S. Afr. J. Chem., 2003, **56**, 5–9,
<http://journals.sabinet.co.za/sajchem/>.

Voltammetric Determination of a Benzimidazole Anthelmintic Mixture at a Poly(3-methylthiophene)-modified Glassy Carbon Electrode

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Received 3 October 2001; revised 17 October 2002; accepted 24 October 2002.

ABSTRACT

The voltammetric determination of benzimidazole anthelmintics at a glassy carbon rotating-disk electrode modified with poly(3-methylthiophene) is presented. The purpose of surface modification was to improve the sensitivity and limits of detection for determination of the compounds in a standard mixture. Thus, five compounds, namely thiabendazole, mebendazole, albendazole, fenbendazole and oxibendazole have been studied using square wave voltammetry. It has been possible to resolve four of the compounds, mebendazole, fenbendazole, oxibendazole and thiabendazole, in a mixture. Investigations of a number of parameters, including the mode of potential application, cathodic reduction *versus* anodic oxidation, the type of electrode, effect of pH and speed of electrode rotation, among others, are reported.

KEYWORDS

Electrochemistry, square-wave voltammetry, benzimidazole anthelmintics, poly(3-methylthiophene) electrode.

1. Introduction

1.1. Anthelmintics

Anthelmintics are drugs used to prevent or treat animal disease such as gastrointestinal nematodes of ruminants.[1] They are usually used in two ways, therapeutic and prophylactic. The former involves the treatment of already infected hosts while the latter involves the epidemiology of the parasites to prevent infections. There are different categories of anthelmintics; piperazines, imidazothiazoles, tetrahydropyrimidines, benzimidazoles, pro-benzimidazoles, avermectins and organophosphates.[2] This work focuses mainly on the benzimidazole anthelmintics. The latter are widely used in both clinical medicine and veterinary practice.[3–5] The potential toxicity of the benzimidazoles to the host organisms has raised concern from the public, hence the need for monitoring of the residue levels of these compounds in food and other samples.[6] Benzimidazoles are broad-spectrum drugs whose mechanism involves interfering with energy metabolism of the parasites, essentially starving them. Other mechanisms include selective binding to the protein tubulin, which results in disruption of cell functions, e.g. cell division, in helminths,[7] and uncoupling of oxidative phosphorylation.[8]

1.2. Methods of Benzimidazole Anthelmintic Determination

Methods based on ELISA[3,9] and immunoassay[10,11] techniques are mainly used for the determination of benzimidazole anthelmintics. Techniques based on chromatographic separation of the various benzimidazole residues are also common.[12,13] However, in general, studies using electrochemical methods for determination of biological molecules, such as benzimidazoles, are few.[14–17] In addition, the few electrochemical studies that have been reported mainly employed polarography,[14,16,17] with little application of solid state voltammetry.[15] For instance, in 1964, Struck and Elving reported[14] a polarographic determination of parabanic acid (imidazolidinetrion) in acetate/phosphate buffer (pH 5.1). Smola and Sontag, in 1985, reported[16] the determination of thiabendazole by differential-pulse polarography (DPP) and sampled DC-polarography. McClean and coworkers[17] used a DPP technique to study the degradation of benzimidazole sulphoxide anticancer drugs (SK- and F-95601) and omeprazole, in acidic media. The limited use of solid state voltammetry is due to poor sensitivity and detection limits on classical surfaces such as metals and carbon. This is partly due to electrode fouling and poor selectivity.[18] Attempts[19] to alleviate these problems have been made by using polymer coating on the surface of the metal or carbon electrode. Conducting polymers such as poly(3-methylthiophene) have been employed[2] for this purpose. Recently, we have reported[20] modification of glassy carbon surfaces using poly(3-methylthiophene) for the determination of sulphonamides. In an effort to explore further the potential applications of this polymer in biological samples, this study reports the determination of benzimidazole anthelmintics. In addition, we have also compared the sensitivities of the differential pulse- and square-wave voltammetry (DPV and SQWV) techniques. The purpose of this study, therefore, is to investigate the effect of electrode surface modification on sensitivity and detection limits as well as the effectiveness of resolving benzimidazole compounds in a mixture of standard solution. Thus, five compounds, namely mebendazole, fenbendazole, oxibendazole, thiabendazole and albendazole were studied. Investigations of a number of parameters, including the mode of potential application, cathodic reduction/anodic oxidation, the type of the electrode surface, effect of speed of electrode rotation and pH, among others, have been carried out.

