leucine *versus* those catalyzed by palladium-on-poly-*S*-valine. The catalyst possessing the α-helical conformation (palladium-on-poly-*S*-leucine) gave a faster rate of hydrogenation than the nonhelical catalyst (palladium-on-poly-*S*-valine).

The hydrogenation rates for α-methylcinnamic acid were faster than those for α-acetamidocinnamic acid with both of the catalysts.

Inhibition studies show that the two substrates, α-methylcinnamic acid and α-acetamidocinnamic acid, occupy entirely different sites on the catalyst surface in both the palladium-on-poly-*S*-leucine- and the palladium-on-poly-*S*-valine-catalyzed reactions.

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DRUG STANDARDS

Use of Ceric Sulfate and Cupric Perchlorate for Titrimetric Analyses of Phenothiazine Derivatives

L. G. CHATTEN, R. A. LOCOCK, and R. D. KRAUSE

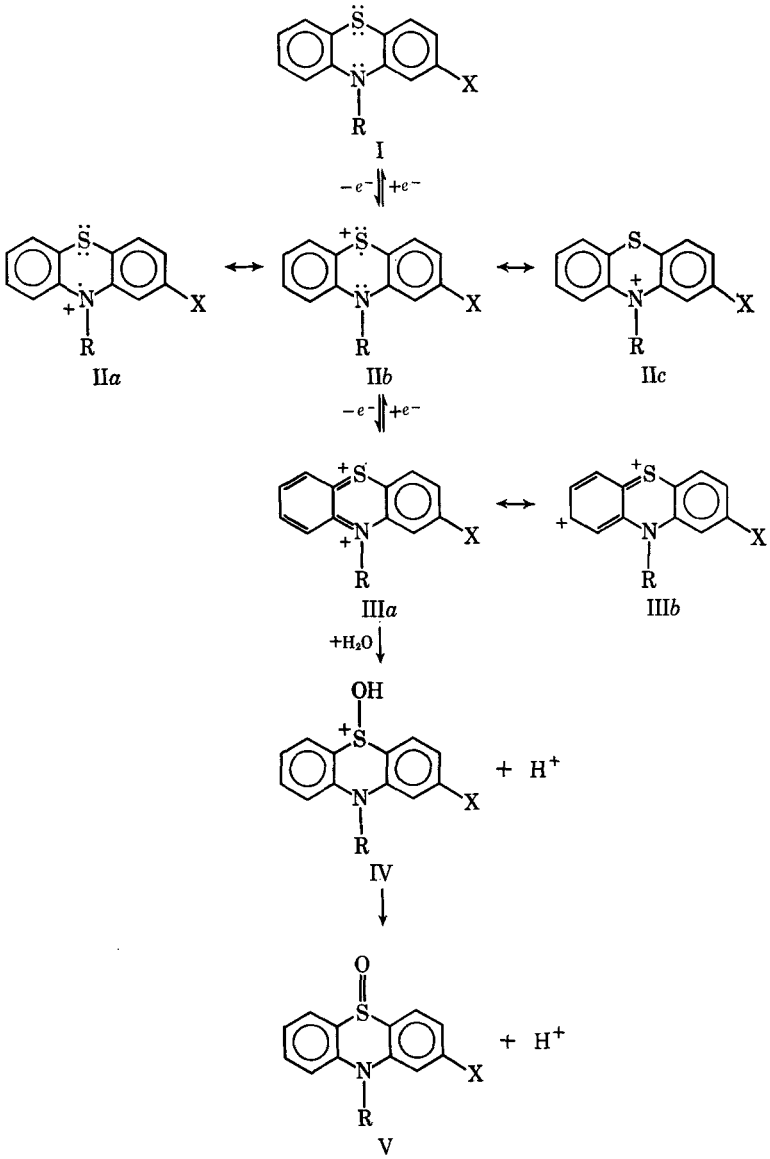
Abstract □ A method was developed in which phenothiazine derivatives were titrated visually to a colorless end-point with ceric sulfate. Quantitative recoveries were obtained only for chlorpromazine, acetylpromazine, trifluoperazine, and triflupromazine. The method was also applied to pharmaceutical dosage forms of these drugs. UV photometric detection of the end-point was found to be applicable only to thiethylperazine and thioridazine. Attempts to develop a quantitative procedure for phenothiazines by photometric titration with cupric perchlorate in acetonitrile were unsuccessful.

