some anionic ion pairing agents such as NaTPB, TPA, TSA, flavianate, reinickate or PMA in the electrode matrixes.

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Different MB ion pairs (MB-TPB, MB-RN, MB-PTA and MB-PMA) were incorporated in the PVC matrix, and the fabricated electrodes were conditioned in  $10^{-3}$  M of MB solution for 24 h. Preliminary experiment declared that PVC electrodes that contain no electroactive material, and plasticized with o-NPOE showed no response towards the MB; while those modified with different ion pairs gave Nernstian responses with different slopes, and sensitivities depend on the nature of the ion pair used. Electrode incorporated with MB-TPB showed the best performance (slope  $51.5 \pm 2.8$  mV decade $^{-1}$  in the concentration range from  $6.7 \cdot 10^{-7}$  to  $10^{-3}$  M) compared to those modified with other MB ion pairs.

Furthermore, the amount of the MB-TPB ion-pair in the electrode matrix was varied from 2.5 to 20 mg, and addition of 10 mg MB-TPB in the membrane matrix was sufficient to achieve reasonable ionic exchange at membrane interface, and the best electrode performance, the corresponding slope was  $58.3 \pm 2.0$  mV decade $^{-1}$  in the tested concentration range ( $10^{-7}$ - $10^{-3}$  M). Increasing the MB-TPB content within the electrode matrix reduced the electrode performance as the slope of the electrode decreased to reach 43.0 mV/decade with 20 mg IP.

### Electrode modified with the ion pairing agents (Insitu)

Incorporation of a suitable ion pairing agent in the electrode matrix followed by soaking in the dye solution may lead to the formation of an ion exchanger at the electrode surface which subsequently extracted by the plasticizer into the electrode bulk. Such technique will reduce the time required for the electrode fabrication as there is no need for IP precipitation.

The effect of the ion pairing agent type was tested as the electrode matrices containing different ion pairing agents (NaTPB, PTA, PMA or RNS) were prepared, soaked in  $10^{-2}$  M of MB solution, and used for the potentiometric determination of MB. The obtained results indicated the super priority of the NaTPB incorporation in the electrode matrix indicated by the highest slope ( $54.2 \pm 4.5$ ,  $49.5 \pm 1.2$ ,  $46.4 \pm 6.1$ ,  $51.4 \pm 6.1$  mV decade $^{-1}$  for NaTPB, RNS, PMA and PTA, respectively). The content of NaTPB within the electrode matrices was varied from 10 to 50 mg, and it was found

that incorporation of 40 mg in the PVC matrix gave the highest slope ( $55.5 \pm 2.0$  mV decade $^{-1}$ ).

### Plain electrodes

In addition to the aforementioned methods for the electrode fabrication, a simple and reliable suggested procedure could be applied by soaking the plain electrodes in the aqueous suspension of the lipophilic IP solutions. The electrode mediator (plasticizer) extracts IPs and becomes gradually saturated with this IP and hence, there is no need to incorporate neither the IPs nor the ion pairing agents into the electrode matrix. The IP concentration in the organic phase increases with increasing both the extractability and the solubility product of the IP formed<sup>[27]</sup>.

The plain electrodes were soaked in the aqueous suspensions of different MB-IPs for 24 h and used for potentiometric measurements. The results obtained showed that the electrodes soaked in the MB-TPB had the best sensitivity indicated by the highest slope ( $55.3 \pm 5.5$  mV decade $^{-1}$ ) when compared with other IPs ( $48.5 \pm 10.1$ ,  $40 \pm 0.9$ ,  $44.7 \pm 2.6$  and  $42.5 \pm 4.3$  for PVC soaked in MB-RAS, MB-PTA and MB-PMA) which is directly related to the solubility products of these IPs.

### Effect of the plasticizer

Plasticizers play an important role in the behavior of ISEs since they improve the solubility of the sensing material and lower the overall bulk resistance of the electrode due to their polarity characteristics. The influence of the type of the plasticizer on the electrode performance has been studied as the electrode plasticized with o-NPOE was compared with those plasticized with TCP, DOS, DBS, DOP and TOTM. The obtained calibration graphs with PVC electrode using different methods of preparation and different plasticizers clarified that, using of o-NPOE as plasticizer showed the highest sensitivity of the membrane electrode indicated with the highest slope and wider linear range specially with the plain electrodes (slope values were  $56.3 \pm 5.5$ ,  $53.0 \pm 5.0$ ,  $50.2 \pm 4.8$ ,  $46.8 \pm 4.5$ ,  $49.7 \pm 14.2$ ,  $47.9 \pm 3.0$ , and  $42.3 \pm 2.2$  mV decade $^{-1}$  for o-NPOE, TCP, DOS, DBS, DOP, DBP, and TOTM, respectively) which may be related to the dielectric constant of these plasticizers ( $\epsilon = 24$ , 17.6, 3.88, 4.5, 5.2, 4.7 and 3.1 for the tested plasticizers in the same order).

