

行政院國家科學委員會補助專題研究計畫 成果報告
 期中進度報告

(計畫名稱)

含硫雜環之化妝品與藥品中四氫噻唑衍生物測定與快速檢測器之研究

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計畫主持人：王來好

共同主持人：

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A novel method for quaternium-73 determination based on electrochemical impedance spectroscopy using metal oxide nano-particles modified carbon paste electrode

奈米結構金屬氧化物修飾碳電極並應用電化學交流阻抗光譜法於化妝品雜環噻唑衍生物含量之測定
(第三年)

中文摘要

製備奈米結構金屬氧化物(氧化錫、氧化鈾、氧化鈷、氧化鐵和氧化鎳)修飾碳電極作工作電極,利用電化學交流阻抗光譜法(EIS)測定雜環噻唑衍生物(quaternium-73)。由交流阻抗光譜法所測得電子轉移阻抗(R_{et})隨 quaternium-73 濃度之增加而降低。電子轉移阻抗倒數 ($1/R_{et}$)對濃度作圖,求得線性關係。此線性定量測量的極限(LOQ)為 3.73 μM 。新發展的 EIS 分析法,可應用於市售化妝品中 quaternium-73 之測定,並可與液相層析紫外光譜法做比較,其結果一致。

關鍵字: 奈米結構金屬氧化物修飾碳電極; 電化學交流阻抗光譜法; 雜環噻唑衍生物; 化妝品。

Abstract

A method is developed for quantitative determination of thiazolium derivative (quaternium-73) using electrochemical impedance spectroscopy (EIS). The method is based on metal oxide nano-particles (tin oxide, cerium oxide, cobalt oxide, iron oxide and nickel oxide) modified carbon paste electrodes (CPEs). An increase in the quaternium-73 concentration results in an increase in the diffusion current density of the ylidene group (=CH-R) oxidation, which corresponds to a decrease in the faradaic electron-transfer resistance (R_{et}) obtained from the EIS measurements.

Quaternium-73 is quantified from linear variation of the sensor response ($1/R_{et}$) as a function of quaternium-73 concentration in solution. The method is straightforward and nondestructive. The dynamic range for determination of quaternium-73 is extended to more than two orders of magnitude. A concentration limit of quantitation (LOQ) 3.73 μM

with a sensitivity of $7.58 \times 10^4 \Omega^{-1} \mu\text{M}^{-1}$ is obtained. The method is applicable to the quantitative determination of quaternium-73 in cosmetics. Findings using EIS determination and high-performance liquid chromatography method and were comparable.

Keywords: Metal oxide nano-particles modified carbon paste electrodes; electrochemical impedance spectroscopy; quaternium-73; cosmetic products.

報告內容

前言

Thiazole and pyrazole derivatives of thiazolylidene as effective inhibitors of cancer cell migration and invasion, anti-HIV activity and potential antimicrobial agents [1-3]. Quaternium 73 is a cyanine dye and chemical name is 3-heptyl-2-[(3-heptyl-4-methyl-3H-thiazol-2-ylidene)methyl]-4-methylthiazolium iodide. Its thiazole structure is created by the two nitrogen-containing heterocyclic molecules, which are bound to identical heptyl groups at the N site. The antimicrobial activity of quaternium 73 is reported to be associated with the length of the N-alkyl groups [4]. It is used as pharmaceutical ingredient including anti-acne, anti-dandruff, conditioning agent, bactericide and whitening agent in personal care products [5-7]. A survey of literature there were only two methods applied in cosmetics and pharmaceuticals which include liquid chromatography [8] and near-infrared

[9]. Electrochemistry and spectral investigation studies of thiazole derivatives on the reaction pathway and in classical organic solvents as well as in room temperature ionic liquids [10-11].

