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Photosensitizing Dye: Its Voltammetric Behavior and Determination in Cosmetics

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Abstract: We developed a method that uses electrochemical impedance spectroscopy (EIS) to choose an electrode to determine photosensitizing dye No. 201 (quaternium-73), a thiazolium derivative, in cosmetics. The method is based on nanoparticle metal oxide (tin, cerium, cobalt, iron, and nickel)-modified carbon paste electrodes. Using differential pulse voltammetry (DPV), we found that the electrocatalytic property of tin oxide was better than that of the other metal oxides tested. When the proposed method was used to determine photosensitizing dye No. 201 in commercial cosmetics, the results were



were comparable to those obtained using high-performance liquid chromatography with ultraviolet detection.

Keywords: Nanoparticle metal oxide-modified carbon-paste electrodes, electrochemical impedance spectroscopy, photosensitizing dye, cosmetics.

1. INTRODUCTION

Thiazole and pyrazole derivatives of thiazolidine effectively inhibit HIV-2 activity [1] and cancer cell migration and invasion [2], and they are potential antimicrobial agents [3]. Photosensitizing dye No. 201 (Quaternium-73) is a cvanine dye with the chemical name 3-heptyl-2-[(3-heptyl-4methyl-3H-thiazol-2-ylidene) methyl]-4-methylthiazolium iodide. Its thiazole structure is created by two nitrogencontaining heterocyclic molecules bound to identical heptyl groups at the N site. The antimicrobial activity of Quaternium-73 is associated with the length of the n-alkyl groups [4]. The dye is used as a pharmaceutical ingredient in a variety of personal care products: bactericides, and anti-acne, anti-dandruff, conditioning, and whitening agents [5-7]. A survey of the literature showed that there are currently only two methods of quantitatively determining the Quaternium-73 concentrations in cosmetics and pharmaceuticals: liquid chromatography [8] and near-infrared [9]. Electrochemistry and spectral investigation studies are also used to determine thiazole derivatives on the reaction pathway and in classical organic solvents as well as in room-temperature ionic liquids [10, 11].

Electrochemical impedance spectroscopy (EIS) is powerful, nondestructive, and informative. It allows access to the complete kinetic characteristics of electrochemical systems, such as rate constants, diffusion coefficients, and electron transfer [12-14]. EIS has recently been used in analytical chemistry to trace the modification steps of chemically modified electrodes based on self-assembled monolayers (SAMs) and to quantify pharmaceutical and biological species in solution. Of all the detection methods, EIS has raised the most interest because of its high sensitivity and label-free characteristics, which are uniquely attractive for biosensor and pharmaceutical analysis [15-25]. To the best of our knowledge, there are no published reports on using metal oxide nanoparticle-modified carbon paste electrodes (CPEs) based on Faradaic impedance sensors to evaluate Quaternium-73. In the present work, we report using the EIS method with metal oxide nanoparticlemodified CPEs to quantitatively determine Quaternium-73 in cosmetics.

2. EXPERIMENTAL

2.1. Apparatus and Materials

All electrochemical measurements were made using a potentiostat/galvanostat (SP-150; Bio-Logic SAS, Claix, France) with a conventional configuration of three working electrodes: a carbon fiber electrode (CFE), an unmodified CPE, and a metal oxide nanoparticle-modified CPE. 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. Highperformance liquid chromatography (HPLC) was used (L-7100 pump, 7125 injector, 20-µl sample loop, L-7455 photodiode array detector; Hitachi, Tokyo, Japan). Chromatograms were acquired and peak areas calculated using a chromatogram data integrator (D-7000; Hitachi).

Quaternium-73 (Scheme 1) was purchased from the Hayashibara Company (Okayama, Japan). The metal oxide nanoparticles (25-100 nm) tested were tin, cerium, cobalt, iron, and nickel oxide (Sigma-Aldrich, St. Louis, MO, USA). All other reagents were locally purchased and were of analytical grade.

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Scheme 1. Photosensitizing dye No. 201 (Quaternium-73) structure.

