using the experimental titration time and the calibration curve determined by regression analysis. The average percent error for these unknowns over five experimental sessions was found to be 4%.

The precision of a single measurement was studied at each concentration by making four injections for each sample. A summary of the data is presented in Table II. Values for relative standard deviation (RSD) for this work ranged from 8% RSD to less than 0.01% RSD with an average of 0.8% RSD.

CONCLUSIONS

Results obtained with this system indicate its potential as a detector for flow injection titrimetry. The measurements themselves are time measurements which can be made precisely and accurately. Once the system is calibrated, samples can be titrated rapidly since speed of analysis is governed by the return to base line of the electrode signal. Implementation of a high-quality antilog circuit may provide some improvement in the stability of the base line and thus yield an overall improvement in the performance of the system.

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Interference in Determination of Ammonia with the Hypochlorite–Alkaline Phenol Method of Berthelot

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The blue color resulting from the formation of indophenol in the Berthelot method of determining ammonia was suppressed by primary and secondary amines, sulfides, thiols, and ascorbic acid, and to a lesser extent by tertiary amines. We postulate that nucleophilic additions of amines, thiols, and other nucleophiles to the quinoid intermediates of the Berthelot reaction decrease the formation of indophenol. It is also possible that reducing agents deplete hypochlorite to suboptimal levels.

The formation of the deep blue color of indophenol from hypochlorite–alkaline phenol solution and ammonia was first reported by Berthelot more than a century ago (*1*). Since then this reaction has been widely used for determining ammonia (*2–9*). A number of modifications of the basic Berthelot reaction have been made, such as changes in the order of adding the reagents (*7*) and substituting different catalysts to accelerate the formation of indophenol. The following catalysts have been used: manganese(II) ion, sodium nitroprusside, and sodium pentacyanonitrosylferate (*7–10*).

A few substances, such as copper, zinc, iron, bromide, hydroxylamine, and *p*-aminophenol (*4*) were reported to interfere with ammonia determination by the Berthelot reaction. In another study (*11*), we noted that a number of commonly used buffers such as Tris [tris(hydroxymethyl)aminomethane], glycylglycine, imidazole, bicine, tricine, etc. interfered with ammonia determination by suppressing formation of the blue color in the Bethelot reaction (*11*).

In view of the wide-spread use of this reaction for determining ammonia in analytical laboratories, hospitals, and industrial facilities, we have systematically investigated commonly used substances that might affect color development in the Berthelot reaction. We present the results of this survey and some possible mechanisms of color suppression.

EXPERIMENTAL SECTION

Chemicals. Chemicals were obtained from commercial sources and used without further purifications.

Abbreviations: Bicine, *N,N*-bis(2-hydroxyethyl)glycine; Caps, (3-cyclohexylamino)propanesulfonic acid; Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)propanesulfonic acid; Pipes, piperazine-*N,N'*-bis(2-ethanesulfonic acid; Taps, (3-((tris(hydroxylmethyl))methyl)-amino)propanesulfonate; Mops, 3-(*N*-morpholino)propanesulfonic acid, Tes, *N*-(tris(hydroxymethyl)methyl)-2-aminoethanesulfonate; Tricine, *N*-(tris(hydroxymethyl)methyl)glycine; Tris, tris(hydroxymethyl)aminomethane.

Reagents. Two reagents were required for ammonia determination by Berthelot alkaline phenol hypochlorite method: (reagent I) 10.00 g/L phenol, 0.05 g/L sodium nitroprusside; (reagent II) 5.00 g/L sodium hydroxide; 0.42 g/L sodium hypochlorite.

