# A Methylene Blue-Selective Membrane Electrode Using Methylene Blue-Phosphotungstate as Electroactive Material and its Pharmaceutical Applications

*Meng Liang Wen, Yi Bin Zhao, Xiang Chen, and Chang Yi Wang\**

*Department of Chemistry, Yunnan University, Kunmimg, Yunnan 650091, People's Republic of China*

A methylene blue poly (vinyl chloride) membrane electrode based on methylene blue-phosphotungstate ion-pair complex as electroactive material is described. The linear response covered the range of $1 \times 10^{-3} - 1 \times 10^{-6}$ mol dm$^{-3}$ methylene blue solution, with a slope of $51.5 \pm 0.8$ mV/decade (pH range 3.0–10.0). The detection limit was $6.79 \times 10^{-7}$ mol dm$^{-3}$. The electrode showed stability, good reproducibility and a fast response. Interferences from common inorganic cations and some organic bases were negligible. These characterstics of the electrode enabled its successful use for determination of methylene blue in injection. There was good agreement for the results of methylene blue content in injection between the potentiometric method and the *United States Pharmacopoeia* standard procedure.

## INTRODUCTION

Methylene blue (3,7-bis(dimethylamino)phenothiazin-5-ium chloride trihydrate) is an antimethemoglobinemic agent and an antidote to cyanide.[1] Various methods have been used for its determination, *e.g.* complexometry,[2] spectrophotometry,[3,4] ion-exchange chromatography,[5] thin layer and high performance liquid chromatography,[6,7] coulometric titration,[8] reductimetric titration,[9] polarography[10–15] and potentiometric titration.[16–22] Compared to

---

\* Author to whom correspondence should be addressed.

the sophisticated methods (*e.g.* ion-exchange chromatography, thin layer and high performance liquid chromatography, fast atom bombardment and tandem mass spectrometry, *etc.*), which usually require several time-consuming manipulation steps, expensive instruments, and professional training, potentiometric analysis with ion-selective electrodes has the advantage of being inexpensive, easy to use, and requiring simple maintenance. Although potentiometry using a suitable indicator electrode has been widely used in pharmaceutical analysis,[23,24] no data have ever been reported about the methylene blue ion selective electrode (ISE).

The present paper describes the construction and performance characteristics of a methylene blue ion-selective electrode based on the ion-pair complex methylene blue-phosphotungstate, embedded in a PVC matrix. Application of the proposed electrode to the determination of methylene blue content in injection is also described.

## EXPERIMENTAL

All reagents were analytical reagent grade and were used as received without further purification unless otherwise stated. All solutions were prepared with distilled and deionized water. The methylene blue injections (Jiangsu Taixing Pharm. Co., Jiangsu, People's Republic of China) were purchased from a local pharmacy.

All E.M.F. (electromotive force) measurements were made at $25 \pm 1$ °C using an Orion microprocessor ionanalyzer (Model 901). The electrode was used in conjunction with a model 801 double-junction calomel electrode (Jiangsu Electroanalytical Instrument Factory, Jiangsu, People's Republic of China). The pH values of all solutions were measured with a Model 231 glass electrode (Shanghai Dian Guang Device Works, Shanghai, People's Republic of China). The USP (*United States Pharmacopoeia*) procedure[25] for the assay of methylene blue injection was carried out using a spectrophotometer (Model 721–100, The Third Analytical Instrument Factory, Shanghai, People's Republic of China).

### *Preparation of the Electrode*

The ion-complex was prepared by mixing stoichiometric amounts of a 0.01 mol dm$^{-3}$ aqueous solution of phosphotungstate with an equimolar aqueous solution of methylene blue. The blue precipitation obtained was sedimented by centrifugation to 3000 g, extensively washed with deionized water and dried over phosphorous pentoxide for 2 days in an evacuated desiccator.

