STUDIES ON THE CHEMICAL PROPERTIES OF METHYLMETHIONINE SULFONIUM DERIVATIVE

I. COLORIMETRIC DETERMINATION WITH POTASSIUM 1, 2-NAPHTHOQUINONE-4-SULFONATE

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Methylmethionine sulfonium derivative (MMSD), one of the anti-peptic ulcer factors (1), has in recent years been increasingly used in stomachic preparations. As to its chemical determination, there have been a few reports (2—4). However, none of them seems to be satisfactorily applied for the determination in complicated, mixed preparations. It has been found that MMSD exhibits a yellowish orange color with potassium 1,2-naphthoquinone-4-sulfonate (NQS) in alkaline medium. Utilizing this coloration in conjunction with the precipitation reaction with phosphotungstic acid (PTA), reaction conditions were investigated and a simple colorimetric method was established.

EXPERIMENTAL AND RESULTS

1. Method of Assay

Reagents—(1) Buffer solution, pH 8.7. This was prepared from 0.2 M H_3BO_3 -KCl and 0.2 M Na_2CO_3 . (2) 15% aq. solution of PTA. PTA used was the special grade product of Ishizu Pharmaceutical Co., Ltd., having the characteristics as shown in Fig. 1 by differential thermal analysis. (3) 10% aq. solution of NQS. (4) Saturated $BaCl_2$ solution in water (ca. 33%). (5) Special grade HCl.

Procedure—(1) Extraction. Sample corresponding to ca. 9.0×10^{-5} mole of MMSD was accurately weighed and transferred to a glass-stoppered centrifugal tube with the aid of 25 ml of water and shaken. The extraction was repeated successively with 10, 10, and 5 ml of water. When the sample contained $NaHCO_3$, the combined aq. extract was transferred to another centrifugal tube in which 5 ml of saturated solution of $BaCl_2$ in water had previously been placed, allowed to stand for a few minutes and was centrifuged. The supernatant liquid was filtered through a G4 glass filter into the third centrifugal tube and centrifuged. The precipitate was washed with three 5 ml portions of water and each washing was filtered through the same filter to combine with the above filtrate.

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FIG. 1 The Differential Thermal Analysis Curve of PTA and its Weight Variation with Temperature

(2) Precipitation of methylnmethionine sulfonium phosphotungstate (MMSP). To aq. extract were added 10 ml of HCl and 10 ml of 15% aq. solution of PTA. The mixture was thoroughly mixed and kept overnight in a refrigerator to make the formation of MMSP complete. When the sample did not contain NaHCO_3 , the aq. extract was filtered through a G4 glass filter, transferred into a centrifugal tube, followed by the addition of 20 ml of water and treated as stated above beginning with "To the combined aq. extract were added 10 ml of HCl and 10 ml of 15%-----".

(3) Colorimetry. MMSP obtained after centrifugation and removal of the supernatant liquid, was once washed with 10 ml of water and dissolved in buffer solution, pH 8.7, to make up the volume to 100 ml. The solution was filtered if necessary. To 10 ml of the solution was added 5 ml of 1% aq. solution of NQS and the mixture was heated at $50 \pm 0.2^\circ$ exactly for 10 minutes. After cooling, it was diluted with buffer solution pH 8.7 to 100 ml. The absorbance of the colored solution thus obtained was measured at $460 \text{ m}\mu$ using 10 ml of buffer solution pH 8.7 treated with the same reagents in the same way as the sample as a blank solution and the content of MMSP was determined by referring to the calibration curve.

2. Investigation of Reaction Conditions

In the following experiments, 9.0×10^{-5} mole of methylnmethionine sulfonium cyclohexylsulfamate (MMSCy) was used.

Effect of the Amount of PTA—The amount of PTA enough to precipitate MMSCy was investigated as shown in Fig. 2. From this result, 10 ml of 15% aq. solution of PTA was employed.

Effect of Concentration of NQS—The result as shown in Fig. 3 was obtained. So 10% solution was employed.

Reaction Temperature and Period—The coloration reaction was carried

FIG. 2 *Effect of Amount of PTA* FIG. 3 *Effect of Concentration of NQS* FIG. 4 *Effects of Reaction Temperature and Period*

●—●, 80°; ····●····, 50°; —·—●—·—, 40°; ×, 20°.

out at 20°, 40°, 50° and 80° for 5, 10 and 20 minutes and the result as shown in Fig. 4 was obtained. The reaction at 50° for 10 minutes was employed.

pH and the Composition of Buffer Solution—MMSP dissolved in a medium having pH more than 8. The effect of pH on the coloration was investigated over the pH range 8.0 to 10.0 using buffer solutions composed of 0.2 M H_3BO_3 -KCl and 0.2 M Na_2CO_3 . In this case, borate ion played a very important role for lowering

FIG. 5 Effects of pH and Components of Buffer Solutions

Compositions of buffer solutions: I= H_3BO_3 , KCl, Na_2CO_3 ; II= KH_2PO_4 , Na_2HPO_4 ; III= NaHCO_3 , Na_2CO_3 . Solid line indicates the value of assay and dotted lines those of blank.

the blank value, because other anion such as phosphate or carbonate was all ineffective in this respect as is evident from Fig. 5.

