a powder is, or more exactly, how much the dissolution behavior deviates from what would be expected if the powder were completely monodisperse. A value close to zero characterizes a nearly monodisperse powder, while higher values indicate increasing "degrees of dispersion." Probably, the most important property of σ is that it is a measure of how long it takes the last fraction of a polydisperse powder to dissolve. For example, it is seen from Eq. 16 that the time for complete dissolution increases exponentially with σ. For this reason, a significant correlation probably exists between σ and systemic availability for very slightly soluble drugs that exhibit low systemic availability due to dissolution rate-limited absorption. Research in this area should be of considerable pharmaceutical interest.

Although the multiparticulate dissolution model (Eqs. 40 and 41) defining K_m^* and σ may seem complex, the interpretation of these parameters is simple and they can be readily obtained. The experimental technique used requires a high precision, flow-through dissolution apparatus that is easy to standardize [*e.g.*, the apparatus described in Ref. 5 or other suitable flow-through system (12)] in combination with a nonlinear regression program.

The method could well become established as a routine procedure in quality control, and further investigation could result in improved standards for drug dissolution.

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Sensitive Assay Procedure for Ethambutol Hydrochloride *via* Charge Transfer Complex Formation

HENRY S. I. TAN ˣ, ERIC D. GERLACH, and ANTHONY S. DIMATTIO

Abstract □ The charge transfer complex formation between ethambutol and iodine was investigated and utilized as the basis for a sensitive spectrophotometric procedure for ethambutol and its dosage forms. The solutions exhibited blue-shifted iodine bands at 293 and 360 nm. A Job's plot of corrected absorbance against the mole ratio of ethambutol to iodine indicated a 1:2 drug–iodine ratio. At 293 nm, the absorbance was linear (r = 0.9998) over the 0.25–15-μg/ml concentration range, but the concentration range for best accuracy is 1.6–5.8 μg/ml. The method can be applied successfully to the analysis of commercially available ethambutol tablets.

Keyphrases □ Ethambutol—spectrophotometric analysis using charge transfer complex formation, prepared samples and commercial tablets □ Spectrophotometry—analysis using charge transfer complex formation, ethambutol in prepared samples and commercial tablets □ Charge transfer complex formation—spectrophotometric analysis of ethambutol in prepared samples and commercial tablets □ Antibacterials—ethambutol, spectrophotometric analysis using charge transfer complex formation, prepared samples and commercial tablets

Ethambutol is a new antitubercular drug included in USP XIX (1). Being a relatively new drug, few procedures have been reported for its determination as a drug substance or in commercial dosage forms. The official assay procedure involves a nonaqueous titration with perchloric acid titrant (1).

Quantitative analyses of small quantities of ethambutol have been based on the chelation properties of the drug with copper in aqueous or nonaqueous media (2, 3). A reineckate method and an acid–dye technique using bromthymol blue were also reported (4, 5). However, these procedures are not feasible for the analysis of microquantities of the drug in biological fluids.

Amines and alcohols are lone-pair (n) electron donors and can interact strongly with sacrificial electron acceptors such as iodine to form charge transfer complexes (6). Recently, a study on charge transfer complexes of iodine with alkaloids was reported (7). This paper reports the charge transfer complex formation of ethambutol with iodine and its application to drug assay.

EXPERIMENTAL

Apparatus—A UV–visible double-beam spectrophotometer[1] with 1-cm cells and dynode voltage of 500 v (slit width 0.80–0.85 mm) and an analytical balance[2] were used.

Materials and Reagents—Ethambutol hydrochloride[3] and 8×10^{-4} M iodine[4] (resublimed) in anhydrous chloroform[4] were used. Other reagents were analytical grade.

Ethambutol Base—Ethambutol hydrochloride (1 g) was dissolved

[1] Beckman Acta V, Beckman Instruments Inc., Fullerton, Calif.
[2] Mettler model H-18, Mettler Instruments Co., Princeton, N.J.
[3] Lederle Laboratories, Pearl River, N.Y.
[4] Fisher Scientific Co., Pittsburgh, Pa.

