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Optical Detection of Formaldehyde

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ABSTRACT

The potential for buildup of formaldehyde in closed space environments poses a direct health hazard to personnel. The National Aeronautic Space Agency (NASA) has established a maximum permitted concentration of 0.04 ppm for 7 to 180 days for all space craft. Early detection is critical to ensure that formaldehyde levels do not accumulate above these limits. New sensor technologies are needed to enable real time, in situ detection in a compact and reusable form factor. Addressing this need, research into the use of reactive fluorescent dyes which reversibly bind to formaldehyde (liquid or gas) has been conducted to support the development of a formaldehyde sensor. In the presence of formaldehyde the dyes' characteristic fluorescence peaks shift providing the basis for an optical detection. Dye responses to formaldehyde exposure were characterized; demonstrating the optical detection of formaldehyde in under 10 seconds and down to concentrations of 0.5 ppm. To incorporate the dye in an optical sensor device requires a means of containing and manipulating the dye. Multiple form factors using two dissimilar substrates were considered to determine a suitable configuration. A prototype sensor was demonstrated and considerations for a fieldable sensor were presented. This research provides a necessary first step toward the development of a compact, reusable, real time optical formaldehyde sensor suitable for use in the U.S. space program.

Keywords: formaldehyde, fluorescence, NASA, sensor, optical

1. INTRODUCTION

The chemical formaldehyde, CH_3O, occurs through various processes. Naturally occurring formaldehyde is primarily the result of incomplete combustion of carbon containing materials and the effect of sunlight on atmospheric methane and hydrocarbons. Formaldehyde is also produced by and used in many anthropogenic processes including the manufacture of permanent resin adhesives and other industrial chemicals, treatment of textiles, and use as a preservative of disinfectant in many common consumer items[1]. Formaldehyde is even generated in harmless trace amounts by the normal metabolic processes of the human body[2]. Anthropogenic sources pervade homes, offices, hospitals, work environments, automobiles, aircraft, military vehicles of all types, and space vehicles/craft[1,3].

Formaldehyde is a colorless, flammable gas in its natural state having a distinctive pungent odor and presenting a serious health hazard at low concentrations. The cause and effect relationship between short term (acute) exposure is immediately observable and well documented. The effects of long term (chronic) exposure have been indirectly tested (assessment of persons believed to be exposed) in humans and directly tested (induced exposure) in animals. These studies show strong correlations between the various effects and exposure levels[2].

1.1 Applicability to space environments

A spacecraft or space installation is a closed environment using sophisticated air cleaning and recycling technologies to provide and maintain support for human crew. In any such closed environment the detection and removal of toxins is of paramount concern. The National Aeronautic and Space Agency (NASA) has specified that crew may be exposed to no more than 0.04 parts per million (ppm) of formaldehyde over 7 to 180 days[3]. As a point of reference, the National Institute for Occupational Safety and Health (NIOSH) reports that the amount of formaldehyde in the earth's atmosphere from both natural and anthropogenic sources ranges from 0.005 ppm to 0.6 ppm near industrial sources or heavy smog[4].

A full analysis of the air composition in a space environment is presently only available with technologies such as mass spectroscopy which detect the individual ions of the analyte material and thus determines the concentration and composition of the air sample with high accuracy. Size, weight, and other constraints inherent to space travel render the use of such technologies infeasible in today's spacecraft. A small, sensor device capable of detecting toxins such as formaldehyde at low, non-hazardous concentrations, however, would provide sufficient capability to protect crew health;

effectively providing an early warning cue to implement hazard containment and cleaning procedures. Such a device must be at a minimum compact, light weight, reusable, and capable of performing in microgravity.

Current commercial formaldehyde detection technologies can detect concentrations in the range of 0.6 to 1.0 ppm using photoelectric photometry, chemical, and other methods[5]. However, these technologies are designed and intended for use in industrial, earth-based settings and rely on relatively large air sample sizes for analysis. It is suggested by this research that an alterative method combining technologies and using smaller sample sizes is better suited to the rigorous demands and constraints of a space environment.

1.2 Research goal - a formaldehyde sensor for space environments

Taking a cue from the highly sensitive chemical detection technologies available in the bio-chemical community, multiple fluorophor reactive dyes were examined to determine the suitability for a formaldehyde sensor. These reactive dyes, designed for bio-chemical assays, are inherently fluorescent and selectively bond with formaldehyde[6]. After binding with formaldehyde the dyes exhibit a detectable shift in the peak wavelength of their characteristic fluorescence spectrum. This research uses the salient chemical properties of the reactive dyes in combination with optical techniques for fluorescence detection and quantification to demonstrate a formaldehyde sensor with a sensitivity of at least 1 ppm that could be adapted and packaged for space environments.

The sensitivity of three reactive dyes were measured and compared for a target detection capability of at least 1 ppm of formaldehyde. The dyes in aqueous solution were used to determine a baseline sensitivity and other relevant characteristics. To facilitate the incorporation of a reactive dye into a formaldehyde sensor, two substrate materials were selected for exploration.

1.3 Optics and Chemistry of the Dye Reaction

Designed for use with fluorescence microscopy, the reactive dyes provide the key to an optical detection of formaldehyde. As such and as demonstrated in this experiment, the dyes are suitable for fluorescence spectroscopy. When stimulated by an appropriate light source, the fluorophor group of the dye undergoes a fluorescence shift (either Stokes or Anti-Stokes); emitting a characteristic fluorescence. Each of the candidate dyes' amine group binds reversibly with an aldehyde. Taking R to represent the reactive dye, the reaction may be described as follows:

$$R\,(l) + CH_3O\,(g) \rightarrow RCH_3O\,(l), \tag{1}$$

or

$$R\,(l) + CH_3(OH)_2\,(l) \rightarrow RCH_3O\,(l) + H_2O\,(l). \tag{2}$$

The RCH_3O (dye + analyte) product molecule is also fluorescent; exciting and emitting near but not at the characteristic wavelength of the fluorophor dye. This effect is due to a change experienced by the outermost electrons/orbitals of the fluorophor upon binding with the analyte. In the majority of observed reactions, the energy emitted as photons by the product molecule was greater than the energy emitted by the fluorophor dye. The product molecule therefore fluoresced at a lower wavelength than the fluorophor dye because of the fundamental relationship between energy and wavelength

$$E = \frac{hc}{\lambda}, \tag{3}$$

where $h \approx 6.626 \times 10^{-34}\,Js \approx 4.135 \times 10^{-15}\,eVs$ is Planck's constant. The excitation sources used to stimulate the fluorophor dye also excite the product molecule. The observed change in the fluorescence spectrum of the product molecule from the characteristic fluorescence spectrum of the fluorophor dye is readily detectable, is a direct result of binding to the analyte, and therefore provides a basis for an optical detection of the formaldehyde analyte. Collectively, there exist reactive dyes that are optically excited and emit in the visible, ultraviolet (UV), and near infrared wavelengths.

The reactive dyes selected for evaluation in this experiment are: 8-aminopyrene- 1, 3, 6-trisulfonic acid, trisodium salt (APTS); Alexa Fluor® 488 hydroxylamine (AF488); Alexa Fluor® 350 hydroxylamine (AF350). The Alexa Fluor® series of reactive dyes are typically used for protein, nucleic acid, and oligonucleotide labeling[6]. Whereas, the APTS reactive dye is typically used for glycoprotein and general sugar labeling[6]. Each dye ships in solid form and must be rendered in aqueous solution in order to fluoresce. In the solid state, APTS is yellow, AF488 is orange, and AF350 is

white. In aqueous solution of concentrations of at least 1 % by weight APTS is green, AF488 is yellow-green, and AF350 is colorless; aqueous solution concentrations below 1% were not explored in this experiment.

1.4 Substrates

Two commercially available substrates were selected for evaluation: sol-gel and silicone elastomer. Both materials accept dopants and are generally considered not to have a deleterious effect on the chemical properties of the dopants. The sol-gel, also known as a silica aerogel, is a silica glass formed by hydrolyzing a precursor chemical for form the sol. There are many recipes for making sol-gel. In this experiment the sol-gel was formulated in the lab using the tetraethoxysilane (TEOS) precursor. The gelation process begins by mixing the precursor with water and a catalyst. Following hydrolyzation, one of several curing methods is then employed to achieve the desired result. The choice of catalyst affects the resulting structure of the sol-gel with an acidic catalyst producing a linear or randomly branched polymer structure and a basic catalyst producing a tighter, clustered polymer structure. The CV-2500 NuSil® product is a commercially available silicone elastomer based on a methyl silicone polymer. The product comes in a two part kit. To form the elastomer, Part A is mixed with Part B in a 10:1 ratio by weight[7]. Curing of the elastomer may be conducted at room temperature or elevated temperature to speed the process. To avoid damaging the dopants, ambient room temperature curing was employed for both substrates in this experiment.

