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Spectrophotometric Determination of Sulphadiazine in Pharmaceutical Preparations Via Arsenazo III - Cerium (III) Reaction

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الخلاصة

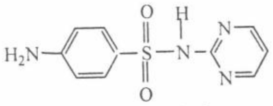
تم اقتراح طريقة طيفية بسيطة وسريعة ودقيقة ومتوافقة لتقدير السلفادايازين بشكله الحر وفي مستحضره الدوائي والبيطري. اعتمدت الطريقة على تفاعل الأكسدة والاختزال بين السلفادايازين و أيون السيريوم الرباعي يتبعها مفاعلة ناتج التفاعل أيون السيريوم الثلاثي مع الكاشف آرسين آزو III في وسط حامضي ليعطي معقداً ذا لون أخضر- مزرّق مستقر وذائب بالمحلول المائي تُقاس شدة امتصاصه عند الطول الموجي 654 نانوميتر وكانت قيمة الامتصاصية المولارية 1.22×10^5 لتر.مول$^{-1}$.سم$^{-1}$ وانطبق قانون بير في مدى التركيز بين 1 و10 مايكروغرام من السلفادايازين في حجم نهائي 25مل. تم تطبيق الطريقة المقترحة بنجاح في تقدير السلفادايازين في مستحضره الدوائي والبيطري.

ABSTRACT

A simple, rapid, accurate and precise spectrophotometric method is proposed for determining sulphadiazine (SDA) in pure form and in its pharmaceutical and veterinary preparations. The method is based on oxidation-reduction reaction between SDA and cerium(IV) ion (ceric ion), then the subsequent reaction of cerium(III) with arsenazo III reagent in acidic medium to produce a green-blue complex which is stable, water soluble and has a maximum absorption at 654 nm with a molar absorptivity of 1.22×10^5 $l.mol^{-1}.cm^{-1}$. Beer's law is obeyed in the concentration range from 1 to 10µg SDA in a final volume of 25 ml. The proposed method has been applied successfully to determine SDA in pharmaceutical and veterinary preparations.

INTRODUCTION

Sulphadiazine (N^1-2-pyrimidinylsulphanilamide) is one of the many known sulphonamides drugs (1). It is well absorbed by mouth with an intermediate $t_{1/2}$ of 10h, and it enters cerebral spinal fluid more readily than other sulphonamides (2).



Sulphadiazine

The important role in chemotherapy played by sulphadiazine and its effects in the various situations makes the assay of sulphadiazine of utmost importance (3).

Several methods for the determination of SDA have been suggested, these methods included diazotization of SDA and coupling the diazotised SDA with α-naphthylamine (4), 3-aminophenol (5), iminodibenzyl (6) and primaquine phosphate (7). Other methods are based on the formation of charge transfer (CT) complex between SDA and alizarin derivatives (8) and phenosafranine (9). An oxidative coupling reaction of SDA with different reagents and oxidizing agents has also been proposed; included reagents are phenothiazine and ceric ion (10), phenothiazine and hypochlorite (11) and 4-amino-N,N-dimethylaniline and dichromate (12).

Arsenazo III undergoes sensitive and selective reactions with several cations, such as reaction with cerium(III) ion in the presence of cerium(IV) ion. This reaction can be used in the determination of some organic compounds which have the ability to undergo oxidation reaction with cerium(IV) ion (13 - 15).

The aim of the present work is to evaluate an indirect, simple, rapid, accurate and precise spectrophotometric method for the determination of SDA based on the oxidation – reduction reaction of SDA with cerium(IV) ion, then the subsequent reaction of cerium(III) ion with arsenazo III reagent in acidic medium to yield a coloured complex whose intensity is indirectly related to the concentration of SDA.

EXPERIMENTAL

All spectrophotometric measurements are performed on Shimadzu UV-Visible Recording Spectrophotometer UV-160 by using 1 cm silica cell. pH meter type Philips PW 9420 is used for pH reading.

Reagents

All chemicals used are of analytical - reagent grade.

Standard SDA solution, 100μg.ml^{-1}. This solution is prepared by dissolving 0.01 g of SDA (SDI- Iraq) in 10 ml ethanol to increase solubility and the volume is diluted to 100 ml with distilled water in a volumetric flask.

Working SDA solution, 10μg.ml^{-1}. This solution is prepared by appropriate dilution of standard solution.

