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Investigation of salicylaldehyde-5-sulfonate as a precolumn derivatizing agent for the determination of *n*-alkane diamines, lysine, diaminopimelic acid, and isoniazid by capillary zone electrophoresis

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Abstract

Reactions of primary amines with salicylaldehyde-5-sulfonate (SAS) lead to the formation of stable Schiff bases at weakly alkaline pH and at moderate temperature in an ethanol-rich aqueous solution. Some alkane diamines were converted to salicylaldimine with SAS, and the products were resolved and detected by capillary zone electrophoresis (CZE). The reactivity of SAS was applied also to the derivatization of amino acids. Optimal conditions for the analysis of lysine in the presence of diaminopimelic acid, the metabolite of lysine, were investigated. Addition of Na_2SO_4 into the migrating solution enhanced the stacking effect, and led to increase in the detection limits. Another advantage of the use of SAS is its ability to form stable water-soluble metal chelate as Schiff base ligands. For practical analysis, the copper chelate of the Schiff base of isoniazid, which is known as the most active antibacterial used in the treatment of pulmonary tuberculoses, was investigated, and the detection limit of isoniazid was improved in the magnitude of 11 times compared with the one of free isoniazid.

Keywords: *n*-Alkane diamines; Salicylaldehyde sulfonate; Lysine; Diaminopimelic acid; Isoniazid; Schiff base; Capillary zone electrophoresis

1. Introduction

Salicylaldimine ligands and their complexes derived from the reaction with salicylaldehyde and 2-hydroxy-1-naphthaldehyde have been extensively studied [1,2]. A number of salicylaldimine have been found to behave as reversible oxygen carrier [3–5], and they have been used as models for biological systems. Some Schiff base complexes have also been used in catalytic reaction as [bis(salicylidene-γ-iminopropyl)methylamine]cobalt(II) to oxidize olefins in the presence of dioxygen or hydrogen peroxide [6], and of chloro

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and carbonyl complexes ruthenium(III) Schiff base derived from salicylaldehyde and picolinaldehyde with several amines used as good homogenous catalysts in the reductive carbonylation of nitrobenzene to phenylurethane and in the oxidation reaction of cyclohexene to its epoxide [7,8]. In our previous investigation [9], we developed the use of salicylaldehyde-5-sulfonate (SAS) for precolumn derivatization for the analysis of alkylamines by capillary zone electrophoresis (CZE). In the present study, a sensitive CZE method with UV–visible detection has been developed first for simultaneous determination of six kinds of *n*-alkane diamines (carbon number: 4–9). The total derivatization procedure was found to be very simple by adding an excess of SAS reagent and using 10 mM phosphate buffer (pH 7.0), the reaction proceeded within 5 min in the ultra-sonic bath and the reaction mixture was directly operated for CZE analysis. The overall procedure was applied to the analysis of amino acids, since a fast, sensitive, and precise analysis is very important in diagnosing the diseases caused by disorder in protein metabolism. In addition, amino acids are measured to determine the quality of the final products in food sciences. Precolumn or post-column derivatization methods have been commonly used in liquid chromatography using UV chromophore or fluorophore reagents [10–15]. Another approach to amino acid analysis is the capillary electrophoresis as an alternative to LC [16], which can provide high-resolution efficiency.

A great advantage in the use of SAS offers an intramolecular hydrogen bonding between phenolic proton and imine moiety, which leads to the formation of stable Schiff bases in a moderate polar solvent. Furthermore in this study, a stable metal complex was investigated for the analysis of isoniazid. In a higher ethanol content in the solution (less polar media), the isoniazid Schiff base formed was not sufficiently stable, while the addition of copper II ion in the sample solution helped total derivatization of isoniazid, and its assay in a commercially available powder as isoniazid Schiff base–copper chelate was investigated.

2. Experimental

2.1. Instruments

Electrphoretic separations were carried out using a Hewlett–Packard (Waldbronn, Germany) ^{3D}CE capillary electrophoresis system with a diode-array detector. A fused silica capillary of 50 μm i.d. (Hewlett–Packard) was attached to the system. The total length of the capillary was 50.5 cm with a 42 cm effective length from the injection point to the detector window. Injection of samples was carried out by applying pressure of 50 mbar for 3 s.

