Dendrimer Nanoparticles for Extending Biosensor Lifetime

Xiaohua Qian, Ph.D. and Mary Beth Tabacco, Ph.D.

Smiths Detection, Boston, MA, USA, xiaohua.qian@smithsdetection.com

ABSTRACT

Smiths Detection has been developing *semi-selective* optical biosensors for the direct airborne detection of biological agents. Here we report a unique sensor formulation that has the active sensing chemistry encapsulated in a reverse micelle structure resulting in extended sensor lifetime. Dendrimers added to the aqueous phase serve as both a host molecule for the sensing chemistry and as a molecular "scaffold" for the reverse micelle structure. The sensors prepared as reverse micelles have demonstrated excellent sensitivity and selectivity for classes of bioagents and immunity to non-biological interferents. We believe this is the first report of using dendrimer-assisted reverse micelle systems in a dry sensor film for detection of airborne biological agents.

Keywords: biosensor, biological warfare agents, fluorescence, reverse micelle, dendrimer

1 INTRODUCTION

Simple, rapid detection of biological threats continues to be an intractable problem. There are urgent needs for low cost instrumentation to provide Detect-to-Warn and Detect-to-Treat systems for homeland security applications. A network of small biological aerosol sensors could be used to protect the nation's critical infrastructure as well as to protect soldiers on the battlefield. Because of the very complexed nature of the biological threat and the need for high selectivity and sensitivity, no such detector exists at present. Identification of microorganisms at the species and strain level still requires sophisticated analysis, sample preparation, user expertise and time.

Smiths Detection has been developing *semi-selective* optical biosensors for the direct airborne detection of the four primary classes of biological warfare agent: vegetative bacteria, bacterial spores, toxins and viruses [1-4]. A suite of fluorescence sensors have been developed that target different biological markers associated with biological agents such as nucleic acid, protein or some other surface materials. Information from multiple sensors is analyzed as an ensemble using chemometric algorithms, thereby providing the unique capability to detect and classify the biological material. The sensors, integrated with a complete detection and data processing system, have been tested at several third-party and government test facilities and have demonstrated excellent sensitivity and selectivity. However, with certain of the current sensor formulations sensor stability and operational lifetime are affected by environmental conditions. Sensors with augmented operational and storage lifetimes would reduce cost and logistic burdens and would also broaden potential application areas. The objective of this project is thus to develop a new sensor formulation for extended lifetime but retain the high sensitivity and selectivity of the existing sensor formulation.

Dendrimers are highly branched, structurally well-defined polymers possessing a very high concentration of surface functional groups [5]. They have been used in gene or drug delivery [5-7] where covalent bonding or host-guest interactions between dendrimers and a variety of target molecules were exploited. Specific types of dendrimers can also be used as scaffolds for presenting vaccine antigens, especially peptides for use in vaccines [7]. The ability of dendrimers to disrupt cell membranes [1, 8] and increase cell permeability may help to transport dye molecules through cell membranes and increase sensor sensitivity. Earlier research showed that incorporating dendrimers into biosensor films extended sensor lifetime for live bacteria detection [1].

In the current sensor formulation the dye molecules have to maintain some degree of freedom so they can interact with either cell membranes or virus capsids. This degree of freedom is best afforded in a fluid environment. Providing this suitable microenvironment in a solid state sensing film is a great challenge. Our solution to this problem is to use the microheterogeneous system of reverse micelles (RMs) supported by dendrimer macromolecules (Figure 1). Reverse micelles are aggregates of surfactant molecules that are generated spontaneously in the oil-water system. They consist of microdomains of water dispersed in a continuous oil domain, stabilized by surfactant molecules present at the interface. Reverse micelles have been applied to oil recovery, preparations of pharmaceuticals, cosmetics and monodispersed nanoparticles.

Cetyltrimethylammonium bromide (CTAB) is among the most widely studied cationic surfactants, and was used because of its positive charge and high water uptake and solubilization capacity [9]. In this paper we report the

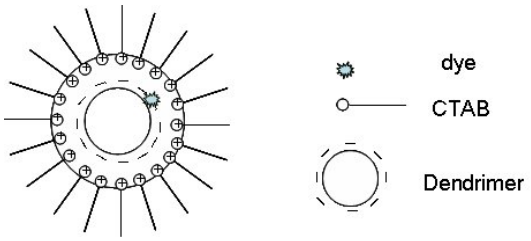

Figure 1 Schematic model of dendrimer-reverse micelle supramolecule

development of a dendrimer-assisted reverse micelle based biosensor formulation that forms a solid sensing film when dried on polycarbonate film. The sensors have demonstrated excellent sensitivity and selectivity for classes of bioagents and immunity to non-biological interferents. The operational lifetime of the sensors based on RMs has increased dramatically from hours to days. We believe this is the first report of reverse micelles in a dry sensor film for detection of airborne biological agents.

