SYSTEMATIC ABSORBANCE ERRORS WITH TECHNICON AUTOANALYZER II COLORIMETERS

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Abstract—*Technicon* AutoAnalyzer II colorimeters have spectrophotometric flowcells with curved optical ends. Light passing through these flowcells is refracted severely at the non-perpendicular ends. Curved ends therefore cause differences in apparent absorbance measured at the phototube which are related to differences in the refractive indices of the solutions in the cells. This can cause significant error in the colorimetric analysis of solutions with different refractive indices (caused, for example, by variations in salinity). This effect is responsible for a systematic error which, for typical AutoAnalyzer determinations of phosphate in seawater or estuarine waters, can approximate $0.2 \,\mu$ M.

INTRODUCTION

In the manufacture of cuvettes to be used in spectrophotometry, great care is taken to assure that the optical ends through which the light beam passes are exactly parallel, of uniform thickness, and perpendicular to the light path. With such cells the light beam strikes both ends of the cell perpendicularly and is not refracted, so that the angle and location of incidence of light on the phototube are unaffected by the nature of the solution. Under these circumstances, variations in the refractive index of samples have no effect on the apparent absorbance, and one need not worry about such differences between samples, standards and blanks.

The cells in *Technicon* AutoAnalyzer colorimeters are designed with curved ends to assist in the continuous flow of fluid through the cell in order to assure complete washout between samples. With these flowcells, solutions with identical absorbances but differing refractive indices cause the light beam passing through the cell to be refracted to a greater or lesser extent. In turn this causes differences in light intensity to be measured at the phototube (Fig. 1). The apparent absorbance error varies with cell length and curvature of the cell ends. The curved ends appear to vary from cell to cell, so that the error differs from flowcell to flowcell. The above effect is significant in analyzing solutions of varying salinity, such as estuarine waters, or where reagent blanks and samples have a different refractive index.

Realization of this problem in the marine literature falls into several categories. Some investigators were aware of an effect (but not the cause) and used seawater as a blank correction; others were aware of an effect and attempted to compensate. Henriksen (1965) suggested determining the true reagent blank manually with artificial or Sargasso seawater in order to correct the recorder response to a true baseline. Various salinity corrections have been published for AutoAnalyzer techniques. In addition to known ionic strength effects (e.g. in the silica method), these corrections likely include refractive index corrections that are applicable only to a particular colorimeter-flowcell combination.

Atlas et al. (1971) realized that absorbance differences between distilled and artificial seawater were apparently due to flowcell geometry and differences in refractive index, yielding a false reagent blank with distilled water. A complete test of their AutoAnalyzer I system indicated that the effect was insignificant. A preliminary test of their AutoAnalyzer II system indicated that the effect was more significant, but systematic results had not been completed. At our



Fig. 1. Diagramatic representation of light paths through a flowcell with curved ends.



Fig. 2. Corrected absorbance versus refractive index of solutions at various concentrations: sucrose = 0; NaCl = •; Sargasso seawater = \triangle ; KCl = \triangle . (a) colorimeter No. 1, flowcell A, 50 mm; (b) colorimeter No. 1, flowcell B, 50 mm; (c) colorimeter No. 2, flowcell A, 20 mm; (d) colorimeter No. 2, flowcell B, 20 mm. Vertical bars indicate the maximum effect of varying slit width, using KCl solution. The various solutions were measured at different times and with various slit widths, thus accounting for the apparent spread of the relationship.

suggestion, Loder & Glibert (1977) have checked for this refractive index error on their AutoAnalyzer II reactive phosphate system and found it to be about $0.2 \,\mu$ M. They have also determined refractive index, salinity and wetting agent errors for other nutrients (Si, NO₃, NO₂ and NH₄).

This paper reports the results of an investigation of the refractive index effect upon the reactive phosphate analysis of seawater in two *Technicon* AutoAnalyzer II Industrial colorimeters.

METHODS

In general, apparent absorbances were determined by manually pouring solutions of varying refractive index through each flowcell and measuring the absorbance observed by the phototube. The true absorbance of the solution was then determined and subtracted from the apparent absorbance to obtain a corrected absorbance which was due only to refraction of the light beam by the curved flowcells.

Aqueous solutions of sucrose, NaCl, KCl. Sargasso seawater, and Sargasso seawater dilutions plus phosphate plus reagents were used to generate curves of corrected absorbance versus refractive index for each colorimeter flowcell. The refractive index of each solution was obtained from either the Handbook of Chemistry and Physics, the International Critical Tables or by hand-held refractometer (American Optical Co., A10419).

These solutions were poured manually through the colorimeter in order to avoid bubbles and the use of wetting agents. Levor IV, for example, the wetting agent recommended by Technicon for the phosphate method, forms an insoluble milky suspension with seawater or artificial seawaters containing magnesium and calcium.

