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Selectivity of Reaction among Chlorine, Ammonia, and Salicylate for Determination of Ammonia

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Spectrophotometric data show that within the pH range 9.5–13.5, the sodium nitroprusside catalyzed reaction among ammonia, chlorine, and salicylate is unaffected by pH; however, the spectrum of the product(s) is. At the most sensitive pH (~13), the reaction can tolerate a wide range of salicylate and nitroprusside concentrations, but the *available* chlorine concentration exhibits a narrow optimum. Additions of (<250 μg ml^{-1}) K, Ca, Mg, Cu, Al, Zn, and Ni are without effect. Manganese causes a slight enhancement (<2%) at concentrations <50 μg ml^{-1}, higher concentrations precipitate. Iron at >50 μg ml^{-1} also precipitates. Precipitation could not be prevented by the complexing agents tested; however, neither of the precipitates interfere if removed before spectrophotometry.

The sodium nitroprusside catalyzed, salicylate–dichloroisocyanurate (SCC) procedure of Reardon et al. (*1, 2*) for the colorimetric determination of ammonia is more sensitive than the phenol–hypochlorite methods (*3*). Color development in the latter varies with pH and is enhanced by Fe, Cr, and Mn ions and inhibited by Cu(II) ions (*3*). However, few interferences have been reported for the SCC method. A substantial drop in sensitivity is known to occur in the presence of (EDTA) (*4*) and a slight enhancement is caused by certain amino acids (*1*). This evidence suggests that the SCC method is sufficiently selective to permit accurate ammonia determinations to be made directly in Kjeldahl digests (*4–6*) and soil extracts (*4*) and in biological fluids (*1*). Direct analysis of such sample matrices, using the phenol–hydrochlorite methods, generally gives inaccurate results; however, accurate results are obtained when the ammonia is distilled from the sample matrix prior to the determination (*7*).

When first testing the SCC method (*2*), we used it to determine ammonia which had been distilled from soil extracts into boric acid solution (2% w/v) and titrated with standardized acid (*8*). Known amounts of ammonia were used to cross-calibrate the procedures and the between method comparison was excellent. However, it was noted that the sensitivity of the colorimetric procedure was depressed in the presence of boric acid. Similar effects were found with other acids and the inhibition was shown to be pH dependent. The pH dependence of the sensitivity of the reaction has since been confirmed in two independent studies (*5, 6*); however, the reported pH optima differ substantially.

We present a more detailed study on the effect of pH than any reported previously. We report the effects caused by adding K, Ca, Mg, Fe, Cu, Mn, Al, Zn, Ni, and Se salts to aqueous ammonia standards at interferent ion concentrations up to the maximum likely to be found in Kjeldahl digests of crop plants and soils. We also report the effect of adding a number of complexing agents in order to lessen the precipitation of certain of the interferent species under the alkaline conditions required for the colorimetric determination (*2*).

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EXPERIMENTAL

A 5 ml aliquot of an ammonia standard (0–5 μg of N ml^{-1}) was pipetted into a 50 ml capacity test tube. Three ml of sodium salicylate and sodium dichloroisocyanurate reagents (*2*) were added. The solutions were mixed, diluted to 25 ml with water, then allowed to stand for 30 min at room temperature before their absorbance was measured.

The effect of pH on the color produced was determined by adjusting the pH of the final 25 ml either before or after color development. The pH was adjusted within the range 9.5–12.3 using boric acid and from 12.3–13.6 with NaOH. The pH measurements were made with a Radiometer G 202B glass electrode (Radiometer, Copenhagen, Denmark) and a saturated calomel reference electrode on an Orion model 801 digital pH meter (Orion Research Inc., Cambridge, Mass.). Measured pH values were corrected for sodium error using the table provided with the glass electrode. Only corrected pH values are presented.

Reagent concentrations were optimized at the optimum pH (determined above) and the effect of spiking the ammonia standard solutions with 0–250 μg of one or more of K, Ca, Mg, Fe, Mn, Cu, Al, Zn, and Ni (as their chloride salts) and Se (as sodium selenate) was measured. The effect of each complexing agent on each interferent was determined with the ligand concentration in the final solution at 0.01 M.