研究目的

The electrochemical impedance spectroscopy (EIS) is a powerful, nondestructive and informative technique, which allows access to the complete of kinetic characteristics of electrochemical systems, such as rate constants, diffusion coefficients and to study the electron transfer [12-14]. Recently, the EIS has been used in analytical chemistry to trace modification steps of chemically modified electrodes based on self-assembled monolayers (SAMs) and to quantify pharmaceutical or biological species in solution. Among all the detection methods, EIS has raised great interest because of its high sensitivity and label free characteristics, which is uniquely attractive for biosensor and pharmaceutical analysis [15-25]. Up to our knowledge, there has been no previous report on using metal oxide nano-particles modified carbon paste electrode (CPE) based on faradaic impedance sensors to evaluate quaternium 73. In the present work, we report on the application EIS method for the determination of quaternium 73 in cosmetics on metal oxide nano-particles modified CPEs.

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- es modified 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. HPLC was performed with a Hitachi model L-7100 pump and model 7125 injector equipped with 20 μ l sample loop and model L-7455 photodiode array detector. Chromatograms were
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- 研究方法**
- Experimental*
- Apparatus and Materials*
- All electrochemical measurements were performed with a potentiostat–galvanostat (SP-150; Bio-Logic SAS, 1 rue de l'Europe 38640 - CLAIIX - FRANCE) with a conventional three-electrode configuration with carbon fiber electrode (CFE), un-modified carbon paste electrode (CPE) and nano-metal oxid
- acquired and peak areas calculated by means of D-7000 chromatograms Data Integrator.
- Quaternium-73 (Scheme 1) was purchased from Hayashibara Company, Limited (Okayama, Japan). The metal oxide nanoparticles (25-100 nm) tested were tin, cerium, cobalt, iron, and nickel oxide (Sigma-Aldrich Co., St Louis, MO). All other reagents were locally purchased and of analytical

grade.

Electrode modification and electrochemical measurements

CPE were prepared as previously described [26]. The metal oxide – nano-particles modified CPE electrode was produced by mixing weighed amounts of graphite powder, paraffin oil, and nano-metal oxides (0.5 -3.0 % w/w) thoroughly in a mortar until they were perfectly homogenized. The body of the composite metal oxide carbon paste working electrode was fabricated from a PTFE rod (o. d. 7 mm) with a 3 mm deep hole bored (diameter 3 mm) into one side for the composite metal oxide carbon-paste filling. The composite metal oxide carbon paste was placed in the body of the electrode, using a PTFE spatula, and smoothed off.

Differential pulse voltammetry (DPV) and EIS were performed in a phosphate buffer (pH 2.20 and 6.66), Britton and Robison buffer (pH 2.93- 6.83), and lithium perchlorate (LiClO_4), tetraethylammonium tetrafluoroborate (Et_4NBF_4) and tetraethylammonium perchlorate (Et_4NClO_4) solutions as supporting electrolytes at modified CPE. In DPV potential were from +0.0V to + 2.0 V with scan rate at 10 mV s^{-1} . The EIS and DPV 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 quaternium- 73 in cosmetics

Stock solution of standard was prepared by dissolving the appropriate amount of quaternium- 73 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 quaternium- 73 content of the anti-acne cleansing gel and spray, samples(approx. 0.05-0.5 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 centrifugated respectively. The supernatant was transferred into a 5 mL calibrated flasks. An aliquot of the solution was filtered through a $0.45 \mu\text{m}$ membrane filter prior to HPLC analysis. We used that a simple dilution process worked for EIS and DPV experiments well as the standard solution. A Phenomenex Luna C_{18} column ($5 \mu\text{m}$, $250 \times 4.6 \text{ mm}$) was used for reverse-phase HPLC. The mobile phase were 20:80 methanol-water (containing phosphate buffer, pH 4.53) at a flow rate of 1.0 mL min^{-1} , the UV detector was operated at 225 nm. By means of the injection value, $25 \mu\text{L}$ 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.

結果與討論

Choice of analytical method

The oxidation of quaternium-73 in 0.1 mol L⁻¹ phosphate buffer (pH 6.66) was studied at a CFE, CPE and Au electrode. The Nyquist plots obtained on CFE, CPE and Au electrodes are shown in Fig. 1. It can be seen from Fig. 1 that CFE and Au electrodes exhibited an almost straight line that is characteristic of a diffusional limiting step of the electrochemical process. It was found that CPE exhibited a pronounced electrochemical Nyquist plots. The charge transfer resistance is enhanced by the adsorption of carbon paste due to a blocking effect at the interface. Differential pulse voltammetric (DPV) experiments further confirm that accumulation of the analyte on the CPE electrode. From Fig. 2 can be seen, selective preconcentration of quaternium-73 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 quaternium-73 at CFE and CPE were 3.61 μ A and 26.9 μ A, respectively. Therefore, the CPE was chosen for use in the determination of quaternium-73 in cosmetics.

