Full Paper

Alkanethiols Modified Gold Electrodes for Selective Detection of Molecules with Different Polarity and Molecular Size. Application to Vitamin B2 Analysis

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Abstract
The cyclic voltammetry behavior of several molecules with different polarity and molecular size on gold electrodes modified with nonfunctionalized alkanethiols of different chain length, usually employed as chromatographic stationary phases, are studied. The redox systems hexacyanoferrate(II/III), ferrocene/ferrocine and hydroquinone/quinone are chosen as template molecules. As modifiers, ethanethiol, 1-octanethiol and di-*n*-octadecyldisulfide are selected. We can conclude that polar molecules can reach the electrode surface through channels created by the modifiers. However, when nonpolar compounds are analyzed, the nonpolar interactions between the analyte and the terminal group of the modifier lead to retention of the compound, retarding its arrival to the electrode surface. A molecule with polar and nonpolar part was used for the application of this conclusion. If the gold electrode is modified with di-*n*-octadecyldisulfide, the electrochemical behavior of vitamin B2 becomes simpler than that observed on a bare one. This result allows a sensitive and selective procedure to be developed for direct determination of vitamin B2 in pharmaceutical formulations.

Keywords: Modified electrodes, Alkanethiols, Chemisorption, Electrochemistry, Vitamin B2

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1. Introduction

The possibility of developing chemically modified electrodes (CMEs) according to the 'user needs' for sensitive and selective analytical applications is the most attractive advantage of the electrochemical techniques. It is well known that self-assembled monolayers (SAMs) provide a way to control the chemical nature of the electrode-solution interface in order to improve the selectivity and sensitivity of gold electrodes. Many self-assembled systems have been investigated, however monolayers of thiolated compounds on gold electrodes are probably the most studied SAMs [1, 2]. These self-assembled monolayers of chemisorbed thiols on gold surface are a well-known example in sorption studies [3–6]. The easy surface modification and the possibility of the attachment of functional groups are the main advantages of this approach. Therefore, physical and chemical properties of self-assembled monolayers as well as different examples of their technical applications are widely reported [7–20]. Chemisorption of not reactive thiols on gold surfaces can lead to the formation of nanochannels through which the transference of material can be carried out so, this methodology can be used as a strategy to control the arrival of electroactive species to the electrode surface.

The existence of defects or pinholes in these monolayers will affect the behavior of the modified electrode. Therefore, the control of the structure of the resulting monolayers is of great relevance to know whether the analyte under research can reach the electrode surface through on pinholes. As stated by Mirsky [21], the author concludes the selective permeability of self-assembled monolayers opens the way to using them as selective filters for chemical sensors.

In this work, we present the electrochemical behavior (measured by Cyclic Voltammetry, CV) of molecules with well-known electrochemical properties [22] but with different size, physical and chemical properties (hexacyanoferrate(II/III), ferrocene/ferrocine and hydroquinone/quinone), on electrodes modified with not electroactive thiols of different length: ethanethiol (C2), 1-octanethiol (C8) and di-*n*-octadecyldisulfide (C18). These modifiers, usually used as solid phases in chromatography columns, have been chosen in an attempt of conferring to the electrode surface the properties that these materials present in chromatographic applications. Since the modifiers are not electroactive molecules, these electrodes are designed with two objectives: to build channels, of different length, allowing the electroactive species to reach the electrode surface and/or to block the presence of other species with some affinity

for the modifier at a fixed distance. After studying the influence of the different modifications, the C18 modification was applied to the determination of a molecule with a polar and nonpolar part such as vitamin B2 (riboflavin, RF).

Analytical methods for the determination of riboflavin including spectrophotometry [23, 24], high pressure liquid chromatography [25–30], capillary zone electrophoresis [29], fluorescence [30, 31] chemiluminescence [32], and electrochemiluminescence (ECL) [33]. In addition, the electrochemical behavior of RF has been examined [34–38]. Due to its adsorptive properties, electroanalytical measurements of riboflavin by adsorptive stripping voltammetry at electrochemically activated glassy carbon electrodes [39] and mercury electrodes [40, 41], have permitted to develop analytical methods with detection limits at the 2.5 ng/mL level. Moreover, recently Wu et al. [42] have reported the electrochemical determination of riboflavin traces by adsorptive stripping square-wave voltammetry (SWV) with an electrically heated graphite cylindrical electrode (HGCE).

