were carried out instrumentally after the sample had decayed for about 10 hours; this is the time required for the decay of ^{18}F produced from the water.

RESULTS AND DISCUSSION

In the solution of the enzyme Stellacyanin, a copper content of 0.89 ± 0.08 μg was measured in samples with volumes of 10 μl. The quantitative removal of the activity from the aluminium foils was experimentally verified using ^{64}Cu samples of known strength which were given the same treatment as the copper enzyme samplex as regards pre- and post-irradiation procedures.

The conversion of liquids into frozen droplets for purposes of neutron activation may also be applied to advantage in the analysis of organic fluids such as oils. In addition, the technique may also be utilized in the determination of neutron cross-sections of nuclides with high absorption probabilities, because such nuclides often require diluting in order to avoid self-shielding effects. Where small volumes of water are concerned, the effects of flux perturbation within the sample can generally be neglected.

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Fluorimetric Determination of Sulfur Dioxide as Sulfite

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ONE OF THE MOST commonly used methods for the determination of atmospheric sulfur dioxide is the Schiff reaction between sulfur dioxide, formaldehyde, and pararosaniline as published by West and Gaeke (*1*). These authors demonstrated that the SO_2 could be successfully trapped in a solution of sodium tetrachloromercurate(II) and subsequently reacted with formaldehyde followed by pararosaniline. The method gave a detection limit of 0.05 μg/ml SO_2 in the trapping solution. Nauman *et al.* (*2*) went on to study the Schiff reaction in detail. Huitt and Lodge (*3*) indicated that multiple reactions can occur between the pararosaniline and the formaldehyde–bisulfite complex. Pate *et al.* (*4*) noted that most pararosaniline samples obtained from various manufacturers contained impurities which led to analytical errors. Despite attempts to purify the dye through a butanol extraction procedure, by Scarengelli *et al.* (*5*), and through recrystallization, by King and Pruden (*6*), the basic problems of poor quality reagent and possible nonstoichiometry on the three active sites of the pararosaniline molecule are still present in the analysis.

The idea of using a Schiff reaction for the determination of SO_2 is, nevertheless, still an excellent one. The previously described West–Gaeke method (*1*) was spectrophotometric. Many sensitive analytical procedures, however, involve the use of fluorescence, and in the work described below the Schiff reaction was combined with fluorimetry to produce a more reliable and sensitive SO_2 test. The HCHO–bisulfite complex reacts with a fluorescent molecule to form a nonfluorescent or a weakly fluorescent species. The fluorescent molecule has only one primary amine site and can be obtained in relatively pure form. Also it offers a sensitivity greater than pararosaniline, and experiments indicate that future modifications of the molecule can potentially have an even higher sensitivity toward SO_2.

EXPERIMENTAL

Apparatus. Fluorescence measurements were made with a Perkin-Elmer 203 Spectrofluorometer equipped with a continuous-spectrum, Xenon lamp source. Low-fluorescence quartz cells (1-cm) were used. The excitation wavelength was set at 405 or 460 nm, and the emission was observed at 515 nm. All measurements were made in a 23 °C, air conditioned room.

Reagents. The 5-aminofluorescein was provided by the Eastman Kodak Co. All other chemicals were of reagent grade, and the water used was first passed through a deionizing column and then distilled.

Procedure. A $1.2 \times 10^{-3}M$, 5-aminofluorescein (5AF) (mol wt 347) working solution was prepared by dissolving the appropriate weight of 5AF in 25 ml of absolute methanol in a 100-ml volumetric flask. After the dye has dissolved, 4 ml of concentrated HCl (12.1*M*) was added and the solution was diluted to volume with distilled water. The HCHO solution was made by placing 1.5 ml of 38% HCHO in a 100-ml volumetric flask and diluting to volume with water. The simulated samples of trapped SO_2 were made by dissolving the appropriate weight of $Na_2S_2O_5$ in a solution which was 0.1*M* in $HgCl_2$ and 0.2*M* in NaCl [0.1*M* tetrachloromercurate(II) or TCM]. Subsequent dilutions were made with 0.1*M* TCM, hereafter referred to as simply TCM. Occasionally, where indicated, 0.01*M* TCM was used.