2. EXPERIMENTAL

All reagents are used as received without further purification. Nucleic acid stains SYTOX Green and SYTO 13 were purchased from Molecular Probes. PAMAM dendrimers with ethylenediamine core are purchased from Aldrich. Water is deionized and distilled and filtered through a 0.2 um filter before use.

Sensors films were made according to these general procedures: dyes (stock solution in DMSO) were diluted into aqueous dendrimer solution. The dye solution was added to a mixture of CTAB in isooctane and 1-hexanol. After thorough mixing and sonication, reverse micelle formation was confirmed by the clear appearance of the resulting solution. A microliter scale aliquot of reverse micelle solution was deposited onto a clear polycarbonate film pretreated with surfactant and allowed to dry and form a continuous film at room temperature protected from light and direct air flow.

The RM-based sensors were tested with BWA simulants in house using the prototype Biological Detection System 2+ (BDS2+, Figure 2). The BDS2+ is an 8-channel detection system designed to detect and classify airborne pathogens in real time. It is intended for stand-alone operation or can be integrated into a multi-detector suite. Laboratory testing was conducted by placing the BDS2+ in a bioaerosol chamber that is inside a biological safety hood (BSL 2). The integrated air sampler on the BDS2+ brings the chamber air to the sensor surfaces. Biological agents and/or inteferents were disseminated into the aerosol chamber with a Meinherd nebulizer and collected on the sensor surface through inertial impaction. Real time data acquisition and processing was done with a Data Combiner and Processing Module (DCPM) that is a part of BDS2+; optionally data can be displayed on a PC with a LabView interface also developed in house.

Figure 2 Prototype BDS2+ system

The sensors were examined post exposure using an epi-fluorescence microscope (Nikon Eclipse E400). The images were taken with a digital camera (Nikon Coolpix 990) mounted on the microscope with a C-mount.

3. RESULTS AND DISCUSSION

Reverse micelles are spherical water droplets surrounded by a monolayer of closely packed surfactant molecules dispersed in a solvent of low polarity [10]. These water droplets are thermodynamically stable due to the presence of the interfacial surfactant layer that prevents unfavorable direct contact between water and organic solvent [11]. The rational for this sensor design is to entrap the sensing chemistry in the aqueous phase of the reverse micelles. When the reverse micelles form solid sensing films, the super structure frame formed will provide the needed mechanical strength and slow down the water loss thus prolonging sensor lifetime. However, it was found that RMs formed with only water in the polar phase do not dry well to form the final dry sensor film. When reverse micelle solutions were deposited on the sensor substrate, both organic solvent (isooctane) and water evaporated albeit at different rates. Under these conditions the RMs collapsed and surfactant molecules dried out on the surfaces (data not shown). Good, stable, optically transparent sensing films were formed after addition of dendrimer (PAMAM) and glycerol to the polar phase. The resultant sensing films demonstrated the desired high sensitivity and selectivity. The robustness of the sensors was evident by the exceptional operational and storage lifetime. The effect of different components of the formulation on sensor performance is discussed in the following sections.

3.1 Effect of Dye on Sensor Performance

The amount of dye in the sensor formulation has a direct impact on the sensitivity of the sensors. In Figure 3 fluorescent microscope images of the RM sensors with

different W_0 are presented after being challenged with *Bacillus subtilis* vegetative cells. W_0 here is the ratio of polar solvents (water plus glycerol) to surfactant molecules (CTAB plus 1-hexanol). As can be seen in Figure 3 as W_0 increases the cells appear brighter under the microscope. We believe that the higher the aqueous component the more dye available on the sensor to stain the cells.

