Full Paper

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Quantifying Trace 2,4,6-Trinitrotoluene (TNT) in Polymer Microspheres

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Abstract: Well characterized test materials are essential for validating the performance of current trace explosive detection systems. Explosive encapsulated microspheres have proven to be a valuable test material for trace explosive detection because of their precise size, shape, and composition. Presented herein is the quantification of explosives in the polymer microspheres by high performance liquid chromatography with UV/Vis detection (HPLC-UV/Vis). A size exclusion separation is employed to quantify the amount of explosive encapsulated in cured microspheres. Complete quantification was achieved by simultaneously separating and quantifying the explosive and polymer components. Results indicate that approximately 30% of the TNT is lost in the manufacturing of the microspheres and subsequent loss from the cured microspheres is minimal if stored at 4 °C.

Introduction

With increasing terrorist activities worldwide, the detection of both trace explosives and illicit narcotics has become a significant area of interest. Typical trace detectors rely on some form of particle or residue sample collection with a swipe, followed by chemical analysis. In swipe-based systems, a screener collects a sample from the surface of interest on a wipe that is then rapidly heated causing vaporization of any trace contraband materials to allow detection. In many cases an ion mobility spectrometer (IMS) is used for the detection and identification of these vapors.

In order to validate the trace detector instrument performance, reliable and well characterized test materials are required. These test materials need to be stable and mimic many of the same properties such as size and shape of real world particle residues. Furthermore, the development of plastic explosive standoff particles is needed to simulate plastic explosives such as composition B. The pharmaceutical industry has used poly(lactic-co-glycolic acid) (PLGA) microsphere encapsulation for site-specific drug delivery because the dissolution of PLGA in the body provides a slow metered release of the drug at a targeted site. Increased stability, safety, and lifetimes of the encapsulated drugs are additional beneficial properties [1–3]. Taking advantage of this technology, we were been able to produce well characterized test materials containing encapsulated low vapor pressure explosive molecules [4,5]. At the National Institutes of Standards and Technology (NIST), microspheres containing trace amounts of high explosives such as 2,4,6-trinitrotoluene (TNT) were prepared by an oil/water emulsion process using a co-flow particle fabrication nozzle [5]. PLGA encapsulation was thought to especially beneficial for TNT because of the relatively high sublimation rate (rel-

ative to other military explosives). Precisely controlled solution microdrops of a known amount of PLGA/TNT dissolved in dichloromethane is injected into a water bath containing a one percent polyvinyl alcohol solution where the microspheres are formed and allowed to cure.

Previous attempts to quantify the amount of trace explosives encapsulated in the microspheres by ion mobility spectrometry indicated a lower than expected response for the calculated values of encapsulated TNT [5]. Presented herein is the quantification of explosives in the polymer microspheres by size exclusion chromatography with UV/Vis detection (SEC-UV/Vis). Quantifying TNT in microspheres using a bench top UV spectrometer has proven ineffective due to the overwhelming interferences from PLGA. Therefore, in order to quantify the amount of explosives, a size exclusion separation was employed to separate and quantify the amount of explosive encapsulated in cured microspheres. By using a SEC separation of TNT and PLGA, quantification of each species' mass fractions can be determined, thus completely quantifying the TNT encapsulated. To the best of the authors' knowledge, this is the first time trace explosive encapsulated within PLGA microspheres have been successfully and completely quantified. Previously, the TNT component but not the PLGA in TNT/PLGA microspheres had been quantified using gas chromatography mass spectrometry (GC-MS) [6]. Moreover, the ability

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to quantify and document the amount of explosive encapsulated in the poly(lactic-*co*-glycolic acid) (PLGA) polymer microspheres made at NIST is essential for the development of a high quality particulate test materials that are simulate plastic explosives.

Materials and Methods

Materials

The copolymer poly(DL-lactide/glycolide) acid (PLGA) (Sigma Aldrich, St. Louis, MO*) was used to encapsulate two different sources of 2,4,6-trinitrotoluene (NIST TNT canine detection training aid) explosive and high purity 2,4,6-trinitrotoluene (Cerilliant, Round Rock, TX) explosive. All response curves were generated using the high purity TNT and PLGA.

