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A Square-Wave Adsorptive Stripping Voltammetric Method for Analysis of Erythrosine B, a Food Additive Dye

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Abstract

Square wave adsorptive stripping voltammetric determinations of trace concentrations of the xanthene coloring agent erythrosine b were described. The analytical methodology used was based on the adsorptive preconcentration of the dye on the hanging mercury drop electrode and then a negative sweep initiated. Hence, in pH 8.5 carbonate supporting electrolyte, erythrosine b gave a well- defined and sensitive AdSV peak at –1000 mV. The electroanalytical determination of this dye was found to be optimized in carbonate buffer (pH 8.5) with the following experimental conditions: accumulation time (150 s), accumulation potential (0.0 V), scan rate (1000 mV s^{-1}), pulse amplitude (100 mV) and frequency (30 Hz). Under these optimized conditions the AdSV peak current was proportional over the concentration range 1×10^{-7}- 2.5×10^{-7} mol l^{-1} (r = 0.99) with a detection limit of 6.96×10^{-10} mol l^{-1}. This analytical approach possessed enhanced sensitivity than the conventional HPLC or spectrophotometry and it was simple and not time-consuming. The precision of the method in terms of RSD%, was 1.7 % whereas the accuracy was evaluated via the mean recovery of 101.8%±1.79. Possible interferences by several substances usually present as food additive azo dyes (E110, E102), allura red, carmine, amaranth, natural and artificial sweeteners, preservers and antioxidant were also investigated. Applicability of the developed electroanalysis method was illustrated via the determination of erythrosine b in soft drink samples.

Keywords: erythrosine b, square wave, adsorptive voltammetry, food dye.

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1. Introduction

Adsorptive stripping voltammetry (AdSV) method of analysis over the last two decades have fulfilled an important role in the accomplishments of analytical chemistry for the determination of trace concentrations of many coloring agents of food, cosmetic and textile significance. In fact, Fogg [1] and his colleagues [2] have reviewed the utilization of stripping voltammetric techniques for the determination and monitoring of various synthetic dyes. In addition, various research workers have investigated the stripping voltammetric behavior and properties of several food additive dyes such as Sunset Yellow (E-110) [3], Tartrazine (E-100) [4], Quinoline Yellow (E-104) [5] and Ponceau 4R (E-124), Allura Red (E-129) and Carmoisine (E-122) [6].

Erythrosine b E-127 (formerly FD&C Red No. 3 -color Index No: 45430) is an iodo derivative of flourescin with distinctly bluish shade whereas eosin is a bromine derivatives of flourescin. Erythrosine b (formula I) is a water-soluble red synthetic dye used as a coloring agent in foodstuffs,

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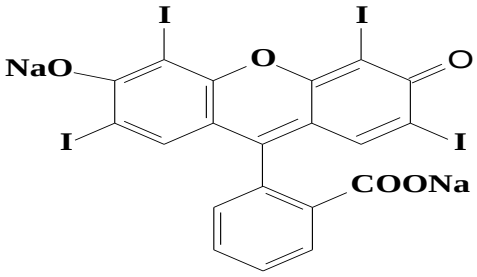
medicines and cosmetics and it is one of the food additive dye in use in many countries. It is used as a plasma stain for nerve cells and staining bacteria in soil. It is used as a phosphorescent triplet probe to detect rotational diffusion of membrane proteins. Although some evidence of carcinogenicity was found in early animal studies, subsequent work failed to confirm these findings, hence, in the UK and other countries this food colorant is considered suitable for use in food industries [7]. It's estimated acceptable daily intake is up to 500 μg/kg body weight [8].

There is a substantial need for analytical methods capable for monitoring synthetic food dyes at low concentration levels, hence, a wide variety of analytical techniques were introduced to determine trace concentrations of many synthetic dyes such as erythrosine b in diverse foodstuff products and clinical formulations. Instrumental methods of analysis successively used for determining erythrosine b include Spectrophotometry [9-11], HPLC [12], micellar capillary [13,14], TLC [15], colormetric methods [16], flourometry [17] and photo kinetic [18]. Furthermore, this food additive dye was analyzed by the electroanalytical techniques such as polarography [19] and voltammetry [20,21].

Aim of the study: Due to the low content of this coloring agent in some foodstuff products and pharmaceutical formulations, a powerful electrochemical procedure capable of ensuring far enhanced sensitivity is needed. In comparison to other analytical procedure, the proposed stripping voltammetric method reduced the length of real time of determination since no pretreatment steps were requested. Another advantage of this electroanalytical approach is its suitability to analysis very diluted samples, a simple an quick strategy to avoid matrix effect in food analysis. Although, multi-verities calibration method with adsorptive voltammetry was used to analyze erythrosine b, yet no especially devoted research study for the analysis of this dye using square-wave adsorptive stripping voltammetric method (SW-AdSV) have been reported in the literature. Considering the above remarks, this work was devoted to a detailed study of the SW-AdSV behavior of this artificial coloring agent in order to develop an effective electroanalysis method for the determination of erythrosine b in commercial products.