Keyphrases □ Phenothiazine derivatives and dosage forms—analysis □ Ceric sulfate—phenothiazine titration □ Titration, visual—phenothiazine derivatives, analysis □ UV photometric titration—phenothiazine derivatives, analysis

In 1940, Michaelis *et al.* (1) showed, by potentiometric titration, that the oxidation of phenothiazine (I) and several of its derivatives proceeds through two successive and distinct one-electron steps. Scheme I illustrates the sequence in the oxidation of an *N*-substituted phenothiazine. A highly colored free radical or semiquinone (II) results from the first oxidation step. The loss of another electron from this intermediate gives a phenazathionium ion (III). The phenazathionium ion from the second step can then react with water to produce a sulfonium base (IV), which will lose a proton to yield the phenothiazine sulfoxide (V).

The formation of colored semiquinone products by the action of a variety of oxidizing agents has been used for the detection and assay of phenothiazines (2–15). The 1963 edition of the British Pharmacopoeia (16) included an oxidimetric assay procedure for chlorpromazine tablets, using ceric sulfate as the titrant and dilute sulfuric acid as the solvent. The end-point was determined visually using the phenanthroline–ferrous complex as indicator. This method is not in the 1968 British Pharmacopoeia (17). During this investigation, Agarwal and Blake (18) reported the titration of phenothiazines and some dosage forms with ceric sulfate. The end-point was determined photometrically by following the reaction at 420 nm., the wavelength of maximum absorbance of ceric sulfate.

The purposes of the present investigation were: (*a*) to develop a simple assay for phenothiazine drugs



Scheme I—Oxidation pathway of an N-substituted phenothiazine derivative

using aqueous ceric sulfate or cupric perchlorate in acetonitrile as the titrant, and (*b*) to apply the procedure to various dosage forms on the Canadian market.

EXPERIMENTAL

Apparatus—The following were used: low actinic volumetric flasks, 5-ml. microburet equipped with a platinum tip, a Beckman model DB spectrophotometer equipped with a Beckman 96160 flow cell, and a titration vessel originally described by Rehm *et al.* (19).

Reagents—The following were used: glacial acetic acid; 0.1 *M* perchloric acid in glacial acetic acid (standardized against primary standard potassium acid phthalate); 6% mercuric acetate in glacial acetic acid; 0.01 *M* disodium ethylenediaminetetraacetic acid; copper metal[1]; 0.1% α-pyridyl-β-azonaphthol in 95% ethanol; acetonitrile; 0.001 and 0.005 *M* cupric perchlorate in acetonitrile; sulfuric acid; ceric ammonium nitrate; arsenic trioxide (primary standard); 0.025 *M ortho*-phenanthroline–ferrous sulfate complex; 1 in 400 solution of osmium tetroxide in 0.05 *M* sulfuric acid; chloroform; and methanol.

Phenothiazine Derivatives and Dosage Forms Investigated—The following phenothiazine drugs were used in this investigation: acetylpromazine maleate, aminopromazine fumarate, chlorpromazine and chlorpromazine hydrochloride, fluphenazine dihydrochloride, levomepromazine hydrochloride, mepazine acetate hydrate, methdilazine, perphenazine, pipamazine, prochlorperazine and prochlorperazine dimaleate, promazine and promazine hydrochloride, promethazine, pyrathiazine, thiethylperazine dimaleate, thiopropazate and thiopropazate dihydrochloride, thioridazine and thioridazine hydrochloride, trifluoperazine and trifluoperazine dihydrochloride, triflupromazine hydrochloride, and trimeprazine tartrate.

The purity of these compounds was established by titration of 0.1 meq. of the phenothiazine derivative dissolved in glacial acetic acid with 0.1 *M* acetous perchloric acid, using crystal violet as the indicator. Mercuric acetate in glacial acetic acid was added for halide salts. All compounds had a purity of 98% or greater, with the exception of pipamazine (97.85%) and pyrathiazine (93.79%).

Dosage forms were analyzed for the following: chlorpromazine[2], fluphenazine[3], triflupromazine[4], and trifluoperazine[5].

[1] British Drug House, Analar.

[2] Chlorpromanyl "10" tablets and Chlorpromanyl injection (Paul Maney Laboratories); Largactil tablets, Largactil Spansule capsules, Largactil injection, Largactil suppositories, and Largactil liquid (Poulenc Ltd.)

[3] Moditen tablets, E. R. Squibb and Sons Ltd.

[4] Vesprin injection, E. R. Squibb and Sons Ltd.

