



DETERMINATION OF ISOTHIAZOLINONES IN COSMETICS USING DIFFERENTIAL PULSE VOLTAMMETRY ON AN ULTRAMICROELECTRODE

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ABSTRACT

A simple electroanalytical method for the simultaneous determination of isothiazolinones has been developed using a carbon fiber electrode. Electron transfer properties of carbon fiber electrode and carbon paste electrode are investigated by electrochemical impedance spectroscopy (EIS). The methylchloroisothiazolinone (MCI) and methylisothiazolinone (MI) can be separated at the carbon fiber electrode easier than carbon paste electrode. For differential pulse voltammetric measurements with suitable methodical parameters, linear concentrations are found over the ranges 4.0 - 230 mg L⁻¹ and 2-260 mg L⁻¹ for MCI and MI, respectively. The method is applicable to the quantitative determination of MCI and MI in cosmetics. Findings using voltammetric determination and high-performance liquid chromatography method and were comparable.

Key Words:- Ultramicroelectrode, Electrochemical Impedance Spectroscopy, Methylchloroisothiazolinone (MCI)/Methylisothiazolinone (MI), Cosmetics.

INTRODUCTION

Isothiazolinones are heterocyclic organic compounds with bactericidal, algicidal and fungicidal activity. The use of isothiazolin-3-ones (which are also referred to as 3-isothiazolones) in such biocidal products is well established, since this class of compound includes highly active biocidal compounds (Baum R *et al.*, 2010). The combination of 5-chloro-2-methyl-4-isothiazolin-3-one (methylchlorois othiazolinone, MCI) and 2-methyl-4-isothiazolin-3-one (methylisothiazolinone, MI), in a 3:1 ratio, commercialized as Kathon CG, has been frequently used as a preservative since the beginning of the 1980s. They widely utilized both in cosmetics and household cleaning products (Lundov MD *et al.*, 2010; Magnano M

et al., 2009). MCI/MI is one of the most frequent causes of preservative contact allergy and early studies showed that both MCI and MI are sensitizers, mainly in cosmetics (Higgins E *et al.*, 2013; Fransway AF *et al.*, 2013; Coelho MV *et al.*, 2012; Wolfgang U *et al.*, 2012; Lundov MD *et al.*, 2011; Kerem Y *et al.*, 2011). Research on MCI/MI is popular; however, almost all studies focus on contact allergy. Few reports focus on analytical method. A survey of literature there were only two methods applied in cosmetics and environmental water which include liquid and gas chromatography (Matissek R *et al.*, 1986; Nakashima H *et al.*, 2000; Rafoth A *et al.*, 2007). Electrochemical impedance spectroscopy (EIS) is a powerful tool for examining many chemical and physical processes in solutions as well as solids such as rate constants, diffusion coefficients and to study the electron transfer (Barsukov Y *et al.*, 2012; Sezgin S *et al.*, 2012; Lupu S, 2011; Ulrich C *et al.*, 2007; Orazema ME and Bernard T, 2008; Zielinska D and Pierozynski B, 2009).

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Carbon fibers were mechanically stable and their surfaces could be easily modified by chemical reactions. Therefore, they were versatile and convenient basis materials for the fabrication of voltammetric and amperometric as well as of potentiometric micro electrodes. Due to the advantages of ultramicroelectrodes their use in electrochemical studies had been an important application of investigation of organic compound in recent years (Wang LH and Chu SC, 2004). Carbon fiber electrodes (CFEs) are commonly used in the *in vitro* and *in vivo* detection of electroactive species, especially in biological systems. The ability to build electrochemical sensor from noble metals instead of carbon fibers may be beneficial in developing inexpensive multiplexed electrochemical detection schemes (Heramb C *et al.*, 2012; Liu X *et al.*, 2012; Munteanu G *et al.*, 2010; Zachek MK *et al.*, 2008; El-Nour KA *et al.*, 2003). In this work, the electrochemical oxidation of MCI and MI using a gold, silver, carbon paste electrode (CPE), and carbon fiber electrode (CFE) has been investigated by impedance and differential pulse voltammetry (DPV). The optimum experimental conditions for the determination of MCI and MI in cosmetic products are described in this paper.