Formula I: Erythrosine B,

2. Experimental

2.1 Apparatus

All adsorptive stripping measurements were carried out with 757 AV computrace (Metrohm, Herisau, Switzerland) in connection with Dell computer Pentium 3 and controlled by (VA computrace 2.0) control software. Stripping voltammograms were printed via a LaserJet 1200 series printer. A conventional three electrode system was used in the hanging mercury drop electrode (HMDE) mode. pH values were measured with Metrohm 632 pH meter. Oxford adjustable micropipette (Ireland) was used to measure microliter volumes of the standard solutions.

2.2 Reagents

All chemicals used were of analytical reagent grade and were used without further purification. Erythrosine b (Sigma-Aldrich) stock solution of 1×10^{-2} mol l^{-1} was prepared by dissolving the

appropriate amount of erythrosine in distilled water in 25 ml volumetric flask. This stock solution was stored in the dark. Standard solutions of this coloring agent with lower concentration were prepared daily by diluting the stock solution with distilled water. Britton-Robinson supporting buffer (pH ≈ 2, 0.04 mol l^{-1} in each constituent) was prepared by dissolving 2.47 g of boric acid (Winlab, UK) in 500 ml distilled water containing 2.3 ml of glacial acetic acid (BDH, UK) and then adding 2.7 ml of ortho-Phosphoric acid (Riedal-deHaen, Germany) and diluting to 1 L with distilled water. The carbonate buffer was 0.1 mol l^{-1} in both sodium hydrogen carbonate (Winlab, UK) and disodium carbonate (BDH, UK), while phosphate buffer was prepared from 0.1 moll^{-1} in both phosphoric acid (Riedal-deHaen, Germany) and sodium dihydrogen phosphate (Winlab, UK). The acetate buffer was prepared from 0.02 M in both sodium acetate (Winlab, UK) and acetic acid (BDH, UK). While, ammonia buffer was prepared by dissolving 4.5 g of ammonium chloride (BDH, UK) in 20 ml distilled water and then adding 35 ml of concentration ammonia (Winlab, UK) and diluting to 1 L with distilled water.

2.3 Procedure

The general procedure adopted for obtaining adsorptive stripping voltammograms was as follows: A 20 ml aliquot of carbonate supporting buffer (unless otherwise stated) at desired pH was pipetted in a clean and dry voltammetric cell and the required standard solutions of erythrosine b were added. The test solutions were purged with nitrogen for 5 minutes initially, while the solution was stirred. The accumulation potential of 0.0 V vs. Ag/AgCl was applied to a new mercury drop while the solution was stirred for 150 seconds. Following the preconcentration period, the stripping was stopped and after 20 seconds had elapsed, cathodic scans were carried out over the range 0.0 to –1.5 V. All measurements were made at room temperature.

In order to apply the developed SW-AdSV method for the analysis of erythrosine b in food samples, and because no commercial food products which contain this food additive dye was found in regional market, erythrosine has been spiked it into a soft drink beverages (7 UP and Miranda). These soft drinks have the following composition: carbonated water, sugar, citric acid, lemon flavor, sodium citrate, sunset yellow and sodium benzoate. A known volume of the commercial drink was added to 20 ml carbonate buffer in order to prepare $1x10^{-4}$ M (with 7 up) and $9.7x10^{-6}$ mol/l (with Miranda) erythrosine solution, which was placed into the voltammetric cell and deoxygenated for 8 minutes. No further pretreatment or extraction steps were needed.

3. Results and Discussion

3.1 SW-AdSV Behavior of Erythrosine b.

Preliminary study of this synthetic xanthene dye indicates that it was adsorbed effectively onto the hanging mercury drop electrode (HMDE) and it can be monitored by AdSV approach after scanning the applied potential in the negative direction. For instance, adsorptive collecting of 1×10-7 mol l^{-1} erythrosine b solution for 60 s in carbonate supporting electrolyte (pH 8.5), yielded a single well-defined voltammetric peak at –1060 mV vs. Ag/AgCl reference electrode. This electrochemical response is thought to have resulted from the cathodic reduction of the xanthene electroactive moiety. It is known that the cathodic reduction process of dye compound containing electron-donating groups such as the carbonyl group is accompanied by reduction of this group and the formation of the corresponding alcohol [22,23]. In fact, in alkaline media, a one-step reduction mechanism was proposed for this xanthene dye. The step is likely a two-electron, two-proton reduction process resulting in the formation of a carbonyl form, which is then, undergoes irreversible reductive reaction to give the corresponding alcohol. The suggested electro-chemical reduction mechanism is schematically shown in Figure 1, while Figure 2

illustrates a typical stripping voltammogram of this food additive dye in carbonate buffer at 1×10^{-7} mol l^{-1} dye concentration.

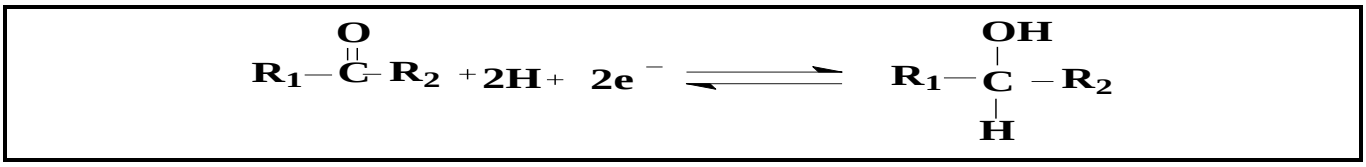


Figure 1: General proposed cathodic reduction mechanism of Erythrosine b.