Characterization of metal oxide nano-particles modified CPEs

EIS of un-modified CPE and metal oxide nano-particles modified CPE were investigated at a

constant concentration of quaternium-73 (1.48×10^{-5} M). Typical Nyquist diagrams of the CPE functionalized by different metal oxide nano-particles immersed in the phosphate buffer (pH 6.66), were shown in Fig. 3. The impedance-plane plots of Fig. 3, are characterized by two distinct regions: (1) a semicircle in the higher frequency range related to the electron transfer process, (2) an inclined line in the complex-plane impedance plot defining a Warburg region of semi-infinite diffusion of species to the modified electrode. The plot exhibited a semicircle near the origin at high frequency followed by a linear tail with a slope of unity. The data can be fitted adequately by the modified Randles circuit, a well-known mixed kinetic and diffusion control model [27]. The presence of metal oxide nano-particles in the CPE enhances the interaction of metal oxide with quaternium-73. Typical impedance results the adsorbed quaternium-73 on CPE electrodes were given in Fig. 3, where the effect of Fe₂O₃, SnO₂ and CeO₂ addition to the CPE, can be observed. These semicircles are related to the electron transfer (i.e. the semicircle's diameter is equal to the electron transfer resistance, R_{et}) resistance and capacitance of electrode/solution interface and are large for the adsorption of quaternium-73 (i.e. in the presence Fe₂O₃, SnO₂ and CeO₂). The R_{et} of Fe₂O₃ modified CPE was higher than the others. Therefore, the Fe₂O₃ modified CPE with different percent ratio

(0.5%- 2.0%) were characterized by EIS experiments. The results were shown in Fig. 4. The 0.5% Fe₂O₃ displayed nearly a linear. When a CPE modified with Fe₂O₃ for different percent ratio, the electron transfer resistance decreased significantly for the 1.0% ($R_{et} = 73 \text{ k}\Omega$), then did not slightly decrease the R_{et} with percent ratio (1.5%-2.0%). It is evident that a saturated nanoparticle of Fe₂O₃ on the CPE; then, the adsorption did not change with the increase of amount Fe₂O₃. Fig. 5A presents the Nyquist diagram for various supporting electrolytes with the development of the Fe₂O₃ modified CPE. We observed that each salt solution: LiClO₄, Et₄NBF₄, and Et₄NClO₄ with similar linear portion, which indicated salt of supporting electrolytes can enhance electron transfer rate between the electrode and solution. However, the electrode in phosphate buffer (pH 6.66) generates an impedance increase. This increase is due to the change of the electric characteristics of electrode/electrolyte interface. DPV was performed to further clarify electrochemical properties, shown in Fig. 5B. The peak potential of quaternium- 73 was less positive and peak shape more sharp in salt of supporting electrolytes than that in phosphate buffer.

Quantification and sensitivity

If the electrode is to be used to quantify the analyte concentration and not just to detect its presence, the range of measurable concentrations is important. The dynamic range is the ratio of the

largest target concentration and the limit of detection. Dynamic range can be extended on the upper end by simply performing measurements with dilution series of the sample [28]. The sensitivity of Fe₂O₃ (nano-particles) modified CPE electrode was investigated by measuring the changes of R_{et} in various concentrations for quaternium- 73. As shown in Fig. 6A and B, the electron transfer impedance R_{et} decreases with concentration of quaternium- 73. $1/R_{et}$ has a good linear relationship with quaternium- 73 concentration c in the range of $3.50 \times 10^{-6} \text{ M} - 1.12 \times 10^{-4} \text{ M}$ with the linear equation $R_{et} = 0.118 \log C - 0.031$ (unit of C , mg L^{-1}), and linear regression coefficient of 0.9943. This observation is opposite of what we have seen for previous EIS examples, where the R_{et} has generally increased with concentrations. The analytical performance of the quaternium- 73 is compared with that of the conventional HPLC methods. It is found that the detection limit of the metal oxide nano-particles modified CPE developed is comparable or even lower.