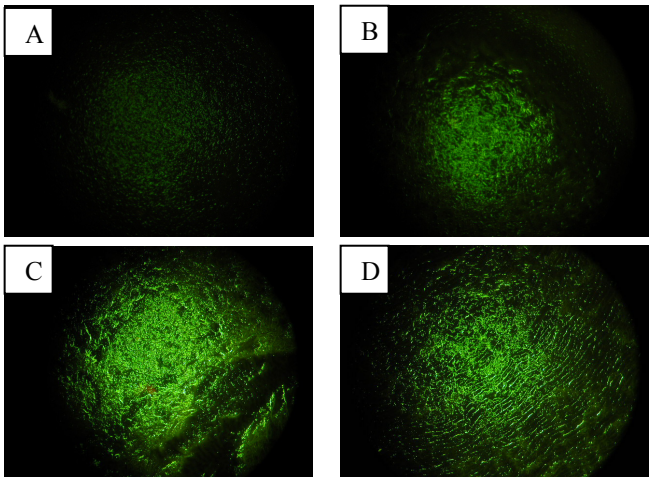

Figure 3 Fluorescence microscope images of sensors after challenge with biological simulants. A) W_0 = 3.1; B) W_0 = 5.7; C) W_0 = 10.2; D) W_0 = 12.5

3.2 Effect of Glycerol on Sensor Performance

Glycerol is an essential part of the sensor formulation. It is believed that glycerol contributes to sensor performance in two ways. First, glycerol determines the amount of polar phase preserved in the sensor film, and hence the dendrimer content. This in turn determines the size and quality of the supramolecular structure that forms the sensor film. This is supported by the appearance of the films shown in Figure 3 and by the data in Table 1. Comparing sensor films in Figure 3D and 3C, both W_0 and water content is higher in 3D than in 3C, but because there is less glycerol in 3D, the quality of the film is poorer and resulting in lower sensitivity (data not shown). In summary too little glycerol and the size of the polar phase is too small for maximum sensitivity; too much glycerol destabilizes the superstructure (data not shown).

[H2O] M	[Glycerol] M	w0	Images
2.48	0.85	3.13	3A
4.67	1.03	5.70	3B
8.55	1.04	10.48	3C
10.52	0.72	12.47	3D

Table 1 Effect of glycerol on sensors performances

Secondly, glycerol in the sensor film serves as a wetting agent and solubilizes dye in the liquid phase within the supramolecular structure. In operation the BDS2+ samples air continuously thereby exposes the sensor surfaces to constant air flow. If not protected, the solvents in the sensor film will be lost over time. When dye molecules in the sensing film start to loss mobility the sensors gradually lose sensitivity. In this new formulation dye in glycerol/water mixture is encased in the supramolecular structure and protected from rapid drying. The sensor film was very stable for a long time until the structure started to break down. Then the sensor started to dry out and lose activity as shown in Figure 4. The operational lifetime increased over 6 times that of the original formulation.

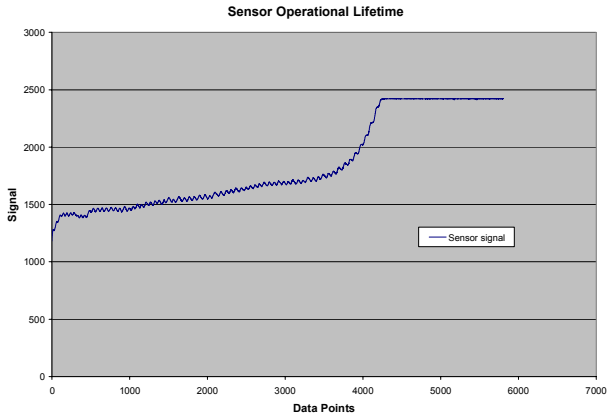

Figure 4 Sensor behaviors when exposed to continuous air sampling

3.3 Effect of Dendrimer PAMAM on Sensor Performance

PAMAM dendrimer with carboxylate surface groups also is an essential component of the current RM sensor formulation. The dendrimer serves as the macromolecular host for the dye molecules. Host-guest binding can either take place in the cavities of the dendrimer core ('endo-receptor'), or at the multivalent surface on the outer shell of the dendrimer ('exo-receptor') [12]. In this case, we believe the dye molecules were bound to the outer shell of the dendrimer as a result of the electrostatic attraction between the carboxylate surface groups of the dendrimer and positive charge of the dye molecules. The host-guest complexation between dendrimer and dye molecules helped to stabilize the micellar structure by mitigating electrostatic repulsion between the dye molecules and the cationic head group of the surfactant molecules in the polar phase. It is also thought that the PAMAM dendrimer serves as scaffold to support the suprastructure formed when the sensor film forms on the sensor substrate. PAMAM dendrimers with their large molecular size and multivalent surfaces serve as good scaffolds for synthetic macromolecular hosts [7]. Ottaviani et al [13-15] applied EPR spin probe technique to

