RESEARCH ARTICLE

Micro Evaluation of Sulphonamide in Biological Samples by Coupling Reaction

Ahmed J. Muklive Al-Ogaidi[1], Aliaa A. Razzak Mohammed[1*], Abeer Abdul Razak Mohammed[2]

[1]*University of Technology, Department of Applied Sciences, Applied Chemistry Section, Baghdad, Iraq*
[2]*Mustansiriyah University, College of Sciences, Department of Chemistry, Iraq*

Received: 27th June, 2020; Revised: 31th July, 2020; Accepted: 29th August, 2020; Available Online: 25th September, 2020

ABSTRACT

Sulphonamide is considered a turning point for therapeutic science. Structural changes in sulphonamide can lead to the formation of various drugs used for combating different diseases. Sulphonamide can be used in different applications, such as, antitumor agents, carbonic anhydrase inhibitors, anti-bacterials, hypoglycaemic agents, protease inhibitors, and diuretics.

The most important thing for this assay is to find a modified approach to assess sulphonamide by utilizing an organic reaction that depends on a process of coupling between our target material (sulphonamide) with 4-amino antipyrine in basic media of phosphate buffer (pH = 11.3), forming a colored complex containing a higher molar absorptivity (wavelength = 457 nanometers). A preliminary investigation test was done to determine the typical condition for this reaction to determine the concentration curve for the interval 8.25×10^{-9} to 1.15×10^{-2} ppm, and the absorptivity molar was 2.1×10^{4} $L.mol^{-1}.cm^{-1}$, RSD value greater than 1.12%, with a percentage of recovery of approximately 99.88%. We obtained the result and got the approved mole ratio for this reaction about 1:1 (sulphonamide:diazotized amino compound); the value of the stability factor reached 2.8×10^{6} $L.mol^{-1}$. This proposal could be used for a fair assessment for sulphonamide determination, which has different advantages, such as, low-cost economy, no need for an expert, simplicity, no need for more time, and high-quality results in the requirement of rapid and excellent determination. This approach can be utilized for validation of sulphonamide in different active biological samples with higher efficiency.

Keywords: Azo dyes, Biological samples, Coupling reaction, Spectrophotometric, Sulphonamide.

International Journal of Drug Delivery Technology (2020); DOI: 10.25258/ijddt.10.3.35

How to cite this article: Al-Ogaidi AJM, Mohammed AAR, Mohammed AAR. Micro evaluation of sulphonamide in biological samples by coupling reaction. International Journal of Drug Delivery Technology. 2020;10(3):509-512.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Sulphonamide, Figure 1, is considered a turning point for therapeutic science. Structural changes in sulphonamide could lead to the formation of various drugs for combating different diseases.[1] Sulphonamide could be used in different applications, such as, antitumor agents, carbonic[2] anhydrase inhibitors, anti-bacterial, hypoglycaemic agents, protease inhibitors, and diuretics.[3]

Various methods have been utilized to determine sulphonamide involved in high-performance liquid chromatography but this technique suffered some drawbacks related to the error in the detection limit.[4] A development study has been done to improve the valuation of this compound[5] in medical research with different patients and disease.[6]

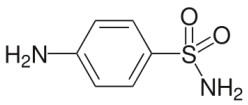

Figure 1: Structure of sulphonamide

For the analytical applications of samples, the most appropriate and easy methods are diazotizing and coupling reactions.[7] This is primarily useful for those problematic samples to determine using the original techniques and the interference in addition to that. A sensitive method to determine various applications as a diazotizing agent is through the formation of colored azo dye[8] that can absorb light at specific wavelengths. Due to the importance of sulphonamide[9] in combating different diseases, we must look for an excellent method[10] to determine this compound, which is present in trace amounts.[11]

A variety of methods and approaches have been used for the assessment of this compound. The methods involved include spectrophotometry,[12] enzyme-linked immunosorbent

**Author for Correspondence: alia.abdulrazzak7@gmail.com*

assay (ELISA),[13,14] liquid chromatography,[15] high-performance liquid chromatography (HPLC),[16] and liquid chromatography-mass spectrometry (LC-MS).[17] Therefore, it is of great significance to develop rapid and straightforward methods for sulphonamide determination.

In this method, our task was to develop a suitable procedure for the ultra micro determination of sulphonamide, depending on the diazotizing reaction of sulphonamide with 4-amino antipyrine in basic media.

MATERIAL AND METHODS

Standard Solutions

Sulphonamide Solution (50 µg/mL)

It was prepared by dissolving a 0.0125-gram of sulphonamide (from BDH ANALAR company) in 50 mL absolute ethanol, then completing it with distilled water to 250 mL volumetric flask.