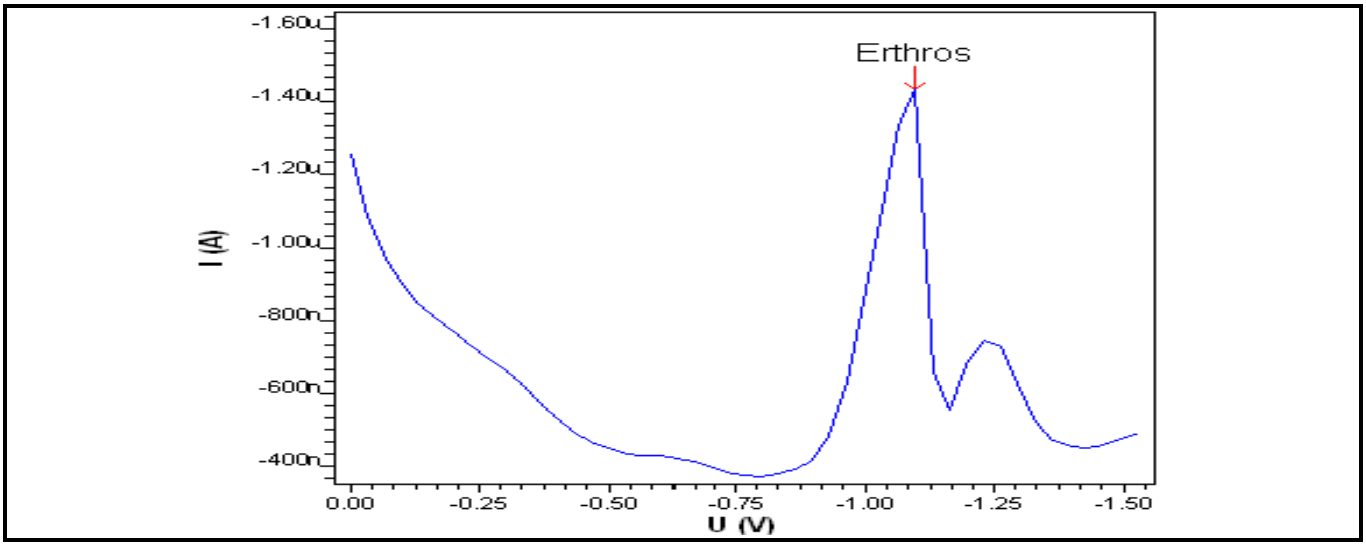


Figure 2: SW-AdSV of Erythrosine b in carbonate buffer at pH 8.5. Experimental conditions: T_{acc}= 150 s, E_{acc}= 0.0 V, scan rate 1000 mV s^{-1} and dye concentration 1×10^{-7} mol l^{-1}.

The irreversible reductive reaction characteristic of dye group was confirmed by cyclic voltammetric measurement of erythrosine solution at a scan rate of 50 mV s^{-1} in various pH values. No anodic peak was observed on any of the measured cyclic voltammograms. A representative cyclic voltammogram of 1×10^{-4} mol l^{-1} erythrosine in B-R buffer at pH 8.5 is shown in Figure 3.

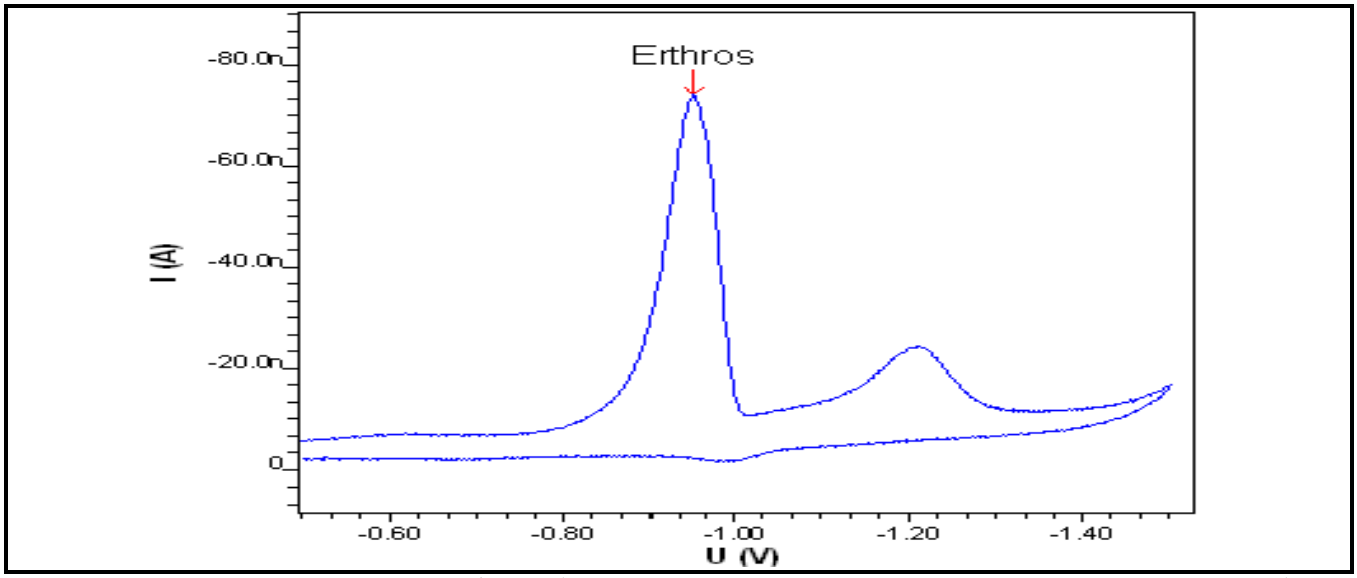


Figure 3: Cyclic voltammetry of 1×10^{-4} mol l^{-1} Erythrosine b at pH 8.5 carbonate buffer, scan rate 50 mV s^{-1}

