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SPECTROPHOTOMETRIC DETERMINATION OF Fe (III) USING AG NANOPARTICLE–AZO REAGENT–CTAB BASED TERNARY COMPLEX

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In this work, a new spectrophotometric method for the determination of Fe(III) ions has been developed. The method is based on ternary complexes formed between green-synthesized silver nanoparticles (AgNPs), a bis-azo reagent (R), and cetyltrimethylammonium bromide (CTAB). AgNPs were synthesized using a starch–glucose system, and the reagent R was synthesized by the reaction of benzidine and pyrogallol. The interaction of Fe(III) with the R, Ag–R, and Ag–R–CTAB systems was studied with respect to pH, reagent concentration, temperature, and time factors. The Ag–R–CTAB system gave maximum absorbance at 487 nm and showed a clear bathochromic shift, and the molar absorptivity was 2.2×10^4 L·mol^{-1}·cm^{-1}, which is much higher than that of the binary systems.

The complex is stable for 24 hours and obeys Beer's law in the range of 0.10–2.24 mg/L. Common ions do not interfere even at high concentrations. The method was successfully applied to fruit samples and showed good accuracy and repeatability. The results show that azo compounds modified with AgNPs significantly increase the sensitivity of Fe(III) determination.

Keywords: silver, nanoparticles, CTAB, spectrophotometric determination, ternary complex

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INTRODUCTION

In recent years, the application of nanomaterials, especially metal nanoparticles, in analytical chemistry has been rapidly developing. Silver nanoparticles (AgNPs), due to their high surface area and unique optical and catalytic properties, create new opportunities for the sensitive and selective determination of various ions, particularly transition metal and heavy metal ions. Owing to their surface plasmon resonance (SPR) effect, the intensity of the absorption signal increases, resulting in a lower detection limit and enhanced sensitivity of analytical methods. On the other hand, azo reagents are classical chromogenic reagents that form stable and intensely colored complexes with metal ions. However, in some cases, these reagents exhibit low selectivity and limited sensitivity [1–5].

Therefore, the modification of azo reagents with nanoparticles has been developed as an important direction in modern analytical chemistry. As a result of such modification, the electron density of the reagent changes, the coordination ability increases, and the stability of complex formation is enhanced. In systems based on azo reagents modified with silver nanoparticles, the optical density (ε) and absorption signal are intensified due to the surface plasmon resonance of the nanoparticles, leading to increased sensitivity. At the same time, the interaction of the nanoparticle surface with functional groups facilitates the specific sorption

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and complexation of metal ions, thereby improving selectivity. Thus, the use of azo reagents modified with silver nanoparticles increases the sensitivity and selectivity of analytical reactions and enables the photometric determination of metal ions with high accuracy in environmental and biological samples.

This direction is also of great scientific and practical importance in terms of developing environmentally friendly analytical methods with low reagent consumption, in accordance with the principles of "green chemistry". Such systems can be widely used in environmental analysis, in the determination of metal ions in biological samples, as well as in the development of new nanosensors [6–10]. The aim of this work is to develop a sensitive and selective spectrophotometric method for the determination of Fe(III) ions based on ternary complexes formed with silver nanoparticles, a bis-azo reagent (R), and CTAB, and to evaluate its applicability to real fruit samples.

EXPERIMENTAL

Materials

Silver nitrate ($AgNO_3$, PLC 141459, 98% chemically pure), soluble starch ($(C_6H_{10}O_5)n$, PLC 121096, 98% chemically pure), β-D glucose ($C_6H_{12}O_6$, CAS No.50-99-7); sodium hydroxide (NaOH, PLC 1416x87), cetyltrimethylammonium bromide (CTAB, AB 117004), ethanol (C_2H_5OH, CAS No.64-17-5, 95%), benzidine ($C_{12}H_{12}N_2$, CAS No.92-87-5), sodium nitrite ($NaNO_2$, CAS No.7632-00-0), hydrochloric acid (HCl, CAS No.-01-0), were used as received.

Synthesis and stabilization of silver nanoparticles

Silver nanoparticles were synthesized using an environmentally friendly (green) chemical approach. Initially, 150 mL of a 1% starch solution was added to 100 mL of a 0.01 M silver nitrate ($AgNO_3$) solution to obtain a homogeneous mixture. During the synthesis process, starch served simultaneously as a reducing agent and a stabilizing agent.

