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DETECTION OF SMALL MOLECULES WITH A FLOW IMMUNOSENSOR

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ABSTRACT

We describe the development of an easy-to-use sensor with widespread applications for detecting small molecules. The flow immunosensor can analyze discrete samples in under 1 minute or continuously monitor a flowing stream for the presence of specific analytes. This detection system is extremely specific, and achieves a level of sensitivity which meets or exceeds the detection limits reported for rival assays. Because the system is also compact, transportable, and automated, it has the potential to impact diverse areas. For example, the flow immunosensor has successfully detected drugs of abuse and explosives, and may well address many of the needs of the environmental community with respect to continuous monitoring for pollutants. Efforts are underway to engineer a portable device for use in the field.

INTRODUCTION

Market demand for a rapid means of detecting small molecules in diverse environments has increased dramatically in recent years. Monitoring for environmental contaminants, screening for drugs of abuse, and controlling fermentation processes all require accurate and sensitive determinations of what compounds are present in a complex sample. Current methods rely on optical sensing, which does not provide specificity, or on more complex analytical techniques, which are time consuming and labor intensive. The Center for Bio/molecular Science and Engineering at the Naval Research Laboratory has developed a sensor which operates in flow and is capable of detecting small molecular weight compounds in the parts per billion range in minutes (1,2).

BACKGROUND

The flow immunosensor capitalizes on the specific recognition between an antibody and its selected target molecule, the antigen. With the current technologies, large quantities of antibody with a defined specificity can be readily produced in the laboratory. This confers a great degree of flexibility on this sensor with respect to the compounds it can be designed to detect.

Though other antibody assay systems are in use, they rely on either direct binding of antigen (i.e. sandwich immunoassays) or a competitive binding of antigen versus labeled antigen. Only the latter configuration has been widely used for the detection of small molecules. The flow immunosensor relies on a distinctly different type of antibody-antigen interaction. This device measures antigen displacement events rather than binding events.

TECHNICAL APPROACH

The key elements of the sensor are: 1) the antibody specific for the target, 2) signal molecules similar to the target, but labeled so they are highly visible to a detector, and 3) a detector. Antibodies are chemically immobilized on a solid support, for example glass beads. All the antibody binding sites are saturated with a fluorescently-labeled analog of the antigen, creating an antibody/labeled antigen complex. The support is packed into a 1/2 inch column and connected to a water stream. The target molecule is introduced via a continuous or pulsed stream under nonequilibrium conditions. Only the target molecules in a complex sample are recognized. When the target molecule is present in the sample, the labeled antigen is displaced from the antibody and moves into the flow stream. The displaced labeled antigen enters the flow cell of a simple fluorimeter and triggers a response.

The signal from the detector will be proportional to the number of target molecules in the sample. No reagent addition is required throughout the assay and analysis time is minimal. A laptop computer is used to control system hardware, as well as handle data acquisition and analysis. A schematic of the system is shown in Figure 1.

EXAMPLE OF FLOW IMMUNOSENSOR USE

The flow immunosensor developed at NRL has successfully detected both drugs of abuse and explosives present at low levels in water samples. For example, to detect cocaine, an antigen to the immobilized antibody was reacted with fluorescein-labelled benzoylecgonine, the major metabolite of cocaine. Samples containing cocaine were introduced into the water stream. Analysis time was rapid, as detection of the cocaine was accomplished within 1 minute. Multiple samples could also be detected on the same column. Figure 2 illustrates the application of 750 ng/ml samples of cocaine to a single column. Over 50 positive samples were detected.

The explosive molecule TNT has also been tested using the flow immunosensor. As shown in Figure 3, when TNT was introduced into the flow stream, the magnitude of the signal was proportional to the amount of TNT introduced into the system. The response was specific for TNT, as a compound with a similar ring structure did not generate a signal.

