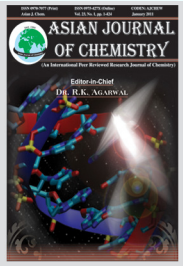
Asian Journal of Chemistry; Vol. 23, No. 3 (2011), 1163-1166

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Indirect Determination of Sulfamethazine Sodium by Cu^{2+} Complexation Extraction and Flame Atomic Absorption Spectrophotometry

XIAOLING WEI*, DONGMEI LI, LIANGWEI DU and QI GONG

College of Chemistry and Chemical Engineering, Guangxi University, Nanning, 530004, P.R. China

*Corresponding author: Tel: +86 771 3236316; E-mail: wxl1651@163.com

(*Received*: 7 April 2010; *Accepted*: 6 November 2010) AJC-9258

Sulfamethazine (SM_2) salt and Cu^{2+} combined to form 2:1 complex in the solution of pH 5.0-6.5. After the complex was extracted with chloroform, the absorbence of Cu^{2+} remaining in water phase was determined by flame atomic absorption spectrometry (FAAS). It was found that the absorbancy difference (ΔA) of Cu^{2+} in raffinate solution of reagent blank solution and sample solution increased linearly with the concentration of sulfamethazine. Under the optimum conditions, the linear range of sulfamethazine was 14.6-144.1 mg L^{-1}, the linear regression equation was ΔA = 0.0025c - 0.0046 (r = 0.9991), the detection limit for sulfamethazine was 5.6 mg L^{-1} and the recovery was 99.3-99.6 %. This method was used to determine the sulfamethazine sodium injection samples with satisfactory results.

Key Words: Sulfamethazine, Cu^{2+} complexation, Flame atomic absorption spectrophotometry, Indirect determination.

INTRODUCTION

Sulfamethazine is widely used in the treatment of infectious diseases such as hemolytic streptococcal, meningococcal and pneumococcal with long-term efficacy. It is particularly suited to those patients who lack tolerance to antibiotics. As feed additives, fowl cholera, fowl typhoid and coccidiosis in chickens can be treated with many advantages such as good absorbability, wide distribution, high antibacterial detoxification ability, long half-lift, rapid effect, not easy to repeat and so on. Great product benefits promoted the rapid development of chemical research and production of the sulfa drugs in the pharmaceutical industry. Many methods for the determination of sulfa drugs have emerged. At present, the determination methods of sulfamethazine are as follows: enzyme-linked immunosorbent assay[1], UV-visible spectrophotometry[2-4], fluorescence polarization immunoassay method[5], capillary electrophoresis[6], efficient solution gas chromatography[7-9], thin layer chromatography[10] and liquid chromatography-mass spectrometry (LC-MS)[11] *etc*. Immunosorbent assay has good selectivity but its operation was complicated and time-consuming. UV-Visible spectrophotometry was simple to operate with poor selectivity. Chromatography and LC-MS was high cost. In this paper, sulfonyl group of sulfamethazine molecules with the excess Cu^{2+} combined to form complex which was extracted with chloroform. The absorbance of copper remained in the aqueous phase was determined by FAAS. It was found that the absorbance value of Cu^{2+} was decreased linearly with the increases of drug concentration in the sample. Accordingly, a simple, rapid and accurate method for the indirect determination of sulfamethazine content was established. To our knowledge, this method has not been reported up to now.

EXPERIMENTAL

TAS-990 atomic absorption spectrophotometer (Beijing Purkinje General Instrument Co. Ltd.) with copper hollow cathode lamp was employed for the absorption spectra. Test conditions was as follows: wavelength, 324.7 nm; spectral passband, 0.4 nm; lamp current, 3.0 mA; burner height, 3 mm and gas flow, 1500 mL min^{-1}. HH-38 thermostatic water-bath (Changcheng Technology and Business Limited Company, Zhengzhou) and Leici pHS-3C pH meter (Shanghai Precision Scientific Instrument Corporation) were used in the experiments.

