2-Aminotoluene——A solution of 2-nitrotoluene (135.5 mg) in 6 ml of AcOH–H_2O (1: 1, v/v) was treated with 5.1 ml (8 mol equiv.) of aqueous $TiCl_3$ (added as a single portion), and the whole was stirred for 7 min at 15°. After usual work-up, 99.5 mg (y. 94.0%) of 2-aminotoluene was obtained. All spectral data were identical with those of an authentic sample.

Acknowledgement The authors are grateful to Prof. Chikara Kaneko for his encouragement throughout this work. Thanks are also due to the staff of the analytical section of our university for the elemental analyses and mass spectra.

Chem. Pharm. Bull.
28(8)2518—2522(1980)

Analytical Studies on Pyrimidine Derivatives. V.[1] Simple and Rapid Spectrophotometric Determination of Silver (I) with 5-*p*-Dimethylaminobenzylidene-2-thiobarbituric Acid[2]

Kenichiro Nakashima and Shuzo Akiyama

Faculty of Pharmaceutical Sciences, Nagasaki University[3]

(Received February 25, 1980)

The spectrophotometric determination of silver(I) with 5-*p*-dimethylaminobenzylidene-2-thiobarbituric acid (DABTB) was studied and two simple and rapid methods are proposed. One is based on photometry of the DABTB-Ag(I) complex in ethanol-buffer (pH 6) solution (method A), and the other is based on measurement of the decrease in absorbance of DABTB due to the complex formation (method B).

Beer's law holds over the range of 0.04—2 μg/ml of silver(I) at 400 nm in method A, and 0—35 μg of silver(I) at 484 nm in method B. The molar extinction coefficient of the complex is $2.5\times10^4 \cdot l \cdot mol^{-1} \cdot cm^{-1}$ in method A. Hg(I,II), Au(III), Pd(II), Pt(IV), and various anions such as Br^-, Cl^-, I^-, SCN^-, CN^-, $S_2O_3{}^{2-}$, and S^{2-} interfered with the determination.

These methods were successfully applied to the determination of silver(I) in commercial preparations such as silver nitrate eye lotion and silver protein.

Keywords——5-*p*-dimethylaminobenzylidene-2-thiobarbituric acid; silver(I); spectrophotometric determination of silver(I); silver nitrate eye lotion; silver protein

The coloration mechanism of 6-aminouracil derivatives with *p*-dimethylaminobenzaldehyde and the application of the coloration in a colorimetric determination method have been reported by one of the authors (K.N.).[4] In the course of these studies it has become apparent that *p*-dimethylaminobenzylidenebarbituric acid derivatives were readily obtained as single reaction products. We investigated the utilization of these compounds as analytical reagents for metal cations. Among these benzylidene derivatives, 5-*p*-dimethylaminobenzylidene-2-thiobarbituric acid (DABTB)[5] efficiently forms complexes with heavy metal cations such as silver (I), mercury (I and II), gold (III), palladium (II), and platinum (IV). Silver (I)-DABTB complex[6] showed the absorption maximum in a wavelength region where the reagent blank

---

1) Part IV: K. Nakashima and S. Akiyama, *Yakugaku Zasshi*, **100**, 515 (1980).
2) A part of this work was presented at the 18th Annual Meeting of the Japan Society for Analytical Chemistry, Koriyama, October 1979, p. 464.
3) Location: *1-14, Bunkyo-machi, Nagasaki 852, Japan.*
4) K. Nakashima, *Yakugaku Zasshi*, **97**, 202 (1977); *idem, ibid.*, **97**, 906 (1977).
5) DABTB has been utilized for the qualitative detection of rare earth elements; T. Pavolini and F. Gambarin, *Anal. Chim. Acta*, **3**, 27 (1949).
6) The structure of this complex is now under investigation.

scarcely absorbed. This led us to develop a rapid and simple spectrophotometric determination method for silver (I). In this paper, we report the results of studies to determine the optimal experimental conditions for the determination of silver (I), and two determination methods have been established. These methods are applicable to the analysis of silver in commercial preparations.

Results and Discussion

Two methods have been devised for the determination of silver (I).

1) DABTB reacts with silver (I) to form an orange complex in both weak acidic and neutral solutions. The absorbance of the complex is proporational to the quantity of silver (I). Consequently, this determination is based on photometry of the complex solution (Method A). However, high concentrations of silver (I) resulted in the formation of colloidal complex solutions. Therefore the photometric procedure had to be done in very dilute solution ($<1.8 \times 10^{-5}$ M).

