

A Review on the Simultaneous Electrochemical Determination of Azo Dyes Used as Colorants in Foods and Beverages

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Received (Geliş Tarihi): 28.06.2025 , Accepted (Kabul Tarihi): 10.09.2025

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ABSTRACT

Synthetic azo dyes such as sunset yellow, tartrazine, allura red, amaranth, and azorubine have been widely used in foods and beverages as coloring agents. These colorants are frequently preferred in the industry due to their attractive bright colors, low cost, easy availability, and outstanding storage stability against pH and light. In addition to these advantages, synthetic dyes are known to have adverse effects on human health, including carcinogenicity, asthma, allergies, anxiety, and hyperactivity, especially in children. Organizations such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the EU Scientific Committee on Food (SCF) have determined that the acceptable daily intake of these colorants must not exceed 7.5 mg per kg of body weight. Therefore, the quantitative determination of synthetic dyes in foods and beverages is crucial, and their levels must be strictly controlled in foods. Because these azo dyes are electroactive, numerous studies have been conducted recently on their single or simultaneous electrochemical determination, and review articles have generally been published on the determination of single dyes. This review presents recent electrochemical studies on the highly sensitive and selective simultaneous determination of these colorants using voltammetric methods on various modified electrodes. The study discusses the general properties of azo dyes used as colorants; their electrochemical behavior and oxidation/reduction reaction mechanisms; the electrodes used and their modifications; and finally, their simultaneous voltammetric determination.

Keywords: Azo dyes, Colorants, Voltammetric determination, Modified electrode, Food and beverages

Gıda ve İçeceklerde Renklendirici Olarak Kullanılan Azo Boyalarının Eş Zamanlı Elektrokimyasal Tayini Üzerine Bir Derleme

ÖZ

Gün batımı sarısı, tartrazin, allura kırmızısı, amarant ve azorubin gibi sentetik azo boya ları, gıda ve içeceklerde renklendirici olarak yaygın şekilde kullanılmaktadır. Bu renklendiriciler çekici parlak renkleri, düşük maliyetleri, kolay bulunabilirlikleri, pH ve ışığa karşı üstü depolama kararlılıkları nedeniyle gıda ve içecek endüstrisinde sıkça tercih edilmektedir. Bu avantajlarına rağmen, sentetik boya ların özellikle çocuklarda kanserojenlik, astım, alerji, anksiyete ve hiperaktivite dahil olmak üzere insan sağlığı üzerinde olumsuz etkilerinin olduğu bilinmektedir. Gıda Katkı Maddeleri Ortak FAO/WHO Uzman Komitesi (JECFA) ve AB Gıda Bilimsel Komitesi (SCF) gibi kuruluşlar, bu renklendiricilerin kabul edilebilir günlük alım düzeyinin 7,5 mg/kg vücut ağırlığı değerini aşmaması gerektiğini bildirmiştir. Bu nedenle, gıda ve içeceklerdeki bu sentetik boya ların belirlenmesi çok önemlidir ve düzeyleri sıkı bir şekilde kontrol edilmelidir. Bu azo boya ları elektroaktif olduklarından, son yıllarda tek veya eş zamanlı elektrokimyasal tayinleri üzerine çok sayıda çalışma yürütülmüş ve genellikle tek boya ların tayini üzerine derleme makaleler yayınlanmıştır. Bu derleme, çeşitli modifiye elektrotlarda voltammetrik yöntemler kullanılarak bu renklendiricilerin oldukça hassas ve seçici eş zamanlı tayini üzerine son zamanlarda yapılan elektrokimyasal çalışmaları sunmaktadır. Bu çalışmada, renklendirici olarak kullanılan

azo boyalarının genel özellikleri; elektrokimyasal davranışları ve yükseltgenme/indirgenme reaksiyon mekanizmaları; kullanılan elektrotlar ve modifikasyonlar ve son olarak eş zamanlı voltametrik tayinleri ele alınmıştır.

Anahtar Kelimeler: Azo boyar maddeler, Renklendiriciler, Voltametrik tayin, Modifiye Elektrot, Gıda ve içecekler

INTRODUCTION

Food dyes are additives used primarily to color food and beverages during production. They are also used to recolor foods and beverages that have lost their color during packaging, storage, and transportation after production [1]. Food dyes also enhance consumer visual appeal by providing bright colors to foods. Moreover, because the human brain positively associates colorful products with flavors, food colors play a significant role in product purchases by influencing people's perceptions of sweets and beverages [1]. Today, there is virtually no food or beverage sector that does not use food dyes. Colorants are added to otherwise colorless beverages or foods (e.g., jelly, candies, bubble tea, ice cream, etc.) to make them more attractive and appetizing, thus increasing the product's appeal to consumers.

Dyes used as food additives are classified into two main categories: natural and synthetic. Natural dyes such as betaine, chlorophyll, flavonoids, β -carotenes, and anthocyanins are found mostly in vegetables and fruits (grapes, red beets, blackberries, red and black carrots, saffron, algae, leaves, etc.) and can be isolated from these products [2-3]. Besides plant sources, natural dyes such as carmine are also derived from animal sources [3]. The use of natural dyes as food additives is limited due to their disadvantages, such as their instability to temperature, light, and pH; their easy loss or degradation of color during processing; and the laborious isolation procedures that result in increased costs [1-4]. Artificial food dyes, developed as alternatives to natural dyes, are widely used in the food and beverage industry due to their relatively low cost; high resistance to air, heat, light, and pH; bright and attractive colors; resistance to degradation during production, packaging, storage, and transportation; ease of access; and high color stability [2-5].

The most important group of artificially synthesized dyes for use in food and beverages is azo dyes with the general chemical structure $R^1-N=N-R^2$. The azo group ($-N=N-$) is more likely a chromophore group and is reducible. Furthermore, the R^1 and R^2 groups are primarily composed of benzene or naphthalene rings, which contain sulfonate groups as well as oxidizable $-OH$ groups [2, 3]. The sodium sulfonate group attached to the aromatic structure increases water solubility, while the $-OH$ groups attached to the aromatic ring exhibit auxochrome properties [6]. The color of azo dyes is determined by the chromophore and auxochrome, along with the azo bonds. Yellow dyes such as Tartrazine (Trz) and Sunset Yellow (Ssy), and red dyes such as Allura Red (Alr), Amaranth (Amr), and Azorubine (Azr)/Carmoisine (Cms), which are named based on the different structures in the R^1 and R^2 groups, are among the most used azo dyes.