2. Experimental

2.1. Apparatus

Voltammetric measurements were carried out with a BAS i-Epsilon potentiostat with a standard three electrode cell. The reference electrode was a Ag/AgCl (3 M KCl) electrode; a platinum wire was the counter electrode and a gold electrode (supplied by BAS) was used as working electrode.

2.2. Reagents

Di-*n*-octadecyldisulfide (C18, >98%) was obtained from Lancaster Synthesis (England), octanethiol (C8, >98%) and ethanethiol (C2, >97%) were obtained from Aldrich Chemical Co. (St. Louis, USA). C18 and C8 stock solutions were prepared in tetrahydrofuran and C2 in methanol, both solvents from Scharlau (Barcelona, Spain). Ferrocene (98%) was supplied by Acros Organics, riboflavin (vitamin B2, 98%) by Fluka and hydroquinone (99%) by Aldrich Chemical Co. (St. Louis, USA). All reagents used were of analytical reagent grade and ultrapure water was purified with a Millipore MilliQ System (Waters). All solutions were prepared just prior to use.

2.3. Procedures

The methodology applied for all measurement consisted of three steps: i) electrode activation (including a cleaning step when required), ii) electrode modification, iii) measurement.

2.3.1. Electrode Activation

In order to prepare the electrode surface for a new experiment and to desorb the alkanethiol monolayers, activation and regeneration of the electrode surface was carried out by successive cyclic voltammetric scans in 0.1 M H_2SO_4 solution between 0.0 V and 1.5 V at 100 mV/s. A piranha solution or an ultrasonic bath was used to clean the electrode surface when it was required before the activation procedure described before.

2.3.2. Electrode Modification

Once the electrode surface is cleaned, the modification of its surface was carried out immersing the clean electrode in a modifier solution at a predefined concentration level and for a prefixed time under constant stirring. Next, the electrode was carefully washed with ultrapure water and set in the analysis cell.

2.3.3. Measurement

The modified electrode was immersed in a deoxygenated solution containing the analyte under investigation and then cyclic voltammograms were recorded.

3. Results and Discussion

Some previous experiments related to the modification conditions (modification time from 1 to 20 min, and modifier concentration, in the $10^{-2}-10^{-5}$ M range) were carried out in 0.1 M sulfuric acid. As expected, a decrease of the current corresponding to the characteristic reduction gold wave was produced in all cases when the electrode was modified with respect to that recorded with the bare electrode. From the comparison between the gold reduction currents, we observed that the largest modifications were obtained at short modification time periods (1 min) and for a modifier concentration of 10^{-2} M.

3.1. Electrochemical Behavior at Alkanethiols-Modified Electrodes

The voltammograms of ferrocene/ferricinium, hexacyanoferrate(II)/(III) and hydroquinone/quinone redox pairs, either at a bare electrode and at modified gold electrode with different alkanethiols under different conditions, are depicted in Figure 1. Voltammetric responses were obtained in all cases at bare Au electrodes.

Regarding alkanethiol modified electrodes different responses for ferrocene/ferricinium and hexacyanoferrate(II)/(III) systems were observed with respect to the hydroquinone pair . In the former case (Fe(II)/(III) systems), the slight differences observed for the peak potential values related to the unmodified electrode led us to think that the supply of substance to the electrode surface