Diazotizing Solution for 4-Amino Antipyrine (100 µg/mL)

It was prepared by weighing a 0.025-gram of 4-amino antipyrine from (BDH ANALAR), then dissolving it in 40 mL of distilled water, then heated the solution to increase the solubility. We then added 4 mL of 0.7 standard hydrochloric acids with cooling to zero degrees using an ice bath and then adding the mixture of 0.0077-gram sodium nitrate. The final diazonium solution was poured into a 250 mL volumetric flask after 5 minutes, then the volume was completed using cold distilled water to 4˚C, and the solution was kept in the freezer. The final solution became stable after 1-hour at room temperature, i.e., 22˚C.

Hydrochloric Acid (0.7M) (from Fluka AG company)

Hydrochloric acid (0.7M) was prepared with an approximate concentration by dilution of concentrated hydrochloric acid, then titrated with standard sodium carbonate to fix the concentration to (0.7µ).

Sodium Carbonate Na_2CO_3 (0.7M) (from BDH company)

Using dried sodium carbonate for an hour in 115°C, in a watch glass, then cooled it and it weighed 3.32449 gm. We then dissolved it in distilled water and completed it to 250 mL in a volumetric flask to the final volume.

Solution (8M) of Sodium Hydroxide (from Fluka AG Company)

It was prepared using the standard ammonia solution. To determine the exact molarity of ammonia, we prepared 8M of NH_4OH according to the dilution equation.

Buffer Solution

The buffer solution was prepared with a pH of 3.2 and 6.4, respectively—prepared 0.1M solution of Na_2HPO_4 and 0.2M solution of citric acid. Table 1 below shows the preparation of a buffer solution (buffer citrate).

The buffer solution was prepared with a pH of 9.8 and 11.1, respectively. Thus, we prepared two solutions: Na_2CO_3 (0.2M) and the other $NaHCO_3$ (0.2M). Table 2 represents the preparation of the buffer solution (carbonate and bicarbonate buffer).

The phosphate buffer solution with a pH of 11.6 was prepared. Took 70 mL of 0.3M Na_2HPO_4 solution and added 5.5 mL of 0.2M sodium hydroxide solution. We then diluted 250 mL by using distilled water.

Experimental Method

Determination of Sulphonamide by Coupling Reaction

The coupling reaction operation is done by mixing 50 µg/mL of sulphonamide with 100 µg/mL of 4-amino antipyrine in the presence of phosphate buffer solution (pH = 10.8). We added 3 mL of the diazotized compound to 6 mL of buffer solution, then added a different sulphonamide volume, then added to it 50 mL distilled water. After that, we measured the absorptivity (A) for this solution against the blank solution at a wavelength of 450 nm, as illustrated in Figure 2. The calibration curve was drawn between the absorbance and the concentration, and a straight line was obtained.

DISCUSSION

Primary Test

We noticed a complex salt formed from mixing sulphonamide compound with 4-amino antipyrine in basic media. The reaction involved two steps, which are:

- 4-amino antipyrine compound reacted in acidic media in 0˚C with an equal amount of sodium nitrate to give diazonium salt.
- Single azo dye, orange in color, was formed when sulphonamide was added to the diazotizing compound in basic media, which gave high absorptivity at a wavelength of 455 nm.

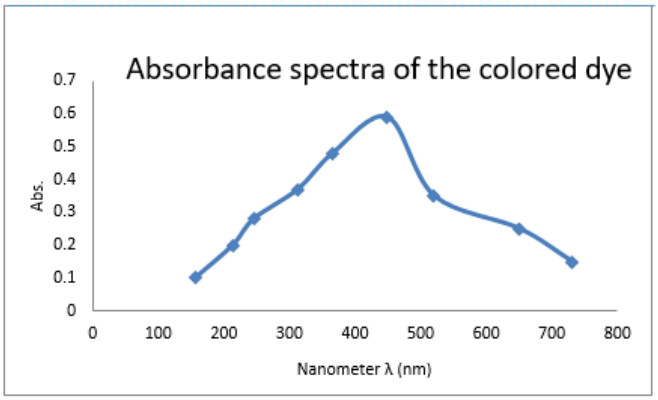

Figure 2: Spectroscopic curve for colored product

Table 1: Buffer solution (buffer citrate)

pH	*0.1M Na_2HPO_4 (mL)*	*0.2M citric acid (mL)*
2.3	2.13	19.65
7.4	18.45	1.59

Table 2: Buffer solution (carbonate buffer)

pH	*Na_2CO_3 (0.2M) (mL)*	*$NaHCO_3$ (0.2M) (mL)*
9.8	4	6
11.1	10	2

Types of Parameters Influencing Reaction

Effect of pH

It was noticed that no azo dye is formed in neutral and acidic media. A study of sulphonamide with diazotizing compound 4-amino antipyrine in neutral, acidic, and basic media was conducted to reach optimum conditions (i.e., high sensitivity, fast reaction, and low absorbance) for the blank solution. The best media for the coupling reaction is the basic media, whose pH = 10.8, as shown in Table 3.