2.2. Chemicals

Alkane diamines, lysine, and diaminopimelic acid were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Isoniazid was from Sigma–Aldrich (St. Louis, MO, USA). Copper chloride and sodium sulfate were from Wako (Osaka, Japan). SAS was synthesized in our laboratory as previously described [9]. The pH values of the stock solution of buffers were adjusted by mixing a potassium dihydrogenphosphate and a disodium hydrogenphosphate with total phosphate concentration of 100 mM.

2.3. Derivatization procedure for the alkane diamines and electrophoretic separation

Di-derivatized alkane diamines were obtained by adding SAS at the concentration of 2.2×10^{-3} M in 60% (v/v) ethanol solution, which also contained 10 mM phosphate buffer (pH 7.8). The reaction proceeds within 5 min in an ultrasonic bath. The solutions, thus, obtained were directly used for CZE analysis. Electrophoretic separation of six kinds of alkane diamines Schiff bases and the validation of the optimized method were carried out using 10 mM phosphate buffer (pH 7.8) in 60% (v/v) ethanol solution. The capillary and the vials for sample solutions were thermostated at 25 °C. The sample solutions were injected for 3 s (50 mbar). A voltage of 30 kV was applied for the CZE separation, and the analytes were detected at 250 nm. The standard sample

solutions contained the *N*,*N*′-substituted bis(salicylaldimine-5-sulfonate) at concentration levels of 10^{-5} M.

2.4. Standard procedure for the determination of lysine and diaminopimelic acid as salicylaldimine derivatives

A sample solution containing lysine, diaminopimelic acid, 10^{-3} M SAS, and 10 mM phosphate buffer (pH 7.0), in 50% (v/v) ethanol–water was prepared in the ultra-sonic bath for 10 min. Then the solutions were stood for 10 min. The running buffer used in CZE analysis was a mixture of 10 mM phosphate buffer (pH 7.0) 10 mM sodium sulfate and 5% (v/v) ethanol. The temperature of the capillary and the vials was fixed at 30 °C, samples were injected by a pressure of 50 mbar for 7 s. The voltage applied for the separation was 25 kV, the wavelength for detection being 250 nm.

2.5. Standard procedure for the determination of isoniazid as copper chelate with SAS

The copper chelate of isoniazid–SAS Schiff base was prepared by mixing corresponding solutions to form 5×10^{-5} M isoniazid, 10^{-3} M SAS and 5×10^{-4} M copper chloride in a solution containing 20 mM phosphate buffer (pH 7.0) and 5% (v/v) ethanol. The CZE analysis was performed by using a migrating solution containing 20 mM phosphate buffer (pH 7.0) and 5% (v/v) ethanol. Capillary electrophoretic conditions were maintained in the same conditions as in amino acids analysis.

3. Results and discussion

3.1. Optimization of derivatization conditions for alkane diamines

Initial experiments for the derivatization reaction was performed by preparing stock solutions of Schiff bases under the same conditions as those previously employed for *n*-alkyl amines: stirring a mixture of 5×10^{-4} M *n*-alkane diamine and SAS (eight times equivalent to the diamine) at 40 °C in 40% (v/v) ethanol solution. The electropherogram obtained showed two peaks for each alkane diamine. This behavior was explained by the fact that two Schiff bases corresponding to mono-derivatized and di-derivatized ones were formed, which indicates that the conditions were not enough for complete derivatization. It was confirmed through the electrophoretic mobility of 1,7-diaminoheptane Schiff base against pH (Fig. 1). Mono-derivatized analyte has neutral (zwitter ionic) character at pH < 7.0 due to the sulfonate and the ammonium moieties on the ends of the molecule. When the pH is higher than 8.5, the ammonium moiety can release one proton and the analyte becomes anionic with the associated sulfonate group, and it migrates slower than the EOF; the mobility increased to 3.9×10^{-5} cm^2 V^{-1} s^{-1}. On the other hand, di-derivatized 1,7-diaminoheptane has anionic character given by two sulfonate groups on both ends of the molecule, and its mobility increased gradually with pH increase.