Separately, 100 mL of a 0.2 M glucose solution was mixed with 100 mL of a 0.07 M sodium hydroxide (NaOH) solution. The resulting NaOH–glucose mixture was then added dropwise to the $AgNO_3$–starch solution under continuous mechanical stirring. The reaction mixture was stirred for 30 min, during which a gradual color change to dark brown was observed, indicating the reduction of Ag^+ ions to metallic silver nanoparticles (Ag^0).

To remove excess reagents and unreacted ions, the obtained colloidal solution was centrifuged using an Eppendorf R 5430 ultracentrifuge at 12 000 rpm. The precipitate was washed several times with a water–ethanol mixture (1:1), ensuring the complete removal of residual ions and reagents from the nanoparticle surface. In this synthesis route, starch functioned as both a reducing and stabilizing agent, glucose acted as an additional reducing agent, and NaOH provided an alkaline medium that accelerated the reduction process [11–14].

Synthesis of bis-[2,3,4-trihydroxyphenylazo]benzidine reagent (R)

For the diazotization process, 3.68 g (0.02 mol) of benzidine was dissolved in 8 mL of hydrochloric acid solution prepared at a volume ratio of 1:2 (v/v) in a 500 mL three-necked flask. The solution was cooled to 0 °C using an ice bath. Subsequently, 1.38 g (0.02 mol) of a 30% sodium nitrite ($NaNO_2$) solution was added dropwise under constant mechanical stirring, resulting in the formation of the corresponding diazonium salt. The reaction temperature was carefully maintained below 0 °C throughout the addition by continuous cooling. The diazotization reaction was allowed to proceed for 30 min at 0 °C. Excess nitrite was eliminated by adding a small amount of urea. In a separate 500 mL three-necked flask, 0.04 mol of pyrogallol was dissolved in 10 mL of a buffer solution adjusted to pH 3 and cooled to 0 °C. The freshly prepared diazonium salt solution was then added slowly to the pyrogallol solution under mechanical stirring. The coupling reaction was carried out at 0 °C for 1.5–2 h. After completion of the reaction, the resulting-colored azo compound precipitate was collected by vacuum filtration using a Büchner funnel, thoroughly washed with distilled water, recrystallized, and dried in a desiccator containing calcium chloride. The overall yield of the synthesized product was approximately 76%.

Synthesis of Ag–R–CTAB-based complexes

The synthesis of Ag^+–R–CTAB-based complexes was performed stepwise. Initially, a 10^{-3} M solution of the azo reagent (R) was prepared by accurately weighing the reagent on an analytical balance. The reagent, a pyrogallol-based azo compound, was synthesized according to previously reported procedures. A calculated amount of R was completely dissolved in ethanol to obtain a homogeneous solution, which was then transferred to a 100 mL volumetric flask. Fifty milliliters of the prepared reagent solution was placed into a 100 mL glass beaker, followed by the addition of 10 mL of a 0.01 M silver nanoparticle (AgNP) solution. The mixture was stirred at room temperature using a magnetic stirrer for 2 h. A noticeable color change during stirring indicated the formation of the Ag^+–R binary complex. In the subsequent step, an additional 10 mL of 0.01 M AgNP solution was introduced into the same reaction medium and stirred further. Thereafter, 5 mL of cetyltrimethylammonium bromide (CTAB) solution was added. The mixture was continuously stirred for another 2 h to ensure complete complex formation. A second color change observed after CTAB addition confirmed the formation of the Ag^+–R–CTAB ternary complex. In this system, CTAB acted as both a stabilizing agent and a surfactant, enhancing the colloidal stability of the complex and facilitating the interaction between silver nanoparticles and the azo reagent. As a result, a stable ternary complex system based on silver nanoparticles, the azo reagent (R), and CTAB was successfully obtained.