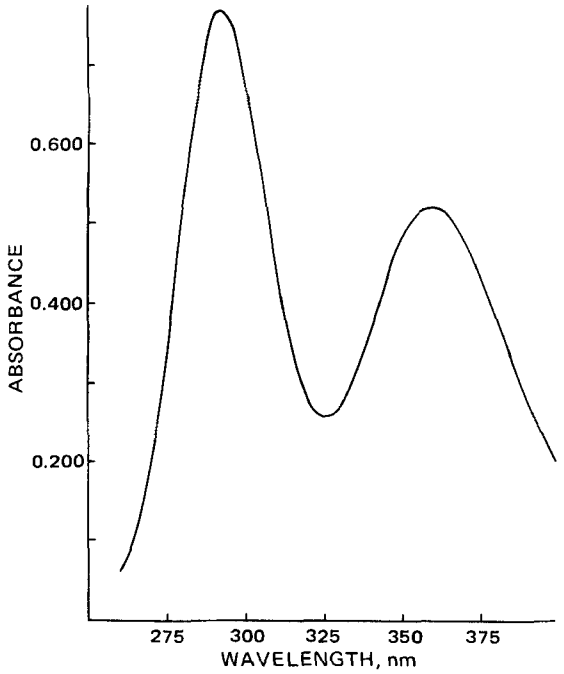

Figure 1—*Absorption spectrum of ethambutol–iodine complex.*

in 50 ml of aqueous sodium hydroxide solution (1:12) and extracted with five 30-ml portions of chloroform. The chloroform extracts were combined, dried with anhydrous sodium sulfate, and filtered, and the filtrate was evaporated to dryness under nitrogen. Recrystallization of the residue (yield 98.9%) from chloroform–hexane afforded white needles (mp 87–88°).

Standard Curve—A 25.0-mg portion of ethambutol base was weighed accurately and dissolved in 100.0 ml of anhydrous chloroform. Further dilutions were then made to prepare standard solutions containing 2.5–150 μg of ethambutol/ml. One milliliter of this solution was utilized for complex formation with the iodine reagent as described under *Assay Procedure for Ethambutol Hydrochloride,* beginning with: "Pipet 1.0 ml of this solution into a 10-ml volumetric flask . . . ."

Assay Procedure for Ethambutol Hydrochloride—*Powder*—Weigh accurately about 50.0 mg of ethambutol hydrochloride and transfer into a 60-ml separator. Dissolve the powder in 3 ml of sodium hydroxide solution (1:12) and extract with five 15-ml portions of anhydrous chloroform. Combine the chloroform extracts, dry them with anhydrous sodium sulfate (5 min), and filter through dry filter paper into a 100-ml volumetric flask. Rinse the sodium sulfate and filter with anhydrous chloroform, and use the washings to dilute the bulk solution to volume.

Measure 5.0 ml (buret or pipet) into a 50-ml volumetric flask and dilute to volume with anhydrous chloroform. Pipet 1.0 ml of this solution into a 10-ml volumetric flask, add 5.0 ml of iodine reagent, and dilute to volume with anhydrous chloroform. Allow the solution to stand in the dark for no less than 1 hr and measure the absorbance at 293 nm against a blank. Determine the concentration of ethambutol from the calibration curve or from the regression equation.

Tablets—Weigh and finely powder 20 ethambutol hydrochloride tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of ethambutol hydrochloride, to a 60-ml separator. Then add 3 ml of sodium hydroxide solution (1:12) and proceed as described for the powder, beginning with: "extract with five 15-ml portions of anhydrous chloroform . . . ."

Stoichiometric Relationship—Master solutions of iodine (1.67×10^{-4} *M*) and ethambutol base (1.67×10^{-4} *M*) in anhydrous chloroform were prepared. A series of 10-ml quantities of mixtures containing a total of 10 ml of the master solution of ethambutol base and iodine in different complementary proportions (from 0:10 to 10:0 inclusive) were made up in 10-ml volumetric flasks. After the flasks were allowed to stand in a water bath at 25° for 60 min, absorbances were measured at 293 nm.

Association Constant and Molar Absorptivity—Ethambutol base solutions in anhydrous chloroform were prepared (1.5, 3.0, and 6.0×10^{-4} *M*), and these solutions and an iodine solution in anhydrous chloroform (8×10^{-5} *M*) were placed in a water bath at 25° for 30 min. Five milliliters of the iodine solution was mixed with the same volume of each ethambutol solution. After quick mixing, the absorbance of each solution was determined immediately at 293 nm.