A higher ethanol content (60% (v/v)) in the reacting solution, lead to the stabilization of the

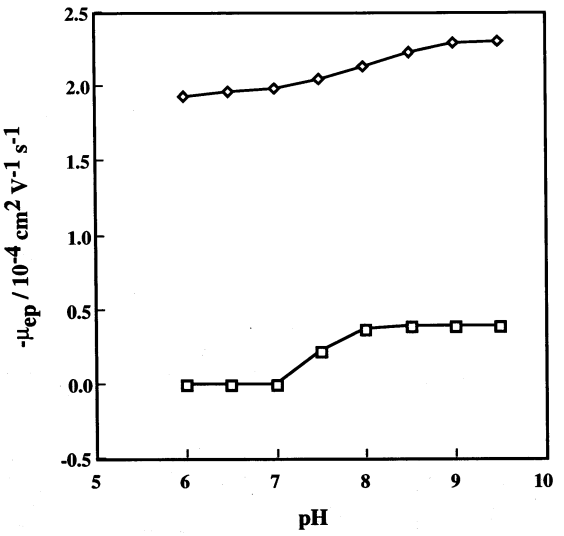

Fig. 1. Change in the electrophoretic mobility of 1,7-diaminoheptane Schiff bases with the pH. Migrating solution: 10 mM phosphate buffer+40% (v/v) ethanol. CZE conditions: capillary temperature, 25 °C; applied voltage, 30 kV; detection wavelength, 250 nm; injection period, 5 s. Analytes: □, mono-derivatized 1,7-diaminoheptane; ◇, di-derivatized 1,7-diaminoheptane.

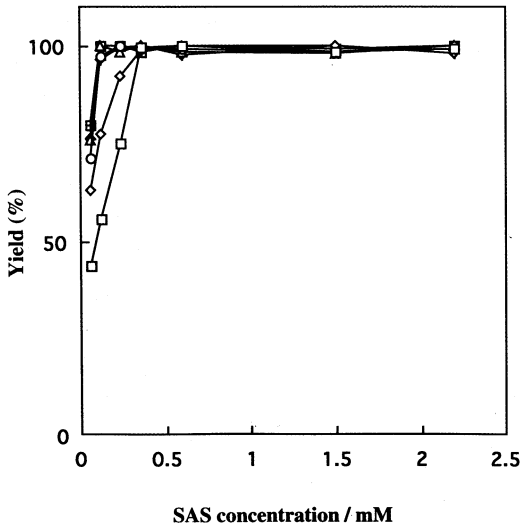

Fig. 2. Yield of the alkane diamines Schiff bases as a function of SAS concentration. Migrating solution: 10 mM phosphate buffer (pH 7.8) + 60% (v/v) ethanol. Sample solutions: 3×10^{-5} M of alkane diamine, SAS, 10 mM phosphate buffer (pH 7.8) + 60% (v/v) ethanol. CZE conditions are the same as in Fig. 1. Alkane diamines: □, 1,4-diaminobutane; ◇, 1,5-diaminopentane; ○, 1,6-diaminohexane; △, 1,7-diaminoheptane; ▽, 1,8-diaminooctane; ◁, 1,9-diaminononane.

Schiff base formed. However, it did not improve the yield of di-derivatized diamines. The influence of the reaction temperature was found to be less effective, and only the concentration of SAS was found to affect the reaction yield. It resulted, the complete derivatization of all alkane diamines tested at the concentrations of 2.2×10^{-3} M (Fig. 2).

3.2. Separation and determination of alkane diamines as Schiff bases by CZE

The requirement for stable di-derivatized alkane diamines was found in 60% (v/v) ethanol solution under slightly alkaline pH condition. Hence, optimal conditions for the derivatization and the determination of di-substituted alkane diamine Schiff bases by CZE were obtained by running with 10 mM phosphate buffer (pH 7.8) containing 60% (v/v) ethanol.

A representative electropherogram showing the good base-line resolution for six kinds of alkane diamines (C_4–C_9) within 16 min is shown in Fig. 3. The optimized conditions for the derivatization and the CZE determination allowed accurate alkane diamines assignments and sensitive quantitation in low micromolar ranges. The results are summarized in Table 1 and indicate the high correlation coefficient (> 0.990) studied in the concentration range of 5–250 μM and a high sensitivity (limit of detection (LOD): 2.7 μM for the 1,7-diaminoheptane to 8.6 μM for the 1,4-diaminobutane) calculated as the concentration corresponding to three times its standard deviation (S.D.).

