Communications to the Editor

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DETERMINATION OF PENICILLINS WITH MERCUROCHROME[1)]

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The reactions between the halogenofluorescein-mercury compounds such as mercurochrome and penicillins such as penicillin G(PCG), ampicillin(ABPC), sulbenicillin(SBPC) and amoxicillin(AMPC) were investigated. Detection tests and fluorometric determinations of these penicilins are proposed using the coloring system of mercurochrome and penicillin reactions. The detection limit of penicillins, as ABPC, was 0.8 μg/ml, and the calibration curves were rectilinear up to about 5 μg/ml (about 1.5×10^{-5} M) of PCG, ABPC, SBPC and AMPC at 535 nm. The relative standard deviation was 2.7% for 1.4 μg/ml of ABPC(n = 5).

KEYWORDS —— fluorometric determination; mercurochrome; coloring reaction; penicillin; quenching; ampicillin; penicillin G; sulbenicillin; amoxicillin

Various methods have been reported for fluorometric determination of sulfur compounds such as sulfide ion, thiourea and thioxine derivatives, and long chain alkylamine compounds such as hexadecylpyridinium chloride and dimethylbenzyltetradecylammonium chloride(zephiramine) using halogenofluoresceins-mercuries and fluorescein-mercury compounds.[2-10)]

Penicillins are a family of β-lactam antibiotics containing a sulfur atom widely used for clinical chemotherapy. Various methods for the determination of penicillins using metal ions such as iron, mercury, copper and vanadium have been reported.[11-15)] However, the color reaction systems of penicillins with the organic mercury compounds have not been discussed. We noticed that the reaction system between the micro amount of penicillins such as penicillin G(PCG), ampicillin(ABPC), sulbenicillin(SBPC), amoxicillin(AMPC) or predecomposed penicillins and mercurochrome as a halogenofluorescein-mercury compound gave a coloring form, and the fluorescence intensity of the mercurochrome solution was quenched in proportion to the concentration of the penicillins in a weakly basic

as a dispersion agent, and the maximum and most constant ΔF was observed upon the addition of more than 1.0 ml of a 1.0% PVA solution to the final volume of 10 ml.

Several series of experiments were carried out to investigate the influence of the mercurochrome concentration at 535 nm. The maximum and most stable ΔF was obtained by adding 0.1 - 0.4 ml of a 0.1% mercurochrome solution. The reaction between mercurochrome and the penicillins was imperfect at room temperature, and its fluorescence intensity was unstable. The effect of temperature was examined by heating Solutions B and A; good results were obtained by heating Solutions B and A at 60°C for 45 min and then cooling them in water to 15 - 25°C for 5 min. With this procedure, the fluorescence intensities of solutions B and A were constant and stable for at least 3 h at room temperature. The fluorescence intensity obtained by heating Solution B at 535 nm was nearly equal to that of the mercurochrome-predecomposed penicillins (=penicilloic acid)[16] solution.

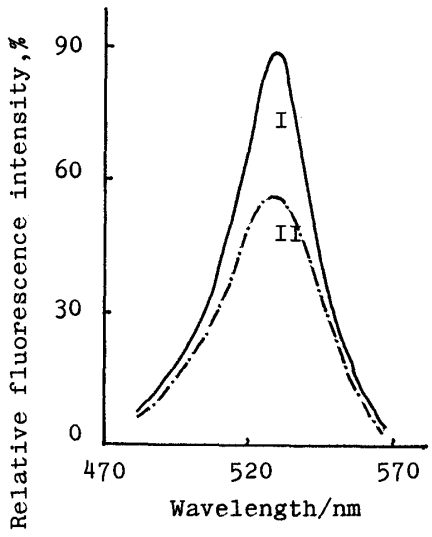

Fig. 1 Fluorescence Spectra of Mercurochrome Solution(Solution A) and Mercurochrome-ABPC Solution(Solution B) at pH 10
Mercurochrome, 0.25 ml of 0.1% mercurochrome solution/10 ml; ABPC, 1.0 X 10^{-5} M; PVA, 0.1%; curve I, Solution A; curve II, Solution B.

The calibration curves of PCG, ABPC, SBPC and AMPC were rectilinear up to about 5 μg/ml (about 1.5 X 10^{-5} M). The relative standard deviation for 14 μg of ABPC in the final 10 ml was found to be 2.7% (5 determinations). A detection test for penicillins in a test tube was also examined. Satisfactory results were obtained when detection tests were carried out according to the procedure described above. The detection limits for ABPC in the test tube was 0.8 μg/ml of ABPC.

