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Expansion of the capabilities of the Person-Portable Analytical Kit (PPAK)

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Abstract

The Person-Portable Analytical Kit (PPAK) was further expanded to allow the quantitative determination of primary amines. *p*-Dimethylaminobenzaldehyde was used to analyse for hydrazine, aniline, *m*-nitroaniline, and 2,4-diaminotoluene in tap water, waste water and sea water at temperatures ranging from 10 °C to 32 °C. Aniline and ethylamine were quantitatively determined in tap water using fluorescamine to form a visible chromophore.

Keywords: Person-portable analytical kit; Emergency response; Analysis of pollutants; Analysis of amines; Colorimetric analysis of pollutants

1. Introduction

As part of its continuing program aimed at enhancing emergency response capabilities at spill sites [1–4], this laboratory developed a Person-Portable Analytical Kit (PPAK) for on-site analysis of chemicals during spill situations [1]. The PPAK was used to analyze pollutants in soil or water matrices in which a single known chemical was involved. Quantitative determination could be made by means of colorimetric and titrimetric techniques. The kit was developed using a commercially available colorimeter. It was field portable and permitted rapid colorimetric detection of pollutants in water and soil. It was limited to visible chromophores which absorb in the 400–900 nm range. For this type of application, the reactions involving chromophores formation must be rapid, sensitive, and specific. Preferably, they can proceed

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in aqueous media without the need to extract the pollutants with an organic solvent. Additionally, the chromophores formed must be reasonably stable over an extended period of time to allow completion of the analysis.

In its early form, the kit was capable of detecting all of the inorganic chemicals from Environment Canada's priority list, with the exception of hydrogen peroxide and titanium dioxide. However, because of potential hazard to human health and ecosystems, the monitoring of the concentration of organic constituents became more important and analytical procedures for organic compounds were in urgent demand [5]. Consequently, the development of the PPAK focused on developing procedures and methodologies for the analysis of spill priority chemicals, with the emphasis on organic compounds [1]. Classes of compounds for which tests were developed include: aliphatic and aromatic hydrocarbons, aldehydes, and ketones. In 1991, with the introduction of the DR-2000 digital spectrophotometer (Hach Company) the PPAK was further improved in that the overlays originally used to "calibrate" the meter according to the substance being analyzed were no longer required and calibration curves could be programmed directly into the memory of the instrument.

The order in which methods for target compounds have been developed is based on a priority list [6] established for emergency spill response. To date, amines represent one class of compounds for which there are still few methods available. Most of the amines found on the priority list are primary amines. Hence, this study focussed on the development of methods for the quantitative analysis of primary amines in aqueous matrices. Methods developed in aqueous media are often applicable to other matrices such as soil and vegetation when combined to an adequate extraction procedure.

There exist methods available to perform the analysis of primary amines. Watt and Chrisp [7] reported the analysis of hydrazine with *p*-dimethylaminobenzaldehyde and a study by Udenfriend et al. [8] showed that 4-phenylspiro[furan-2(3H), 1′-phthalan]-3,3′-dione (fluorescamine) reacts with primary amines in the picomole range to form fluorophors with excitation and emission bands at 390 and 475 nm, respectively. Felix et al. [9] studied the reaction of fluorescamine with primary amino acids to produce highly fluorescent pyrrolinones and its reaction with secondary amines to produce non-fluorescent aminoenone chromophores. The main difference between these methods lies in the fact that the fluorescamine reagent was prepared in dioxane (2.0 μmol/ml). The fluorometric reagents fluorescamine and *o*-phthalaldehyde are used to react with primary amines and are not restricted to amino acids. Fluorescamine reacts rapidly at room temperature and exhibits low ammonia interference but is unstable in aqueous solutions and the fluorescence intensity varies for each compound [10]. *o*-Phthalaldehyde is stable in aqueous solution, reacts rapidly at room temperature to form a highly fluorescent product but shows ammonia interference [11].