Although azo dyes provide a more appealing flavor to foods and beverages due to their bright colors and taste, numerous studies have shown that these dyes have toxicological effects on the kidney, brain, lungs, liver, urinary system, and thyroid gland [7-9]. The widespread human consumption of azo dye-containing foods and beverages, their toxicity, and the numerous reported adverse effects make the situation even more alarming regarding their potential risks. In addition to health concerns such as carcinogenicity, cytotoxicity, and mutagenicity, these dyes have also been reported to cause neurological disorders, diarrhea, urticaria, angioedema, allergies, asthma, vomiting, gastric annoyance, reproductive system problems, and behavioral disorders [2, 7-10]. It is also a fact that children consume excessive amounts of these dye-containing beverages and foods. Therefore, studies on children have linked ingestion of these azo dyes to behavioral disorders (hyperactivity, attention deficit, etc.), raising awareness about the use of these dyes [4, 11]. Organizations such as the United States Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) regulate the authorization of azo dyes, ensuring people consume safer food and beverages [12, 13]. EFSA has established acceptable daily intakes (ADIs) for Ssy (E 110), Trz (E 102), Alr (E 129), Amr (E 123), and Azr/Cms (E 122) as 4.0, 7.5, 7.0, 0.15, and 4.0 mg/kg body weight per day, respectively [14-18]. The ADI represents the amount of a substance (mg) that can be safely consumed daily per kg of body weight throughout a lifetime without causing any health problems, making it an important parameter to consider for our health [12, 19]. Furthermore, the letter "E" (for Europe) numbering system is another indicator used for these dyes, and designating a colorant with this code defines its safety under potential exposures. E and the three-digit numbers (International Numbering System) next to it not only define the risk of the substance but also provide information that will facilitate the reduction and control of the risk [19].

As mentioned in previous sections, azo dyes in food and beverages require rigorous control due to their widespread use and toxic effects. Numerous analytical methods have been developed to determine these substances, and research in this area continues apace. Chromatographic and electrophoretic methods, which provide adequate sensitivity (changed based on the detector used) and highly selective analysis, are widely used in this field [20, 21]. For example, azo dyes have been selectively and simultaneously determined using high-performance liquid chromatography with UV (diode array detector) (HPLC-DAD) [22, 23], reverse phase-HPLC (RP-HPLC) [24], high-performance thin-layer chromatography (HPTLC) [25], ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) [26], and liquid chromatography/mass spectrometry (LC/MS) [27] methods. However, chromatographic devices have

some drawbacks, such as their high cost, bulkiness, and weight; the need for specialized columns specific to the analytes; the expensive auxiliary apparatus; time-consuming sample preparation procedures; and the need for well-trained personnel [2, 12, 28].

Molecular spectroscopic methods using fluorometric, spectrophotometric, and colorimetric techniques have also been developed for the analysis of azo dyes [29-31]. Despite their advantages, including simplicity, cost-effectiveness, and ease of determination, the application of spectrophotometric and colorimetric methods is limited by relatively low sensitivity compared to other techniques. Moreover, the spectral interference caused by coexisting azo dyes, narrow linear ranges, and the necessity of lengthy extraction methods to separate analytes in samples have also been reported as other drawbacks [32]. Furthermore, such substances have been determined in foods and beverages using expensive and time-consuming methods such as enzyme-linked immunosorbent assay (ELISA) [33] and surface-enhanced Raman scattering (SERS) [34].

Developing simple, rapid, low-cost, highly sensitive, and selective alternatives to the chromatographic and spectroscopic methods is of great importance, and developing new methods has always been a topic of interest for analytical chemists. Electrochemical methods have become an important alternative for the determination of azo dyes due to their electroactive groups. Recently, the number of studies on azo dye determinations based on electroanalytical methods has significantly outpaced both chromatographic and spectrophotometric methods. The widespread use of electroanalytical methods is attributed to their advantages. For example, electrochemical devices are relatively lower cost and simpler than chromatographic methods, provide quicker response times than other methods, allow for electrode modification, enable highly selective and sensitive analyses, have a wide range of applications, and are more amenable to miniaturization than other techniques [2, 12, 28]. In addition, remarkable enhancement in the sensitivity and selectivity in the electrochemical analyses has been achieved by using electrodes modified with a wide variety of nanomaterials or materials, such as metal or metal oxide nanoparticles, graphene oxide, carbon nanotubes, metal-organic frameworks, quantum dots, and conductive polymers [35-40]. Due to these superior properties, the analysis of artificial dyes in foods, beverages, cosmetics, and pharmaceuticals based on electrochemical methods has attracted the attention of many researchers, and numerous studies have been conducted in this area [40-45]. A few review articles have been published by Vladislavić et al. [2], the Ozkan research group [12], and Georgescu State et al. [3] on the electrochemical determination of azo dyes, particularly sunset yellow and tartrazine, in foods and beverages. Following the publication of these articles, researchers developed numerous electrochemical methods using various types of modified electrodes. This review summarizes the simultaneous determination of azo dyes, considering recent studies (last decade), unlike previously published reviews.

GENERAL PROPERTIES of AZO DYES USED AS COLORANTS in FOODS and BEVERAGES

Numerous studies have been conducted on the simultaneous voltammetric determination of the azo dyes Ssy, Trz and Alr in their binary and ternary dye mixtures. Therefore, the chemical structure and toxicity of these three azo dyes are discussed in this section, and their electrochemical behavior and mechanisms will be discussed in the following section.

Sunset Yellow

Ssy (E110) one of the most widely used azo dyes, is also known as 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalenesulfonate ($C_{16}H_{10}N_2Na_2O_7S_2$, MW: 452.38 g/mol) (Figure 1a) and is produced by the reaction of diazotized 4-aminobenzene sulfonic acid and 6-hydroxy-2-naphthalene-sulphonic acid [46]. Ssy with an orange-red color tone has become one of the indispensable additives of foods and beverages because it has some advantages, such as outstanding stability against light, heat, and acidity; water solubility; and a reasonable price [47]. Besides food and beverages, it is also used in cosmetic products such as perfumes, colognes, shampoos, lotions, and creams, and in the pharmaceutical industry as a coating or ingredient in pharmaceutical tablets, ointments, and capsules [47, 48]. In this context, the necessary regulations have been established regarding Ssy. For example, the maximum permissible level of Ssy in foods and beverages is set by the Codex Alimentarius International Food Standard at between 50 and 400 mg/kg (e.g., 100 mg/kg for water-based flavored beverages and 300 mg/kg for confectionery, sauces, and dairy products) [47, 48]. Furthermore, as mentioned earlier, the EFSA has set the ADI for Ssy at 4.0 mg/kg/body weight/day [14]. Although Ssy consumed within established limits is considered safe, excessive consumption of foods and beverages containing high concentrations of Ssy or prolonged exposure to Ssy can lead to serious health problems. For example, potential health problems include attention deficit hyperactivity disorder in children, asthma, allergies, and possible xenoestrogenic, genotoxic, immunotoxic, and carcinogenic effects [12, 49]. Considering all this information, conscious consumption of Ssy is important.