3.2 Factors Affecting Adsorptive Stripping Response.

3.2.1 Effect of supporting electrolyte and pH.

The choice of a suitable medium is an important parameter for the adsorptive stripping determination of the synthetic dyes. Thus, 1×10^{7} mol l^{-1} solutions of erythrosine b were studied by SW-AdSV method in Britton-Robinson, acetate, ammonia, carbonate and phosphate buffers at different pH values (3, 7 and 9) after 60 s preconcentration times at 0.0 V accumulations potential. The ideal adsorptive stripping response in terms of peak shape and current and the smoothness of the baseline was observed when utilizing carbonate buffer, which was selected as optimum condition for subsequent works. In general, it was noticed that the peak height of the obtained AdSV signal reached it maximum values in alkaline media. The influence of the variation of pH over the range 7.5-10.5 on the peak height and potential of the dye single was investigated further. This is exhibited in Figure 4, in which the stripping voltammetric peak height of 1×10^{-7} mol l^{-1} erythrosine solution was plotted as a function of pH. Variations of pH values over the range 7.5-8.5 gradually enhanced the monitored AdSV peak current, while beyond pH 8.5, the peak current decreased steadily and become constant. For analytical purposes, the optimum pH value for the determination of this food additive dye seems to lie around pH 8.5.

3.2.2 Effect of accumulation conditions.

Preconcentration of the analyzed food additive dye on the surface of the HMDE is another essential condition for highly sensitive determination since the amount of the adsorbed color agent depends on the length of the time over which adsorption process allowed to proceed, in addition to the intensity of stirring and applied accumulation potential. The reduction current of 1×10^{-7} mol l^{-1} erythrosine b solution was measured as a function of accumulation time as shown in Figure 5. In this diluted solution, an almost quasi-linear relationship between the AdSV peak current and accumulation time was observed up to 150 s and decreased there after possibly due to competitive adsorption of any impurities in the sample or buffer solution. A 150 s time collection was adopted as optimum for the following SW-AdSV studies.

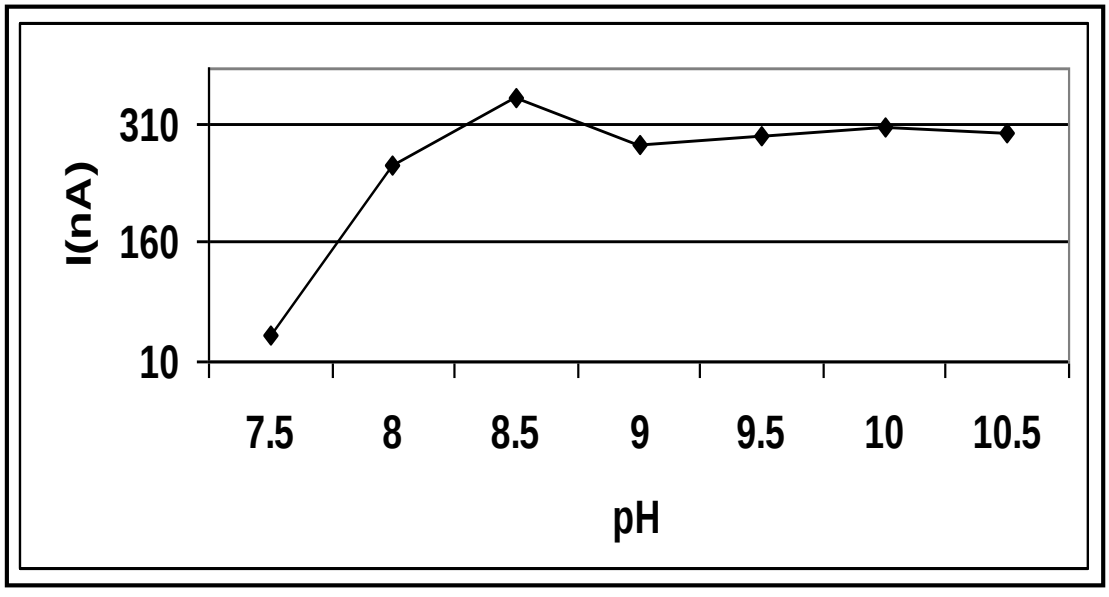


Figure 4: Effect of pH on the peak current of erythrosine b at carbonate buffer pH 8.5 buffer, scan rate 50 mV s^{-1}. T_{acc}= 150 s, E_{acc}= 0.0 V, scan rate 1000 mV s^{-1} and dye concentration 1×10^{-7} mol l^{-1}.