To clarify what action borate ion did, polarographic behaviors of NQS in various buffer solutions were examined. It was thereby found that reduction wave due to vicinal carbonyl radical of NQS disappeared only in the presence of borate ion. Thus it was assumed that borate ion stabilized the reagent, forming a chelation with the carbonyl radicals of NQS.

Absorption Spectrum of Colored Solution—The colored solution had a peak at 460 $\text{m}\mu$.

Stability of Color—The intensity of color was almost constant from 15 minutes to 18 hours after the coloration.

Calibration Curve—MMSC, the product of Nihon Kayaku Co., Ltd., was further purified with water and ten times the volume of ethanol and dried at 40° under reduced pressure. Five hundred and thirteen mg of it was accurately weighed and dissolved in water to make the volume to 250 ml. Five, 10, 15, 20 and 25 ml of this solution, which corresponded to 3, 6, 9, 12 and 15×10^{-5} mole of MMSC respectively, were placed in centrifugal tubes and the total volume in each tube was made up to 70 ml with water. Then 10 ml of HCl and 10 ml of 15% aq. solution of PTA were added to each tube. The mixture was treated as described in the article (2) of procedure beginning with "the mixture was thoroughly mixed and kept overnight----". The absorbances were plotted against the concentration and the good linearity was obtained over this concentration range as shown in Fig. 6. Coefficient of variance at the concentration level of 9.0×10^{-5} mole was 2.20% for 19 experiments.

3. Recovery Rates of MMSD in Known Pharmaceutical Preparations

These were examined using known pharmaceutical preparations containing 75 mg of MMSCy in their daily dose. The dosage forms were all powder. The results were shown in Table 1.

TABLE 1
Recovery of MMSD in Known Stomachic Preparations

No.	Ingredients	Recovery
		<i>per cent</i>
1	Na bicarbonate, Mg carbonate, Al Mg silicate, vitamins, amino acids, enzymes, crude drugs, MMSCy, <i>etc.</i>	101.2
2	Na bicarbonate, Mg carbonate, Al Mg silicate, enzymes, MMSCy, <i>etc.</i>	102.0
3	ditto	100.1
4	Mg carbonate, Al hydroxide, enzymes, MMSCy, <i>etc.</i>	98.8
5	Mg carbonate, Al hydroxide, Al Mg silicate, MMSCy, <i>etc.</i>	95.5
6	Mg carbonate, Al hydroxide, amino acids, MMSCy, <i>etc.</i>	103.0

4. Reaction Mechanism

Formation of MMSP—As to the composition of MMSP, there has been a report of Lavine *et al.* (4). In the present experiment, the combining ratio between MMSD and PTA was examined through the measurement of conductivity of excess PTA after precipitation. It was found that 3 moles of MMSD combined with 2 moles of PTA as shown in Fig. 7.

On the other hand, MMSP dissolved in acetone was made to react with tetramethylammonium bromide, thereby producing 3 protons, which was ascertained

FIG. 6 Calibration Curve

FIG. 7 Variation of Conductivity with the Concentration of PTA

by potentiometric titration with NaOH. From these findings, it was considered that the reaction of MMSD with PTA proceeded as in the following.

From the fact that tetraalkylammonium salt forms phosphotungstate having the composition of $(\text{R}_4\text{N})_3(\text{PO}_4 \cdot 12\text{WO}_3)$ (5), the reaction of MMSP with tetramethylammonium bromide could be interpreted to proceed as shown below.

Coloration — By the reaction of MMSCy with NQS in a cold alkaline medium, followed by acidification, a reddish brown precipitate was obtained, which was purified with dimethylformamide and acetone, then washed several times with hot acetone. This substance did not melt below 270° and showed the existence of sulfur by sodium fusion but negative result for Beilstein test. In the IR absorption spectrum of this substance, a strong absorption band due to stretching vibration of carbonyl radicals of the reagent completely disappeared, whereas those due to sulfonate radical around $1,200\text{ cm}^{-1}$ as well as at $1,040\text{ cm}^{-1}$ still remained. New appearance of the absorption at $1,500\text{ cm}^{-1}$ might be attributed to the formation of carboxylate radical in the compound. The compound produced a precipitate with phosphotungstic acid, while the reaction product obtained from homoserine and NQS in a similar way with MMSC did not. Furthermore, the sulfur analysis of the compound resulted in smaller contents than expected from the structure (I). On the other hand, glycine, one of amino acids, has been

reported to react with carbonyl radical of NQS with its amino radical (6). These facts might lead to the assumption that the condensation between amino radical of MMSCy and carbonyl radicals of NQS took place, giving the product (I) shown below, which was then partially hydrolysed to another compound (II).

DISCUSSION

The assay method of MMSD reported here has the following advantageous points.

1. Through the conversion to phosphotungstate, MMSD could be separated from its decomposition product and other co-existing substances. Moreover, MMSP dissolved preferably in buffer solution pH 8.7, this fact contributed further to its purification from its co-precipitated substances.

2. The use of borate as one of the components of buffer solution could markedly lowered the blank value and improved the recovery rates.

3. MMSD in preparations containing antacids, enzymes and others could be determined with good recovery rates.

SUMMARY

A simple assay method of methylmethionine sulfonium derivative was established utilizing the precipitation reaction with phosphotungstic acid and the coloration with potassium 1,2-naphthoquinone-4-sulfonate. This method could satisfactorily be applied for the determination in rather complicated, mixed preparations.

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