The tetrahydrofuran (THF) solution (5.0 mL) containing 11.9 mg ion-complex together with 150 mg of PVC and 300 μL of dibutyl phthalate were poured into a small glass ring (diameter 46 mm) lying on the glass cover slip. The ring was covered with a sheet of filter paper and a second cover slip to obtain slow evaporation of the solvent over a period of 24 h at room temperature.

The electrode was prepared by gluing the out cut of the PVC membrane disc onto the cleaned ends of PVC modules by means of a PVC-THF solution (62 mg PVC in 1.0 mL THF). The modules were made of PVC tubes, were 4 cm long, and had a

thread fitted to the electrode bodies. Screwing the membrane modules onto the electrode bodies, which contained an Ag-AgCl internal leadout (Laboratory made), assembled the complete electrodes. The electrode body was filled with an inner filling solution containing $1 \times 10^{-1}$ mol dm$^{-3}$ NaCl and $1 \times 10^{-3}$ mol dm$^{-3}$ methylene blue solution (saturated with AgCl). The electrode was preconditioned overnight by soaking it in a $4 \times 10^{-3}$ mol dm$^{-3}$ methylene blue solution. The schematic construction of the methylene blue selective electrode is presented in Figure 1. When not in use, the electrodes were stored in the same methylene blue solution and thoroughly washed with deionized water between measurements. The assembled membrane modules were stored dry at room temperature before use.
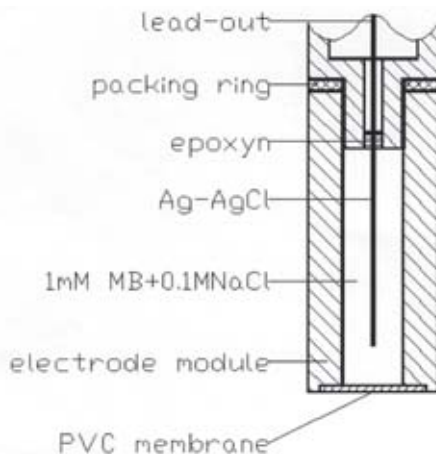


Figure 1. Schematic construction of the methylene blue selective electrode.

## Standard Addition Method

Standard methylene blue solutions and methylene blue injection were analyzed using the standard addition method.[26] Six batches of 5 cm$^3$ methylene blue injection were each transferred into a calibrated flask (50 cm$^3$) and diluted to mark. The standard addition method was performed by spiking 200 mm$^3$ of standard $2 \times 10^{-3}$ mol dm$^{-3}$ methylene blue solution into 20 cm$^3$ of the test solution and measuring the change in potential.

## RESULTS AND DISCUSSION

Typical calibration curves for the methylene blue membrane electrode in Figure 2 show that the electrode was linear in the range of $1 \times 10^{-3} - 1 \times 10^{-6}$ mol dm$^{-3}$ for the citrate buffer solution. The calibration curve equation is $E = E^\circ + 51.5 \pm 0.8 \times \log$ [MB] with a correlation coefficient of 0.9999. The
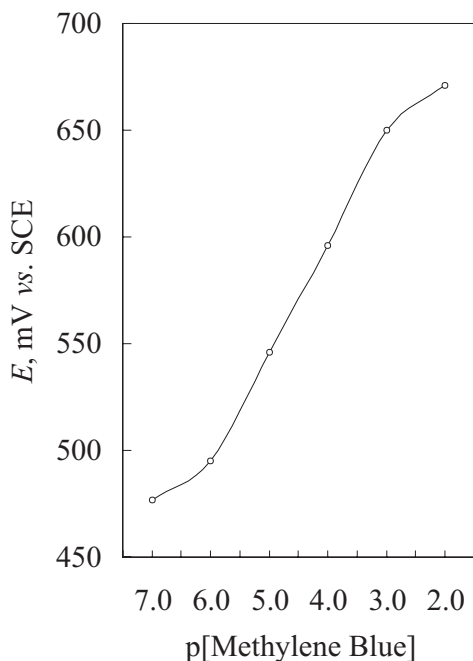
Figure 2. Calibration graph for the methylene blue selective electrode.