[5] Stelazine tablets and Stelazine injection, Smith Kline & French.

Table I—Variation of Recovery with 2-Position Substituent in Titration of Phenothiazine Derivatives wih Ceric Sulfate

Drug	Average Percent Recovery[a]	2-Position Substituent
Acetylpromazine maleate	101.7	—$COCH_3$
Trifluprom azine hydrochloride	101.9	—CF_3
Trifluoperazine dihydrochloride	103.9 (4 *M*)	—CF_3
Fluphenazine dihydrochloride	106.0 (2 *M*)	—CF_3
Pipamazine	104.4	—Cl
Chlorpromazine	104.1 (2 *M*)	—Cl
Mepazine acetate hydrate	109.6	—H
Levomepromazine hydrochloride	113.3	—OCH_3
Thioridazine	120.4	—SCH_3
Thiethylperazine dimaleate	163.0	—SCH_2CH_3

[a] Solvent used was 0.2 *M* H_2SO_4 unless otherwise indicated.

Preparation and Standardization of 0.05 *M* Ceric Sulfate—The procedure followed was as outlined in the USP (20) for 0.1 *M* ceric sulfate. Appropriate adjustments were made in quantities of ceric ammonium nitrate and sulfuric acid.

Visual Titration of Phenothiazine Derivatives with Ceric Sulfate—About 10 mg. of the phenothiazine was accurately weighed into a 10-ml. beaker and dissolved in 5 ml. of dilute sulfuric acid. The titrant was 0.05 *M* ceric sulfate. The solution was titrated with the aid of a magnetic stirrer and a titration lamp. The end-point was taken at complete decolorization of the solution.

Visual Titration of Pharmaceutical Dosage Forms with Ceric Sulfate—*Tablets*—Twenty tablets were weighed and powdered. A sample of powder approximately equivalent to 10 mg. of the active ingredient was accurately weighed into a 10-ml. beaker, dissolved in 5 ml. of 1 *M* sulfuric acid, and titrated as already described.

Injections and Solutions—An aliquot of the liquid preparation containing about 10 mg. of the active ingredient was accurately pipeted into a 10-ml. beaker. After dilution to 5 ml. with 1 *M* sulfuric acid, titration proceeded as for tablets.

Suppositories—Ten suppositories were weighed and reduced to fine particles. An amount of material approximately equivalent to 10 mg. of the active ingredient was accurately weighed into a 10-ml. beaker and dissolved in 3 ml. of chloroform. Three milliliters of methanol was added, and the titration was completed as for the tablets.

Photometric Titration of Phenothiazine Derivatives with Ceric Sulfate—An aliquot containing about 0.25 mg. of the drug in 1 *M* sulfuric acid was transferred to the photometric titration vessel and diluted to 25 ml. with 1 *M* sulfuric acid. The titrant, 0.005 *M* ceric sulfate, was added in 0.05-ml. increments from a 5-ml. microburet; the absorbance at 270 nm. (275 nm. for thiethylperazine or thioridazine) was read 30 sec. after the addition of each increment. The absorbance was plotted against the volume of titrant added, and the end-point was taken at the change of slope of the graph.

Standardization of Cupric Perchlorate Titrant in Acetonitrile—A 0.01 *M* solution of the disodium salt of ethylenediaminetetraacetic acid in water was standardized against pure copper metal. The indicator employed was a 0.1% α-pyridyl-β-azonaphthol solution in ethanol, which changed at the end-point from violet to yellow.

The standardized ethylenediaminetetraacetic acid solution was then used to standardize 0.005 and 0.001 *M* solutions of cupric perchlorate in acetonitrile. A 5-ml. aliquot of the acetonitrile solution was treated in the same manner.

Photometric Titration of Phenothiazine Derivatives with Cupric Perchlorate—An aliquot containing about 4 mg. of the drug in acetonitrile was transferred to the photometric titration vessel and diluted to 25 ml. with acetonitrile. One milliliter of 0.005 *M* cupric perchlorate in acetonitrile was added to the solution, and the visible spectrum was scanned. The wavelength of maximum absorbance was used for the photometric titration. The titration vessel and flow-through cell were then drained and rinsed with acetonitrile; another aliquot of drug was added and diluted. The titrant was added from a 5-ml. microburet in 0.4-ml. increments, and the absorbance was read 30 sec. after the addition of each increment. The absorbance was plotted against the volume of titrant added, and the end-point was taken at the change of slope of the graph.

