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Indirect Micro-determination of Primary and Secondary Amines by Copper(II)-catalyzed Oxidation of Pyrocatechol Violet

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An indirect method is described for the micro-determination of primary and secondary amines in the presence of tertiary amine by means of a catalytic indicator reaction. The copper bis(dithiocarbamate) complex formed from primary or secondary amine and carbon disulfide in the presence of copper was removed by extraction into chloroform. The remaining copper in aqueous solution was determined by measuring the rate of oxidation of pyrocatechol violet by hydrogen peroxide. A simplified complementary tristimulus colorimetry (SCTS method) was used for determination of the reaction rates, since it has the advantage of permitting accurate determination of the rate constants in the presence of color impurities. Quantitative recoveries were obtained of microamounts of primary and secondary amines with good accuracy (from -2.9% to $+2.9\%$ error). The method presented is sensitive and does not require a separate calibration curve for each primary and secondary amine.

Keywords——primary and secondary amine; carbon disulfide; catalytic indicator reaction; pyrocatechol violet; hydrogen peroxide; copper; simplified complementary tristimulus colorimetry

It is important to develop rapid, selective methods for the determination of primary and secondary amines in pharmaceutical mixtures.[2] In this connection, the reaction of carbon disulfide with secondary amines, which was first investigated by Dowden,[3] results in the formation of dialkyl dithiocarbamic acids (DTCH), which form colored copper bis(dithiocarbamate) [$Cu(DTC)_2$] with copper(II). These compounds are soluble in organic solvents such as benzene and chloroform. Primary amines are less suitable for spectrophotometric determination, because the complexes formed have low color intensities. Tertiary amines do not react with carbon disulfide. This reaction has been utilized for the direct determination of secondary amines in the presence of primary or tertiary amines.[4] There are, however, only a few reports dealing with the quantitative microanalysis of secondary amines.[5]

The purpose of the present investigation was to determine microamounts of primary or secondary amines in the presence of tertiary amine by employing a catalytic indicator reaction; the concentration of the amine was determined indirectly from the amount of copper remaining after the reaction, based on its catalytic effect on the oxidation of pyrocatechol violet (PV) with hydrogen peroxide. In order to minimize the error associated with the determination of the rate constants, the rate of oxidation of PV was measured at the time when the mol fraction of PV was equal to $1/e$,[6] which was calculated by simplified complementary tristimulus colorimetry (SCTS method)[7] without knowledge of the initial concentration of PV.

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Experimental

Materials——All the amines used were obtained from commercial sources and were used without further treatment. All other reagents used were of analytical reagent grade or the purest grade commercially available. PV (Dojindo Co.) solution was prepared daily by dilution of a 0.1% stock solution with distilled water. Standard copper(II) solution (90 μg/ml) was prepared by dissolving $CuCl_2 \cdot 2H_2O$ in a 1 : 1 (v/v) mixture of pyridine and water. CS_2–pyridine solution was prepared by mixing 25 ml of CS_2, 25 ml of pyridine and 65 ml of isopropyl alcohol.

Apparatus——The spectrophotometric equipment used in the present studies has been described previously.[7] Temperature (25±0.1°) was controlled with a Sharp TE-10K circulator bath.

Procedure——Copper solution was added to a mixture containing 2 ml of a sample solution and 3 ml of CS_2–pyridine solution. After agitation for about 30 min, 2 ml of 20% acetic acid and 2 ml of chloroform were added. The mixture was shaken for several minutes and the phases were then allowed to separate. 0.3 ml of the upper (aqueous) layer was injected into a solution containing 3 ml of 0.4 M borate buffer (pH 9.2), 1.5 ml of 1.0 M hydrogen peroxide and 0.5 ml of PV solution.

SCTS Method——The absorbances at wavelengths u (580 nm), v (440 nm) and w (350 nm) were recorded against a reagent blank with time. The color point corresponding to wavelength u was calculated automatically from the relation

$$Q_u = A_u/J \qquad (1)$$

where A_u is the absorbance at wavelength u, and J is the sum of absorbances at the three wavelengths. A pseudo first-order rate constant was determined using the following equation,

$$-\ln q = k \cdot t \qquad (2)$$

where q is the mol fraction of PV. The value of q at any time could be calculated from the color points of a mixture of PV and the product at the same time, Q_{tu}, as follows

$$q = E_P(Q_{Pu}-Q_{tu})/[E_R(Q_{tu}-Q_{Ru})+E_P(Q_{Pu}-Q_{tu})] \qquad (3)$$

where E_R and E_P are the overall absorptivities, and Q_{Ru} and Q_{Pu} are the color points of PV and the product at wavelength u. Analogous equations can be obtained for wavelengths v and w.