Sulfamethazine of analytical pure standard substance was obtained from National Institute for the Control of Pharmaceutical and Biological Products. Copper sulphate of analytical pure was purchased from Shanghai Xinbao Fine Chemical Plant. Chloroform of analytical pure was purchased from Shanghai Reagent Plant. Sodium hydroxide of analytical pure was purchased from Shanghai Chemical Reagent Co. Ltd. Water used in the experiments was distilled water.

Sulfamethazine sodium standard solution preparation: 0.2783 g sulfamethazine was accurately weighed, then dissolved in 10 mL 0.1 mol L^{-1} sodium hydroxide solution, transferred to 50 mL brown volumetric flask, added distilled water to the

scale and mixed. The concentration of solution was 2×10^{-2} mol L^{-1}. The solution was diluted to 2×10^{-3} mol L^{-1} before use.

Cu^{2+} standard solution preparation: 0.4994 g $CuSO_4 \cdot 5H_2O$ was accurately weighed and dissolved in 100 mL distilled water. The concentration was 2.00×10^{-2} mol L^{-1}. It was diluted to 2.00×10^{-3} mol L^{-1} before use.

Procedure: 1.20 mL Cu^{2+} standard solution and a certain amount of sulfamethazine sodium test solution were added into a 25 mL colorimetric cylinder with plugs, then diluted by distilled water to 10 L scale and mixed well. After the reaction solution was placed in 40 ºC water-bath for 10 min, 10 mL chloroform was added to extract for 10 min. After static stratification, 2.00 mL upper liquid (aqueousphase) was carefully sucked to place into a 10 mL volumetric flask and diluted with distilled water to the scale, then shaken up. This solution was injected into Air C_2H_2 flame and the absorbance value A of Cu^{2+} in raffinate solution of the test solution was measured. Under the same condition, absorbance value A_b of Cu^{2+} in raffinate solution of reagent blank solution was measured and $\Delta A = A_b - A$ was calculated.

RESULTS AND DISCUSSION

Extractant selection and dosage study: The extraction capacity of different extractant to complexes was not the same. In this section, chloroform, carbon tetrachloride, dichloroethane and dichloromethane were chosen as extractants for the test. 2.00 mL sodium sulfamethazine standard solution and 1.00 mL Cu^{2+} standard solution were added, respectively into four 25 mL colorimetric cylinder with plugs, then fixed volume with distilled water to 10 mL and shaken to ensure thorough mixing. After reacted for 0.5 h at room temperature, 10 mL chloroform, carbon tetrachloride, dichloroethane, dichloromethane were added for extraction, respectively. The absorbance value of Cu^{2+} in the aqueous phase was determined by FAAS. The smaller A with the more complete extraction indicated the stronger extraction ability of extractants. Experimental results showed that the high-to-low order of their extraction ability was chloroform, dichloromethane, tetrachloride, dichloroethane. Thus chloroform was chosen as extractant in this experiment. When the organic solvent of ether was used as extractant, a small amount of organic solvent into the aqueous phase that was sprayed into the combustion flame caused a serious background absorption and the unstable flame. Therefore ether was not selected.

In this experiment, the amount of extractant was also studied. We fixed the amount of Cu^{2+} solution and drug solution. After extraction with chloroform by different volume, the absorbance of Cu^{2+} in raffinate solution was measured. The results showed that the absorbance reduced with the increase of chloroform volume. A remained stable after the volume of chloroform increased to 10 mL, which indicated that the complex extraction has been complete. Therefore, 10 mL chloroform was selected in this experiment.

Amount of Cu^{2+} solution: 2.00 mL sodium sulfamethazine standard solution was placed into 25 mL colorimetric tube. We changed the amount of Cu^{2+} standard solution with other conditions fixed, then ΔA was determined according to the experimental method. The results were shown in Fig. 1. When the volume ratio of Cu^{2+} solution to sodium sulfamethazine was greater than 1:2, ΔA achieved maximum and stable, which proved that the reaction has been complete. In this study, the volume of Cu^{2+} solution was chosen as 1.20 mL.

