

Figure 5. Diffuse reflectance spectrum of heated packing material after subtraction of the Chromosorb W spectrum.

at this point, is really just a mixture of the two components. Upon heating of the system, several changes occur. The loss of intensity at 2950 cm⁻¹ and from 3600 to 3000 cm⁻¹ indicates a condensation reaction involving silica hydroxyls and Carbowax 20M hydroxyls, resulting in a Si-O-C bond. Simultaneously, intensity of the combination bands at 2742 and 2695 cm^{-1} is lessened. This indicates a change in the distribution of conformers in the polyethylene glycol. These overtone bands are much more intense for ethers in a gauche conformation. In dioxane, where the conformation is locked in, these bands are quite strong, but in 1,2-dimethoxyethane they are extremely weak. Before heating, the Carbowax 20M has some ether units in a gauche conformation. Upon heating, the hydroxyl groups react and the polymer spreads to form a thin film. To cover the silica surface and maximize interaction between polymer and support, the chains stretch out, taking trans conformations. Thus the overtone bands lessen in intensity. These results suggest a thin film of Carbowax 20M, strongly adsorbed onto the silica, as proposed by Aue, but in addition the end groups have tied to the silica surface by a condensation reaction.

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Aromatic Disulfides in the Detection of Thiols

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Seven dithiodianil compounds are compared with 3,3'-dithiobis[6-nitrobenzoic acid] (Ellman's reagent) in a spot test for the detection of thiols. The limits of detection vary considerably with the thiol being detected and the detector reagent utilized, but the sensitivity of the detection of 1-butanethiol with any of the dithiodianils is at least five times that achievable with Ellman's reagent. Color changes (detection responses) produced in the tests utilizing the dithiodianils are very sensitive to solvents. The solvatochromic effects of one of the dithiodianils gave a good correlation (correlation coefficient, 0.94) with a dual polarity scale using Gutmann's acceptor and donor numbers (Gutmann, V. "The Donor-Acceptor Approach to Molecular Interactions", Plenum Press: New York, 1978) for the solvent.

While numerous methods for the detection and estimation of thiols have been reported (1-3), a procedure described by Ellman (4) which depends upon the formation of yellow colored 3-mercapto-6-nitrobenzoic acid anion by a displacement reaction between a thiol and 3,3'-dithiobis[6-nitrobenzoic acid] (Ellman's reagent) has proven to be exceptionally valuable and has very often been cited. This is largely because the detection test is extremely sensitive, the reaction of the reagent with many thiols is very rapid, and the reaction lends itself to spectrophotometric measurements and quantitative analyses.

While Ellman's method is an attractive one for many instrumental thiol analyses, its utility for detection tests which depend upon visual observation of a color change is limited because both Ellman's reagent and the displaced anion are yellow. Although the molar absorptivities of the reagent and its anion are vastly different, there are difficulties in interpreting data by visual observation under conditions where the thiol is present in very low concentrations and the reagent in relatively high concentrations. For visual detection tests, we have sought a color change corresponding to a difference between the absorption maxima of the reagents and the colored bodies of ca. 100 nm. Hence, several new disulfide reagents (4,4'-dithiodianiline derivatives $1\mathbf{a}-\mathbf{g}$, Table I) were synthesized which would hopefully (a) react similarly as



Ellman's reagent with thiols but produce thiolate anions of increased conjugation and (b) absorb light at longer, i.e., >550 nm wavelengths. In the course of this study (which was to investigate the potential of these compounds for the detection of thiols), it was found that the wavelength of absorption of the anion was markedly affected by the solvent in which the thiolate anion was formed and the effect of the solvent on the absorption maxima was not readily correlatable with empirically derived solvent parameters (e.g., E_t , Z, ...). The details of our studies, which include the development of a detection test, and correlation of the observed spectral maxima in different solvents with various solvent polarity scales form the gist of this report.