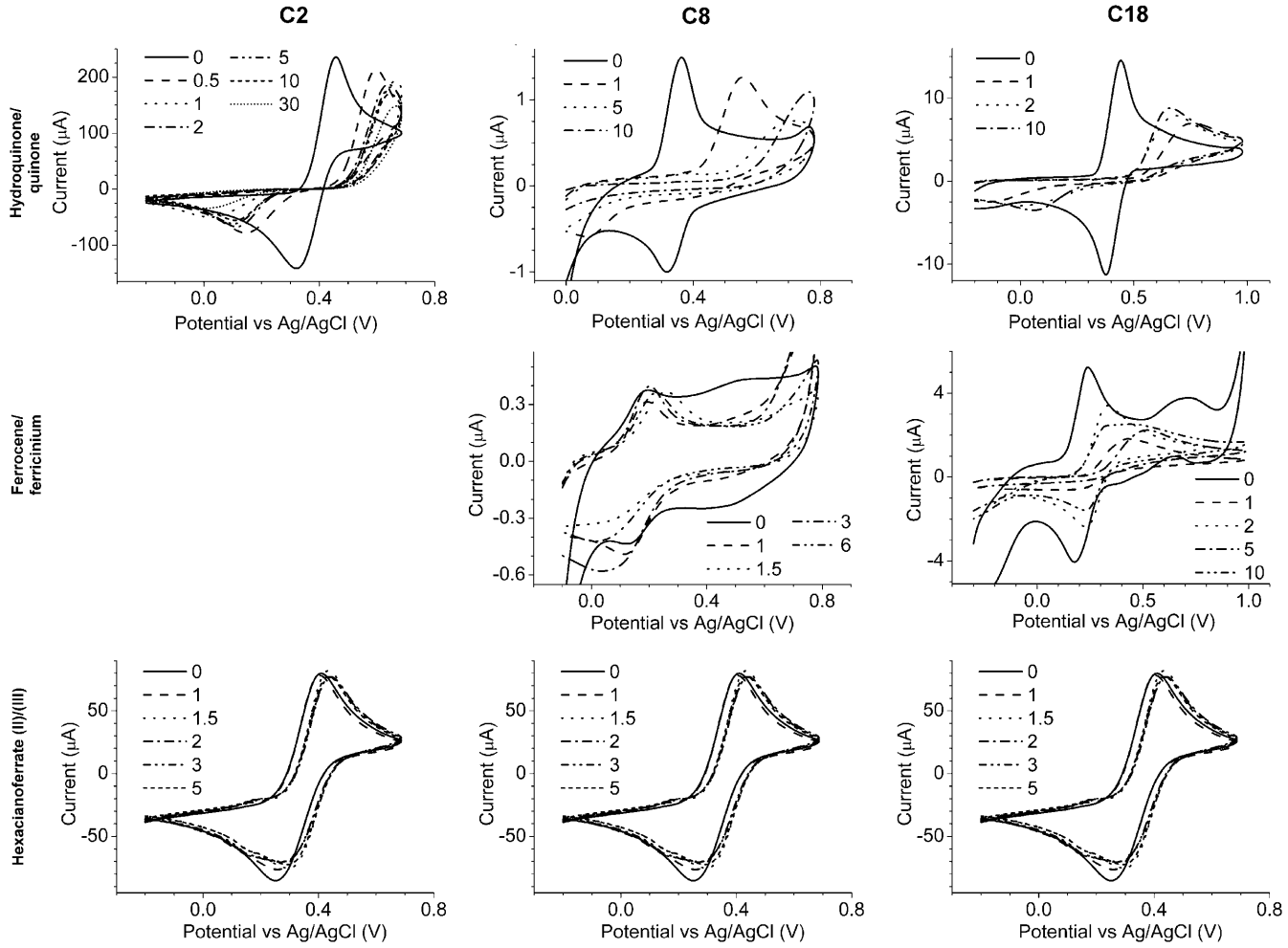


Fig. 1. Cyclic voltammograms for 1×10^{-4} M hexacyanoferrate(II), 1×10^{-3} M hydroquinone, in sulfuric acid 0.1 M, and for 1×10^{-3} M ferrocene, in sulfuric acid 0.1 M-methanol (1:1, v:v) at bare gold electrode and at C2, C8, and C18 modified electrodes. Figures indicate the modification time (in minutes).

was produced through the channels created by the modifier irrespective of the alkanethiol employed as modifier. Moreover, as expected, neither increasing the modification time nor the alkanethiol length chain produced significant differences in the voltammograms recorded for these polar electroactive species.

However, hydroquinone peak potential values were severely affected upon electrode modification. In all cases there was a significant shift in the oxidation potential value (Fig. 1), thus suggesting an increase in the energy required to produce the oxidation reaction due to the indering produced for the electroactive species to reach the electrode surface as a result of the modification. Therefore, one of the factors controlling the electrochemical reaction kinetics has been significantly modified. In addition to the variation in the potential values, a decrease of the peak current is also show. These results indicate a decrease in the mass transport rate to the electrode surface. These changes are more dramatic upon when increasing the alkanethiol chain length. According by, the electrochemical behavior of these systems shifts from reversible to irreversible when the number of carbon atoms (chain length) of the modifier increases. When the electrode is modified, the electroactive species can reach the electrode surface through the channels created as a result of the monolayer organization, which is better observed for long length chain alkanethiols, or it can be retained on the alkanethiol terminal group, therefore electron transference occurring by tunneling, or both. The nonpolar side of the hydroquinone molecule allows it to be retained at the end of the modifier as a consequence of dipole – dipole interactions.