5mL of buffer solution was used for every 25 mL of the final solution. Table 3 shows that the best buffer solution for the coupling reaction is a phosphate buffer.

Type of Buffer Solution

The buffer solution's effect after the perfect value for the pH equal to 10.8 was known to study the solution's effect on the absorptivity of phosphate buffer NaOH-Na_2HPO_4 and KCL-NaOH buffer. Table 4 represents the kind of buffer solution.

Intensity of Absorbance Measurements against pH

After fixing the optimum condition for the reaction, an experiment was made to give the perfect volume for the buffer solution, which gives high sensitivity. Table 5 shows that 4 mL is the ideal volume for the buffer solution, which gives a high sensitivity. Table 5 also shows that 4 mL is the perfect volume for the buffer solution.

Measuring Quantity of Diazotized Compound

For measuring the amount of diazoting compound for the 4-amino antipyrine compound, an experiment was conducted to show the size effect of the diazoting solution on the absorptivity. Table 6 shows the final result. The diazoting agent's perfect size is found to be 3 mL.

Priority Addition

With the use of a phosphate buffer solution, order addition was measured after fixing the diazotizing agent for the p-amino antipyrine compound. Order addition measurement is vital for the solution and its effect on the formed azo day compound's intensity color. Thus, we studied order addition. Table 7 represents the result.

The reaction component was considered on order number one based on its high sensitivity.

Table 3: Effect of pH

Kind of buffer	*pH*	*λ_{max} (in nm)*	*Absorbance*
Na_2HPO_4	2.6	317	0.18
Na_2HPO_4	7	430	0.29
Na_2CO_3-$NaHCO_3$	9.3	366	0.328
Na_2CO_3-$NaHCO_3$	10.4	444	0.368
Phosphate buffer	10.8	449	0.661
Hydroxide-chloride buffer	13.8	433	0.285

Table 4: Buffer solutions

Kind of buffer	*pH*	*Λ_{max} (in nm)*	*Absorbance*
Na_2HPO_4-NaOH	10.8	449	0.661
KCL-NaOH	10.8	449	0.357

Color Stability

The color stability for the formed complex from the reaction was studied. An experiment was done to explain the stability of the complex's color formed due to the reaction between the sulphonamide and 4-amino antipyrine compound in the presence of phosphate buffer with optimum conditions, as shown in Table 8. The stability time for the color of the complex was found to be 30 minutes.

Calibration Curve Assay

The calibration curve to a series of the volumetric flask (250 mL) was measured. We added 2 mL of diazotizing agent solution, followed by 4 mL of phosphate buffer solution. Then, poured 2 to 12 mL of 50 μg/mL sulphonamide solution. Then, completed with distilled water until it reached the mark. Then, we measured the absorbance against the blank solution at 450 nm after 30 minutes, from reaching the final solution (250 mL). Figure 3 shows the standard curve.

Table 5: Typical buffer solution volume

Different solutions for buffer (in mL)	*Absorbance*
2	0.214
2	0.312
3	0.254
4	0.661
5	0.362
6	0.245

Table 6: Effect of diazoting solution size on absorptivity

Size of diazotizing solution (in mL)	*Absorbance*
1	0.254
3	0.661
5	0.153
6	0.321

Table 7: Effect of order addition on absorptivity

Order number	*Reaction component*	*Absorbance*
1	A + D + W	0.661
2	W + D + A	0.243
3	W + A + D	0.352

D = buffer solution; W = sulphonamide; A = diazotizing agent

Table 8: Color stability

Absorbance	*T (minutes)*
0.177	5
0.267	10
0.423	15
0.324	20
0.421	25
0.661	30
0.581	1-hour
0.426	2 hours
0.325	24 hours

Table 9: Result of accuracy

Represent value	*Found value*	*Error%*	*Recovery%*	*RSD%*
10	10.55	0.56	96.7	±1.07
60	60.45	2.78	101.1	±1.23
100	98.45	0.89	99.3	±1.12

Table 10: Sulphonamide assessment in biological media

	Sulphonamide assessments				
Samples	*First*	*Second*	*Third*	*Fourth*	*Fifth*
Reading	4.65 ± 0.01	4.34 ± 0.08	4.48 ± 0.09	3.99 ± 0.02	4.44 ± 0.07

