

Research Article

Design of a Flow-Through Voltammetric Sensor Based on an Antimony-Modified Silver Electrode for Determining Lithol Rubine B in Cosmetics

Wang Lai-Hao and Huang Shu-Juan

Department of Medical Chemistry, Chia Nan University of Pharmacy and Science, 60 Erh-Jen Road, Section 1, Jen Te, Tainan 71743, Taiwan

Correspondence should be addressed to Wang Lai-Hao, e201466.wang@msa.hinet.net

Received 4 October 2010; Revised 16 December 2010; Accepted 21 January 2011

Academic Editor: Juan F. Garcia-Reyes

Copyright © 2011 W. Lai-Hao and H. Shu-Juan. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Lithol Rubine B (LRB; the disodium salt of 3-hydroxy-4-[(4-methyl-2-sulfophenyl) azo]-2-naphthalenecarboxylic acid) was detected using high-performance liquid chromatography with an electrochemical (antimony film on silver) detector (HPLC-ECD). For direct current (DC) mode, with the current at a constant potential, and measurements with suitable experimental parameters, a linear concentration from 0.125 to 1.80 µg/mL was found. The detection limit of our method was approximately 2.0 ng/mL. An antimony-modified silver detector was used to demonstrate that LRB is electrochemically reduced in acidic media and to analyze commercial cosmetics to determine their LRB content. Findings using HPLC-ECD and HPLC with an ultraviolet detector were comparable.

1. Introduction

Cosmetics often contain dyes to make them more attractive to consumers. Some of the substances used for these dyes are either allergenic or have other side effects in some persons. Lithol Rubine B (D&C Red no. 6), the disodium salt of 3-hydroxy-4-[(4-methyl-2-sulfophenyl) azo]-2-naphthalenecarboxylic acid, which is an anionic azo compound (CI: 15850), is one such substance. Lithol Rubine B is mutagenic because of intermediate reactions or by-products found in commercial samples [1]. There are several methods for determining trace concentrations of Lithol Rubine B in cosmetic products. Some use high-performance liquid chromatography (HPLC) with ultraviolet visible (UV/Vis) or mass spectrometry (MS) detection [2–6], Raman spectra [7], and voltammetry [8, 9]. Most methods for the quantitative detection and characterization of azo dyes use HPLC analysis [10–14]. UV visible spectrometry is a useful tool, but the azo dyes are largely affected by solvent influence, and require a photodiode array detector. Detection using mass spectrometry is a more sensitive, but more expensive, approach and is not yet available for every laboratory. LC/MS has been recommended for analyzing disperse and low-molecular (<300) azo dyes, but it is not applicable to sulfonated azo dyes. When the mixture sample contains more than two azo dyes, the voltammetric waves of these dyes are seriously overlapped, which makes their quantification difficult without any preanalysis separation and purification. Recent research on the electrodeposition of antimony (Sb) focuses on (a) Sb/Sb2O3 particles for lithium-ion battery anodes [15]; (b) binary and ternary alloys for use in thermoelectric elements [16]; (c) the electrodeposition of Sb on tin oxide (SnO2) electrodes [17], gold (Au) electrodes [18], glassy carbon electrodes (GCEs) [19, 20], and mercury (Hg) electrodes [21]; (d) one ternary film, (antimony/tellurium) (Sb2Te3), on a silver (Ag) electrode [22]. Ag [23] and metal-Sb alloys [24, 25] cause the reduction of electrocatalytic activity and oxidation in organic compounds. However, there are no published studies on using an electrocatalytic sensor with liquid chromatography-electrochemical detection (LC-EC) and flow-injection analysis (FIA) determination of Lithol Rubine B in cosmetic products. Directive 76/768/EEC
allows Lithol Rubine B to be used in cosmetic products as a coloring agent at a level of 0.2–2.0%. In the present study, we developed a more sensitive and low-cost method for detecting Lithol Rubine B in cosmetic products. We also report the development of a flow-through voltammetric sensor to catalyze the electroduction of Lithol Rubine B.