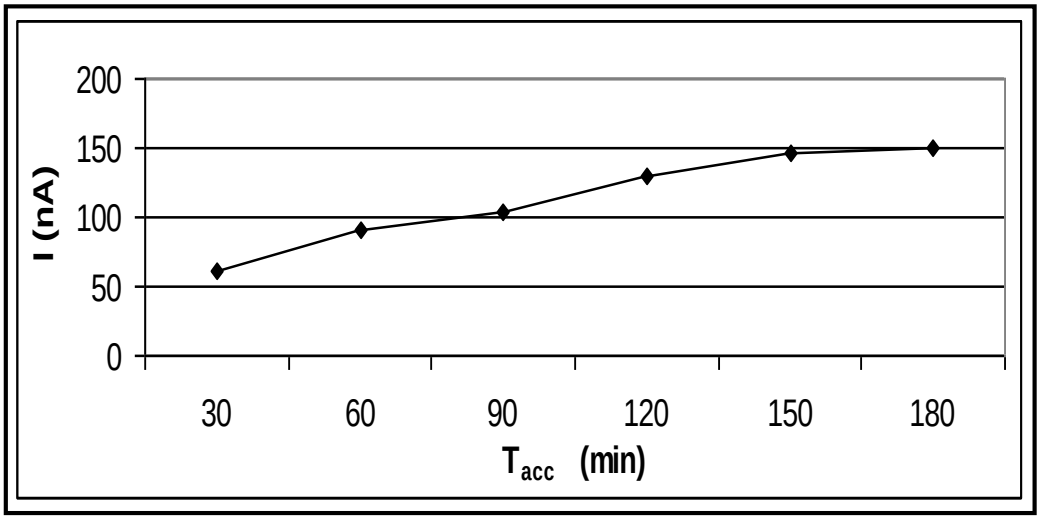


Figure 5: Effect of accumulation time on the peak current of erythrosine b at carbonate buffer pH 8.5, E_{acc}= 0.0 V, scan rate 1000 mV s^{-1} and dye concentration 1 ×10^{-7} mol l^{-1}.

On the other hand, very slight effect on the peak current of dye moiety was recorded due to the variation of accumulation potential from +0.2 V to –0.4 V (see fig. 6). In fact, over the range -0.4 – 0.0 V, there is linear relationship between the AdSV peak current and accumulation potential was observed, while beyond 0.0V, the peak current decreased steadily. As a result, 0.0 V value was selected as optimum in order to ensure adequate sensitivity with absent of any competitive electrostatic attraction emerge from ionic constituents or impurities, which may compete, with the analyte of interest for the adsorption sites on the HMDE. However, the alteration of accumulation time and potential did not cause significance shifts in the AdSV peak potential.

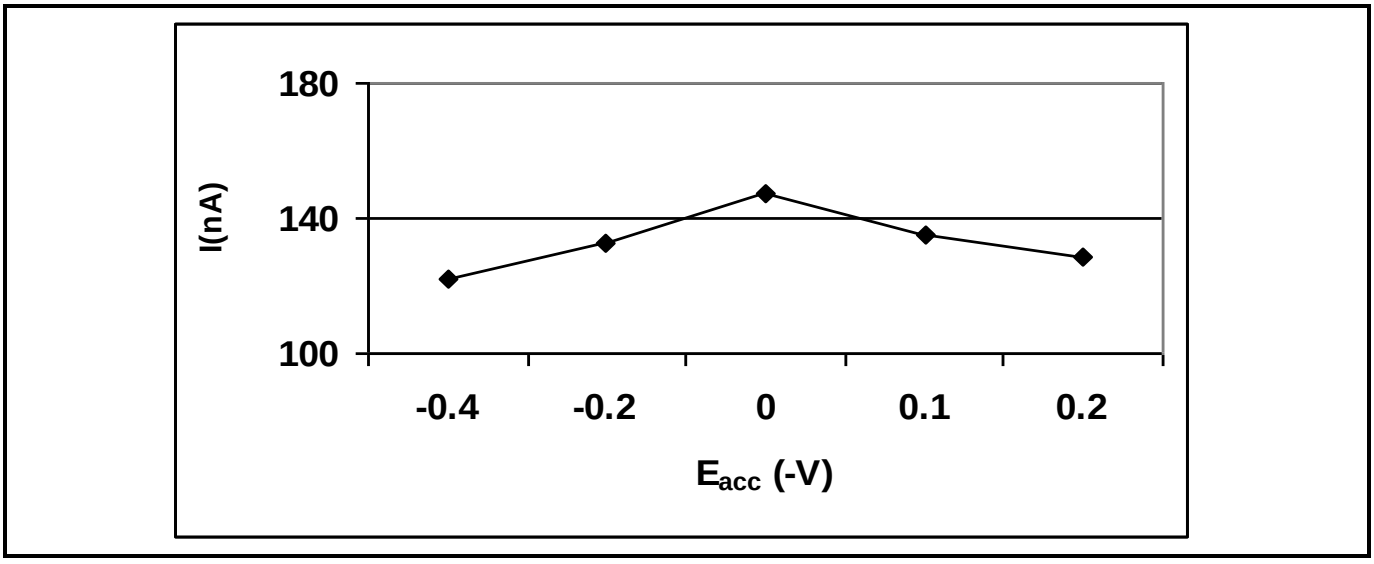


Figure 6: Effect of accumulation potential on the peak current of erythrosine b at carbonate buffer pH 8.5, T_{acc}= 150 s, scan rate 1000 mV s^{-1} and dye concentration 1 ×10^{-7} mol l^{-1}.