response characteristics of the electrode are shown in Table I. The stability of the electrode response was checked over a period of 6 months. The response time of the electrode depended on the concentration of methylene blue (less than 1 min for $10^{-2} - 10^{-4}$ mol dm$^{-3}$ methylene blue solutions and 1–2 min for $10^{-5} - 10^{-6}$ mol dm$^{-3}$ methylene blue solutions). The electrode response displayed good stability and reproducibility during the test, as shown by the relative standard deviation values.

TABLE I

Response Characteristics of Methylene Blue Selective Membrane Electrode

| Parameter | Response |
|---|---|
| Slope (mV per log $a$)* | $51.5 \pm 0.8$ |
| Intercept, $E°$ (mV)** | $803 \pm 1.4$ |
| Linear range (mol dm$^{-3}$) | $1 \times 10^{-3} - 1 \times 10^{-6}$ |
| Detection limit (mol dm$^{-3}$) | $6.79 \times 10^{-7}$ |

\*   Standard deviation of average slop values for multiple calibration ($n$=50)
\*\* Standard deviation of values recorded over a period of three months ($n$=90)

The effect of pH ($1 \times 10^{-3} - 1 \times 10^{-5}$ mol dm$^{-3}$ methylene blue solution) on the electrode potential was investigated by observing the changes in the potential readings with pH of the solution after addition of small volumes of HCl and/or NaOH (0.1 mol dm$^{-3}$ or 1 mol dm$^{-3}$). The investigated electrode gave a useful pH range from 3.0–10.0, as shown in Figure 3. In alkaline media, measurements were hindered due to the formation of a precipitate in test solutions.
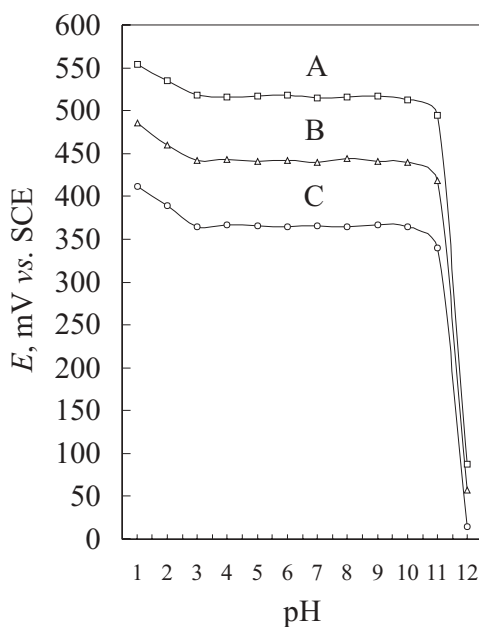


Figure 3. Effect of pH on the response of the methylene blue electrode (A: $1 \times 10^{-3}$ mol dm$^{-3}$; B: $1 \times 10^{-4}$ mol dm$^{-3}$; C: $1 \times 10^{-5}$ mol dm$^{-3}$ methylene blue solution).

Selectivity coefficients of the methylene blue–phosphotungstate electrode determined against a number of dyes and alkaloids using the separate solution method[27] are given in Table II. The results show that the electrode was reasonably selective towards methylene blue. Interference from metal cations, glucose and fructose was generally negligible. However, with the exception of acid chrome blue K and xylenol orange, other dyes such as methyl violet, crystal violet, brilliant and malachite green caused significant interference. Fortunately, these interferences were absent in methylene blue injection.