RESULTS AND DISCUSSION

Spectrophotometric determination of Fe (III)

Figure 1 illustrates the UV–Vis absorption spectra of silver nanoparticles (a), the reagent R (b), the Ag–R binary complex (c), and the Ag–R–CTAB ternary complex (d). The absorption spectrum of silver nanoparticles exhibits a characteristic maximum at 415 nm, which is attributed to the surface plasmon resonance of AgNPs (Figure 1a). The absorption band of the reagent R is observed in the range of 250–400 nm, showing distinct maxima at 216 nm, 365 nm, and 432 nm (Figure 1b). Upon the addition of AgNPs, the formation of the Ag–R binary complex leads to noticeable changes in the spectral profile, with absorption peaks appearing at 199 nm, 267 nm, 365 nm, and 400 nm. These changes are accompanied by a bathochromic shift compared to the free reagent (Figure 1c).

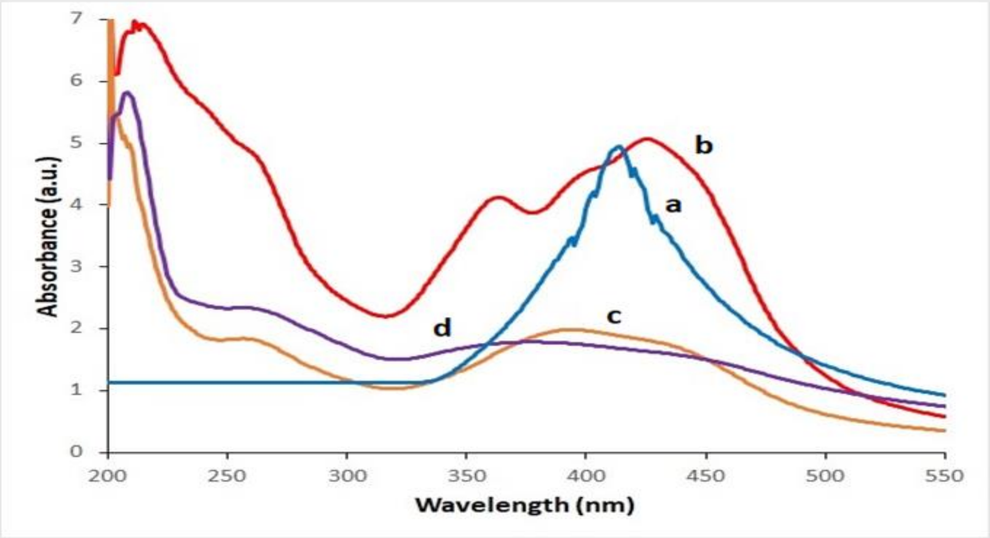

Figure 1. UV–Vis absorption spectra of silver nanoparticles (a), the reagent R (b), the Ag–R binary complex (c), and the Ag–R–CTAB ternary complex (d).

The formation of this binary complex is also visually confirmed by a color change from dark brown to a brownish-yellow shade. In the case of the Ag–R–CTAB ternary complex, the absorption maxima are further shifted to 211 nm, 271 nm, and 405 nm (Figure 1d). These spectral shifts can be attributed to the modification of the nanoparticle surface by the surfactant, as well as to changes in the surface plasmon resonance behavior of AgNPs upon interaction with CTAB. The observed results confirm the successful formation of the ternary complex and its distinct optical characteristics [15–18].

The spectrophotometric determination of Fe(III) ions was carried out using a ternary complex composed of silver nanoparticles, the reagent R, and CTAB. The absorption spectra of the complex were recorded at different wavelengths and under various pH conditions, and the dependences of optical density on wavelength and pH are presented in Figures 2 and 3, respectively.

