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A single novel PVC membrane for dual determination of sulphadimethoxine and malachite green in aquatic environment

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Abstract A novel ion-pair of a quaternary ammonium compound; malachite green (MG), and an anionic drug sulphadimethoxine sodium (SDM) was prepared. The developed ion pair was incorporated into poly (vinyl chloride)-based membrane sensors for the dual quantification of sulphadimethoxine (sensor 1) and malachite green (sensor 2) in aquatic environment. Linear responses of SDM and MG were obtained within a concentration range of $10^{-5}–10^{-2}$ and $10^{-5}–10^{-3}$ M, respectively. The slopes of -29.8 ± 0.31 and 35.5 ± 0.20 mV/decade were obtained over pH range of 6–8 and 4–8 using sensors 1 and 2, in order. The proposed sensors displayed useful analytical characteristics for determination of SDM in its pharmaceutical formulation and in aquatic environment with average recoveries of 100.92 ± 0.19 and 102.47 ± 4.63, respectively. The recovery of MG in aquatic environment was 101.32 ± 3.64.

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1. Introduction

Malachite green (MG) is a cationic dye that belongs to the triphenylmethane group commercially available as the oxalate salt (Fig. 1). It is well known as an effective, inexpensive topical fungicide and disinfectant used in the aquaculture

industry. MG and its metabolite leucomalachite green were reported to cause human carcinogenesis and mutagenesis (Culp and Beland, 1996), and thus have been banned for use in aquaculture by both the US FDA and European Union. However, due to its low cost and high efficacy, this harmful dye is still used and will probably continue to be used in the aquaculture in some parts of the world and there is still concern about its illegal use.

Malachite green was determined by several methods for the detection and determination of both the dye and its leuco form in various matrices, such as water, urine, fish tissue, and animal feed by HPLC methods (Van de Riet et al. 2005; Mitrowska et al.,2005; Xu et al., 2012). Other methods also include the spectrophotometric method (Yuan et al., 2009) in

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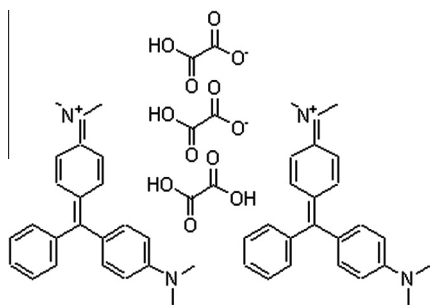E-mail address: h.marzouk@yahoo.com (H.M. Marzouk).

Figure 1 Structural formula of malachite green oxalate [$C_{46}H_{50}N_4\cdot3C_2H_2O_4$, formula weight 929.02 g/mol].

addition to electrochemical methods (Yi et al., 2008; Elmorsy et al., 2001).

Sulfadimethoxine (SDM) (Fig. 2) belongs to sulfonamides, which is commonly used in food-producing animals as growth promoters and as therapeutic and prophylactic drugs (Crosby, 1991), it is used in combination with trimethoprim. The presence of residues of sulfonamides in fish is of toxicological and regulatory concern as some of them could be carcinogenic and cause allergic hypersensitivity reactions and therapeutic ineffectiveness in human beings (Nue, 1992).

Many analytical methods were developed for the determination of sulphadimethoxine in different matrices including HPLC methods (Boison and Keng, 1995; Furusawa and Mukai, 1994; Granja et al., 2013), electrochemical methods (Soto-Chinchilla et al., 2006; Yao et al., 1987), and immunoassays (Ploum et al., 2005).

In the present study, the cationic malachite green (MG) reacts with the anionic sulphadimethoxine (SDM) with the formation of water insoluble ion association complex. The very high lipophilicity and remarkable stability of this complex (MG–SDM) suggest for its use as a novel electroactive material in PVC membrane. The developed membrane can be used in fabrication of two sensors for dual determination of the cationic MG and anionic SDM, selectively, in the presence of different interferents and in aquatic environment.

2. Experimental

2.1. Instruments

Potentiometric measurements were made at 25 ± 1 °C with a Jenway digital ion analyser model 3505 (Essex, UK) with Ag/AgCl double junction reference electrode No. Z113107-1EAPW (Aldrich Chemical Co.). A Jenway pH glass electrode No. 924005-BO3-Q11C (Jenway, Essex, UK) was used for pH measurements. A magnetic stirrer (Jenway 1000), membrane
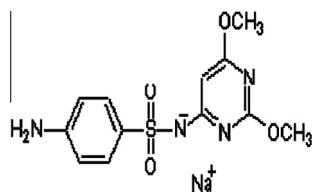

Figure 2 Structural formula of sulphadimethoxine sodium [$C_{12}H_{13}N_4NaO_4S$, formula weight 332.32 g/mol].

filter (sartoriusstedim biotech, 0.45 μm, Lot No. 16555 00867103) were used.