In order to test the applicability of the developed Fe₂O₃ modified CPE electrode to cosmetics was analysed by the standard addition method. The cosmetic sample was diluted with phosphate buffer, and the analytes was spiked with different concentrations of quaternium- 73. A representative EIS of commercial cosmetics was shown in Fig. 7A and B. Analytical results were given in Table 1. These results agree with those obtained by a

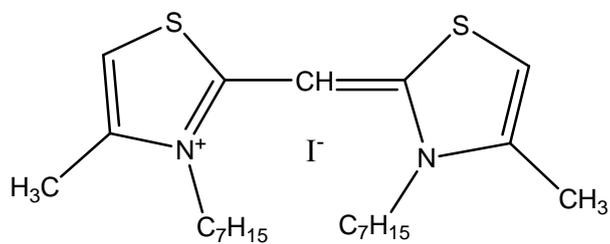
high-performance liquid chromatography method.

致謝

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Scheme 1 Quaternium-73 structure

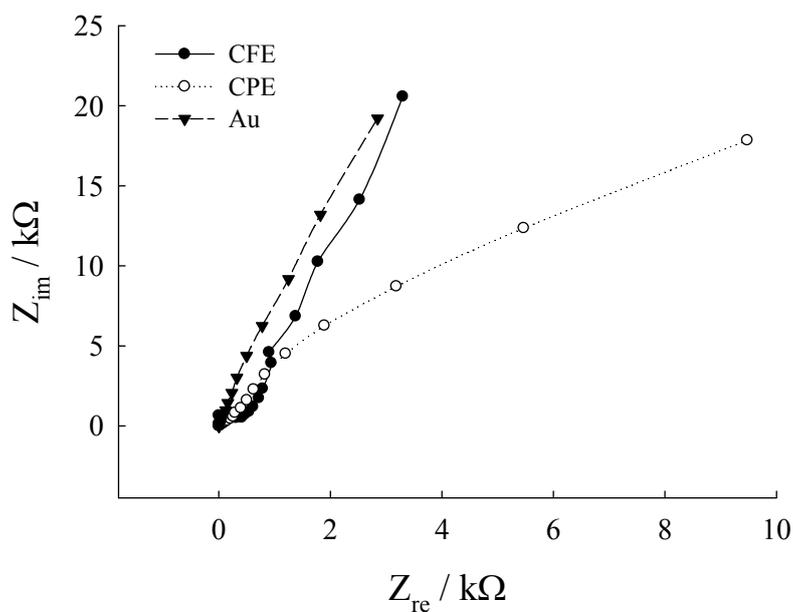


Fig. 1. Electrochemical impedance spectroscopy (EIS) of the different electrodes: carbon fiber electrode (CFE), carbon paste electrode (CPE) and gold wire electrode (Au) in phosphate buffer (pH 6.66) containing quaternium-73 (1.48×10^{-5} M). Scanning voltage amplitude = 5 mV, the frequency ranging from 0.1 Hz to 10 kHz.