MATERIALS AND EQUIPMENT

Synthesis of 4,4'-Dithiodianiline Derivatives 1a-g. 4,4'-Dithiodianiline (10 g, 0.04 mol) from Aldrich Chemical Co. was dissolved in hot methanol (300 mL) and filtered through a bed of decolorizing carbon to obtain an amber solution. The desired aldehyde (0.10 mol) from Aldrich Chemical Co. was dissolved in hot methanol (250 mL). While hot, the 4,4'-dithiodianiline solution and the aldehyde solution were combined and then refluxed for 15 min causing the product to precipitate. After the mixture was cooled to ambient temperature, the product was filtered. In two cases (1c and 1d) the products separated as liquids. These mixtures were stirred 16 h (overnight) at ambient temperature to crystallize the products. The products were recrystallized from dimethylformamide (DMF). In several cases where crystallization did not occur immediately after cooling the dimethylformamide to ambient temperature, methanol was added to precipitate the products. The crystalline products were filtered, washed with methanol, and air-dried. Yields varied from 80 to 100%. The melting points (uncorrected) and analytical data (from Schwartzkopf Microanalytical Laboratory, Woodside, NY) for the 4,4'-dithiodianiline derivatives 1a-g are reported in Table I.

Solvents. High-grade or freshly distilled solvents were used. The organic solvents were dried for a minimum of 3 days over Type 3A molecular sieves (Grace Chemical Co.). (Drying efficiencies of aprotic solvents using molecular sieves are reported in ref 5.) Solvents that are known to form peroxides when anhydrous (6) were also passed through a small column of neutral alumina (Woelm, Activity Grade 1) immediately prior to use.

RESULTS AND DISCUSSION

Detection of Thiols with Reagents 1a-g. Comparison with Ellman's Reagent. Important factors in a visual detection test are the detection limit and the stability of the colored body. Studies were made with three thiols of varying pK_a 's: viz., 2-naphthalenethiol ($pK_a = 6.5$), thiocholine ($pK_a = 8.2$), and 1-butanethiol ($pK_a = 11.5$). The studies included (a) determination of the visible spectra (λ_{max} and molar absorptivity) and stability of the anions formed from the detection reagents in alkaline dimethylformamide solution and
 Table II. Effect of Anion Structure on the Absorption

 Spectra and Autoxidation in Alkaline Dimethylformamide

compd from) nm	rate of oxidn		
which anion was produced	$[10^{-3}\epsilon, M^{-1} \text{ cm}^{-1}]$	$\frac{10^2 k_1,^b}{\min^{-1}}$		
1a	609 [5.6]	4.3		
1b	605 [8.2]	6.6		
1c	538 [3.6]	3.6		
1d	538 [4.0]	3,5		
1e	713 [10.4]	15.4		
1f	538 [6.2]	2.4		
1g	(420^{a}) [0.2]	6.3		
Ellman's reagent	502 [26.9]	с		

^a Shoulder. ^b The kinetic studies were carried out at 25 ± 1 °C. Decomposition was found to autocatalytic. However, approximately first-order kinetics were observed during the loss of about half of the original color intensity and, for the purpose of making a comparison, first-order rate constants were calculated. ^c Under the conditions of these experiments (i.e., when the solution was kept in the dark cell compartment of the Cary 14 spectrophotometer between measurements) less than 10% of the original color intensity was lost in 1 h. However, in a room with fluorescent lighting the rate of fading was increased to 3.0%/min (see text).

(b) optimization of spot test conditions and determination of the visual detection limits using the reagents 1a-g with the three thiols.

For studies under (a) the anions of 1a-g and Ellman's reagent were formed in situ by the reaction of the disulfides with 2-naphthalenethiol. The procedure was as follows: To 1 mL of a solution of 1a-g and Ellman's reagent (5×10^{-5} M) in DMF containing 0.5% v/v of pH 8 phosphate buffer was added 1 mL of a DMF solution of 2-naphthalenethiol (10^{-3} M). After the solution was mixed, the spectrum was obtained in a 1-cm cell. Repeated scans (at 5-min intervals with the solutions being kept in the closed cell compartment of the Cary 14 spectrophotometer between scans) provided the data for determining the wavelength of maximum absorbancy, the molar absorptivity at the wavelength of maximum absorbancy, and the stability of the anions. These data are shown in Table II.

Under these conditions, the anion from Ellman's reagent was the most stable, decomposing at less than 0.2%/min. Decomposition of the anions produced from 1a-g was found to be autocatalytic, but the rate was approximately first order during the first 50% of the decomposition. First-order rate constants are reported in Table II to allow a comparison of the relative stability of the anions to be made. Thus, of the anions produced from 1a-g, the anion produced from 1f was

Table III.Solvent Effects on the Absorption Maxima of1a and on the Anion Produced from 1a inAlkaline Solution