EXPERIMENTAL

Apparatus and Materials

All electrochemical measurements were performed with an EG&G Princeton Applied Research Model 394 Polarographic Analyzer or a potentiostat-galvanostat (SP-150; Bio-Logic SAS, 1 rue de l'Europe 38640 - CLAIX - FRANCE) with a conventional three-electrode configuration with a gold, silver, carbon fiber electrode (CFE) and carbon paste electrode (CPE) as working electrodes. Potentials were measured versus the Ag/AgCl electrode (RE-1; Bioanalytical Systems, West Lafayette, IN, USA), and a platinum wire was used as the auxiliary electrode. The HPLC system used consisted of a Hitachi Model L-7110 pump with a Rheodine 7125 injection valve with 20 μ l sample loop. 2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one (Scheme 1) were purchased from Fluka and Santa Cruz Biotechnology Company, respectively. The cosmetics investigated were purchased at a local

department store. All other reagents were locally purchased and of analytical grade.

Electrochemical measurements by EIS and DPV

CPE and CFE were prepared as previously Described (Wang LH *et al.*, 2001; Wang LH *et al.*, 2012). Differential pulse voltammetry (DPV) and EIS were performed in a Britton and Robison buffer (pH 2.76-8.87) and lithium perchlorate (LiClO₄) solutions as supporting electrolytes at CFE. In DPV potential were from +0.5V to + 2.0 V with scan rate at 10 mV s⁻¹. The EIS data acquisition were performed using SP-150; Bio-Logic SAS and EC-Lab® softwares. The impedance spectra were recorded over a frequency range of 0.1 Hz to 10 kHz, using a sinusoidal excitation signal, superimposed on a *dc* potential of + 0.180 V. Excitation amplitude of 5 mV was used throughout.

Determination of MCI and MI in cosmetics

Stock solution of standard was prepared by dissolving the appropriate amount of MCI and MI in methanol. A set of standard solutions were produced by diluting aliquots of the stock solutions with methanol to 10 mL in calibrated flasks. Taking into account about the MCI and MI content of the anti-dandruff shampoo and whitening body lotion, samples (approx. 0.05-0.6 g) of the latter were weighed accurately in a 15 mL beaker, diluted to about 10 mL ethyl acetate, dichloromethane, ethyl acetate-dichloromethane (3:2, v/v) and ethyl acetate-dichloromethane (3:1, v/v), dissolved and centrifuged respectively. The supernatant was transferred into a 5 mL calibrated flasks. An aliquot of the solution was filtered through a 0.45 μ m membrane filter prior to HPLC analysis. We used that a simple dilution process worked for DPV experiments well as the standard solution. A Phenomenex Luna C₁₈ column (5 μ m, 250 x 4.6 mm) was used for reverse-phase HPLC. The mobile phase was 20:80 methanol-water (containing lithium perchlorate, pH 7.14) at a flow rate of 1.0 mL min⁻¹, the UV detector was operated at 270 nm. By means of the injection value, 20 μ L of the prepared sample solution and standard solution was chromatographed under the operating conditions described above. Quantitation was based on the peak area of the sample.

Table 1. Effect of pH and supporting electrolytes on the differential pulse voltammetric peak potential and peak current of 5-chloro-2-methyl-4-isothiazolin-3-one (MCI) at carbon fiber electrode (CFE) and capillary carbon paste electrodes CPE (i.d. 0.8 mm)

Supporting electrolytes	pH	CFE		CPE	
		Potential (V)	Current (μ A)	Potential (V)	Current (μ A)
Phosphate Buffer	2.32	1.478	2.236	1.357	2.854

Phosphate Buffer	6.60	1.370	3.213	1.323	3.721
BR Buffer	4.19	1.353	0.346	— ^a	— ^a
BR Buffer	4.62	1.445	0.644	— ^a	— ^a
BR Buffer	5.64	1.414	0.701	— ^a	— ^a
BR Buffer	6.55	1.400	2.314	— ^a	— ^a
BR Buffer	7.21	1.349	0.815	— ^a	— ^a
BR Buffer	9.90	1.279	0.540	1.447	1.276
LiClO ₄	6.04	1.405	3.987	1.641	6.112