2.2. Materials

2.2.1. Reference sample

Sulphadimethoxine sodium was kindly supplied from PHARMA SWEDE-Egypt and its purity was certificated to be 99.7%. Malachite green oxalate salt was obtained from Sigma–Aldrich, Germany with certificated purity of 96.9%.

2.2.2. Pharmaceutical formulation

Trimethoxin® was manufactured by Chemifarma and was purchased from local market. Each 1 mL was claimed to contain 200 mg sulphadimethoxine and 40 mg trimethoprim, Batch number (B.N.): A120009.

2.3. Reagents

All chemicals and reagents used throughout this work were of analytical grade and bidistilled water was used. Tetrahydrofuran (THF) 99%, polyvinylchloride (PVC) powder, and dioctyl phthalate (DOP) were obtained from Aldrich, Germany. Potassium chloride, sodium hydroxide, hydrochloric acid were purchased from El-Nasr Pharmaceutical Chemical Company, Cairo, Egypt.

2.4. Standard solutions

2.4.1. Stock solutions of SDM and MG (each, 1×10^{-2} M)

They were freshly prepared by transferring 0.166 g of SDM and 0.4645 g of MG oxalate, separately, into two 50-mL volumetric flasks. Each compound was separately dissolved in 10 mL bi-distilled water, and the volume was then completed and protected from light.

2.4.2. Working solutions of SDM and MG (1×10^{-7} to 1×10^{-3} M)

Different solutions of each compound were freshly prepared by serial dilution of its corresponding stock solution using bi-distilled water. The prepared solutions were kept in well-closed tight containers and protected from light.

2.5. Procedures

2.5.1. Aquatic sample preparation and collection

2.5.1.1. Sample collection. The present study was carried out on few numbers (five to nine) of Oriochromus Niloticus (Nile tilapia) randomly collected from private freshwater farms at Abbassa–Sharkia. The fish were stocked in full glass aquarium filled with dechloronized tap water and supplied with air stone for aeration. The fish were fed (3% of their body weight) from commercial pellets containing at least 30% proteins. Water samples were collected three days from fish stocking in amber glass bottles.

2.5.1.2. Sample preparation. The collected water samples were filtered through Whatmann filter paper to eliminate suspended matter and then filtered through 0.45 μm nylon membrane

filter to eliminate fine particulate matter. Samples were stored at 4 °C in order to avoid any degradation or deterioration.

2.5.2. Precipitation-based technique for the preparation of PVC-membrane sensor

A volume of 20 mL aliquot of saturated aqueous sulphadimethoxine sodium (SDM) solution was mixed with 20 mL of saturated aqueous malachite green oxalate salt (MG) with continuous stirring. The resultant precipitate (ion pair complex) formed was filtered using Whatmann No. 42 paper, washed with bi-distilled water, dried at room temperature (about 25 °C) and grinded to fine powder. In a glass Petri dish (5 cm diameter), 10 mg of MG–SDM ion pair was thoroughly mixed with 0.4 mL of DOP and 0.19 g of PVC. The mixture was dissolved in 5 mL of THF. The Petri dish was covered with a filter paper and left to stand overnight to allow solvent evaporation at room temperature. A master membrane with a thickness of 0.1 mm was obtained. From the master membrane, two disks (8 mm diameter) were cut using a cork borer and pasted using THF, to two interchangeable PVC tips that were clipped into the end of the two electrode glass bodies to construct sensors 1 and 2.

Sensor 1 was then filled with an internal solution of equal volumes of 10^{-2} M SDM and 10^{-2} M KCl, while sensor 2 was filled with 10^{-2} M MG and 10^{-2} M KCl. Ag/AgCl wire (1 mm diameter) was used as an internal reference electrode. Each sensor was conditioned by soaking in 10^{-2} M of its aqueous solution for 24 h, and then stored in the same solution when not in use.