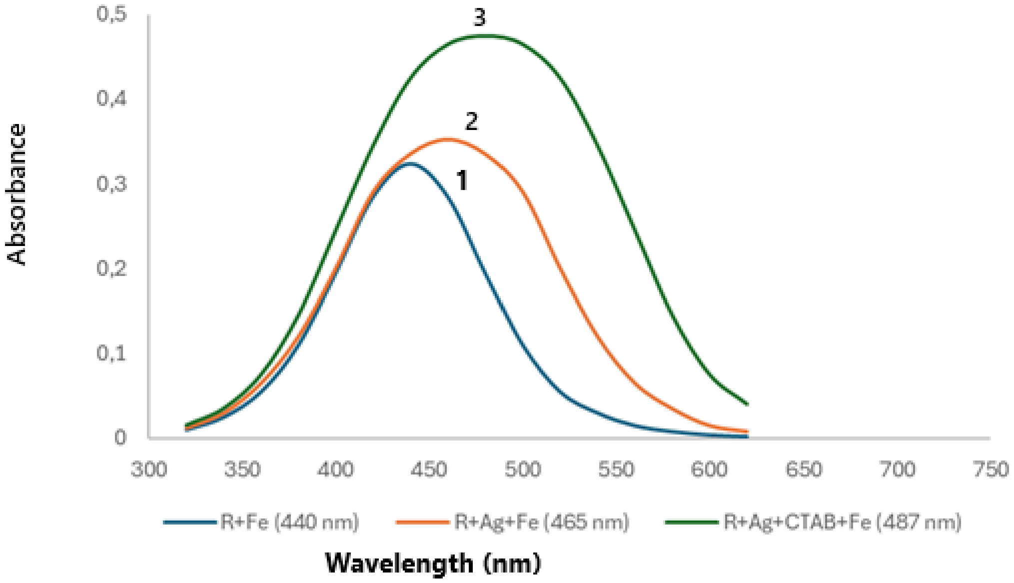

Figure 2. Dependence of optical density on wavelength for the R–Fe (1), R–Ag–Fe (2), and R–Ag–CTAB–Fe (3) systems.

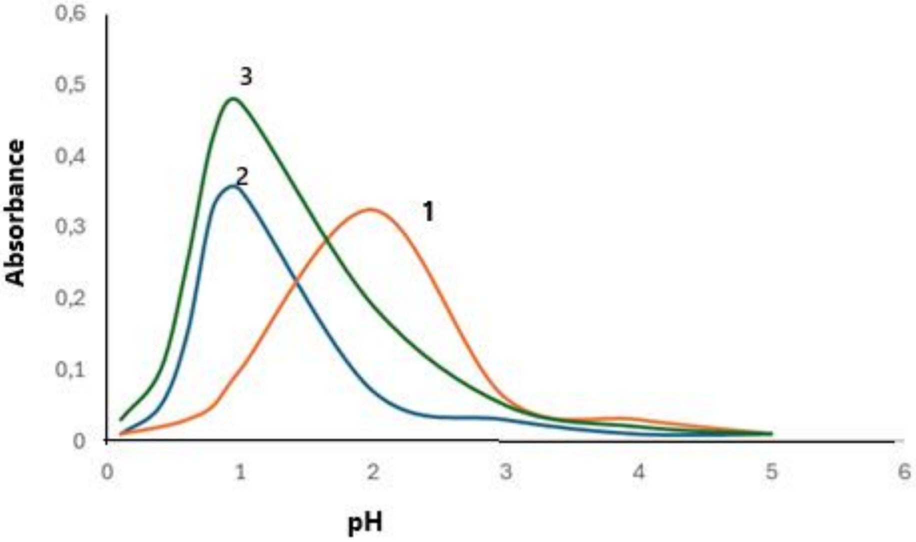

Figure 3. Dependence of optical density on pH for the R–Fe (1), R–Ag–Fe (2), and R–Ag–CTAB–Fe (3) systems.

A spectrophotometric method for the determination of Fe(III) was developed using the benzidine-based azo compound R through the Ag–R and Ag–R–CTAB complexes. Previous studies have investigated the complex formation of Fe(III) with the R reagent, and the optimal conditions for complex formation were determined to be pH_{opt}-2, λmax = 440 nm [18–20].

The pH dependence of complex formation between Fe(III) and the Ag–R and Ag–R–CTAB systems was also examined. Based on the obtained results, the optimal conditions for the formation of these complexes were found to be: for Ag–R, pH_{opt}-1, λmax -465 nm, and for Ag–R–CTAB, pH_{opt}-1 λmax -487 nm.

Bathochromic shifts were observed in the absorption spectra of the Ag–R–Fe and Ag–R–CTAB–Fe complexes, with the shifts occurring toward acidic conditions at the optimal pH. At this pH, the maximum absorption of Ag–R and Ag–R–CTAB was found to be 400 nm and 405 nm, respectively. The effect of reagent concentration on complex formation was also investigated under optimal conditions. It was determined that, for Fe(III) at a concentration of 8×10^{-5} M, the Ag–R system requires a reagent concentration of 4.8×10^{-5} M, whereas the Ag–R–CTAB system requires 3.2×10^{-5} M to achieve complete complex formation. The influence of temperature and reaction time on complex formation was studied. The optical density of both Ag–R–Fe and Ag–R–CTAB–Fe complexes reached a maximum at 60 °C. The stability of the complexes overtime was also assessed, showing that their optical densities remained unchanged for up to 24 hours.