2. Experimental

2.1. Standards and Chemicals

All the chemicals were of analar grade (AR). The 3-methylthiophene and benzimidazole compounds, (i) fenbendazole [methyl 5-(phenylthiol)-2-benzimidazolecarbamate], molar

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mass 299.35 g mol^{-1}, (ii) oxibendazole [methyl (5-propoxy-1H-benzimidazol-2-yl)carbamate], 249.27 g mol^{-1}, (iii) thiabendazole [2-(4-thiazolyl)benzimidazole], 201.25 g mol^{-1}, (iv) mebendazole (5-benzoyl-2-benzimidazolecarbamic acid methyl ester), 295.30 g mol^{-1}, and (v) albendazole [methyl-5-(propylthiol-2-benzimidazolecarbamate)], 265.34 g mol^{-1}, were obtained from Sigma (St Louis, MO, USA). Boric acid was obtained from SAARCHEM (Halfway House, South Africa) and acetic acid, phosphoric acid and sodium hydroxide from N.T. Laboratory Supplies (Midrand, South Africa). Stock solutions (1000 ppm, $\sim 3.895 \times 10^{-3}$ M) were prepared by dissolving 10 mg of the benzimidazole anthelmintic in 10 cm^3 of methanol-formic acid mixture (95:5). The working standard solutions were prepared from the stock solution by serial dilution. For most of the experiments on optimization of parameters, a 1.00 ppm ($\sim 3.895 \times 10^{-6}$ M) solution was used. High-quality ultrapure water was obtained by passing de-ionized water through a purifier (ELGASTAT, UHPQS, UK). Stock solutions were stored in brown glass bottles and kept at 4°C. Solvents were filtered through a cellulose membrane (0.45 μm). The liquid monomer, 3-methylthiophene, was first vacuum distilled in a nitrogen gas atmosphere before use. A solution of purified 3-methylthiophene (0.05 M) was prepared in tetramethylammonium tetrafluoroborate supporting electrolyte (0.1 M) dissolved in acetonitrile. Current measurements for the benzimidazole standards were done in a supporting electrolyte of Britton-Robinson (BR) buffer.[21] The buffer solution was composed of phosphoric acid (5.3 cm^3 of 85%), glacial acetic acid (4.6 cm^3) and boric acid (4.94 g) made up to 2 dm^3 with ultra high purity (UHP) water. By adding varying amounts of sodium hydroxide solution (0.2 M) to the buffer solution, a pH range of 2.55 to 9.54 was obtained.

2.2. Instrumentation

For all electrochemical measurements, namely cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SQWV), an electrochemical analyser (AUTOLAB PGSTAT10, Ecochemie, Netherlands) running under a general-purpose electrochemical system (GPES) was used. Cyclic voltammetry (+0.5 to +2.0 V) was used for electropolymerization of 3-methylthiophene. The cathodic and anodic behaviour (potential range –4.0 to +4.0 V) of benzimidazole compounds was investigated using DPV and SQWV techniques at Pt and glassy carbon (GC) rotating disc electrodes (RDE), with a surface area of 0.280 cm^2, *versus* Ag/AgCl (3 M KCl) and Pt sheet (2 × 2 cm^2) as auxiliary electrode. The RDEs were polished using a suspension of alumina powder in water, rinsed with UHP water and dried with soft tissue paper. All the electrodes and accessories were purchased from Metrohm, Switzerland.

3. Results and Discussion

The polymerization of 3-methylthiophene, effect of pH on the redox behaviour of the compounds, comparison between DPV and SQWV techniques, cathodic *versus* anodic processes, and response characteristics of the benzimidazole anthelmintics, including Levich behaviour of GC-RDE, are discussed in the following sections.