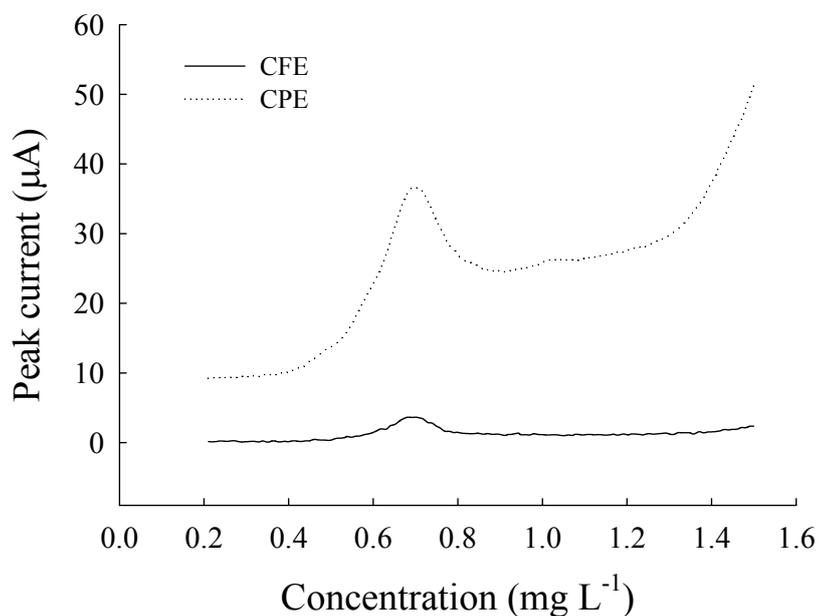


Fig. 2. Differential pulse voltammograms of quaternium-73 ($30 mg L^{-1}$) in phosphate buffer (pH 6.66). The solid line is for a carbon fiber electrode (peak at 0.689 V, 3.61 μA); the dotted line is for a carbon paste electrode (peak at 0.699 V, 26.9 μA).

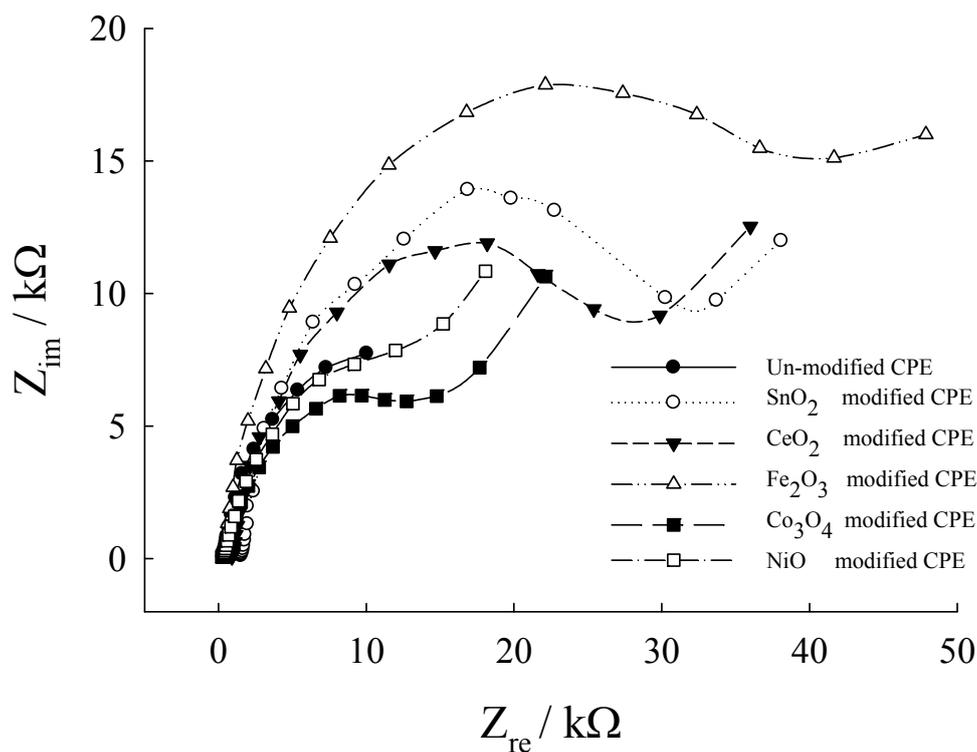


Fig. 3. Electrochemical impedance spectra: un-modified carbon paste electrode (CPE) and 1.5% metal oxide nano-particles (SnO_2 , CeO_2 , Fe_2O_3 , Co_3O_4 , and NiO) modified CPE in phosphate buffer (pH 6.66) containing quaternium-73 ($1.48 \times 10^{-5} \text{ M}$). Scanning voltage amplitude = 5 mV, the frequency ranging from 0.1 Hz to 10 kHz.