Heat buildup in the flowcell compartment is appreciable. Since the refractive index of aqueous solutions is temperature dependent, the flow rate through the cells was kept constant at about 4 ml min⁻¹ to maintain a constant solution temperature in each flowcell.

Absorbances for all experiments were measured at 880 nm in both colorimeters (*Technicon* AutoAnalyzer II single channel colorimeters). Colorimeter No. 1 contained two 50 mm cells; No. 2, two 20 mm cells. True absorbances of test solutions were determined at 880 nm in a Beckman DU spectrophotometer with 10-cm cells. Factors for equating true absorbance with AutoAnalyzer absorbance units were determined empirically by measuring the absorbance of phosphate standards in distilled water with both instruments (AutoAnalyzer and Beckman DU) after developing the phosphomolybdate blue color with the manual technique (Murphy & Riley, 1962).

Adjustment of sample and reference slit widths affects the path of the light beam through the cell, and thus creates variations in the magnitude of the refractive index effect. The maximum range of variation due to slit width adjustment was therefore estimated by measuring the effect of varying slit width on the corrected absorbances of 2N KCl solutions in all four flowcells. Perhaps because of the slit width effect, our experience has been that the usual procedure for maximizing the colorimeter response (optical peaking) tends also to maximize the error due to variations in refractive index.

RESULTS

Figure 2a-d displays graphs of corrected absorbance versus refractive index for all four flowcells. The absorbance units along the ordinate of Figs. 2 and 3 are AutoAnalyzer units at a range expansion ("standard calibration") setting of 6.91 on both colorimeters. This gives a 100% scale deflection over the range 0-3.5 μ M phosphate for colorimeter No. 1, and 0-9 μ M for colorimeter No. 2. The refractive index effect was obviously larger for colorimeter No. 1



Fig. 3. Corrected absorbance versus refractive index for diluted Sargasso seawater containing 1 μ M phosphate. The phosphomolybdate color was developed manually. Flow-cell No. 1-A = O; Flowcell No. 1-B = \bullet .



Fig. 4. Equivalent phosphate (absorbance due only to refractive index) versus sample salinity. Colorimeter No. 1. Technicon Method No. 155-71W.

(50 mm cells) than No. 2 (20 mm cells). Flowcell No. 2-A even displays a slightly negative effect. The slopes and magnitudes of these effects must depend upon the exact geometry of each flowcell and its orientation within the cell compartment. Thus there is no assurance that the data presented here represent any maximum or minimum effect, although it appears reasonable to conclude that longer cells will display a larger deviation.

Additional experiments were performed with flowcells 1-A and 1-B. Several dilutions of Sargasso seawater (phosphate-free) were prepared with distilled water, and then spiked with $1 \mu M$ phosphate. The phosphomolybdate blue color was developed with the manual technique. The refractive index of the final reaction mixture was measured, but did not differ significantly from that of the original solution, since only 4 ml of mixed reagent was added to 40 ml of sample. When compared with Fig. 2, the results (Fig. 3) indicated that, as would be expected, the effect of refractive index was independent of absorbance.

Next we determined the magnitude of this error with the Technicon phosphate methodology. Solutions were pumped through colorimeter channel 1-A with the phosphate cartridge (Technicon 1973), but without wetting agents or intersample bubbles ("steady-state peaks"). Dilutions of Sargasso seawater (36%) spiked with 1 μ M phosphate were analyzed while pumping the phosphate mixed reagent and diluent (without Levor IV) as specified in the Technicon method. Corrected absorbances of these solutions were determined as above and converted to equivalent phosphate concentrations. The refractive indices of these solutions were quite different from the original sample solutions, since pumping introduces 23 ml of reagent plus 32 ml of diluent (distilled water) for every 42 ml of sample.

The results (Fig. 4) indicate that an error of about 0.2 μ M phosphate can result from the refractive index effect over the salinity range 0-35 ‰. The magnitude of this error in other AutoAnalyzer methods will be

dependent upon the refractive index of reagents and samples, their relative proportions when mixed in the flowcell, and the geometry, orientation and length of flowcells.

DISCUSSION

Many people analyzing marine and estuarine samples using *Technicon* AutoAnalyzer II systems are not aware of the effects of refractive index (salinity) upon colorimeter response. Since open ocean phosphate concentrations range from 0 to $3 \mu M$, this 0.2 μM error can be very significant, particularly in surface waters where values are often less than 0.2 μM .

If the analyst is careful to prepare a true reagent blank of the same refractive index (salinity) as his samples, and if all samples are of similar salinity (+3%), then no error will occur. Confusion may arise from the use of distilled-water reagent blanks. For instance, addition of 0, 1, 2, and 3 μ M phosphate to phosphate-free seawater will yield a linear plot of absorbance versus concentration. However, the $0-\mu M$ seawater will appear about 0.2 µM higher than a distilled-water reagent blank. The analyst may incorrectly assume this value to be the true phosphate content of the seawater. The extra $0.2 \,\mu M$ is of course fictional, being entirely due to the refractive index effect of seawater versus distilled water. Thus, sample absorbance minus the distilled water reagent blank absorbance will yield corrected absorbances that are systematically $0.2 \,\mu M$ too high.