A solution of NH_4Cl (0.5 mmol/L) was prepared daily in sodium phosphate buffer (50 mmol/L), pH 7.0. All compounds to be

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Table I. Compounds Tested for Possible Interference in Ammonia Determination by the Berthelot Method

no.	compounds	% absorbance[a]	no.	compounds	% absorbance[a]
1	water	100	37	glycine	26
2	benzoic acid	102	38	glycylglicine	22
3	boric acid	99	39	guanidine	9
4	calcium chloride	103	40	1,6-hexanediamine	0
5	cacodylic acid, sodium salt	100	41	imidazole	7
6	3,3-dimethylglutaric acid	98	42	lysine	0
7	glucose	95	43	methionine	0
8	lithium hydroxide	110	44	Caps	4
9	magnesium chloride	106	45	Taps	0
10	potassium chloride	99	46	Tes	0
11	potassium phthalate	100	47	Trigine	0
12	sodium acetate	100	48	Tris	0
13	sodium azide	99	49	creatine	84
14	sodium bicarbonate	100	50	creatinine	99
15	sodium chloride	99	51	ethylenediaminetetraacetate	98
16	sodium citrate	100	52	triethylamine[b]	90
17	sodium nitrate	96	53	Bicine	93
18	sodium phosphate	99	54	Hepes	94
19	sodium pyrophosphate	100	55	Mes	97
20	sodium tartrate	100	56	Mops	95
21	succinic acid	99	57	Pipes	76
22	sucrose	99	58	cupric sulfate	65
23	bovine serum albumin[b]	101	59	lauryl sulfate[b]	5
24	*N,N*-dimethylformamide[b]	98	60	manganese sulfate	142
25	dioxane[b]	98	61	potassium dichromate	112
26	dextran, mol wt 40 000[b]	102	62	sodium metabisulfite	99
27	ethylene glycol[b]	97	63	sodium nitrate	80
28	glycerol[b]	99	64	sodium sulfate	112
29	human serum[b]	96	65	sodium sulfide	64
30	triton X-100[b]	99	66	sodium sulfite	103
31	twin-20[b]	99	67	ascorbic acid	0
32	1-amino-8-naphthol-3,6-disulfonic acid	44	68	dithiothreitol	0
33	arginine	20	69	mercaptoethanol	0
34	cysteine	2	70	dimethyl sulfoxide[b]	5
35	ethanolamine	9	71	thiourea	0
36	ethylenediamine	4	72	urea	100

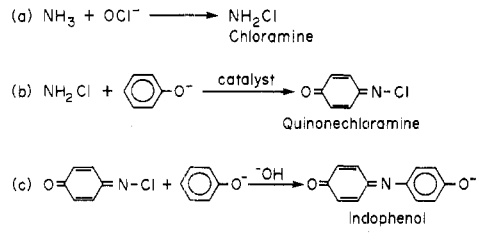
[a] The values reported were average of three determinations. The absorbance was reported as percentage of that obtained with ammonium chloride (0.5 mM) dissolved in water, i.e., ((absorbance with NH_3 + test compound)/absorbance with NH_3 alone) × 100%. A value of 100 means no interference; a value of 0 means total interference, i.e., no color formation at all, and values greater than 100 mean the test compound enhances the absorbance of the solution. [b] A solution of 0.1% w/v or v/v was used. Unless otherwise stated, all test compounds were prepared at 10 mM.

tested were prepared at 10 mmol/L and their pHs were adjusted to approximately 7 using either 1 mol/L NaOH or 1 mol/L HCl at 25 °C.

Procedure. Ammonia was determined by the method of Charney and Marbach (*9*). To 0.5 mL of 0.5 mmol/L NH_4Cl, 0.5 mL of a solution containing the test compound was added and mixed well. Next, 2.5 mL each of reagent I and II were added successively, the solution was mixed and incubated at 25 °C for 30 min, and the absorbances were taken at 625 nm. The blank solution used to balance the spectrophometer was prepared identically as to the test solution except that no NH_4Cl solution was included.

RESULTS AND DISCUSSION

The formation of indophenol from ammonia, hypochlorite, and alkaline phenate in the Berthelot reaction is postulated (*4*) to proceed through the following series of reactions:



The absorption spectrum of the reaction product, indophenol, has a maximum at 625 nm (Figure 1a). A solution of authentic indophenol gives the same absorption spectra.