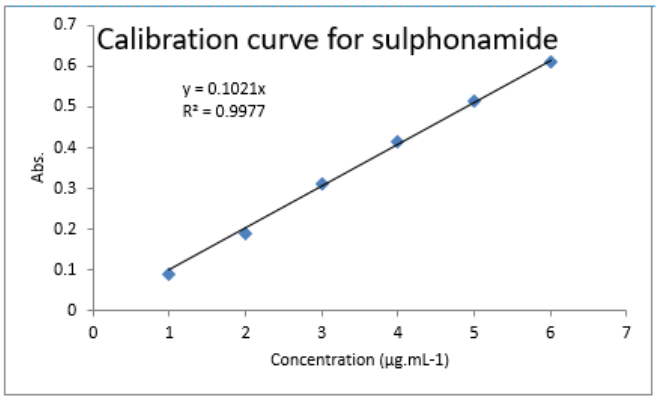

Figure 3: Calibration curve of sulphonamide

Study of Accuracy and Compatibility of Method

The method's accuracy and compatibility were studied at different concentrations of sulphonamide (10, 60, and 100 µg/mL), using an optimum condition. Table 9 represents the result.

Analytical Application

The results obtained for the coupling reaction for micro determination of sulphonamide involved all the typical conditions for this determination. We can then use this approach to determine sulphonamide for persons taking this medicine. Therefore, we obtained results using the coupling reaction between the diazotized compound of 4-amino antipyrine and target material (sulphonamide). It also illustrated how this method is considered an excellent technique for the determination of sulphonamide. Table 10 explains the results.

CONCLUSION

The proposed method showed that this technique is an excellent procedure to determine sulphonamide, especially in trace amounts. We can also consider this method, an advice procedure, to determine sulphonamide using a coupling reaction of diazotized 4-amino antipyrine with sulphonamide.

REFERENCES

1. Velisek J, Cejpek K. Vitamins.: part 1-a review. Czech. J. Food Sci. 2017;25:49–64.
2. Linster CL, Van Schaftingen E. Vitamin C-Biosynthesis, recycling and degradation in mammals. Febs J. 2018;274:1–22.
3. Davey MW, Van Montagu M, Inze D, Sanmartin M, Kanellis A, Smirnoff N, Benzie IJJ, Strain JJ, Favell D, Fletcher JJ. Sci. Food Agric. 2018;80:825–860.
4. Noctor G, Foyer CH. Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant molec. Biol. 2018;49:249–279.
5. Ahmed Jassim Muklive AL-ogaidi, Ultra Micro Determination of Ascorbic Acid in Biological Samples by Coupling Reaction, 2nd International Conference on Materials Engineering & Science (IConMEAS 2019).
6. Meister A. Glutathione Ascorbic-Acid Antioxidant System in Animals. J. Biol. Chem. 2017;269:9397–9400.
7. Hart,R.D, scutz, "Organic Chemistry A Short Course", 5th Ed. Oughon Mifflin Comp., Boston, USA., 2017;19:25-36.
8. Zolliningar, Azo and Diazo Chemistry, Inter Sience Publishers, New York. 2018;7:20-31.
9. Englard S, Seifter S. The Biochemical Functions of Ascorbic-Acid. Annu. Rev. Nutr. 2017;6:365–406.
10. Levine M., Rumsey S.C., Daruwala R., Park J.B., Wang Y.H. Criteria and recommendations for vitamin C intake. JAMA-J. Am. Med. Assoc. 2018;281:1415-1423.
11. Gomez-Romero M, Arraez-Roman D, Segura-Carretero A, Fernandez-Gutierrez A. Analytical determination of antioxidants in tomato: Typical components of the Mediterranean diet. J. Sep. Sci. 2017;30:452-461.
12. Vinas P, Balsalobre N, Cordoba MH. Analytical Chimica Acta. 2016;558:11–15.
13. Bogusz MM, Hassan H, Al-Enazi E, Ibrahim Z, Al-Tufail M, Journal of Chromatography, 2016, B (807), 343- 356.
14. Gantverg A, I Shishani I, Hoffman M. Analytica Chimica Acta, 2015, 483, 125-135.
15. Scortichini G, Annunziata L, Haouet MN, Benedetti F, Krusteva L, Galarini R. Analytica Chimica Acta. 2016;535, 43-48.
16. Kai Y, Wang XH, Zhang W, Yang L, Liu P. International Journal of Science Innovations and Discoveries. 2016;2(6):610-616.
17. Shelke SP, Thorat M. International Research Journal for Inventions in Pharmaceutical Sciences. 2015;1(1):27-29.