RESULTS AND DISCUSSION

Titrations with Ceric Sulfate—The use of ceric ion in aqueous solutions as a titrant for the analysis of phenothiazines has been extensively investigated (4–13). Since ceric ion is a very strong oxidizing agent (E° = 1.44 v. in 1 *M* H_2SO_4), phenothiazines are oxidized to the corresponding sulfoxides; thus, the equivalence point in the titrations corresponds to the addition of 2 moles of ceric ion per mole of phenothiazine.

The titrations were carried out in aqueous solutions of sulfuric acid where the free radical intermediate is stabilized. During the titrations, the color of the solution, due to the presence of the free radical species, increased to a maximum after 1 equivalent of titrant was added; then the color decreased in intensity and disappeared entirely after 2 equivalents were added. It appears, therefore, that a simple procedure could be devised in which this self-indicating property of phenothiazine derivatives might be utilized for their analysis. Accordingly, a series of titrations were carried out in which a pure phenothiazine derivative in dilute sulfuric acid was titrated to a colorless end-point with a standard solution of ceric sulfate. Each derivative was titrated in several different concentrations of sulfuric acid, but a consistent correlation could not be found between the results and the concentration of acid in the solvent.

The recoveries obtained were invariably greater than 100%. A correlation was seen between the extent of the error and the electron-withdrawing power of the substituent in the 2-position of the phenothiazine nucleus. The best results were obtained when this substituent was strongly electron withdrawing. Typical results are listed in Table I.

From these results, it appears that ceric sulfate is such a strong oxidizing agent that the phenothiazine derivatives are oxidized past the sulfoxide stage during the titration. When the end-point, chosen by the disappearance of the color due to the free radical species, is reached, some titrant already has been consumed in converting the sulfoxide to a higher oxidation state, probably the corresponding 3-hydroxyphenothiazine or 3-phenothiazone derivative. This subsequent reaction would be expected to proceed to a greater extent in compounds that are more easily oxidized. Kabasakalian and McGlotten (21), in a polarographic study of phenothiazines, found that oxidation was more difficult when electron-withdrawing groups were substituted in the 2-position of the phenothiazine nucleus.

Analysis of Pharmaceutical Dosage Forms—The visual titration method with ceric sulfate was also applied to certain pharmaceutical

Table II—Results of Visual Titration of Phenothiazine Dosage Forms with Ceric Sulfate

Dosage Form	Percent Recovered
Chlorpromazine hydrochloride, 11.2 mg./tablet	101.8
Chlorpromazine hydrochloride, 55.8 mg./tablet	99.1
Chlorpromazine hydrochloride, 30.0 mg./tablet	109.0
Chlorpromazine, 25.0 mg./suppository	109.0
Chlorpromazine hydrochloride injection, 27.9 mg./ml.	99.1
Chlorpromazine hydrochloride injection, 5.58 mg./ml.	96.7
Chlorpromazine hydrochloride liquid, 27.9 mg./5 ml.	127.6
Fluphenazine hydrochloride, 1 mg./tablet	108.1
Trifluoperazine dihydrochloride, 10 mg./tablet	101.6
Trifluoperazine dihydrochloride injection, 1 mg./ml.	110.4
Trifluoperazine hydrochloride injection, 20 mg./ml.	100.5

Table III—Results of Photometric Titration of Phenothiazine Derivatives with Cupric Perchlorate

Drug	R[a] at End-Point: Without Perchloric Acid, First End-Point	R[a] at End-Point: Without Perchloric Acid, Second End-Point	R[a] at End-Point: With Perchloric Acid
Chlorpromazine	1.23	2.82	1.49
Levomepromazine	0.85	2.54	1.49
Prochlorperazine	0.72	2.76	1.57
Promethazine	0.86	2.04	1.34
Thioridazine	0.92	2.64	1.58
Trifluoperazine	1.14	2.73	1.46
Methdilazine	1.16	2.65	1.47

[a] R = (moles cupric perchlorate/mole drug).

dosage forms of those phenothiazine derivatives for which the best results were obtained. Results of these titrations are shown in Table II.

Compressed tablets were analyzed simply by stirring a quantity of the powdered tablet material with dilute sulfuric acid for several minutes and then titrating to the disappearance of color with ceric sulfate. Although insoluble tablet excipients remained suspended throughout the titration, there was no difficulty in determining the decolorization end-point. Good results were obtained for chlorpromazine and trifluoperazine tablets. The recovery obtained for fluphenazine tablets was high, but only slightly higher than the recovery obtained when the pure drug was analyzed by this method.