3.3. Use of SAS as a derivatizing agent for lysine and diaminopimelic acid

In order to study the applicability of derivatization to di-substituted Schiff bases, we selected lysine (LYS) as an amino acid and its metabolite diaminopimelic acid (DAPA) possessing amino groups in their structures; the structural formula are given in Fig. 4.

For the complete conversion of amino groups of each analyte to Schiff bases, we first mixed 5×10^{-5} M of each one with an excess of SAS (2×10^{-3} M) in 60% (v/v) ethanol solution containing 10 mM phosphate buffer (pH 7.8), the mixture was left in the ultra-sonic bath for 10 min then standing for 10 min. When the solution was analyzed by CZE, only one peak identified as LYS derivative was detected and any signal corresponding to DAPA was not observed within 60 min. The behavior should be explained by the suppressed EOF caused by the high ethanol percentage attributed together with the fact that DAPA Schiff base posses two carboxylate groups as well as two sulfonate groups, conferring highly anionic character; and, therefore, DAPA Schiff base migrated to the anodic end and the analyte would not have reached the detector.

The ethanol percentage in the sample and the migrating solutions was varied in the ranges from 5 to 60% (v/v). It can be noticed that the complete derivatization of both analytes requires high percentages of ethanol ($> 50\%$ (v/v)) in the sample solution, however, smaller percentages of ethanol in the migrating solution did not affect the stability of the Schiff bases, allowing rapid migration in the

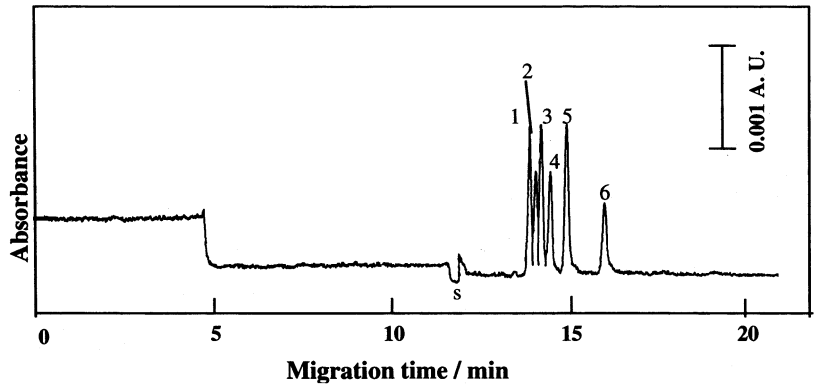

Fig. 3. Electropherogram for alkane diamines after derivatization with SAS. Signals: 1, 1,9-diaminononane; 2, 1,8-diaminooctane; 3, 1,7-diaminoheptane; 4, 1,6-diaminohexane; 5, 1,5-diaminopentane; 6, 1,4-diaminobutane. Migrating solution: 10 mM phosphate buffer (pH 7.8)+60% (v/v) ethanol. Sample solution: 3×10^{-5} M of each alkane diamine, 2.2×10^{-3} M SAS, 10 mM phosphate buffer (pH 7.8)+60% (v/v) ethanol. CZE conditions are the same as in Fig. 1.

Table 1
Linearity and sensitivity for alkane diamines by the proposed method

Diamines	Correlation coefficient[a]	LOD (μM)[b]
1,4-Diaminobutane	0.990	8.6
1,5-Diaminopentane	0.998	4.8
1,6-Diaminohexane	0.990	7.6
1,7-Diaminoheptane	0.997	2.7
1,8-Diaminooctane	0.995	2.9
1,9-Diaminononane	0.995	3.1

[a] Concentration range: 5–250 μM.
[b] Concentration corresponding to three times its S.D.

capillary toward the cathodic end. Despite the favorably fast migration of the anionic derivatives, the peaks showed asymmetric shapes due to the difference between the sample and the buffer matrix. In order to shapen the signals, the conductivity of migrating solution was increased using sodium sulfate at the concentration of 10 mM; the analytes were allowed to be stacked in a narrow zone. Improvements in the analysis time, the peak shapes, and the LOD were observed by running with the migrating solution containing 10 mM pH buffer (pH 7.0) and 10 mM Na_2SO_4 and 5% (v/v) ethanol.