3.1. Electropolymerization of 3-Methylthiophene

3-Methylthiophene monomer was electropolymerized through oxidation, at about +1.505 V and +1.404 V on Pt and GC electrodes, respectively, against Ag/AgCl (3 M KCl) reference. The difference in results (oxidation potentials of 3-methylthiophene) between the two electrodes illustrates the importance of the nature of the electrode surface in electron-transfer processes. The polymer, on the other hand, was reduced at about +0.940 V and +0.842 V using Pt and GC electrodes, respectively. These potential values are comparable with those obtained by Galal.[2] The poly(3-methylthiophene)-modified electrodes were then used in subsequent experiments.

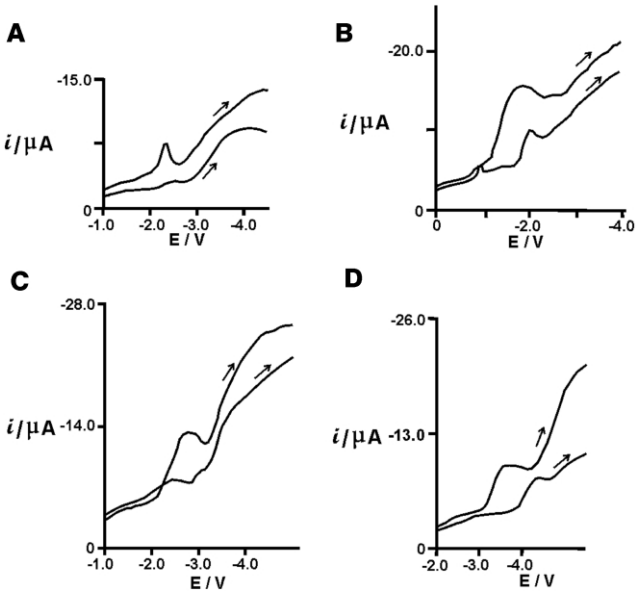

Figure 1 Effect of surface modification of GC electrode (RDE, 300 rpm) using SQWV in cathodic reduction (0 to –4.0 V) of benzimidazole compounds (1.0 ppm in BR buffer, pH 4.93): (1) modified electrode and (2) unmodified electrode. **A**, fenbendazole; **B**, mebendazole, **C**, oxibendazole, and **D**, thiabendazole. Arrows indicate the direction of potential scan.

3.2. Effect of Electrode Surface Modification

In order to study the effect of surface modification, both the modified and the unmodified GC electrodes were used (see Fig. 1) for the detection of (A) fenbendazole, (B) mebendazole, (C) oxibendazole and (D) thiabendazole. It was observed that the peak currents measured at the modified electrode were significantly higher than those at the unmodified one. This is due, as expected, to the presence of the conducting polymer film, known[1] to catalyse electron-transfer processes across solution interfaces.

3.3. Effect of pH on Cathodic Peak Current (I_{pc}) and Potential (E_{pc})

The effect of pH was investigated by preparing solutions of benzimidazoles in BR buffer at different pH values (2.55 to 9.54). The results obtained (see Fig. 2), show cathodic currents to be lowest (least negative) between pH 4.0 and 6.5, hence this is the best pH region for most of the compounds (Fig. 2A). However, there appears to be only a slight difference in cathodic potentials for most of the compounds. Thus only two compounds (fenbendazole and albendazole) appear to have slightly higher (negative) potential values in the pH region 4.0 to 6.5 (Fig. 2B). The pH effect could be explained on the basis of the pK_a values. All the compounds have two pK_a values (pK_{a1} and pK_{a2}); albendazole[23] 2.80 and 10.26; thiabendazole[24] 5.82 and 12.79; oxibendazole[25] 5.74 and 11.20; mebendazole[25] 4.50 and 10.40; and fenbendazole[26] 5.14 and 11.80 for pK_{a1} and pK_{a2}, respectively.