Microspheres Manufacturing

The PLGA-TNT microspheres have been described in detail [4,5]. Briefly, a known amount of TNT and PLGA is dissolved in dichloromethane (DCM) and microspheres were prepared by an oil/water emulsion process using a precision particle fabrication nozzle. Precisely controlled microdrops of a known amount of PLGA/TNT in dichloromethane were injected into a water bath containing a 1% polyvinyl alcohol solution where the microspheres were allowed to cure. After curing, the PLGA/TNT microspheres were separated from the water bath by permitting the spheres to settle and decanting the water. The microspheres were transferred to a smaller vessel and were repeatedly washed with pure water to remove any TNT residue that may be resident on the microsphere surface. After at least three washings, the PLGA/TNT microspheres were dried, bottled, and stored in the refrigerator or in the laboratory.

High Performance Liquid Chromatography Instrumentation

The mass of annealed PLGA-TNT polymer microspheres was determined gravimetrically using a microbalance. The sample was dissolved in known mass of DCM. A size exclusion separation method was developed for the separation of the PLGA and TNT constituents using a Varian* SEC column on a Dionex 3000 Ultimate HPLC with UV/Vis detection. A Varian guard column (PLgel 5 μm) and a SEC analytical column (Varian PLgel 5 μm Mixed-D −7.5 mm id) were used to separate a 20 μL injection of TNT from PLGA in dichloromethane at a flow rate of 1.0 mL min^{-1}. The instrument's column oven and auto-sampler temperatures were set to 20 °C. Detector wavelengths were monitored at (233 and 254) nm. Explosives are known to show a strong absorbance at 254 nm and DCM has an absorbance below 230 nm. Due to this solvent absorbance no measurements below 230 nm were included.

Results and Discussion

Separation Method Development

The first step in quantifying the TNT in the polymer is ensuring baseline separation of the TNT from the PLGA. Figure 1 shows the size exclusion chromatograms of PLGA, TNT, and PLGA/TNT mixture at UV wavelength of 254 nm and 233 nm.
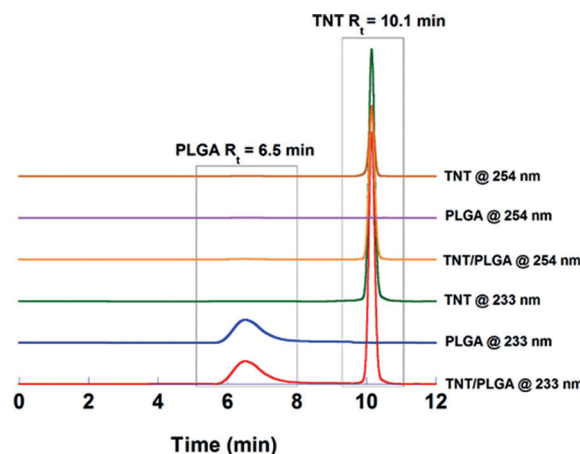

Figure 1. Size exclusion separation of PLGA, TNT, and PLGA/TNT at UV/Vis wavelengths 254 nm and 233 nm. Solvent dichloromethane at flow rate of 1.0 mL min^{-1}. Injection volume is 20 μL.

Since PLGA is a larger molecule, it elutes from the column first followed by the TNT. The broad PLGA peak shape is due the polymer polydispersity. Using either wavelength (233 nm or 254 nm) both PLGA and TNT absorbance peaks are baseline resolved with retention times (R$_t$) of approximately 6.5 min and 10.1 min, respectively. By monitoring the wavelength 254 nm for TNT, we further reduce the possibility for interference from PLGA since PLGA does not absorb effectively at this wavelength.