The MCI concentration is 4 mg L⁻¹; —^a Not determine; BR: Britton—Robinson buffer

Table 2. Recoveries of isothiazolinones in spiked shampoo sample for DPV and LC-UV

Analytes	DPV			LC-UV		
	N = 3 ^a			Added (μg L ⁻¹)	Found(μg L ⁻¹)	Recovery(%)
	Added(μg L ⁻¹)	Found(μg L ⁻¹)	Recovery (%)			
MI	20.0	19.3	96.5(5.5%) ^b	20.0	19.6	97.8(3.3%)
MCI	40.0	39.8	99.5 (3.2%)	40.0	39.1	97.7 (2.3%)

^a Number of determination.

^b Values in parentheses indicate R. S. D.

Table 3. Analytical results of determination of MCI and MI in commercial cosmetic preparations by differential pulse voltammetry (DPV) and liquid chromatographic with ultraviolet detection (LC-UV)

Samples	Concentration (% , w/w)			
	MI		MCI	
	DPV	LC-UV	DPV	LC-UV
	N = 3 ^a			
Shampoo	---- ^c	---- ^c	2.75 x 10 ⁻³ (5.9%)	2.15 x 10 ⁻³ (0.5%)
Cream	---- ^c	---- ^c	5.08 x 10 ⁻³ (2.5%)	4.03 x 10 ⁻³ (0.3%)
Cosmetic raw	0.271(4.6%)	0.302(0.2%)	1.38(3.0%)	1.42(0.48%)

^a Number of determination.

^b Values in parentheses indicate R. S. D.

^c Not determined

Fig 1. Electrochemical impedance spectroscopy (EIS) of the different electrodes: carbon paste electrode (CPE), carbon fiber electrode (CFE), gold wire electrode (Au), and silver (Ag) electrode in lithium perchlorate containing methylisothiazolinone (8.0 x 10⁻⁵ M). Scanning voltage amplitude = 5 mV, the frequency ranging from 0.1 Hz to 10 kHz

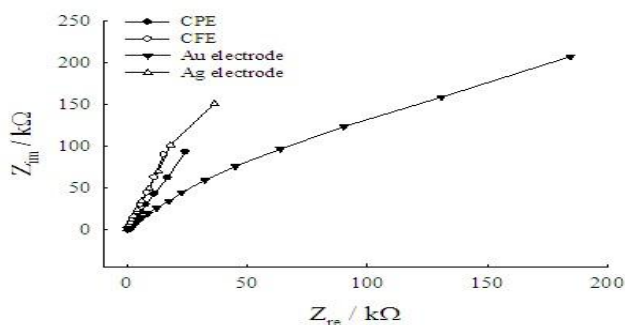


Fig 2. Nyquist impedance spectra of a CFE electrode in several supporting electrolytes containing methylisothiazolinone (5.0 x 10⁻⁶ M). The impedance spectra were measured at: Britton-Robinson buffer (pH 2.76, 5.19, and 8.87) and lithium perchlorate (LiClO₄) solutions as supporting electrolytes

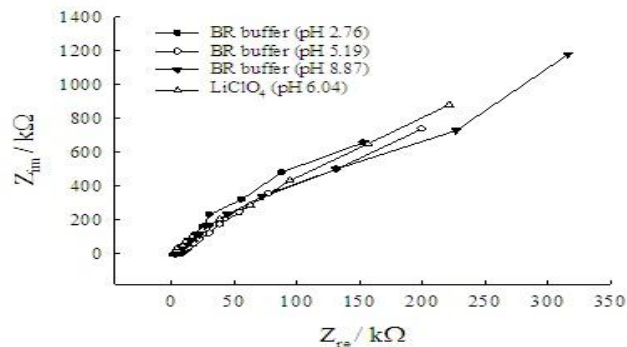


Fig 3. The effect of pH on the response current of 4 mgL⁻¹ methylchloroisothiazolinone (MCI) in Britton-Robinson buffer at a carbon fiber electrode; scan rate 10 mV/s.