Tartrazine

Another frequently used azo dye is Trz (E 102), also known as 3-carboxy-5-hydroxy-1-(4'-sulfonatophenyl)-4-(4'-sulfonatophenylazo)-H-pyrazole-3-carboxylate ($C_{16}H_9N_4Na_3O_9S_2$, MW: 534.36 g/mol) (Figure 1b). Trz can be synthesized in two steps: the first is the formation of an intermediate product by the reaction of phenylhydrazine-p-sulfonic acid with oxalacetic diethyl, and the second step is the reaction of the intermediate product with diazotized sulfanilic acid and then hydrolyzing with NaOH [50]. Trz, which provides a lemon-yellow color, has been frequently preferred in food and beverages due to its high solubility in water (20.0 g/100 mL at 25°C), low cost, and high stability. Trz is also used in fruit juices, candies, jellies, cakes, and soups, as well

as in the pharmaceutical, drug, and cosmetic sectors [50]. The EFSA has approved an ADI value of 7.5 mg/kg body weight/day for Trz [15], and the maximum concentration in alcoholic and non-alcoholic beverages is 200 and 100 mg/L, respectively. Depending on the food product, Trz can be used at levels ranging from 50 to 500 mg/kg [50]. Studies on the toxicity of Trz indicate that it negatively impacts our health by damaging some of our organs. Trz can be converted into aromatic and nitrous agents in the intestinal flora, leading to the release of reactive oxygen species (ROS), ultimately leading to numerous ROS-based health problems [51]. It has also been reported to cause asthma, allergies, urticaria, rashes, migraines, hyperactivity disorder in children, cytotoxic and xenoestrogenic effects, and neurobehavioral and skin problems [12, 50].

Allura Red

Allura Red (E 129), also known as disodium 2-hydroxy-1-(2-methoxy-5-methyl-4-sulfonato-phenylazo) naphthalene-6-sulfonate ($C_{18}H_{14}N_2Na_2O_8S_2$, MW: 496.42 g/mol) (Figure 1c), is synthesized by the reaction of diazotized 5-amino-4-methoxy-2-toluenesulfonic acid with 6-hydroxy-2-naphthalene sulfonic acid [52]. Alr is a red-orange dye, and its application areas range from dark red to brown. Easily soluble in water, Alr has a wide range of uses in candies, jams, jellies, fruit juices and beverages, sauces, chips, sausages, seafood, and meat products for the aim of coloring, coating, and decoration [53]. It is also frequently used in medications such as painkillers, vitamin tablets, and syrups. EFSA has approved an ADI of 7.0 mg/kg body weight/day for Alr [15], and the maximum concentration is 50 mg/L for soft drinks, while up to 500 mg/kg is permitted in coatings, decorations, and sauces [53]. As with the other two azo dyes (Ssy and Trz), studies on the toxicity of Alr have been conducted, and adverse effects such as liver and kidney toxicity, neurotoxicity, DNA damage, and carcinogenesis have been reported [53].

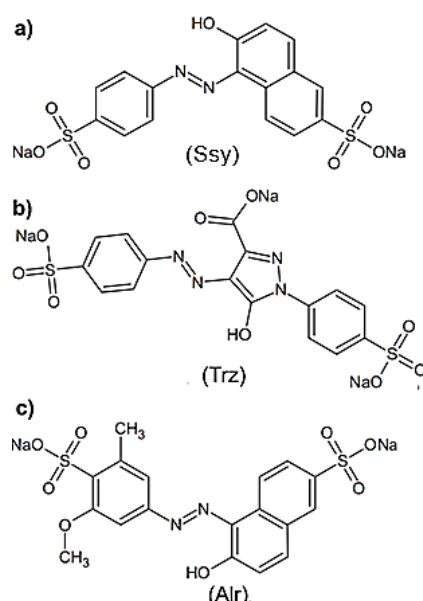


Figure 1. The chemical structure of Ssy (a), Trz (b) and Alr (c)

ELECTROCHEMICAL BEHAVIOR of AZO DYES

As seen in Figure 1, three different azo dyes contain two electroactive groups in common: a reducible azo group ($-N=N-$) and an oxidizable aromatic hydroxy group ($-OH$). Studies have been conducted on the oxidation of the $-OH$ group, as it is generally more stable and resilient in the electrochemical determination of these substances. A comprehensive study of the electrochemical behavior and mechanism of azo dyes was conducted by Durigon and colleagues [54]. Ssy was chosen as the model in this study because it is the most widely used azo dye in the world.

To summarize the electrochemical behavior of azo dyes using Ssy, its aromatic hydroquinone group first converts to a phenoxy radical by losing one H^+ and one e^- and then rapidly oxidizes to a quinone group [2, 54]. Cyclic voltammetric studies indicate that an irreversible oxidation peak is observed at positive potential values varying between +0.7 and +1.2 V, depending on the type of reference and working electrodes (especially the performance of the modified electrode) used and the pH of the supporting electrolyte. It can appear as a distinct single peak or a pair of closely spaced peaks, as noted by Durigon et al. [54]. The shape of the peak generally depends on the electrode type and working conditions. This difference is explained by the sequential or simultaneous occurrence of proton loss and electron transfer during quinone formation between benzenic carbon and oxygen [54]. An illustration of this mechanism is given in Figure 2. Figure 3 shows the cyclic voltammogram of 5.0×10^{-4} M Ssy (a), Trz (b), and Alr (c) in a pH 2.0 Britton Robinson buffer solution at a pencil graphite electrode. A peak in this CV, designated Ox_1 , is attributed to the irreversible oxidation of Ssy, Trz, and Alr, as discussed in the mechanism. The same mechanism has been reported in numerous studies, as well as in the review written by Vladislavić et al. [2]. While Trz is oxidized at approximately 1.08 V and separates from both Ssy and Alr, the oxidation peaks of Ssy and Alr are observed at the same potential (at 0.82 V). This phenomenon means that while Ssy and Trz or Trz and Alr can be quantified simultaneously, simultaneous determination of Ssy and Alr is quite difficult because they are oxidized at the same potential.

In the cathodic scan of the CV recorded in Figure 3, a reversible reduction of Ssy and Alr is observed at approximately +220 mV, and an irreversible peak is observed at -90 mV. On the other hand, the irreversible reduction peak of Trz (Red_2) was observed at -310 mV, while the reversible reduction peak (Red_1) was not observed for Trz (Figure 3b). The first peak, designated Red_1 , is attributed to the reduction of the azobenzene group to hydrazobenzene by the oxidized quinone form (Ox_1) by accepting two e^- and two H^+ [2, 54]. It is reported that the carbonyl in the ortho position is an electron-withdrawing group, thus reducing the electron density in the ($-N=N-$) group and promoting the easy cleavage of the double bond [54].

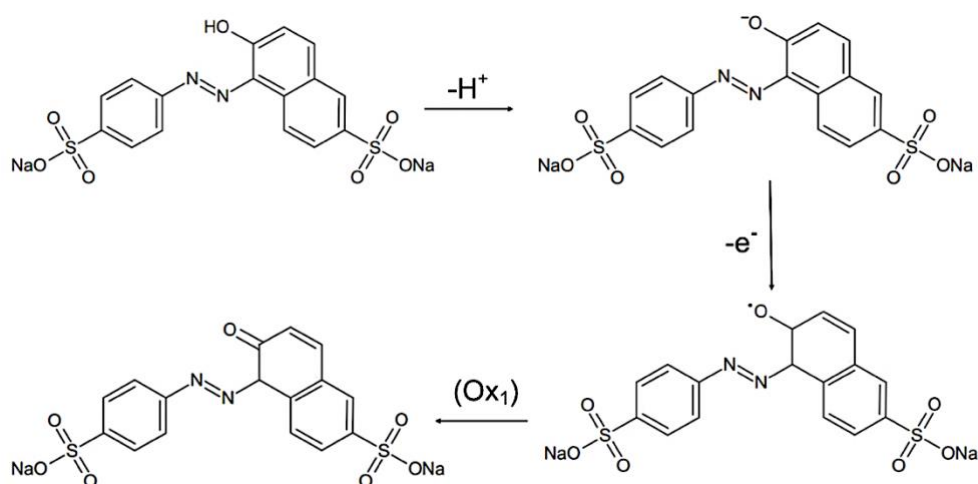


Figure 2. The oxidation mechanism of aromatic hydroxy group in the Ssy structure (Modified from [54])

The second reduction peak, observed irreversibly at a highly negative potential and designated Red2 in the CV (Figure

3), is attributed to the cleavage of the nitrogen bond in hydrazobenzene, accepting two e^- and two H^+ .