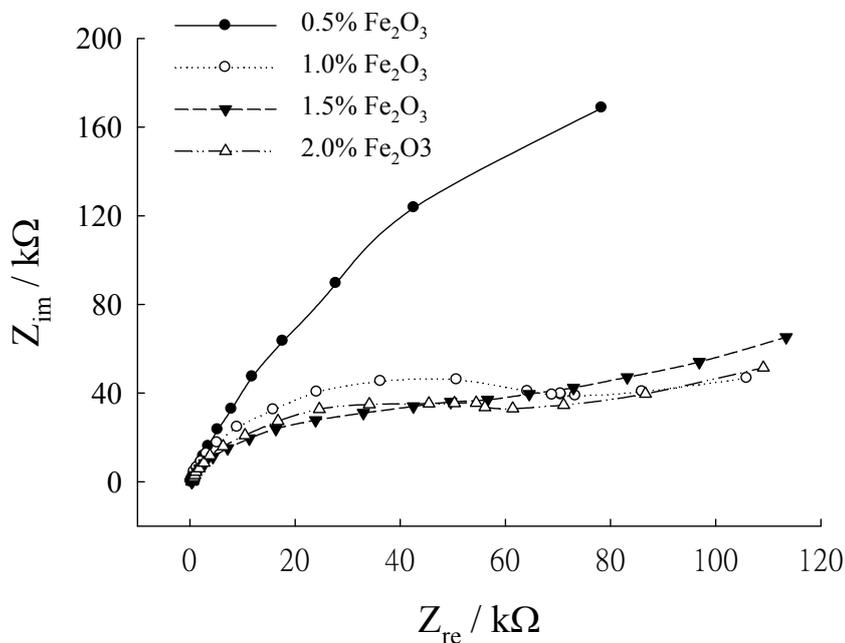


Fig. 4 Electrochemical impedance spectroscopy (EIS) of the different ratio of Fe_2O_3 modified CPE electrodes in phosphate buffer (pH 6.66) containing quaternium-73 ($7.44 \times 10^{-6} \text{ M}$). Scanning voltage amplitude 5 mV, the frequency ranging from 0.1 Hz to 10 kHz.

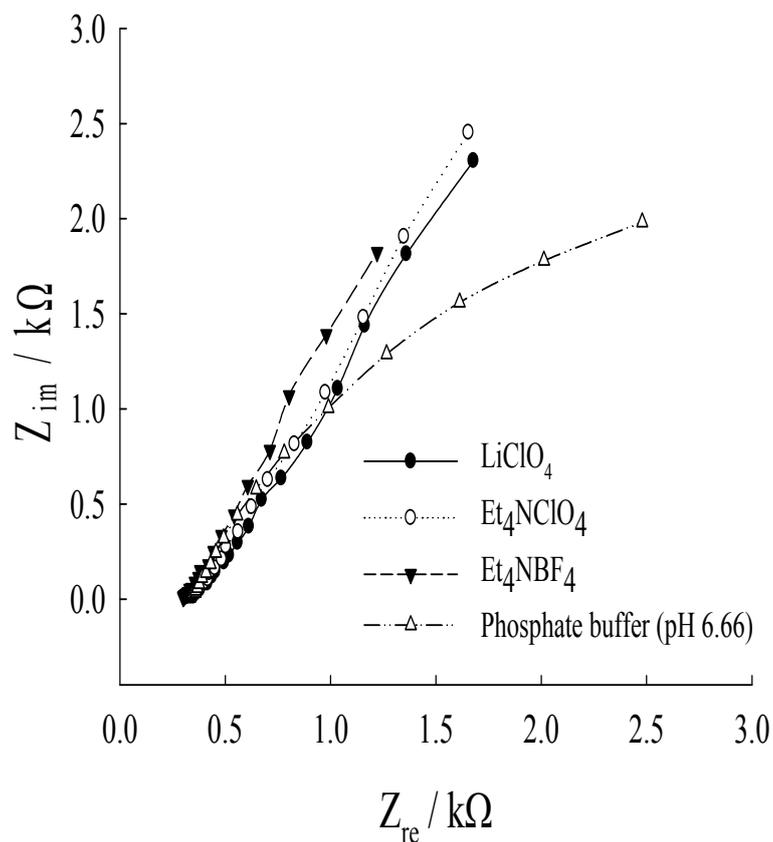


Fig. 5A Nyquist impedance spectra of a 2% Fe_2O_3 modified CPE electrode in several supporting electrolytes containing quaternium-73 ($1.12 \times 10^{-4}\text{M}$). The impedance spectra were measured at: lithium perchlorate (LiClO_4), tetraethylammonium tetrafluoroborate (Et_4NBF_4), tetraethylammonium perchlorate (Et_4NClO_4) and phosphate buffer (pH 6.66) solutions as supporting electrolytes.