The aliquot of solution containing various amounts of foreign substances and 14 μg of ABPC was tested exactly as described in the recommended procedure. The limiting values of the concentration of foreign ions or substances were taken as the value which caused an error of not more than ± 3% in the fluorescence intensity. The coexistence of caffeine or sulfisomidine did not interfere in equimolar amounts with respect to the penicillins. Though the coexistence of zinc(II), copper(II) and iron(III) interfered in 5- to 100-fold excess of ABPC, these metal ions could be masked by addition of nitrilotriacetic acid.

On the other hand, the coexistence of other organic substances containing nitrogen atom such as riboflavine and pyridoxine in micro amounts and the

medium.

In this paper, a color reaction between the penicillins and halogeno-fluorescein-mercury compounds, mercurochrome, is discussed and the reaction conditions are established for a detection test and fluorometric determination of penicillins using mercurochrome.

The recommended procedures for the detection and fluorometric determination of total penicillins are as follows. Detection test - Put in a test tube 0.1 ml of a 0.1% mercurochrome solution, 0.5 ml of 1.0% polyvinylalcohol (PVA) solution and 1.0 ml of Sörensen buffer solution (borax-sodium hydroxide, pH 10). To this solution add a drop of penicillin solution containing more than 4.0 μg and dilute the mixed solution to 5.0 ml with water. Then keep the solution at 60°C for 45 min. Compare the intensity of the coloring (red color) and fluorescence (quenching of fluorescence) of the mercurochrome and penicillin mixture with a blank in parallel. Fluorometric determination - Place a sample solution containing up to about 50 μg of a penicillin such as PCG, ABPC, SBPC and AMPC in a 10 ml calibrated flask. To this solution add 2.0 ml of a 1.0% PVA solution, 0.25 ml of 0.1% mercurochrome solution and 2.0 ml of Sörensen buffer solution (borax-sodium hydroxide, pH 10). Dilute the mixture to 10.0 ml with water (Solution B), and keep it at 60°C for 45 min. After Solution B and the blank solution (Solution A) are cooled to 15 - 25°C in water for 5 min, measure the difference of relative fluorescence intenstiy(= ΔF) between Solutions B and A at an emission wavelength of 535 nm and an excitation wavelength of 365 nm.

In this color reaction system between halogenofluorescein-mercury compounds such as 2,7-dichlorofluorescein-mercury compound,[9,10] 2,4-dichlorofluorescein -mercury compounds[9,10] and 3',4',5',6'-tetrachlorofluorescein mercury compounds[9,10] and pencillins such as PCG, ABPC, SBPC and AMPC, the use of mercurochrome was the most effective and optimal in terms of sensitivity and reproducibility. The color reaction rarely occurred at room temperature, but it was clearly elicited when the mixtures were heated or when predecomposed penicillins (e.g., penicilloic acid) were used.[16] The detection limit of ABPC in the test tube was 0.8 μg/ml in this procedure.

Figure 1 shows the fluorescence spectra of the mercurochrome solution (Solution A) and the mercurochrome-ABPC solution (Solution B), respectively. The maximum emission wavelength of the mercurochrome solution in a basic medium was about 535 nm. The maximum excitation wavelength of the solution was about 470 nm, but the excitation wavelength of 365 nm was used because a middle pressure mercury lamp generally was used as a light source.

The maximum and almost constant ΔF at 535 nm was obtained over the pH range of 9.6 to 10.5 with 1.5 - 4.0 ml of Sörensen buffer solution, and was proportional to the concentration of the penicillins. Among various surfactants tested, polyvinylpyrrolidone (PVP, K-30), PVA (n = 500, 2000), gum arabic, Tween 20, gelatin, etc, PVA (n = 500), a nonionic surfactant, was found to be the best

coexistence of large amounts of thiocyanate, thiosulfate, iodide and sulfide ions interfered seriously.

The proposed method was applied to the determination of ABPC in artificial 1.0% ABPC ointment. Recovery of ABPC from the artificial ointment was satisfactory (98.0 - 105.3%).

In conclusion, the proposed method was about 6 times more sensitive than the imidazole-mercury method,[8] and may be used for the simple fluorometric determination of penicillins without organic solvent extraction. Since the mercurochrome solution gradually reacted with penicillins at room temperature to form coloring materials (mercurochrome-penicillins), this must be kept in mind on the use of mercurochrome together with penicillins.

Although further investigation is necessary, the proposed method should be useful for separative assays of total penicillins and decomposed penicillins (e.g., penicilloic acid), and then for the examination of the effectiveness of the penicillins.

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