New Diaz Coupling Reaction, Cloud Point Extraction Spectrophotometric Determination of Sulphadimidine Soudium in Pure form and Pharmacetical Preparation with Salicylic Acid as the Coupling Reaction

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Abstract

In this research we study two simple methods, rapid of use spectrophotometric determination of sulphadimadine sodium (SDMS). The first method based on diazotization of drug by sodium nitrite at 5Cº followed by coupling with Salicylic Acid in basic medium to form yellow color. The product was measured at 453 nm. Beer's law is obeyed in the concentration range of (1-16) $\mu g \cdot ml^{-1}$. Sandell's sensitivity was 0.07575 $\mu g \cdot cm^{-1}$, the detection limit was 0.3992 $\mu g \cdot ml^{-1}$, and the limit of Quantitation was 1.07879$\mu g \cdot ml^{-1}$. The second method was used cloud point extraction (CPE) with used Triton X-114 as surfactant. Beer's law obeyed in the range of concentration was (1-12) $\mu g \cdot ml^{-1}$. Sandell's sensitivity was 0.04 $\mu g \cdot cm^{-1}$, the detection limit was 0.0259 $\mu g \cdot ml^{-1}$, and the limit of quantitation was 0.029563 $\mu g \cdot ml^{-1}$. All variables were study including of the reagent concentration, reaction time. The composition of product (1:1). The methods were effectively useful to the determination of SDMS in pharmaceutical dose form, and the attained results were in good agreement with the official and other methods in literature .No interference was observed from the commonly encountered additives and recipients.

Keyword: *Cloud Point Extraction, Salicylic acid , Triton X-114 , Sulphdimidine Sodium.*

Introduction

Sulfa drug (sulfonamide) were the fist antibiotics , discovered in 1935 by Gerhard Domagk [(1)]. Above 5400 products of sulfanilamide were formed, however around 20 products formulas are usually obtainable the structure of sulfonamide [(2)] .

Generally, sulfonamides are weak organic acids, relatively insoluble in water, more soluble in alkaline than acid [(3)]. Over-all characterizes of sulfadimidine Sodium are certain in table 1 [(4)].

Table (1) : General properties of SDMS :

Chemical structure	
Nomenclature	2,4,6-(Aminobenzensulfonamido- dimethylpyrimidine)

Cont... **Table (1) : General properties of SDMS :**

Other names	Sulfamethazine Sodium, Sulfadimethylpyrimidine sodium, Sulfamethiazine sodium, Sulfadine sodium, sulfodimesin sodium, ect.
Formula	$C_{13}H_{14}N_4O_2SNa$
Molecular Weight	300.312

Instrumentation and Apparatus:

Instruments:

UV-Vis spectrophotometer: SHIMADZU, Double beam UV-Vis, model UV-1800 made in Japan. The range of wavelength (190-1100) nm, cell quartz with path 1cm.,Water Bath : A thermostat water bath, Memmert, made in Germany, Electric Balance: Sartorius (0.0000), made in Germany, Centrifuge: Triup International corp, TRIU 800 Centrifuge, made in Korea & PH meter: HANNA, PH meter, HI 83141.

General procedure for Azo coupling:

The prepared Azo Coupling product are added in volumetric flask (10ml) in ice bath , 1ml of Sulphadimidine Sodium (SDMS) (1000 µg ml^{-1}) ,1ml for hydrochloric acid , 1ml for sodium nitrate (1%) ,1ml for sulphamic acid (1%), 1ml for salicylic acid (1000 µg ml^{-1}) ,at last added 1ml for sodium hydroxide and complete the volume by distilled water .Then absorbance is measured by UV-VIS. And the maximum wave length show in figure:(1-1).