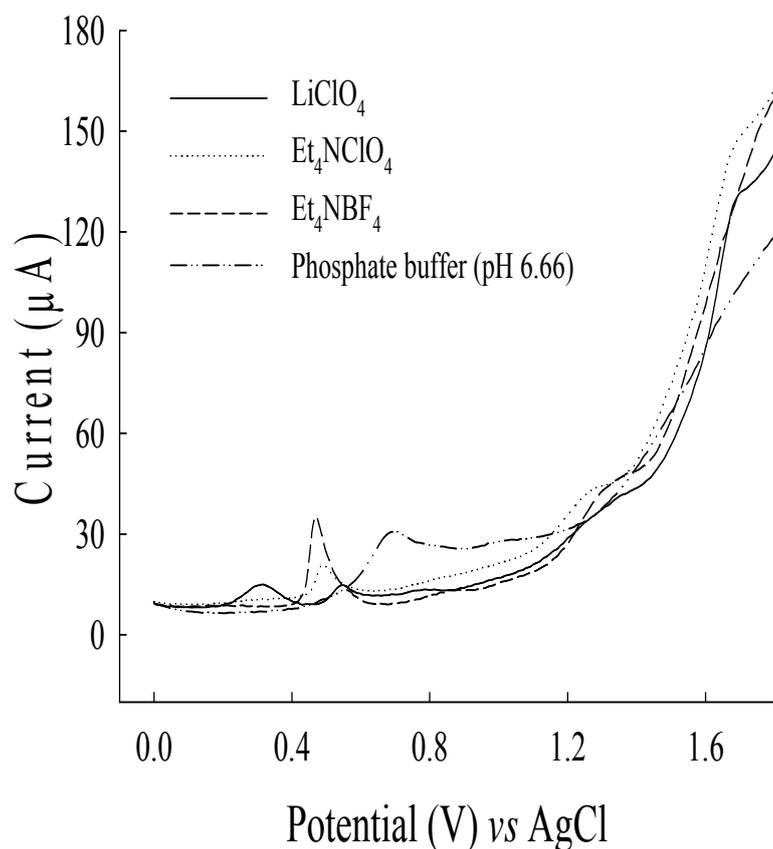


Fig. 5B DPV curves of a 2% Fe_2O_3 modified CPE electrode in several supporting electrolytes containing quaternium-73 ($1.12 \times 10^{-4}\text{M}$). The impedance spectra were measured at: lithium perchlorate (LiClO_4), tetraethylammonium tetrafluoroborate (Et_4NBF_4), tetraethylammonium perchlorate (Et_4NClO_4) and phosphate buffer (pH 6.66) solutions as supporting electrolytes.