The proposed electrode was employed for the assay of methylene blue content in injection by the potentiometric method and by the USP standard

TABLE II

Selectivity Coefficients for the Methylene Blue-Selective Membrane
Electrode at 25 °C

| Interferent | $K_{MB,J}^{pot}$ | Interferent | $K_{MB,J}^{pot}$ |
|---|---|---|---|
| $Li^+$ | $5.2 \times 10^{-3}$ | Oxylenol orange | $4.9 \times 10^{-5}$ |
| $Na^+$ | $7.1 \times 10^{-3}$ | Brilliant cresyl blue | 1.79 |
| $K^+$ | $3.9 \times 10^{-2}$ | Crystal violet | $3.7 \times 10^3$ |
| $Ba^{2+}$ | $2.6 \times 10^{-4}$ | Malachite green | $6.3 \times 10^2$ |
| $Mg^{2+}$ | $6.1 \times 10^{-4}$ | Methylene violet | $1.9 \times 10^3$ |
| $Ca^{2+}$ | $1.3 \times 10^{-4}$ | Berberine | 3.22 |
| $Cu^{2+}$ | $4.7 \times 10^{-4}$ | Brucine | $2.4 \times 10^{-3}$ |
| Fructose | $5.2 \times 10^{-2}$ | Caffeine | $2.8 \times 10^{-3}$ |
| Glucose | $5.6 \times 10^{-2}$ | Deoxyephedrine | $5.4 \times 10^{-2}$ |
| Maltose | $4.9 \times 10^{-2}$ | Phenacetin | $4.8 \times 10^{-4}$ |
| Acid chrome blue K | $2.8 \times 10^{-5}$ | Theobromine | $9.8 \times 10^{-4}$ |
| Bromo cresol green | $7.2 \times 10^{-4}$ | Theophylline | $7.7 \times 10^{-4}$ |

procedure.[25] The results of the potentiometric methods as compared with
the USP standard procedure are shown in Table III. As can be seen from Ta-
ble III, high precision was obtained (RSD%) by the potentiometric method.

TABLE III

Comparison of the Mean Values of Conventional and Potentiometric Methods
for the Assay of Methylene Blue Injection

| Sample | Potentiometric Method | | USP Method | |
|---|---|---|---|---|
| | Recovery (% of nominal value)* | RSD (%) | Recovery (% of nominal value)* | RSD (%) |
| 1 | 97.76 | 0.63 | 101.10 | 1.32 |
| 2 | 99.58 | 1.07 | 102.61 | 1.19 |
| 3 | 98.04 | 0.82 | 100.45 | 1.02 |
| 4 | 100.51 | 0.74 | 103.11 | 0.89 |
| 5 | 96.24 | 1.61 | 98.29 | 1.87 |
| 6 | 100.05 | 0.36 | 99.34 | 1.63 |

* All values are the average of 5 determination

The methylene blue–selective plastic membrane electrode, based on the methylene blue–phosphotungstate ion–pair complex in a PVC matrix, exhibited useful analytical characteristics for the determination of methylene blue in pharmaceuticals.