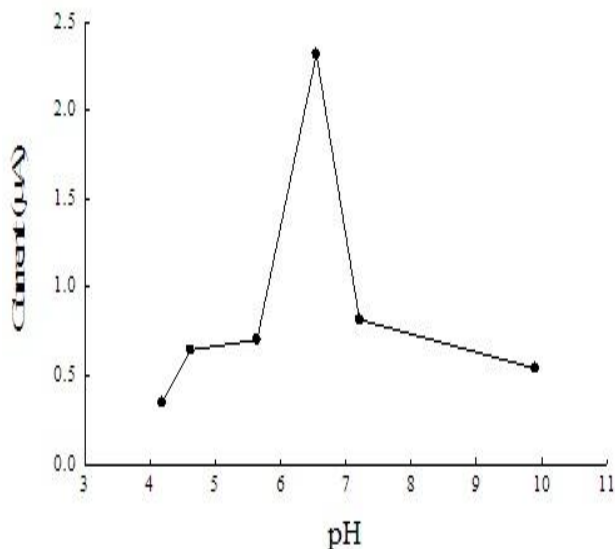


Fig 4. Calibration curves for methylisothiazolinone/methylchloroisothiazolinone (MI / MCI) at a CFE in 0.1 M lithium perchlorate. Peaks: 1a, 1.22 V, 0.748 µA (15.7 mg L⁻¹ MI added); 1b, 1.23 V, 1.09 µA (31 mg L⁻¹ MI added); 1c, 1.29 V, 1.56 µA, 2c 1.49 V, 0.95 µA (60 mg L⁻¹ MI / MCI added); 1d, 1.33 V, 2.65 µA, 2d 1.51 V, 1.27 µA (113 mg L⁻¹ MI / MCI added); 1e, 1.36 V, 4.77 µA, 2e 1.56 V, 2.25 µA (204 mg L⁻¹ MI / MCI added); Scan rate 10 mV, pulse height, 0.05 V.

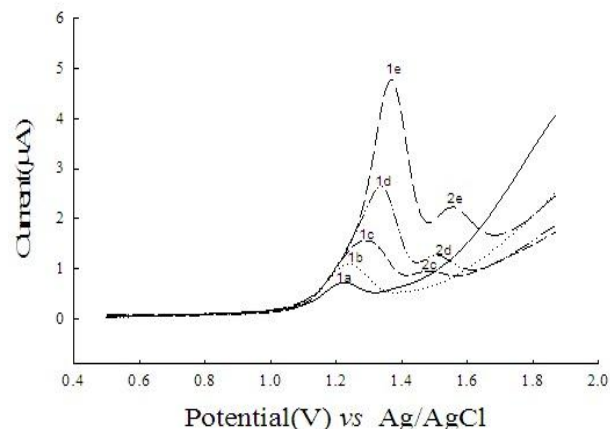


Fig. 5 DPV traces for methylisothiazolinone/methylchloroisothiazolinone (MI / MCI) from commercial cream at a CFE in 0.1 M lithium perchlorate. Peaks: 1a, 1.40 V, 0.297 µA; 2a, 1.59 V, 0.792 µA (0 mg L⁻¹ MI / MCI added); 1b, 1.43 V, 1.01 µA, 2b, 1.64 V, 1.07 µA (60 mg L⁻¹ mg L⁻¹ MI / MCI added); 1c, 1.44 V, 1.61 µA, 2c 1.65 V, 1.36 µA (113 mg L⁻¹ mg L⁻¹ MI / MCI added); Scan rate 10 mV, pulse height, 0.05 V.