	λ_{\max}, nm			
solvent	for 1a	for anion pro- duced from 1a		
tetrahydrofuran	375	628		
N,N-dimethyl- acetamide	374	625		
1-methyl-2- pyrrolidinone	379	620		
pyridine	375	612		
N,N-dimethyl- formamide	375	609		
dimethyl sulfoxide	379	587		
ethyl ether	373	579		
benzonitrile	382	579		
nitrobenzene	411	575		
acetone	370	566		
benzene	380	563		
propanediol 1,2- carbonate	373	557		
acetonitrile	370	544		
nitromethane	372	525		
<i>n</i> -butanol	375	501		
ethanol	371	477		
formamide	370	472		
methanol	372	454		

the most stable, decomposing at only 2.4% /min. The anion from 1e was the least stable, decomposing at 15.4% /min while the anions produced from the remaining disulfides decomposed at 6.6% /min or less. While it was found that Ellman's anion was much more stable than the ones made from 1a-gwhen the solution of the anion was kept in the dark cell compartment of the spectrophotometer between scans, exposure of the solution to light accelerated the degradation. Under fluorescent light of 430 lx illumination the decomposition rate of Ellman's reagent anion increased to 3.0% /min. Light appeared to be less accelerating to the anions from reagents 1a-g than to the anion from Ellman's reagent. Under laboratory illuminated light, fading of the colored bodies appeared visually to occur at similar rates with the anions from all of the disulfides including Ellman's reagent.

The wavelength maxima and the molar absorptivities in alkaline DMF for the anions from the reagents 1a-g and Ellman's reagent are also reported in Table II. In three compounds in which the only change was the para substituent, both the wavelength maxima and the molar absorptivities were found to increase with increasing electron-withdrawing capability of the para substituent, i.e., $F < CN < NO_2$, in com-

pounds 1g, 1f, and 1b. Also, the o,p-dinitro derivative had a higher molar absorptivity and absorbed at an even longer wavelength than the para-substituted compound. The anion from the o,p-dinitro compound (1e) displayed the longest wavelength maximum (713 nm) and the largest molar absorptivity ($1.04 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) of the anions generated from 1a-g. The anions of 1a, 1b, and 1e had similar and desirable visual colors, viz., green-blue in alkaline DMF, but the anion from 1a was more stable than the anions of the other two.

Conditions for the spot test were optimized (studies under (b)) by using 1a as the analytical reagent and with 1-butanethiol as the test thiol. The strongest color changes (detection signals) were obtained when the solvent contained 0.5% of an aqueous buffer in the 8–12 pH range. A pH 8 buffer was selected for the procedure to minimize adverse effects of alkalinity on the test blanks. The strength of the detection signal was found to be independent of the type of aqueous buffer used, and a phosphate buffer was selected for convenience. Of 18 solvents (see Table III) tested in the spot test procedure, DMF was chosen as the preferred solvent because it combined the highest test sensitivity with the most stable test blanks.

The spot detection tests were performed as follows: Into each cavity of a white ceramic spot plate was placed one $30-\mu L$ drop of a DMF solution containing 0.5% v/v of pH 8 phosphate buffer and 10⁻³ M with respect to the disulfide. To each of the above drops was added a $30-\mu L$ drop of the thiol (also in DMF) to be tested, the concentration of the thiol in the $30-\mu L$ drop decreasing until a color change was no longer visible. The results of the tests are shown in Table IV. The detection limits varied from 0.5 to 10 μ g with 1-butanethiol, from 2 to 5 μ g with 2-naphthalenethiol, and from 5 μ g to more than 100 μ g with thiocholine iodide. Interestingly, tests with 1a and Ellman's reagent were found to have the same sensitivity to 2-naphthalenethiol, while in tests with 1-butanethiol 1a was found to be 20 times as sensitive as Ellman's reagent, and with thiocholine iodide Ellman's reagent was twice as sensitive as 1a. While it is clear from these data that there are basic differences between the exchange reaction with the new reagents and with Ellman's reagent, the cause of these differences is not apparent. Nevertheless, the data clearly indicate that while Ellman's reagent produced an anion with a higher molar absorptivity and perhaps even better stability (Table II) than the new disulfides, under spot test conditions several of the new reagents (viz., 1a, 1b, 1d, 1e, and 1f) produced more striking color changes and all gave more sensitive tests when 1-butanethiol was the test thiol. Under the conditions of this test, a difference in color was noted immediately after addition of either of the two more acidic thiols and within 1 min after the addition of the very weakly acidic butanethiol.