Analytical Responses and Evaporation Effects

For complete quantification of the microspheres, the individual components need to be accurately measured. Therefore, reliable analytical calibration response curves for TNT and PLGA are needed to quantify the trace explosives in polymer microspheres. We used pure crystalline TNT and PLGA to make the solutions that form the basis of the calibration response curves. All the analytical response curves relate the mass to mass (weight to weight) ratios of analyte (TNT or PLGA) to solvent determined gravimetrically using mass to mass dilutions. It was noticed early on in the experiments that using this approach would be troublesome since DCM is extremely volatile and every time the sample is opened the mass fraction changes due to evaporation loss. Therefore, the evaporation loss of DCM was taken into
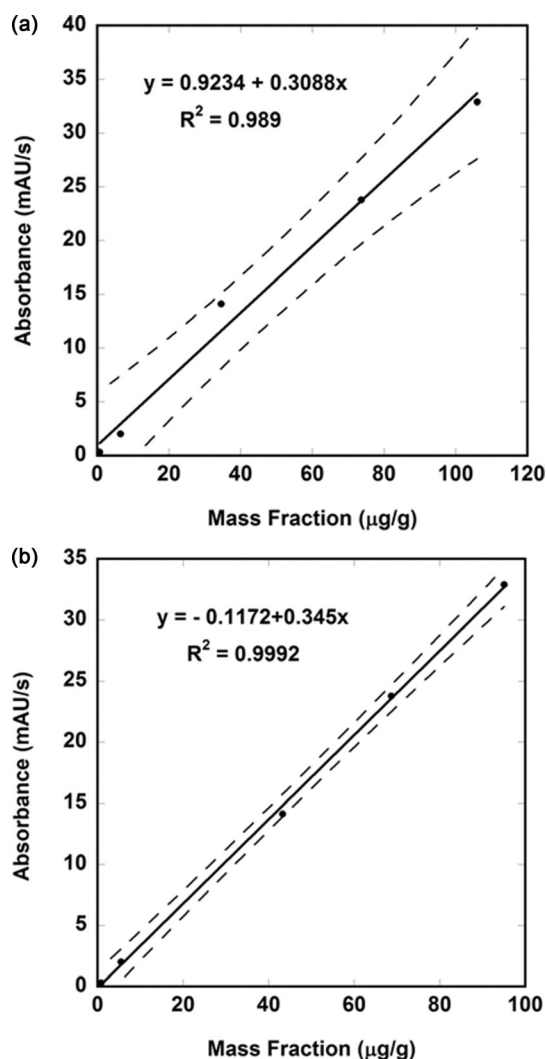
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Figure 2. (a) TNT response curve at 254 nm without evaporation correction. Solvent is dichloromethane at a flow rate of $1.0 \, mL \, min^{-1}$ with injection volume of 20 μL. Absorbance uncertainties (y) are expressed as standard deviation (SD). Uncertainties in the fitted line is expressed by 95 % confidence interval (dashed lines). (b) TNT response curve at 254 nm corrected for evaporation loss. Solvent is dichloromethane at a flow rate of $1.0 \, mL \, min^{-1}$ with an injection volume of 20 μL. Absorbance uncertainties (y) are expressed as standard deviation (SD). Uncertainty in the fitted line is expressed by the 95 % confidence interval (dashed lines).

account by weighing all calibration and analyte vials before, during, and after dilution. Figure 2(a) and 2(b) show the evaporation effect DCM has on the analytical response curves, linear correlation (R^2), and concentration. The data represent triplicate 20 μL injections over the mass fraction range 0.703 to 106 $μg \, g^{-1}$. On the basis of linear regression statistics of the response curves and the use of triplicate solvent blank injections, limits of detection (LOD) = $3\sigma_{blank}/m$ (where σ_{blank} is the standard deviation of the blank values and m is the slope of the response function) for TNT without and with evaporation loss are 69.4 and 62.1 $ng \, g^{-1}$, respectively. The response curve for Figure 2(a) has a linear correlation of 0.989, whereas Figure 2(b) has a linear correlation approaching unity and all datum points on the calibration curve are on the line. The dotted lines represent the confidence intervals for the mass fraction measured values (x values). The confidence intervals for the measured mass fractions are determined according to Ref. [7] and represent 2σ (> 95 %) confidence intervals. Table 1 shows the calculated TNT mass fraction using each calibration curve for nominal 1 % TNT polymer microspheres. As can be seen in Table 1, uncorrected evaporation loss will result in approximately an 8 % systematic bias in the TNT concentration. For relative measurements such as determining the amount of TNT lost or gained by the microspheres, the bias does not affect the calculation as long as the same calibration curve is used. But for actual TNT mass fraction determinations, the evaporative correction is necessary. For all subsequent analyses with the explosive microspheres, the calibration curve from Figure 2(b) is used to calculate percentage loss and lifetime concentrations.