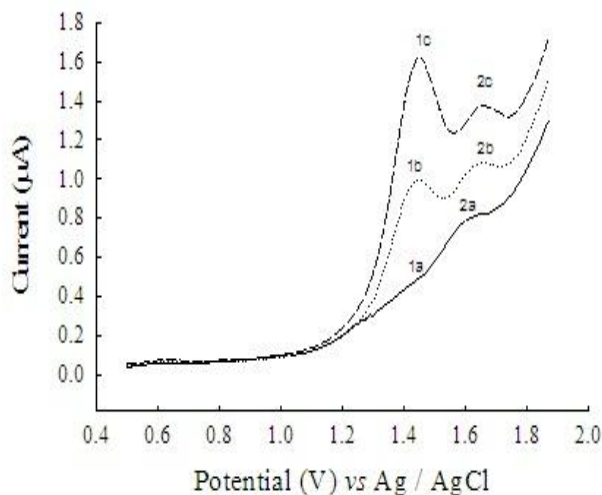
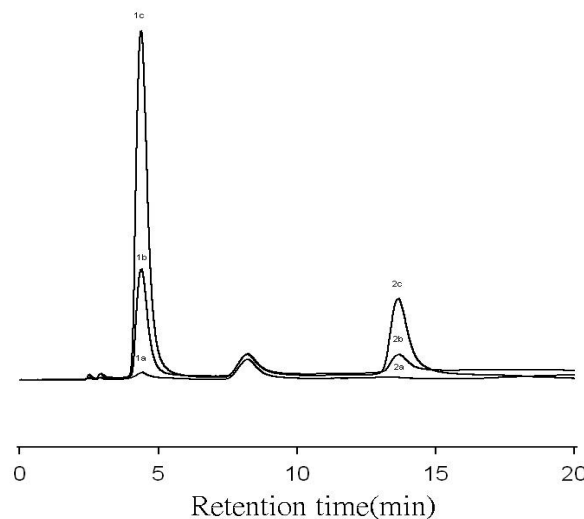
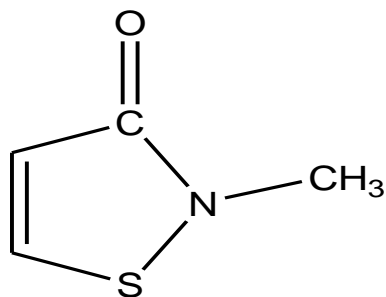


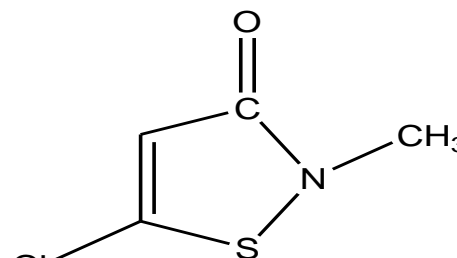
Fig 6. Chromatograms of commercial sample on a Phenomenex Luna 5u C₁₈ (250 x 4.6 mm) column. Peaks: 1a, MI; 2a, MCI(0 mg L⁻¹ MI / MCI added); 1b, MI; 2b, MCI(10 mg L⁻¹ MI and 40 mg L⁻¹ MCI added); 1c, MI; 2c, MCI(40 mg L⁻¹ MI and 160 mg L⁻¹ MCI added). Mobile phase methanol-water (20: 80, v/v) containing 0.1mM lithium perchlorate, pH 7.14) at a flow rate of 1.0 mL min⁻¹, the UV detector was operated at 270 nm.



Scheme 1: Structure of MI and MCI



2-methyl-4-isothiazolin-3-one(methylisothiazolinone, MI)



5-chloro-2-methyl-4-isothiazolin-3-one(methylchloroisothiazolinone, MCI)