Ammonium ceric sulphate [cerium(IV) ion solution], 4×10^{-5}M. This solution is prepared by dissolving 0.00632 g of ammonium ceric sulphate dihydrate (BDH) in 250 ml of distilled water in a volumetric flask, this solution is freshly prepared daily.

Arsen azo III reagent solution, 2×10^{-4}M. This solution is prepared by dissolving 0.0411 g of arsenazo III (Fluka) in 250 ml distilled water in a volumetric flask.

Sulphuric acid solution, 0.05 N. This solution is prepared by appropriate dilution of 1.4 ml of the concentrated sulphuric acid (35.5 N) solution to 1000 ml with distilled water in a volumetric flask.

Sulphaprim (Veterinary injectable solution , 20% SDA), 10μg.ml^{-1}. A suitable volume (0.5 ml) of the veterinary injectable solution containing exactly 0.1 g of SDA is treated with 10 ml of ethanol and diluted to 1000 ml with distilled water to obtain solution of 100 μg.ml^{-1} of SDA. The tested solution is prepared by appropriate dilution .

Flammazine cream(1% Ag.SDA), 100μg.ml^{-1}. The 100 μg.ml^{-1} Ag.SDA solution is prepared as described (16). Less dilute solution (10 μg.ml^{-1} Ag.SDA) is prepared by appropriate dilution.

Procedure and calibration graph

To a series of 25- ml calibrated flasks, an increasing volume (0.1-1.6 ml) of 10 μg.ml^{-1} SDA solution are transferred, followed by 5 ml of 4×10^{-5}M cerium(IV) ion solution and 0.7 ml of 0.05 N sulphuric acid solution, standing for 25 minutes, then 3 ml of 2×10^{-4}M arsenazo III reagent solution is added. After dilution the flasks with distilled water, the absorbances are measured at 654 nm against the reagent blank. Beer's law is obeyed over the range of concentration 1 to 10 μg SDA in 25 ml (Fig. 1). A negative deviation from Beer's law is occurred beyond the upper determination limit. The apparent molar absorptivity, referred to SDA, has been found to be 1.22×10^{5} l.mol^{-1}.cm^{-1}.

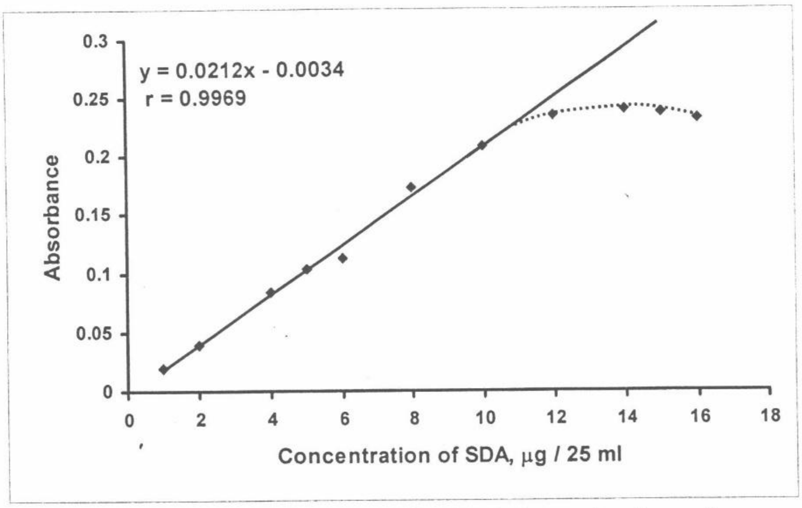


Fig. 1: The calibration graph for SDA determination using the reaction between arsenazo III and cerium(III).

RESULTS AND DISCUSSION

During the investigation , 10 µg of SDA is taken and the final volumes are brought to 25 ml with distilled water.

Optimization of variables

The effect of various parameters on the absorption intensity of the coloured complex is studied and the reaction conditions have been optimized.

Effect of pH

The effect of pH on intensity of the coloured complex is examined. Different volumes (0.01-6 ml) of 0.05 N sulphuric acid solution is added to an aliquot of solution containing 10 µg of SDA. The intensities of absorption are read against the reagent blank. The results are shown in table 1.