## REFERENCES

1. Z. Y. Chen, T. P. Liu, and Y. Yang, *Handbook of Clinical Pharmaceuticals*, Shanghai Science and Technology Publishing House, Shanghai, P. R. China, 3rd Edition, 1995, p. 36.
2. P. S. Kovtun, A. A. Kozyreva, and T. Y. Bagrii, *Farm. Zh*. **5** (1981) 59–61.
3. U. Muralikrishna, G. V. Ramanadham, and R. N. Someswara, *Fresenius'Z Anal. Chem*. **279** (1976) 208.
4. A. M. Kotenko and T. V. Koval'chuk, *Farm. Zh*. **1** (1978) 59–63.
5. I. P. Koka, *Mater S'ezda Farm. B SSR*. **3rd** (1977) 130–132.
6. G. Balansard, G. Schwardrohn, E. Vidal-Oillvier, and R. Elias, *Ann. Pharm. Fr.* **46** (1988) 129–132.
7. W. Baeten, J. Claereboudt, M. Claeys, B. M. Liedekerke, and A. P. De Leenheer, *Spectroscopy* (Ottawa) **7** (1989) 113–122.
8. H. J. Li, D. Y. Liu, and Y. Wang, *Fenxi Huaxue* **13** (1985) 603–605.
9. V. V. Senkevich, Y. D. Sister, and Y. S. Lyalikov, *Zh. Ana. Khim*. **27** (1972) 380–385.
10. V. Stuzka and O. Ruzicka, *Mikrochim. Acta* **2** (1980) 77–83.
11. H. L. Ye, H. Xiang, and P. Wei, *Fenxi Huaxue* **14** (1986) 928–930.
12. S. R. Sagi and T. B. Babu, *Talanta* **23** (1976) 465–466.
13. T. Ogawa, *Denki Kagaku* **25** (1957)613.
14. V. Stuzka and O. Ruzicka, *Acta Univ. Palacki. Olomuc., Fac. Rerum. Nat*. **61/65** (1979/80) 161.
15. V. Stuzka and O. Ruzicka, *Acta Univ. Palacki. Olomuc. Fac. Rerum. Nat*. **76** (1983) 61.
16. K. Vytras, *Ion-Sel. Electrode Rev*. **11** (1989) 111.
17. M. N. Rukmini, V. S. N. P. Kavitha, and R. Y. Pulla, *Talanta* **26** (1979) 409–410.
18. C. Burgess, A. G. Fogg, and D. T. Bums, *Lab. Pract*. **22** (1973) 472–475.
19. Y. M. Dessouky, K. Töth, and E. Pungor, *Analyst* **95** (1970) 1027.
20. K. Vytras, *Collect. Czech. Chem. Commun*. **42** (1977) 3168–3174.
21. J. C. Vire, G. J. Patriarche, and G. D. Christian, *Mikrochim. Acta* **1** (1977) 17–22.
22. W. Selig, *Fresenius'Z Anal. Chem*. **308** (1981) 21–23.
23. Z. R. Zhang and V. V. Cosofret, *Sel. Electr. Rev*. **12** (1990) 35–135.
24. V. V. Cosofret and R. P. Buck, *Pharmaceutical Applications of Membrane Sensors*, CRC Press Inc., Boca Raton, FL, USA, 1992.
25. *United States Pharmacopoeia XX*, US Convention, Rockville, MD, USA, 1980, p. 519.
26. J. Koryta and K. Stulik , *Ion-Selective Electrodes*, Cambridge University Press, Cambridge, UK, 2nd Edition, 1983, p. 100.
27. V. V. Cosofret and R. P. Buck, *Analyst* **109** (1986) 1321.

## SAŽETAK

### Farmaceutska primjena membranske elektrode izrađene od metilensko plavo-fosfovolframata kao elektroaktivne tvari i selektivno osjetljive na metilensko plavilo (MB)

*Meng Liang Wen, Yi Bin Zhao, Xiang Chen i Chang Yi Wang*

Opisana je membranska elektroda od metilenskog plavila – polivinilklorida kojoj je elektroaktivna tvar ionski par metilensko plavilo – fosfovolframat. Elektroda je selektivno osjetljiva na metilensko plavilo (MB). Potencijal elektrode je linearna funkcija logaritma koncentracije MB u rasponu od $1 \times 10^{-6}$ do $1 \times 10^{-3}$ mol dm$^{-3}$, s nagibom od $51,5 \pm 0,8$ mV (unutar pH područja od 3,0 do 10,0). Granica detekcije MB je $6,79 \times 10^{-7}$ mol dm$^{-3}$. Elektroda se odlikuje stabilnošću, dobrom reproducibilnošću i brzim odzivom. Uobičajeni anorganski kationi i neke organske baze izazivaju zanemarive smetnje. Zbog takvih značajki, elektroda se može uspješno koristiti za određivanje koncentracija MB u injekcijama. Rezultati potenciometrijskih mjerenja koncentracija MB u injekcijama dobro se slažu sa rezultatima mjerenja standardnom procedurom propisanom Farmakopejom SAD.