2.2. Electrode Modification and Electrochemical Measurements

CPE were prepared as previously described [26]. The metal oxide nanoparticle-modified CPE electrode was produced by thoroughly mixing weighed amounts of graphite powder, paraffin oil, and nanometal oxides (0.5-3.0% w/w) in a mortar until they were perfectly homogenized. The body of the composite metal oxide carbon paste working electrode was fabricated from a PTFE (polytetrafluoroethylene) rod (optical density [o.d.]: 7 mm) with a hole 3-mm deep (diameter: 3 mm) bored 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 then smoothed off.

Differential pulse voltammetry (DPV), at a scan rate of 10 mV s⁻¹, and EIS were done in phosphate buffer (pH 2.20 and 6.66), Britton and Robison buffer (pH 2.93-6.83), lithium perchlorate (LiClO₄), tetraethylammonium tetra-fluoroborate (Et₄NBF₄), or tetraethylammonium perchlorate (Et₄NClO₄) solution as a supporting electrolyte at the modified CPE. DPV showed potentials from +0.0 V to +2.0 V. EIS and DPV data were acquired using SP-150 (Bio-Logic SAS) and EC-Lab software. The impedance spectra were recorded over a frequency range of 0.1 Hz to 10 kHz, using a sinusoidal excitation signal, superimposed onto a direct current (DC) potential of +0.180 V. An excitation amplitude of 5 mV was used throughout.

2.3. Determining Quaternium-73 in Cosmetics

A stock standard solution was prepared by dissolving the appropriate amount of Quaternium-73 in methanol. A set of standard solutions was produced by diluting aliquots of the stock solutions with methanol to 10 mL in calibrated flasks. Taking into account the Quaternium-73 content of the antiacne cleansing gel and spray, samples (ca. 0.05-0.5 g) of the latter were accurately weighed in a 15-mL beaker, diluted to about 10 ml-with ethyl acetate, dichloromethane, ethyl acetate-dichloromethane (3:2, v/v), and ethyl acetatedichloromethane (3:1, v/v)—dissolved, and then centrifuged. The supernatant was transferred to a 5-mL calibrated flask. An aliquot of the solution was filtered through a 0.45-µm membrane before the HPLC analysis. That simple dilution process worked well for producing the standard solution for EIS and DPV experiments. A C₁₈ column (5 μ m, 250 × 4.6 mm) (Phenomenex Luna; Scientific Hightek Co., Taipei, Taiwan) was used for reverse-phase HPLC. The mobile phase was 20:80 methanol-water (containing phosphate buffer [pH 4.53]) at a flow rate of 1.0 mL min⁻¹: the ultraviolet (UV) detector was operated at 225 nm. Using the injection value, 25 µL 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.

3. RESULTS AND DISCUSSION

3.1 Choice of Analytical Method

The oxidation of Quaternium-73 in 0.1 mol L^{-1} phosphate buffer (pH 6.66) was studied using CFE, CPE, and gold (Au) electrodes. CFE and Au electrodes showed almost straight lines, characteristic of a diffusion-rate-limiting step of the electrochemical process, and CPE showed a pronounced electrochemical Nyquist plot (Fig. 1). The charge transfer resistance increased because of the adsorption of carbon paste due to a blocking effect at the interface. DPV experiments confirmed that analyte accumulated on the CPE. Selective preconcentrations of Quaternium-73 were placed on the carbon paste surface, and the surface-bound species



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 photosensitizing dye No. 201 (Quaternium-73) (1.5×10^{-5} M). Scanning voltage amplitude = 5 mV (frequency range: 0.1-10 kHz).

was measured, which showed a peak current higher for the CPE than for the CFE. The DPV peak currents of Quaternium-73 at the CFE and CPE were 3.61 μ A and 26.9 μ A, respectively (Fig. 2). Therefore, we chose to use the CPE to determine the concentrations of Quaternium-73 in cosmetics.