Several physical aspects of the substrates drive their effectiveness as a host to the reactive dye solution. As the reactive dyes must remain in solution to be used in fluorescence spectroscopy, the removal of water during the substrate curing process is of critical importance. Additionally, surface features, porosity, and sample geometry affect the degree to which an analyte may enter and diffuse in the doped substrate.

2. METHODOLOGY

To assess each of the candidate dyes and substrates for suitability in a future formaldehyde sensor, testing centered on two main activities: determining the sensitivity of the reactive fluorophor dyes and comparing the performance of the doped substrates. To begin the characteristic fluorescence of each dye in aqueous solution was measured. While the manufacturer publishes reference values for the dye's fluorescence peaks, the actual fluorescence peaks observed during testing varied slightly due to factors such as differences in the formulation of the dye solution and differences in the calibration of the manufacturer's and the experimenter's spectrometric sensors. The measured characteristic fluorescence spectrum for each dye is shown in Figure 1.

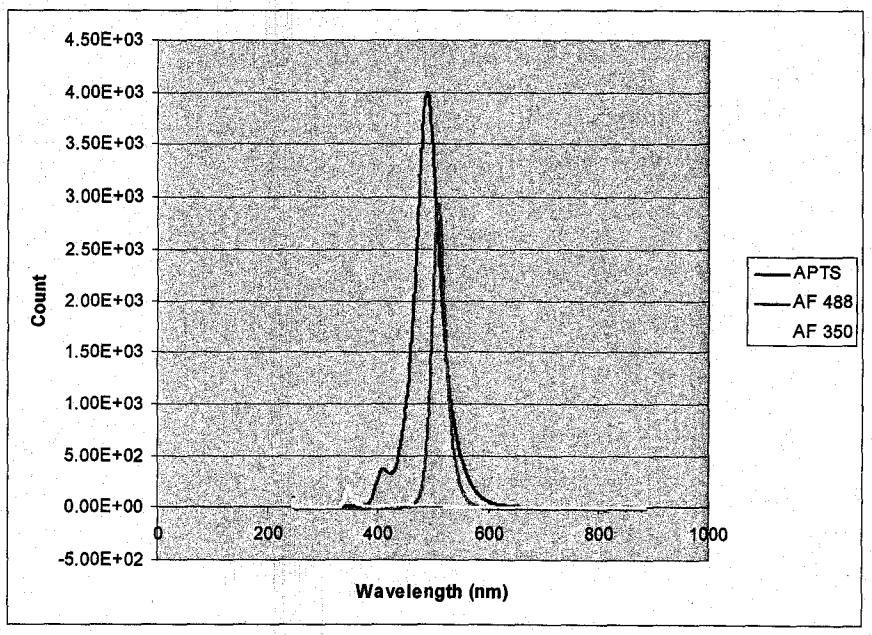

Fig. 1. Characteristic fluorescence of APTS, AF488, and AF350. Peak emission wavelengths: APTS, 490 nm; AF488, 508 nm, AF350, 447 nm.

As is obvious from Figure 1, the magnitude of fluorescence from AF350 is significantly less than that given by APTS and AF488. This is due at least in part to the relative weakness of the UV LED. A suitable alternative UV LED source was not commercially available.

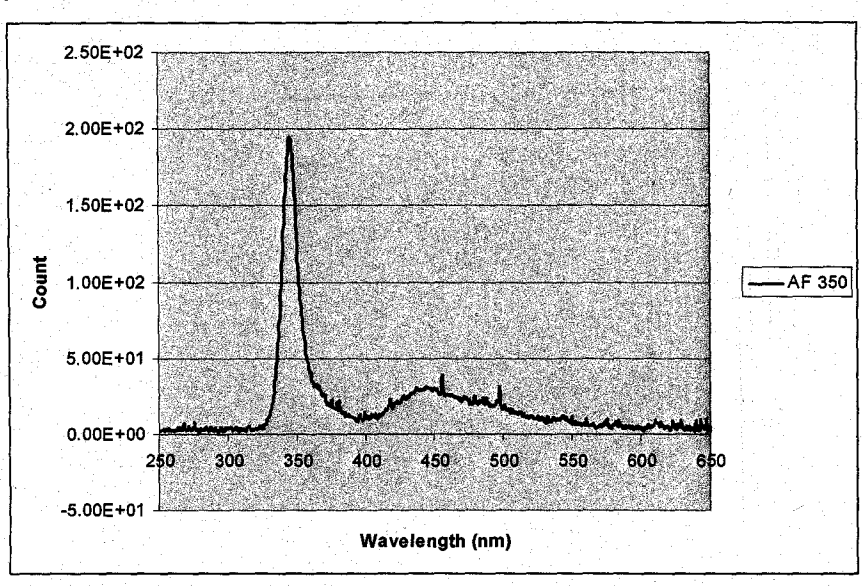

Fig. 2. Characteristic fluorescence of AF350. The large peak near 350 nm is bleed through from the UV source used to stimulate the AF350 solution. The fluorescence peak for AF350 occurs at 447 nm in this graph; outlier values at 456 nm and 497 nm are considered anomalies.

Initial testing of the dye solutions was performed with aqueous formaldehyde solution in varying concentrations. Testing of doped substrates was performed with both aqueous formaldehyde and gaseous formaldehyde. Two primary form factors were evaluated for the doped substrates: a film of the doped substrate applied to a glass microscope slide and an ampoule containing the doped substrate. While a film clearly presents the greatest surface area for reaction it also presents the greatest opportunity to lose needed water. Evaluation of the form factors yielded important insights into the practicalities of packaging for a sensor.

The essential optical components of the sensor (stimulating light source, detector, etc.) were replicated in the experimental setup and may be seen in Figure 3: a stimulating light source, sample, and detector.

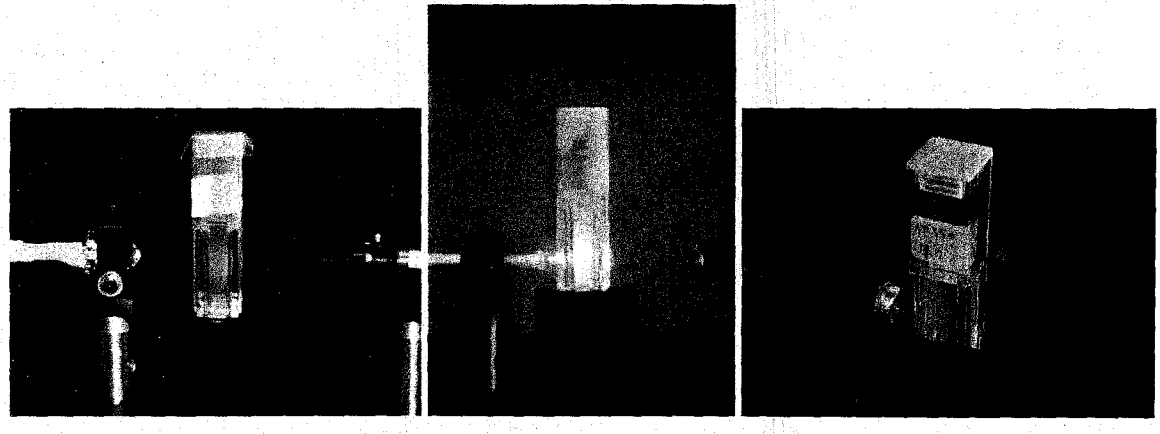

Fig. 3. Picture of characteristic fluorescence of reactive dyes. From the left, APTS stimulated by a violet LED, AF488 stimulated by a blue-green LED, and AF350 stimulated by an UV LED.

Analysis of these test results provides the basis for identifying an optimal configuration of dye, substrate, sample geometry, and the relative concentrations of the chemicals. Using the selected configuration, the remaining components of a sensor may then be assembled into a prototype device for demonstration of the concept and further study.

3. DATA COLLECTION AND ANALYSIS TECHNIQUE

For each reactive dye and substrate multiple samples were generated. Multiple spectra were captured for each test run using the spectrometer's native software, SpectraWiz®. Prior to exposing a sample to formaldehyde, a reference spectrum was captured. After exposure, spectra were captured over a determined time period to evaluate the speed and reversibility of the dye to formaldehyde binding. Samples were subjected to multiple test runs over a period of 12 months. This permitted evaluation of the expected shelf life of the dye. Test data was tabulated and grouped into populations for comparison. Test populations were determined by the type of dye, concentration of dye in the sample, substrate presence, and concentration of formaldehyde used in the test. Analysis of the spectral data was performed graphically and numerically in Microsoft® Excel. For each population the arithmetic mean (average), median, mode, standard deviation and variance of the measured fluorescence peaks were calculated.