Because of an early observation that buffers containing amino groups interfere with ammonia determination by the Berthelot reaction (*11*), a number of compounds commonly used in analytical laboratories were tested for possible interference with the reaction (Table I). It is clear that compounds (compounds 1–31) without an amino functional group do not interfere. Aliphatic compounds with primary or secondary amino groups (compounds 33–48), on the other hand, strongly suppress the development of blue color. Aromatic and tertiary amines depress the color to a lesser degree (compounds 32, 49–57). Other compounds that depress the color in the Berthelot reaction are sodium sulfide, ascorbic acid, thiols, thiourea, dimethyl sulfoxide (compounds 65, 67–71). Unlike thiourea, urea does not inhibit color development (compound 72). Some compounds, such as potassium dichromate, LiOH, $MgSO_4$, and Na_2SO_4 (compounds 61, 8, 60, 64) cause an increase in absorbance.

POSSIBLE MECHANISMS OF INHIBITING COLOR FORMATION

Amines (Compounds 32–48). The formation of blue color was transient when ammonia was determined by the Berthelot reaction in the presence of amines. The transient blue color indicates the formation of quinonechloramine and indophenol. Quinones are very susceptible to nucleophilic attack by amines or other nucleophiles (*12–15*). For example, the following well-characterized reaction between benzoquinones and

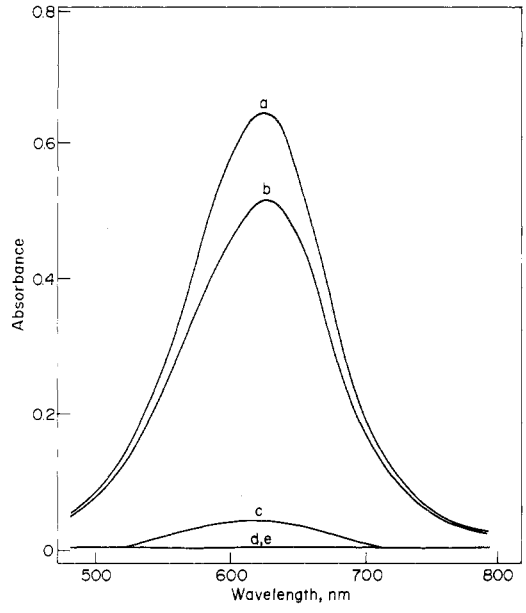
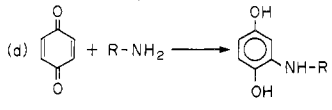
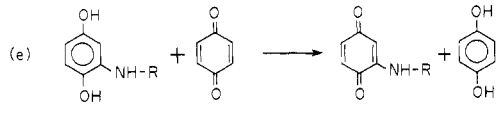


Figure 1. The absorption spectra of the reaction products obtained by reacting ammonia, hypochlorite, alkaline phenol, and sodium nitroprusside without any additive (curve a) or with sodium sulfide (curve b), with dimethyl sulfoxide (curve c), with thiourea (curve d), and with Tris (curve e).

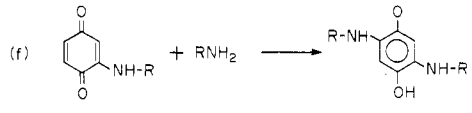
amines show that amines readily combine with benzoquinones (reaction d).



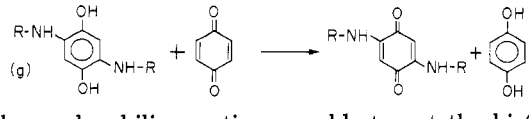
Monoalkylhydroquinones have lower oxidation potentials than the parent benzoquinones and are immediately oxidized by excess benzoquinone to give monoalkylaminobenzoquinones (reaction e).



The monoalkylaminobenzoquinones can undergo further reactions with amines forming bis(alkylamino)hydroquinones (reaction f).



The bis(alkylamino)hydroquinones have oxidation potentials which are even lower than the monoalkylaminohydroquinones and thus are readily oxidized by the unreacted benzoquinones (reaction g).



The nucleophilic reactions would stop at the bis(alkylamino)quinone (reaction f) due to the presence of two electron-donating groups on the ring which lowers considerably its susceptibility to further nucleophilic attack by amines. Since the products of the amine–benzoquinone reaction possess conjugated systems which are similar to the reactant benzoquinones, the absorption spectra of the products would not differ much from the reactants.