General procedure for CPE:

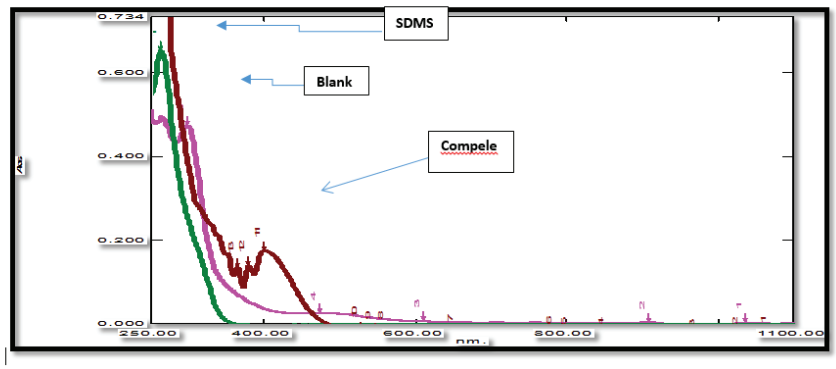
A typical experiment of cloud point include the following steps: taking the volumetric flask (10ml) and added the optimum condition of azo coupling and added 1ml for surfactant (10%) and complete the volume by distilled water . The contain of volumetric flask transfer to centrifuge test tube then added the mixture in water bath 60 C^0 at 20 min and separated by centrifugation 4000 rpm at 20 min. Test tube taken in ice bath to increased viscosity micelles layer 1min. then become easily separated . The separated sediment s dissolved by 1ml of ethanol and measured the absorbance by UV-VIS.

Result and Discussion

First methods: Spectrophotometric determination of sulphadimidine sodium (SDMS) by oxidation coupling reactions .

Optimization Parameters for Reaction .

All of the factors that affect to the absorbance of formation of azo dye product are optimized to improve the sensitivity and detection limit for the determination of the drugs .All optimization work under wavelength at 473 nm.



Figure(1) : Absorbance spectra of the Resulting Dye.SDMS

Effect of Acid Type.

In this study, made series experiment using (0.1 – 0.5 and 1 M) of different acid [HCl , H_2SO_4 , HNO_3 ,H_3PO_4 and CH_3COOH] that follow the same procedure that [1ml of drug SDMS , 1 ml of each acid , 1ml of $NaNO_2$, 1ml of H_3NSO_3, 1ml salsilic acid and 1ml of NaOH] in volumetric flask 10 ml and complete the volume by distilled water to formation diazonium salt. After that measuring the absorbance at 453 nm .

It is clear from this study that the phosphoric acid (0.5 M) gives higher absorbance for SDMS , this acid is a few of use in subsequent experiments.

Optimum Volume of 0.5M phosphoric acid .

The same addition for SDMS is [1ml drug , with varying volumes of 0.5 $H_3PO_4$from (0.1-1) ml , 1 ml $NaNO_2$, 1ml H_3NSO_3, 1ml Salsilic acid and 1ml of NaOH in 10 ml volumetric flask and complete the volume by distill water .Then measured the absorbance at 453 nm and the optimum volume for higher absorbance were fixed for sequence experiment 0.7 ml.

It is obvious that absorbance increased with increased of the volume of acid, suddenly the absorbance decrease because the primary amine becomes inactive [5] .

Base Type.

We used different bases [NaOH, KOH, K_2CO_3, Na_2CO_3, NH_4OH, $NaHCO_3$] with different concentration (0.1,0.5 and 1) M and that follow the addition in 10 ml volumetric flask. Then see the higher of absorbance for the base is KOH 90.5 M)

It is clear the potassium hydroxide give the higher absorbance , this base it is fixed in subsequent [6].

Optimum Volume of 0.5M KOH.

The same addition for SDMS in volumetric flask of 10 ml with different volume of KOH (0.1-1 ml) and complete the volume by distill water .Then the absorbance were measured at 453 nm and higher absorbance that fixed at 0.9ml.

It is clear that absorbance increased with increased the volume of KOH , but rapidly decrease the absorbance because the formation diazotate ions and decomposition of complex happen when increase basicity and agreement with previous studies [7] .

Optimum Volume of 1% Sodium nitrite.

The same additions were added with varying volume of 1% $NaNO_2$ from (0.1-1.5) ml and the absorbance were measured of optimum volume and fixed at 1.1 ml .

It is obvious the absorbance increase when the volume of $NaNO_2$ increase ,suddenly the absorbance decrease because the nitrate toxic became a high rate of pollutants affected on diazonium salt [8] .

Optimum Volume of 1% Sulphamic acid .

The additions were added of [1ml for SDMS,**0.7** ml H_3PO_4, **1.1** ml $NaNO_2$ with varying volume of 1% H_3NSO_3 from (0.1-1) ml, 1 ml salsilic acid and **0.9** ml KOH] in volumetric flask 10 ml and the higher absorbance were measured of optimum volume and fixed at 0.4 ml .