Fluorescence spectra were measured with a StellarNet® EPP2000 UV to visible range spectrometer. A fiber optic cable ran from the spectrometer to a small single lens which collected the fluorescence output of the sample. Loss along the lens and cable were negligible for APTS and AF488 but not for AF350. Although the AF350 fluorescence was clearly visible to the naked eye (see Figure 3), it was necessary for the emitted light to be incident directly on the spectrometer aperture and to isolate the system from ambient light to distinguish the weak AF350 signal from noise. An alternate UV enhanced Ocean Optics spectrometer was used in the hopes of improving clarity. However, the results were the same: the luminous intensity of the AF350 was simply insufficient for good measurement with available equipment.

4. RESULTS

To provide a foundation for analysis of test results, the various components of the experiment were individually tested for consistency. The output of the LEDs used to excite the reactive fluorophor dyes and the reactions of those dyes to stimulation were characterized. Both the LED sources and the dyes were sufficiently self consistent with respect to emission peak and wavelength range to pose no detriment to the experiment. Small variations in the characteristic fluorescence peaks were observed and are summarized in Table 1.

Table. 1. Summary of variability of the characteristic fluorescence of the reactive dyes in aqueous solution by dye concentration.

Dye %	Average Emission Peak (nm)	Median	Mode	Standard Deviation	Variance
APTS 2.77%	484.26	487.00	488.00	9.68	93.77
AF 488 2.77%	521.71	522.75	523.75	3.08	9.51
AF 350 2.77%	444.05	444.00	444.00	5.14	26.42
APTS 8.33%	504.79	504.63	504.75	0.81	0.65
AF 488 8.33%	527.94	527.88	527.75	0.21	0.04

A variability was observed in the number of counts (relating to the luminous intensity) measured by the spectrometer. While the intensity variation did not interfere with identifying a formaldehyde detection in a lab environment, they could potentially complicate operations in a future sensor. Variation in intensity was due primarily to the normal variation in LED output intensity and changes in alignment of the optical components between test runs which were performed over a 12 month period.

An examination of the average (arithmetic), median, and mode clearly indicate that the distribution of peak emission values is not Gaussian. Comparing to the manufacturer's reference values for the emission peak (APTS 505 nm, AF 488 519 nm, and AF350 442 nm) one sees that the closest correlation of values occurs for the 8.33% concentration APTS, the 2.77% concentration of AF 488, and the 2.77% concentration of AF 350, respectively. Looking at the standard deviation and variance, AF 488 is more consistent than APTS at each respective concentration. Although, APTS and AF488 are more self consistent at the 8.33% concentration. Due to the poor performance of in early tests AF350 was not formulated at the 8.33% concentration. A similar evaluation was performed for the LED excitation sources which is summarized in Table 2.

Table. 2. Summary of variability of the characteristic emission spectrum of the LED excitation sources. When duplicate values are not present there is no mode; denoted by "N/A".

LED	Average Emission Peak (nm)	Median	Mode*	Standard Deviation	Variance
Violet	425.83	426.00	426.00	0.24	0.06
Blue-green	504.13	506	N/A	3.86	14.92
UV	364.40	363.50	363.50	1.20	1.44

The output from the UV and violet LED's are very consistent, while the blue-green LED's have a larger standard deviation and variance (due to one atypical LED that was not used). It should be noted that the violet and blue-green LED's are classified as bright (i.e., at least 5 mW output) while the UV LED's provide nominally 0.5 mW output. The spectrometer's integration time parameter was used to maximize the number of counts without saturation; ensuring a sufficient number of counts from a LED was received by the spectrometer to obtain a measurement.

As is shown in the following sections the APTS and AF 488 gave clear detections of formaldehyde at all tested concentrations. The AF 350 fluorescence was too weak to provide a clear detection and was therefore excluded from further testing. The majority of fluorescence shifts observed were Anti-Stokes. The other tests resulted in either Stokes shifting of the measured fluorescence or a mixture of Anti-Stokes and Stokes shifts.

4.1 Testing with Aqueous Dye Solutions

Prior to testing with gaseous formaldehyde the sensitivity of the reactive fluorophor dyes were examined using various concentrations of aqueous formaldehyde. The concentration of dye in the samples was varied to determine appropriate limits on detectable reactions. The testing of 1%, 0.1%, 0.01%, and 0.001% aqueous formaldehyde showed a progression of the dye sensitivity from an assumed capability, based on suitability of the dye to its intended commercial use, below the experimental objective range of 1 ppm.

The reaction of the samples to the introduction of formaldehyde was immediately observable on the spectrometer software display; within the time span in which the computer/software refreshed and therefore well within the 10 second objective limit. Spectral data was measured pre-test and subsequently at intervals of 30 seconds from 30 to 180 seconds for each test run. During tests, the sample containers contain more dye than formaldehyde; only those dye molecules bound to formaldehyde experience a shift in fluorescence and hence some characteristic fluorescence is still present in the optical signal. To remove the effects of this residual characteristic fluorescence the pre-test signal is subtracted from the test signal as background noise. Representative test data for APTS and AF 488 are shown. Figure 4 depicts the reacted spectrum for test run number 44: 2.77% AF 488 dye solution exposed to 0.01% formaldehyde solution. The intensity of the pre-test fluorescence is on the order of 3.65 X 103 counts while the reacted fluorescence is on the order of 2.81 x 102 counts. For comparison, the peak wavelength of the pre-test fluorescence is approximately 525 nm and the average reacted peak is 517 nm.

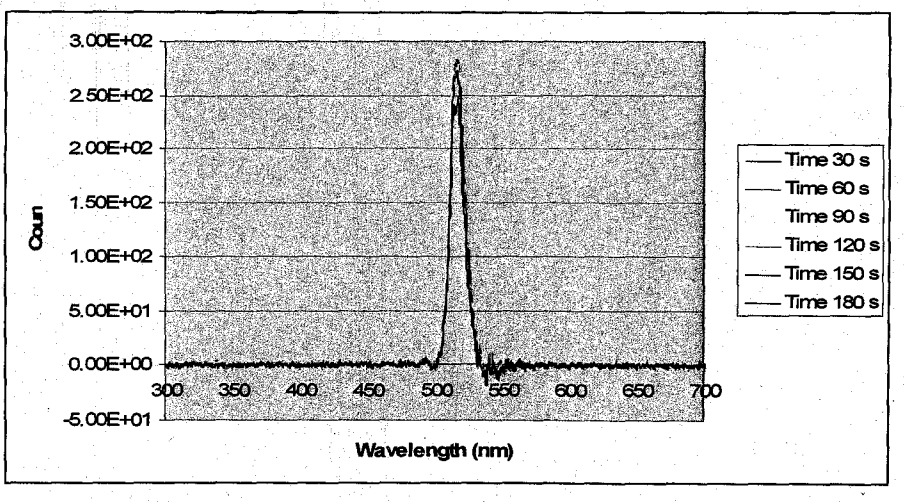

Fig. 4. Graph of measured fluorescence: test run 44, 2.77% AF 488.

The change in peak and number of counts constitutes a clear reaction to the presence of formaldehyde. Tables 3 and 4 summarize the quantities calculated to compare the shifted fluorescence peaks for the AF 488 dye solution populations.

Table. 3. Summary of variability of the shifted fluorescence spectrum of AF 488 2.77%.

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
7	515.83	515.50	515.50	1.13	1.27
10	516.18	516.00	515.00	0.97	0.95
14	514.71	514.50	514.50	0.83	0.68
16	512.74	510.50	509.50	4.70	22.11
20	513.72	513.75	512.50	1.02	1.03
22	511.67	511.00	510.50	2.23	4.99
26	514.24	515.00	515.00	0.72	0.52
28	510.71	511.00	511.50	1.00	1.00
29	514.58	514.50	514.00	0.75	0.56
31	510.17	510.00	509.50	0.67	0.45

Table. 4. Summary of variability of the shifted fluorescence spectrum of AF 8.33%.

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
15	523.24	520.50	520.50	4.27	18.21
21	520.81	520.50	520.00	0.76	0.58
27	521.00	521.25	522.00	1.08	1.17
30	520.81	521.00	521.50	0.67	0.45

The AF 488 dye exhibited a low amount of variation within populations for both concentrations. For each concentration the average peak was reasonably consistent between populations. Figure 5 depicts the reacted spectrum for test run number 39: consisted of 2.77% APTS exposed to 0.01% formaldehyde. The peaks of the pre-test and reacted spectrum are at 489 nm (at 3.78×10^3 counts) and 458 nm on average (at 1.91×10^2 counts), respectively.