The analysis of the simulated samples was as follows: to the TCM–SO_2 solution, 1 ml of the HCHO solution was added, mixed, and allowed to stand for 5 min, and then 1 ml of the working dye solution was added. The solution was again allowed to stand for a minimum of 20 min, and the fluorescence was measured. The concentration was determined from the working curve. The 0% blank used was TCM and the 100% blank was made from TCM, HCHO, and dye-treated identically with the samples.

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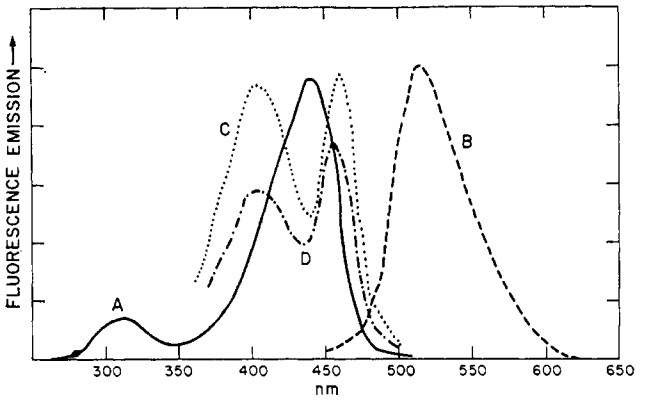


Figure 1. Schematic representation of 5AF excitation and emission spectra in TCM. (Magnitudes of each peak are individual and are not comparable to each other)

A. **$10^{-6}M$ 5AF excitation**
B. **Typical emission spectrum which was the same for all excitation wavelengths**
C. **$10^{-4}M$ 5AF excitation with HCHO but with no HSO_3^- present**
D. **$10^{-4}M$ 5AF excitation after reaction with HCHO and 0.25 × $10^{-4}M$ HSO_3^-**

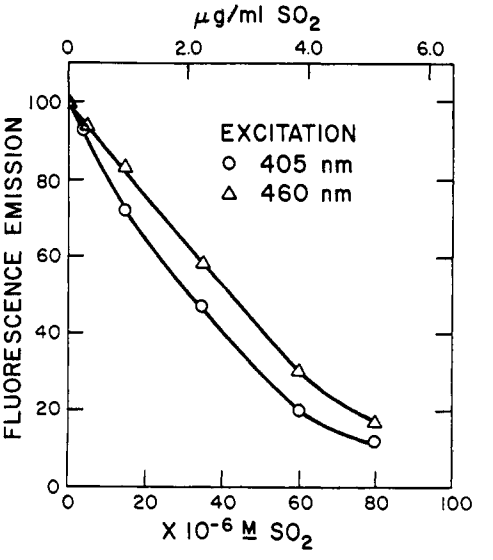


Figure 2. Standardization of $10^{-4}M$ 5AF using different excitation wavelengths, 515 nm emission

RESULTS

Fluorescence Spectra. The excitation and emission spectra for 5AF were obtained point by point with the spectrofluorometer (Figure 1).

The excitation maxima appear to be concentration dependent, since 1 × $10^{-4}M$ 5AF has maxima at 405 nm and 460 nm and, upon dilution to $10^{-5}M$ or $10^{-6}M$, the 405-nm and 460-nm peaks disappear and the 440-nm peak is present. The 515-nm emission maximum was the same regardless of which excitation maximum was used. (For the Xenon source, corrections were not made for variations of light intensity with wavelength.)

Acidity and HCHO Optimization. The optimum acid concentration was determined by varying the HCl concentration in the final solution and measuring the maximum fluorescence suppression per SO_2 concentration. For $10^{-4}M$ dye, the suppression is independent of the acidity from 0.02–0.05M. For 10^{-5} and $10^{-6}M$, the acidity must be 0.05–0.1M. Likewise for the HCHO, using $10^{-4}M$ 5AF, the suppression is independent of the HCHO in concentration of 0.2–0.7%, whereas the 10^{-5} and $10^{-6}M$ 5AF require HCHO concentrations of 0.6–1.5%.

Dye Standardization. All concentrations of 5AF are standardized with various $Na_2S_2O_5$ concentrations. The $10^{-4}M$ 5AF standardization curves are shown in Figure 2. The sensitivity, as can be noted, depends upon the excitation wavelength used. The 405-nm curve is more sensitive but is less linear. Using 5 × $10^{-5}M$ 5AF, the lower limit for determination is approximately 0.02μg/ml SO_2 in TCM.