2) The color of DABTB benzene solution fades rapidly in proportion to the amount of silver (I) added, and can be determined indirectly at the absorption maximum at 484 nm in benzene without any observable interference from the formed Ag (I) complex (Method B).

Method A

(1) **Absorption Spectrum of the Complex**——Fig. 1 shows that the absorption spectrum of the DABTB–Ag (I) complex in ethanol–buffer (pH 6) solution has an absorption maximum at 400 nm which is hardly affected by the reagent blank (DABTB).

(2) **Effect of pH**——The effect of pH on the complex formation was examined using a citric acid–phosphate buffer solution (pH 2.2—8), and the optimum pH was found to be 5—6.8.

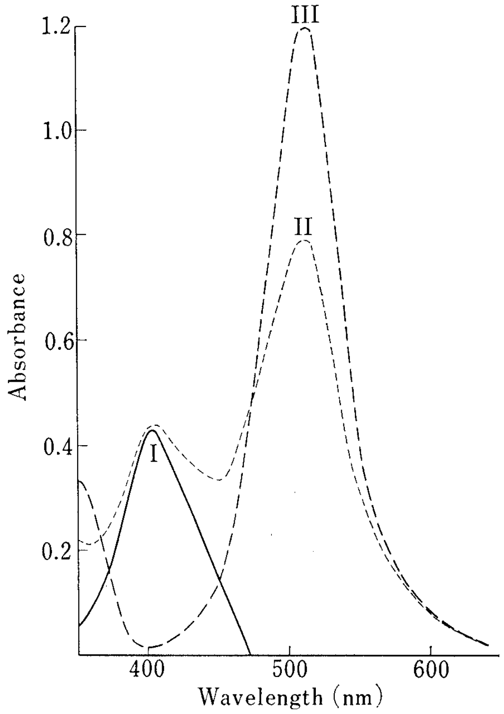

Fig. 1. Absorption Curves of Ag–DABTB Complex

I: Ag–DABTB against reagent blank.
II: Ag–DABTB against water.
III: Reagent blank against water.
Ag(I): 45 μg; DABTB: 3×10^{-5} M; Buffer: pH 6.

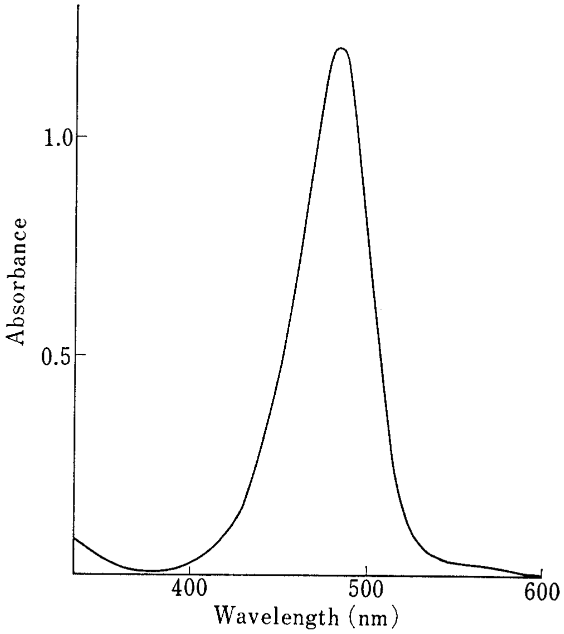

Fig. 2. Absorption Curve of DABTB

DABTB: 1.5×10^{-5} M benzene-DMSO (99: 1) soln.
Reference: benzene.

(3) **Effect of Concentration of DABTB**——Studies of the effect of DABTB concentration in DMSO (from 0.5×10^{-5} to 6.0×10^{-5} M) on the complex formation showed 3×10^{-5} M solution to be the optimum. Five ml of ethanol was added to dissolve DABTB, which is not sufficently soluble in water.

(4) **Stability of the Color**——The change of absorbance of the complex was examined at 400 nm to check the stability of the color. The absorbance was constant up to 10 minutes after color development then gradually decreased. Thus, absorbance measurements should be done within 10 minutes.

(5) **Calibration Curve**——Conformity to Beer's law over a range of 0.04—2.0 μg/ml of silver (I) was obtained by using the recommended procedure described in "Experimental." The absorptivity of the complex as estimated from the calibration curve was $2.5\times10^{4}\cdot\mathrm{l}\cdot\mathrm{mol}^{-1}\,\mathrm{cm}^{-1}$.

Method B

(1) **Absorption Spectrum of DABTB**——As shown in Fig. 2 the absorption maximum appears at 484 nm in benzene and shows no appreciable change on storage for a month.