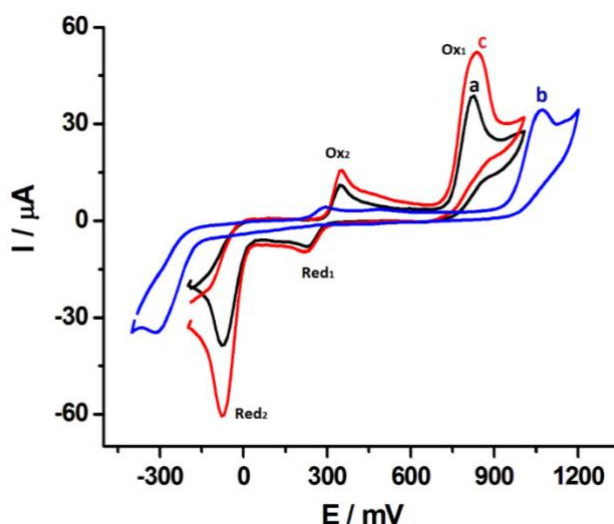


Figure 3. Cyclic voltammograms of 5.0×10^{-4} M Ssy (a), Trz (b) and Alr (c) in pH 2.0 BRB solution in PGE at 50 mv/s. (This figure is obtained from our laboratory results and has not been published anywhere.)

One of the two aromatic amine groups formed after this reduction step contains an aromatic hydroxyl group as well. Therefore, when the anodic cycle occurs again, a second oxidation peak, identified as Ox₂, is observed at +0.35 V for Ssy and Alr, while Trz is oxidized at +0.29 V. This oxidation, like the first oxidation, has been attributed to the formation of a phenoxy radical with one e^- and one H^+ , followed by its conversion to quinone. If we express the reduction step as a total reaction, Ssy in the oxidized form formed by the first oxidation was reduced by accepting a total of four e^- and four H^+ . As a result, two different amines were formed by cleaving both the double and single bonds in the azobenzene group. The mechanism for both the reductions and the second oxidation is presented in Figure 4. It has been reported that apart from oxidation of the aromatic hydroxyl group in one of the amines, oxidation can also occur via the amino groups of both amines.

Alr is structurally more similar to Ssy than to Trz, and it is expected that Alr will exhibit a very similar behavior to the oxidation/reduction mechanisms proposed for Ssy. The electrochemical determination of Alr was carried out at a glassy carbon electrode (GCE) modified with a composite material obtained from poly(diallyldimethylammonium chloride)-functionalized graphene and nickel nanoparticles [55]. It was noted that the modified electrode gave a reversible oxidation peak at approximately 900 mV vs. SCE at pH 3.0, and that this peak was due to the oxidation of the aromatic -OH group. The same mechanism shown in Figure 3 was also proposed for Alr.

In a reduction study, differential pulse voltammetric determinations of the Alr together with three different azo dyes (Amr, Azr/Cms, and ponceau 4R) were performed in 0.10 M NaCl media using Bi-film-modified GCE [56]. Irreversible reduction peaks were observed for each azo

dye between approximately -400 and -500 mV. This study also indicated that the (-N=N-) group in these azo dyes was reduced to the (-HN-NH-) group by $2e^-$ and $2H^+$. Although Trz is structurally slightly different from Ssy and Alr, its electrochemical determination was based on a similar oxidation/reduction mechanism. The hydroxyl group attached to the pyrazole ring in Trz is oxidized similarly to the aromatic -OH group in Ssy but is observed at a positive value 200–300 mV higher than that of Ssy. This small difference provides a significant advantage for the simultaneous determination of both dyes. For example, Ssy and Trz were determined simultaneously on a carbon paper electrode modified with graphene powder, and an oxidation mechanism very similar to that described for Ssy was proposed [57].

In a study on Trz, the reduction of Trz was investigated using direct current voltammetry using GCE [58]. It was

shown that the electrochemical reduction signal of tartrazine was most pronounced in an acidic environment at pH 2.0. As with Ssy, the reduction occurs in two steps: to the hydrazo compound and then to the aromatic amines, and the activity of these two reductions was assessed to be influenced by the ambient pH and substituents. It was noted that in the presence of electron-donating groups such as hydroxy and amino, cleavage of the azo bond is more effective, resulting in reduction to the main. Conversely, in the absence of electron-withdrawing groups, azo compounds are reduced to hydrazo compounds. Therefore, in Trz, it was reported that the highly acidic pH, combined with the electron-donating OH in the para-position relative to the azo bond, causes the (-N=N-) bond to break, resulting in a more efficient reduction to two different aromatic amines [58]. Figure 5 depicts both the oxidation and the total reduction mechanism of Trz.

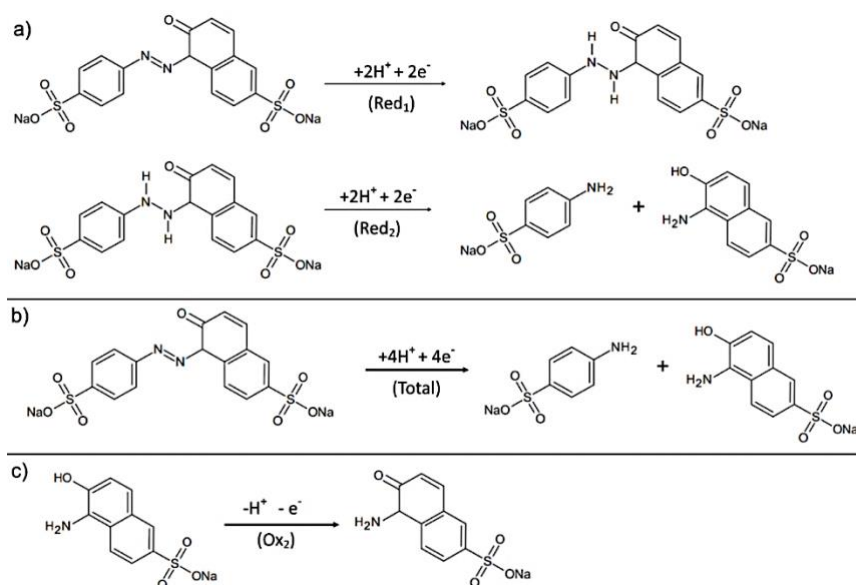


Figure 4. The mechanisms for reduction of Ssy (a), and total reduction reaction (b) and second oxidation of Ssy structure (c) (Modified from [54])