The accurate analysis of low-level reactive phosphate in seawater is inherently blank-limited. A common difficulty with preparing reagent blanks of the same refractive index as the samples is the possibility of contamination with phosphate. Many reagent grade salts at the appropriate dilutions contain phosphate at concentrations comparable with surface seawater. There seems to be no way to avoid making a manual check of either artificial seawater or lownutrient seawater when used as a blank.

We use two methods in our lab to correct for this refractive index problem. With open ocean samples (salinities within $\pm 3\%$ of 35 ‰) we establish the reagent blank with surface Sargasso seawater which is phosphate-free (checked manually to be less than 0.01 μ M). This Sargasso seawater is sampled from a sample cup for several minutes to establish a good steady-state plateau. Alternatively, we establish the reagent blank with distilled water (from a sample cup, as before) and mathematically correct for the refractive index error of the blank from graphs such as Fig. 4 (see below). We prefer this second method, because it avoids hiding (and therefore often ignoring) the refractive index problem.

For estuarine samples, where salinities may range from near 0 to almost 35 %, the simplest solution is to construct a refractive index correction graph such as Fig. 4 (it may preferably be called a salinity correction graph), and from this obtain a correction for each sample of known salinity.

The salinity correction graph can easily be prepared for any phosphate channel. Add reagents and diluents to both distilled water and seawater in the ratios called for in the method. Measure the refractive indices of both reaction mixtures. Next, prepare two KCl solutions having refractive indices coinciding with the above values. Pump each of these solutions through all lines (i.e. undiluted in the flowcell) while measuring the absorbance at 880 nm. Convert the absorbances to phosphate concentrations using the factor appropriate for that standard calibration setting and the KCl solution corresponding to the distilled water reaction mixture as the "blank correction." Then plot the salinity of the original two solutions (distilled water and seawater) versus the calculated phosphate concentrations (= equivalent phosphate). Corrections for each sample salinity may be found by interpolation between these points. Note that if slit widths or flowcell orientations are altered. the plot should be remade.

The resulting graph allows one to use distilled water as the true reagent blank, a practice that is much more desirable than the use of low-phosphate seawater or artificial seawaters in which the phosphate concentration must be independently measured. In principle, this procedure can be used to generate a refractive index correction graph for any methodology, provided the true absorbances (if any) of the KCl solutions at the wavelength of interest are taken into account.

An example calculation is given below to illustrate the use of the above plot for samples of any salinity, based on Fig. 4.

$$[\mathbf{PO_4^{\pm}}] = \{F \times (A_s - A_{DW})\} - 0.20 \ (S/35)$$

where

A,

S

- [PO[‡]] = phosphate concentration (µM); F = slope of standard concentration versus "absorbance" (in AA II units);
 - = sample "absorbance" (in AA II units);
- A_{DW} = distilled water reagent blank "absorbance" (in AA II units);
- 0.20 = refractive index correction at 35 ‰ salinity from Fig. 4. Note that this value is *independent* of scale expansion (standard calibration setting);

= salinity of sample in %.

CONCLUSIONS

The curved ends on *Technicon* AutoAnalyzer II colorimeter flowcells cause an apparent absorbance to be measured at the phototube which is a function of the refractive index of the solution. The error is larger for longer cells and must be dependent upon the geometry of each system. This effect can lead to systematic errors of about $0.2 \,\mu$ M in the automated determinations of phosphate in seawater.

We recommend that users of the AutoAnalyzer II methodology for low-level phosphate in seawater calibrate each colorimeter for the refractive index effect we have described here. True reagent blanks can then be readily determined with distilled water and corrected for the refractive index error. This avoids the problem of using artificial seawater or Sargasso seawater as blanks, which must be manually blanked against distilled water routinely to establish them as determinant-free solutions.

REFERENCES

Atlas E. L., Gordon L. I., Hager S. W. & Park P. K. (1971) A practical manual for use of the *Technicon* AutoAnalyzer in seawater nutrient analyses; revised. Technical Report 215, reference 71-22 of the Department of Oceanography, Oregon State University. 49 p.

- Henriksen A. (1965) An automatic method for determining low-level concentrations of phosphate in fresh and saline waters. *Analyst Lond.* **90**, 29–34.
- Loder T. C. & Glibert P. M. (1977) Blank and salinity corrections for automated nutrient analysis of estuarine and seawaters. UNH Sea Grant No. UNH-5G-JR-101 and WHOI No. 3897. 29 p.
- Murphy J. & Riley J. P. (1962) A modified single solution method for the determination of phosphate in natural waters. Analytical chim Acta 27, 31-36.
- Technicon Industrial Systems (1973) Orthophosphate in water and seawater. Industrial Method No. 155-71 W/Tentative. Tarrytown, N.Y. 10891.