Considering these results, which support the idea that polar molecules reach the electrode surface through channels, whereas partially nonpolar molecules interact with the modifier, mixtures of both types of molecules were tested. Interesting results were obtained working with C18 as a modifier. On the one hand, the long chain of the alkanethiol leads to an ordered submonolayer with channels to the electrode surface; on the other hand the different interactions of C18 with polar and nonpolar molecules allows the electrochemical response of a mixture of electroactive species to better resolved without any previous separation

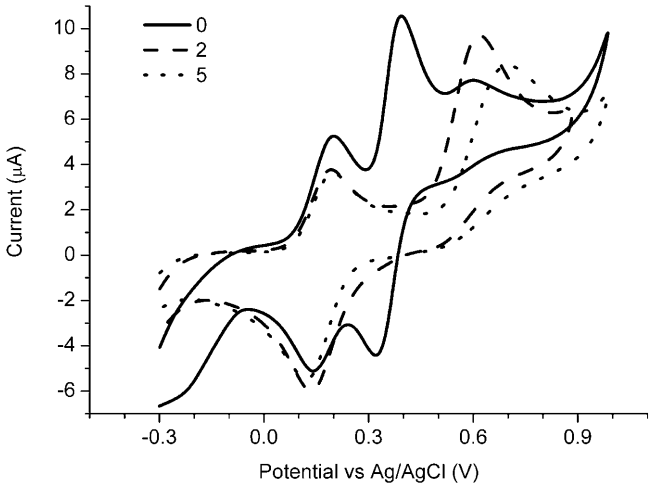


Fig. 2. Cyclic voltammograms for a mixture of 1×10^{-3} M hydroquinone and 1×10^{-3} M ferrocene, on a gold electrode modified with C18 for different times (the numbers in the legend, in minutes), in sulfuric acid 0.1 M-methanol (1:1, v:v), and at 100 mV/s.

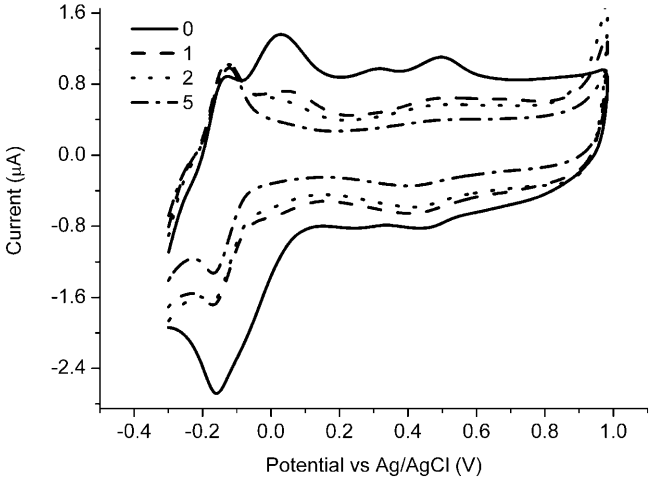


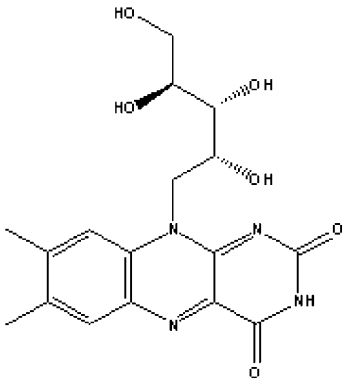
Fig. 3. Cyclic voltammograms for 5×10^{-5} M vitamin B2, at a C18 modified gold electrode for different modification periods of time (numbers shown in the legend, in minutes), 0.1 M sulfuric acid as supporting electrolyte; scan rate of 100 mV/s.

as it can be seen in Figure 2: a mixture of ferrocene and hydroquinone was resolved when the modified electrode was used.

Taking into account all these results, we decided to study the electrochemical behavior of a bigger molecule than those previously studied, with both, polar and nonpolar parts (vitamin B2) at a C18-modified gold electrode.

3.2. Electrochemical Behavior of Vitamin B2 at a C18-Modified Gold Electrode

Vitamin B2 is an electroactive polynuclear aromatic compound with the following structure:



Scheme 1. Vitamin B2 structure.

Figure 3 shows cyclic voltammograms of 5×10^{-5} M vitamin B2 in 0.1 M sulfuric acid. As it can be seen, riboflavin exhibits a complex electrochemical behavior at the bare electrode, due to the high number of electroactive groups in its structure (Fig. 3, solid line).