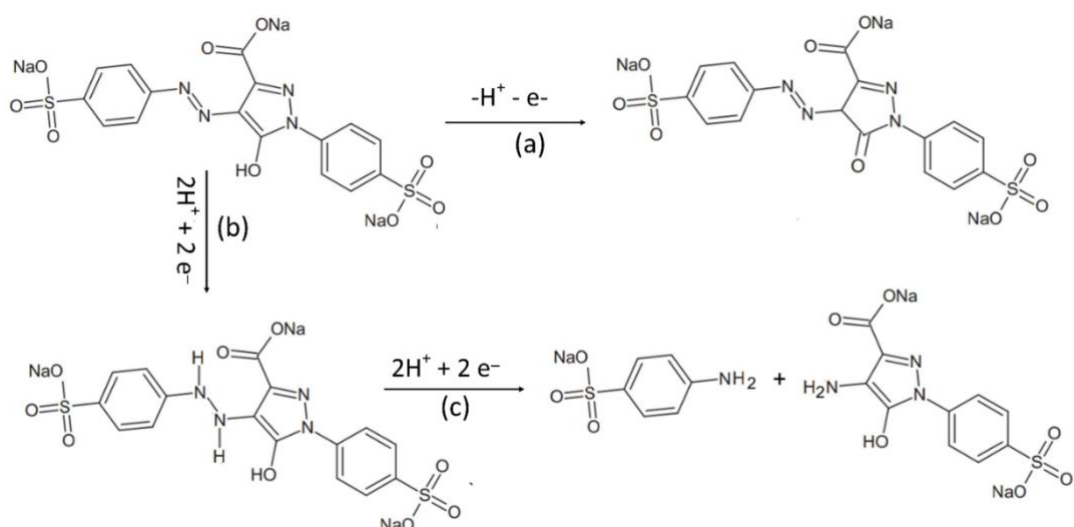


Figure 5. The mechanisms for oxidation (a) and reduction (b and c) of Trz (Modified from [57, 58])

Simultaneous Voltammetric Determination of Azo Dyes

Researchers have developed numerous methods based on electrochemical techniques, and they report numerous publications annually in the sensor and biosensor fields. As previously mentioned, the increasing interest in electrochemical sensors and biosensors stems from the advantages that electrochemical methods offer over other methods, such as chromatography and spectroscopy. These advantages include low cost, instrumental simplicity, portability, straightforward miniaturization and automation, ease of implementation, and short response times [2, 12]. Furthermore, the preparation of modified electrodes with a wide range of materials, such as metal nanoparticles, metal oxides, carbon-based nanomaterials (graphene, carbon nanotubes), polymers, redox mediators, metal-organic frameworks, quantum dots, ionic liquids, or composite structures, provides a wide range of applications in the field of electrochemical sensors and biosensors [59,60]. Modified electrodes prepared with such materials provide for more sensitive and selective analysis of low-level analytes in samples containing complex matrices, significantly improving the analytical performance of electrochemical sensors and biosensors developed for azo dyes, as well as for all other analytes [12, 59, 60].

Different electrochemical techniques can be used in electrochemical analysis, depending on the targeted analyte and sample matrix. For example, potentiometric sensors, based on the measurement of the potential difference between the analyte-selective working electrode and the reference electrode at a constant current; amperometric sensors, where the current is measured at a constant potential in a three-electrode electrochemical cell; conductometric sensors, where electrical conductivity is measured; coulometric sensors, based on the measurement of electric charge; impedimetric sensors, based on the measurement of the electrical charge transfer resistance at the electrode surface; and voltammetric sensors, where the current is monitored versus the potential change over time in a three-electrode electrochemical cell, are used in electrochemical sensor and biosensor design [3, 12].

Among these methods, voltammetric techniques are frequently preferred for the determination of azo dyes and other toxic chemicals in food and beverages [61]. This benefit is due to their excellent efficiency, robustness, accuracy, and stability. Another advantage of voltammetric methods is that they are more sensitive and selective than some electrochemical techniques, allowing for the simultaneous determination of these substances in a coexisting environment, i.e., single-step detection [61]. Differential pulse voltammetry (DPV) and square wave voltammetry (SWV) have been frequently used in the electrochemical analysis of azo dyes due to their greater sensitivity compared to other voltammetric techniques. In addition to these techniques, cyclic voltammetry (CV) is also a very distinct voltammetric technique, particularly for mechanism elucidation. Linear sweep voltammetry (LSV) has also been used in studies

in conjunction with CV for the determination of azo dyes [3, 58, 62].

As mentioned in the previous section, azo dyes exhibit similar electrochemical behavior because they have the same electroactive groups. Therefore, the oxidation and reduction peaks obtained in the voltammetric responses of azo dyes may overlap, or a potential difference of 100-200 mV may be observed between the peaks in the presence of electron-withdrawing/donating groups in the structure. In foods and beverages, these substances can be used either individually or in combination to obtain different colors. These properties can lead to significant challenges, including interference in the electrochemical analysis of samples containing different azo dyes. To overcome these challenges, bare electrode surfaces have been modified with different materials, both to increase the resolution of closely spaced peaks and to enable simultaneous sensitive determination of these azo dyes. As with the electrochemical determination of azo dyes, improving the analytical performance of all other electrochemical sensors and biosensors is crucial.

In addition to the voltammetry techniques mentioned above, modified electrodes and method improvements have contributed significantly to improving analytical performance. One of the most significant advantages of electrochemical analysis is that these improvements allow for the simultaneous determination of analytes with similar properties. Thus, numerous studies have been conducted on the simultaneous determination of azo dyes using various modified electrodes and voltammetric methods. Due to the more widespread use of Ssy and Trz in the food industry, researchers have focused primarily on the simultaneous determination of these two azo dyes. Furthermore, studies on the simultaneous electrochemical determination of other dyes such as Alr, Amr, and Azr have also been conducted. In this context, simultaneous voltammetric sensors developed recently for binary or ternary mixtures of Ssy, Trz, and Alr, as well as their mixtures with other azo dyes, are presented in Table 1.

As seen in Table 1, the most used azo dyes in their simultaneous voltammetric determination are Ssy and Trz. This is an expected situation, as these two dyes are widely used in foods and beverages. Furthermore, simultaneous determinations were performed using the oxidation of the aromatic -OH group (Ox_1), rather than resulting from the reduction and oxidation of first reduction products. These results can be explained by the fact that the potentials observed for both the reduction of the (-N=N-) group to the (-NH-NH-) group (Red_1) and the reduction of the (-NH-NH-) group to amine groups (Red_2) for azo dyes are close to each other. Due to structural differences, the potentials of oxidation peaks observed for the aromatic -OH group (Ox_1) may differ. Furthermore, when looking at the oxidation potentials associated with the aromatic -OH group in the table, Trz is generally oxidized around +1.0 V, while Ssy is oxidized around +0.7 V. The potential difference of approximately 250-300 mV reflects the possibility of simultaneously determining these two substances without interfering with each other's oxidation peaks.