2.5.3. Effect of pH

The effect of pH on the potential values of the two sensors was studied over pH range of 3–10 at 1 pH interval by using 10^{-4} and 10^{-3} M solutions for sensors 1 and 2, respectively. The pH was gradually increased or decreased by adding aliquots of dilute sodium hydroxide or dilute hydrochloric acid solutions. The potential obtained at each pH value was recorded.

2.5.4. Sensor calibration

Each sensor and the double junction Ag/AgCl were immersed in its corresponding aqueous solutions in the range of 1×10^{-7} to 1×10^{-2} M. They were allowed to equilibrate while stirring and recording the EMF readings within ± 2 mV. The membrane sensors were stored in distilled water between measurements. The mV-concentration profiles were plotted.

2.5.5. Sensor selectivity

The potentiometric selectivity coefficient $- \log K^{pot}_{(Primary\ ion, interferent)}$ was used to evaluate the extent to which a foreign ion would interfere with the response of an electrode to its primary ion.

Selectivity coefficients were calculated by the separate solution method (IUPAC, 2008), where potentials were measured for 10^{-3} M aqueous solutions for sensors 1 and 2, respectively and then for 10^{-3} M aqueous interferent solution separately then potentiometric selectivity coefficients were calculated using the following equation:

$$\log K^{pot}_{A,B} = (E_B - E_A)/S + (1 - Z_A/Z_B)\log a_A$$

where $K^{pot}_{A,B}$ is the selectivity coefficient, E_A and E_B are the potentials of the drug and the interferent solutions respectively, S is the slope of the calibration plot, a_A is the activity of the drug, Z_A and Z_B are the charges on the drug and the interfering ions, respectively.

2.5.6. Direct potentiometric determination of SDM in its pharmaceutical formulation (Sensor 1)

From Trimethoxin® bottle, suitable dilutions were taken using bi-distilled water to obtain serial of 10^{-4}–10^{-3} M SDM. Procedure was then completed as under Section 2.5.4. From the recorded potential calculate the concentration of SDM from its corresponding regression equation.

2.5.7. Direct potentiometric determination of SDM and MG in fish water

In volumetric flasks of 25 mL, the filtered fish water was spiked with standard drug solutions and the volume was completed to the mark with the fish water. Then the EMF values of these spiked water samples were recorded and the concentrations of SDM and MG were calculated from the corresponding regression equation.

3. Results and discussion

The use of antimicrobial agents in the aquatic environment would cause concern, in terms of both potential environmental impact and potential human health implications. Both malachite green and Sulphadimethoxine are on the Food and Drug Administration's priority list for fish drugs that need analytical methods of development (Rushing and Hansen, 1997; Gehring et al., 2006).

Over the last two decades, ISEs in drug analysis have acquired increasing prominence. This technique also offers a lot of promise as an analytical tool in analysis of certain compounds in aquatic environment and coupled with the reliability of the analytical information leads to an attractive approach for the assay of aquatic compounds.

It is well known that the performance characteristics of IESs based on ion-exchangers depend to a large extent on

Table 1 General characteristics of the two investigated sensors.

Parameter	Sensor 1	Sensor 2
Slope (mV/decade)[a]	−29.8	35.5
Intercept (mV)	−1.8	229
LOD (mol L^{-1})[b]	9.7×10^{-6}	2.4×10^{-6}
Response time (s)	40	30
Working pH range	6–8	4.5–8
Concentration range (mol L^{-1})	10^{-5}–10^{-2}	10^{-5}–10^{-3}
Stability (days)	30	25
Average recovery (%) ± S.D.[a]	99.91 ± 1.21	99.98 ± 0.63
Correlation coefficient (r)	0.9994	0.9997
Precision (%)[c]	100.37	100.02