The reaction products of the Berthelot reactions have an extended conjugated system of electron distribution. Compounds with such an extended conjugated system would have an absorption spectra distinctly different from the parent compounds lacking an extended conjugation.

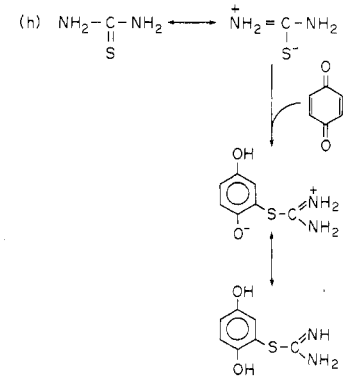
Nucleophilic addition of alkylamine to quinonechloramine would generate 2,5-bis(alkylamino)quinonechloramine which is more sterically crowded at the ortho position than the quinonechloramine itself. Such a steric crowding alone would retard the formation of indophenol and could explain the inhibition of color formation by these amines in Berthelot reactions. For example, tris(hydroxymethyl)aminomethane at 10 mmol/L totally abolished the absorption of indophenol (Figure 1d).

Tertiary alkylamines are much less inhibitory than primary or secondary alkylamines because the bulky trialkyl group of the tertiary alkylamine is likely to sterically hinder the approach of quinonechloramine or indophenol by the amine. In contrast to the claim made by Bolleter et al. (*4*) that aliphatic amines do not interfere with the Berthelot reaction for ammonia determination, our results conclusively and consistently show that all primary and secondary aliphatic amines severely suppress color formation in the Berthelot reaction.

Sulfide, Thiols, and Ascorbic Acid (Compounds 65, 68, 69). Sulfide and thiols are good nucleophiles at alkaline conditions. They readily undergo nucelophilic reaction with quinoid compounds such as benzoquinone, quinonechloramine, and indophenol. Furthermore, because sulfide and thiols are good reducing agents, reduction can take place at all of the steps leading to the final quinoid structures in the Berthelot reactions (*a*–*e*). Either the nucleophilic or the reduction reaction alone or in combination would lower the concentration of indophenol. Furthermore, reducing agents may deplete the concentration of hypochlorite which is required for the formation of quinonechloramine. The absorption spectrum of the product of the Berthelot reaction carried out in the presence of sodium sulfide is shown as curve b (Figure 1b). The absorbance at 625 nm is significantly reduced.

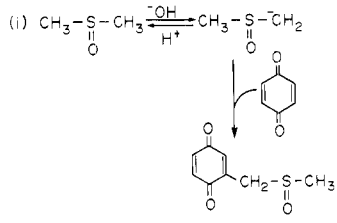
Ascorbic acid may act as other reducing agents in inhibiting color formation.

Thiourea and Dimethyl Sulfoxide (Compounds 70, 71). The electronegativity of sulfur in thiourea contributes to the resonance structures which are responsible for the low basicity of $-NH_2$. The $-NH_2$ resonance effect also renders the $-S^-$ very nucleophilic. Therefore, a nucleophilic addition of thiourea to the quinoids of the Berthelot reaction may take place (reaction h).

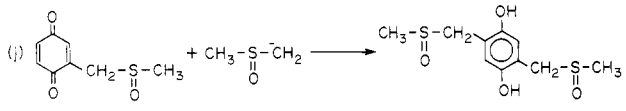


It is known that the $-S^-$ in thiourea is more nucleophilic than the $-O^-$ of urea. For example thiourea readily undergoes a nucleophilic reaction with alkyl halides (*16*–*18*), whereas no such comparable reactions between urea and alkyl halide were reported.

Under the extremely alkaline conditions, such as those encountered in the Berthelot reactions, a carbanion of dimethyl sulfoxide can be formed. The carbanion can further undergo a nucleophilic reaction with quinoids according to the following reaction scheme (reaction i). Another carbanion



can add on to the monosubstituted hydroquinone forming a disubstituted hydroquinone (reaction j). The formation of



these hydroquinones lowers the amount of quinoids that would otherwise form indophenol. As shown in Figure 1, curve c, dimethyl sulfoxide greatly reduces the absorbance of the solution.