The complex electrochemistry of riboflavin becomes simpler at the C18 modified electrode even upon the softest modification conditions (Fig. 3, dashed and dotted lines). In this case, just one of the voltammetric signals recorded at the bare electrode can be clearly observed. Furthermore, this wave, corresponding to a reversible system with no changes in the peak potentials values irrespective of the modification conditions, is better defined than that recorded at the unmodified electrode. The most noticeable changes in the riboflavin behavior are observed for a modifier concentration of 10^{-3} M and with short modification times (see Table 1). This behavior suggests that riboflavin is better oriented to the electrode surface due to the interactions between its nonpolar side and the nonpolar modifier.

With the aim of evaluating the effect of pH on the electrochemical behavior of riboflavin, different 0.1 M buffer solutions (phosphate, acetate and borate) were assayed maintaining a constant ionic strength of 0.1 M (Fig. 4).

As expected, both anodic and cathodic peak potentials shift to more negative potentials when pH was increased according with the equations:

$$E_{p,an}\ (V) = -0.049\ pH - 0.0519 \quad R^2 = 0.9912$$

$$E_{p,cat}\ (V) = -0.044\ pH - 0.0110 \quad R^2 = 0.9912$$

The slope values suggest that equal number of electrons and protons are involved in the redox reaction. In fact, it is reported that the reduction of RF from the isoalloxazine ring involves a reversible two-electron redox process due to two overlapping one-electron steps [36]. First, RF accepts one proton and one electron to its unsaturated nitrogen to

Table 1. Variation of peak parameters obtained by cyclic voltammetry for vitamin B2 for different C18 concentrations and modification times, $v = 100$ mV/s.

$-\log C$ of C18	Modification time (min)	E_{an} (mV)	E_{cat} (mV)	$E_{an} - E_{cat}$ (mV)	I_{an} (μA)	I_{cat} (μA)	I_c/I_a
Bare gold electrode		−137	−159	22	1.0	2.3	2.19
5	1	−133	−159	26	1.3	1.8	1.42
	2	−132	−157	25	1.2	1.7	1.40
	5	−130	−158	28	1.2	1.5	1.21
	10	−130	−151	21	1.3	1.7	1.27
	15	−141	−166	25	1.3	1.9	1.51
	20	−138	−170	32	1.2	2.0	1.65
4	1	−139	−155	16	1.3	1.4	1.06
	2	−140	−165	25	1.1	1.1	0.96
	5	−140	−165	25	1.1	1.1	0.96
	10	−135	−155	20	1.4	1.3	0.96
	15	−134	−157	23	1.3	1.2	0.95
	20	−135	−155	20	1.3	1.4	1.12
3	1	−111			0.4		
	2	−111	−172	61	0.6	0.7	1.10
	5	−121	−193	72	0.5	0.6	1.08
	10	−119	−176	57	0.9	0.9	1.02
	15	−123	−176	53	0.5	0.7	1.30
	20	−131	−163	32	0.7	0.9	1.37

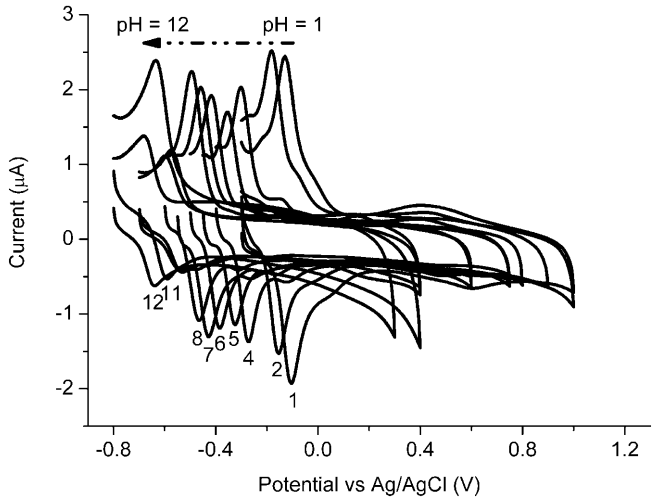


Fig. 4. Cyclic voltammograms of 5×10^{-5} M riboflavin with at a C18 modified gold electrode for different pHs (the numbers close to each voltammogram), with a constant concentration of buffer (0.1 M), and at a scan rate of 100 mV/s.

form a semiquinoid free radical; then, another one further proton and one electron can approach to other unsaturated nitrogen to form the reduced form of RF (RFH2).