The chlorpromazine hydrochloride injections contained a preservative which was more easily oxidized by ceric sulfate than was chlorpromazine. Thus, initial addition of titrant did not result in the production of a red coloration, but a color did appear after all the preservative was oxidized. Readings were taken at the first appearance of color and at complete decolorization of the solution. The difference was the amount of titrant consumed by the drug. Recoveries obtained for several dosage forms were higher than expected, probably because of the presence of other oxidizable substances in the product.

UV Photometric Titrations with Ceric Sulfate—UV absorption spectra of dilute sulfuric acid solutions of phenothiazine derivatives substituted with —H, —Cl, —OCH_3, or —CF_3 in the 2-position show a strong peak at 250–255 nm. and a weaker, broad peak centered around 300 nm. Derivatives substituted with —SCH_3 or —SCH_2CH_3 in the 2-position also showed a weak, broad peak at 300 nm., but the position of the stronger peak was shifted to 260 nm. Addition of 2 equivalents of ceric sulfate to a solution of chlorpromazine converted the chlorpromazine spectrum to that of chlorpromazine sulfoxide, which exhibited absorption maxima at 240, 273, and 299 nm. Similarly, the addition of 2 equivalents of ceric sulfate to solutions of all phenothiazine derivatives with —H, —Cl, —OCH_3, or —CF_3 substituents in the 2-position resulted in spectra with strong absorption in the 240-nm. region and absorption peaks between 269 and 273 nm. The position of this latter peak was shifted to 275 nm. when the 2-substituent was a thioalkyl group.

Since the sulfoxide and the free radical species derived from thioalkyl substituted phenothiazines absorb at different wavelengths in the UV regions, the absorption due to the sulfoxide species produced during the titration can be measured without interference from the free radical intermediate. Photometric titrations of these compounds at a wavelength of 275 nm. resulted in a curve in which the absorbance increased linearly with the addition of ceric sulfate until 2 equivalents of titrant were added and then remained constant with the addition of further increments of titrant. Recoveries of thiethylperazine and thioridazine obtained by this method were 98.3 and 98.1%.

Photometric titrations with ceric sulfate were also attempted for phenothiazine derivatives with substituents other than thioalkyl. With these compounds, the absorption of the free radical interfered with that of the sulfoxide at 270 nm. Thus, a peak was produced in the photometric titration curve after the addition of 1 equivalent of titrant, corresponding to the maximum intensity of the free radical absorption. The absorption at 270 nm. decreased with the addition of the 2nd equivalent of titrant as the free radical was being converted to the sulfoxide. After 2 equivalents of ceric sulfate were added, the absorbance, now due only to the sulfoxide, remained constant with the addition of further increments of titrant. Recoveries obtained for the latter class of compounds ranged from 84 to 125%.

Titrations with Cupric Perchlorate—Although the use of nonaqueous solvents as media for acid–base reactions in analytical chemistry is now well established, little work has been done to investigate the use of these solvents in oxidation–reduction reactions. A large proportion of the work reported in this area involved the utility of glacial acetic acid as the solvent. Some interest has been focused on acetonitrile as a solvent because it is highly resistant to oxidation or reduction. Its moderately high dielectric constant promotes ionization of solutes and, therefore, more rapid electron transfer between species. Kratochvil *et al.* (22) found that cupric perchlorate is a powerful oxidizing agent in acetonitrile. Consequently, the titration of the free bases of several phenothiazine derivatives in acetonitrile with a standard solution of cupric perchlorate was attempted. The end-point was determined photometrically by following the absorbance at the wavelength of maximum absorption of the colored free radical intermediate produced upon partial oxidation of a phenothiazine derivative, which occurs at 510 nm. in phenothiazines not substituted in the 2-position, at 500 nm. if the 2-substituent is a —CF_3 group, at 525 nm. if —Cl, at 575 nm. if —OCH_3, and at 645 nm. in thioridazine where the substituent is —SCH_3.

Photometric titrations were attempted both with and without the prior addition of perchloric acid. If acid was not added to the solution before titration, initial addition of cupric perchlorate did not result in the production of any color. The free radical is stabilized by the presence of hydrogen ions; apparently when none is added to the solution, the free radical intermediate does not exist. Under these conditions, the oxidation does not stop at the free radical stage but continues to the formation of the phenazathionium ion. However, after the addition of 0.7–1.2 moles of cupric perchlorate per mole of phenothiazine, a stable color was produced in the solution, resulting in a linear increase in absorbance with the addition of more titrant, and an apparent photometric end-point. Stabilization of the free radical form is thought to result from accumulation of hydrogen ions produced in the reaction of phenazathionium ion and traces of water present in the solvent.