Diazotization with *N*-(1-naphthyl)-ethylenediamine as coupling agent for determination of aminophenols, phenylenediamines, dinitroanilines, trichloroanilines and tetrachloroaniline was investigated by Norwitz and Keliher [12]. All targets yielded violet to cherry red products which have maximal absorbance in the range of

521–582 nm. Unfortunately, most of the amines studied were either insoluble in water or produced unstable products.

This paper reports on the value of *p*-dimethylaminobenzaldehyde and fluorescamine in colorimetric determination of primary amines. Both reagents form reasonably stable visible chromophores. Fluorescamine, however, is insoluble in water, and is utilized as a solution in a water miscible solvent, such as dioxane or acetone. Methods were developed to analyze for different concentrations of hydrazine, aniline, *m*-nitroaniline, ethylamine and 2,4-diaminotoluene in water under varying temperature conditions. Experiments were carried out with tap water, waste water and sea water to determine the scope of applicability of the method.

2. Experimental: materials and methods

Absorbance measurements were done on a DR 2000 spectrophotometer (Hach) using 25 ml cuvettes. 1,4-Dioxane (99% spectroscopic grade, Aldrich chemical company), unless otherwise specified, was dried and distilled over calcium hydride. Glass-distilled acetone (Caledon) was dried over self-indicating calcium sulphate, decanted, and then re-distilled. Aniline (Fisher Scientific) was distilled twice under reduced pressure and used as is without prior standardization. Other materials employed in this study were used without prior purification: hplc-grade ethanol, anhydrous reagent-grade hydrazine, ACS-grade concentrated hydrochloric acid, *p*-dimethylamino-benzaldehyde, *o*-, *m*- and *p*-nitrotoluene (all of the preceding were obtained from Fisher Scientific), fluorescamine (98%), ethylamine (70% wt:wt in water), 2,4-diaminotoluene (98%), 2,4-dicyanotoluene (80%) (all of the preceding were obtained from Aldrich), reagent-grade sodium tetraborate (Merck and Co), potassium phosphate and sodium borate (both Metrepak, Microessential Laboratory).

Potassium phosphate–sodium borate buffer: One capsule dissolved in 100 ml deionized water, pH = 9.

Boric acid buffer: 0.05 *M* $Na_2B_4O_7 \cdot 10\,H_2O$ in deionized water, pH = 9.31. The pH of the solution was adjusted to pH = 7–9 by titrating with concentrated hydrochloric acid.

Hydrazine 800 ppm stock solution: 7.97 μl of neat hydrazine was diluted to 10 ml with 1 *N* hydrochloric acid.

Aniline 9300 ppm stock solution: 91 μl of distilled aniline was diluted to 10 ml with the potassium phosphate–borate buffer.

Ethylamine 450 ppm stock solution: 80.9 μl of 70% (wt:wt) ethylamine in water was diluted to 100 ml with boric acid buffer.

m-Nitroaniline: 138.2 mg of *m*-nitroaniline was dissolved in 200 ml of pH 7.5 boric acid buffer (solution of 691 ppm).

2,4-Diaminotoluene: 122.5 mg of 2,4-diaminotoluene was dissolved in 100 ml of pH 7.5 boric acid buffer (solution of 1225 ppm).

p-Dimethylaminobenzaldehyde: 4 g *p*-dimethylaminobenzaldehyde was dissolved in a mixture of 200 ml of ethyl alcohol and 1 ml of concentrated hydrochloric acid. An excess (10 ml) of this reagent was used in colour development for all the amines

analyzed, and at all spike levels. In the analysis of hydrazine, the colour was developed by adding the reagent to a mechanically or magnetically stirred 50-ml sample of aqueous hydrazine solution of 0.02 ppm to 0.8 ppm. After stirring for 10 min, the intensity of each solution was measured at 458 nm. A mixture of 50 ml of test water and 10 ml of the colour reagent were stirred for 10 min and the intensity was measured at 458 nm to afford an absorbance for a blank determination. The absorbance for each spike level in each calibration curve was obtained by subtracting the absorbance of the blank determination from the absorbance of each spike level. The analysis of aniline, *m*-nitroaniline, and 2,4-diaminotoluene used 25 ml of water samples instead of the 50 ml samples used in the analysis of hydrazine.