3.2.3 Effect of dye concentration.

The concentration of the analyzed xanthene food dye has a profound effect on the examined SW-AdSV peak current. The increase in the concentration of erythrosine (5 ×10^{-8}-5 ×10^{-7} mol l^{-1}) is accompanied by a quasi-linear enhancement in the electroanalytical peak height, which indicates the

suitability of the applied approach for the measurement of the studied dye by calibration graph method. The variation in the AdSV peak height with continuous addition of the tested dye is illustrated in Figure 7. The quasi-linear correlation was observed over the range 5×10^{-8}-3×10^{-7} mol l^{-1} and then start steadily to level off at higher concentrations owing to the saturation of the working electrode surface area. A 3×10^{-7} mol L^{-1} erythrosine concentration was adequate for further investigations.

3.2.4 Effect of potential sweep conditions.

Generally, the SW-AdSV response depends on various parameters related to the way the applied potential was scanned. For instance, the cathodic peak current of erythrosine was found to be proportional to the scan rate. As can be seen from Figure 8, the alteration of scan rate between 100 and 1200 mV s^{-1}, caused the SW-AdSV peak current to increase over the range 100-1000 mV/s and then it levels off. For the subsequent work, scan rate value of 1000 mV s^{-1} was selected because it ensured adequate sensitivity with short practical time.

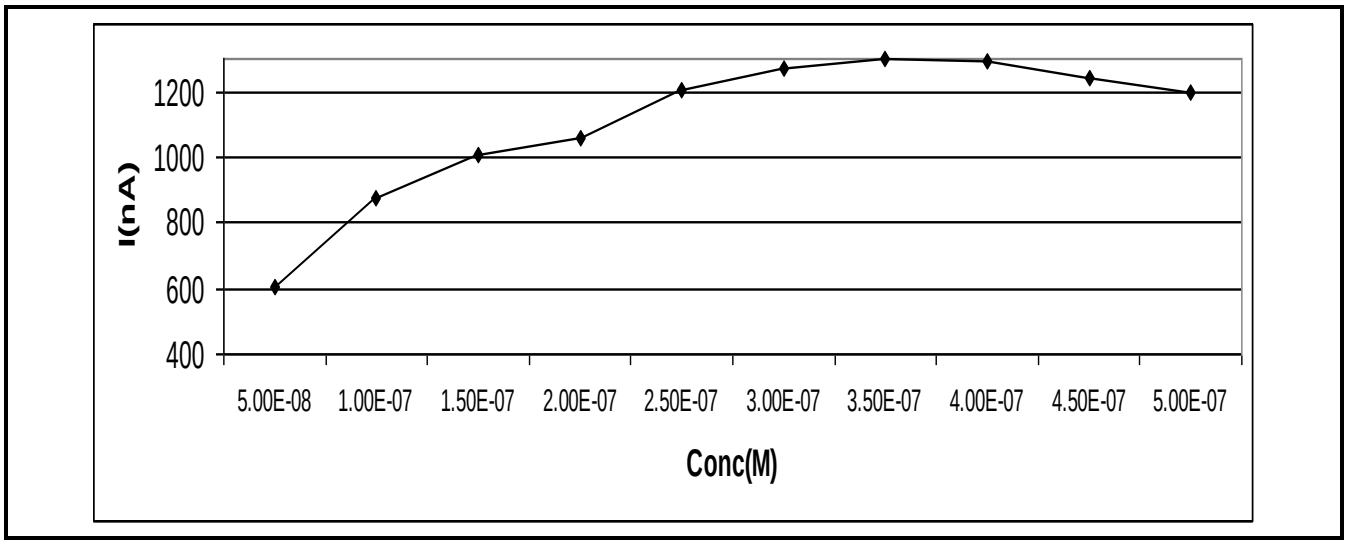


Figure 7: Effect of erythrosine b concentration on the peak current at carbonate buffer pH 8.5 T_{acc}= 150 s, E_{acc}= 0.0 V and scan rate 1000 mV s^{-1}