Table 1. Effect of pH on absorbance

ml of 0.05 N H_2SO_4	Absorbance	Final pH
0.00	0.020	4.39
0.01	0.042	4.06
0.10	0.086	3.60
0.20	0.136	3.44
0.30	0.139	3.22
0.50	0.170	3.04
0.70	0.214	2.80
1.00	0.186	2.60
2.00	0.128	2.50
6.00	0.067	2.15

The results shown in table 1 indicate that the pH of 2.80 is considered optimum. A pH 2.8 is selected for subsequent investigation because of good sensitivity. Five buffer solutions of pH 2.8 with different composition have been tested, tartaric acid-NaOH (B_1), citric acid-NaOH (B_2), KH phthalate-HCl (B_3), glycine-HCl (B_4) and formic acid-NaOH (B_5), (Table 2).

Table 2. Effect of buffer solutions on absorbance

ml of buffer solution	Absorbance/ml of buffer added				
	B_1	B_2	B_3	B_4	B_5
1	0.040	0.046	0.057	0.018	0.093
2	0.024	0.020	0.094	0.022	0.097
3	0.021	0.033	0.160	0.038	0.085
4	0.020	0.019	0.130	0.043	0.077
5	0.017	0.012	0.093	0.046	0.048

The results in table 2 indicate that all types of buffer solutions decrease the intensity of absorption of the coloured complex, so that the use of buffer solutions is not recommended. A 0.7 ml of 0.05 N sulphuric acid solution has been recommended for subsequent experiments.

Effect of oxidizing agent [cerium(IV) ion] amount

Different amounts of cerium(IV) ion solution are added and the optimum amount which gives higher intensity of coloured complex and higher value of correlation coefficient (Table 3) has been selected.

Table 3. The effect of ceric ion amount on absorbance

ml of 4×10^{-5}M cerium(IV) ion solution	Absorbance/µg SDA in 25ml					Absorbance of reagent blank	r
	1	3	5	7	10		
3	0.030	0.018	0.031	0.052	0.070	0.067	0.8927
5	0.029	0.073	0.106	0.131	0.206	0.079	0.9935
7	0.027	0.062	0.078	0.096	0.113	0.180	0.9743
10	0.012	0.044	0.068	0.081	0.106	0.194	0.9843

The results shown in table 3 indicate that the volume of 5 ml of 4×10^{-5}M cerium(IV) ion solution is an optimum amount because of highest value of correlation coefficient and therefore recommended for subsequent experiments.

Effect of time on reduction of cerium(IV) ion

The effect of time needed to complete the reduction of cerium(IV) ion to cerium(III) ion is studied by standing of the solutions after adding cerium(IV) ion solution for different times, then the other reagents are added and the absorbances measured against the reagent blank (Table 4).

Table 4. Effect of time on reduction process

Time (min.)	0	5	10	15	20	25	30	35	40	45
Absorbance	0.010	0.052	0.070	0.145	0.178	0.210	0.158	0.135	0.121	0.114

The results indicate that complete reduction of cerium(IV) ion occurred after 25 minutes and the intensity decreased above 25 minutes because the intensity of reagent blank solutions increased. Therefore the standing time 25 min. is recommended for the subsequent experiments.

Effect of arsenazo III reagent amount

The effect of the amount of arsenazo III reagent on maximum formation of the coloured complex is investigated. The results is shown in table 5.

Table 5. Effect of arsenazo III reagent amount on absorbance

ml of 2×10⁻⁴M arsenazo III reagent	Absorbance/µg SDA in 25ml					r
	1	3	5	7	10	
1	0.019	0.030	0.048	0.076	0.098	0.9912
3	0.025	0.064	0.105	0.136	0.208	0.9979
5	0.009	0.013	0.039	0.052	0.090	0.9816
7	0.002	0.010	0.029	0.051	0.088	0.9866

The results shown in table 5 indicate that 3 ml of arsenazo III reagent solution give the higher sensitivity and higher value of correlation coefficient, therefore it has been selected for subsequent experiments.

Effect of order of addition of reagents

The effect of order of addition of reagents on intensity of coloured complex is investigated (Table 6).

Table 6. The order of addition of reagents

Reaction components	Order number	Absorbance
SDA + cerium(IV) ion (O) + sulphuric acid (S) + arsenazo III (R)	I	0.206
SDA + O + R + S	II	0.097

The results indicate that the acidic medium is necessary for the redox reaction, so that order of addition of reagents should be followed as given under procedure (order I).