Fluorescamine stock solution: 99.8 mg of fluorescamine was dissolved in 10 ml of dry distilled dioxane or acetone. Unless specified, colour development used excess fluorescamine in a total volume of 5 ml dioxane for each spike level. When the volume of solvent was too small, fluorescamine precipitated as soon as the solution came into contact with water. The excess fluorescamine was added very rapidly to an aqueous mixture of the amine, while the solution was being agitated vigorously by magnetic stirring. After about one minute of stirring, the intensity of the solution was measured at 402 nm. A blank determination was run for each spike level in a calibration. In the analysis of amines with fluorescamine, the intensity of the blank depends upon the initial quantity of fluorescamine. The intensity increases with an increase in the quantity of fluorescamine. A blank solution was made up of 25 ml of water, 2 ml boric acid buffer, 5 ml dioxane, and a volume of deionized water equivalent to the volume of the amine spike, where a relatively large volume of spike was used, and would affect the overall absorbance reading. In the analysis of aniline, the spike sample was relatively small and was considered negligible. The absorbance employed in calibration curve preparation were obtained by subtracting the absorbance of the blank from the absorbance of the sample.

3. Principle

In this study, *p*-dimethylaminobenzaldehyde was used for the quantitative determination of hydrazine, aniline, *m*-nitroaniline and 2,4-diaminotoluene to the nanomole level. The analyses are based upon the reaction shown in Fig. 1. When an acidic solution of *p*-dimethylaminobenzaldehyde in a mixture of ethanol and concentrated hydrochloric acid is added to a solution of each amine in water, a deep yellow colour is formed. The intensity of the yellow chromophore was measured at 458 nm (hydrazine), 455 nm (aniline), and 445 nm (*m*-nitroaniline and 2,4-diaminotoluene). This method is applicable to the analysis of other aromatic primary amines capable of forming a chromophore.

In a study by North [13] fluorescamine was used to perform the rapid fluorometric measurement of primary amino compounds in seawater. The procedure includes the following steps: the sample solution is buffered to $pH = 9$ with 0.5 ml of a sodium borate solution @$pH = 9.6$ to maximize fluorescence, 0.5 ml of fluorescamine in acetone (20 mg/100 ml) is added while shaking. Vigorous mixing is important as it

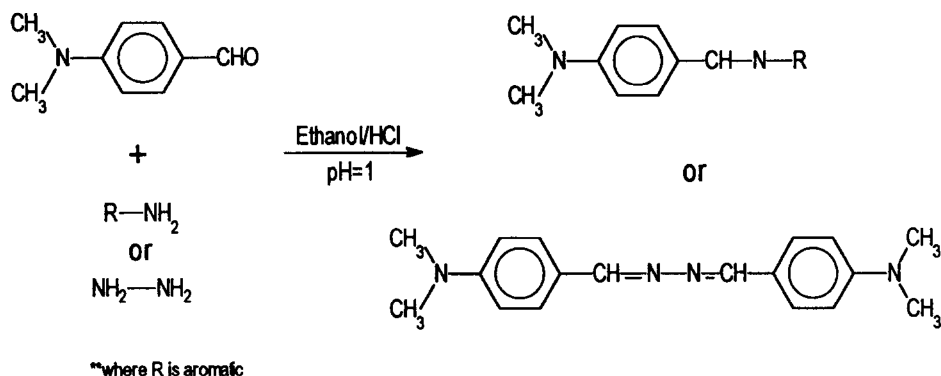

Fig. 1. Reaction of hydrazine with *p*-dimethylaminobenzaldehyde.