Results and Discussion

A precipitate of $Cu(DTC)_2$ was separated from copper remaining in the aqueous solution by extraction with chloroform. The addition of 20% acetic acid during the procedure, as pointed out by Stanley *et al.*,[8] forced the extraction of the complex from the aqueous layer into chloroform to completion. Pyridine appears to act as a proton acceptor for the amine–amine salt equilibrium in the carbamate reaction.[9] Addition of 2.5 ml or more of CS_2–pyridine solution was found to be necessary for the formation of $Cu(DTC)_2$ to reach approximately 90 to 95% completion. For most of the primary and secondary amines studied, the formation of the complex was completed within 10—20 min and the amount obtained was constant after 2 hr at room temperature.

The oxidation of PV with hydrogen peroxide in the presence of copper was investigated by Birmantas and Jasinskiene,[10] and by Janjić and Milovanović.[11] It was found that the oxidation reaction obeyed the following kinetic equation (when$[PV] \ll [H_2O_2]$):

$$-d[PV]/dt = (k_1[Cu^{2+}]_0+k_2)[PV] \qquad (4)$$

where [PV] and $[Cu^{2+}]_0$ are the concentration of PV and the initial concentration of copper, and k_1 and k_2 are the observed rate constants of the catalyzed and uncatalyzed reaction, respectively. Under the conditions used, it was found that k_2 was very small ($6.5 \pm 0.32 \times 10^{-5}$ min^{-1}). The apparent pseudo first-order rate constant, K, for the oxidation of PV was determined as a function of the concentration of copper by means of Eq. (2) and (3) from the color points of the reaction mixture of PV and the product. The calibration curve (K *vs.* $[Cu^{2+}]_0$)

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Table I. Determination of Primary and Secondary Amines in Mixtures with Tertiary Amine

Amines	Amount used ($\times 10^6$ M)	Amount found ($\times 10^6$ M)[a]	Error (%)
N-Methylbenzylamine in N,N-dibenzylaniline[b]	2.09	2.06±0.15	−1.40
Di-*n*-butylamine in *tert*-butylamine[c]	3.88	3.83±0.71	−1.30
	7.70	7.60±0.05	−1.30
Diethylamine in triethylamine[d]	1.37	1.33±0.34	−2.90
Monoethanolamine in triethanolamine[e]	1.60	1.59±0.62	−0.60
	8.27	8.51±0.11	+2.90
Isoniazid in N,N-dimethylaniline[f]	3.67	3.61±0.08	−1.60

a) The mean values±S.D. of five determinations.
b) 10^{-5} M $(C_6H_5CH_2)_2NC_6H_5$.
c) 2×10^{-5} M $(CH_3)_3CNH_2$.
d) 1.5×10^{-5} M $(C_2H_5)_3N$.
e) 5×10^{-5} M $N(CH_2CH_2OH)_3$.
f) 5×10^{-5} M $C_6H_5N(CH_3)_2$.

Table II. Application to Pharmaceuticals

Amines	Amount used ($\times 10^6$ M)	Amount found ($\times 10^6$ M)[a]	Error (%)
DL-Norepinephrine Hydrochloride in 2-dimethylaminoethanol[b]	2.44	2.50±0.04	+2.50
L-Epinephrinebitartrate	2.73	2.68±0.53	−1.80
in 2-dimethylaminoethanol[c]	6.56	6.55±0.09	−0.20
DL-Phenylephrine hydrochloride in N,N-dimethylaniline[d]	2.46	2.52±0.05	+2.40

a) The mean values ±S.D. of five determinations.
b) 1.2×10^{-5} M $(CH_3)_2NCH_2CH_2OH$.
c) 1.5×10^{-5} M $(CH_3)_2NCH_2CH_2OH$.
d) 10^{-5} M $C_6H_5N(CH_3)_2$.

was linear in the range of 3.3 μg/ml to 14 μg/ml and passed through the origin. The coefficient of variation for 5 μg/ml of copper was 0.4% ($n=5$).

A list of the primary and secondary amines tested is given in Table I. This method is clearly suitable for the micro-determination of primary and secondary amines in the presence of tertiary amine with good accuracy (from −2.9% to+2.9% error). $Cu(DTC)_2$ from L-epinephrine and L-norepinephrine remained in the aqueous layer containing copper even after extraction with chloroform under the present conditions. As the SCTS method has the advantage of permitting accurate determination of the reaction rate in the presence of color impurities, the value of K could be determined by the same approach using Eq. (2) and (3) from the color points of the reaction mixture in the presence of the corresponding complex. The coefficients of variation for 2.73×10^{-6} M L-epinephrine and 2.44×10^{-6} M DL-norepinephrine were 0.90% ($n=5$) and 1.10% ($n=5$), respectively (Table II).