[a] Result of five determinations.
[b] Limit of detection (measured by interception of the extrapolated arms of Fig. 3).
[c] Average recovery percent of determining 10^{-3} and 10^{-4} mol L^{-1} SDM and MG for the studied sensors using different runs.
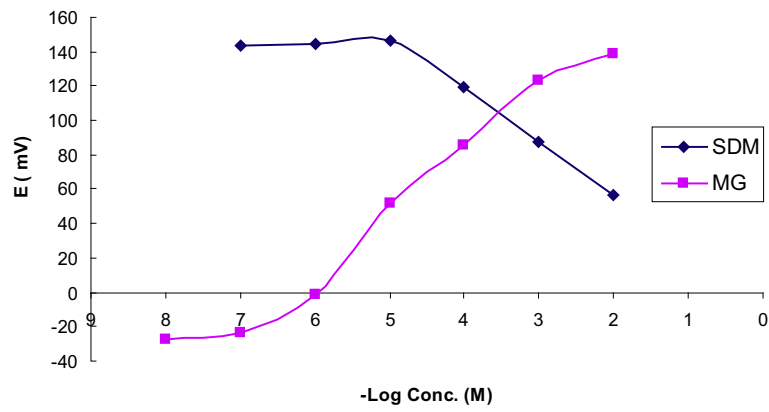
Figure 3 Profile of the potential in (mV) versus −log concentration of SDM and MG in mol/L.
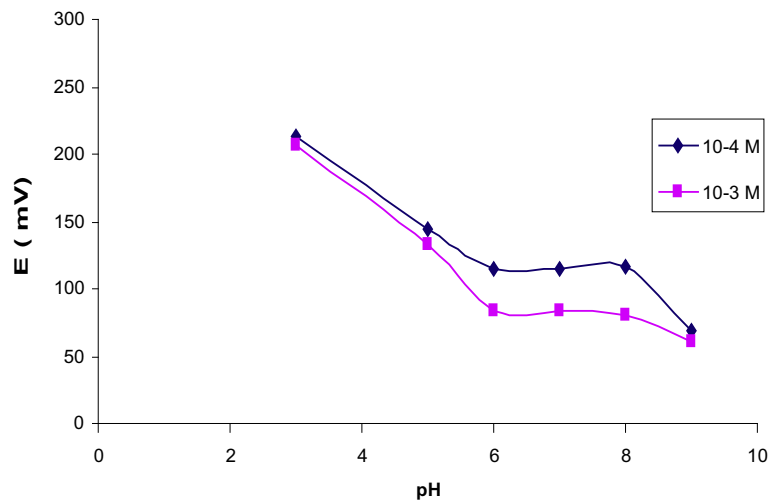

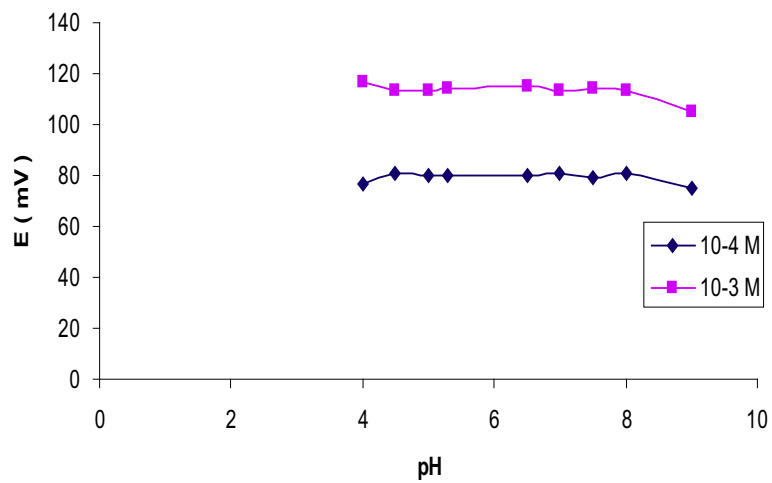**Figure 4** Effect of pH on sensor 1.

Figure 5 Effect of pH on sensor 2.

Table 2 Potentiometric selectivity coefficients of the proposed sensors by the separate selectivity method (SSM).

Interferent[a]	Selectivity coefficient	
	Sensor 1	Sensor 2
KCl	1.3×10^{-2}	2.1×10^{-5}
Lactose	5.6×10^{-3}	6×10^{-5}
Glucose	6.6×10^{-3}	3.5×10^{-5}
Urea	7.2×10^{-3}	1.3×10^{-5}
NaCl	9.1×10^{-2}	3.4×10^{-6}
CaCl$_2$	1.6×10^{-2}	2.7×10^{-5}
Sulphadiazine sodium	2.4×10^{-1}	1.1×10^{-6}
NaNO$_3$	7.1×10^{-3}	–
NaNO$_2$	2.2×10^{-2}	–
NaF	1.7×10^{-3}	–
Trimethoprim[b]	6.1×10^{-2}	–
Malachite green oxalate	1.0×10^{-9}	–
Sulphadimethoxine sodium	–	2×10^{-7}

[a] Aqueous solutions of 1×10^{-3} M were used.
[b] Was dissolved in least amount of methanol and the volume was then completed with bi-distilled water.

the nature of these ion-exchangers and their lipophilicities (Ibrahim et al., 2007).