	Table IV.	Detection	Limits and	Observed	Color	Changes in	Spot	Test for	Thiols
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detection limit, ^{b,c} µg (min)			color obsd		
compd	NT	TCI	BT	initial	final
1a	2(2.5)	10(1)	0.5(2)	pale yellow	greenish blue-green
1b	2(1)	10 (2)	1(1.5)	yellow	greenish blue-green
1c	2(1.5)	10 (0.5)	1(1)	colorless	orange
1d	2(1)	10 (1)	1(1)	yellow	red
1e	5 (10)	10(0.5)	1(2)	yellow	green
1f	2 (2)	10(2.5)	0.5(1)	pale yellow	red
1g	5(2)	>100 (d)	2(1)	colorless	yellow-greenish yellow
Ellman's reagent	2(1)	5(1)	10 (5)	colorless	orange

^a Tests were carried out at 25 ± 1 °C with illumination (430 lx) from artificial fluorescent lights. ^b Code refers to the following thiols: NT, 2-naphthalenethiol; TCI, thiocholine iodide; BT, 1-butanethiol. ^c Values in parentheses are the times of return to the original color of the detector solution after maximum color had been reached using the indicated level of thiol. The detector reagent was observed for a positive test immediately after addition of the solutions of 2-naphthalene-thiol or thiocholine iodide and 1 min after addition of solutions of 1-butanethiol. ^d Color fades within a few seconds after addition of the thiocholine iodide solution.

Thus, the rate of the displacement reaction [which in some measure is dependent upon the pK_a of the thiol (7)] will most likely be adequate for any thiol.

Solvent Effects on Absorption Maxima of the Thiolate Anion Produced from 1a. Compound 1a was judged to have overall the best characteristics for spot detection of thiols. It was therefore deemed advisable to study the spectral behavior of **1a** and its anion in various solvent systems to determine the effects of different solvents in the detection system. Table III shows the wavelengths of maximum absorption of the anion produced from 1a and 1a itself. The anion was produced by mixing 25 μ L of a solution of 0.01 M 1a in DMF with 4 μ L of an aqueous solution containing 10% tetrabutylammonium hydroxide. (Formation of thiolate anion of Ellman's reagent by alkaline decomposition is described in ref 8.) After the mixture was shaken to ensure complete mixing, 1 mL of the solvent to be tested was added. Two separate determinations were made for each solvent system. For determination of the λ_{max} of 1a, the step involving addition of the tetrabutylammonium hydroxide was omitted. The anion of 1a shows marked negative solvatochromism (hypsochromic shift with increasing solvent acidity). Shifts in the absorption maxima in conjugated organic anions in different solvents and in the same direction as that of our study are not unique, but generally shifts have been observed to be small. For example, Sawicki et al. (9) observed shifts of conjugated organic anions in change of solvent from ethanol to DMF as being less than 50 nm, although some very highly conjugated anions, e.g., the anion of 4'-nitro-4-hydroxyazobenzene, showed a λ_{max} displacement of 95 nm. The displacement found in our system using these solvents was 132 nm.

The marked negative solvatochromism shown by the anion of 1a can also be seen by comparison with other systems. In our system, a solvent change from pyridine to methanol results in a hypsochromic shift of the wavelength of maximum absorption of 158 nm and a solvent change from acetone to methanol of 112 nm. By contrast, the intermolecular charge-transfer absorption of 1-ethyl-4-methoxycarbonylpyridinium iodide (which is considered to show a marked solvatochromism) is shifted only 105 and 92 nm for the same solvent systems (10, 11). A shift of 162 nm has been found for a change of solvent from acetone to methanol in the absorption maximum of N-(2,6-diphenyl-4-phenoxide)pyridinium betataine, whose solvatochromism is reputed to be the largest on record (12).

Correlations of Spectrophotometric Data with Empirical Solvent Polarity Scales. Correlations of the λ_{max} of the anion of 1a with Kosower's Z(10, 11) as well as the Dimroth-Reichardt E_t and RPM (13) polarity scales were poor, the correlation coefficients of the regression lines being 0.77, 0.78, and 0.78, respectively. A correlation using Gutmann's AN (acceptor numbers) (14) was better (correlation coefficient, 0.86) but still not acceptable. A slightly better correlation (correlation coefficient, 0.88) was obtained by using eq 1 as suggested by Krygowski and Fawcett (15) where both the Dimroth-Reichardt acidity parameter, E_{t} , and the Gutmann donor number, DN, are used for assessing the solvent effect on the wavelength of absorption. In eq 1, Q is the