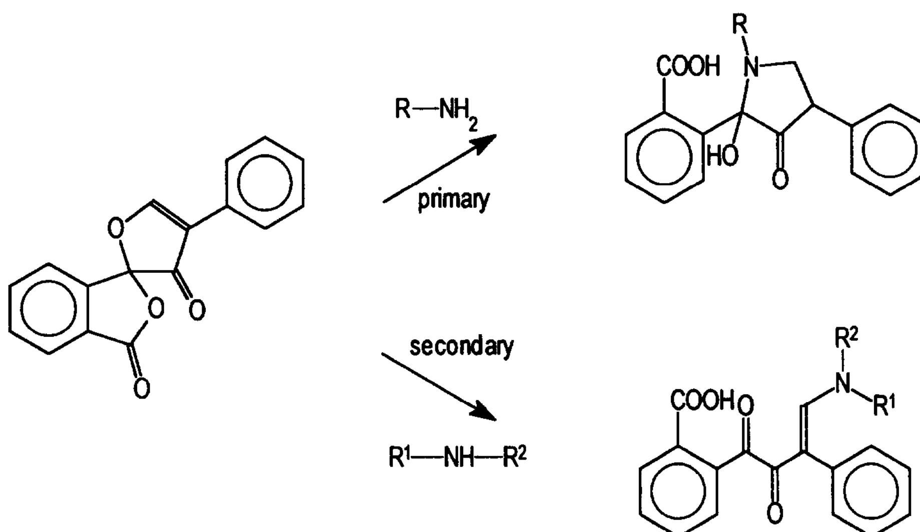

Fig. 2. Reaction of primary and secondary amines with fluorescamine.

promotes interaction between fluorescamine and the amino compounds while minimizing the destruction of the fluorescamine by the water. The reaction proceeds instantaneously at room temperature and yields highly fluorescent compounds as well as non-fluorescent degradation products when secondary amines are present (note that fluorescamine itself is non-fluorescent). The absorbance of the sample is then measured at 480 nm. It was found that the compounds naturally present in seawater (organic compounds, metals) did not appear to interfere with the reaction. Neither ammonia nor urea produced detectable fluorescence.

The assay is based upon the reaction shown in Fig. 2, and requires a spectrofluorometer to measure the intensity of the fluorophores formed. Even though

spectrofluorometry is generally more sensitive than colorimetry, it is desirable to limit the field instrumentation to a single apparatus. Hence, based upon earlier reports showing that fluorescamine can be used in colorimetric assay of amino acids [8], we investigated the use of fluorescamine in the colorimetric assay of aniline and ethylamine to the nanomole level. Furthermore, colorimetric measurements are less sensitive to interferences from the blanks than their fluorometric counterparts [14].

For quantitative analysis in water, a large excess of fluorescamine is required, and during colour development, the mixing of reagents has to be done rapidly. The initial quantity of fluorescamine required for colour development depends upon the reactivity of the amine being analyzed. To minimize the hydrolysis of fluorescamine, dry distilled dioxane or acetone was used as the solvent. The light yellow colour formed when a solution of fluorescamine in dioxane or acetone is added to a basic solution of aniline or ethylamine in water forms the basis for a quantitative assay. The intensity of the yellow chromophore was measured at 402 nm.

4. Results and discussion

According to the method developed by Watt and Chrisp [7] , hydrazine reacts with *p*-dimethylaminobenzaldehyde in the presence of HCl to form a yellow chromophore. The calibration curves for the quantitative analysis of hydrazine with *p*-dimethylaminobenzaldehyde in 50 ml of deionized water, tap water or wastewater at ambient temperature are shown in Fig. 3. These results compare well to those reported by Watt and Chrisp for the analysis of hydrazine in distilled water. The curves can be considered to have a linear response for hydrazine concentrations ranging between 0.03 and 0.5 ppm.