The 5AF undergoes a visible color change as well as a fluorescence change. The original dye color is yellow, but reaction with HCHO–bisulfite produces an orange-brown color. With reference colors, one can determine approximate SO_2 concentration levels with the naked eye.

Time Dependence and Reproducibility of the Reaction. Solutions were prepared with different concentrations of bisulfite and 5AF, and the fluorescence intensities were measured at various times after the addition of the dye. From Table I, the values appear to be stable after 1 hr of reaction time for the dilute (10^{-5}–$10^{-6}M$) 5AF solutions and 20 min for the more concentrated (0.5–1 × $10^{-4}M$) 5AF solu-

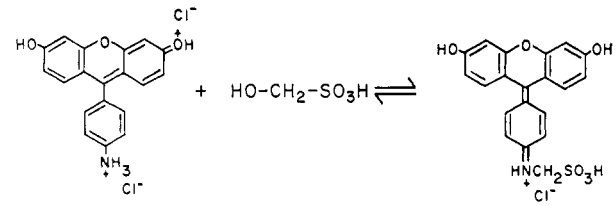
Table I. Reaction Time of 5AF with HSO_3^-, HCHO Fluorescence Emission *vs.* Time[a]

$10^{-4}M$ 5AF *t* min	5[b]		25[b]		50[b]
5	97[c]		69[c]		45[c]
10	95		62		...
15	94		60		32
20	...		58		29
30	94		58		28
45	94		59		27
60	...		57		...
75	95		58		28
105			58		
135			58		

$10^{-5}M$ 5AF *t* min	0.5[b]	1.0[b]	3.0[b]	10[b]	20[b]
15	98[c]	97[c]	89.5[c]	65[c]	44[c]
30	99	96	83.5	55	30
45	99	94	84	52	27
60	97	94	82	51	26
75	98.5	94	84	51	26.5
90	97	94	84	52	27
105	97.5	94	83.5	52	29
120	98.5	95	83.5	53.5	30

$10^{-6}M$ 5AF *t* min	0.1[b]	0.3[b]	1.0[b]	3.0[b]	10.0[b]
15	101[c]	100[c]	97[c]	87[c]	62[c]
30	101	100	95	81.5	49.5
45	101	99.5	93.5	79	44
60	101	99.5	93.5	78.5	42
75	101	99.5	94	78	42
90	101	100	94	78.5	43
120	101	99.5	94	78.5	44

[a] Solutions with no HSO_3^- read 100.
[b] ×$10^{-6}M$ HSO_3^-.
[c] Fluorescence emission.



5-AMINOFLUORESCEIN (FLUORESCENT) (NONFLUORESCENT PRODUCT)

Figure 3. Proposed mechanism of the 5AF, HCHO–bisulfite reaction

tions. Most remain stable within 2% for more than 2 hr. In the dilute 5AF reacted solutions, the fluorescence intensity begins to rise after about 2 hr indicating some decomposition, whereas the more concentrated 5AF reacted solutions remain stable. An attempt was made to decrease the reaction time for the dilute 5AF by placing the samples in a 60 °C water bath, but this experiment was not successful. A 50% water–dioxane solution was also tried without success.

A $10^{-7}M$ 5AF solution, although it fluoresces visibly, did not react with HCHO–bisulfite and the $10^{-6}M$ solution required HCHO–bisulfite concentrations equal to or greater than the dye. Triplicate analysis of simulated SO_2 samples in TCM showed deviations of 0.5% absolute on the 0–100% fluorometer scale.

TCM Concentration. Scarengelli *et al.* (*5*) reported that an increase in sensitivity of the pararosaniline method can result by reducing the concentration of TCM. A similar effect was noted with 5AF, but the sensitivity increased by only 5% when the TCM concentration was lowered from 0.1 to $0.01M$ using $5 \times 10^{-5}M$ 5AF. A 25% sensitivity increase was noted for $10^{-4}M$ 5AF, but the $5 \times 10^{-5}M$ 5AF still remains the more sensitive of the two dye concentrations regardless of the amount of TCM used.