Table I—Effect of Time on Absorbance of Ethambutol–Iodine Complex

Minutes after Mixing	Absorbance (293 nm)
0	0.823
10	0.930
20	0.923
30	0.917
40	0.908
50	0.896
60	0.881
70	0.880
80	0.879
90	0.880
120	0.878

RESULTS AND DISCUSSION

Charge transfer complex formation is distinguished from other slow oxidation or substitution reactions of iodine with amines by being practically instantaneous, analogous to ionic reactions. Upon reaction of ethambutol with a chloroform solution of iodine, the violet color of iodine is changed immediately to yellowish purple or yellow, depending on the amount of ethambutol present. Further confirmation of the charge transfer nature of the complex was obtained by extracting the drug from the complex by shaking with aqueous hydrochloric acid, whereby the violet color of iodine was restored in the chloroform layer. The regeneration of the drug from the charge transfer complex is easy, since the binding energy of charge transfer complexes is usually small; addition of a solvent of relatively high polarity causes complete decomposition (8).

Therefore, the chloroform used must be checked for its suitability. With some spectrograde samples of chloroform, no complex was formed, as evidenced by a lack of absorbance in the wavelength region studied. This result was due presumably to relatively high concentrations of water. Unfortunately, the water content levels in these spectrograde brands of chloroform were not furnished by the manufacturers. *Anhydrous* chloroform[4], which contains the same amount of ethanol preservative but a low water content of 0.02%, was suitable as a medium for the ethambutol–iodine complex formation.

The absorption spectrum of iodine in chloroform showed only one peak, with a maximum absorption at 515 nm. However, the charge transfer complex with ethambutol exhibited blue-shifted iodine bands at 293 and 360 nm, with the first peak being more intense than the second (Fig. 1). The absorption at these wavelengths is due solely to the complex because ethambutol itself is nonabsorbing.

Although the complex was formed rapidly, constant absorbance readings were obtained only after no less than 1 hr of standing in the dark (Table I). The readings remained constant for at least 1 additional hr.

At 293 nm, a linear relationship ($r = 0.9998$) was obtained between absorbance and concentration over the 0.25–15-μg/ml range. The regression equation[5] of the line was $A_{293} = 0.1648C - 0.0079$, where A_{293} is the absorbance at 293 nm, and C is the concentration of ethambutol base expressed in micrograms per milliliter. Conversion to the concentration (micrograms per milliliter) of ethambutol hydrochloride can be obtained by multiplying C by 1.357. The standard deviations ($n = 3$) of the slope and y-intercept were 4.1×10^{-3} and 5.5×10^{-3}, respectively.

The Ringbom plot (9) indicates that the concentration range for best accuracy is 1.6–5.8 μg of ethambutol base/ml in the final test solution (Fig. 2). Deviations from linearity in the Beer–Lambert plot result when the concentrations of donor and acceptor differ in magnitude (8, p. 171). For this reason, the concentration of iodine reagent was kept at the same order of magnitude as that of the ethambutol.

The determination of the mole ratio of reactants in the complex is based on the method of continuous variation (10). The plot (Fig. 3) shows a 1:2 molar ratio of ethambutol–iodine in the complex. No attempts were made to predict the orientation of the donor and acceptor moieties in the charge transfer complex.

In analogy to equations for the equilibria involved in a 1:1 charge

[5] Wang computer 700C and plotting output writer 2202.

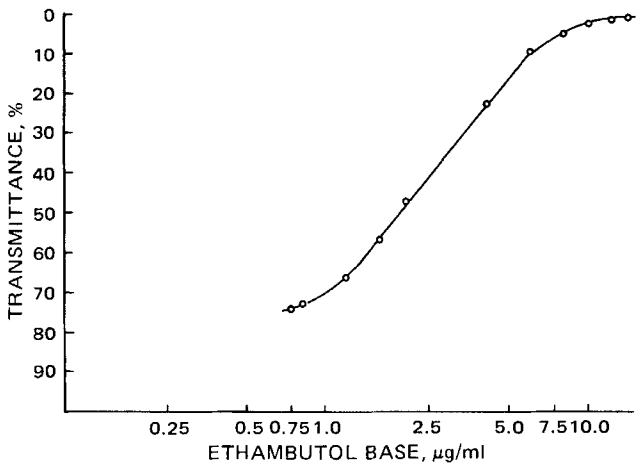

Figure 2—*Ringbom plot of ethambutol–iodine complex.*

transfer complex formation (8, 11, 12), it can be postulated that for a 1:2 ethambutol–iodine ratio the reaction for the equilibrium involved is shown in Scheme I:

$$E + 2I_2 \rightleftarrows E \cdot 2I_2$$

Scheme I

where E stands for ethambutol. Based on this equilibrium reaction, the association constant, K, for the complex $E \cdot 2I_2$ can be easily derived:

$$K = \frac{[E \cdot 2I_2]}{[E][I_2]^2} \qquad \text{(Eq. 1}a\text{)}$$

$$K = \frac{[E \cdot 2I_2]}{([E]_0 - [E \cdot 2I_2])([I]_0 - [E \cdot 2I_2])^2} \qquad \text{(Eq. 1}b\text{)}$$

where $[E]_0$ and $[I]_0$ indicate the total, *i.e.*, free and complexed, concentrations of ethambutol and iodine, respectively. Then:

$$\frac{1}{K} = \frac{[E]_0[I]_0^2}{[E \cdot 2I_2]} + [E \cdot 2I_2]([E]_0 + 2[I]_0) - [E \cdot 2I_2]^2 - [I]_0(2[E]_0 + [I]_0) \qquad \text{(Eq. 2)}$$

Since the absorbance, A, at 293 nm is due entirely to the complex, which obeys the Beer–Lambert law, in a 1-cm cell:

$$[E \cdot 2I_2] = \frac{A}{\epsilon} \qquad \text{(Eq. 3)}$$

where ϵ is the molar absorptivity of the complex. Substituting Eq. 3 into Eq. 2 yields:

$$\frac{1}{K} = \frac{[E]_0[I]_0^2\epsilon}{A} + \frac{A([E]_0 + 2[I]_0)}{\epsilon} - \frac{A^2}{\epsilon^2} - [I]_0(2[E]_0 + [I]_0) \qquad \text{(Eq. 4)}$$

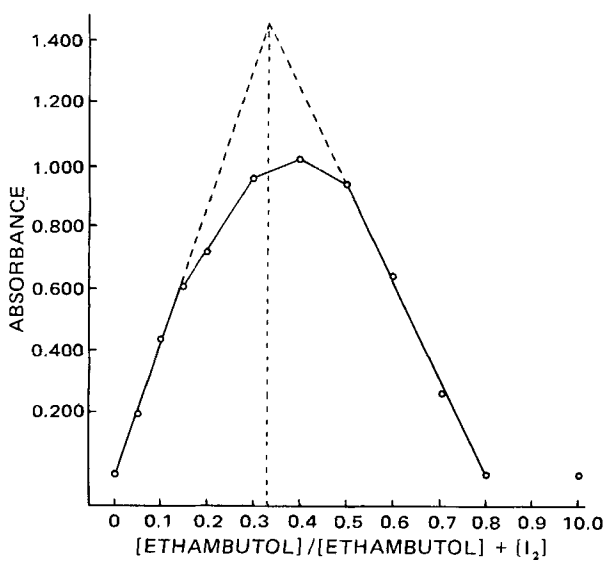

Figure 3—*Continuous variation plot obtained from solutions of ethambutol and iodine in chloroform (1.67×10^{-4} M).*

Table II—Precision Study of the Method at the 4.1-μg of Ethambutol Base/ml Concentration Level

Sample	Absorbance (293 nm)
1	0.630
2	0.611
3	0.614
4	0.629
5	0.631
6	0.618
7	0.618
8	0.619
9	0.624
10	0.624
11	0.626
12	0.633
13	0.616
Average	0.622
RSD	0.0112

Equation 4 contains two unknowns, K and ϵ. If two equations like Eq. 4 are set up, each representing different experimental trials (*i.e.*, $[E]_0$ and $[E]_0'$ and A and A'), an analytical solution can be derived specifically for ϵ. Because this expression was awkward to use, a graphical procedure was developed.

Evaluation of Eq. 4, which is a nonhomogeneous polynomial of the third order, by substituting random values of ϵ for a given set of experimental data generates corresponding values of K^{-1}. The values of K^{-1} and ϵ for *each* given set of experimental data are plotted. The intersect of the curves yields the association constant and molar absorptivity of the system. The results (Fig. 4) show that the association constant, K, for the ethambutol–iodine complex is $2.07 \pm 0.22 \times 10^8$ and that the molar absorptivity is $6.10 \pm 0.15 \times 10^3$.

The reproducibility of the procedure was determined by running 13 replicate samples, each containing 4.1 μg of ethambutol base/ml in the final test solution. At this concentration level, the relative standard deviation was 0.0112 (Table II).