3.2. Characteristics of Metal Oxide Nanoparticlemodified CPEs

The EIS of unmodified CPE and of metal oxide nanoparticle-modified CPE were investigated at a constant concentration of Quaternium-73 $(1.48 \times 10^{-5} \text{ M})$. Electrochemical impedance data of the CPE functionalized by different metal oxide nanoparticles immersed in phosphate buffer (pH 6.66) are shown in (Fig. 3). The presence of metal oxide nanoparticles in the CPE improved the interaction of metal oxide with Quaternium-73. Typical impedance results of the adsorbed Quaternium-73 on CPEs are given in (Fig. 3), where the effect of tin oxide (SnO₂), cerium oxide (CeO₂), and iron oxide (Fe₂O₃) added to the CPE can be observed. The electron-transfer resistance (R_{et}) was lower for the SnO₂modified CPE than for the others. For comparison, CPEs were modified with five kinds of nanometal oxides. The DPV of (Fig. 4) show a more pronounced electrocatalytic effect on the SnO₂ (\triangle)-, CeO₂ (\triangledown)-, and Fe₂O₃ (\square)-modified CPEs than on the unmodified CPE because the peak currents of SnO_2 , CeO_2 , and Fe_2O_3 were higher than those of the others. Therefore, EIS experiments were used to characterize the SnO₂-modified CPEs with different percentage ratios (0.5-3.0%). The semicircles (Fig. 5A) are related to the electron transfer resistance (i.e., the semicircle's diameter is equal to the electron transfer resistance [Ret]) and capacitance of the electrode/solution interface and are large for the adsorption of Quaternium-73. When a CPE had been modified with SnO₂, the R_{et} was significantly lower at the 1.5% ratio ($R_{et} = 20 \text{ k}\Omega$), but not at the 2.0% and 3.0% ratios relative to the 1.5% ratio. It is evident from the lower Ret values (Fig. 5A) that a saturated SnO_2 nanoparticle was on the CPE. DPV was used to clarify the electrochemical catalytic properties of the electrodes (Fig. 5B). The peak potential and peak current closely depended upon the pH of the buffer solution and various supporting electrolytes. DPV was used to clarify the electrochemical properties of the supporting electrolytes (Fig. 6). The peak current of quaternium-73 was higher in the phosphate buffer (pH 6.66) than in the other supporting electrolytes.



Fig. (2). Differential pulse voltammograms (DPV) of photosensitizing dye No. 201 (Quaternium-73) (5.6×10^{-5} M) in phosphate buffer (pH 6.66). The solid line is for a carbon fiber electrode (CFE) (peak at 0.689 V, 3.61 µA); the dotted line is for a carbon paste electrode (CPE) (peak at 0.699 V, 26.9 µA).



Fig. (3). Electrochemical impedance data: unmodified carbon paste electrode (CPE) and 2% metal oxide nanoparticle (SnO₂, CeO₂, Fe₂O₃, Co₃O₄, and NiO)-modified CPE in phosphate buffer (pH 6.66) containing photosensitizing dye No. 201 (Quaternium-73) (1.48×10^{-5} M). Scanning voltage amplitude = 5 mV (frequency range: 0.1-10 kHz).



Fig. (4). The catalytic effect of 2% metal oxide nanoparticle-modified carbon paste electrodes (CPEs) on photosensitizing dye No. 201 (Quaternium-73) in phosphate buffer (pH 6.66).



Fig. (5). A). Electrochemical impedance spectroscopy (EIS) of the different ratios of SnO_2 -modified carbon paste electrodes (CPEs) in phosphate buffer (pH 6.66) containing photosensitizing dye No. 201 (Quaternium-73) (3.0×10^{-5} M). Scanning voltage amplitude 5 mV (frequency range: 0.1-10 kHz). **B**). Differential pulse voltammetry (DPV) curves of photosensitizing dye No. 201 (Quaternium-73) (3.0×10^{-5} M) at 1-2% SnO₂-modified carbon paste electrodes (CPEs) and an unmodified CPE in phosphate buffer (pH 6.66) solutions as supporting electrolytes.