Interferences. Various anions and cations were added to TCM followed by the addition of HCHO and $10^{-4}M$ 5AF. The fluorescence of this solution was compared to a blank and the error noted. The presence of NO_3^-, SO_4^{2-}, H_2O_2, Fe^{2+}, and NH_4^+ in $10^{-2}M$ concentration gave no significant error; K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, $C_2H_3O_2^-$, and NO_2^- at $10^{-2}M$ caused a ±5% error; and a solution of $10^{-3}M$ I^- caused a similar error. Solutions of $10^{-2}M$ Fe^{3+} caused a 45% reduction in the fluorescence whereas $10^{-4}M$ Fe^{3+} reduced the initial intensity by only 2%.

These errors are not significant because they are caused by very high concentrations that are certainly not typical of impurities either in the chemicals or in the trapping solutions. Even the concentration of $10^{-4}M$ Fe^{3+} is a rather high value. Thus, in practicality, the dye does not appear to be affected by other ions. However, this is not meant to conclude that the SO_2 itself is stable in the presence of these various materials.

Nitrogen dioxide has been reported to interfere in the SO_2 analysis with pararosaniline (*1*). Pate *et al.* (*7*) recommended the addition of sulfamic acid to the TCM just prior to analysis. As noted above, NO_2^- in high concentrations reacted minimally with the 5AF. The interference of NO_2 was also investigated; 0.1 ppm NO_2 was bubbled through a $5 \times 10^{-5}M$ HSO_3^-–TCM solution at 2.2 l./min for 30 min. The samples were analyzed for HSO_3^- with and without the addition of sulfamic acid. (For the analysis, 1.25 g of sulfamic acid was added to the HCHO solution.) Table II shows the results of this experiment. The sulfamic acid appeared to lower the sensitivity of the 5AF just as this acid did with pararosaniline (*7*). In addition, the only indication that NO_2 might interfere occurred during the sampling because, in both cases, the bubbled samples had less HSO_3^- than the bench samples. The sulfamic acid did not appear to offer any utility and should not be used.

Table II. NO_2 Interference

	Fluorescence emission, %
5AF—no HSO_3^-	100
Blank—TCM	0
Bench sample, no sulfamic acid	29.5
Bubbled sample, no sulfamic acid[a]	35.5
Bench sample, sulfamic acid	49.5
Bubbled sample, sulfamic acid[a]	55

[a] 0.1 ppm NO_2 bubbled through $5 \times 10^{-5}M$ HSO_3^-–TCM at 2.2 l./min, 30 min.

A possible error to consider is the trapping of any fluorescent organic materials during sampling. This effect was noted in a H_2S fluorescence method (*8*).

DISCUSSION

Mechanism of Reaction. A mechanism parallel to the one presented by Nauman *et al.* (*2*) can be proposed for the 5AF, HCHO–bisulfite reaction (Figure 3). Since the fluorescence in 5AF is derived from the resonance quinone structure in the upper rings and the product is either nonfluorescent or very weakly so, the probable result of the Schiff reaction is the formation of the quinoid structure. This structure shifts the double bond on the central (pyran) carbon atom so that the quinone in the upper ring is no longer the dominant structure and the resulting compound is no longer fluorescent.

Reaction Equilibrium. For low 5AF concentrations (10^{-5}–$10^{-6}M$), the standardization graph is markedly curved, and, therefore, indicates an incomplete reaction. Calculations give for this reaction a formation constant of $K_f = 10^5$ l.-mole^{-1}. This is based upon the assumptions that the product is nonfluorescent and that the reaction is complete after 1 hr.

Although the lower 5AF concentrations show this tendency toward an incomplete reaction, the curvature in the high dye concentration calibration graphs cannot be attributed totally to the K_f since, in contrast to the lower dye concentrations, the 5AF appears to be destroyed in amounts greater than stoichiometric. This is especially indicated by measurements at the 405-nm excitation wavelength. For some fluorescing compounds, Sawicki (*9*) observed the excitation spectra to be dependent upon concentration. He noted that split maxima will occur at higher concentrations of the fluorescing reagent. In Figure 1, curve *C* shows the 405-nm and 460-nm excitation maxima about equal, but after the 5AF has reacted with the HCHO–bisulfite, the 405-nm curve has markedly dropped (curve *D*), the distortion again being due to the changing 5AF concentration. This distortion causes the reaction to appear to be greater than stoichiometric.

The fluorescence of the dye can be measured easily in concentrations of $10^{-7}M$ but the reaction does not occur. Proper

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modification of the dye could result in a 100-fold increase in the sensitivity of this reaction.