Effect of Time

The effect of time on the development and stability of the coloured complex for different amounts of SDA is investigated under the optimum experimental conditions established. Complete colour formation occurs immediately after all reaction mixtures are added and the absorbance of the complex remains constant for at least 1 hour (Table 7).

Table 7. Effect of time on the absorbance of complex

μg of SDA in 25 ml	Absorbance/min. standing time*												
	2	5	10	15	20	25	30	35	40	45	50	55	60
3	0.064	0.064	0.064	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.064	0.06
5	0.106	0.106	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.104	0.104	0.10
7	0.166	0.164	0.161	0.161	0.161	0.161	0.161	0.161	0.161	0.160	0.160	0.160	0.15

*After 25- min. reaction time of SDA with Ce(IV)

The results shown in table 7 indicate that the stability period is sufficient to allow several measurements to be performed sequentially.

Absorption spectra

Absorption spectra of the coloured complex formed from the reaction between cerium(III) ion with arsenazo IIII reagent in acidic medium against its corresponding reagent blank show maximum absorption at 654 nm in contrast to the arsenazo reagent blank which shows a weak absorption at the same wave length (Fig. 2).

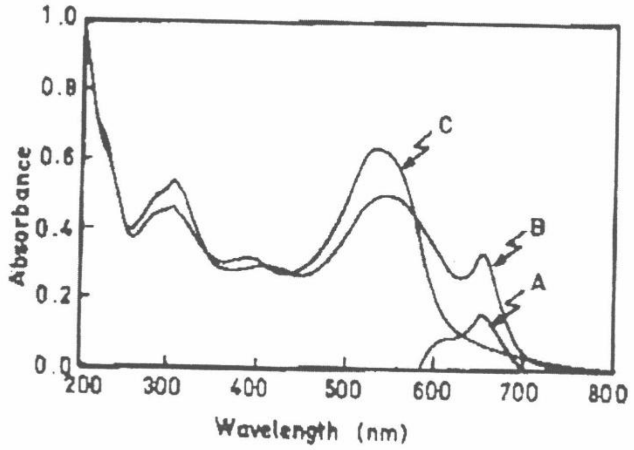


Fig.2: Absorption spectra of 10 μg sulphadiazine / 25 ml treated according to the optimum conditions and measured against (A) blank, (B) distilled water and (C) blank measured against distilled water.

Accuracy and precision

To check the accuracy and precision of the calibration graph, SDA is determined at three different concentrations. The results shown in table 8 indicate that the calibration graph is satisfactory.

Table 8. Accuracy and precision

Amount of SDA taken, μg/25 ml	Relative error*, %	Relative standard deviation*, %
2	-0.50	±2.15
5	+0.38	±1.75
10	+1.70	±1.84

* Average of five determinations

Nature of the reaction between SDA and cerium(IV) ion

Job's method has been used in the determination of the reaction ratio of SDA with cerium(IV) ion. The obtained results (Fig. 3) showed that a 1:4 SDA to cerium(IV) ion ratio is obtained.

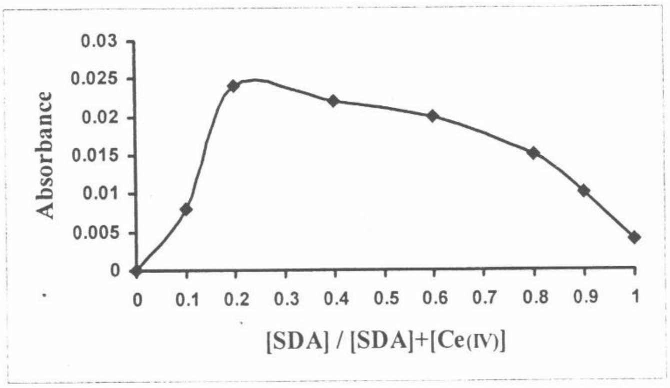


Fig.3: Job's plot for SDA-cerium (IV) ion

Nature of arsenazo III-cerium(III) ion complex

The stoichiometry of the reaction is investigated using the Job's method under the optimized conditions. The obtained results (Fig. 4) showed that a 1:1 arsenazo III to cerium(III) ion ratio is obtained.