The novelty of the work arises from the use of malachite green as a bulky, lipophilic cationic exchanger that allows its minimal leaching and longer lifetime. The resultant ion pair incorporated into the fabricated PVC membrane allows the dual determination of both the anionic SDM and the cationic MG using the same membrane.

3.1. Performance characteristics of the sensors

The electrochemical performance characteristics of the two investigated sensors were systematically evaluated according to the IUPAC standards in Table 1.

The slope of the calibration plots was typically -29.8 and 35.5 mV/concentration decade for sensors 1 and 2, respectively. Typical calibration plots are shown in Fig. 3.

3.2. Dynamic response time

Dynamic response time is an important factor for analytical applications of ion selective electrodes. In this study, practical response time was recorded by increasing the SDM and MG concentrations by up to 10-fold. The required time for sensors to reach values within ± 1 mV of the final equilibrium potential was nearly 30 s for sensors 1 and 2, respectively.

3.3. Effect of pH

Studies were carried out to reach the optimum experimental conditions. The potential obtained at each pH value was recorded. It was apparent from the potential–pH profile (Fig. 4) that the response is fairly constant over the pH range (6–8) for sensor 1, while the response is constant over a wider pH range (4.5–8) for sensor 2 (Fig. 5).

3.4. Sensors selectivity

The potentiometric selectivity coefficients of the proposed sensors (1 & 2) were evaluated in the presence of a co-formulated drug (trimethoprim), organic additives and some inorganic ions ($K^+, Na^+, Ca^+ +, NO_3^-, NO_2^-, F^-$) that are usually present in pharmaceutical dosage forms, in biological fluids or in fish water as shown in Table 2. The results reveal that the proposed membrane sensors display high selectivity and lower response for the potentially interfering species and co-formulated drug.

3.5. Potentiometric determination of SDM in its pharmaceutical formulation

Sensor 1 was successfully applied for the selective analysis of SDM in its pharmaceutical formulation with fairly no

Table 3 Determination of sulphadimethoxine sodium in pharmaceutical formulation by the suggested potentiometric procedures (sensor 1).

Pharmaceutical formulation	Recovery% ± SD[a]	
	Sensor 1	Official method (USP, 2011)
Trimethoxin B.N. (A120009)	*100.92 ± 0.19*	*100.65 ± 0.36*
Student's *t*-test (2.306)[b]	1.483	–
F-value (6.39)[b]	3.59	–

[a] Average of five determinations for either the proposed sensor or the official method.
[b] The values between parentheses are the corresponding theoretical values of *t* and *F* at the 95% confidence level.

Table 4 Determination of SDM and MG in spiked fish water sample using the proposed sensors.

Sensor 1		Sensor 2	
SDM measured in fish water sample (ppm)	Recovery%	MG measured in fish water sample (ppm)	Recovery%
10^{-5} (3.3232)	–	10^{-5} (9.2902)	104.3%
10^{-4} (33.232)	105.75%	10^{-4} (92.902)	97.26%
10^{-3} (332.32)	99.2%	10^{-3} (929.02)	102.4%
Mean ± SD	102.47 ± 4.63		101.32 ± 3.64

interference from trimethoprim. The results prove the applicability of the sensor for the determination of the studied drug in pharmaceutical formulation. Results obtained by the proposed sensor for the determination of SDM in the pharmaceutical formulation were statistically compared to those obtained by the official method (USP, 2011) as shown in Table 3.

3.6. Potentiometric determination of SDM and MG in the spiked fish water

For the application to fish water, it was found that the two sensors are reliable and give stable results with high accuracy and precision without preliminary extraction procedures, which is shown in Table 4. The pH of these samples was measured before spiking and was found to be 6.5 ± 0.5, which is within the pH working range of the two proposed sensors.

4. Conclusion

The novelty of these fabricated sensors was raised from that, MG was used for the first time as a cationic exchanger for an anionic drug such as SDM. The very high lipophilicity and remarkable stability of the proposed ion-pair (MG–SDM) suggest its use as a novel electroactive material in PVC membrane sensors. The developed membrane was used for dual determination of the cationic MG and anionic SDM, selectively. The electrodes were easily prepared with high sensitivity, wide dynamic range and remarkable long lifetime. High selectivity and rapid response make these electrodes suitable for measuring the concentration of MG and SDM in a wide variety of samples without the need for pretreatment steps and without significant interference from other anionic or cationic species present in the aquatic environment.

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