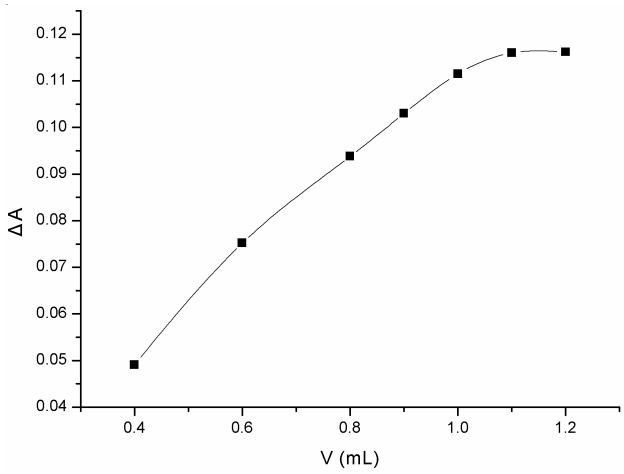


Fig. 1. Effect of amount of reagent on ΔA

Acidity effect: According to the experimental method, we fixed other conditions and regulated solution acidity with H_2SO_4 (0.1 mol L^{-1}) and NaOH (0.1 mol L^{-1}). The effect of solution acidity on the complex reaction was studied. The experimental results were shown in Fig. 2. When pH is less than 5, ΔA was small. This result showed that the remaining Cu^{2+} in aqueous phase was more and the complex may be difficult to generate in the acidic medium. ΔA was large and stable at pH = 5.0-6.5, which indicated the more complete complex reaction. When pH was more than 6.5, the test solution and blank solution could generate $Cu(OH)_2$ precipitation, then ΔA was decreased. Therefore, pH of the complex reaction was about 6 in this study. The pH of the as-prepared test solution was just 6 or so. Thus, experimental procedure was more simplified without the addidation of buffer solution.

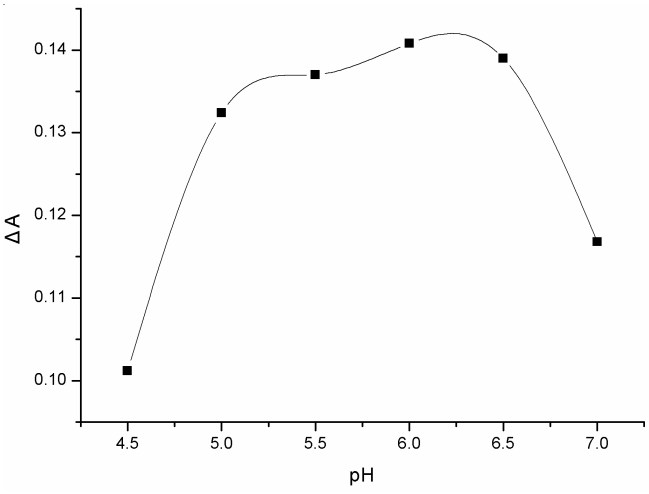


Fig. 2. Influence of pH on ΔA

Temperature influence: According to the above-mentioned experimental method, we took 2.00 mL sodium

sulfamethazine standard solution and fixed other conditions. The influence of temperature on complex formation was studied in the temperature range from 25-60 ºC. The results were shown in Fig. 3. At first, ΔA was small and increased with the increase of temperature, which indicated incomplete complexation at lower temperature. Then, the largest ΔA at 40 ºC indicated the most complete complex reaction. When the temperature exceeded 40 ºC, ΔA was decreased, which may be caused by the gradual decomposition of complex. Thus, the optimum complex temperature was 40 ºC.