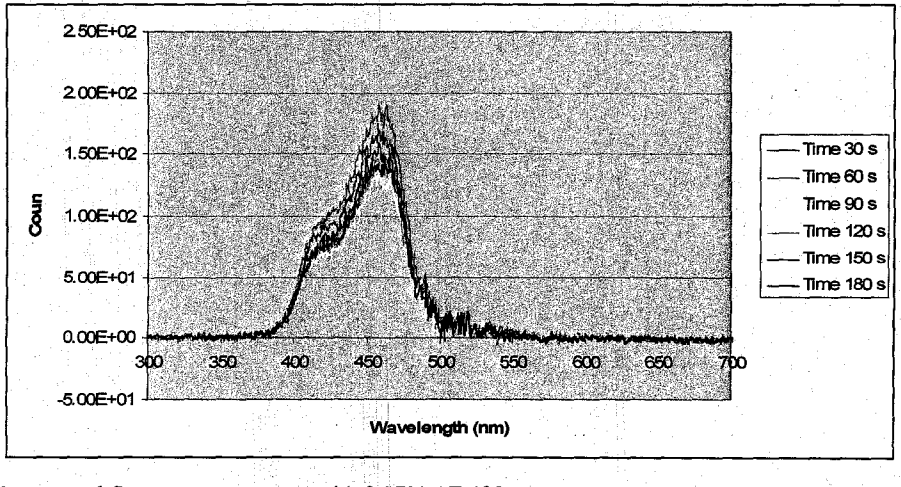

Fig. 5. Graph of measured fluorescence: test run 44, 2.77% AF 488.

As with AF 488, the change in peak and counts constituted a clear reaction to the presence of formaldehyde. Tables 5 and 6 summarize the quantities calculated to compare the shifted fluorescence peaks for the APTS dye solution populations.

Table. 5. Summary of variability of the shifted fluorescence spectrum of APTS 2.77%.

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
3	470.31	469.50	467.00	3.38	11.44
6	461.89	461.50	463.50	3.71	13.79
9	462.13	461.75	480.00	10.25	105.05
11	455.60	455.75	456.00	2.26	5.10
13	425.36	421.50	418.00	8.18	66.84
17	452.23	446.00	446.00	7.24	52.48
19	441.59	443.25	443.50	6.54	42.83
23	416.26	408.00	407.50	19.52	380.92
25	416.79	416.25	414.50	3.37	11.37

Table. 6. Summary of variability of the shifted fluorescence spectrum of APTS 8.33%.

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
12	504.54	504.50	504.50	0.17	0.03
18	492.58	492.75	493.00	0.45	0.20
24	494.21	494.00	492.00	2.38	5.66

4.2 Testing with Doped Substrates

Multiple samples were generated with each substrate at varying concentrations of the APTS and AF 488 dye solution. After curing, it was obvious that the silicone elastomer had expelled the majority of the water in the dye solution rendering the dyes unable to fluoresce. Thin films made with the doped elastomer completely dehydrated due to the hydrophobic nature of the elastomer: all water was expelled from the elastomer and then able to evaporate. Ampoules made with the doped elastomer exhibited surface dehydration of the dye and isolated droplets of dye solution. Attempts to induce fluorescence in the droplets failed. The silicone elastomer was therefore excluded from testing with aqueous and gaseous formaldehyde. Sol-gel samples were made using a modified Stöber method with ethyl alcohol as a solvent and ammonia as a catalyst. The doped sol-gel in thin film also completely dehydrated; however, this was due to the porous nature of the sol-gel rather than hydrophobia. The water content of doped sol-gel in ampoules was readily controlled. Some water was expelled during gelation; however, sufficient water remained to keep the dyes in solution and permit fluorescence. Samples of doped sol-gel were generated for test with aqueous and gaseous formaldehyde at various concentrations of sol and dye (Figure 6).

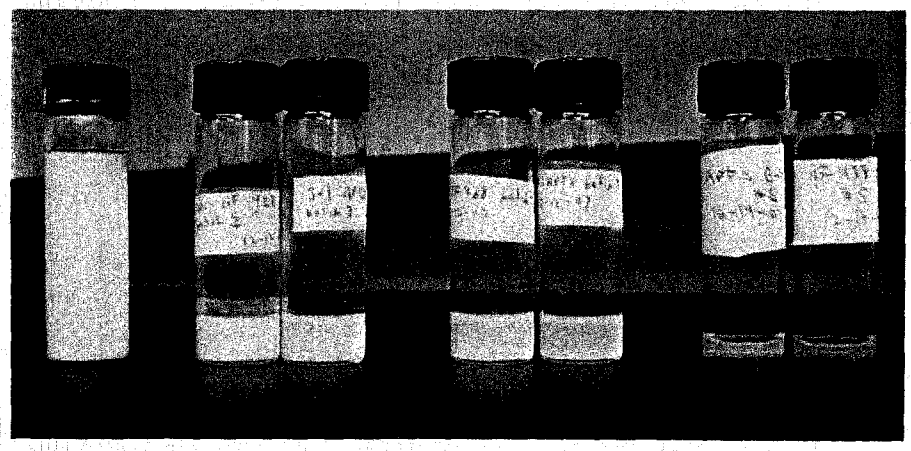

Fig. 6. Doped Sol-gel. From left to right: undoped sol-gel, AF 488 (0.9% dye), APTS batch (0.9% dye), AF 488 (1.9% dye), APTS (1.9% dye), APTS (1.38% dye), and AF 488 (1.38% dye).

Spectral data was measured and assessed using the same methods as the dye solution tests with the exception that the pre-test fluorescence was not subtracted from the measured test fluorescence as it was anticipated that the peaks and intensities would be close with such low concentrations of formaldehyde. Testing of the doped sol-gel with aqueous formaldehyde was performed with 0.1%, 0.01%, and 0.001% concentrations. Reactions were observed at all concentrations demonstrating detection to 0.5 ppm. Tables 7 and 8 summarize the measured data.

Table. 7. Summary of variability of the shifted fluorescence spectrum of APTS doped sol-gel (all concentrations).

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
38	497.21	495.25	494.50	4.25	18.06
39	494.67	495.00	497.00	2.72	7.38
40	496.13	494.75	493.50	15.49	239.96
41	511.67	511.25	520.50	8.10	65.68
42	490.17	491.00	491.50	1.77	3.14
43	487.96	488.50	488.50	0.88	0.77
48	473.58	473.25	458.00	12.96	168.08

Table. 8. Summary of variability of the shifted fluorescence spectrum of AF 488 doped sol-gel (all concentrations).

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
32	510.00	511.50	513.50	4.61	21.25
33	516.58	516.00	516.00	1.77	3.12
34	512.42	508.50	504.50	8.38	70.20
35	513.08	514.50	521.00	7.53	56.66
36	501.13	502.00	502.50	2.41	5.80
37	505.50	503.50	503.50	8.02	64.31
44	478.56	486.25	486.50	12.19	148.50
45	513.71	513.25	513.50	2.02	4.06
46	513.04	508.25	500.00	12.53	157.10

Several of the test populations experienced outlier data causing large values for standard deviation and variance. In particular, populations 40, 44, 46, and 48 have extreme variances. While previous tests with the dye solution have shown that some anomalous data will occur, it appears from the doped sol-gel data that incorporating the reactive dye into a sol-gel process affects dye performance. This may be due to pH sensitivities, dye bleaching, or possibly damage to the fluorophor during the process of making the sol-gel samples.

The formulation of doped sol-gel that yielded the most consistent detections was used for gaseous formaldehyde tests. Two gas sources were utilized for testing: gas calibration tube producing an ambient concentration of 0.08 ppm and evaporation of aqueous formaldehyde producing an ambient concentration of 0.5 ppm in the test chamber. Table 9 provides a summary of the data.

Table. 9. Summary of variability of the shifted fluorescence spectrum of doped sol-gel (all concentrations). Populations 49 and 52 are APTS (1.38%). Populations 50 and 51 are AF 488 (1.38%).

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
49	488.00	488.00	488.00	0.00	0.00
50	517.34	515.75	515.50	3.16	10.01
51	429.67	430.75	431.50	2.25	5.06
52	520.75	521.00	522.50	1.63	2.65

Figure 7 depicts the reacted spectrum for test run number 181: 1.38% AF 488/doped sol-gel to 0.5 ppm formaldehyde gas (evaporation). The pre-test fluorescence peak is 520.5 nm at 6.87 x 10^2 counts and the reacted fluorescence is on average 519 nm at 4.60 x 10^2 counts.

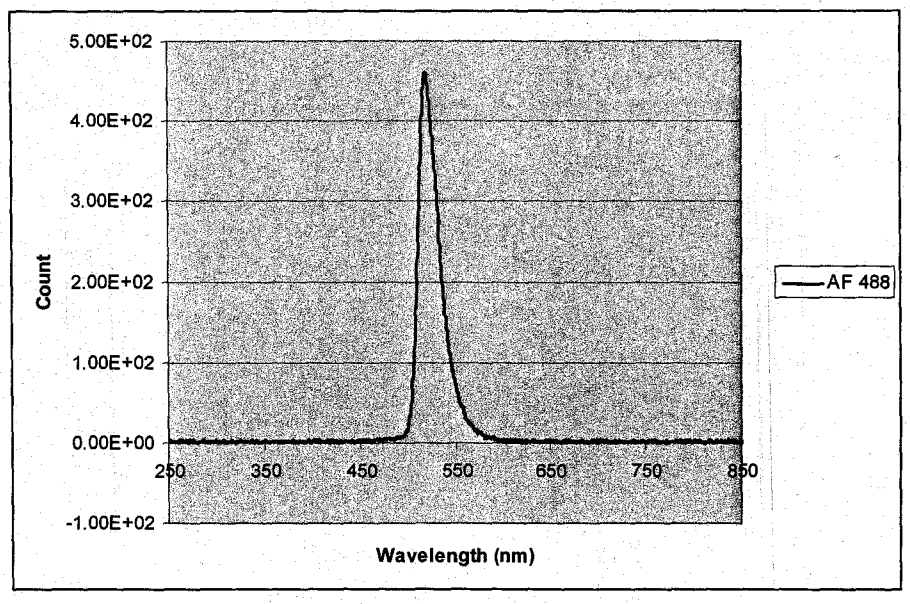

Fig. 7. Graph of measured fluorescence: test run 181, 1.38% AF 488 in sol-gel.

