Beside this potential source of errors, deviations may result from the very rapid decrease of the sensitivity of the formaldehyde determination which occurs when the S solution is used for a longer period: after 4 days the absorbance does not increase any more upon addition to a formaldehyde solution together with a P reagent. This is due to the decrease of the sulfite concentration owing to oxidation to sulfate in the weakly basic S solution (*8*).

Lyles et al. (*3*) found that when separate TCMS and P reagents are used, better results are obtained in the determination of formaldehyde concentrations than when using TCMSP combined reagent. In this strongly acidic combined reagent the $[HgCl_2SO_3]^{2-}$ complex is not stable. Though no oxidation of the sulfite ion occurs, its concentration decreases by liberation of sulfur dioxide gas. Since in an acidic medium the tetrachloromercurate consequently has no stabilizing effect, it was justified to expect that the stability and therefore the sensitivity pattern in the formaldehyde determination would be the same for the TCMSP solution as for the SP solution. This indeed proved to be the case.

When a freshly prepared SP combined reagent is used, the same sensitivity is found as with the TCMS–P method (mean value from five measurements over a period of 2 months: 0.480 AU mL μg^{-1}, $V = 2\%$, $r = 0.999\,96$). However, after a period of 6 h the sensitivity of formaldehyde determination with this reagent has decreased by 2%, after 1 day by 10%, and after 4 days by 30%.

Thus, whenever it is undesirable to use the environmentally harmful tetrachloromercurate reagent in the determination of formaldehyde by the pararosaniline method, a freshly prepared sulfite–pararosaniline combined reagent is the best alternative.

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Registry No. P, 569-61-9; Na_2SO_3, 7757-83-7; TCM, 14024-34-1; Hg, 7439-97-6; Formaldehyde, 50-00-0.

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Low-Temperature Filter Paper Phosphorescence

Sir: During an investigation of X-ray excited low-temperature (XLTP) and X-ray excited room-temperature (XRTP) luminescence of drugs (*1*), it was noticed that a dilute solution of *p*-aminobenzoic acid was enhanced (compared to room temperature measurements) by a factor of 10 when the sample was cooled to 90 K. In the case of theophylline with no heavy atom present, a luminescence signal was seen for XLTP (at 90 K) when no luminescence signal could be seen at room temperature unless a heavy atom perturber was present. Because of the considerably greater detection power of UV excited phosphorescence compared to X-ray excited phosphorescence (*1*), we felt an evaluation of UV-excited low-temperature filter paper phosphorescence (LTFpP) would be interesting and a comparison with UV-excited room-temperature filter paper phosphorescence (RTfpP) was worthwhile, especially because of the recent research into the analytical uses of RTfpP (*2–6*).

It should be noted that Miller et al. (*7, 8*) have described a thin-layer phosphorimeter for scanning thin-layer chromatographic plates at 77 K. However, in their work (*8*), the sample (5 μL) was separated by TLC, the TLC plate was dried and wrapped around the sample holder drum, the plate was sprayed with a solvent, like ethanol, until it was wet, and the drum was inserted into its compartment, filled with liquid

Table I. Comparison of the Signal Enhancement Ratios and the LOD Ratios of RTFpP and LTFpP

compound	concn, μg/mL	I_{LTFpP}/I_{RTFpP}	LOD_{RTFpP}/LOD_{LTFpP} [b]
privine HCl	200	9.4	10
6-methylmercaptopurine	10	84	8.0
L-tryptophane	1	2.4	1.7
chloroquine diphosphate	2	12.5	6.7
p-aminobenzoic acid	1	21.3	6.0

[a] The luminescence intensity was measured at the emission maxima for each compound. Signals given in this table have been corrected for background emission. [b] The values for this ratio were taken from Table II.

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Table II. Comparison of Several Figures of Merit for RTFpP and LTFpP

	RTFpP						LTFpP					
			LOD[b]						LOD[b]			
compound	λ_{ex}, nm	λ_{em},[a] nm	μg/mL	ng	LLS[c]	CORR[d]	λ_{ex}, nm	λ_{em}, nm	μg/mL	ng	LLS	CORR
privine HCl	290	*485*, 520	3.0	15	0.59	1.00	285	*475*, 510	0.3	1.5	0.68	0.999
6-methyl-mercaptopurine	295	458	0.04	0.2	0.96	0.999	290	450	0.005	0.025	0.95	0.999
L-tryptophane	270	450	0.001	0.005	1.15	0.998	275	455	0.0006	0.003	0.86	0.977
chloroquine diphosphate	330	480	0.04	0.2	0.91	0.999	330	460	0.006	0.03	0.77	0.996
p-aminobenzoic acid	280	430	0.003	0.015	1.19	0.999	275	430	0.0005	0.0025	0.67	0.982

[a] Italicized wavelength used for LOD determination if more than one is given. The solvent work in all cases is water. [b] LOD defined as that concentration necessary to produce a signal equal to three times the standard deviation of 16 blanks. [c] LLS is the slope of the plot of the log of signal vs. the log of concentration. It was calculated by the method of least linear squares from those points used in determining the calibration curve of each compound. [d] CORR is the correlation coefficient of the least linear square analysis.