2. Experimental

2.1. Apparatus and Materials. The HPLC system consisted of a Hitachi Model L-7110 pump with a Rheodyne 7125 injection valve with a 20 µL sample loop and was coupled with an EG&G PARC 400 controlled potentiostat. The flow-through electrolysis cell was designed with the following electrodes: an Ag/AgCl/0.1 M KCl reference electrode (BAS), a platinum auxiliary electrode, and modified silver electrodes as working electrodes for detecting Lithol Rubine B azo dye. All solvents and the analyte were filtered through 0.45 µm cellulose acetate and polyvinylidene fluoride (PVDF) syringe membrane filters, respectively. A chromatogram of Lithol Rubine B was acquired, and the peak height was calculated using an SISC Chromatogram Data Integrator. Lithol Rubine B (D&C Red 6) pure dye content (>90%) was purchased from Unipure LC Sensient Cosmetic Technologies/LCW (Milwaukee, WI, USA). Cosmetics samples were bought from local department stores or retail outlets in southern Taiwan. Other chemical reagents used were of analytical grade.

2.2. Preparing a Thin-Film Antimony Electrode Surface. A thin-film antimony electrode was produced using the following method. Before the analysis, the silver wire electrode (4 cm long, 3 mm in diameter) was mirror-polished sequentially with aqueous suspensions of 1.0, 0.5, and 0.05 µm alumina. The electrode was then rinsed with deionized water and electrolytically plated with antimony ions (1.0 × 10−3 to
4.0 × 10⁻³ M) from 10 mL of acetate buffer (pH 4.5). Plating time was 8 min according to a potential scan of between −1.0 and 0 V (versus Ag/AgCl; at 10 mV/s).

2.3. Constructing a Flow-Through Voltammetric Detector.

A flow-through electrolysis cell was used for DC-mode electrochemical detection. The detection cell (Scheme 1) was constructed in the laboratory. The antimony-modified silver electrode (0.3 mm diameter) was constructed from a length (∼8 cm) of Teflon tubing (1/32 in., i.d.; 1/16 in., o.d.), inserted into one end of the Teflon tube, and then sealed with acrylic resin (Struers). A small copper wire was placed at the other end of the Teflon tube to allow an electrical connection to the antimony-modified silver wire electrode. The platinum wire, which served as a counter, and the Ag/AgCl wire, a reference electrode, were then attached in series with the Teflon tube. For stability, the cell compounds were secured with tape to an insulated plastic box. The voltammetric detector, that is, the eluate, was fed to the antimony-modified silver wire electrode, which had been...
placed in an overflow vessel containing counter-electrodes and reference electrodes.

2.4. Preparing the Cosmetics. The cosmetics (rouge, lipstick, and nail polish) contained oils (mineral oil, petrolatum, and lanolin), ester (isopropyl myristate), waxes (paraffin and carnauba), and a powder base (zinc oxide, titanium dioxide, inorganic pigments, and talc) as the primary constituents of the base. The base was bound with the required color. Raw samples received for analysis were converted into a form suitable for making useful measurements by pretreatment, separation, and preconcentration. Taking into account the content of Lithol Rubine B azo dye in each sample, about 1.0 g of each sample was accurately weighed in a 50 mL beaker, diluted to about 10 mL with methanol, dissolved, and then stirred for 2 h. The mixture solution was concentrated, 9 mL of acetate buffer was added, and then the mixture was centrifuged at 6000 × g for 30 min. The supernatant was transferred into a 10 mL calibrated flask; acetate buffer was added to ensure that the flask contained 10 mL of liquid. The sample (1 mL) was loaded onto a C_{18} cartridge and washed with 1 mL of ethyl acetate, and then the eluent was discarded. The resulting solution was extracted twice with methanol, and the organic layer was evaporated at 40 °C under nitrogen. The dried extract was reconstituted with 1 mL of methanol and filtered through 0.45 μm membrane filters before HPLC analysis.