Absorbance of all colored solutions was recorded between 640 and 700 nm against distilled water, using a Varian Techtron model 635D spectrophotometer (Varian Techtron, Melbourne, Australia). A 1-cm light path was used with a bandpass of 1 nm.

Available chlorine was determined during the color development period after the method of Kolthoff and Sandell (*9*).

RESULTS AND DISCUSSION

pH Effect. By raising or lowering the pH of solutions before color development, it was found that maximum color development occurred in the pH range 12.8–13.1 (Figure 1 (a)). Below pH 12.8, the depressed color intensity was accompanied by a shift in λ_{max} (Figure 1 (b)). Above pH 13.1, no wavelength shift was associated with the decline in color intensity. The results were independent of ammonia concentration and the data presented (Figure 1) were obtained with 25 μg of NH_3–N. Identical results were obtained when the pH was adjusted after color development, just prior to spectrophotometry. Therefore, the action of pH is a direct effect on the chromophore and not on the extent or rate of its production or degradation.

The occurrence of an inflection in the color intensity/pH relationship at about pH 11.5, in addition to a maximum at about pH 13 suggests the existence of at least two chromogenic species which differ considerably in ϵ (Figure 1 (a)).

Of the pH studies reported (*5, 6*) only one (*6*) was taken through a sufficiently high pH range for an optimum to be observed. The value reported (12.48) in the other study (*5*) is therefore meaningless. Furthermore, in both these experiments, absorbance was observed at only one wavelength (near 660 nm). A colorimeter with a nominal 18-nm bandpass interference filter system was used (*10*). This technique would have measured the net effect of the concomitant pH dependent changes in ϵ and λ_{max} (Figure 1 (a) and (b)). This source of experimental error was fortuitously minimized because the chromophore has a broad absorbance band and because both d λ_{max}/d pH and d A/d pH approach zero near the optimum

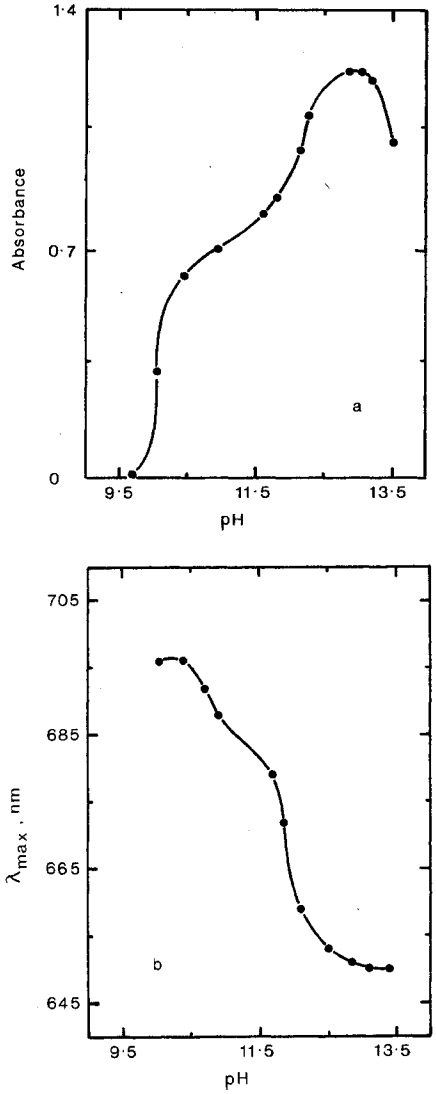

Figure 1. The effect of pH on (a) the absorbance and (b) the wavelength at which maximum absorbance occurred (λ_{max}). Data are for 25 μg of ammonia nitrogen

pH (Figure 1 (a) and (b)). The optimum pH (12.8–13.0) observed by Conetta et al. (*6*) is therefore similar to the value we obtained.

