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BIOCOMPATIBLE FLUORESCENT MICROSPHERES: SAFE PARTICLES FOR MATERIAL PENETRATION STUDIES

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ABSTRACT:

Biocompatible polymers with hydrolyzable chemical bonds are being used to produce safe, non-toxic fluorescent microspheres for material penetration studies. The selection of polymeric materials depends on both biocompatibility and processability, with tailored fluorescent properties depending on specific applications. Microspheres are composed of USFDA-approved biodegradable polymers and non-toxic fluorophores and are therefore suitable for tests where human exposure is possible. Micropheres are being produced which contain unique fluorophores to enable discrimination from background aerosol particles. Characteristics that affect dispersion and adhesion can be modified depending on use. Several different microsphere preparation methods are possible, including the use of a vibrating orifice aerosol generator (VOAG), a Sono-Tek atomizer, an emulsion technique, and inkjet printhead. The advantages and disadvantages of each method will be presented and discussed in greater detail along with fluorescent and charge properties of the aerosols. Applications for the fluorescent microspheres include challenges for biodefense system testing, calibrants for biofluorescence sensors, and particles for air dispersion model validation studies.

INTRODUCTION:

The use of biocompatible polymer microspheres has been proposed for use in drug delivery, dispersal model verification and as a surrogate for threat agents in instrument validation [1,2]. The ability to have a polymer sphere with a broad range of property tuning will allow for safe low cost use for homeland security and defense applications. Three main challenges are present in the production of such spheres. The first challenge is the reproduction of the fluorescent and other chemical properties of interest. Secondly, the particles must have the size and charge attributes needed for the specific application and testing. Finally, in order to reproduce testing and evaluation criteria a rapid, reliable, scalable and low cost production method must be available. This paper and related poster will address these challenges in studies focused on poly (D,L)-lactide co-glycolide (PLGA) microspheres.

In order to simulate the fluorescent properties of threat agents several non-toxic biological chemicals are available that cover narrow emission ranges. Dipicolinic acid,

tyrosine and tryptophan were evaluated for use to simulate a *B. anthracis* emission spectrum. Initial production of biosafe simulant microsheres at LLNL was done with an emulsion technique and a VOAG aerosol generator to combine the fluorophores tryptophan and poly-tryptophan.

The accurate and reproducible production of single size microspheres, or microspheres within a range of sizes, is important for reproducible studies and for rapid mass production since target bio-threats have specific size ranges determined by intrinsic properties and aerosolization and handling methods. If a sample particle has the correct chemical properties such as fluorescence but falls outside of the desired size range the particles may not be suitable as a simulant.

Aerosolization and the inherent chemical properties of particles will determine the ability to carry charge. If charge on a particle changes so do many critical properties. The settling time and filtration efficiencies of commercial air handling equipment can be effected by charge. At LLNL it has been determined that something as simple as a plastic air handling "jug" used in experiments can remove half of the charged particles. Unfortunately the charge on a plastic surface can vary with time and both positive and negative charged particles will be removed at different rates as the experiments progress. This is attributed to the particles being rapidly adsorbed by the charge on the plastic surface.

It was determined that the VOAG[3], Sono-Tek[4] and emulsion[5] methods were capable of producing microspheres with simulated fluorescence properties but low cost mass production was outside the capabilities of the techniques. Ink jet print heads are capable of combining the size specific attributes of the VOAG while meeting the mass production goal. The inkjet technique produces very specific size droplets and is capable of mass parallel production. Future inkjet work will build upon the fluorescence successes to further enable charge matching in evaluation this low cost, mass production technique.

EXPERIMENTAL:

Fluorescence:

Biocompatable microspheres were produced by the emulsion method and VOAG generator. The emulsion-based microspheres were PLGA/tryptophan and the VOAG microspheres were produced using poly-tryptophan. The resulting spheres were tested with total luminescence spectroscopy (TLS) for ability to simulate a threat agent fluorescence spectrum. TLS is commonly used as excitation emission matrices where both the excitation and intensity of the emission are recorded[6]. This measurement results in a unique matrix that can be used to fingerprint and identify the fluorescent compound. When a suitable TLS simulant was constructed additional measurements were taken for size and charge properties.

Size Determination:

Several commercially available techniques are available for aerosol size measurement in the 1-10μm size range. The microsphere size profile was measured with an Aerosol Particle Sizer (APS), SEM, and LLNL's Single Particle Aerosol Mass Spectrometer.

Charge:

Particle charge was measured with the charge detector recently developed by the Single Particle Aerosol Mass Spectrometry Group at LLNL. A single particle enters a conductive tube and induces a charge on the tube. The mirror image charge is amplified and recorded. In order to calibrate the charge detector a small electrical pulse is injected into the tube through a capacitor.

RESULTS:

Fluorescence:

Total luminesence spectroscopy was performed on both emulsion-PLGA/tryptophan and the VOAG-PLGA/poly-tryptophan microspheres. The resulting emission matrix was compared to that for *B. anthracis*. Figure 1 shows a comparison of the TLS spectra. The bottom frame shows the TLS spectra for *B. anthracis Stern* spores. This spectrum was used as a target for microsphere spectral features. In the top frame a TLS is shown for the emulsion PLGA/tryptophan spheres. It is important to note that the area with the highest signal in frame a. is slightly shifted to higher wavelengths than the spectra for *B. anthracis*. This shift is on the order of 50 nm. In Figure 1b a TLS spectrum is shown for VOAG produced poly-tryptophan microspheres. The use of the poly-tryptophan reduces the spectal maxima shift to ~25nm. Additional fluorescent measurements were collected with the single particle aerosol mass spectrometer. Although no quantative measurements were made it was found that the biosafe microspheres had flourecent qualities that triggered the system to collect mass spectra (they were determined to be biological like by the automatic detection system).