Pocklington reported that although solvent extraction of amino acids from river, lake or other low salinity waters works well, the same is difficult to achieve in high salt content water [15]. It was suggested that the cations present in seawater may affect the extraction of highly polar compounds, such as amines, and that the best methods might only yield an 80% efficiency. To evaluate this potential limitation, similar analyses were performed in seawater samples. For a given set of experimental conditions, the results obtained for seawater samples compared well with those obtained for tap water and wastewater samples. In fact, the calibration curves obtained for seawater fell within 5% of their tap water and wastewater counterparts, as it can be seen from Fig. 3. It can be concluded that the presence of salt in an aqueous solution has no significant effect on the method reported herein.

Absorption maxima for products arising from the reaction between *p*-dimethylbenzaldehyde and aniline, *m*-nitroaniline, and 2,4-diaminotoluene were obtained empirically prior to the actual analytical experiments. They were observed at 455, 445, and 445 nm, respectively. This overlap implies that the method can be used for a single-component analytical protocol only. Typical calibration curves for aniline, *m*-nitroaniline, and 2,4-diaminotoluene in various water samples at ambient temperature are shown in Figs. 4 and 5. The aniline calibration curve can be considered linear only

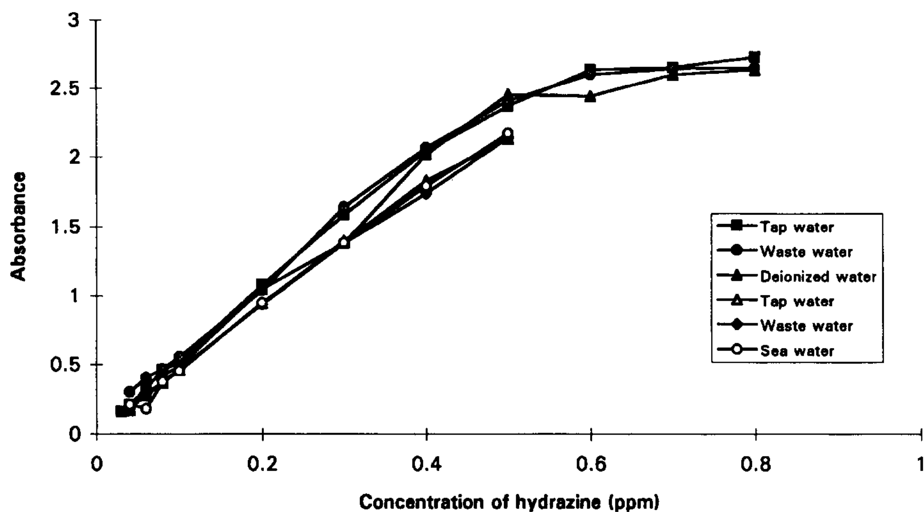

Fig. 3. Absorbance values of the chromophore obtained by the reaction of hydrazine with *p*-dimethylaminobenzaldehyde in different matrices.