When the color development was carried out at the optimum pH, we detected, on standing, a time-dependent decrease in color intensity (~50% in 24 h); whereas the chromophore had been reported as being stable for at least 20 h (*1*). The decline in color intensity was associated with the pH fall accompanying absorption of atmospheric CO_2. The color was stable for 24 h (the longest time tested), when CO_2 absorption was prevented.

From these results it is obvious that strict pH control is required if absorbance is to be used as a quantitative measure of ammonia and that the most desirable pH is near 13. At this pH, maximum color intensity is achieved and the intensity is relatively unaffected by small pH differences. In practice, these conditions would be more easily maintained if the pH were buffered (e.g., using equimolar KCl and NaOH) and the determinations carried out in a closed system such as an AutoAnalyzer (Technicon Corporation, Tarrytown, N.Y.), from which atmospheric CO_2 could be excluded.

Reagent Concentrations. In a recent study (*6*), optimum reagent concentrations were determined; however, the v/v proportions of sample, buffer and reagents were not given. We therefore re-examined the reagent concentrations required for maximum color development when color formation and

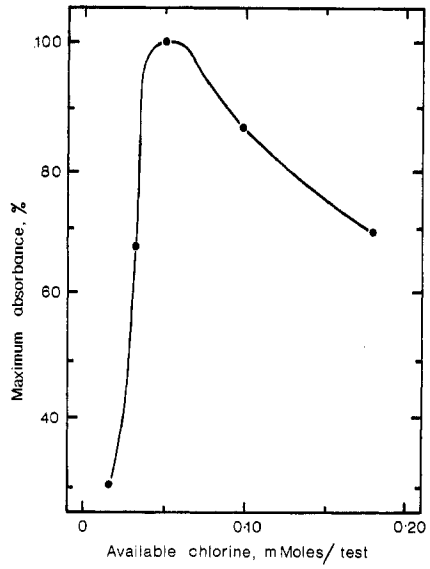

Figure 2. Change in sensitivity as a function of *available* chlorine level

measurement were carried out at pH 13. The concentrations of salicylate, nitroprusside, and cyanurate recommended by Reardon et al. (*1, 2*) were optimal. The sensitivity of the test was relatively unaffected by small changes in salicylate and nitroprusside concentrations; however, the cyanurate concentration was critical. Cyanurate concentrations at least six times above optimum have been used (*12*); however, above 1.5 times Reardon et al.'s recommended cyanurate concentration, we find a color depression (Figure 2). Identical results were obtained when NaOCl was the chlorine source. Similar trends were reported by Conetta et al. (*6*), but the chlorine levels causing the effect were not documented.

The same order of addition of reagents is used in all (*2, 4–6, 11*) but one (*12*) of the published procedures, i.e., salicylate followed by the chlorine source. We found no difference when the reverse order was used, provided that reagents were added in rapid succession; however, when a few minutes elapsed between the reagent additions, a time-dependent decrease in color intensity resulted. This did not occur when the salicylate addition preceded the cyanurate addition. The latter procedure is therefore preferable for manual methods.

Chemical Interferences. Of the 10 ions tested only Mn and Fe affected ammonia determination. As the quantity of Mn(II) was increased, the absorbance for a given quantity of ammonia was progressively enhanced. Enhancement reached a maximum of 2% at 50 μg Mn. Above this level, a brown precipitate, presumably MnO_2 was formed. Manganese added as permanganate caused less interference. Iron(III) did not cause interference at levels below 50 μg, the level above which a brown solid, presumably ferric hydroxide, precipitated. Even above these levels, the interference was eliminated by centrifuging to remove the particulate matter. This result confirms an earlier report (*11*).

The volume of the precipitates produced when Fe(III) and Mn(II) levels were approaching those found in Kjeldahl digests of soils and certain Mn accumulating plants (such as bananas) and in soil extracts made following anaerobic incubations (*8*), were such that they prevented liquid flow through an AutoAnalyzer manifold several minutes after commencement of the analysis. To decrease the precipitation we tried complexing Mn and Fe with 0.01 M sodium cyanide, di- and tetra- sodium EDTA, disodium diethylenetriaminepentaacetic acid, triethanolamine, sodium fluoride, trisodium citrate, and disodium tartrate during color development. However, none of the ligands reduced precipitation to a level at which continuous filtration was possible.

