

COMMENTS & LETTERS TO THE EDITOR

Letter to the Editor on "Using the Berthelot Method for Nitrite and Nitrate Analysis"

The indophenol-blue or Berthelot method for NH_4^+ analysis can be used indirectly for microscale determination of NO_2^- and NO_3^- after initial reduction of these species with Devarda's alloy (Sims et al., 1995). This approach subjects NO_3^- determination to interferences normally associated with NH_4^+ analysis, thus improvements in the chemistry of the Berthelot reaction that have been published since Sims et al. (1995), should be carefully considered when using this application for NO_3^- . Rhine et al. (1998) described the use of the sodium salt of 2-phenylphenol (PPS) as a substrate for the Berthelot reaction for improved NH_4^+ analysis in the presence of organic and inorganic interferences expected in environmental samples, and included a version of the assay for microplate analysis. Though Rhine et al. (1998) dealt only with NH_4^+ analysis, the PPS chemistry presented therein is better suited to NO_3^- analysis than is the salicylate-based method of Sims et al. (1995), owing to reduced susceptibility to interference, and improved analytical performance as described below. The reduction step (Devarda's alloy) used in the salicylate method can be directly applied to the PPS method without further modification. This provides the user with all the analytical advantages of the PPS reagent for NH_4^+ analysis and the added ability to determine NO_2^- and NO_3^- .

Microscale reduction of NO_3^- and NO_2^- to NH_4^+ with Devarda's alloy as described in Sims et al. (1995) requires tedious introduction of finely powdered Devarda's alloy (0.05–0.2 mm) into small microplate wells. To improve throughput, the reductant may be suspended (with constant stirring) in methanol (0.2 g mL^{-1}), and dispensed into microplates (100 $\mu\text{L well}^{-1}$) with a multi-channel pipette (tips may be trimmed to enhance delivery), after which the solvent can be evaporated under a stream of N_2 . Other polar, volatile solvents, such as ethanol, isopropanol, acetonitrile, or acetone work equally as well, however aggressive solvents that might attack the polystyrene microplate, or solvents containing water (which oxidizes the alloy) should be avoided. This approach can be done in mass, with large numbers of plates produced in advance and stored in a desiccator (to reduce oxidation of the alloy) until they are needed. Alloy added in this manner tends to adhere to the plate and provides cleaner samples for subsequent analysis than when added in a dry state. Figure 1 shows the relationship between the amount of alloy used and recovery of NO_3^- as NH_4^+ , as well as associated experimental error.

The Berthelot reaction requires a high reaction pH, thus acidic samples, such as those treated with sulfamic acid to destroy NO_2^- or traces of other acids added to retain NH_4^+ formed by reduction of NO_3^- or NO_2^- (Sims et al., 1995), occasionally adversely affect assay performance. Since absorbance of the indophenol chromophore is a function of pH, variability among samples in NO_3^- or NO_2^- concentration will result in inversely proportional variation in residual acidity, which may (if concentrations exceed buffer capacity) lead to underestimation when analyte concentration is low. By far, most queries we receive regarding use of the technique described by Sims et al. (1995) are related to such pH problems, often attributable to exceeding recommended concentrations of added acids or buffering reaction pH below optimal. Such procedural variability tends to be less important when PPS

is used, as the resulting chromophore produces color over a wider pH range, and both the acid and base forms of the PPS chromophore exhibit significant absorbance at 660 nm. Sample variability can be further reduced for either of the cited methods by doubling the concentrations of both Na_3PO_4 and NaOH relative to the published concentrations when making the buffered hypochlorite solution. This increases the buffering capacity of the reagent, maintaining the desired reaction pH even with slightly acidic samples.

Since publication of the paper by Rhine et al. (1998), we have become aware of a possible human carcinogen risk associated with PPS (Brusick, 2005). Thus, though PPS is an analytically superior reagent for this application, salicylate may yet prove the reagent of choice for laboratories with high sample throughput, serious waste disposal concerns, or samples not expected to exhibit interferences. As with most colorimetric methods, toxicity concerns for the waste stream are based on the added reagents, as no toxicology information is available for the complex indophenol-blue chromophores formed in Berthelot reactions. The use of micro-scale methods, such as those mentioned herein, thus becomes especially important to minimize toxicity of the waste stream.

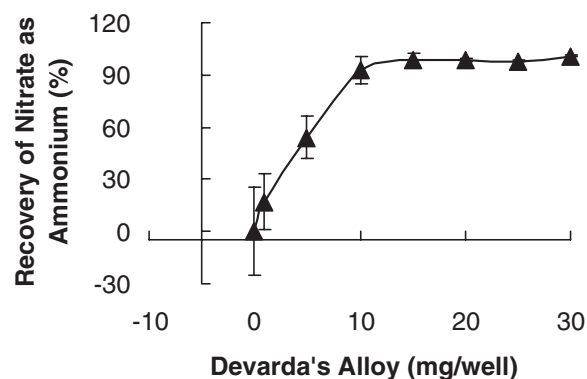


Fig. 1. Recovery of 10 mg NO_3^- -N L^{-1} (KNO_3 in water) as NH_4^+ following reduction with Devarda's alloy (Sims et al., 1995) in a microplate. Ammonium analysis performed according to Rhine et al. (1998). Error bars indicate coefficient of variation for three replicate samples.

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