## Sensors performance

The potentiometric response characteristics of different methylene blue sensors prepared with different methods (modification with MB-TPB, modification with NaTPB or plain) were evaluated according to IUPAC recommendations. The data obtained (Figure 2) indicated that the developed sensors can be successfully applied for the potentiometric determination of MB in concentration range  $7 \times 10^{-8}$  to  $10^{-3}$  M with Nernstian cationic slopes depend on the method of electrode fabrication. The MB-TPB modified electrode showed the best performance (slope  $58.3 \pm 2.0$  mV/decade) compared with the plain electrodes or modified with NaTPB.

For analytical applications, the response time of a new fabricated sensor is of critical importance. The response times of all fabricated electrodes were measured according IUPAC recommendation. Both the modified, and plain electrodes showed faster response time (10s) than insitu one ( $\sim 14$ s). The useful lifetime of the different fabricated electrodes was tested by performing day to day calibration. PVC electrodes showed useful lifetime of 14-28 days during which the Nernstian slopes did not change significantly ( $\pm 2$  mV/decade), while the detection limit was shifted from  $10^{-8}$  to  $10^{-6}$  M MB at the end of this period.

The effect of pH on the electrode potential was investigated by recording the changes in the potential readings with the pH after the addition of small volumes of HCl, and/or NaOH (0.1 or 1 M). The investigated electrode gave a useful pH range from 3.0–10.0. In alkaline media the measurement were hindered owing to the formation of a precipitate in the test solutions.

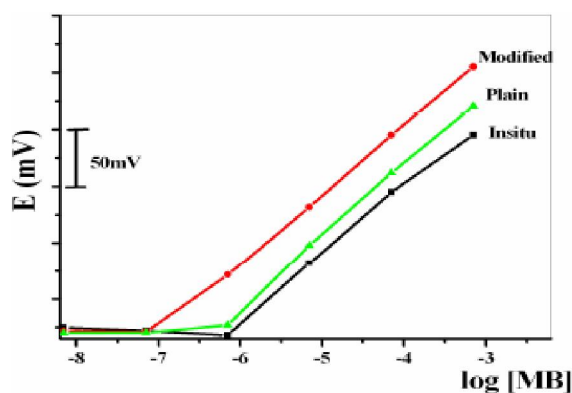


Figure 2 : Potentiometric determination of MB using different PVC electrodes

## Potentiometric titration

In contrast to direct potentiometric measurements requiring careful calibrations of measuring cells, the potentiometric titration techniques offers the advantage of high accuracy and precision; although the cost of increased time and consumption of reagents used as titrants. Parallel to the studying of the factors affecting the electrodes performance under the batch conditions, the effect of these factors was also investigated under potentiometric titration of MB with NaTPB. When ISEs are used to monitor the titration based on IP formation, the magnitude of both the potential break and sharpness at the inflexion point of the titration curve is predetermined by the solubility of the corresponding IP in membrane solvent (IP modified mode) and connected also with the extractability of the IP into the membrane mediator (insitu and plain mode). The effect of the electrode plasticizer on the titration process was investigated using membranes plasticized with different plasticizers (Figure 3). Generally, the electrodes plasticized with *o*-NPOE gave the highest total potential change ( $\Delta E = 400$  mV) compared with those plasticized with TCP, DOP, DOS, DBS, DBP or TTOM ( $\Delta E$  were 348, 264, 310, 297, 332, and 40 mV for the plasticizers in the same order) which can be explained by higher extractability of the MB-TPB ion pair into the membrane solvent due to the relative higher dielectric constant of *o*-NPOE.

The effect of the electrode fabrication techniques on the titration process was investigated. The plain electrodes showed the best titration curve compared with the modified with either the MB-TPB ion pair or with the ion pairing agent regarding the total potential change or the potential break at the end point.

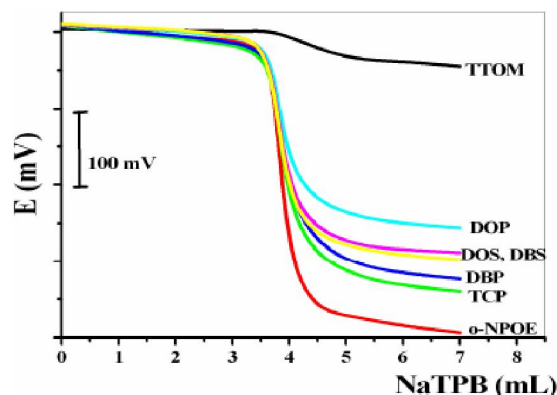


Figure 3 : Effect of the plasticizer on the potentiometric titration of MB with NaTPB

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The effect of the precipitants (ion pairing agent) on the performance of the potentiometric titration of MB was also investigated as NaTPB was replaced with RAS, PTA or PMA (Figure 4). Titration curves performed using NaTPB showed the highest total potential change followed by PTA and PMA, respectively. Generally, NaTPB was selected as the titration curves were highly reproducible and the end point showed formation of 1:1 IP compared and 1:3 complexes were formed between MB and PTA or PMA which agreed with the stoichiometric ratios obtained from the elemental analysis and conductometric measurements.