ADVANTAGES OF THIS APPROACH

The flow immunosensor has many advantages over existing technologies. Operation of the sensor is straightforward and fast, and does not require a skilled operator or extensive training. The prototype now in use requires only two computer keystrokes. In its simplest version, the user introduces the sample at the beginning of the system and records the results within 1 minute of sample introduction. Again, this is in contrast to the user intensive and time consuming operation of currently available detection devices. The widely used methods often require addition of different reagents throughout the assay and lengthy incubation times, or demand the use of large, sophisticated instruments. In the NRL sensor, all the components required to recognize the target and release a signal are contained within a small column.

The flow immunosensor is also well-characterized. Experimental parameters, including column size and flow rate, have been studied extensively. Using equations derived at NRL, we are able to predict the behavior of the sensor for a given antibody-analyte pair. In addition, because the immunosensor is antibody-based, detection is extremely specific for the target molecule.

System manufacturing costs and portability are also important considerations. The components of the current system are inexpensive and off-the-shelf. Cost for the laboratory prototype is under $10,000, and the sensor can be engineered to fit into a single briefcase with microprocessor control.

An additional strength of the NRL detector is that it can be used in a variety of environments. It is readily adaptable for use with individual samples injected by hand, air samplers that extract vapors into water, or super sipper systems that rapidly inject samples from hundreds of vials.

Finally, the detection limit of the flow immunosensor is already comparable to established, more complicated systems. Using the NRL sensor, cocaine and TNT in water have been detected at levels of less than 5 parts per billion (equivalent to 5 ng/ml). This level of sensitivity is well-below that obtained using precipitation, dip stick, ELISA and fluorescence polarization methods, and is comparable to radioimmunoassays.

CONCLUSION

A flow immunosensor has been developed and tested at NRL which will successfully detect both drugs of abuse and explosives present at low levels in water samples. This detection system relies on the ability of sample antigen to displace labeled antigen from antibody under flow conditions. The laboratory prototype is currently being used to detect cocaine, heroin, and TNT. The advantages of the NRL sensor include its speed, specificity, and versatility with regard to measurement of a range of analytes and sampling conditions.

NOTES AND ACKNOWLEDGEMENTS

Two patents (3,4) have been filed covering this technology and are available for license. This work was supported by the Federal Aviation Administration and the U.S. Customs Service. The authors thank Reinhard Bredehorst, Robert Ogert, Paul Charles, Jeffrey Chilla, and James Whelan for their scientific contributions. The views expressed here are the authors' own and do not reflect the policy of the U.S. Navy, Department of Defense or United States Government.

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4. Flow Immunosensor Method and Apparatus, Patent Application # 07-486024 (submitted 2/90)

FIGURES

Figure 1. Flow immunosensor operation
Figure 2. Repetitive detection of cocaine samples.
Figure 3. Detection of TNT with the flow immunosensor