To study the quantitative possibility of the derivatizing reaction on direct injection of LYS and DAPA by CZE analysis, experiments were carried out with the analytes at the concentrations of 8, 10, 50, 100 and 500 μM, considering the average peak area of three injections. Calibration graphs showed linearity and the correlation coefficients were larger than 0.993 for both analytes (Table 2). The relative standard deviation (R.S.D.) values for the electrophoretic mobility, peak area, and peak height were less than 3.5%. The LOD calculated as the concentration corresponding to three times its S.D. are found to be 0.7 μM for LYS and 1.0 μM for DAPA.

3.4. Investigation of analysis of isoniazid as Shiff base–copper chelate

On the basis of the results discussed above, the reaction between isoniazid and SAS was tried by adding an excess of SAS in 60% (v/v) ethanol solution mixed with 10 mM phosphate buffer (pH 7.0), no peak corresponding to isoniazid Schiff base was observed; and the product should be decomposed still in higher ethanol percentage and at high reaction temperature. The derivatization reaction showed very slow rate (1 day to complete) at the room temperature in 5% (v/v) ethanol solution. In order to accelerate the reaction rate and to get stable water-soluble Schiff base, copper chloride was added to the reaction mixture. A binuclear structure of copper chelate was formed by ultra-sonicating for 10 min and then standing

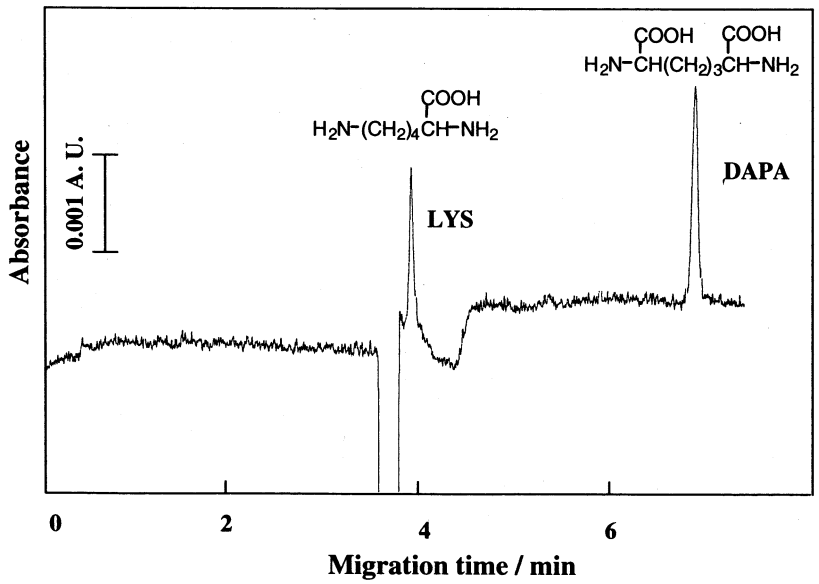

Fig. 4. Application of the proposed method to the analysis of lysine and diaminopimelic acid. Migrating solution: 10 mM phosphate buffer (pH 7.0)+10 mM Na_2SO_4+5% (v/v) ethanol. Sample solution: 5×10^{-5} M of each analyte, 10^{-3} M SAS, 10 mM phosphate buffer (pH 7.0)+50% (v/v) ethanol. CZE conditions are the same as in Fig. 1 except for the injection period, 7 s.

for additional 10 min. In Fig. 5, electropherograms corresponding to isoniazid as free compound and as SAS–Schiff base copper chelate are obtained. As is expected, the signal of the chelate was much higher than that of the free one. A commercial powder of isoniazid was analyzed for the quantitative determination of its active ingredient. Results are summarized in Table 3. The analytical results obtained by both methods were within the specification required, while the LOD was greatly improved by derivatizing isoniazid as Shiff base–copper chelate. In order to validate the developed method further, the precision was evaluated through six measurements in a day (Table 4). For the same concentrations of isoniazid, the obtained R.S.D.s of the peak area and the peak height indicate that the determination of isoniazid as Schiff base–copper chelate is more precise, owing to the higher sensitivity. The optimized derivatization is proved to be more suitable for the analysis of isoniazid.