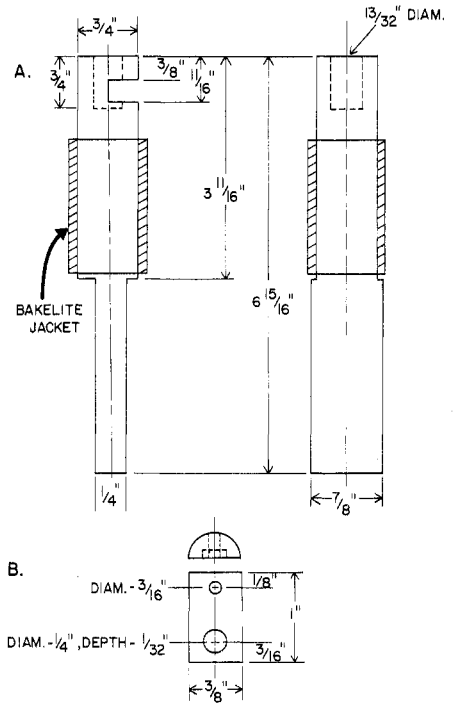

Figure 1. (A) Copper conduction cooling bar. (B) Copper sample holder.

nitrogen, and scanned slowly. In their case, the solvent had a large effect upon the phosphorescence signals. The Miller et al. (*8*) approach is probably more correctly called low-temperature thin-film (glass) phosphorimetry.

EXPERIMENTAL SECTION

All measurements were made on an Aminco-Bowman spectrofluorometer fitted with a 150-W xenon arc lamp (Schoeffel, Westwood, NJ) and a potted 1P21 photomultiplier tube powered by a high-voltage power supply (Princeton Applied Research, Princeton, NJ). Signals were processed with a DC electrometer (*9*).

The conduction cooling bar designed for the LTfpP studies (shown in Figure 1) is a modification of the one described by Ward et al. (*10–12*). The sample compartment and sampling procedure were identical with those used by Ward et al. (*11, 12*). The sample holder (Figure 1B) consists of a copper hemispherical rod with a small hole ($^3/_{16}$ in.) for alignment and a large hole or well ($^1/_4$ in. diameter, $^1/_{32}$ in. deep) for the filter paper disk with sample. The temperature achievable at the sample holder is 90 K (measured by a thermocouple).

The instrumental conditions were as follows: photomultiplier tube voltage, 705 V; monochromator slits, 0.5 mm; phosphoroscope speed, 250 rps; spectral scan rate, 50 nm/min; and RC final filter time constant, 1 s.

All analytes were prepared in stock solutions of 2000 μg/mL in water. The chemicals were obtained from the following companies: privine hydrochloride, Ciba Geigy Corp., Summit, NJ; 6-methylmercaptopurine, Nutritional Biochemicals Corp., Cleveland, OH; L-tryptophane, Eastman Kodak, Rochester, NY; chloroquine diphosphate, Aldrich Chemical Co., Milwaukee, WI; and *p*-aminobenzoic acid, Fisher, Fairbanks, NJ. All chemicals were used as received (no purification). The sampling procedure consisted of pipetting 5 μL of the sample solution onto the filter paper disk, drying under an (IR) heat lamp for 10 min, and then pressing by tweezers into the sample holder. The sample holder was then plunged into liquid nitrogen for 30 s before being placed in the conduction cooling bar; this process minimized frost build up on the sample head by decreasing the sampling time by a factor of 2.

The sampling time of LTfpP was 11 min (including the 10 min drying time) as compared to 20 min for conventional RTP which required 10 min flushing time of the sample compartment of N_2 to minimize O_2 quenching. Of course, by use of more sample holes (wells) in the sample bar, e.g., four, the time per sample in RTP can be reduced considerably (*13*).