In this figure is obvious when the volume of Sulphamic acid increase , the absorbance increase however the absorbance decrease rapidly because this volume remove nitrite and escape as nitrogen gas[9].

Optimum Volume of (100 µg mL^{-1}) Reagent .

The same additions are for[1ml SDMS,**0.7** ml H_3PO_4, **1.1** ml $NaNO_2$ with varying volume of 1% H_3NSO_3 from (0.1-1.5) ml, 1 ml salicylic acid and **0.9** ml KOH] in volumetric flask 10 ml and the absorbance of optimum volume were measured **1.1 ml** at maximum wavelength and fixed for sequence experiment .

In this graph was clear when The volume of reagent increase, the absorbance increase but , gradually decrease because this volume was essential to coupling with SDMS [10] .

Reaction Time on Stability Color Product.

When the optimum volumes of parameters were complete we study effect of stability color product of SDMS [1ml SDMS,**0.7** ml H_3PO_4, **1.1** ml $NaNO_2$,1.1 ml 1% H_3NSO_3 , 1.1 ml salsilic acid and **0.9** ml KOH] in volumetric flask 10 ml . The stability of product was one of significant factor that diazotization and clouding reaction were depended on it, as a result the time needed was (0-60) min. Then the absorbance were measured and fixed the high reader at high maximum wavelength .

This apparent the best time of product stay stable for SDMS was 50 min [11].

Order Addition.

All additions of diazotization and coupling reaction are added for SDMS with optimum condition . Then followed diluted by different polar solvent [water , ethanol , methanol ,1- propanol ,acetonitrile & acetone] in volumetric flask 10 ml ,at maximum wavelength for each drug the absorbance are measured and recorded for the best solvent.

. It is obvious the greatest addition is the order two because it is give the higher absorbance [12] .

Type of Solvent :

All the compounds of the optimal parameters for SDMS were dissolved in volumetric flask and completed this volume for different solvent to study the best of solvent and measured the absorbance.

In this experiment shown the best of solvent was water because gave higher absorbance . It is sensitive , no expensive , economically and nontoxic[13].

Temperature:

In this experiment study the effect of different temperature on the color product have been (5-60 C^O) . the absorbance were measured and the higher absorbance was recorded .

It is clear the best temperature is 20C^O because was given the higher absorbance [14] .

Stoichiometric Determination of Product. :

Continuous Variation Method [15] **.**

A series of different volumes of reagent and drug are prepared (0.1-0.9) ml ,with concentration ($4x10^{-4}$ M) in volumetric flask 10 ml .The additions are optimal condition and complete the volume by distilled water (10). Then absorbance are measured by UV-VIS at λmax =473 nm . the stoichiometric ratio between reagent[R] and drug[D] result 1:1 .

Mole Ratio Method

In this method the volume of drug is fixed at I ml with concentration ($4x10^{-4}$M) and the volume of reagent is change (0.5-4.5 ml) . The optimum of addition is complete by distill water in volumetric flask 10 ml and the absorbance is measured by UV-VIS at λmax =473 nm. The stoichiometric ratio between reagent[R] and drug[D] result 1:1 .

3- Calibration Curve for complex of SDMS - α-Naphthol .

In this study the solution are prepared in volumetric flask 10 ml continue of different volume of SDMS (1-12) µg mL^{-1} by taken [0.7 ml H_3PO_4 , 1.1 ml **NaNO2** ,0.4 ml **H2NSO3** , 1.1 ml **Salicylic acid,** 0.5 ml **KOH**] . The volume complete by distill water and measured the absorbance by UV-VIS at maximum wave length against a blank solution prepared same condition without drug . Linear calibration graph is established by blotting absorbance against concentration of SDMS ,it found (1-12) µg mL^{-1} obeys the Bear Law . The molar absorption coefficient of product equals ($9,970x10^3 L.mol^{-1}.cm^{-1}$) and sandal's sensitivity (0.03012 µg mL^{-2}).

3- Effect of interference.

In this study effect of interference expected present with SDMS by added 1ml (1000 ppm), and the rest of optimum addition in volumetric flask 10 ml and complete by distill water. Then measured the absorbance by UV-VIS .

Table (2): Data of Absorbance of interference.