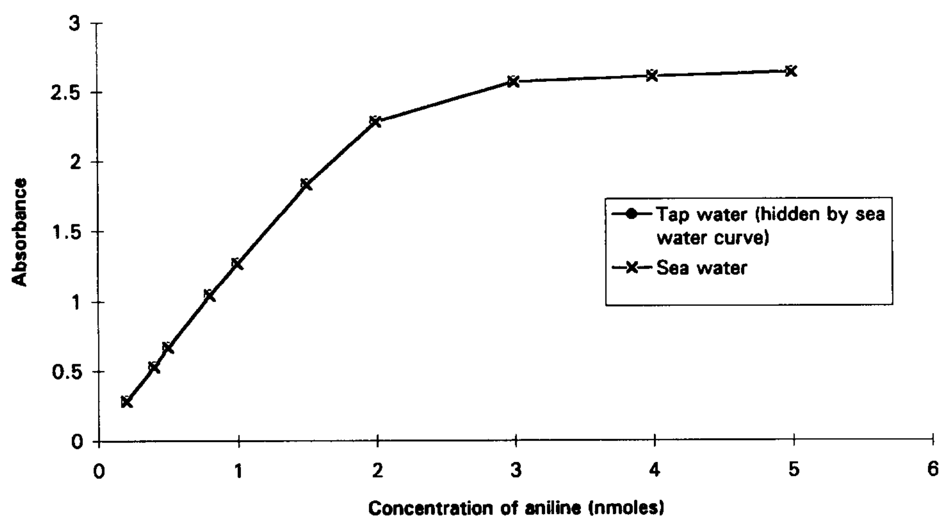

Fig. 4. Calibration curves for the quantitative analysis of aniline in tap and seawater.

over the 0.2–2 n mol range, whereas the *m*-nitroaniline and 2,4-diaminotoluene each have linear responses over the 25–800 n mol range.

Colour development during chromophore formation occurred in less than ten minutes, which is faster than the colour development observed in the analysis of

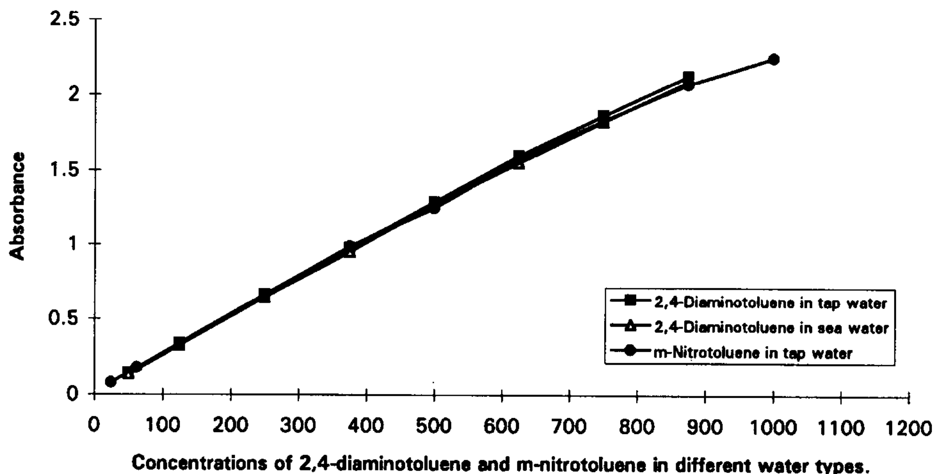

Fig. 5. Calibration curves for the quantitative analysis of 2,4-diaminotoluene in tap water and of *m*-nitrotoluene in tap and seawater.

hydrazine. This can be explained by the fact that there are two reaction sites in hydrazine compared to one amino group in each of aniline and *m*-nitroaniline. Although 2,4-diaminotoluene has two reactive amino groups, it appears that only the amino group *para* to the methyl group reacts; this is likely the result of a steric hindrance.

The reaction could not be extended to the analysis of *ortho*- or *para*-nitroaniline because solutions of *ortho*- or *para*-nitroaniline in water are intensely coloured and indistinguishable with naked eye from the solution of the chromophore with *p*-dimethylaminobenzaldehyde. The distinction is possible spectrophotometrically, but the difference in the measured intensity between each spiked solution and its corresponding solution of the chromophore is very small. The difference decreases with an increase in the spike level and, consequently, a linear response could not be obtained. Porter [16] observed that *o*- and *p*-nitroaniline and *p*-nitrotoluene develop an orange, red, or purple colour in dimethyl formamide upon addition of tetraethylammonium hydroxide. Unfortunately, the reaction is not good to use in water because at a water content of 5% or greater, the absorbance of the reaction product is reduced. A solution would be to extract the contaminants in an organic solvent.