2.5. Determining Lithol Rubine B Using a Flow-Through Voltammetric Detector. A stock standard sample solution was prepared by dissolving 10 mg of Lithol Rubine B in 10 mL of water, because Lithol Rubine B is a soluble sulfonated salt. Working standard solutions in the range 0.05–1.2 mg/L were prepared from the stock standard solution. Reversed-phase- (RP-) HPLC was done on a Phenomenex HyperClone C_{18} 5 μm (250 mm × 4.6 mm) column eluted with methanol-water (30 : 70, v/v) and acetonitrile-water (30 : 70, v/v), respectively, containing 0.1 mM of K_{2}HPO_{4} (pH 4.08) as the mobile phase, at flow rates of 0.7, 0.5, and 0.3 mL/min. After the azo dye components in the cosmetics sample had been separated on the HyperClone C_{18} column, they were examined using an ultraviolet detector set at 235 nm. The electrochemical detector was operated at −0.6 V. A chromatogram was obtained with 20 μL of the prepared sample solution and a standard solution under the operating conditions described above. Quantitation was based on the peak height of the sample.

3. Results and Discussion

3.1. Voltammetric Measurement. Azo linkages are reducible; a typical two-electron, two-proton reduction was found for azobenzene [26]. Azobenzene undergoes a fast ECEC reduction to hydrazobenzene which is then oxidized at approximately the same potential as azobenzene is reduced [27]. Azobenzene was reduced to the corresponding anilines using controlled potential coulometry since the initially formed intermediate hydrazobenzene decomposes to aniline. The proposed pathway for the electrochemical reduction of Lithol Rubine B is shown in Scheme 2. For both the GCE and the Sb/Ag electrodes, good linearity was observed between the peak height (current) and the square root of the scan rate (Figure 1). The peak potential became more negative as the scan rate increased. These results showed that the electrode mechanism of Lithol Rubine B is EC reduction.

3.2. Optimization LC Analysis. Two different eluents, methanol-water (30 : 70, v/v) and acetonitrile-water (30 : 70, v/v) containing 0.1 mM of K_{2}HPO_{4} (pH 4.08) as the mobile phases of both solvent systems, were investigated at various flow rates. The retention times of acetonitrile-water were 9.8,
The operational stability of the bare Ag and Sb/Ag sensors was tested and compared by continuously exposing them to the flow stream, and by monitoring the amperometric response (at −0.6 V versus Ag/AgCl) of acetonitrile-water (30:70, v/v) containing 0.1 mM K$_2$HPO$_4$ (pH 3.97) over several hours of repetitive injections. Scanning electron microscope (SEM; at 15 kV) images of 4 mM antimony (Sb) particles distributed (Figure 4(a)) at the bare Ag working electrode surface (0.3 mm i.d.) as the working electrode in the flow cell after (b) 0 h and (c) 6 h. Figure 4(c) illustrates the stability of the sensor after 6 h of repetitive injections.

3.4. Precision and Accuracy. The calibration plots obtained by plotting the peak area against the concentration of Lithol Rubine B show good linearity over the range 10–80 mg/L. The regression equations were $y = 1.17x + 0.387$ (correlation coefficient $r = 0.9999$); the range 0.30–1.2 mg/L and $y = 20x + 10.0$ (correlation coefficient $r = 0.9999$) for LC-UV and LC-ED, respectively (Figures 5(a) and 5(b)). Subsequently, we developed a simple and sensitive green electrochemical procedure for determining Lithol Rubine B in real samples. Commercial lipstick was spiked with 0.1, 0.2, or 0.3 mg/L and then analyzed. The calibration plot (Figure 6) shows good linearity. Because it is less expensive than LC-UV analysis, HPLC should be done using a conventional variable wavelength detector to achieve some selectivity and close to the optimum sensitivity of all the colorants. Thus, our proposed analytical method offers a valid and economical alternative to LC-UV detection of Lithol Rubine B.

3.5. Application to Lithol Rubine B. Our proposed LC-EC method was used to determine Lithol Rubine B in cosmetic products. Chromatograms of a comparison of LC-UV (Figure 7(a)) and LC-EC (Figure 7(b)) for Lithol Rubine B in cosmetics show that the sensitivity for the Lithol Rubine B investigated was about two orders of a magnitude higher with
LC-EC than with LC-UV detection; however, there were no significant differences in the values obtained using both types of analysis (Table 1).