Addition of perchloric acid to the solution before titration changes the shape of the titration curve. In this instance, the first addition of titrant results in the formation of a color since the free radical is being stabilized by the acid added. As more titrant is added, a linear increase in absorbance is seen. The end-point is selected as that place where the slope of the graph changes from highly positive to zero or slightly negative. The ratio of moles of cupric perchlorate added at the end-point to moles of phenothiazine present in solution is about 1.5:1 for most of the phenothiazines titrated (Table III).

That this ratio is not integral is probably a result of more than one reaction proceeding at the same time. Initially, addition of titrant produces the free radical species which is stabilized by the presence of acid in the solution. If this was the only reaction, the photometric curve would increase to a maximum after 1 equivalent of cupric perchlorate was added and then decrease at the same rate as more titrant was added, since the additional titrant would oxidize the free radical to the colorless phenazathionium ion. However, it appears that these reactions occur simultaneously; by the time the absorbance due to the free radical species reaches a maximum, some of the free radical has reacted further.

If this hypothesis is correct, an increase in acid concentration would tend to decrease the rate of the second reaction relative to that of the first reaction because of the increased stability of the free radical formed by the first reaction. Thus, the ratio of moles of cupric perchlorate to moles of drug found at the end-point should decrease to a limit of unity with increasing acid concentration. The effect of the concentration of perchloric acid on the molar ratio obtained in the titration of methdilazine was investigated and the results do support the theory proposed. From a value of 1.47 at a perchloric acid concentration of 0.005 *M*, the molar ratio decreased to a value of 1.42 at an acid concentration of 0.1 *M*, 1.27 at an acid concentration of 1.0 *M*, and 1.18 at an acid concentration of 2.0 *M*. As the acid concentration was increased, the photometric titration curve became increasingly rounded in the end-point region. If the acid concentration in the solvent was raised to more than 2 *M*, this curvature made it impossible to determine an end-point in the titration.

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Determination of Zinc in Pharmaceutical Products by Neutron Activation Analysis

MICHEL MARGOSIS and JOSEPH P. F. LAMBERT*

Abstract □ Nondestructive neutron activation analysis was applied as a rapid, efficient, and specific method for the determination of zinc at either low or major concentration levels in various pharmaceutical products in bulk or dosage forms. This technique yielded results that compare well with more conventional methods and offers decided advantages over them.

Keyphrases □ Zinc in pharmaceuticals—determination □ Impurity interference—zinc determination □ Neutron activation analysis—zinc

Official methods for the analysis of zinc involve ashing followed by gravimetry (1) or complexometric (2, 3) or alkalimetric titration (1). Zinc in insulin is determined by a dithizone colorimetric procedure subsequent to extraction (2); in zinc bacitracin, it is determined by complexometric titration with edetic acid (3). However, there is no official procedure for determining zinc in various zinc bacitracin dosage forms such as ointments. The advent of atomic absorption spectroscopy has increased the efficiency and specificity of the determination of zinc, but in many laboratories this technique has been limited to simple dilute aqueous systems.

Table I—Nuclear Properties of Zinc for Activation with Thermal Neutrons

Stable Nuclide	Abundance, %	Cross Section, Barns	Radionuclide Formed	Half-life ($t_{1/2}$)	Energy of Principal γ-Ray, Mev.
^{64}Zn	48.89	0.46	^{65}Zn	245 days	1.115
^{66}Zn	27.81				
^{67}Zn	4.11				
^{68}Zn	18.56	1.0	^{69}Zn	52 min.	None
^{68}Zn	18.56	0.1	^{69m}Zn	13.8 hr.	0.439
^{70}Zn	0.62	0.10	^{71}Zn	2.3 min.	0.12
^{70}Zn	0.62	0.01	^{71m}Zn	4.1 hr.	

The application of neutron activation analysis (NAA) to zinc in pharmaceutical products offers increased utility, specificity, and accuracy (4). Advantages of NAA accrue from the facts that the technique: (*a*) is normally unaffected by the complex organic matrixes composing drug systems; (*b*) requires no physicochemical separation procedure; and (*c*) is both qualitative and quantitative in a presumptive assay method at either microconcentration or macroconcentration levels. This study shows the application of NAA for the deter-