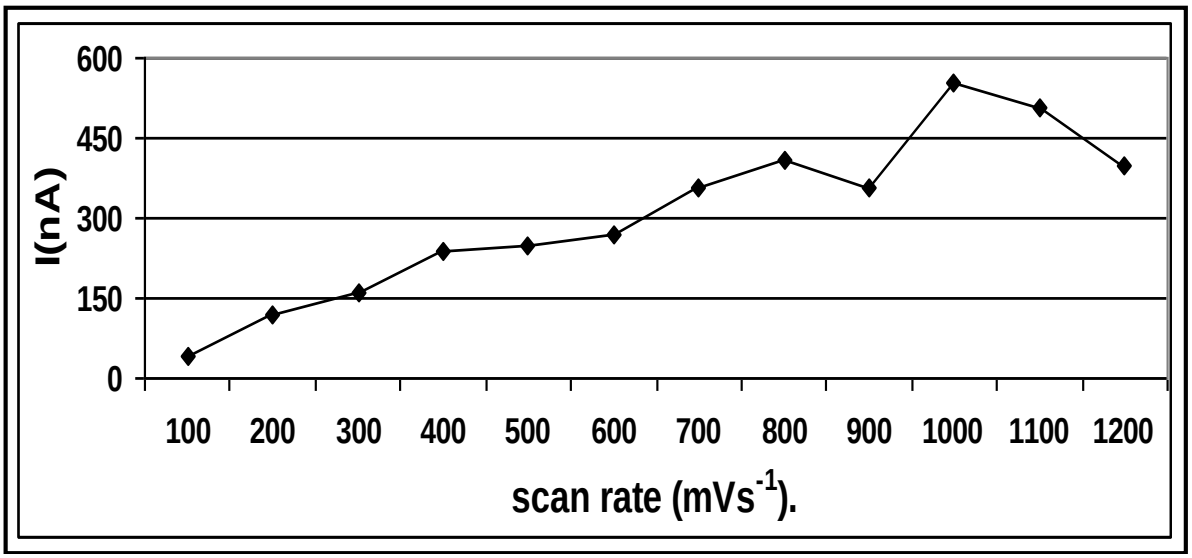


Figure 8: Effect of scan rate on the peak current of erythrosine b at carbonate buffer pH 8.5, T_{acc}= 150 s, E_{acc}= 0.0 V and dye concentration 1×10^{-7} mol l^{-1}.

Furthermore, the effect of changing the pulse amplitude on the square-wave voltammetric current was also evaluated. The peak current of erythrosine almost increased linearly with pulse amplitude over the range 50-100 mV (from studied range 20-150 mV) and at higher values than 100 mV, the dye peak current reached a leveling off stage. Accordingly, 100 mV pulse amplitude valve was adopted as optimum value. In order to estimate the influence of square wave frequency on SW-AdSV peak current, the value of this parameter was varied over the 20-90 Hz range. An initial proportional relationship was observed up to 30 Hz and sharply declined afterward. Hence, for further studies a 30 Hz frequency was chosen.

3.2.5 Effect of instrumental parameters.

The observed electrochemical signal can be further enhanced by optimizing other instrumental factors that can influence the adsorption accumulation process of erythrosine. The enlarge of the surface size of the working electrode over the range 0.15-0.60 mm^2 yielded, as expected, a linear enhancement in the analytical signal and did not affect the value of the stripping voltammetric potential. an enhancement in the stripping voltammetric peak current. Hence, this mercury drop size (0.60 mm^2) was considered as optimum valve. Similarly, the adsorptive stripping peak current can be maximized by selecting faster stirring rate, yet, to reduce any possible competitive adsorption from other surface-active interferences, a moderate 2000 rpm stirring speed was chosen as optimum value.

3.3 Quantitative Utility.

3.3.1 Calibration graph.

Under the optimum experimental conditions a good linear correlation was obtained between erythrosine electrochemical response and its concentration in the range 1- 2.5×10^{-7} mol l^{-1} (see fig.9). The parameters of the dye concentration-current straight line were calculated by the least-squares method. The regression equation of the calibration line has the form:

i_p (nA) = 1143.5 + 7.32×10^{9} C (mol l^{-1}) r = 0.99 n = 4

where i_p is the SW-AdSV peak current, C is the erythrosine b concentration and r is the correlation coefficient.

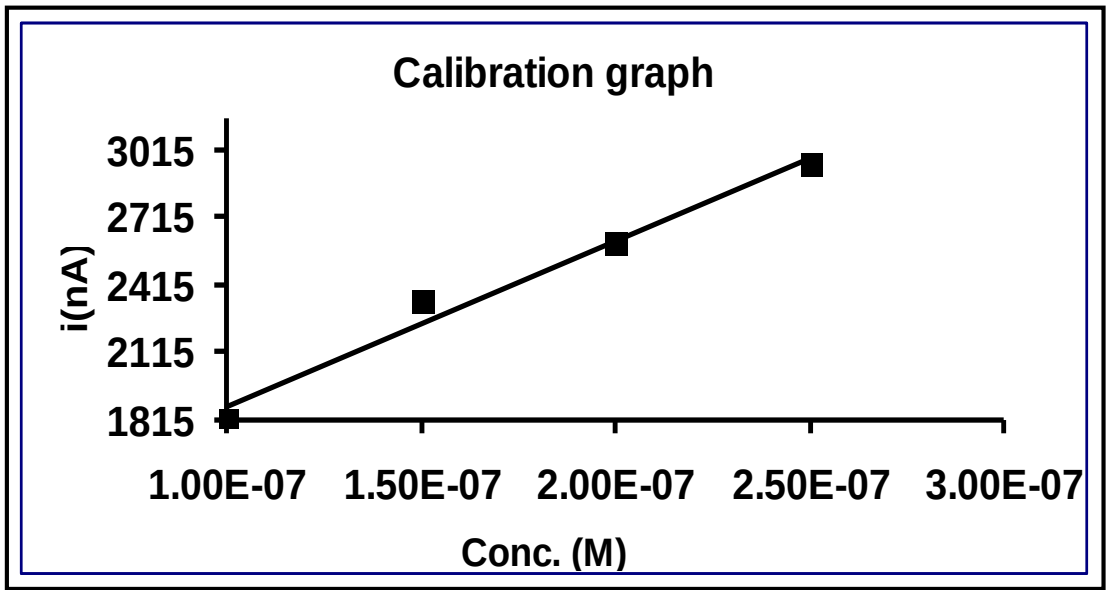


Figure 9: Calibration graph of erythrosine b at carbonate buffer pH 8.5, T_{acc}= 150 s, E_{acc}= 0.0 V and scan rate = 1000 mV/s.

