Real Time Detection of Biological Aerosols

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Interest in the environmental impact of biological aerosols has increased due to the implications of aerosolized bio-materials in indoor building pollution; the release of genetically-engineered organisms into the environment; and the release of potentially pathogenic organisms downwind from sewage treatment plants. Efforts to date for the real time detection of biological aerosols have proven unsuccessful due to the lack of the technology to discriminate between potentially hazardous materials and background materials.

The integration of rapid immunoassay technology with a real-time air sampling capability is presently under investigation. A two-stage air sampler with impingement capability has been developed and integrated with an immunologically-based biosensor to effect a real-time aerosol detection capability. The sampler concentrates and impinges 100 liters of air into 100 ul of fluid. The impinged sample is then mixed with the immunoreagents, and the resulting immune complex is trapped onto a nitrocellulose filter while unreacted materials are washed away. Urease, an enzyme which effects a pH change in the substrate buffer, is used as the enzymatic tag. The slope of the resultant pH change is then determined through the use of the Light-Addressable Potentiometric Sensor.

Mass spectrometry offers an alternative means of detection of aerosols. A small, field portable mass spectrometer, based on Quadrapole Ion Storage (QUISTOR) technology, is also being developed and integrated with an air-sampling capability. The concentrated sample will then be pyrolyzed, with the resulting pyrolysates being analyzed by MS or MS/MS. The resulting spectrum will be compared against an onboard library. An artificial intelligence capability will allow unknown materials to be analyzed and retained for future reference.

Efforts to date have centered on materials which are of military interest. Detection levels as low as 1 ng have been achieved in a one minute assay time for the immunoassay system. Non-military uses of this technology can be developed, depending on the applications of the customer and the development of appropriate antibody reagents or mass spectral libraries.

INTRODUCTION

The contributions of biological aerosols to both indoor and outdoor pollution problems have often been neglected. This has been largely due to the difficulty one has in the detection and characterization of the aerosol. Past attempts at detection have relied upon measuring changes in bulk properties, such as heme content or the presence of specific reactions. These attempts failed because they did not possess the sensitivity and/or specificity to distinguish between hazardous and non-hazardous materials. Recent advances in both biosensor and mass spectroscopic technologies are leading to the development

of small, lightweight, and rugged instruments which can be introduced into field applications for detection of biological materials.

THE BIOCHEMICAL DETECTOR

The field assay of biological materials is difficult due to the need for detection of often minute quantities of specific material in the presence of a large background. Immunological-based sensors are being developed which have the capability to differentiate between analytes and background. Antibodies are bound to either optical or electrochemical transducers, and the binding event is measured due to the generation of an optical or an electrochemical event. Typically, fluorescent dyes or chromogenic enzyme substrates are used for the transduction of an optical signal while enzyme-substrate combinations which yield electrochemically active species are used with the electrochemical sensor. Both types of sensors were initially evaluated in this project. The electrochemical one was chosen for further development.

This sensor is referred to as the "Light-Addressable Potentiometric Sensor" (LAPS), and is marketed by Molecular Devices Corporation, Menlo Park, CA, as the Threshold@ system. It is simple in design, consisting of a silicon wafer, on which a layer of silicon oxide/silicon nitride has been grown. This silicon oxide/silicon nitride serves as an electrical insulator and makes the surface impervious to ion migrations from solutions in contact with the surface, imparting a neutral pH sensitivity to the sensor. The transducer is used to monitor the activity of enzyme-labelled antibodies which are used in the reaction. Presently urease, which catalyzes the hydrolysis of urea to carbon dioxide and ammonia, is used.

The immunological reaction takes place on a nitrocellulose filter which is later placed on the sensor surface. A controlling electrode and a reference electrode back-bias the insulator/silicon junction, creating a depletion layer at the junction (absence of charge carriers). A light emitting diode (LED), driven at 10 kHz, illuminates a small area of the silicon chip, creating charge carriers in the depletion layer at that point, which results in an alternating current between the controlling electrode and the bulk silicon. The magnitude of this current is dependent on the surface potential of the silicon oxide/silicon nitride. This surface potential responds in a Nernstian manner to changes in the surface pH. Several LED's can be used on the chip so that multiple sites can be addressed in succession. When coupled with the appropriate immunological reagents, a sensor can be obtained which has a detection capability for several materials in a small area.

The Bio-Chemical Detector, currently under development by the Army, utilizes this sensor technology in conjunction with an aerosol sampler. The sampler impinges aerosols into a liquid medium. This liquid is then transferred to a reaction manifold where the reagents are added. The resulting immune complexes are then filtered through an active membrane which captures the complex. Unreacted reagents are then washed away, and the filter is transferred to the reading module where the pH change is obtained.

This detection system has as a design goal the detection of six different classes of biological materials- bacteria, rickettsia, viruses, and small, medium, and large molecular-weight toxin materials. Other goals include a total sample acquisition time of two minutes with capability of repetitive analysis over a 24 hour period. Initial results have been encouraging with detection limits of nanograms per milliliter of toxin materials being realized with the sensor. Detection limits of 10^5-10^6 organisms per ml have been realized with two of the microbial materials. These detection limits are realized in the 3-4 minute time frame. The next phases of development include better integration of modules and improvements to the immunoassay format.

Although this system is being developed for materials which are of military interest, the use of this system can be extended to other materials through the development of appropriate antibody reagents. It is conceivable that this type of detection system could be utilized where real-time detection of hazardous biological materials is required.

THE CBMS SYSTEM

There is much interest in the development of small, portable mass spectrometer units for field analysis of hazardous materials. Although these units are significantly smaller than their laboratory counterparts, compromises are made with respect to capability and resolution. In addition, the system must be capable of identifying trace quantities of hazardous materials often in the presence of other interfering compounds. This can often be accomplished through the use of a GC/MS system; however, the complexity and logistical burden of this type of system may make it too cumbersome for routine use in the military environment.

The CBMS is an attempt to develop a small, portable mass spectrometer which has the capability to detect trace quantities of materials in the midst of significant amounts of interferents. It utilizes Quadrapole Ion Storage technology (QUISTOR) to accomplish this goal. This technology has allowed for the development of a small, sensitive mass spectrometer which has an MS/MS capability and can be fitted with a variety of probes to enable sampling of ground contaminants or the detection of biological aerosols. The ground sampling probe is commercially available and will not be described in detail here. The biological aerosol sampling capability was the result of an in-house effort at CRDEC.

The aerosol material is impacted into a quartz tube with subsequent pyrolysis by IR radiation. The resultant vapor is then introduced into the instrument and analyzed. In the case of bacteria where similar primary spectra are obtained, the unit is then switched into an MS/MS mode where daughter ion spectra are obtained. This has allowed bacterial identification to the Genus level; species level should be possible with the development of the appropriate spectral libraries. In addition, an artificial intelligence capability is being built into this unit so that it will be capable of analyzing spectra of unknown materials and trying to "best guess" what they are.

SUMMARY

Both these systems offer viable approaches to the real time detection of biological aerosols. They also demonstrate two of the principles which can be used to achieve this: one being the specificity of antibody molecules, while the other being the chemical signature; a material would give in the mass spectrometer. The bio-specificity approach requires a lot of up front work in the development of the biological reagents (although the hardware development is not trivial) while the other requires a significant amount of work in the development of appropriate spectral libraries. Both systems are in the early stages of development and show great promise.

Second International Symposium

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February 12–14, 1991

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