investigate the supramolecular structures formed when surfactants are added to aqueous solutions of dendritic polymers. It was found that dendrimers (PAMAM generation 3.5) facilitated the formation of surfactant aggregates (CTAB), by offering a surface conducive to a cooperative interaction of the surfactants, even at low surfactant concentration [13]. We believe at the higher surfactant concentration we used, CTAB and PAMAM molecules formed supramolecular structures in isooctane solution as proposed in Figure 1. When the solution was deposited on the sensor substrate the polar phase with glycerol/water and dendrimer surface groups stabilizes the head groups of the surfactant molecules while the tail groups of the surfactant molecules are stabilized by a secondary surfactant, applied earlier to the substrate surface. Thus a semi-rigid structure was formed on the substrate to become the dry sensor film. This hypothesis is supported by the results showing the relationship between the concentration of PAMAM dendrimer and RM formation. When the PAMAM concentration was decreased the reverse micelle still formed in solution, but the sensor films formed were significantly less stable (data not shown).

4. CONCLUSIONS

A dry biosensor film formulation was developed based on reverse micelles supported by dendrimer PAMAM nanoparticles. The sensors demonstrated high sensitivity towards BWA simulants and excellent immunity to non-target biological simulants as well as common interferents. Best of all the RM-based sensors were capable of extended operational lifetime from hours to a few days. The storage lifetime of the sensors also improved to longer than 4 months.

Acknowledgement The authors thank Marines Corps Systems Command for financial support under SBIR Contract Number M67854-05-C-6501.

REFERENCES

1. Chang, A.C., Gillespie, J.B., Tabacco, M.B., "Enhanced Detection of Live Bacteria Using a Dendrimer Thin Film in an Optical Biosensor", *Anal. Chem*. 73, 462-466 (2001)
2. Ji, J, Schanzle, J.A. and Tabacco, M.B., "Real Time Detection of Bacterial Contamination in Dynamic Aqueous Environments Using Optical Sensors", *Anal. Chem.*, 76, 1411-1418 (2004)
3. Chuang, H., Macuch, P., Tabacco, M. B., "Optical Sensors for Rapid Determination of Bacteria (I): General Concepts and Initial Developments", *Anal. Chem*., 73, 467-470 (2001)
4. Taylor, L.C., Tabacco, M.B., Gillespie, J.B., "Sensors for Detection of Calcium Associated with Bacterial Endospore Suspensions", *Anal. Chim. Acta*, 435, 239-246 (2001)
5. Zeng, F.W., Zimmerman, S.C., *Chem. Rev*., 97, 1681 (1997)
6. Maksimenko A.V.; Mandrouguine, V.; Gottikh, M.B.; Bertrand, J.R.; Majoral, J.P; Malvy, C. *J Gene Med* 5(1), 61-71 (2003)
7. Boas, U. Heegaard, P.M.H. *Chem.Soc.Rev*. 33, 43-63 (2004)
8. Block, S., "Disinfection, Sterilization and Preservation", 3rd ed., Lea & Febiger: Philadelphia, PA, (1983)
9. Fang, X., and Yang, C., *J. of Colloid and Interface Science* 212, 242-251 (1999)
10. Luisi, P.L.; Giomini, M., Pileni, M.P., and Robinson, B.H., *Biochim. Biophys. Acta* 947, 209-246 (1988)
11. Gomez-Puyou, M.T. and Gomze-Puyou, A, Critical Reviews in Biochemistry and Molecular Niology, 33(1), 53-89 (1998)
12. Zeng, F. and Zimmerman, S.C., Chem. Rev., 97, 1681-1712, (1997)
13. Ottaviani, M.F., Andechaga, P., Turro, N.J. and Tomalia, D.A. J. Phys.Chem. B, 101, 6057-6065 (1997)
14. Ottaviani, M.F., Turro, N.J., Jockusch, S., and Tomalia, D.A. J. Phys.Chem., 100, 13675 (1996)
15. Ottaviani, M.F., Turro, N.J., Jockusch, S., and Tomalia, D.A. Colloids Surf. 115, 9 (1996)