3.4. Effect of Rotation Speed of the Electrode (RDE)

A plot of inverse current (i^{-1}) *versus* inverse square root of rotation speed ($\omega^{-1/2}$) indicates the current produced to be

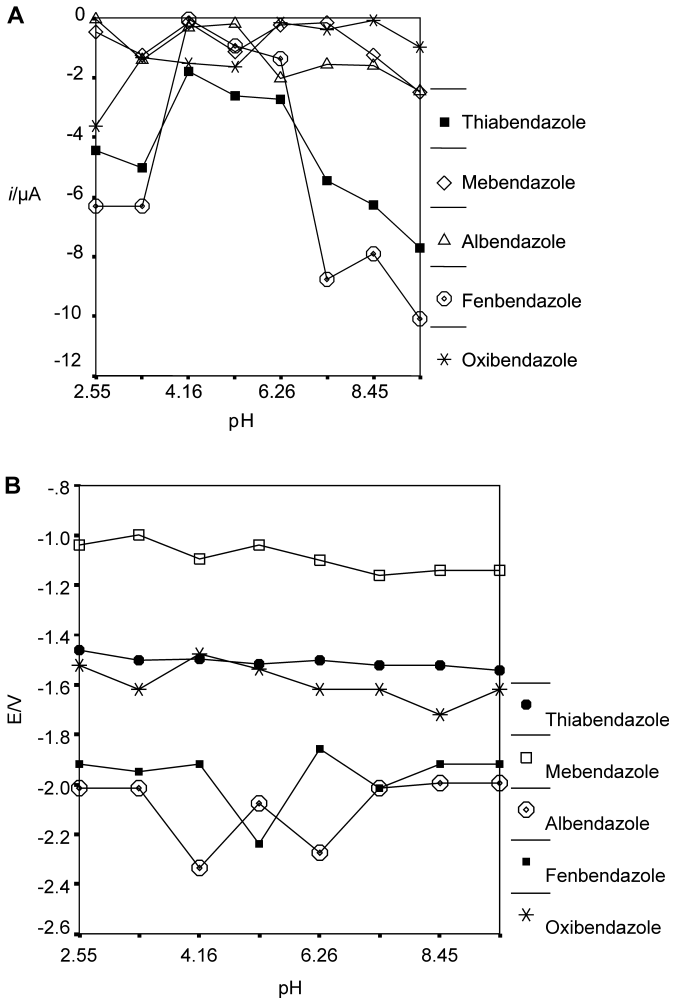

Figure 2 Effect of pH on electrochemical behaviour of benzimidazole anthelmintics. (**A**) peak currents and (**B**) peak potentials of thiabendazole, mebendazole, albendazole, fenbendazole and oxibendazole (1.0 ppm in BR buffer).

proportional to the rate of stirring in a given benzimidazole solution (see Fig. 3). These observations show Levich behaviour, particularly for albendazole and thiabendazole, which also showed least sensitivity in terms of the slopes of the calibration plots (discussed below). Stirring the solution therefore had a relatively significant effect on the response signal, particularly for these two compounds. Levich behaviour is expected due to the fact that the measured current is controlled by the rate of diffusion of analytes to and from the electrode surface. The rate of diffusion is proportional to the difference in concentration at

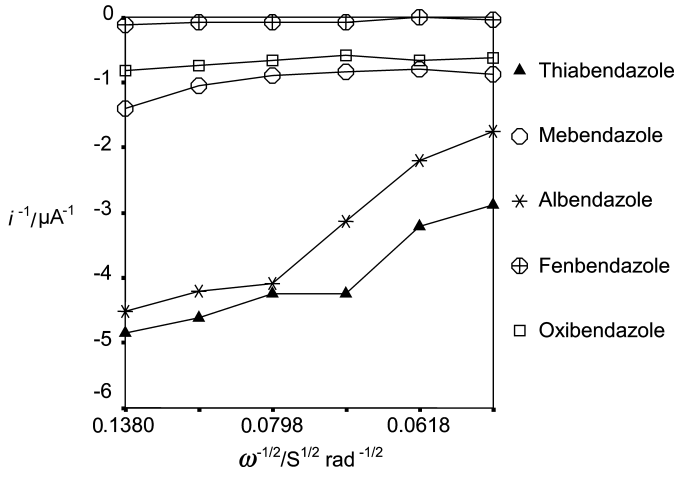

Figure 3 A plot of inverse current *versus* inverse square root of rotation speed at modified GC electrode, for benzimidazole compounds: thiabendazole, mebendazole, albendazole, fenbendazole and oxibendazole.