$$Q = Q_0 + \alpha E_t + \beta(DN) \tag{1}$$

wavelength of maximum absorption in a solvent and E_t and DN are the Dimroth-Reichardt and Gutmann values assigned to these solvents, respectively. In this equation, the sign associated with α and β indicates direct (+) and reverse (-) proportionality of the direction of the shift with the solvent acidity and the basicity in this system. The equation of the line is eq 2. A better and acceptable correlation (correlation

$$\lambda_{\max} = 842 - 7.32E_{t} + 2.4(DN) \tag{2}$$

coefficient, 0.94) was obtained with an acid-base polarity system using Gutmann's AN and DN values in eq 3. ln

$$\lambda_{\max} = 607 - 4.47(AN) + 2.16(DN)$$
(3)

Gutmann's solvent scales, the donor number (DN) is derived from the heat of the reaction of a dilute solution of a particular solvent with SbCl₅ (a reference acid) in 1,2-dichloroethane while the acceptor number (AN) is derived from the ³¹P NMR shift produced by the 1:1 Et₃PO:solvent adduct in 1,2-dichloroethane. Solvent acidity in both dual polarity equations is thus seen to be more important than solvent basicity in this system, although both play a role. For selection of solvents in which the mercaptan will show an absorption at long wavelengths, one selects a dipolar aprotic solvent which has a highly basic (e.g., C=0, S \rightarrow 0, P=0) group, although the choice of a solvent could be limited by solubility of the disulfide in the solvent.

The positions (as well as the intensity and even the shape) of absorption maxima are affected by solvents, since they can affect transition energies in electronic transitions ensuing from the absorption of light energy. In the case of the anion of **1a**, it is thought that a transition from the anionic to the quinoidal structure $(1aa \rightarrow 1aq)$ is likely upon absorption of light energy.

$$O_2N - O_2N - CH = CH - N = CH - O_2N + O_$$

Miller and Parker (16) suggested that the polarizable quinoidal excited-state structure in a similar system will be more highly solvated in a solvent such as DMF than it will be in ethanol (and thus stabilize the excited state). Miller and Parker's hypothesis is corroborated somewhat by studies (17) on the IR spectra of *p*-(iminomethyl)benzenethiols in several nonpolar solvents and Me₂SO (an aprotic solvent like DMF). These investigators conclude that the benzenethiols exist almost exclusively in the quinoidal structure in Me₂SO and almost totally as the anion in nonpolar solvents. On the other hand, a protic solvent can stabilize the anionic state more efficiently than it can the polarizable quinoidal state and cause a hypsochromic shift in the λ_{max} . The poor correlation found when using only the polarity scales based upon acidity and the much better correlations using the dual polarity scales argue for both effects, that is, stabilization of the ground (anionic) state by hydrogen bonding and solvation of the excited state by aprotic solvents.

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Spectrophotometric Determination of Micro Amounts of Trithionate via Mercury(II) Thiocyanate Reaction

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A method has been developed for the determination of trithionate. It is based on the formation of thiocyanate by the reaction of trithionate with mercury(II) thiocyanate and on the spectrophotometric determination of the thiocyanate formed with iron(III). The reaction of trithionate with mercury(II) thiocyanate was elucidated in reference to a system containing the phosphate buffer, and its chemical equation was balanced. The apparent molar absorptivity for trithionate at 460 nm is 7370 L mol⁻¹ cm⁻¹. The present method can be applied to the determination of trithionate in the range of 3 imes 10⁻⁶ to 1.4 imes10⁻⁴ M (2.9–134.5 μ g in 5 mL) and gives a standard deviation of 0.0044 absorbance unit and a relative standard deviation of 0.60% for 1×10^{-4} M trithionate solution.

Polythionates are usually present as mixtures, because the individual polythionates are very similar in their chemical and physical properties. When 1 mol of polythionates reacts with cyanide, sulfite, sulfide, or hydroxide, the mole number of the thiosulfate formed is different in each instance. These reactions of polythionates must, therefore, be studied in detail in order to determine a specific polythionate in the presence of the other polythionates. Many investigations have been made of the cyanolysis of polythionates (1-6), for the thiocyanate formed can easily be determined. The cyanolysis of polythionates has played a critical role in the development of methods for the analysis of mixtures of polythionates (7-12).