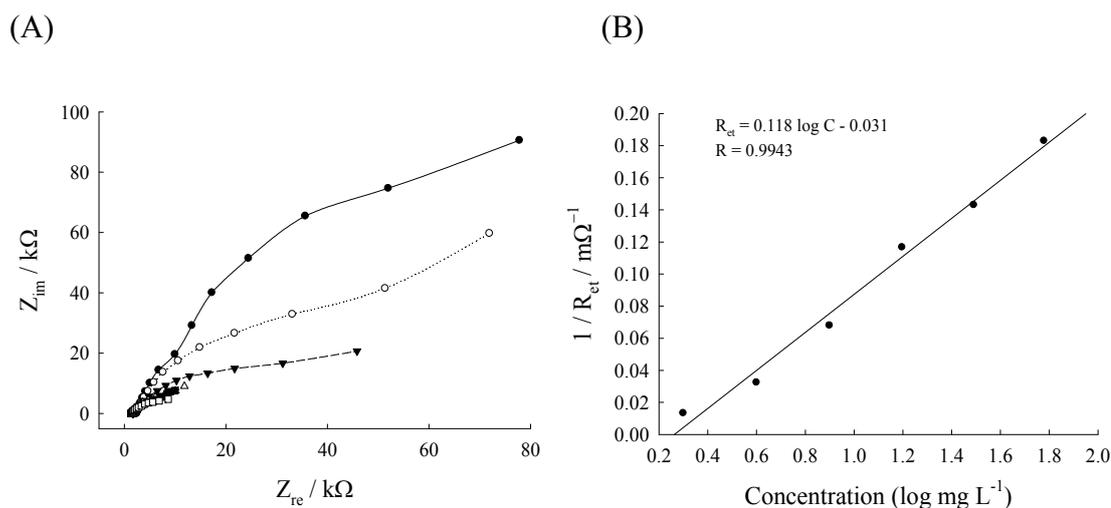
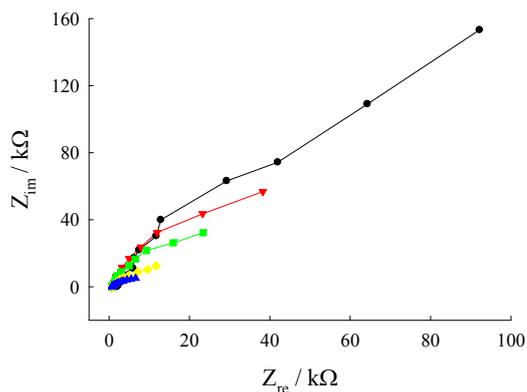


Fig. 6. (A) Nyquist plots of impedance spectra obtained on Fe_2O_3 modified CPE electrode for different concentrations of quaternium-73 in phosphate buffer (pH 6.66). (B) The calibration curve obtained using $1/R_{ct}$ as a function of quaternium-73 concentration at $E_{DC} = 0.18 \text{ V vs. Ag/AgCl}$, linear regression equation: $Y \text{ (k}\Omega\text{)} = 0.118 \log C - 0.031 \text{ (mg L}^{-1}\text{)}$, $R = 0.9943$.

(A)



(B)

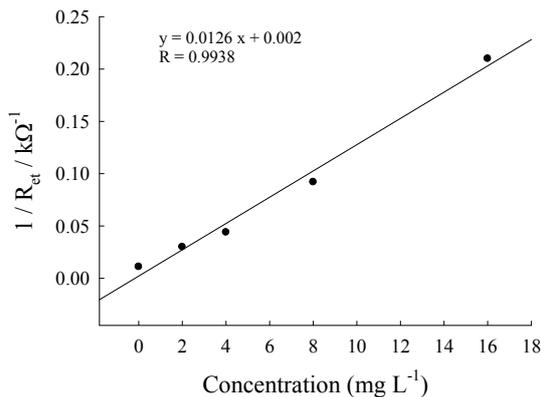


Fig. 7 (A) Nyquist plots of impedance spectra obtained from commercial cosmetics after spiked with quaternium-73 solutions of 0 mg L^{-1} (circle), 2 mg L^{-1} (triangle down), 4 mg L^{-1} (square), 8 mg L^{-1} (diamond), 16 mg L^{-1} (triangle up); (B) the calibration plot.

Table 1 Analytical results of determination of quaternium-73 in commercial cosmetic preparations by electrochemical impedance spectroscopy (EIS) and liquid chromatographic with ultraviolet detection (LC-UV)^a

Samples	Concentration (% w/w)	
	Quaternium-73	
	EIS	LC-UV
	N=3 ^b	
Cleansing gel	3.85×10^{-3} (3.8%)	3.69×10^{-3} (5.5%)
Water spray mist	2.37×10^{-3} (2.0%)	2.25×10^{-3} (6.7%)
Acneless cleansing gel	2.83×10^{-3} (5.8 %)	2.36×10^{-3} (1.8%)

^a Number of determination.

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