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Nonionic Surfactants as Polarographic Maximum Suppressors

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USE OF IONIC surfactants as polarographic maximum suppressors of difficultly suppressible maxima of simple and complex metal ions has been reported (*1–3*). Comprehensive studies, however, have not been done in the case of nonionic surfactants (*4, 5*), although in view of their strong wetting and foaming properties they are expected to act as more effective maximum suppressors. The present communication describes the comparative results of our studies on the effect of Nonidet P40, P42, and Nonex 501 with Triton X-100 on the maxima of a number of simple and complex metal ions and on the electrocapillary curves.

EXPERIMENTAL

Nonionic surfactants—namely Nonidet P40 (100% polyethylene oxide condensate), Nonidet P42 (condensation product of dioctyl phenol and ethylene oxide), and Nonex 501 (methoxy polyethylene glycol laurate) Triton X-100—were all B. D. H. products and were used without further purification. Biuret (*6*) was prepared in the laboratory. Analytical grade reagents and chemically pure reagents were used in all of the investigations. Doubly distilled water (all glass) was used for preparing the solutions.

The procedure used in these investigations is described in detail elsewhere (*5*). All of the measurements were carried out at 25 ± 0.1 °C in a thermostated water bath. A Beckman pH meter model H was used for pH measurements.

For studying the effect of surfactants on the electrocapillary curves, 10.0 ml of 0.1*N* KCl solution was deaerated by bubbling purified nitrogen in the polarographic cell. At least 20 drops were counted and droptime was measured by means of a stop watch. Each set of measurements at a constant potential was repeated three times. The electrocapillary data obtained from 0.0 to −1.2 V (SCE) were repeated in the presence of different concentrations of the surfactant.

RESULTS AND DISCUSSION

The data on the suppression of maxima of both positive and negative types, observed during the reduction of simple and complex metal ions, are summarized in Table I.

Considering the above data, the nonionic surfactants investigated are superior to the anionic surfactants in that a very small amount of the former is required to suppress difficultly suppressible maxima—namely, the Ni^{2+}–Co^{2+} mixture in pyridine and the KCl, CdI_2–KI complex. However, the behavior of nonionic surfactants as maximum suppressors is not different from that of cationic surfactants, although in a few cases, e.g., Ni^{2+}, Co^{2+}, and Pb^{2+}, a smaller quantity of the nonionic surfactant is required. Furthermore, the nonionic surfactants have the extra advantage of not undergoing chemical interaction with the depolarizer. For example, the CdI_2–KI complex maximum is easily suppressed with the nonionic surfactants, whereas the precipitation of the depolarizer takes place on adding cationic surfactants (cetyl trimethyl ammonium bromide and cetyl pyridinium bromide).

The nonionic surfactants differ among themselves in their suppressive action. For example, the amount of Nonidet P40 and Nonex 501 required to suppress the maximum is much lower (order 10^{-3} g/l.). The efficacy of nonionic surfactants as maximum suppressors follows the order:

Nonex 501 > Nonidet P 40 > Nonidet P 42

The amount of nonionic surfactants required to suppress the maximum depends on how far the $E_{1/2}$ is removed from the electrocapillary zero (e.c.z.). Thus for Cu-biuret complex, the amount of the surfactant required for suppressing the maximum is quite small because the $E_{1/2}$ of Cu-biuret complex, −0.50 V, is very close to the e.c.z. value, −0.55 V, in KOH. Likewise, the amount required for the suppression of Pb^{2+} maximum is larger because its $E_{1/2}$, −0.40 V is a little removed from the e.c.z.; U^{6+} with $E_{1/2}$ −0.15 V requires the largest amount for the suppression of its maximum. Similar type of behavior was also found in the case of Triton X-100.

U^{6+} gives reduction waves with pronounced maximum in phosphoric acid and potassium chlorate. Gelatin, even in small quantity (0.001%), distorts the polarographic wave and even changes its nature from the reversible to the irreversible one. Higher concentration of the supporting electrolyte (1*M* $KClO_3$) no doubt suppresses the maximum but in its place a second maximum appears in the polarographic wave at a more negative potential, thereby making the polarographic analysis difficult. On the other hand the use of nonionic surfactants suppresses the maximum of U^{6+} completely without any insidious effect and well defined waves are realized even with dilute solutions of the supporting electrolyte (0.05*M* $KClO_3$).

From the comparative data given in Table I, it is evident that although Triton X-100 is much better than ionic sur-

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