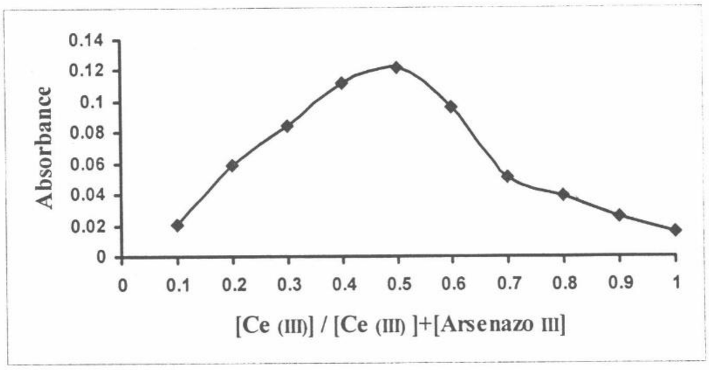
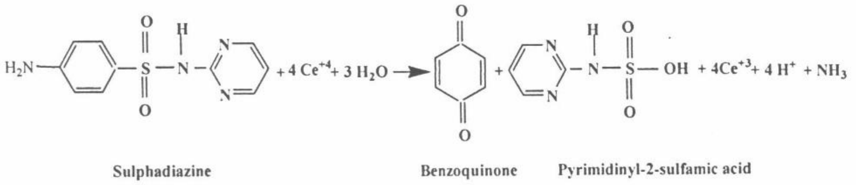


Fig. 3: Job's plot for arsenazo III-cerium(III) ion complex

The probable mechanism might be the following:



The above equation indicates a 1 : 4 sulphadiazine to cerium(IV) reaction ratio.

Analytical applications

The proposed method is applied to determine SDA in flammazine (burn cream containing 1% silver sulphadiazine) and in sulphaprim (veterinary injectable solution containing 20% sulphadiazine sodium). On applying proposed procedure, good recovery is obtained as shown in table 9.

Table 9. Analytical applications

Drug	Pharmaceutical preparation	Supplier	Certified value (g)	µg SDA present/25 ml	µg SDA measured/ 25 ml	Recovery (%)
Flammazine	Cream	Burn cream / Argentique pharma. company / France	1 % silver sulphadiazine	1	0.94	94.0
				4	4.10	102.5
				6	5.90	98.3
Sulphaprim	Injection	Veterinary injection / The arab pesticides and veterinary drugs / Jordan	20 % sodium sulphadiazine	2	2.05	102.5
				5	5.14	103.0
				8	7.86	98.0

The performance of the proposed method is assessed by calculating the student's t – test compared with the standard method (17). At the 95% confidence limit for four degree of freedom , the calculated t - values do not exceed the theoretical value (Table 10) , indicating that there is no significant difference between the proposed method and the standard method.

Table 10. Analysis of sulphadiazine pharmaceuticals by proposed and standard method

Drug	**Drug content (µg / 25 ml)**			**Recovery* (%)**		**t-exp.**
	Present	**Found**				
		Present method	**Standard method**	**Present method**	**Standard method**	
Flammazine	10	9.80	9.98	98.00	99.80	0.52
Sulphaprim	10	10.26	10.02	102.60	100.20	0.96

*Average of five determinations

Comparison of the methods

Table 11 shows the comparison between some of analytical variables obtained from the present method with that of a recent spectrophotometric method.

Table 11: Comparison of the methods

Analytical parameters	**Present method**	**Literature method**[(18)]	**Literature method**[(9)]
pH	2.80	3.19	3.0
Temperature (°C)	At room temperature	At room temperature	At room temperature
Development time (minutes)	5	10	10
λ_{max} (nm)	654	440	273
Medium of reaction	Aqueous	Aqueous	Aqueous
Beer's law range (ppm)	0.04 - 0.4	0.4 – 8.0	1.25 – 15.02
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	1.22×10^5	4.30×10^4	4.53×10^4
Relative error (%)	$\leq +1.7$	$\leq +0.02$	—
RSD (%)	$\leq \pm 2.15$	$\leq \pm 2.34$	$\leq \pm 0.490$
Colour of the dye	Green - blue	Yellow	—
K ($Molar^{-1}$)	5.60×10^5	5.28×10^4	—
Application of the method	Determination of Sulphadiazine in two drugs	Determination of sulphadiazine in one drug	Determination of sulphadiazine in one drug

The results in table 11 shows that the suggested method for the determination of sulphadiazine is more sensitive and the method has been applied to determine sulphadiazine in two drugs.

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