The linear range of Fe (III) determination and the molar absorptivity values were calculated and are summarized in the corresponding table, providing the basis for quantitative analysis.

Table 1. Main spectrophotometric characteristics of Fe (III) complex compounds.

Complex	pH_{opt}	Λ_{max}	$\Delta \lambda$	$\varepsilon \cdot 10^{-3}$	Proportion of component	Linear range according to Beer's law
Ag-R-Fe	1,0	465	65	15,0±0,02	1:1	0,10-2,24
Ag-R-CTAB-Fe	1,0	487	87	22,0±0,03	1:1	0,10-2,24
Fe-R	2,0	436	100	8,5± 0,01	1:1	0,90-5,38

The composition of the colored complex compounds was determined using equimolar series and the relative yield method of Starik and Barbanel. The effect of interfering with ions on complex formation was also investigated [16]. It was found that the determination of Fe(III) as Ag–R–Fe and Ag–R–CTAB–Fe complexes is not affected by alkali and rare earth elements at concentrations higher than 5000-fold, Cu(II), Co(II), Zn(II), Ni(II), Cd(II), Mg(II), Mn(II), Pb(II), and Cr(III) at concentrations higher than 3000-fold, Al(III), Th(IV), Co(III), Ti(III), Sb(III), and Bi(III) at concentrations higher than 2000-fold, or Ti(IV), V(V), Mo(V), and Te(V) at concentrations higher than 1000-fold. The selectivity of these methods is significantly higher than that of the Fe(III) determination using the R reagent alone. The developed methodology was applied for the determination of Fe(III) in fruit samples. For this purpose, 200 g of sour cherries, 150 g of sweet cherries, and white cherries were collected, dried in a graphite crucible, and then ashed in a muffle furnace at 600–700 °C. The obtained residue was dissolved in a mixture of 5 mL HNO_3 and 15 mL HCl and heated with the addition of 5 mL HCl at 70–80 °C until the evolution of nitrogen oxides ceased. The resulting solution was diluted with distilled water, filtered, and transferred to a 100 mL volumetric flask, then diluted to the mark with distilled water. An aliquot of the prepared solution was taken and placed into a 25 mL volumetric flask. Then, 1 mL of 10^{-3} M Ag–R–CTAB was added, and the solution was adjusted to pH 1 and diluted to the mark with distilled water. The optical density of the resulting colored complex was measured at 490 nm against the Ag–R–CTAB blank using a 1 cm cuvette. The Fe(III) concentration was determined from a previously constructed calibration graph. The accuracy of the determination was confirmed by atomic absorption spectrometry, and the obtained results are summarized in the corresponding table.

Table 1. Determination of iron content in selected fruit samples (n = S, p = 0.95)

Analyzed fruit samples	Reagent	Found Fe %	Sr
Apple(simerinka)	Ag-R-CTAB	(1,46± 0,04) 10^{-3}	0,035
	AAC	(1,47 ± 0,06) 10^{-3}	0,044
Sweet cherry	Ag-R-CTAB	(3,47 ±0,03) 10^{-3}	0,042
	AAC	(3,44 ±0,03) 10^{-3}	0,058
Sour cherry	Ag-R-CTAB	(4,24 ±0,18) 10^{-3}	0,024
	AAC	(4,25 ±0,20)10^{-3}	0,041

CONCLUSION

The newly developed spectrophotometric method demonstrated the effective use of AgNP-modified bis-azo reagent (R) and CTAB-based ternary complexes for the determination of Fe(III) ions. The resulting complexes exhibited high stability, showed maximum absorbance in acidic media, and possessed high molar absorptivity, which enhances the sensitivity of the method. The influence of common coexisting ions was minimal, and the method was successfully applied to fruit samples. The experiments revealed that the participation of nanoparticles and the surfactant strengthens complex formation with Fe(III) and improves the selectivity of the method. Therefore, this approach provides a promising strategy for the precise, sensitive, and environmentally friendly determination of metal ions in analytical chemistry.

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