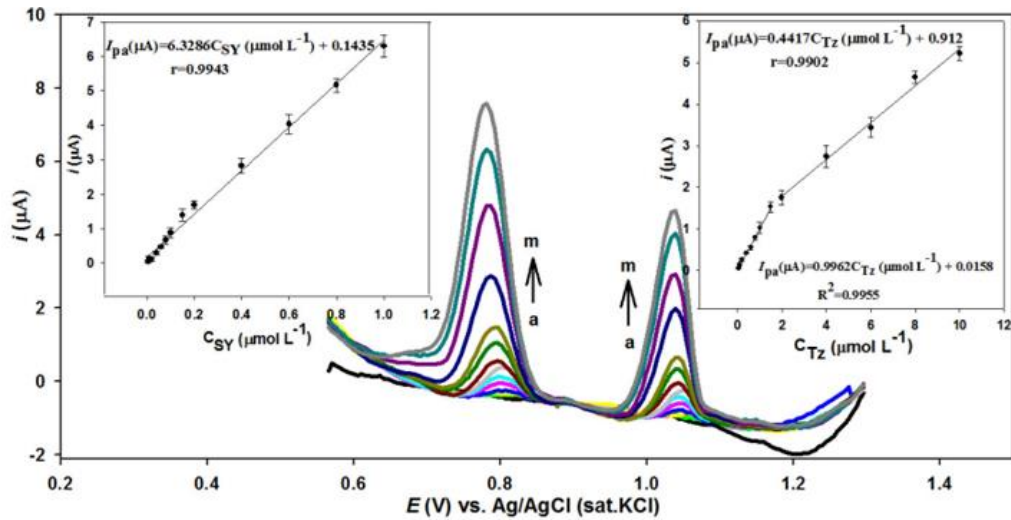


Figure 6. SWV of Ssy and Trz depending on the increasing concentrations (a–m: 0.006–1.0 μM for Ssy and 0.06–10.0 μM for Trz. Inset figures show related calibration curves. Reprinted from ref. (90) with permission from TÜBİTAK.

Table 1. Voltammetric studies reported on the simultaneous determination of azo dyes used as colorants in food and beverages on various types of modified electrodes

Azo dye	Tech.	Electrode	Oxidation Peaks	LR (μM)	LOD (μM)	Real sample application	Ref.
Ssy and Trz	DPV	GrP/CP	0.76 V ¹ and 1.1 V ² vs. Ag/AgCl at pH 5.0 PBs	0.005-1.0 ¹	0.00078 ¹	Alcoholic, non-alcoholic and carbonated drinks	[57]
		rGO/NiBTC/SPCEs	0.68 V ¹ and 0.92 V ² vs. Ag/AgCl at pH 8.0 PBs	0.02-7.5 ²	0.0082 ²	Orange and yellow sport drink	[63]
		sparkled Mo-SPEs	0.765 V ¹ and 1.03 V ² vs. Ag/AgCl at pH 5.0 AcBs	0.05-5.0 ¹	0.025 ¹	Orange and green sweetie; Cocktail Margarita	[64]
		ZnCrFeO ₄ /CPE	0.765 V ¹ and 1.03 V ² vs. Ag/AgCl at pH 5.0 AcBs	0.075-5.0 ²	0.050 ²	Fanta, Mirinda	[65]
		ePAD	0.71 V ¹ and 0.92 V ² vs. Ag/AgCl at pH 2.0 BRBs	0.005-0.25 ^{1,2}	0.002 ^{1,2}	Isotonic, juice powder, freeze pop and gelatin	[66]
		AuNPs/PDDA-Gr/GCE	0.68 V ¹ and 0.9 V ² vs. Ag/AgCl at pH 7.0 PBs	4.0-35 ^{1,2}	2.38 ¹	Three kinds of drinks	[67]
		Cu-BTC/COOH-MWCNTs/GCE	0.75 V ¹ and 0.95 V ² vs. Ag/AgCl at pH 7.0 PBs	40-60 ^{1,2}	2.09 ²	Fanta, Sprite beverages	[68]
		La ³⁺ /Ni _{0.75} Zn _{0.25} Fe ₂ O ₄ @rGO/CPE	0.76 V ¹ and 1.0 V ² vs. Ag/AgCl at pH 6.0 PBs	2-200 ^{1,2}	0.73 ¹	Orange and pineapple powder, mango juice	[69]
		rGO-BaMoO ₄ /GCE	0.70 V ¹ and 0.95 V ² vs. Ag/AgCl at pH 5.0 AcBs	1-140 ^{1,2}	0.0067 ¹	Four beverages	[70]
		MnO ₂ /MWCNT/GCE	pH 7.0 PBs	1.0-80 ^{1,2}	0.0048 ²	Carbonate drinks	[71]
		poly (4-ABA)/GCE	0.713 V ¹ and 0.945 V ² vs. Ag/AgCl at pH 4.8 PBs	0.06-10 ^{1,2}	0.005 ¹	Orange-flavored drinks.	[72]
		Fe ₃ O ₄ /CoFe@N-CSs/SPE	0.63 V ¹ and 0.89 V ² vs. Ag/AgCl at pH 6.0 PBs	0.010-0.75 ^{1,2}	0.0023 ¹	Minute maid, fanta, mirinda	[73]
		YFO/f-CNF/GCE	0.63 V ¹ and 0.88 V ² vs. Ag/AgCl at pH 7.0 PBs	0.75-5.0 ^{1,2}	0.0030 ²	Soft drinks, juices, cheese snacks, candies, jelly, ice cream, red wine, chips, cookies, Mirinda, jelly and candy	[74]
		CuS microflowers/GCE	0.82 V ¹ and 1.02 V ² vs. Ag/AgCl at pH 2.4 PBs	0.008-5.0 ¹	0.002 ¹	Powder Orange Juice, Green Soft Drink	[75]
		Fe ₂ Zr ₂ O ₇ /GCE	0.8 V ¹ and 1.0 V ² vs. Ag/AgCl at pH 2.0 BRBs	0.02-10.0 ²	0.004 ²	Orange and fruit juice, peach gelatin	[76]
		ZnO/Cysteic acid/GCE	0.76 V ¹ and 0.98 V ² vs. Ag/AgCl at pH 5.0 PBs	0.05-466.15 ^{1,2}	0.0015 ¹	Powdered orange juice, Orange juice beverage	[77]
		IL/CCE	0.77 V ¹ and 1.0 V ² vs. Ag/AgCl at pH 5.0 PBs	0.07-1.86 ²	0.0032 ²	Orange juice and soda, jelly	[78]
		ERGO-AuNRs/GCE	Oxidation at 0.68 V ¹ and 0.92 V ² vs. Ag/AgCl at pH 5.0 PBs	0.1-3 ¹	0.0024 ¹	Soft drinks	[79]
		Cu-BTC/CPE	0.62 V ¹ and 0.75 V ² vs. Ag/AgCl at pH 8.0 PBs	0.03-6.0 ²	0.0086 ²		[80]
		PDDAGr-Pd/GCE	0.88 V ¹ and 1.07 V ² vs. Ag/AgCl at pH 3.0 PBs	0.0003-0.050 ¹	5.0x10 ⁻⁵⁽¹⁾	Soft drinks	[81]
		COF/CNTs/SPCE	0.7 V ¹ and 0.9 V ² vs. Ag/AgCl at pH 6.0 PBs	0.001-0.1 ²	1.4x10 ⁻⁴⁽²⁾	Soda, health supplement, p opsicles	[82]