4. Conclusions
We constructed an antimony-modified silver electrode to use as an electrocatalytic sensor liquid chromatography-electrochemical (LC-EC) detection and flow-injection analysis (FIA) determination of Lithol Rubine B in cosmetics. The electrode not only exhibited catalytic activity toward this analyte, but also provided a stable, quantitatively reproducible performance in the chromatographic stream. Thus, the proposed analytical method offers a valid, and economical, alternative to UV detection and mass spectrometry detection of Lithol Rubine B.

Acknowledgment

Financial support of this paper by the National Science Council of Taiwan is gratefully acknowledged (NSC 95-2113-M-041-002).

References

[1] M. Miyagoshi, Y. Hayakawa, M. Nagata, and T. Nagayama, "Studies on the origin of mutagenic impurities in commercial Lithol Rubine B," Eisei Kagaku, vol. 33, no. 4, pp. 276–282, 1987.
[2] B. P. Harp, A. L. Scher, H. H. W. Yang, D. L. Brodie, M. P. Sullivan, and J. N. Barrows, "Reversed-Phase LC determination of two manufacturing intermediates, the unsulfonated subsidiary color, and 4-Methyl-Sudan i in D&C Red No. 6, D&C Red No. 7, and their lakes," Journal of AOAC International, vol. 92, no. 3, pp. 888–895, 2009.
[3] S. C. Rastogi, V. I. Barwick, and S. V. Carter, "Identification of organic colourants in cosmetics by HPLC-diode array detection," Chromatographia, vol. 45, pp. 215–228, 1997.
[4] T. Shibata, S. Tsuji, K. Inamori, M. Inoue, and Y. Ito, "Rapid determination of various dyes in cosmetics," Journal of the Society of Cosmetic Chemists Japan, vol. 23, pp. 190–194, 1988.
[5] J. W. Wegener, J. C. Klamr, H. Govers, and U. A. T. Brinkman, "Determination of organic colourants in cosmetic products by high-performance liquid chromatography," Chromatographia, vol. 24, no. 1, pp. 865–875, 1987.
[20] S. Chen, S. Xia, and S. Sun, “Studies of surface alloy as high efficient electrocatalyst for glyoxylic acid synthesis,” Dian-huaxue, vol. 4, pp. 130–134, 1998.

[21] M. V. Mirkin, A. P. Niloţ, and G. G. Kadisova, “Antimony electrodeposition on the mercury electrode,” Journal of Electroanalytical Chemistry, vol. 274, no. 1-2, pp. 319–324, 1989.

[22] C. Wang, Q. Wang, L. Chen, X. Xu, and Q. Yao, “Electrodeposition of SbTe films on Si(100) and Ag substrates,” Electrochemical and Solid-State Letters, vol. 9, no. 9, pp. C147–C149, 2006.

[23] A. A. Isse, G. Berzi, L. Falciola, M. Rossi, P. R. Mussini, and A. Gennaro, “Electrocatalysis and electron transfer mechanisms in the reduction of organic halides at Ag,” Journal of Applied Electrochemistry, vol. 39, no. 11, pp. 2217–2225, 2009.

[24] H. Matsui, K. Saitou, and K. Kashu, “Electrocatalytic activity of Pt-Sb alloys in oxidation of formic acid and methanol,” in Proceedings of the 211th ECS Meeting of Electrochemistry of Novel Electrode Materials for Energy Conversion and Storage, pp. 225–230, May 2007.

[25] V. Climent, E. Herrero, and J. M. Feliu, “Electrocatalysis of formic acid and CO oxidation on antimony-modified Pt(111) electrodes,” Electrochimica Acta, vol. 44, no. 8-9, pp. 1403–1414, 1998.

[26] J. A. Plambeck, Electroanalytical Chemistry: Basic Principles and Applications, John Wiley & Sons, Somerset, NJ, USA, 1982.

[27] P. H. Rieger, Electrochemistry, Prentice-Hall, Upper Saddle River, NJ, USA, 1987.