The effect of temperature on colour development and stability of the chromophore formed in the analysis of hydrazine with *p*-dimethylaminobenzaldehyde was investigated at three different temperatures; 10 °C (cold room), 23 °C (ambient), and 32 °C (water bath). The choice of the minimum and maximum temperatures was based upon the assumption that the tests would normally be performed in the field, but inside an emergency response vehicle. The results are presented in Fig. 6 and show that, over this temperature range, there is no significant difference on the stability of the

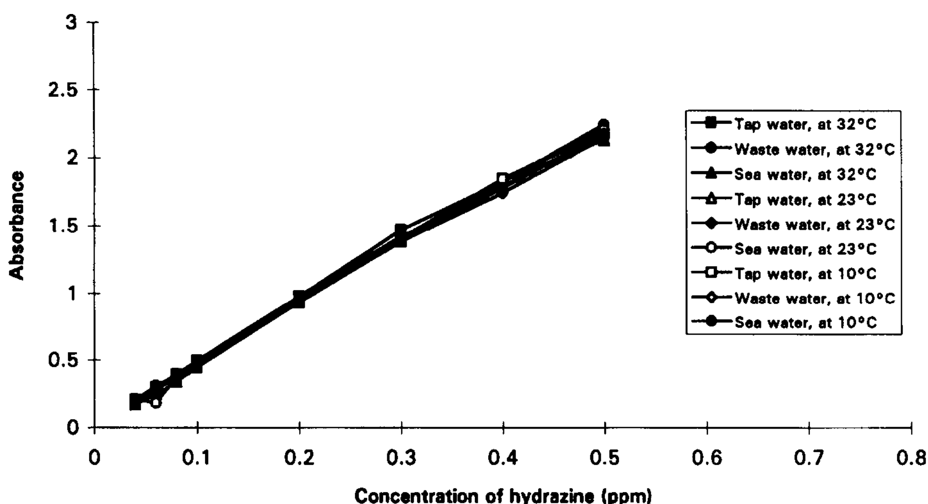

Fig. 6. Effect of temperature on the reaction of hydrazine with *p*-dimethylaminobenzaldehyde in different types of water.

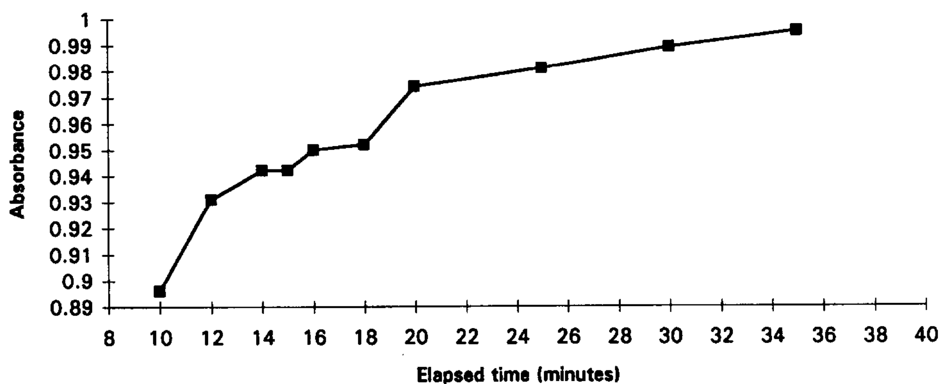

Fig. 7. Absorbance of hydrazine after reaction with *p*-dimethylaminobenzaldehyde as a function of elapsed time.

chromophore, as evidenced by small variation in the measured intensities. The kinetics, however, was affected and there was a significant variation in the time required to yield a complete colour development. The data presented in Fig. 7 show the extent of the effect the temperature had on the colour development time. The absorbance versus time curve rises steadily from 10–20 min, at which points it levels off. According to these results, a minimum of approximately 20 min is necessary for complete colour development at 10 °C.