Not only did the ligands fail to control precipitation; they also affected sensitivity. A 20–90% sensitivity reduction was caused by all but fluoride, citrate, and tartrate, which did not affect sensitivity. Crooke and Simpson (*4*) had observed decreased sensitivity when disodium EDTA was added to prevent precipitation of mercury during analysis of mercury-catalyzed Kjeldahl digests. They did not ascertain how EDTA depressed color formation; however, we found that only those compounds which inhibited color development significantly reduced the amount of *available* chlorine during the reaction. The extent of the inhibition was an inverse function of the residual *available* chlorine content as described above (Figure 2).

From the foregoing, we infer that any substance which reduces the amount of *available* chlorine below the optimum range for color development, will reduce color production. This effect could be overcome by increasing the cyanurate concentration, if the chlorine-reducing capacity were known. However, where the chlorine-reducing capacity is unknown, or expected to vary widely, e.g., in biological fluids, in surface and underground waters, and in extracts of plants and soils, this approach is invalidated by the color depressive effect of excess chlorine (Figure 2).

Although the SCC method is more selective than the alternative phenol–hypochlorite methods, sample matrices in which one or more of the foregoing interferences is known to be operating will nonetheless degrade the accuracy of results. However, both methods perform equally well when ammonia is separated from the sample matrix and any preference for one or the other should be based on their substantially different sensitivities (*4*). Ammonia separation has been attempted by two techniques, both of which take advantage of the volatility of ammonia from alkaline solution, i.e., distillation (*7*) and gas diffusion through pores in a "stretched" polypropylene film (*12*). Both of these processes are continuous, having been developed for use with an AutoAnalyzer. From our preliminary evaluation, gas diffusion appears superior: it requires neither custom made equipment nor an extended sample/wash cycle time.

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Evaluation and Calibration of the Model T Coulter Counter Used in the Population Mode

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Accuracy of a Coulter Counter Model T used in the population mode is tested by number and mass balance experiments. It is demonstrated that the counts are accurate for 50- and 100-μm aperture tubes over the entire operable size ranges. Spurious counts in the lowest three sizes were detected when a 200-μm aperture tube was employed. These spurious counts are thought to be due to secondary coincidence error.

Coulter particle counters are now widely used for size distribution measurements of fine particles, especially in the sub-sieve size ranges. These counters work on the electrolytic sensing zone principle. An electrolytic suspension is forced through a small aperture having two electrodes on either side of it. As a particle in the suspension moves through the aperture, it replaces its own volume of electrolyte and momentarily changes the resistance between the electrodes. This change in resistance causes a voltage pulse of magnitude very nearly proportional to the volume of the particle (*1, 2*). Thus, for a large number of particles having a size distribution, a train of pulses is produced at the aperture. These are amplified, counted, and discriminated in a number of size channels. In the Model T version of the machine, once the size range of measurement is set, the entire number size distribution is obtained at one time.

Recently, Coulter Counters have been used in fundamental studies of crystallization (*3–5*). Population of crystal nuclei in suspension are counted in such studies. Larson and Randolph (*6*) discussed how useful crystallization kinetics can be conveniently obtained from reliable population vs. size data. However, when counting nuclei by the Coulter Counter below ca. 10 μm in a mixed-discharge crystallizer of low magma density, apparently excessive nuclei, increasing rapidly as size decreased, were observed (*5, 7*). The crystal size distributions (expressed as population vs. size) thus obtained were significantly different from expected theoretical distribution (see Ref. 6). This apparent discrepancy instigated the present investigation of the possible existence of any spurious counts in the low end of the size range of measurement. Cooper and Parfitt (*8*) noted a similar build-up of counts in the low sizes due to spurious signals. They concluded that the spurious counts were characteristic of the particular 30-μm aperture tube used. In the present work, the veracity of the counts obtained from the Model T Counter was tested by number and mass balance experiments of polymeric latex particles and glass beads of narrow size distributions.

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