For comparison, Figure 8 depicts test run 173: 1.38% APTS/doped sol-gel to 0.08 ppm formaldehyde gas (calibration tube). The pre-test fluorescence peak is 487 nm at 2.03 x 10^3 counts and the reacted fluorescence is on average 488 nm at 3.47 x 10^2 counts.

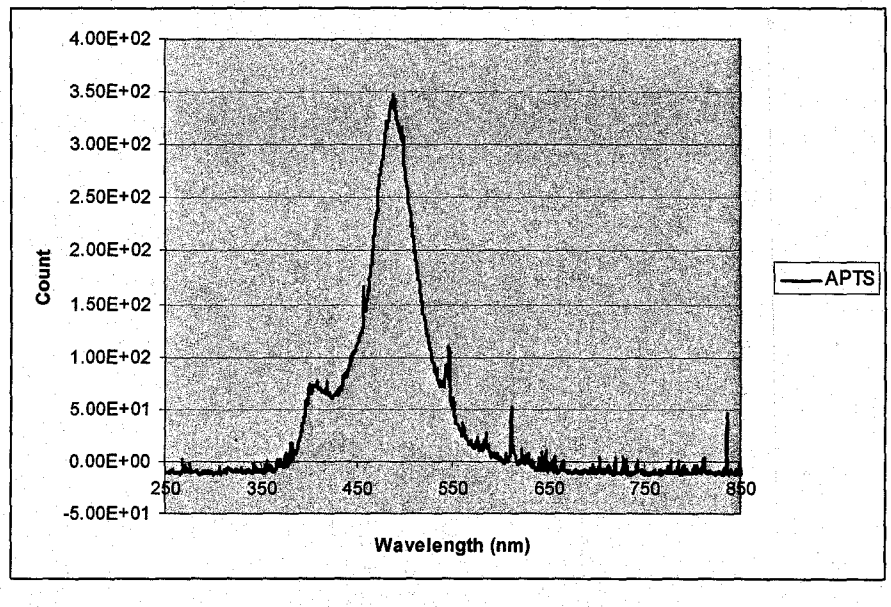

Fig. 8. Graph of measured fluorescence: test run 173, 1.38% APTS in sol-gel.

4.3 Discussion – Prototype Sensor Design

The general criteria for a space environment formaldehyde sensor may be ascertained in terms of basic constraints on the design: a self contained, handheld, and reusable device that can be certified for a space environment. By contrast, the criteria for a prototype/proof of principle device are considerably less rigorous. To demonstrate the feasibility of an

optical formaldehyde sensor, a prototype must both replicate the lab tests and give a clear path toward a future fieldable sensor. The optics of such a prototype are simple. At issue is the containment and/or packaging of the testing chemicals; namely, the reactive dyes. This was the primary motivation behind substrate testing. Aside from the requirement to detect formaldehyde, the physical form and components for an initial prototype are essentially unconstrained. Size did not pose an issue as commercially available optical components were readily available. For the assembled prototype it was not possible to construct a self contained device prior to publication (i.e., one that measured and analyzed all in one package). Rather a bench-top version of the sensor was constructed for testing and an external computer used to analyze captured spectrum data. The components of a future, handheld prototype are shown in Figure 9.

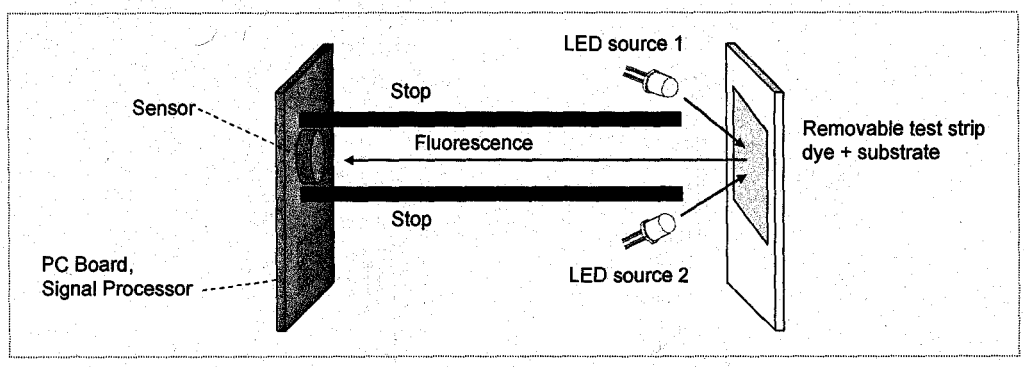

Fig. 9. Handheld prototype components.

The inclusion of two LED sources would permit either APTS or AF 488 test strips to be used. The use of a surfactant coating applied over a doped sol-gel film would retain the water in the sample while still permitting diffusion of the analyte into the sample. Alternatively, small ampoules of doped sol-gel or aqueous dye solution could be used in conjunction with an injection system that would deliver the analyte to the test sample. The detection ability and manufacture cost of each configuration would need to be explored.

5. CONCLUSIONS

Central to this experiment was merging existing technologies in a new way to demonstrate the feasibility of an optical formaldehyde sensor. Such a sensor has significant potential to serve the space industry by detecting formaldehyde before it reaches a hazardous level. The general problem of detecting chemical agents has broader implications. Beyond the clearly understandable difficulties of supporting human life in a space environment, there are also earth-based environments in which formaldehyde and similar hazards accrue and which could benefit from the development of a sensor as postulated in this experiment. This experiment was the first step toward such a sensor device; demonstrating the detection of formaldehyde in gas and liquid form below the objective level of 1 ppm and showing the ability to reach levels on the scale of 0.08 ppm.

The preliminary dye solution tests provided key insight into the design of the substrate tests. Both APTS and AF 488 performed as expected and produced clear detections. The AF 350 dye showed potential provided that a sufficient excitation source could be obtained either by a frequency doubling technique of a bright LED or through the use of a laser diode. The reversibility of the dyes' reactions was confirmed through successful sample reuse over a 12 month period. While the dyes did experience some degradation in performance, they remained sufficiently stable to provide detections over this time frame suggesting that with proper handling the dyes could have a usable shelf life of up to 12 months. Tests with doped sol-gel demonstrated the ability to detect formaldehyde in aqueous form down to 0.5 ppm and in gaseous form down to 0.08 ppm. This exceeded the objective mark of 1 ppm detection and suggests that the NASA threshold of 0.04 ppm is achievable using this method.

Armed with the test results, one may address the question of which of the dyes performed best. The criteria for evaluating the dyes may be summarized as: consistency of performance; strength of reaction; stability of the dye over time. Each of these criteria serve the ultimate goal of developing a reliable sensor device for the detection of formaldehyde at low concentrations. In the dye solution tests, AF 488 proved more consistent than APTS, APTS showed a larger shift than AF 488, and both performed the same over time. The tie breaker in this case is the fact that, while APTS had a larger shift, the AF 488 shift was easily detected and sufficiently strong to provide a clear cut indication that formaldehyde was present. In the doped substrate tests, APTS was numerically more consistent than AF

488, AF 488 provided a larger shift than APTS, and both showed the same performance over time. As with the dye solution test, the sol doped with AF 488 dye edged out APTS. Since the performance of each dye was similar and the dyes emit in the green wavelengths, it would be reasonable to consider using both dyes simultaneously in a sensor device. Such a tactic is unlikely to increase the size and cost of a device significantly and would provide essentially two tests runs at a time thereby reducing the possibility of a detection error.

The successful use of the bench-top prototype demonstrated that an optical formaldehyde sensor was feasible. The lessons learned from the experiment and prototype have led to considerations for further development. Being able to detect formaldehyde with this technique, it remains to establish a spectral response correlation curve for one or both of the dyes. The correlation curve will assess the amount of formaldehyde in the analyte and significantly enhance the utility of the sensor. While such correlation may be calculated in part with existing data, it relies on the intensity of emitted fluorescence. As was discussed, the intensity may vary with several factors, including: the formulation of the sample and concentration of dye, the intensity of excitation source, and degree to which the dye has degraded (dye age). Further testing is needed to clearly establish limits on the dye reaction that can be interpreted for both presence and concentration of formaldehyde.