Table III shows the accuracy of the proposed method. Recovery studies were performed on five different amounts of ethambutol hydrochloride,

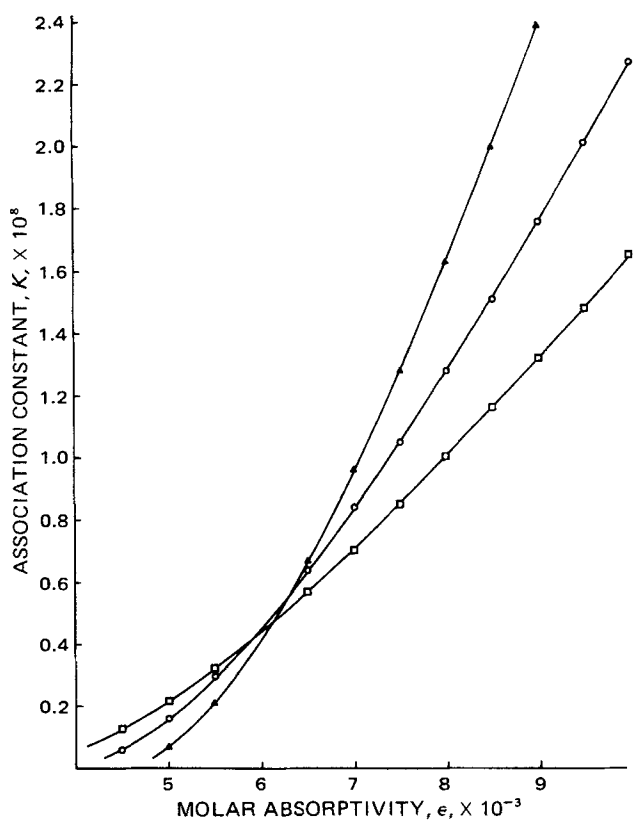

Figure 4—*Graphical solution of Eq. 4 for a series of ethambutol and 8×10^{-5} M iodine solutions in chloroform. Key: □, 1.5×10^{-4} M ethambutol; ○, 3.0×10^{-4} M ethambutol; and △, 6.0×10^{-4} M ethambutol.*

Table III—Recovery Studies of Known Ethambutol Hydrochloride Samples and Commercially Available Tablets

Amount Weighed, mg, or Claimed, mg/Tablet	Concentration Level, μg/ml	Amount Found, mg or mg/Tablet		Percent Recovery or Percent of Claim	
		Proposed Method	USP XIX Method	Proposed Method	USP XIX Method
		Known Samples			
42.1	3.10	42.6	—	101.2	—
46.4	3.42	46.2	—	99.6	—
50.6	1.86	48.7	—	96.2	—
50.7	3.74	50.2	—	99.0	—
63.8	9.40	62.8	—	98.4	—
201.6	—	—	192.8	—	95.6
203.1	—	—	195.3	—	96.2
Overall recovery, %				98.9	95.9
SEM				0.82	
		Commercial Tablets			
100.0	3.73	99.1	99.3	99.1	99.3
100.0	3.67	98.0	97.4	98.0	97.4
400.0	1.86	376.0	388.2	94.1	97.1
400.0	1.87	385.9	391.7	96.5	97.9

and each was analyzed at different concentration levels. The overall percent recovery of the five samples was 98.9% with a standard error of the mean of 0.82. Results obtained by applying the method to the analysis of commercially available ethambutol hydrochloride tablets are in agreement with those obtained by the compendial method.

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GLC Determination of Lasalocid and Its Bromo Analog as Their Silyl Derivatives

GERALD MANIUS and V. VISWANATHAN [x]

Abstract □ A GLC method is presented for the determination of lasalocid and its bromo analog. The method is based on the quantitative trimethylsilylation of the compounds without any molecular cleavage, followed by chromatography on a nonpolar silicone column. Silylation was carried out directly without any extraction or prior cleanup, despite the complexity of the dosage forms. This procedure was used for the assay of pure substances, pellets, premixes, experimental ampul solutions, and mycelial filter cakes. The results were in good agreement with data obtained by microbiological procedures.

Keyphrases □ Lasalocid and bromo analog—GLC analysis, various preparations and dosage forms □ GLC—analysis, lasalocid and bromo analog, various preparations and dosage forms □ Coccidiostatic antibacterials—lasalocid and bromo analog, GLC analysis, various preparations and dosage forms

The isolation of lasalocid (I), an antibiotic, was reported by Berger *et al.* (1). The structural configuration and the existence of four homologs for this antibiotic were described subsequently (2–4). Recently, a microbiological cylinder-plate assay (5) and a spectrofluorometric procedure for the determination of lasalocid in premixes (6) were reported. A GLC procedure also was reported in which the antibiotic was assayed after thermal degradation to yield the retroaldol ketone (7).

The bioassay procedure is a good indicator of the mi-