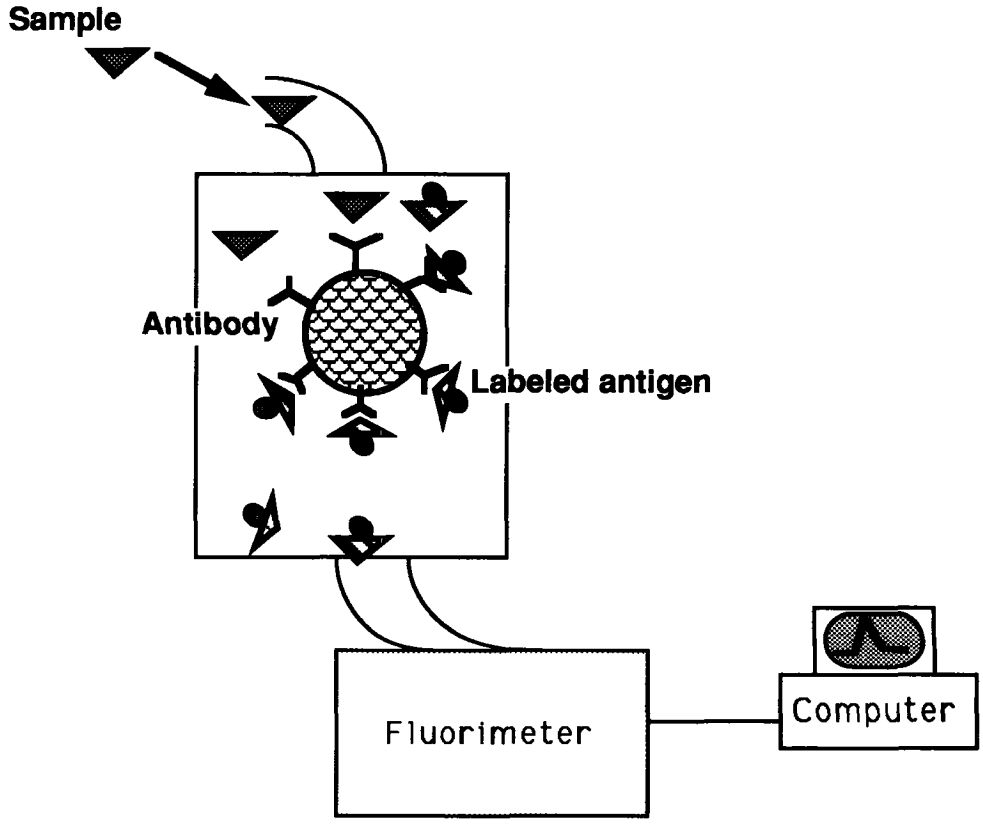

Figure 1 - Flow immunosensor operation. A sample is added to a small column containing immobilized antibodies saturated with labeled antigen molecules. A proportional amount of labeled antigen is displaced by the sample and is moved by the flow stream to the fluorimeter. Signal output from the fluorimeter is transmitted to the computer and analyzed.