the diffusion layer (C_s) and solution bulk (C_o).[22] Thus, peak current ∝ rate of diffusion ∝ (C_o–C_s), since increasing the speed of electrode rotation increases the rate of mass transfer, which in turn increases the Faradaic current. The latter should increase to a maximum when $C_o = C_s$ and reduces when $C_s > C_o$.[22] An increase in signal magnitude with stirring is also a way of confirming the absence of electrode fouling.[2]

3.5. Calibration Curves for Mebendazole and Albendazole: SQWV *versus* DPV

Calibration curves (Fig. 4 A and B) using SQWV and DPV are shown for two of the five compounds, (A) mebendazole and (B) albendazole. The plots indicate a working concentration range of 3.895×10^{-6} M to 3.895×10^{-5} M (~1.0 to 10.0 ppm), deduced from the full calibration curves, 3.895×10^{-7} M to 3.895×10^{-5} M (~0.1 to 10.0 ppm), initially prepared for the five compounds (curves not displayed). A comparison of the R^2 values[27] for both linear and quadratic equations (3.895×10^{-6} M to 3.895×10^{-5} M) were used to determine the best fit to the calibration data (see Table 1). The quadratic equations gave better values (R^2 closer to 1.0) than the linear plots. The working range was therefore worked out on the basis of the R^2 values obtained from the regression plots. Based on the slopes of the regression lines, calculated as change in current (i) per change in concentration (c), $\frac{\Delta i}{\Delta c}$, (Table 1), mebendazole showed the highest sensitivity value among the five compounds, while albendazole showed the lowest.

Table 1 Results of regression and error analysis for determination of benzimidazoles (pH 4.93).

Compound	Slope[a] /μA dm^3 mol^{-1}	(R^2)[b] (linear)	(R^2)[c] (quadratic)	DL[d] /10^{-7} mol dm^{-3}	% RSD[e] (n = 5)
Mebendazole	–16.114	0.9899	0.9954	4.968	2.5
Fenbendazole	–3.185	0.9910	0.9963	4.011	2.1
Oxibendazole	–0.530	0.9760	0.9993	3.768	1.5
Thiabendazole	–0.336	0.9913	0.9978	3.386	1.9
Albendazole	–0.221	0.9880	0.9983	3.334	1.7

[a] Linear slopes calculated for concentrations 3.895×10^{-6} to 2.931×10^{-5} M.
[b] Coefficient-of-determination (R^2) values for linear regression plots, in the concentration range 3.895×10^{-6} to 3.895×10^{-5} M.
[c] Coefficient-of-determination (R^2) values for curvilinear regression plots, in the concentration range 3.895×10^{-6} to 3.895×10^{-5} M.
[d] Detection limit.
[e] Percentage relative standard deviations, %RSD (n = 5) for the DL values.

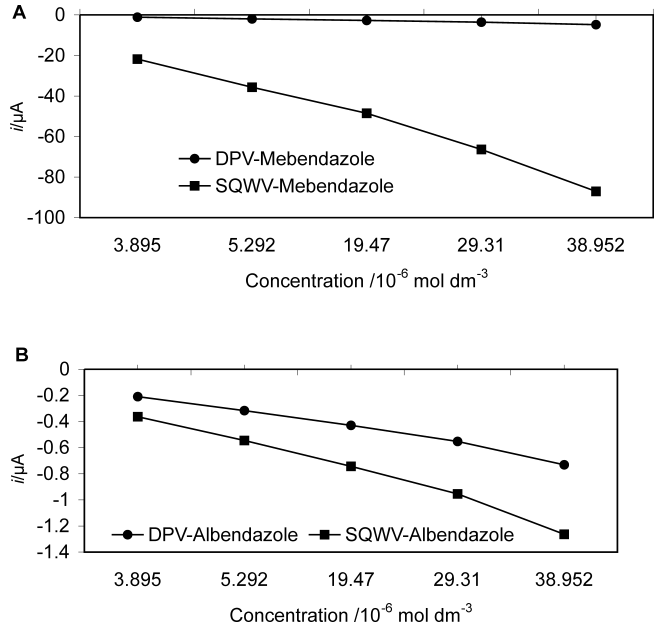

Figure 4 Calibration plots showing current peak heights *versus* concentration using SQWV and DPV techniques: (**A**) mebendazole and (**B**) albendazole, using modified GC electrode. Concentration range 3.895×10^{-6} M to 3.895×10^{-5} M (in BR buffer, pH 4.93).