Under the optimum conditions, the titration curves were symmetrical with a very well defined potential jump indicating the high sensitivity of the electrode. Concerning the titration process, the total potential changes and the potential breaks at the end point were large ( $\Delta E =$

400mV) allowing the application of the electrode to determine MB reaching down to 0.32 mg (Figure 5). The titration process was highly reproducible (for 9.6mg MB, the end point was  $2.156 \pm 0.034$  ml,  $n = 5$ ).

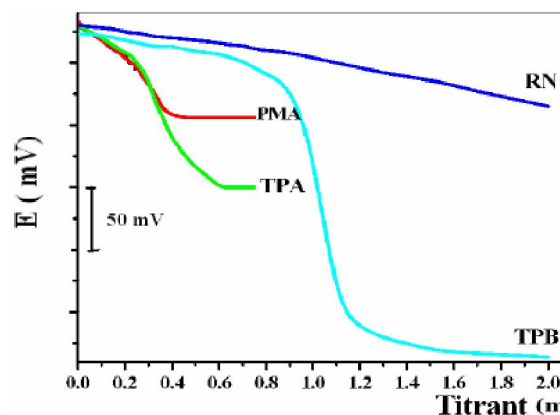


Figure 4 : Effect of the titrant on the potentiometric titration of MB with NaTPB

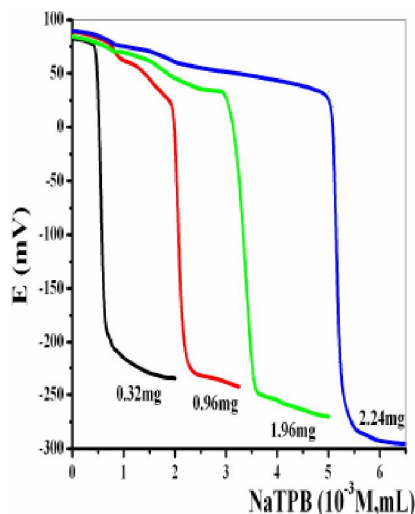


Figure 5 : Potentiometric titration of MB with NaTPB using MB PVC electrode

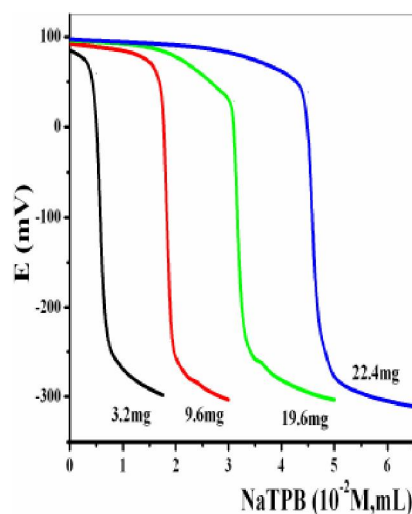


TABLE 2 : Determination of MB in pure form, and in pharmaceutical preparations via potentiometric titration with NaTPB

Sample	Taken (mg)	Found (mg)	Recovery (%)	SD*
Pure	0.32	0.222	69.41	2.80
	0.96	0.648	67.48	2.40
	1.6	1.126	70.37	2.0
	2.24	1.59	71.06	1.60
	3.2	2.125	66.42	1.80
	9.6	6.444	67.13	1.4
	16.0	11.322	70.76	1.20
	22.4	15.178	67.76	1.10
Sample	5.0	3.25	65.0	2.2
	7.5	4.65	62.0	1.8
	10	6.3	63.0	2.1

\*Average of five titration process

## Analytical application

The proposed electrode was successfully employed for the assay of MB in their authentic samples as well as pharmaceutical formulations applying standard addition method and potentiometric titration. The data given (TABLE 2) clearly indicated satisfactory agreement between the MB contents in different samples determined by the proposed sensor and elemental analysis method as there is no official methods.

## CONCLUSION

The present work investigates the fabrication of MB-selective electrode fabricated with different methods The

proposed electrode can be successfully applied for the potentiometric determination of MB in concentration range  $10^{-7}$  to  $10^{-3}$  M with Nernstian cationic slopes depend on the method of electrode fabrication. In addition, the fabricated can also be used as end point indicator electrode in the potentiometric titration of MB in pure, and pharmaceutical preparation with good accuracy, and precision. Compared with the previously published MB-PVC electrode<sup>[20]</sup> the present electrode was characterized by higher sensitivity, and faster response time (10s compared with 1min for the published one) which give the present fabricate electrode the priority for application in following the catalytic reactions involving MB as redox indicator.

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