RESULTS AND DISCUSSION

The enhancements of the luminescence signals of analytes on paper substrates measured at 90 K (LTfpP) compared to room temperature filter paper phosphorescence (RTfpP) are given in Table I for several drugs. Also shown in Table I are the ratios of the limits of detection (LODs) for the two techniques. In order to fairly evaluate the two techniques, we performed both sets of measurements with the same sample holder (the one used for LTfpP). The concentrations used for the signal ratios, I_{LTfpP}/I_{RTfpP}, were chosen from the linear portions of the analytical calibration curve for each compound. The limit of detection ratios were calculated by using the limits of detection for the same compounds listed in Table II. It is clear that the signal ratios and LOD ratios vary considerably from compound to compound (signal ratios of 2.4 for L-tryptophane to 84 for 6-methylmercaptopurine; LOD ratios of 1.7 for L-tryptophane to 10 for privine hydrochloride). However, the enhancement ratios are a factor of ~10 or greater for all except L-tryptophane and the LOD ratios are ~10 (or slightly less) for all except L-tryptophane. We do not know why the LOD of L-tryptophane changed so little by going from room temperature to 90 K. The detection limits by LTfpP are considerably lower than values obtained by Miller et al. (*8*) using low-temperature thin-layer phos-

phorimetry; however, it should be noted that different compounds were measured by Miller than by us except for *p*-aminobenzoic acid where the latter group obtained an LOD of 0.6 ng.

The phosphorescence spectra at 90 K were shifted slightly to lower wavelengths and had slightly narrower bandwidths than those at 298 K. This agrees with previous work where the phosphorescence spectra taken in organic solvents at 77 K are slightly narrower and blue shifted compared to RTfpP spectra.

The reasons for the lack of linearity of the analytical calibration curves (log–log slopes >1.05 or <0.95) for L-tryptophane, privine hydrochloride, chloroquine diphosphate, and *p*-aminobenzoic acid by both LTfpP and RTfpP are not known.

As Ward et al. (*14*) and Bateh and Winefordner (*15*) have pointed out, the major limitation to RTfpP is the inherent paper background (400–600 nm). This is also true for LTfpP. However, it is very encouraging that a factor of ~10 lower detection limits occur with LTfpP. Further evaluation of LTfpP is required, but the potential for drug analysis, toxic chemical monitoring, and analysis of narcotics appears to be good. Work is currently in progress in optimizing LTfpP for use in these application areas.

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AIDS FOR ANALYTICAL CHEMISTS

Determination of Alkyl Quaternary Ammonium Compounds by Liquid Chromatography with Indirect Photometric Detection

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Quaternary ammonium salts (QUATS) are widely used as phase transfer catalysts, disinfectants, and surfactants. Aliphatic QUATS are difficult to determine since they are very polar and lack ultraviolet (UV) absorbance. Gas chromatography (*1–3*), gas chromatography/mass spectrometry (GC/MS) (*4*), and pyrolysis GC/MS (*5*) have been applied to the determination of quaternary ammonium compounds. Normal phase ion-pair chromatography with UV-absorbing ions in the aqueous stationary phase and reversed-phase ion-pair chromatography with UV-absorbing ions in the mobile phase have also been used for determination of QUATS (*6, 7*). However, none of these approaches are totally satisfactory for determining QUATS in the wide variety of matrices in which they are used.

More recently Small and Miller (*8*) used UV absorbing ions in an ion-exchange mode to detect inorganic ionic species. In this technique, which has been named indirect photometric chromatography (IPC), inverted peaks are observed in an elevated base line as the transparent sample ions are selectively displaced from the column by the UV absorbing ion.

This paper describes an indirect photometric chromatographic procedure for determination of aliphatic quaternary ammonium salts in which an aromatic quaternary salt is employed as the UV absorbing species. The effects of various analysis parameters such as eluent composition and concentration are discussed.

EXPERIMENTAL SECTION

Reagents. Acetonitrile distilled in glass was obtained from Burdick and Jackson Laboratories, Inc., Muskegon, MI. Quaternary ammonium salts 98–99% pure were obtained from Chemalog, South Plainfield, NJ, and ICN Pharmaceuticals, Inc., Plainview, NY. Other compounds used were ACS Reagent grade.

Apparatus. The liquid chromatograph used consisted of a Perkin-Elmer LC-75 variable wavelength detector, Rheodyne 7125 injector, Laboratory Data Control Constametric III pump, and 10-mV strip chart recorder. The column used in all examples cited was a 4.6 × 250 mm Partisil-10 SCX strong cation exchanger available from Whatman, Inc., Clifton, NJ.

Chromatographic Conditions. *A. Short-Chained ($<C_4$) QUATS.* The LC operational parameters were as follows: mobile phase, acetonitrile/water (70:30) with 0.025 M benzyltriethylammonium chloride and 1% acetic acid (pH 3.7); mobile phase flow rate, 2.0 mL/min; column temperature, ambient; detector wavelength, 270 nm; detector sensitivity, 0.08 AUFS; injection, 50 μL of 1.0 mg/mL of each QUAT in acetonitrile/water (70:30).

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