The mechanism of inhibition of color formation caused by the presence of guanidine and $NaNO_2$ in the Berthelot reactions is less readily explained. Possibly the reduction of quinoids by these compounds is involved.

Potassium dichromate, manganese sulfate, or sodium sulfate form turbid materials in the Berthelot reactions. The turbidity of the solution, in turn, increases its absorbance.

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Wet Ashing of Organic Matter for the Determination of Antimony

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The wet ashing of organic matter for Sb determination was investigated by using a radioactive tracer technique. Wet ashing with HNO_3 + $HClO_4$ mixtures leads to the formation of insoluble Sb compounds. All the Sb remains in solution when a HNO_3 + $HClO_4$ + H_2SO_4 mixture is used. The influence of the ashing vessel (glass and Teflon) and the oxidation state of Sb were also studied.

The wet ashing of organic matter for trace element determination is a well-established technique (*1-5*).

Problems have been met, however, in the determination of Sb in organic matter after wet ashing with a HNO_3 + $HClO_4$ mixture; part of the Sb remains fixed on the walls of the glass vessel used for this step. Only one observation of such "losses" has been found in the literature (*6*).

The purpose of the present investigation was to look for a reliable method for the wet ashing of organic matter for Sb determination. The element, marked with the appropriate γ-emitting isotope, was studied in the "carrier-free" to 100 μg range. The oxidation state after wet ashing was studied by liquid-liquid extraction with zinc diethyldithiocarbamate.

EXPERIMENTAL SECTION

Reagents. The acids used were HNO_3 (65%), $HClO_4$ (60%), and H_2SO_4 (95-97%). The ^{122,124}Sb(III) solutions were prepared from neutron-irradiated high-purity metallic Sb by dissolving it in hot H_2SO_4. The solutions used for the experiments were 1.0 mg Sb(III)/mL in 5 M H_2SO_4 and ≤0.10 mg Sb(III)mL in 1.5 M H_2SO_4. The "carrier-free" ^{125}Sb(III + V) solution was in 4 M HCl. The solution of Cr(III) (10 mg/mL) was prepared by dissolving $Cr(NO_3)_3 \cdot 9H_2O$ in 1 M HNO_3. The solution of zinc diethyldithiocarbamate ($Zn(DDC)_2$) was 1.7×10^{-3} M in $CHCl_3$ (*7*).

The organic matter for the wet ashing experiments were "fat-free" milk powder and wheat flour. The dry-ashing residues (700 °C) were 7.5% and 0.42% in weight, respectively.

Apparatus. Some wet ashings were made in 100-mL Pyrex conical flasks on a hot plate (380 × 180 mm; 1400 W). Others were carried out in Teflon tubes (outer diameter, 23 mm; height, 170 mm; wall, 1 mm thick) in a temperature programmable aluminum heating block with holes (diameter, 27 mm; depth, 120 mm) type RNS2HR4 (Gebr. Liebisch, Bielefeld, GFR).

Glass fiber Whatman GF/B filters (32 mm) in "chimney" holders were used.

The recovery measurements and the liquid-liquid extractions were made as described in ref 8.

Counting was done with a well-type NaI (Tl) crystal.

Wet-Ashing Experiments. Organic matter (1 g), marked Sb(III) (100 μL), Cr(III) solution (100 μL, only in some experiments), and the acid mixture (HNO_3 + $HClO_4$ or HNO_3 + $HClO_4$ + H_2SO_4) were poured into the conical flasks or the Teflon tubes. Cr(III) was employed as an indicator of total oxidation of the organic matter. Its color changes from green (Cr(III)) to orange (Cr(VI)) near the boiling point (203 °C) of the azeotropic mixture of $HClO_4$-H_2O (72.5% $HClO_4$).

The conical flasks were placed on the hot plate, the surface temperature of which was raised from 160 to 260 °C over 1-2 h. The Teflon tubes were introduced into the aluminum heating block. The following heating program was used: 1 h from 20 to 150 °C, 7 h at 150 °C, and 1 h from 150 to 230 °C. This was followed by 0.5 h (experiments with HNO_3 + $HClO_4$ mixture) or 2 h (experiments with HNO_3 + $HClO_4$ + H_2SO_4 mixture) at 230

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