(2) **Effect of pH**——The optimum pH range was found to be 3—6 by examination of the effect of pH on the absorbance of DABTB, both in the absence and presence of Ag (I) (10 μg).

(3) **Effect of Shaking Time**——It was found to require two minutes to complete the complex formation of silver (I) with DABTB by shaking.

(4) **Calibration Curve**——A straight line was obtained for 0—35 μg of silver from the calibration curve.

TABLE I. Absorption Maxima of Metal Complexes of DABTB

Metal ions	λmax
Hg(I)	454 (2.4×10^{4})
Hg(II)	454 (5.4×10^{4})
Au(III)	458 (2.7×10^{4})
Pd(II)	456 (3.4×10^{4})
Pt(IV)	515 (1.7×10^{4})

Numbers in parentheses under λmax are apparent molar extinction coefficients.

TABLE II. Effects of Various Ions on Ag Determination

Ions	Added (μg)	Form	Ag found (μg)
Bi(III)	100	$Bi(No_3)_3\cdot5H_2O$	29.4
Fe(II)	100	$FeSO_4(NH_4)_2SO_4\cdot6H_2O$	29.4
Fe(III)	100	$Fe(NO_3)_3\cdot9H_2O$	28.8
Co(II)	1000	$Co(NO_3)_2\cdot6H_2O$	29.5
Cu(II)	1000	$CuSO_4\cdot5H_2O$	30.3
Ni(II)	1000	$NiSO_4\cdot7H_2O$	30.7
Al(III)	300	$K_2Al_2(SO_4)_4\cdot24H_2O$	28.0
Cr(VI)	100	$K_2Cr_2O_7$	31.1
Cl^-	5	NaCl	21.0
I^-	5	KI	27.3
Br^-	5	KBr	24.9
F^-	500	NaF	29.1
SCN^-	5	KSCN	21.6
CN^-	5	NaCN	21.7
$S_2O_3^{2-}$	50	$Na_2S_2O_3$	27.8
S^{2-}	5	Na_2S	21.0

Ag used: 30.0 μg

Effects of Other Ions

Since DABTB reacts not only with Ag (I) but also with mercury (I and II), gold (III), palladium (II), and platinum (IV), the effects of various ions on the determination of silver were examined using method A. The absorption maxima of these complexes are summarized in Table I; the mercury (II) complex had a relatively large molar absorptivity, but it was not sufficient to permit the photometric determination of Hg (II) in the same way as that of silver (I) due to overlapping of the absorption with that of the reagent blank. The results with various ions examined in the standard procedure are listed in Table II; various anions such as Br^-, Cl^-, I^-, SCN^-, CN^-, $S_2O_3^{2-}$, and S^{2-} interfered with the determination of silver (I).

Application to the Determination of Silver in Commercial Preparations

The recommended methods described in "Experimental" were successfully applied to the determination of silver in commercial preparations such as silver nitrate eye lotion and silver protein. The results obtained are listed in Table III.

Table III. Analyses of Silver in Commercial Preparations

Sample		Silver content (%)	
		Method A	Method B
Silver nitrate eye lotion		1.01	1.08
		1.05	1.10
		1.04	0.97
		1.03	1.06
		1.05	1.02
	Mean	1.03	1.04
	s.d.	0.02	0.05
	c.v.	1.9	4.8
Silver protein		7.8	8.2
		8.1	8.5
		8.0	8.1
		8.2	8.2
		8.0	
	Mean	8.0	8.2
	s.d.	0.15	0.18
	c.v.	1.9	2.2

In this work we established two simple and rapid photometric determination methods for silver (I). Method A is supeior to method B in accuracy and precision but is inferior in simplicity and stability. The sensitivities of these methods are superior to those of the diethyldithiocarbamate,7) pyrogallol red,8) and 2-amino-6-methylthio-4-pyrimidinecarboxylic acid9) methods, compare favorably with those of the *p*-dimethyl (or diethyl)aminobenzylidenerhodanine10) and dithizone11) methods, and are inferior to those of the 1,10-phenanthroline/bromopyrogallol red,12) 1,10-phenanthroline/tetrabromofluorescein13) and 4,4-bis-(dimethylamino) thiobenzophenone14) methods.