Table 1. Voltammetric studies reported on the simultaneous determination of azo dyes used as colorants in food and beverages on various types of modified electrodes (Continue)

Azo dye	Tech.	Electrode	Oxidation Peaks	LR (μM)	LOD (μM)	Real sample application	Ref.
Ssy and Trz	DPV	3DrGO–MoS ₂ /GCE)	0.79 V ¹ and 1.03 V ² vs. Ag/AgCl at pH 6.0 PBs	0.05-10 ¹ , 10-60 ¹ 0.1-6 ² , 6-60 ²	0.018 ¹ 0.037 ²	Drink sample	[83]
		In ³⁺ /NiO RLHNSs/GCE	0.771 V ¹ and 0.961 V ² vs. Ag/AgCl at pH 5.0 PBs	0.01-700.0 ^{1,2}	0.0027 ¹ 0.0031 ²	Peach and mango jelly, orange, strawberry and fruit juice	[84]
		ERGO-SPCE	0.41 V ¹ and 0.71 V ² vs. Ag/AgCl at pH 6.0 PBs	0.01-20 ¹ 0.02-20 ²	0.0005 ¹ 0.0045 ²	Beverages, soft drinks, juice and energy drinks and thirst quencher	[85]
		TX-100/MPE	0.682V ¹ and 0.937 V ² vs. Ag/AgCl at pH 7.4 PBs	1-6 ¹ 1-8 ²	0.17 ¹ 0.67 ²	-	[86]
Ssy and Trz	LSV	GO/MWCNTs/GCE	0.82 V ¹ and 1.05V ² vs. Ag/AgCl at pH 5.0 PBs	0.09-8.0 ^{1,2}	0.025 ¹ 0.01 ²	Orange Juice	[87]
Ssy and Trz	SWV	Fe ₃ O ₄ @SiO ₂ /MWCNT-CPE	-0.21 V ¹ and -0.31 V ² vs. Ag/AgCl at pH 5.0 PBs (reduction for Red ₁)	0.5–100 ^{1,2}	0.05 ¹ 0.04 ²	Powdered juice, Gelatin, Nutrient-enhanced sports drink	[88]
		La-TiOx/CPE	0.82 V ¹ and 1.1 V ² vs. Ag/AgCl at pH 2.0 PBs	-	0.02 ¹ 0.03 ²	synthetic orange juice, orange juice powder	[89]
		n-BA/G/PPAP/GCE	0.8 V ¹ and 1.04 V ² vs. Ag/AgCl at pH 4.0 BRBs	0.006-1.0 ¹ 0.06-10.0 ²	0.0026 ¹ 0.025 ²	tropical fruit isotonic drink Syrup, juice powder, candies	[90]
		Pr ₆ O ₁₁ @Dy ₂ O ₃ -CNFs/GCE	0.710 V ¹ and 0.947 V ² vs. Ag/AgCl at pH 7.0 PBs	0.001-0.035 ¹ 0.0015-0.040 ²	0.00031 2 ¹ 0.00053 5 ²	Powder aromatic drink, carbonated lemon-flavored drink and pediatric vitamin syrup	[91]
		AgNP-CNF/CPE	0.66 V ¹ and 0.89 V ² vs. Ag/AgCl at pH 8.0 PBs	0.0382-12.9 ^{1,2}	0.0176 ¹ 0.0309 ²	Synthetic samples	[92]
		CPE/SG/CPCI	0.960 V ¹ and 1.171 V ² vs. Ag/AgCl at pH 2.0	0.02-1 ¹ 0.04-1 ²	0.005 ¹ 0.008 ²	Model solution, soft drink, orange and fruit juices	[93]
		NdOx /CPE	Oxidation at 0.3 V ¹ and 0.58V ² vs. Ag/AgCl at pH 3.0 PBs	0.20 - 2.0 ¹ 0.05-0.5 ²	0.09 ¹ 0.02 ²	Orange juice, tangerine juice powder, pharmaceutical dosage	[94]
		ILRGO-Au/GCE	0.65 V ¹ and 1.1 V ² vs. Ag/AgCl at pH 7.0 BRBs	0.004–1.0 ¹ 0.007–1.5 ²	0.52 ¹ 0.83 ²	Fanta, Mirinda, RIO, cocktail	[95]
Ssy and Trz	FIA-M.Amp.	GCE/ReS ₂ /DNPs	Applied potential: 0.8 V ¹ and 1.2 V ^{1,2} pH 5.0 AcBs	-	0.35 ¹ 0.24 ²	Pineapple jelly	[96]
Trz and Alr	DPV	Poly(MY)-MPGE	0.962 V ² and 0.77 V ³ vs. Ag/AgCl at pH 5.8 PBs	1-5 ^{2,3}	0.47 ² 0.27 ³	-	[97]
		Co ₃ O ₄ -Gr/CVE	1.1 V ² and 0.83 V ³ vs. Ag/AgCl at pH 5.0 PBs	0.05-0.15 ² 0.01-0.15 ³	0.016 ² 0.003 ³	Fruit jelly (Mango and Pineapple), fruit drink	[98]
Trz and P4R	DPV	Alumina microfibers/CPE	1.01 V ² and 0.67 V ⁴ vs. Ag/AgCl at pH 3.6 AcBs	0.005-0.14 ² 0.001-0.1 ⁴	0.002 ² 0.0008 ⁴	Drink sample	[99]
Trz and Cms	DPV	CPE/CaMgFe ₂ O ₄	0.82 V ² and 0.60 V ⁵ vs. Ag/AgCl at pH 3.0 PBs	10–900 ^{2,5}	0.92 ^{2,5}	Rose water syrup, wastewater	[100]
		Ni–Co LDH NSs/SPGE	1.08 V ² and 0.380 V ⁵ vs. Ag/AgCl at pH 7.0 PBs	0.3–125.0 ⁵	0.09 ⁵	Powdered and lemon juice	[101]
Trz and Amr	SWV	CPE/1-M-3BIBr/NiO/CNTs	1.1 V ² and 0.65 V ⁵ vs. Ag/AgCl at pH 7.0 PBs	0.1–750 ² 0.07–650 ⁵	0.06 ² 0.02 ⁵	Powdered, fruit, and lemon juice	[102]
		H-SWCNT/SPCE	0.88 V ² and 0.65 V ⁶ vs. Ag/AgCl at pH 2.3 PBs	1.00- 8.50 ² 0.057-0.40 ⁶	0.06 ² 0.03 ⁶	Synthetic orange juice, soft drinks	[103]
Ssy and Trz, or Trz and Alr	SWV	CoC/CPE	0.88 V ¹ 1.13 V ² and 0.92 V ³ vs. Ag/AgCl at pH 3.1 PBs	-	0.9 ¹ 0.3 ² 0.08 ³	Strawberry, orange cherry gelatin, pax orange flavor, syrup	[104]
Ssy, Trz and TMS	SWV	MoO/CPE	0.80 V ¹ 1.07 V ² and 0.95 V ⁷ vs. Ag/AgCl at pH 2.1 PBs	0.16-2.0 ^{1,2}	0.06 ¹ 0.04 ² 0.10 ⁷	Vitamin C, Pax orange flavor (powder), pharmaceutical dose	[105]
Trz, Alr and P4R	SWV	IL-GO-MWCNT/GCE	0.90 V ² , 0.65 V ³ and 0.59 V ⁴ vs. Ag/AgCl at pH 5.0 CitBs	0.02-0.13 ² 0.005-0.45 ³ 0.008-0.15 ⁴	0.01 ² 0.003 ³ 0.006 ⁴	RIO cocktail, Bacardi Breezer	[106]
Trz, Amr, and P4R	DPV	PSY/MWCNTs/GCE	0.83 V ² , 0.7 V ⁶ and 0.53 V ⁴ vs. Ag/AgCl at pH 5.0 CitBs	0.50–30 ^{2,4,6}	0.12 ² 0.11 ⁴ 0.21 ⁶	Jelly belly and powder, tap water	[107]