A comparison of peak currents of the voltammograms (see Fig. 5A) of mebendazole (which showed the highest calibration sensitivity) obtained using both SQWV and DPV, confirms the superiority[22] of SQWV over DPV. A regression curve (Fig. 5B) of SQWV on DPV peak currents shows the two methods to give

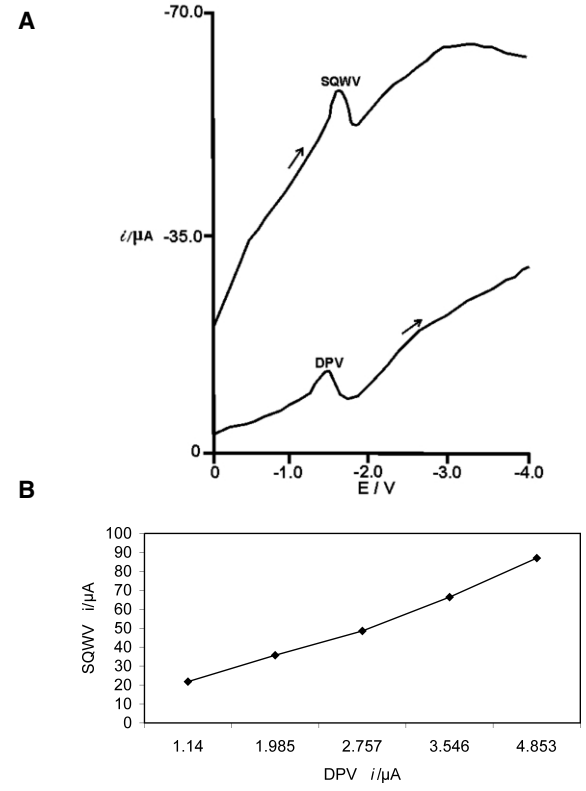

Figure 5 Comparison of SQWV and DPV techniques for determining mebendazole (1.0 ppm in BR buffer, pH 4.93) at poly(3-methylthiophene)-modified GC electrode (RDE, 3000 rpm): (**A**) voltammograms by SQWV and DPV; and (**B**) regression curve of peak current data from SWQV on those obtained by DPV. Arrows in (A) indicate the direction of potential scan.

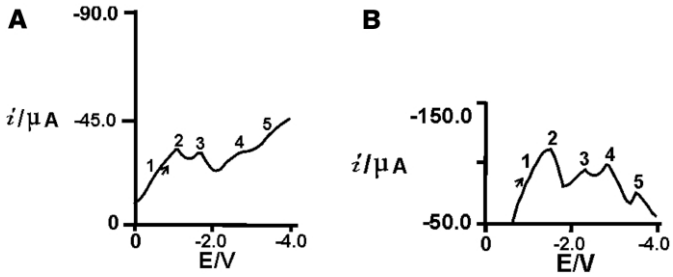

Figure 6 Determination of a mixture of benzimidazoles using (**A**) DPV and (**B**) SQWV techniques: (1) albendazole, (2) mebendazole, (3) fenbendazole, (4) oxibendazole, and (5) thiabendazole. Note: peaks (1) and (2) are not well resolved. Arrows indicate direction of potential scan.

significantly different data (slopes not equal to 1.0). The peak potentials and currents for the five compounds, obtained at similar concentrations, are given in Table 2. The peak potentials obtained with the two techniques (SQWV and DPV) show some differences for most of the compounds, particularly for thiabendazole, which showed the highest reduction potential. The calculated ratios of peak currents, SQWV/DPV, gave values greater than 1.5 for all the compounds. In addition, a statistical *t*-test ($n = 5$, $P = 0.05$) performed on each pair of current values showed a significant difference between the two sets of data (SWQV *versus* DPV). The difference in sensitivity values for the five compounds at the same concentration suggests different stoichiometric values for the reduction reaction involving the imidazole functional group. Thus mebendazole showed the highest current ratio while albendazole gave the lowest. This may also imply the existence of different chemical environments for the functional group(s) in these compounds.