NO.	100ppm interference	Abs.	Recovery %	Erel%
1	Lactose	0.162	103.7878	3.78
2	Starch	0.171	110.606	10.606
3	Arabic Gum	0.156	99.2424	-7.6
4	Glucose	0.160	102.2727	2.272
5	Talc	0.159	101.515	1.515
6	Tri methyprine	0.161	102.78	2.98
7	Without interference	0.158	100.7575	0.757

This result show in table (1-13) there is no interaction between interference and SDMS [16].

4- The Stability Constant of Color Product.

The stability constant K Show in the table (1-14) .

Table (3) : Data of The Stability Constant of Color Product of SDMS.

Volume of 4x10-4M of SDMS / ml	Final con. SDMS /M	As*	Am*	A	K(L..Mol-1)	Mean of K (L.Mol-1)
0.3	1.2X10-3	0.064	0.066	0.03030	3.1811X106	2.39X106
0.5	2X10-3	0.093	0.096	0.03123	2.99 X105	
0.7	2.8X10-3	0.121	0.123	0.016260	0.999 X106	

It is clear the stability constant is high ,so the dye formed is very stable .

Am= the high absorbance , As = the few absorbance .

5- Accuracy and Precision Test .

The table (1-15) show the accuracy and precision of SDMS ,which study at different concentration (12,9.6,3) . It is clear this result has a good accuracy and precision.

6- Application:

The proposed method applied on [Montajat Pharmaceuticals. Saudi Arabia] injection (SULJAT) that contains (200mg) from Sulphadimidine Sodium in100ml) .The result is good quality and summarize in table (1-16).

Table (4): Data of Accuracy and Precision Test.

Amount of SDMS / µg mL-1	*Found	Recovery %	Average Recovery %	Erel%	Average Erel%	RSD%
12	11.8179	98.482	99.5017	-1.5175	0.2525	0.9630
9	9.12118	101.3464		1.3465		1.3890
6	5.0605	101.2116		1.2116		1.9514
3	2.9090	96.9668		-0.03033		4.3554

*= Average for five determination.

Second Method: Spectrophotometric determination of Sulphadimidine Sodium (SDMS) with using Cloud Point Extraction Technique.

Effect Type of Surfactant with SDMS.

The surfactant have an important part in cloud point extraction process .The basic of practical depended of micells for extraction .In this experimental we added all the last parameters with added 1ml of different surfactant in volumetric flask 10 ml and the volume completed by distilled water at $60C^{O}$ for 20 min. then by centrifugated separated at 4000rpm for 20 min.and dissolved in 1 ml ethanol and considered by UV-VIS at λmax=453nm .

The Volume of Triton X-114.

Effect of Equilibrium Temperature .

In series used varied temperature (35-70°) for 20 min. to formed cloud point and separation by centrifugation at 4000rpm for 20 min then dissolved by 1ml ethanol and measured by UV-VIS at λmax=453nm and fixed .

The best of Temperature were fixed at 50 C^0.

The Incubation Time.

The solution prepared of all the parameter and the incubation time for(5-35min) to form cloud point and separated by centrifugation then measured by UV-VIS at λmax=453 nm.

The best of incubation time is 20 min.

Preparation of Calibration Curve in CPE.

The set of experimental prepared by increasing concentration of SDMS (1-12 µg mL^{-1}) in volumetric flask 10 ml and the volume complete by distilled water then measured by UV-VIS at λmax=453nm .

Application:

The proposed method applied on (silphdimidine sodium33.3% injection . Jorden) .That contain (33.3 gm each 100gm) .The result is good and summarized in table (5).

Table (5): Data of Accuracy and Precision Test .

Amount of SDMS / µg mL-1	*Found	Recovery %	Average Recovery %	Erel%	Average Erel%	RSD%
12	11.968	99.7333	99.6444	-0.0266	-0.1155	0.2796
9	8.968	99.6444		-0.3555		0.3336
6	5.952	99.2		-0.08		0.5622
3	3	100		0		1.1925

*= Average for five determination.

Conclusion

The method of Cloud point extraction is demeans, good, safe and use pre-concentration technique to determine Sulphadimidine Sodium by UV/VIS. In designed method is a gentleness, selectivity and gave a low limit of detection and good RSD.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of chemistry, College of science for women, University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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