Full name of modified electrodes has been generally written in article title given in the reference list. PBs: Phosphate buffer solution, AcBs: Acetate buffer solution; BRBs: Britton Robinson Buffer solution; CitBs: Citrate buffer solution; DPV: Differential pulse voltammetry; SWV: Square wave voltammetry; FIA-M.Amp: Flow injection analysis multisense amperometry; LSV: Linear sweep voltammetry; ¹: Ssy (Sunset Yellow); ²: Trz (Tartrazine); ³: Alr (Allura red); ⁴: P4R (Ponceau 4R); ⁵: Cms (Carmoisine); ⁶: Amr (Amaranth); ⁷: TMS (Thiomersal).

It is also noteworthy that no studies have been found on the simultaneous determination of Ssy and Alr. This is attributed to the fact that these two substances are generally oxidized at the same potential values due to their structural similarities, as mentioned earlier. This issue limits the simultaneous determination of these two dyes. To offer an example for the simultaneous determination of Ssy and Trz, in a study conducted by Dursun and Afşar, a modified glassy carbon electrode made of graphite/n-butylamine/poly(4-aminophenol) composite was used for the simultaneous determination of Ssy and Trz [90]. The SW voltammograms of Ssy and Trz recorded simultaneously with this electrode at pH 4.0 are shown in Figure 6. As seen in the figure, Ssy was oxidized at 0.80 V and Trz at 1.04 V, and the peak currents of both azo dyes increased proportionally with increasing analyte concentrations. The detection limit values for Ssy and Trz were found to be 0.0026 and 0.025 μM , respectively, and real sample applications were performed in syrup, fruit juice powder, and candy using the developed simultaneous voltammetric method [90].

There are also studies on the simultaneous voltammetric determination of Trz with some other azo dyes, such as Alr, Amr, Cms, and ponceau 4R (P4R). The fact that Trz is oxidized at more positive potential values (between +1.0 and 1.2 V) than other dyes provides a significant advantage in terms of simultaneous determination and avoiding interference from other substances. For example, Martius Yellow (MY) was electropolymerized on a pencil graphite electrode surface using CV, and the resulting Poly-MY was used for the simultaneous voltammetric determination of Trz and Alr [97]. The DPVs recorded show that the peak current of Trz proportionally increased depending on the increase in its concentration in the presence of constant Alr at pH 5.8. In addition, the peak current of Alr also increased depending on the increase in its concentration in the presence of Trz, which remained constant. Alr oxidized at approximately 0.7 V, while Trz oxidized at 0.9 V. This evidence shows that both dyes can be determined simultaneously. LOD values for Trz and Alr were reported as 0.28 and 0.47 μM , respectively [97].

Additionally, a limited number of simultaneous determinations of three different azo dye mixtures have been performed [104-107]. For example, the simultaneous voltammetric determination of Ssy, Trz, and Alr using the SW voltammetric technique was performed using a cobalt-decorated carbon paste electrode [104]. In this study, the analysis of the triple mixture cannot be determined due to the overlapping potential of Alr and Ssy; only the simultaneous determinations of Trz and Ssy or Alr and Trz have been simultaneously determined. Furthermore, the simultaneous voltammetric determination of Ssy, Trz, and the active pharmaceutical ingredient thiomersal (TMS) in pharmaceutical samples was performed at a molybdenum oxide-modified carbon paste electrode [105]. In a study on the ternary mixture, Ssy was attached to the multi-walled carbon nanotube-modified GCE surface by its electropolymerization, and the obtained Poly Ssy/MWCNT/GCE was used for the simultaneous electrochemical determination of three

synthetic additive dyes (P4R, Amr, and Trz) [107]. DPVs recorded in pH 5.0 citrate buffer showed that P4R, Amr, and Trz were oxidized at 0.59, 0.65, and 0.90 V, respectively, and acceptable separations between the peaks for the oxidation of each were obtained. The LOD values for P4R, Amr, and Trz in Poly Ssy/MWCNT/GCE, which enabled the simultaneous determination of the three azo dyes, were found to be 0.11, 0.21, and 0.12 μM , respectively. With the developed electrochemical sensor, three azo dyes were successfully determined in Jelly belly, jelly powder, and tap water [107]. Simultaneous voltammetric methods for mixtures containing ternary azo dyes are more difficult and demanding than those involving binary mixtures due to potential interference.

CONCLUSION

Food dyes have long been used in the food, beverage, pharmaceutical, and cosmetic industries and will continue to be used until less toxic alternatives are found. However, we cannot ignore that azo dyes used in food and beverages can harm human health. Therefore, numerous methods have been developed for the analysis of these substances. One of these methods is the electrochemical method, which has recently gained considerable attention. The simultaneous determination of these dyes in the same matrix is crucial because azo dyes may coexist. If the oxidation or reduction peaks of these dyes are observed at different potentials, their simultaneous determination can be achieved using electrochemical methods.

The simultaneous determination of Ssy and Trz, the most commonly used azo dyes, has been successfully performed using various types of modified electrodes because the oxidation peaks of these dyes can be separated from each other by a potential difference of approximately 200-300 mV. In addition, studies on the simultaneous determination of Trz and other azo dyes have been successfully conducted. On the other hand, the simultaneous voltammetric determination of azo dyes with similar oxidation or reduction potentials remains limited.

This review highlights the importance of simultaneously determining coexisting azo dyes, considering the recent voltammetric studies on azo dyes. Although electrochemical methods, especially voltammetric techniques, offer excellent analytical performance, the most significant problem is selectivity, which arises from interference between azo dyes and other substances with the signals of azo dyes in the presence of complex matrices. Although attempts have been made to address these problems using modified electrodes or new approaches, such studies remain limited and are a topic requiring further study.

In the future, it is anticipated that more studies will be conducted to develop new strategies, particularly in electrode modification and separation for the simultaneous detection of food dyes. It is expected that advances in nanotechnology and nanoscience will provide significant contributions to electrode modification

and, consequently, to the simultaneous determination of azo dyes. It can be concluded that focus should be placed on the design of new modified electrodes, the development of new analytical procedures, miniaturization and integration into automation, and multiple detection systems for the simultaneous determination of azo dye.

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