RESULTS AND DISCUSSION

Choice of analytical method

The oxidation of MCI and MI in 0.1 mol L⁻¹ lithium perchlorate (pH 6.04) was studied at a CFE, CPE, Ag and Au electrode. The Nyquist plots obtained on CFE, CPE, Ag and Au electrodes are shown in Fig. 1. It can be seen from Fig. 1 that CFE and CPE electrodes exhibited an almost straight line that is characteristic of a diffusional limiting step of the electrochemical process. The results show that CFE and CPE decrease the resistance of the electrode and hold higher electron transfer efficiency. It was found that Au exhibited a pronounced electrochemical Nyquist plots. The charge transfer resistance is enhanced by the adsorption of Au due to a blocking effect at the interface. Fig. 2 presents the Nyquist diagram for various supporting electrolytes with the development of the CFE. We observed that each acid solution: Britton-Robinson buffer (2.76 and 5.19) and lithium perchlorate (pH 6.04) with similar linear portion, which indicated acid of supporting electrolytes can enhance electron transfer rate between the electrode and solution. However, the electrode in Britton-Robinson buffer (pH 8.87) generates an impedance increase. This increase is due to the change of the electric characteristics of electrode/electrolyte interface. Differential pulse voltammetric (DPV) experiments further confirm that accumulation of the analyte on the CPE electrode. From Table 1 can be seen, selective preconcentration of MCI at the carbon – paste surface, followed by measurement of the surface-bound species had resulted in peak current higher than that CFE. The DPV peak currents of MCI in LiClO₄ at CFE and CPE were 3.987 μA and 6.112 μA, respectively. However, the oxidation potentials of MCI were 1.405 V and 1.641V at CFE and capillary CPE, respectively. Due to the resolution of ultramicroelectrode CFE higher than that CPE which CFE can simultaneous determination of MCI and MI. Therefore, the CFE was chosen for use in the determination of MCI and MI in cosmetics.

pH and supporting electrolyte effects

Table 1 summarizes data obtained from differential pulse voltammetric studies of 4.0 mg L⁻¹ of MCI solution in various other solutions on CFE and CPE. Our analyses of the effect of pH and supporting electrolytes on the oxidation peak current and peak potential of MCI in Britton-Robinson buffered solution in the pH range of 4.19- 9.90, phosphate buffer (pH 2.32 and 6.62), and lithium perchlorate (pH6.04) showed that peaks shifted more to less positive potential in Britton-Robinson buffer (pH 9.90) and that the peak current in Britton-Robinson buffer was lower than in the others (Table 1 and Fig. 3). This indicates that the oxidation of isothiazolinones is strongly pH-dependent. In Table 1, it can be seen that the lithium perchlorate is the most suitable since the peak current of MCI is higher than the others, and as can be seen in Fig.4, it can improve the separation of the MCI and MI peaks.

Analytical application to cosmetics

Methods of quantitation of MCI and MI on the basis of the above- voltammetric studies were described in this section. Differential pulse voltammograms (DPV) obtained using the standard addition method on a CFE electrode (Fig. 4, the regression equations used was $y = 0.021x + 0.35$ the correlation coefficient was $r = 0.9983$ for first wave 1.23 V; and $y = 0.009x + 0.32$; $r = 0.9912$ for second wave 1.49 V, respectively) showed two well-defined oxidation peaks.

Recovery tests were carried out on cosmetic products to evaluate the reproducibility and accuracy of the proposed DPV method. Recoveries of two isothiazolinones experiments on liquid-liquid extraction (LLE) with different solvents were carried out. Best results for MCI and MI were found for LLE with dichloromethane. Cosmetic product was spiked with the amounts reported in Table 2 and subjected to the whole procedure. As shown in Table 2, excellent recoveries and

precision were observed (recoveries ranging from $96.5 \pm 5.5\%$ to $99.5 \pm 3.2\%$).

In order to test the applicability of the CFE electrode to cosmetics was analysed by the standard addition method. The cosmetic sample was diluted with lithium perchlorate, and the analytes was spiked with different concentrations of MI and MCI. A representative DPV of commercial cosmetics was shown in Fig. 5. Chromatograms of a comparison of LC-UV Fig. 6 in cosmetics show that chromatograms obtained from commercial cosmetics after they had been spiked with MI and MCI standard solutions; their retention time were 4.42 min and 14.1 min, respectively. Analytical results were given in Table 3. These results agree with those obtained by a high-performance liquid chromatography method.

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CONCLUSION

The EIS measurements of MI were performed on four types of working electrodes. In this study has proved the EIS as a better tool for the selection of suitable conditions such as electrode and supporting electrolyte. We have successfully demonstrated a CFE as a feasible electrode for MCI and MI determination in commercial cosmetics. Furthermore, the cost of the presented electrode is lower than that of currently used method.

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