---

7) E. Sudo, *Nippon Kagaku Zasshi*, **73**, 626 (1952).
8) R.M. Dagnall and T.S. West, *Talanta*, **8**, 711 (1961).
9) O.K. Chung and C.E. Meloan, *Anal. Chem.*, **39**, 383 (1967).
10) E.B. Sandell and J.J. Neumayer, *Anal. Chem.*, **23**, 1863 (1951); G.C.B. Cave and D.N. Hume, *ibid.*, **24**, 1503 (1952).
11) M. Kawahata, H. Mochizuki, and T. Misaki, *Bunseki Kagaku*, **11**, 1017 (1962).
12) R.M. Dagnall and T.S. West, *Talanta*, **11**, 1627 (1964).
13) M.T. El-Ghamry and R.W. Frei, *Anal. Chem.*, **40**, 1986 (1968).
14) K.L. Cheng, *Microchim. Acta*, **5**, 820 (1967).

Experimental

Apparatus——Absorbance at various wavelengths was recorded with a Hitachi 323 recording spectrophotometer. Absorbance measurements at fixed wavelengths were made with a Shimadzu 150 double beam spectrophotometer. Matched glass cells of 10 mm path length were used. A Toa HM-5A pH meter with a saturated calomel-glass electrode system was used for all pH measurements. An Iwaki KM shaker was used to shake the separatory funnels.

Reagents——DABTB: The reagent was prepared by the condensation of 2-thiobarbituric acid with *p*-dimethylaminobenzaldehyde in EtOH, and the crude product thus obtained was recrystallized from MeOH.[4] DABTB was dissolved in DMSO to give a 1.5×10^{-3} M solution for method A. The solution should be used within 3 days. DABTB-DMSO solution (10 ml) was diluted with benzene to 1 l and used as the reagent for method B.

Standard Silver(I) Solution: Standard silver solution (1 mg/ml, $f=1.00$, Wako) was diluted when necessary.

Buffer Solution: McIlvaine buffer was used.[15]

pH 6: 73.7 ml of 0.1 M citric acid solution and 126.3 ml of sodium phosphate solution were mixed and checked with the pH meter.

pH 5: 97.0 ml of 0.1 M citric acid solution and 103 ml of 0.1 M sodium phosphate solution were mixed and checked with the pH meter.

All the other reagents used were of reagent grade.

Recommended Procedures——Method A: The solution containing 1—50 μg of silver(I) is pipetted into a 25 ml volumetric flask, 5 ml of buffer (pH 6) and 5 ml of EtOH are added, and the whole is diluted to about 24 ml with water. After adding 0.5 ml of DABTB–DMSO solution (1.5×10^{-3} M) to this, it is diluted to 25 ml with water and mixed. Absorbance measurements are made at 400 nm with matched 10 mm glass cells against the reagent blank similarly prepared but free from silver(I).

Method B: The solution containing 1—35 μg of silver(I) and 5 ml of buffer (pH 5) are pipetted into a 100 ml separatory funnel and diluted to about 50 ml with water. After adding 25 ml of DABTB–benzene solution, the mixture is shaken for 2 minutes using a shaker. The separated organic phase is filtered and the absorbance is measured with matched 10 mm glass cells against benzene.

Analyses of Commercial Preparations——Sample Solution: Silver nitrate eye lotion; One-half ml of silver nitrate eye lotion in an ampoule (1 w/v%) was diluted to 250 ml with water. Silver protein;[16] Three hundredth grams of silver protein powder (7.5—8.5 w/w%) was dissolved in 1 ml of conc.HNO_3 and it was heated until the solution became clear in the presence of 0.6 ml of conc.H_2SO_4. The mixture was allowed to cool to room temperature and was then diluted to 250 ml with water.

The absorbances of all sample solutions were measured by the recommended procedures and the contents of silver were estimated from the calibration curves obtained with standard silver solutions.

---

15) Editorial committee of tables of chemical constants (ed.), "Tables of Chemical Constants for Laboratory Use," 6th ed., Hirokawa, Tokyo, 1971, p. 403.

16) E. Saitō, *Eisei Shikensho Hokoku*, **81**, 44 (1963).

[Chem. Pharm. Bull. 28(8)2522—2527(1980)]

Synthesis of N,N,3,9-Tetraalkyladeninium Halides by Alkylations of N,N,3- and N,N,9-Trialkyladenines[1]

Taisuke Itaya, Kazuo Ogawa, Hiroo Matsumoto, and Tomoko Watanabe

Faculty of Pharmaceutical Sciences, Kanazawa University[2]

(Received March 10, 1980)

The reactions of N,N,9-trialkyladenines (II) with alkyl halides in N,N-dimethylacetamide gave N,N,3,9-tetraalkyladeninium halides (IV) in good yields. N,N,3-Trialkyl-

---

1) A part of this work was reported in preliminary form.[3a]

2) Location: *13-1 Takara-machi, Kanazawa 920, Japan.*