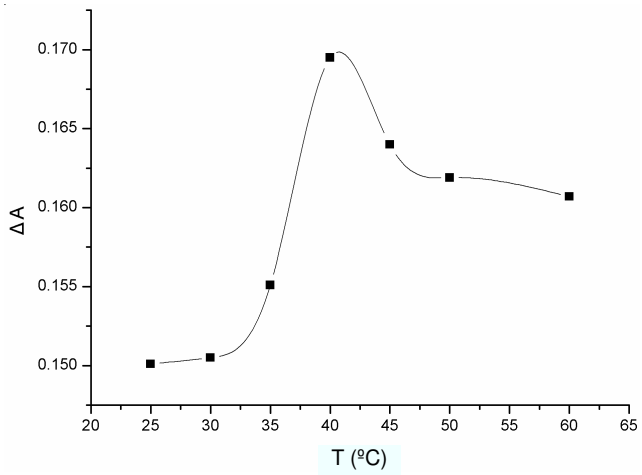


Fig. 3. Influence of temperature on ΔA

Choice of reaction time and extraction time: In this section, we changed the reaction time, but fixed the amount of sulfamethazine sodium and Cu^{2+} standard solution. Under this condition, ΔA was measured according to the experimental method. When the reaction time was more than 5 min, ΔA was stable and the complexation reactions reached equilibrium. In this study, 10 min of the reaction time was selected.

After 10 min of complex reaction, extractant was added to extract. We changed the extraction time and ΔA was measured. Experiments showed that the complex can be extracted completely in 5 min. In this study, 10 min of the extraction time was selected.

Precision: 2.00 mL sulfamethazine sodium standard solution was placed into eleven 25 mL colorimetric cylinder. The eleven ΔA values were subsequently determined and used to calculate relative standard deviation (RSD) with the result of 0.6 %.

Association ratio and stability constant of complex: Fig. 4 shows the plot of the ΔA values *versus* C_{drugs}/C_{Cu}. The association ratio of sulfamethazine sodium and Cu^{2+} is approximate 2:1 (Fig. 4).

In the point of C_{drugs}:C_{Cu} = 2:1, the total concentration of metal ions in solution before extraction was same with that in blank solution. While, Cu^{2+} in aqueous phase after extraction can be regarded as production by complex dissociation. Therefore, complex dissociation degree α can be calculated by:

$$\alpha = \frac{A}{A_b}$$

in which A is the absorbance value of Cu^{2+} in aqueous phase after extraction and A_b is the absorbance value of Cu^{2+} in blank solution. The stability constant K is calculated by the equation as follows:

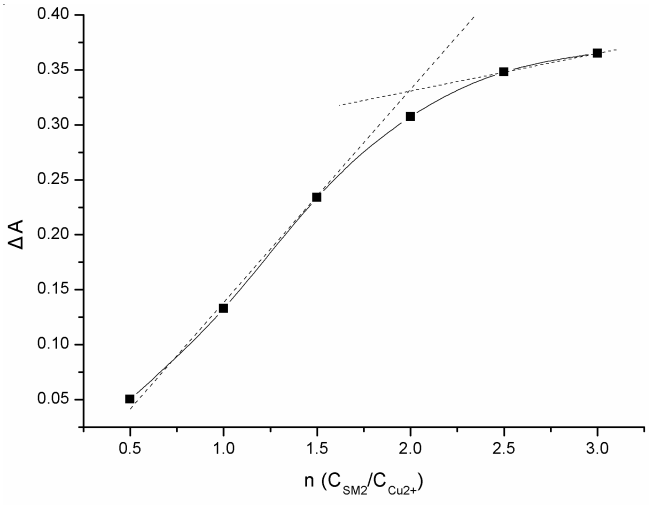


Fig. 4. Complex compound molar ratio

$$K = \frac{[MLn]}{[M][L]^n} = \frac{C_M(1-\alpha)}{C_M\alpha(2C_M\alpha)^2}$$

The calculated results are α = 0.229 and $K = 3.99 \times 10^8$.

Linear range and detection limit: The working curve was obtained by plotting the ΔA values with the concentrations of sulfamethazine sodium solution. The results established that the ΔA values are proportional to the sulfamethazine sodium concentration in the range from 14.6-144.1 mg L^{-1}. The equation educed by regression analysis is $\Delta A = 0.0025C_{drugs} - 0.0046$ with a correlation coefficient of 0.9991. The detection limit calculated by the IUPAC method is 5.6 mg L^{-1}.