4. Conclusion

The derivatization of alkane diamines with SAS, as well as hydrazine, was found to be simple and easy. The optimal conditions for the analysis of

Table 2
Precision, linearity and sensitivity of the analysis of lysine and diaminopimelic acid by the optimized method

Analyte	R.S.D. (%)[a]			Correlation coefficient[b]	LOD (μM)[c]
	Area	Height	Mobility		
Lysine	2.8	3.2	3.4	0.993	0.7
Diaminopimelic acid	3.3	2.0	2.9	0.997	1.0

[a] Values analyzed with six measurements.
[b] Concentration range: 8–500 μM.
[c] Concentration corresponding to three times its S.D.

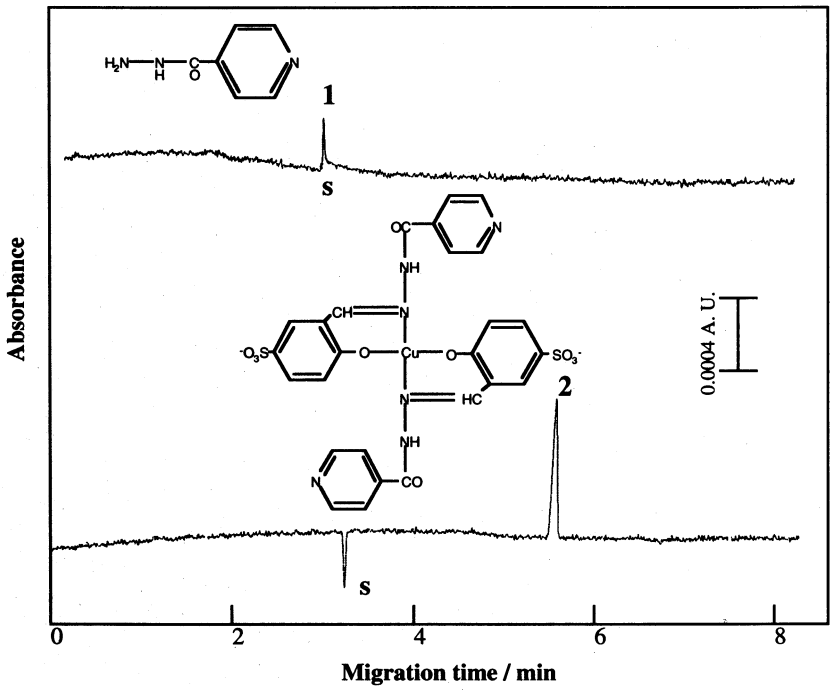

Fig. 5. Electropherograms for isoniazid in the absence and the presence of copper chloride using salicylaldehyde sulfonate as a derivatizing reagent. Migrating solution: 20 mM phosphate buffer (pH 7.8)+5% (v/v) ethanol. Sample solution: (a) 5×10^{-5} M isoniazid, 20 mM phosphate buffer (pH 7.0)+5% (v/v) ethanol; (b) 5×10^{-5} M isoniazid, 10^{-3} M SAS, 5×10^{-4} M $CuCl_2$, 20 mM phosphate buffer (pH 7.0)+5% (v/v) ethanol. CZE conditions are the same as in Fig. 1 except for the injection period, 7 s. Signals: 1, free isoniazid; 2, isoniazid as Schiff base–Cu chelate; s, EOF.

Table 3
Comparison of analytical results for isoniazid in commercial powder with and without chelation

Method	Percent claim found (%)
CZE (free isoniazid)	105.4
CZE (isoniazid chelate)	103.7

Table 4
Precision and sensitivity for free isoniazid and isoniazid Schiff base–copper chelate by the CZE method

Analyte	R.S.D. (%)[a]		LOD (μM)[b]
	Area	Height	
Free isoniazid	0.15	1.33	10.0
Isoniazid chelate	0.05	1.10	0.9

[a] Values analyzed with six measurements of 5×10^{-5} M isoniazid solution.
[b] Concentration corresponding to three times its S.D.

alkane diamines, amino acids, and isoniazid as hydrazine compound showed that SAS is hopeful to develop the analysis of a great variety of amino compounds. Furthermore, successful improvement in the LOD and the quantitative feature of the assay method, in addition to the use of SAS coupled with copper ion, were achieved with the Schiff base–copper chelate and the determination of hydrazine compounds.

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