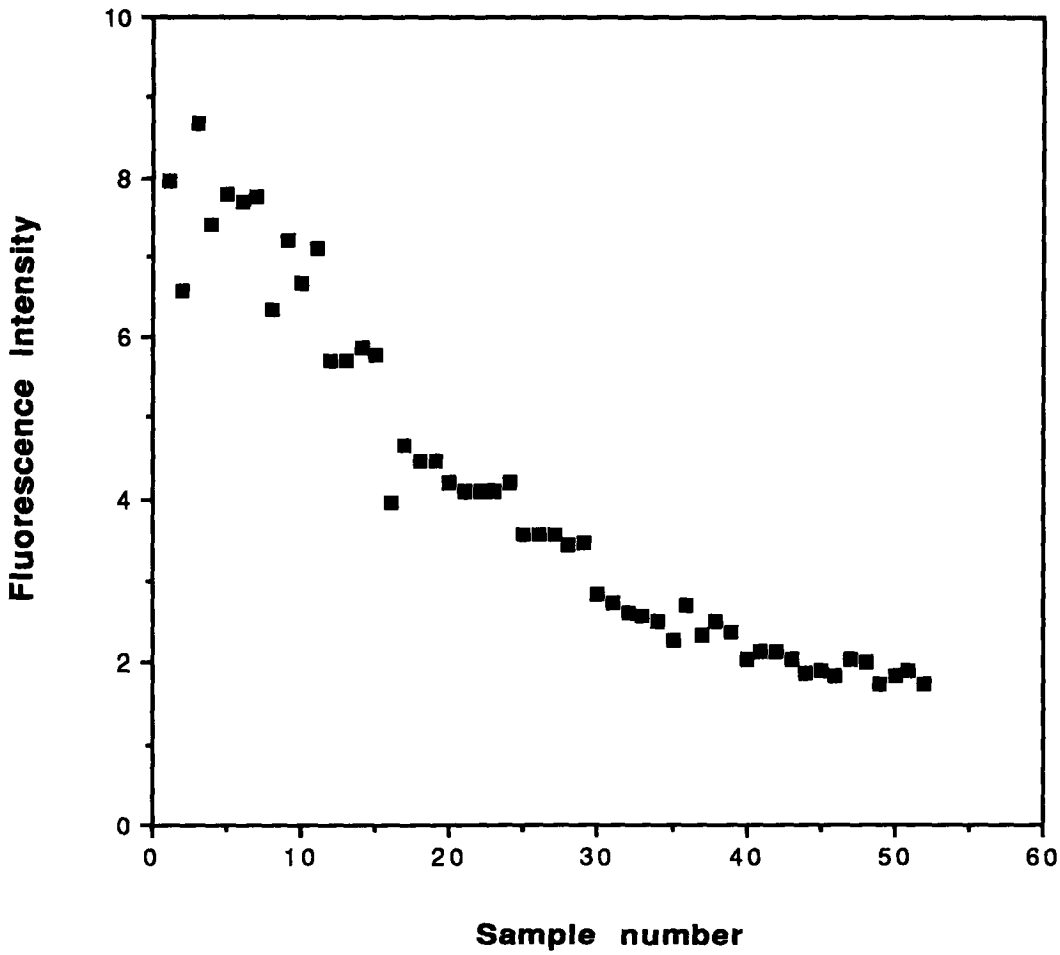

Figure 2 Repetitive detection of cocaine samples. Consecutive samples of cocaine, 200 μl at 750 ng/ml, were applied to the flow immunosensor. The integrated peak area of fluorescence intensity was calculated for each sample and plotted.

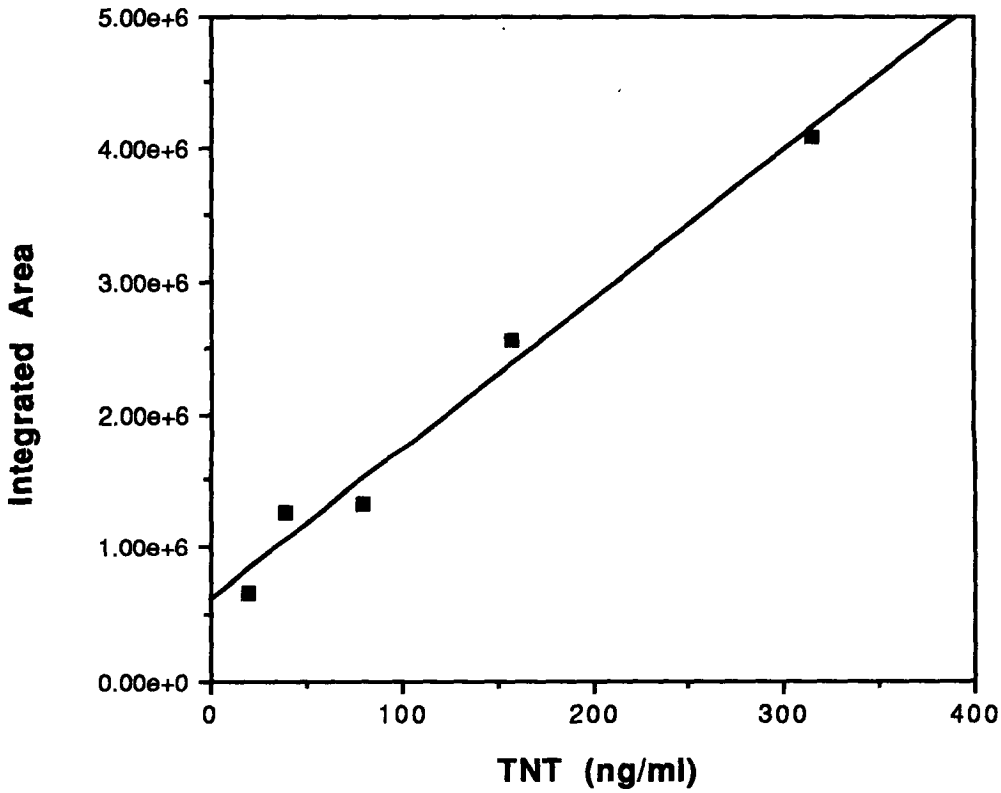

Figure 3 Detection of TNT with the flow immunosensor