3.3. 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 simply by taking measurements with dilution series of the sample [27]. The sensitivity of the SnO₂ nanoparticlemodified CPE was investigated by measuring the changes in R_{et} in various concentrations of Quaternium-73. 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 the linear variation of the sensor response (1/Ret) as a function of the concentration of Quaternium-73 in solution. The method is



Fig. (6). The catalytic effect of 2% SnO₂ nanoparticle-modified carbon paste electrodes (CPEs) on photosensitizing dye No. 201 (Quaternium-73) in various supporting electrolytes. The DPV curves were measured for lithium perchlorate (LiClO₄), tetraethylammonium tetra-fluoroborate (Et₄NBF₄), tetraethylammonium perchlorate (Et₄NClO₄), phosphate buffer (pH 6.66), and Britton and Robison (BR) buffer (pH 2.06 and 3.38) solutions as supporting electrolytes.

straightforward and nondestructive. The dynamic range for determining Quaternium-73 is extended to more than two orders of magnitude.

A concentration limit of quantitation (LOQ) 3.73 μ M with a sensitivity of 7.58 × 10⁴ $\Omega^{-1} \mu$ M⁻¹ is obtained. The method is applicable to the quantitative determination of Quaternium-73 in cosmetics.

To test the applicability of the developed SnO₂ nanoparticle-modified CPE, a cosmetic was analyzed using the standard addition method. The cosmetic sample was diluted with phosphate buffer, and the analytes were spiked with different concentrations of Quaternium-73. The analytical performance of the Quaternium-73 is compared with that of the conventional HPLC methods (Fig. 7A and B). We found that the detection limit of the metal oxide nanoparticle-modified CPE developed was comparable or even lower. A representative EIS of commercial cosmetics is shown in (Fig. 8A and B); analytical results are given in Table 1. These results agree with those obtained using HPLC.

CONCLUSION

The EIS measurements of Quaternium-73 were done for various types of metal oxide (SnO₂, CeO₂, Fe₂O₃, cobalt ox-



Fig. (7). Chromatograms obtained to produce a calibration graph for photosensitizing dye No. 201 (Quaternium-73) (A) standard solution, and (B) from commercial cosmetics. Stationary phase, Phenomenex Luna C_{18} column (particle size 5-µm, 250 mm × 4.6 i.d.); mobile phase, mobile phase was 20:80 methanol-water (containing phosphate buffer [pH 4.53]); flow rate 1.0 mL/min.



Fig. (8). (A) Nyquist plots of impedance spectra obtained from commercial cosmetics after they had been spiked with photosensitizing dye No. 201 (Quaternium-73) solutions of 0 mg L^{-1} (black circle), 2 mg L^{-1} (red triangle), 4 mg L^{-1} (green square), 8 mg L^{-1} (yellow diamond), or 16 mg L^{-1} (blue triangle). (B) The calibration plot ($R_{et} = 0.0126 \text{ x} + 0.002$; R = 0.9938).

Table 1. Analytical results of using electrochemical impedance spectroscopy (EIS) and High-performance liquid chromatography with ultraviolet detection (HPLC-UV) to determine photosensitizing dye No. 201 in commercial cosmetic preparations.

Samples	Concentration (%, w/w) photosensitizing dye No. 201	
	EIS	HPLC-UV
	n ^a = 3	
Cleansing gel	$3.85 \times 10^{-3} (3.8\%)^{b}$	$3.69 \times 10^{-3} (5.5\%)$
Water spray mist	$2.37 \times 10^{-3} (2.0\%)$	$2.25 \times 10^{-3} (6.7\%)$
Acneless Cleansing Gel	$2.83 \times 10^{-3} (5.8\%)$	$2.36 \times 10^{-3} (1.8\%)$

^aNumber of determinations.

^bValues in parentheses indicate relative standard deviation (RSD).

ide $[Co_3O_4]$, and nickel oxide [NiO]) nanoparticle-modified CPEs. We showed that a SnO₂ nanoparticle-modified CPE is a feasible electrode for determining Quaternium-73. Its analytical performance for the impedance detection of Quaternium-73 with a low limit of quantitation was good: its response was rapid, its linear range satisfactory, and its stability and selectivity were good. The SnO₂ nanoparticle-modified CPE successfully determined Quaternium-73 in commercial cosmetics. The method is simple and easily performed. Furthermore, the cost of using this method is lower than that of currently used methods.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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