Interference test: The interfering tests were carried out with the allowable error ≤ ± 5 %, in which 1.0 mL 2.0×10^{-3} mol/L sulfamethazine sodium solution was chosen. We studied the possible interfering substances which were often used in drugs such as lactose, glucose, starch, stearic acid, Ca^{2+} and Mg^{2+} *etc*. Results showed that 150 times' starch, stearic acid, glucose and 30 times' lactose, Mg^{2+} had no interference on the measurement result. The Ca^{2+} had a great interference on the system, but sulfamethazine sodium samples did not contain Ca^{2+}. Thus, the interference of Ca^{2+} can not be considered.

Analytical application: This method was used to determine sulfamethazine sodium injection. The test solution of sulfamethazine sodium was prepared as follows: 0.60 mL sulfamethazine sodium injection with the labeled amount of 1 g/10 mL was placed into 100 mL volumetric flask, then distilled water was added to the scale and shaken up.

Taken 1.00 mL sample solution into 25 mL colorimetric cylinder and added 1.20 mL 2.00×10^{-3} mol L^{-1} Cu^{2+} standard solution, absorbance difference of Cu^{2+} in raffinate of blank solution and test solution was measured according to the experimental method. The measured ΔA was substituted into the regression equation of standard curve to calculate sulfamethazine sodium content. At the same time, recovery experiment was carried out by standard addition method. The determination results of smaples were illustrated in Table-1.

TABLE-1
RESULTS OF SAMPLES (n = 5)

Sample code	Labeled amount (mg)	Measured value (mg)	Equivalent to labeled amount (% ± SD)	Added (mg)	Measured value (mg)	Recovery (% ± SD)
1	0.600	0.603	100.6 ± 1.4	0.240	0.841	99.2 ± 1.2
2	0.600	0.603	100.6 ± 0.8	0.240	0.839	98.3 ± 1.6

Notes: Sample 1 is also named with the specification of 1 g/10 mL and the lot number of 20090802, which was purchased from Chongqing Fang Tong animal Pharmaceutical Co. Ltd. Sample 2 has the local name of, which was obtined from Tongcheng Cole Pharmaceutical Co. Ltd. with the specification of 1 g/10 mL and the lot number of 09050201. Labeled amount is the measured value of manufacturers according to the national standard method.

Conclusion

In this study, drug and metal ions combined to form complex which was extracted by the organic solvent, then the metal ions in raffinate solution was measured by flame atomic absorption spectrometry. Based on this, an indirect method for the determination of drug content has been established. This method is simple and used for the determination of sulfamethazine sodium content with reliable and accurate results. This method break the limit that atomic absorption can only determined inorganic sample, expand the application scope of atomic absorption and offer a new analytical processing for the drug analysis.

REFERENCES

1. S.L. Wei, J.H. He and J.H. Yang. *Chin. Feed*., **14**, 32 (2007).
2. Y.F. Wang, W.J. Shi, Y.H. Sun and J.Z. Yang, *Chin. J. Anal. Lab*., **26**, 29 (2007).
3. Y.N. Ni, Z.B. Qi and S. Kokot, *Chemom. Intell. Lab. Syst.*, **82**, 241 (2006).
4. P. Nagaraja, H.S. Yathirajan, C.R. Raju, R.A. Vasantha, P. Nagendra and M.S. Hemantha Kumar, *Farmaco*, **58**, 1295 (2003).
5. Z.H. Wang, J.H. Shen and S.X. Zhang, *Chin. J. Veterinary Med.*, **43**, 72 (2007)
6. G. Font, A.J. Garcia and Y. Picó, *J. Chromatogr. A*, **1159**, 233 (2007).
7. C. Li, H.-Y. Jiang, S.-J. Zhao, S.-X. Zhang, S.-Y. Ding, J.-C. Li, X.-W. Li and J.-Z. Shen, *Chromatographia*, **68**, 117 (2008).
8. C.Y. Lin and S.D. Huang, *Anal. Chim. Acta*, **612**, 37 (2008).
9. N. Furusawa, *J. AOAC Int*., **85**, 848 (2002).
10. E. Kilinc, B. Gumgum, C. Hamamci and and F. Aydin, *J. Anal. Chem.*, **64**, 714 (2009).
11. T.A.M. Msagati and M.M. Nindi, *Talanta*, **64**, 87 (2004).