Detection limits (DLs), using the SQWV technique (Table 1), were each calculated as the analyte concentration giving a signal equal to the blank signal (y_B) plus three standard deviations ($n = 5$) of the blank (s_B), that is, $y_B + 3s_B$.[27] Mebendazole showed the lowest (best) DL value and albendazole the highest (poorest). This trend of results is similar to that displayed by the calibration sensitivity (slope) values (Table 1). The corresponding percentage relative standard deviations (%RSD) for the DL values were also calculated ($n = 5$) (Table 1). The %RSD values roughly indicate a larger error for higher than for lower concentrations.

3.6 Resolution of Benzimidazole Mixture in SQWV and DPV

Prior to determination of components in the mixture of benzimidazole standards (1.0 ppm of each compound in BR buffer, pH 4.93), a scan of the blank buffer solution was obtained followed by one of each of the individual compounds. Both DPV and SQWV techniques were used for comparison. The SQWV technique (Fig. 6B) showed relatively better resolution than the DPV (Fig. 6A). Thus, while in SQWV, four compounds (mebendazole, fenbendazole, oxibendazole and thiabendazole) were resolved in a mixture, only three (mebendazole, fenbendazole and oxibendazole) were resolved using DPV.

4. Conclusions

The results in this study have shown that modification of the surface of a classical electrode has potential in the analysis of veterinary drug residues. The effect of surface modification of the electrode has been investigated using poly(3-methylthiophene). The modified electrode gave better sensitivity values (slopes of the linear calibration plots) than those obtained

Table 2 Comparison of SQWV and DPV methods for both peak potentials and currents of benzimidazoles (1.0 ppm in BR buffer, pH 4.93) obtained at poly(3-methylthiophene)-modified GC electrode (RDE, 3000 rpm).

Compound	SQWV		DPV		Current ratio (SQWV/DPV)
	Potential /V	Current /μA	Potential /V	Current /μA	
Mebendazole	–1.41	–3.480	–1.55	–0.934	3.7
Fenbendazole	–2.25	–1.790	–2.37	–0.534	3.4
Oxibendazole	–2.77	–1.620	–3.05	–0.804	2.0
Thiabendazole	–3.35	–2.180	–3.25	–1.140	1.9
Albendazole	–1.32	–0.363	–1.48	–0.210	1.7

with the unmodified one. This suggests that the conducting polymer catalyses an electron-transfer process across an electrode-solution interface. The square-wave signal excitation method has been proved to be better, in terms of detection limits, sensitivity and resolving power, than the differential pulse technique. Most of the compounds showed maximum (negative) peak currents in the pH range 3.0 to 7.0, which roughly falls between the pK_{a1} and pK_{a2} values of the compounds. In contrast, the effect of pH on the peak potentials (negative) was insignificant for most of the compounds, except for fenbendazole and albendazole, which showed slightly higher negative potentials in this pH region. The observed detection limits of concentrations around 0.1 ppm ($\sim 3.895 \times 10^{-7}$ M) suggest the suitability of the polymer-modified electrode for determination of benzimidazole compounds in biological samples, as this value (0.1 ppm) falls within the residue range of 10 ppb to 1.0 ppm frequently encountered in veterinary samples.[28] Further studies are being carried out to determine benzimidazoles in multi-residues of veterinary drugs used in Botswana.

Acknowledgements

T.A.M.M. is grateful to the German Academic Exchange Programme – Natural Products for East and Central Africa Association (DAAD-NAPRECA) for a M.Phil. scholarship. He is thankful to M.M. Nindi for initial studies of anthelmintic compounds with electrospray ionization–mass spectrometry (ESI-MS). The authors thank the University of Botswana for providing the necessary facilities.

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