We have found that trithionate reacts with mercury(II) thiocyanate to yield thiocyanate according to the following equation:

$$4S_{3}O_{6}^{2^{-}} + 6Hg(SCN)_{2} + HPO_{4}^{2^{-}} + 10H_{2}O = Hg_{6}S_{4}(OH)_{2}HPO_{4} + 8SO_{4}^{2^{-}} + 18H^{+} + 12SCN^{-} (1)$$

A detailed examination of this reaction led to the development of an accurate spectrophotometric method for the determination of trithionate. The purpose of this work is to establish the optimum conditions under which the reaction of trithionate proceeds to stoichiometric completion according to eq 1 and to clarify the reaction with special reference to the determination of trithionate.

EXPERIMENTAL SECTION

Apparatus. All spectrophotometric measurements were made at 460 nm with a Hirama Model 6B spectrophotometer with 10-mm glass cells. The precipitate formed by the reaction of trithionate with mercury(II) thiocyanate was collected on the interface between aqueous and organic phases with a Kokusan Model H-100B1 centrifuge. Desired temperatures were controlled

by a Taiyo Coolnit Model CL-15 thermoregulator. pH was measured with a Hitachi-Horiba Model M-7 pH meter.

Reagents. All other chemicals used, besides trithionate and mercury(II) thiocyanate, were of an analytical grade and used without further purification. Potassium trithionate was prepared as described by Stamm et al. (13). The trithionate so obtained was recrystallzied with redistilled water at a temperature below 35 °C and then dried in a desiccator containing concentrated sulfuric acid at room temperature, before storage at -10 ± 2 °C. The water physically adsorbed on the trithionate was determined to be 0.14% by the Karl Fischer method. The trithionate was confirmed to be sufficiently pure for present purposes; its purity was calculated from the total potassium and sulfur contents (2). Standard trithionate solution $(1 \times 10^{-3} \text{ M})$ was prepared by dissolving 135.4 mg of the potassium trithionate (water content 0.14%) in redistilled water and diluting it to 500 mL, and it was stored at 5 ± 2 °C. A stock solution of thiocyanate was standardized by Volhard's method (14), and working solutions were prepared by suitable dilution. These standards were used to confirm the stoichiometry and completion of the reaction of trithionate with mercury(II) thiocyanate. Mercury(II) thiocyanate was prepared by adding 250 mL of 0.4 M potassium thiocyanate to 250 mL of 0.2 M mercury(II) nitrate in 1 M nitric acid, in small portions and with vigorous stirring. The precipitate of mercury(II) thiocyanate formed was filtered off by suction with a sintered-glass filter, washed with distilled water and small amounts of methanol, and then air-dried at room temperature. Methanol solution of mercury(II) thiocyanate $(3.5 \times 10^{-3} \text{ M})$ was prepared by dissolving 280 mg of the mercury(II) thiocyanate in methanol and diluting the mixture to 250 mL with methanol. Buffer solutions as required for adjusting the pH of reaction solutions to 2.7-8.1 were prepared by mixing phosphoric acid (0.11 M) with sodium dihydrogen phosphate (0.11 M), acetic acid (0.2 M) with sodium acetate (0.2 M), sodium dihydrogen phosphate (0.11 or 0.2 M) with sodium monohydrogen phosphate (0.11 M) or sodium hydroxide (0.2 M), and boric acid (0.2 M) with sodium tetraborate (0.05 M), in various ratios. The buffer solution of pH 7.4 used in the procedure section was obtained by mixing 50 mL of 0.2 M sodium dihydrogen phosphate with 39.5 mL of 0.2 M sodium hydroxide. A solution of iron(III) nitrate in perchloric acid was prepared by dissolving 306.1 g of iron(III) nitrate [Fe(NO₃)₃·9H₂O] in 217.6 mL of 60% perchloric acid and diluting to 500 mL with redistilled water to give a 1.5 M solution of iron(III) nitrate in 4 M perchloric acid.

Procedure. Place 1 mL of phosphate buffer solution (pH 7.4), 5 mL of trithionate solution (up to 1.4×10^{-4} M), and then 1 mL of methanol solution of 3.5×10^{-3} M mercury(II) thiocyanate in a 15-mL glass-stoppered tube. The pH of the solution is thereby brought to 7.7. Allow the mixture to stand at temperatures ranging from 20 to 35 °C for 10 min, to drive the reaction to completion prior to the spectrophotometric measurement. In this case, the reaction solution becomes turbid owing to the formation of a yellow precipitate. To this mixture, add 2 mL of 1.5 M iron(III) nitrate in 4 M perchloric acid and about 1 mL of carbon tetrachloride. Shake the mixture vigorously by hand, and centrifuge