3.3. Determination of Vitamin B2

All the parameters affecting the square-wave voltammetric response of riboflavin at the C18-modified gold electrode were optimized in order to electrochemically quantify riboflavin. The selected conditions were immersion of the Au electrode in a 10^{-4} M C18 solution for 1 min; 0.1 M phosphate buffer of pH 2 as supporting electrolyte, step = 5 mV, square-wave frequency = 300 Hz and square-wave amplitude = 30 mV. A linear relation between the current and riboflavin concentration was observed according to the equation: I_p (μA) = 2.77 C (μg/mL) − 6.2111; $R^2 = 0.9959$. A *LOD* of 2.3 μg/mL and LOQ of 2.6 μg/mL were calculated according to the 3σ and 10σ criteria respectively.

Before determining vitamin B2 in a pharmaceutical formulation, a study of vitamin B1, vitamin B6, vitamin B12, vitamin C, nicotinamide, L-lysine and glucose as possible interferences was carried out. It was found that even for a 100-fold interferenting riboflavin concentration ratio, no interference was produced by any of these compounds.

The method was applied to the analysis of the pharmaceutical compound Ton Was of Chiesi Parma (Italy) Laboratory with the following composition: thiamine chlorhydrate (vitamin B1) 2.24 mg, cyanocobalamin (vitamin B12) 0.01 mg, riboflavin (vitamin B2) 2.73 mg, and sorbitol 100 mg.

The powder from one vial was dissolved in 10.0 mL of ultrapure water, and sonicated for 10 min. After addition of 4 mL of 0.1 M phosphate buffer pH 2, the solution was diluted to 25.0 mL with ultrapure water. Finally, an aliquot of 200 μL was diluted to 10.0 mL with the supporting electrolyte and transferred to the measurement cell. The standard additions method was applied to determine vitamin B2 (Fig. 5) with a relative error of 2.6% ($n = 3$) The calculated recoveries ranged from 98% to 104% and the amount of vitamin B2 found (2.80 mg/tablet) is in agreement with that specified by the supplier . This reveals that the proposed method is precise and accurate enough and can be applied for a fast, selective and sensitive determination of vitamin B2 in pharmaceutical formulations without any sample treatment.

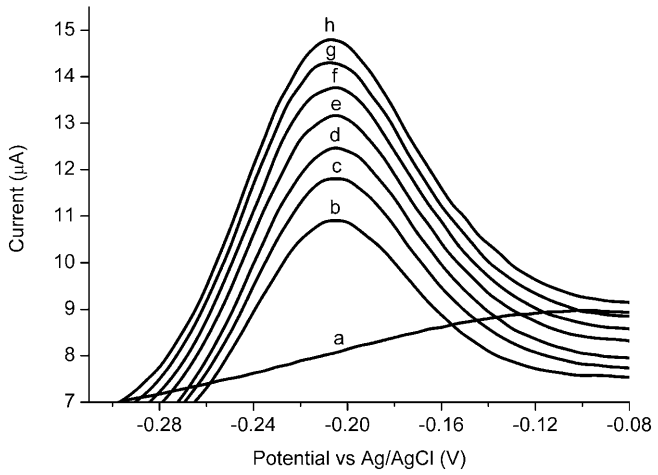


Fig. 5. Square-wave voltammograms for the determination of vitamin B2 using the standard additions method and with a C18-modified gold electrode. 0.1 M phosphate buffer of pH 2 as supporting electrolyte. SQW parameters: step = 5 mV, frequency = 300 Hz; amplitude = 30 mV. Background voltammogram (a); pharmaceutical sample (b); 1.45, (c); 2.78, (d); 4.00, (e); 5.12, (f); 6.15, (g); 7.11(h) μg/mL addition of riboflavin.

4. Conclusions

Modification of gold electrodes with compounds commonly used for refills of chromatography columns provides the electrodes surface of the chromatographic properties of these materials. The arrival of the electroactive compound to the electrode surface is produced through the channels formed as a result of the submonolayer built. In addition, nonpolar interactions between the alkanethiols terminal groups and some electroactive species can also condition the electrochemical response of these compounds. Both factors allow the resolution of mixtures of electroactive species with a similar electrochemical response without previous separation. A gold electrode modified with C18 senders the electrochemical behavior of Vitamin B2 simpler and allows its selective and sensitive determination in pharmaceutical compounds without any additional treatment.

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