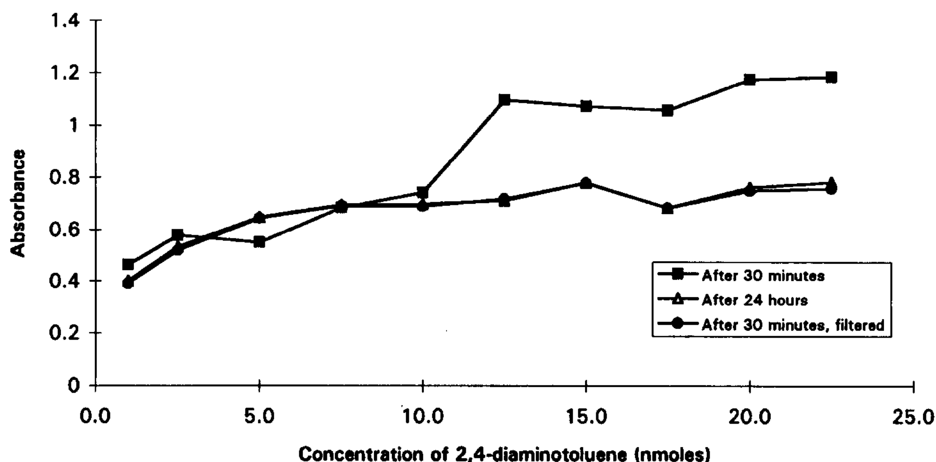

Fig. 8. Absorbance of aniline after reaction with fluorescamine with and without filtration over Whatman filter paper.

Mixing of the reagents to the sample must be done rapidly because fluorescamine undergoes hydrolysis within one minute. The agitation efficiency decreases with an increase in volume – 5 ml test tube sample versus 25 ml cuvette sample (respectively by vortex or magnetic mixer). However, as the resulting precipitate settled, the absorbance reading decreased to a constant value. To overcome this problem, filtration was used. The 25 ml sample was mixed with a magnetic stirrer and excess fluorescamine added with a syringe. An absorbtion versus time study was done on the direct solution and on the solution after filtration over one Whatman #42 ashless filter paper. Filtration makes a significant change to the absorbance value if filtered immediately – Fig. 8 presents the relevant data.

The requirement for filtration seems to arise from the use of the buffer. In fact, in tap water, the phosphate–borate buffer produces metal phosphate precipitates, which interferes with the absorbance measurements if not filtered. The boric acid buffer, however, does not precipitate with tap water and does not require filtration.

Since ethylamine has a boiling point of 17 °C, at normal room temperature and during warm days, it exists in gaseous form. At higher temperature than its boiling point, ethylamine must be analyzed largely in the atmosphere. In order to minimize the loss of ethylamine by evaporation while increasing reproducibility, the fluorescamine stock solution was prepared and stored at 10 °C (cold room). Any residue of an aliquot used daily was discarded at the end of each day.

Samples containing 0.4 μmol of ethylamine in 2 ml boric acid buffer (pH = 8.9) were treated with 5–35 mol equivalents of fluorescamine in 1 ml dioxane. The volume of each sample was adjusted to 25 ml with tap water at 10 °C. A blank solution for each sample was prepared the same way. The absorbance of each sample was

measured at 402 nm. The data show that 5 mol equivalents of fluorescamine is sufficient for complete colour development. In order to allow for a comfortable margin of error, ten mol equivalents of fluorescamine were used to prepare calibration curves for the analysis of ethylamine at room temperature in tap and seawater. The curves were characterized by linear responses over the 400–1200 n mol range.

5. Conclusion

The PPAK developed by Environment Canada was further expanded for the quantitative analysis of some primary amines. The quantity of reagents that are required are very small thus making for a highly portable kit for on-site analysis. *p*-Dimethylaminobenzaldehyde can be used to perform the colorimetric determination of hydrazine, aniline, *m*-nitroaniline and 2,4-diaminotoluene to the nanomole level in tap water, wastewater and seawater at temperatures ranging from 10 °C to 32 °C. Fluorescamine was also used successfully to analyze aniline in tap water and ethylamine in tap water and seawater at ambient temperature.

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