REFERENCES

[1] Occupational Health and Safety Administration, "Regulations (Preambles to Final Rules): Section 3 - III. Properties, Manufacture, and Uses of Formaldehyde," 1992. http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=PREAMBLES&p_id=923

[2] Department of Health and Human Services: Agency for Toxic Substances and Disease Registry, "Toxicological Profile for Formaldehyde," pp. 9-182, 1999. http://www.atsdr.cdc.gov/toxprofiles/tp111.html

[3] JSC, Toxicology Group, "Spacecraft Maximum Allowable Concentrations for Airborne Contaminants," National Aeronautics and Space Administration JSC 20584, 1999. http://hefd.jsc.nasa.gov/files/SMACSDB.pdf

[4] NIOSH, "Current Intelligence Bulletin 34," 1981. http://www.cdc.gov/niosh/81111_34.html

[5] RKI Instruments. Inc. "Product information webpage: Formaldehyde Gas Detector", 2007. http://www.formaldehydedetector.com/index.html?gclid=CMaqgfG0m44CFSCTWAodxmNjZA

[6] R. Haugland, et al. *The Handbook: A Guide to Fluorescent Probes and Labeling Technologies, 10th Edition.* Invitrogen/Molecular Probes, Inc, 2005.

[7] NuSil Technology, LLC. "Product Documentation for Controlled Volatility Silicone Elastomer CV-2500" 2006.

MSFC -748- PRESENTATION

Optical Detection of Formaldehyde

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SPIE Defense and Security 2008

June 11, 2005: Tropical Storm Arlene, seen from the International Space Station within two hours of landfall near Gulf Shores, AL. (NASA/JSC)

Introduction

Research objective: demonstrate a method for optical detection of formaldehyde at a concentration of 1 ppm

- Primary interest is early detection to protect crew from dangerous exposure levels
- NASA has established a maximum permitted concentration of 0.04 ppm long term exposure in all space craft
- Spacecraft and space installations
  - Closed environments with potential for accumulation of formaldehyde
  - Use sophisticated air cleaning and recycling technologies
  - Detailed *in situ* air analysis currently unavailable
- Solution method incorporates fluorescent reactive dyes
  - Dyes bind selectively with formaldehyde
  - Dye fluorescence spectrum shifts after binding
- Prototyping to develop considerations for a formaldehyde sensor

Source of the Problem

- Formaldehyde sources
  - Natural and manmade
  - Primarily via incomplete combustion of carbon containing materials
  - Manmade sources include resin adhesives, textile treatments, preservatives and disinfectants
  - Present in homes, offices, hospitals, automobiles, aircraft, and military and space vehicles
- The National Institute for Occupational Health and Safety (NIOSH)
  - 0.0005 ppm in typical environments (e.g., homes, outdoors)
  - 0.6 ppm near industrial sources or heavy smog
- Primarily a problem for environments in which formaldehyde can accumulate
  - Contain multiple/strong formaldehyde sources
  - Are unable to exchange or vent air

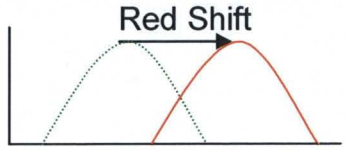
Effects of Formaldehyde Exposure

- Studied in humans and animals
  - Short term
  - Long term
- Effects depend on the concentration of formaldehyde
- Short term exposure
  - Low concentration (up to 3 ppm) causes ocular and respiratory tract irritation
  - Mid concentration (10 to 20 ppm) causes severe respiratory effects and heart palpitations
  - High concentration (over 50 ppm) causes pulmonary edema
  - Above 100 ppm is lethal
- Long term exposure
  - Low concentration (0.4 ppm) causes ocular and respiratory tract irritation
  - Mid concentration (14 to 20 ppm) causes cancer of the respiratory tract
  - Above 40 ppm is lethal

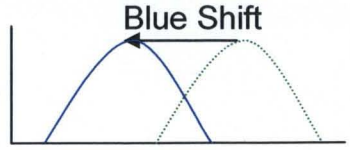
Optics and Chemistry Basis

- Reactive dyes are designed primarily for use with fluorescence microscopy and are also suited for fluorescence spectroscopy
- Three reactive dyes were evaluated
  - 8-aminopyrene- 1, 3, 6-trisulfonic acid, trisodium salt (APTS)
  - Alexa Fluor® 350 hydroxylamine (AF 350)
  - Alexa Fluor® 488 hydroxylamine (AF 488)
- Dye Reaction
  - R representing the dye
  - Dye amine group binds with formaldheyde
    - Formaldehyde gas $R\ (l) + CH_3O\ (g) \rightarrow RCH_3O\ (l)$
    - Aqueous formaldehyde $R\ (l) + CH_3(OH)_2\ (l) \rightarrow RCH_3O\ (l) + H_2O\ (l)$
- After binding with formaldehyde the fluorescence peak shifts (Stokes or Anti-Stokes)

Stokes



Anti-Stokes



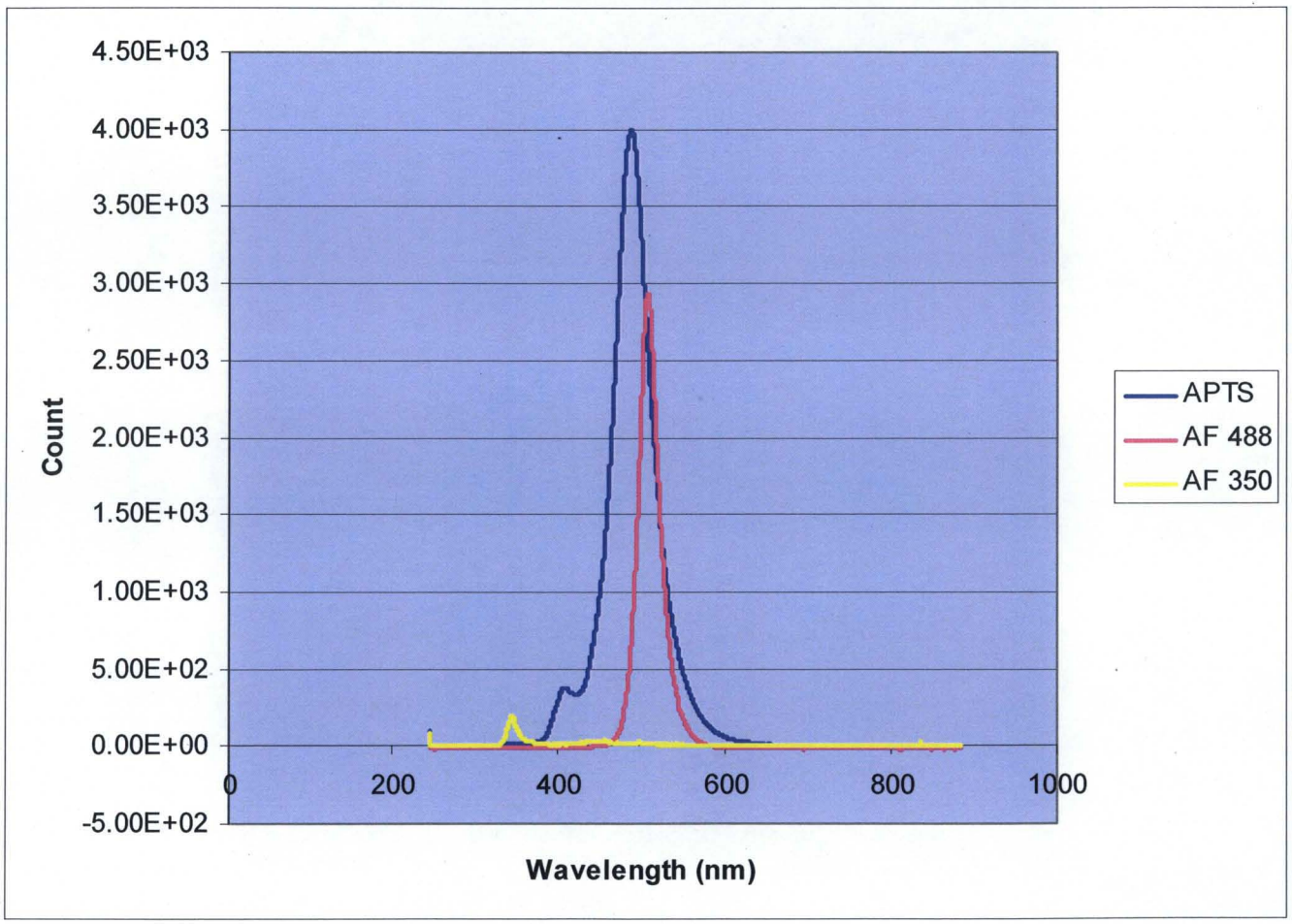
Reactive Dyes

- Dyes reversibly bind with formaldehyde
- Dye excitation (dye in aqueous solution)
  - APTS near 424 nm
  - AF 488 near 495 nm
  - AF 350 near 346 nm
- Dye characteristic fluorescence peak (dye in aqueous solution)
  - APTS near 490 nm
  - AF 488 near 508 nm
  - AF 350 near 447 nm
- Dye concentration and solvent affect the fluorescence peak observed