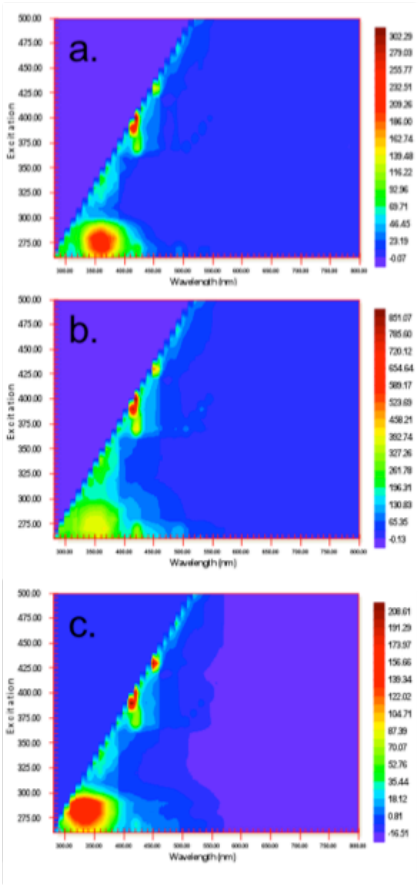

Figure 1: Total lumensence spectra. a) Emulsion-PLGA/tryptophan microspheres. b) VOAG-PLGA/polytryptophan microspheres c) *B. Anthracis Ames* spores

Micro-sphere size determination:

The two methods of producing microspheres had very different size distributions. As expected the emulsion method produced particles ranging from 0.7- 4.7 μm. The VOAG method produced a much narrower size distribution of 4-5 μm. Figure 2 shows SEM images of microspheres produced by emulsion and VOAG methods. It was also determined that particles produced by the Sono-Tek aerosol generator had a poly-dispersed size range of ~ 0.7 to 5 μm.

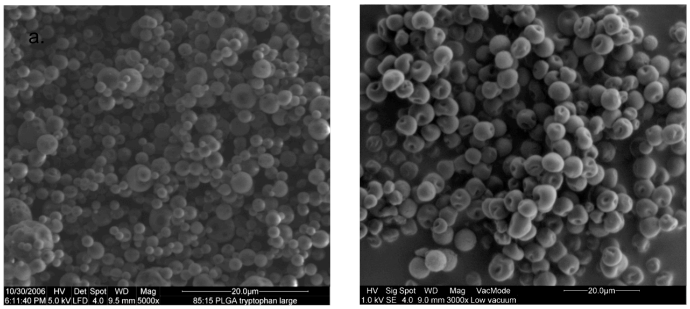

Figure 2. SEM images of microsperes. Left: Emulsion-synthesized PLGA/tryptophan microspheres; size range 0.7– 4.7 mm. Right: VOAG/solvent evaporation-synthesized PLGA/poly-tryptophan microspheres; size range 4-5 μm.

Micro-sphere charge determination:

Histograms were produced of the single particle charge measurements for bio-safe micro-spheres, *B. anthracis* and *Yersinia.* Charge histograms are shown in figure 3. The figure clearly show the slightly wider charge distribution for the engineered micro-spheres compared to the simulated threat agents. The small gap in recorded charged near the zero charge line is an artifact of the amplifiers and digitizers used in the current apparatus and should not be interpreted to mean that there are no particles with zero charge.

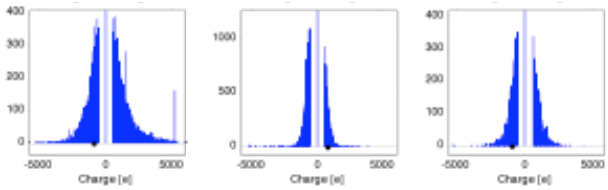

Figure 3. Charge histograms. Left: PLGA microspheres. Middle: *B. anthracis*. Right: *Yersinia*

CONCLUSIONS:

The production of bio-safe micro-spheres with a VOAG, Sono-Tek and emulsion techniques was demonstrated to reproduce many of the desired characteristic of a simulated threat agent. By using tryptophan and poly-tryptophan as the only florophore a close match to the TLS of *B. anthracis* was achieved. Fine-tuning of the fluorescent properties with additional chemicals should prove the ability to match most if not all threat agents.

The tested methods show the ability to produce particles in a size range that would be applicable for threat agent simulants. However, with poly dispersed methods such as the

emulsion technique, and Sono-Tek aerosol generator costly and time consuming size sorting would be required. The VOAG has the capability of producing a mono-dispersed particle type but has significant drawbacks in the ability to mass produce particles at a low cost.

Future and ongoing work is being conducted to determine if the inkjet method can be used for a high throughput production method. Inkjets have the ability to tightly control droplet size and have been proven to be durable by consumers. These properties along with the ability to run them in a parallel system may prove to be ideal for the production of bio-safe microspheres.

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