3.3.2 Detection Limit.

The detection limit, defined as three times the signal-to-noise ratio (S/N =3) reached in the optimum conditions for monitoring this dye was 6.96 $\times 10^{-10}$ mol l^{-1}. Such remarkable sensitivity illustrates the preference of this electrochemical technique over the conventional analytical techniques. Obviously, the applied stripping voltammetric approach enhanced the sensitivity by 2-3 orders of magnitude in contrast to the cited analytical methods.

3.3.3 Reproducibility.

The analytical precision of the developed method was verified from the reproducibility of 10 determinations of 1×10^{-7} mol l^{-1} erythrosine in carbonate buffer at pH 8.5. A relative standard deviation (RSD) of 1.7% was calculated, which indicates reproducible accumulation and monitoring of the studied food additive dye.

3.3.4 Recovery.

The recovery of the developed procedure, which reflects the accuracy of the method, was evaluated by analyzing spiked buffer solution containing 1×10^{-7} mol l^{-1} erythrosine via the optimized SW-AdSV procedure. The mean recovery of five measurements was found to be 101.8%±1.79.

3.3.5 Stability.

The stability of 1×10^{-7} mol l^{-1} erythrosine solution was investigated by monitoring the SW-AdSV signal at the optimum analytical conditions every twenty minutes and the measured electrochemical response seemed to be stable for a period of two hours at least.

3.4 Interferences.

The effects of some possible interfering substances usually present as ingredients or food additives such as sweetness, preservers and coloring agents, on the adsorptive voltammetric determination of erythrosine were also investigated. The interferences by diverse materials were evaluated by adding appropriate amounts of these substance solutions to 20 ml of carbonate buffer containing 1×10^{-7} mol l^{-1} erythrosine. Considerable interference impact can be caused by other co-existing food additive azo dyes, hence, Sunset Yellow (E110) and Tartrazine (E102) azo dyes and another dyes (such as carmine, allura red and amaranth) were selected to modulate the competitive present of artificial coloring matters. Indeed, the presence of 1×10^{-7} mol l^{-1} tartrazine azo dye caused the adsorptive voltammetric response to decrease by 40% probably due to the competitive co-adsorption of this interfering dye on the adsorption sites of HMDE. In comparison, the addition of 5×10^{-7} mol l^{-1} amaranth caused the voltammetric signal to enhance by 30%. While, another dyes caused no significant effects on the SW-AdSV response of erythrosine b.

Many commercial food products usually contained single food additive dye, however, if binary mixture of colorant matters were existed and there analytical signals were overlapped, this interference limitation can be overcame by applying the multi-verities calibration approach [24].

Moreover, additions of increasing concentration of the natural sweeteners (sucrose or glucose) and artificial sweetener (aspartame) yield no effect on the SW-AdSV peak of erythrosine b. In addition, the possible interfering influence of citric acid and sodium benzoate (food additive preserve) was also studied. The voltammetric peak height was decreased by 15% after the addition of $5x10^{-6}$ mol/l of citric acid. Sodium benzoate no effect on the current peak of erythrosine b dye. Finally, slight peak height reduction was observed at all additions of the ascorbic acid antioxidant.

3.5 Practical Applications.

The proposed SW-AdSV method has been applied to the determination of erythrosine b in spiked commercial drinks (7 up and Miranda) and ice cream. In order to avoid matrix effect, the standard additions approach was used for analyzing the spiked erythrosine b at all concentrations ($1x10^{-4}$M with 7 up or $9.7x10^{-6}$M with Miranda and ice cream) after appropriate sample dilution. The results corresponding to four SW-AdSV determinations are summarized in Table 1. As can be noticed from this table, the analytical results obtained by this recovery test for erythrosine have a recovery mean of 100.3% with a standard deviation of ±0.25% for ice cream. In addition, the applied SW-AdSV method was also used to the recovery of erythrosine spiked in commercial soft drinks (7 UP and Miranda). The results obtained by this recovery tests for erythrosine have a recovery mean of 104.25% with a standard deviation of ±4.71% for 7 up and 99.47% ±1.83% for Miranda.

Table 1: comparative determination of erythrosine b in spiked soft drinks and ice cream by the proposed SW-AdSV method.

	Ice cream	**Miranda**	**7 up**
	% Recovery	**% Recovery**	**% Recovery**
Spiked Sample	100.2	102.06	111
	100	99.38	100
	100.7	98.55	103
	100.3	97.9	103
Mean	100.3	99.47	104.25
Standard Deviation	±0.25	±1.83	±4.71

Conclusion

The proposed SW-AdSV method developed for the determination of erythrosine b (E127) food additive dye (and clinical dye) was found to be simple, sensitive (DL ≈ $6.96x10^{-10}$ mol/l) and rapid. It also offer several advantages in terms of low cost, good precision (1.7 RSD%) and adequate accuracy (Recovery 101.8%).

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgement

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