Dye %	Average Emission Peak (nm)	Median	Mode	Standard Deviation	Variance
APTS 2.77%	484.26	487.00	488.00	9.68	93.77
AF 488 2.77%	521.71	522.75	523.75	3.08	9.51
AF 350 2.77%	444.05	444.00	444.00	5.14	26.42
APTS 8.33%	504.79	504.63	504.75	0.81	0.65
AF 488 8.33%	527.94	527.88	527.75	0.21	0.04

Reactive Dyes

- Measured characteristic fluorescence of APTS, AF 488, and AF 350



Substrates

- A substrate is needed to facilitate the inclusion of the reactive dyes into a sensor
  - Contain and protect the dye
  - Provide convenient access to the dye when needed
  - Form factors considered
    - Film
    - Ampoule
- Two commercially available substrates were evaluated
  - Sol-gel using the precursor tetraethoxysilane (TEOS)
  - NuSIL® silicone elastomer
  - Dye added as dopant to substrate
  - Performance of substrates can be affected by
    - Surface features
    - Porosity in terms of diffusion out of and into the substrate
    - Sample geometry (e.g., surface area)
- A bench top prototype was assembled for testing

Methodology

- Dyes were stimulated with commercially available LEDs
  - Violet/indigo, peak 424 nm
  - Blue-green, peak 495 nm
  - UV, peak 346 nm
- Consistency of LED output was tested

LED	Average Emission Peak (nm)	Median	Mode*	Standard Deviation	Variance
Violet	425.83	426.00	426.00	0.24	0.06
Blue-green	504.13	506	N/A	3.86	14.92
UV	364.40	363.50	363.50	1.20	1.44

- Initial tests of the dyes in aqueous solution were performed to
  - Determine sensitivity to formaldehyde
    - Concentrations of 1%, 0.1%, 0.01% and 0.001%
  - Determine the optimal concentration of dye in the sample
- Substrates were doped with the reactive dyes and tested
- Sol-gel was custom made using a modified Stöber method
- Silicone elastomer was mixed per manufacturer's instructions

Data Collection and Analysis

- For each dye and substrate multiple samples were generated and tested
- Spectra was captured for each sample over time
  - Pretest spectrum as a baseline
  - 30 to 180 seconds in 30 second intervals after exposure
- Tests were grouped into test populations for analysis
  - Dye type and concentration
  - Substrate
  - Formaldehyde concentration and state (gas, liquid)
- Spectra were analyzed in MS Excel®
  - Measured peak
  - Calculation of shift
  - Population calculations
    - Arithmetic average
    - Median
    - Mode
    - Standard Deviation
    - Variance

General Results

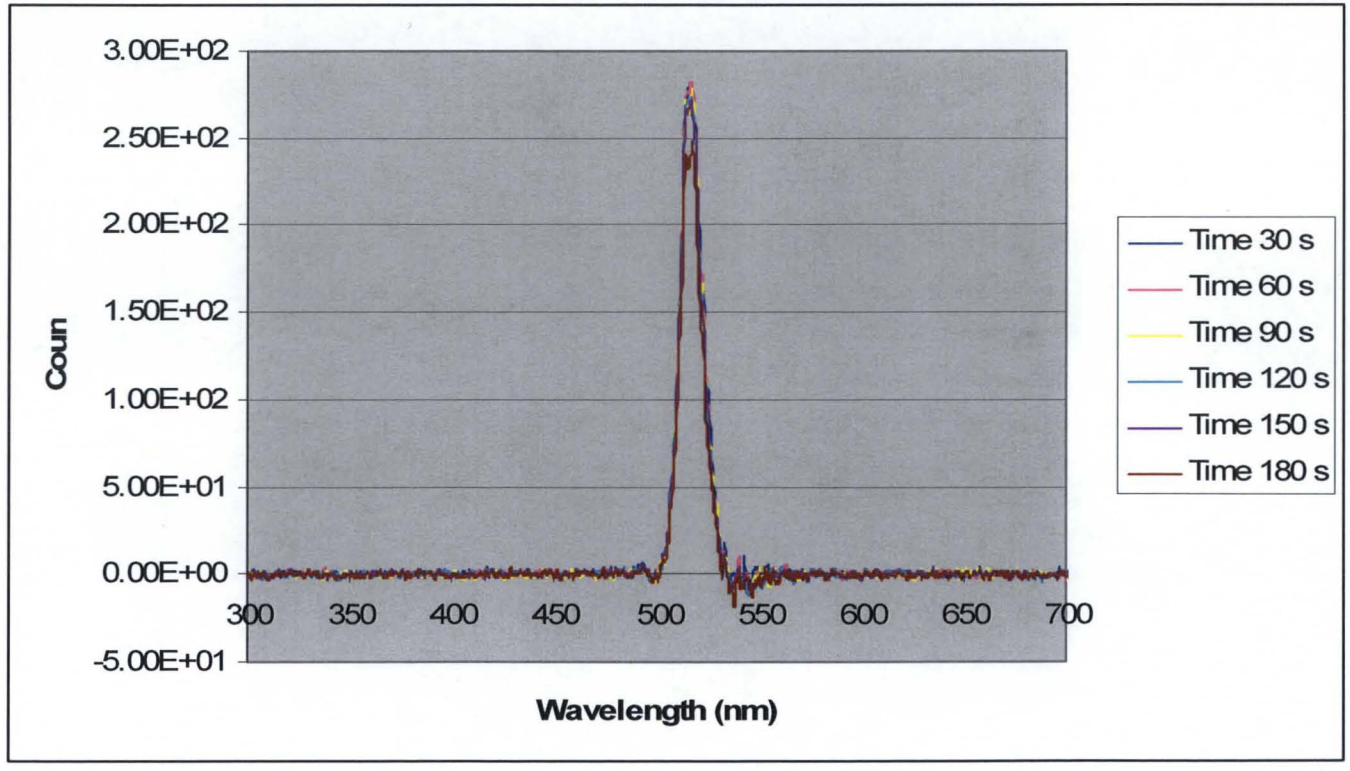
- APTS and AF 488 gave clear detections below objective of 1 ppm
- AF 350 fluorescence was too weak to provide a clear detection
- Measured emission was primarily Anti-Stokes
  - Some Stokes emission was observed
  - Some samples yielded both Stokes and Anti-Stokes emissions
- Dyes must remain in solution to fluoresce
  - Excessive evaporation rendered films impractical without the use of a surfactant to preserve moisture
  - Ampoules avoided this problem
- Doped sol-gel performed consistent with dye solution tests
- Doped silicone elastomer failed to perform
  - Hydrophobic elastomer expelled or isolated the dye solution leaving only dry dye on the surface and isolated beads of dye solution in the interior
- Over time dye performance degrades
  - Process was slow in aqueous solution and faster in substrate
- Detections are clearest when the ratio of dye molecule receptors to formaldehyde molecules approaches a 1 to 1 ratio

Dye Solution Results

- Dye solution tests were performed with aqueous formaldehyde
- Aqueous dye solutions detected formaldehyde at all concentrations
  - 0.001% corresponding to 0.5 ppm concentration
- Example 1: Test 44, 2.77% AF 488 dye solution exposed to 0.01% formaldehyde

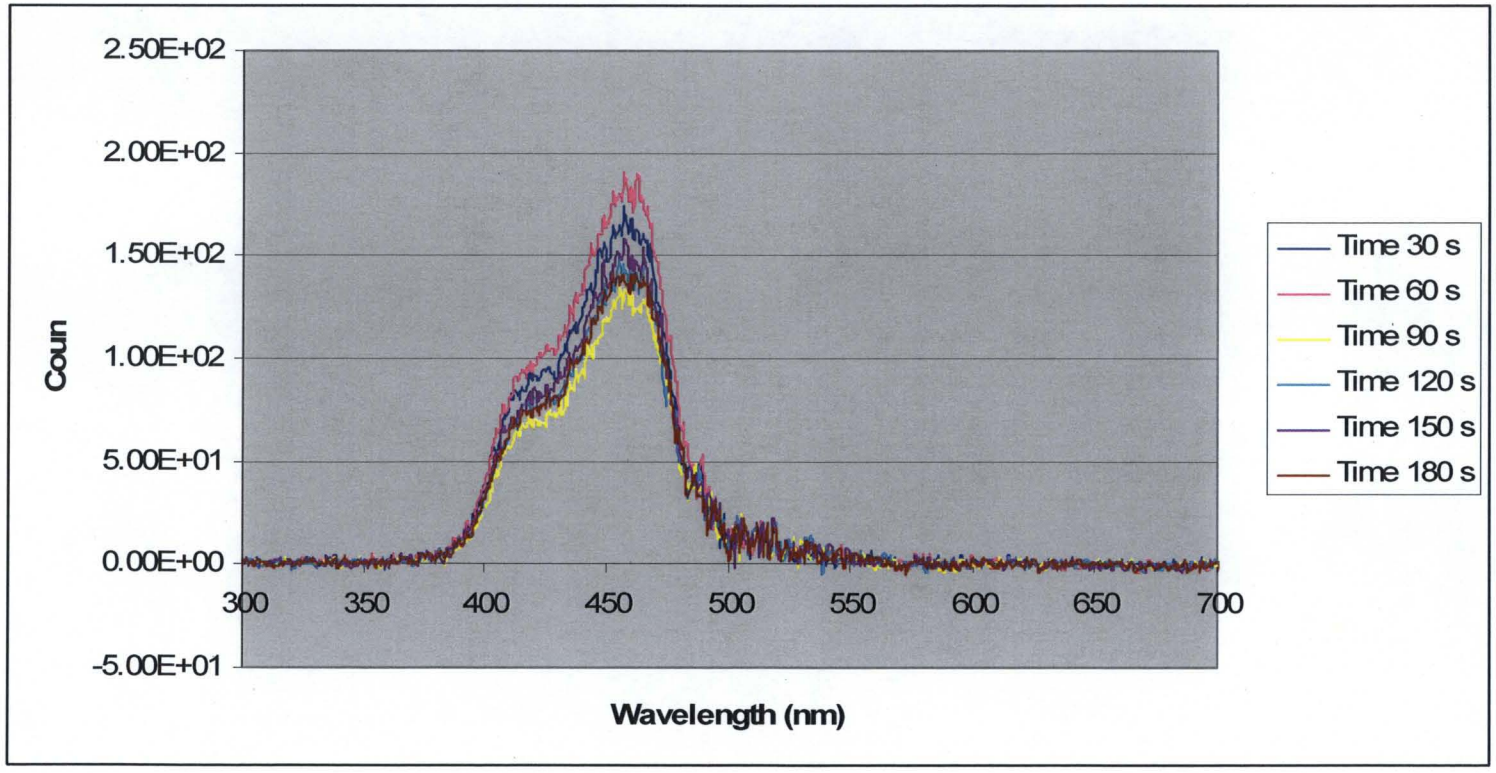
Pre-test Peak: 525 nm, 3.65 x10^3 counts

Test Peak: near 517 nm, 2.81 x10^2 counts



Dye Solution Results

- Example 2: Test 39, 2.77% APTS dye solution exposed to 0.01% formaldehyde
  - Pre-test Peak: 489 nm, 3.78 x103 counts
  - Test Peak: near 458 nm, 1.91 x102 counts



Dye Solution Results

- Measured peaks were compared within populations to determine the consistency with which dyes performed
- Calculated quantities for APTS 2.77% and 8.33% dye solution

At the lower concentration APTS was more variable within populations with populations 9 and 23 performing poorly.

At higher concentrations APTS was more consistent across each population with population 12 performing best.

APTS 2.77%

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
3	470.31	469.50	467.00	3.38	11.44
6	461.89	461.50	463.50	3.71	13.79
9	462.13	461.75	480.00	10.25	105.05
11	455.60	455.75	456.00	2.26	5.10
13	425.36	421.50	418.00	8.18	66.84
17	452.23	446.00	446.00	7.24	52.48
19	441.59	443.25	443.50	6.54	42.83
23	416.26	408.00	407.50	19.52	380.92
25	416.79	416.25	414.50	3.37	11.37

APTS 8.33%

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
12	504.54	504.50	504.50	0.17	0.03
18	492.58	492.75	493.00	0.45	0.20
24	494.21	494.00	492.00	2.38	5.66

Dye Solution Results

- Calculated quantities for AF 488 2.77% dye solution

AF 488 exhibited low variation in the majority of populations for both low and high concentrations of dye.

AF 488 was generally more consistent than APTS both within populations and across populations.

AF 488 2.77%

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
7	515.83	515.50	515.50	1.13	1.27
10	516.18	516.00	515.00	0.97	0.95
14	514.71	514.50	514.50	0.83	0.68
16	512.74	510.50	509.50	4.70	22.11
20	513.72	513.75	512.50	1.02	1.03
22	511.67	511.00	510.50	2.23	4.99
26	514.24	515.00	515.00	0.72	0.52
28	510.71	511.00	511.50	1.00	1.00
29	514.58	514.50	514.00	0.75	0.56
31	510.17	510.00	509.50	0.67	0.45

AF 488 8.33%

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
15	523.24	520.50	520.50	4.27	18.21
21	520.81	520.50	520.00	0.76	0.58
27	521.00	521.25	522.00	1.08	1.17
30	520.81	521.00	521.50	0.67	0.45

Doped Sol-gel

- Tested with aqueous formaldehyde and formaldehyde gas
- Multiple concentrations of TEOS were explored with dye concentrations between 0.9% and 1.38%
- Thin films of doped sol-gel dehydrated and did not fluoresce
- Ampoules containing doped sol-gel permitted control of water loss during and after gelation
- Spectra were analyzed using the same methods as for the dye solution

Doped Sol-gel

- Summary of tests with aqueous formaldehyde

Both dyes experienced variability within populations. AF 488 was slightly more consistent than APTS but not appreciably so.

In general, the doped sol-gel was more variable than the dye solutions.

APTS/Sol-gel

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
38	497.21	495.25	494.50	4.25	18.06
39	494.67	495.00	497.00	2.72	7.38
40	496.13	494.75	493.50	15.49	239.96
41	511.67	511.25	520.50	8.10	65.68
42	490.17	491.00	491.50	1.77	3.14
43	487.96	488.50	488.50	0.88	0.77
48	473.58	473.25	458.00	12.96	168.08

AF 488/Sol-gel

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
32	510.00	511.50	513.50	4.61	21.25
33	516.58	516.00	516.00	1.77	3.12
34	512.42	508.50	504.50	8.38	70.20
35	513.08	514.50	521.00	7.53	56.66
36	501.13	502.00	502.50	2.41	5.80
37	505.50	503.50	503.50	8.02	64.31
44	478.56	486.25	486.50	12.19	148.50
45	513.71	513.25	513.50	2.02	4.06
46	513.04	508.25	500.00	12.53	157.10

Doped Sol-gel

- The formulations of doped sol-gel that performed best with aqueous formaldehyde were used to determine the concentration of dye and TEOS to make samples for testing with formaldehyde gas
- Two populations were generated for gas testing
  - 49, APTS
  - 50, AF 488
- Gas testing was performed with a commercially available gas calibration tube
  - Gas released into a sealed bag chamber containing the samples and test apparatus
  - Ambient concentration of formaldehyde gas was calculated to be 0.08 ppm
  - Permitted 10 minutes for gas to diffuse through chamber prior to exposing samples
  - Captured spectra at 4 and 10 minutes after exposure
- Observed fluorescence shift was present but smaller in magnitude than with aqueous formaldehyde

Doped Sol-gel

- Summary of calculated quantities, APTS and AF 488 doped sol-gel tested with gas calibration tube

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
49	488.00	488.00	488.00	0.00	0.00
50	517.34	515.75	515.50	3.16	10.01

- The measured fluorescence shift was smaller than for the 0.5 ppm test with aqueous formaldehyde
- Retested with formaldehyde gas produced by evaporation – calculated at an ambient concentration of 0.5 ppm

Population	Average (nm)	Median (nm)	Mode (nm)	Standard Deviation (nm)	Variance (nm)
51	429.67	430.75	431.50	2.25	5.06
52	520.75	521.00	522.50	1.63	2.65

- Retesting confirmed detection of formaldehyde gas at low concentrations

Conclusions & Discussion

- Key to experiment was the successful merger of established technologies and techniques
  - Fluorescent reactive dyes
  - Fluorescence spectroscopy
  - Substrate materials
- Formaldehyde was detected in aqueous form down to 0.5 ppm and in gaseous form down to 0.08 ppm
- AF 488 performed more consistently than APTS
- Use of inexpensive LED excitation sources, common dyes, and portable EPP-2000 spectrometer in the tabletop prototype demonstrated the feasibility of a compact, low cost sensor
- While dye performance was reasonably consistent, variations within populations raise practical considerations for future sensor design

Future Work/Discussion

- Possible additional experimentation
  - Correlation of dye fluorescence intensity to formaldehyde concentration
  - Demonstration of detections below 0.08 ppm
  - Exploration of new form factors and surfactants to enable the use of thin films
- Considerations for building a sensor
  - Optical component selection for multiple dye test samples
  - Improved manufacturing techniques for samples
  - Reusability of the device and of test samples