Additionally, in order to completely quantify the TNT in PLGA microspheres in terms of mass fraction, the PLGA species components need to be quantified. Therefore response curves were generated for PLGA using the same methodology as TNT, whereby the response curves take into account the evaporative loss of DCM (shown in Figure 3). The data represent triplicate 20 μL injections over the mass fraction range 16 to 27.8 $mg \, g^{-1}$. On the basis of linear regression statistics of the response curves and the use of triplicate solvent blank injections, limit of detection ($3\sigma_{blank}/m$) for PLGA with evaporation loss is 1 $mg \, g^{-1}$.

## Quantification of TNT in Polymer Microspheres by SEC-UV/Vis

Using the response curves that take into account evaporative loss, quantification of the TNT in the polymer microspheres was achieved. Table 2 presents the TNT concentra-

Table 1. Calculated TNT concentrations (mass fraction of TNT in DCM) for the curves presented in Figure 2(a) and 2(b). The TNT mass fraction is calculated for a constant absorbance value of 32.7 mAU/s^{-1} that corresponds to a nominal 1 % TNT in PLGA. Absorbance uncertainties (y) are expressed as standard deviation (SD). Uncertainties in the mass fraction are expressed at the confidence interval.

	Response curve	TNT mass fraction [μg g^{-1}]
Response curve without evaporation correction	y = 0.3088x + 0.9234	102.9 ± 10.0
Response curve corrected for evaporation loss	y = 0.345x − 0.1172	95.14 ± 2.5

Table 2. Results of SEC-UV/Vis analysis for the nominal 5% and 1% TNT-PLGA microspheres.

Method	Nominal TNT mass fraction [%]	Mass fraction TNT in the microspheres [%]	Standard deviation [%]
SEC-UV/Vis	5	3.5	0.0088
SEC-UV/Vis	1	0.86	0.0034
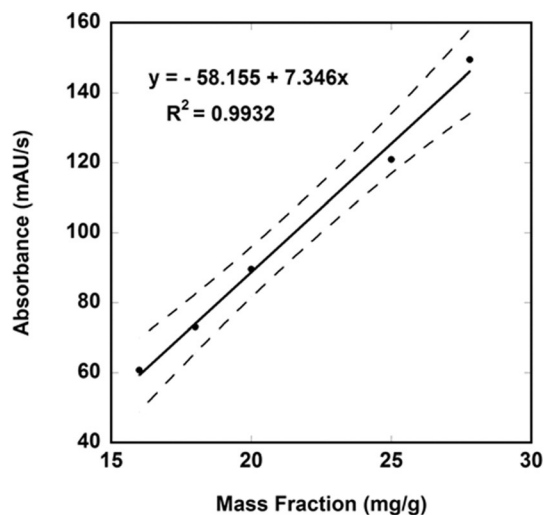
Figure 3. PLGA response curve at 233 nm with evaporation correction. Solvent is dichloromethane at flow rate of 1.0 mL min^{-1} with injection volume of 20 μL. Absorbance uncertainties (y) are expressed as standard deviation (SD). Uncertainty in the fitted line is expressed by the 95% confidence interval (dashed lines).

tions in terms of mass fraction of TNT in the microspheres found by SEC-UV/Vis. The uncertainties correspond to random variation in the measurements (Type A).

Calculations for Determining TNT Loss in Spheres

Early measurements using ion mobility spectrometry and GC-MS indicated that incorporation of TNT into the PLGA microspheres was not complete [5]. Based on calculations during manufacturing of the spheres, it was assumed that 100% TNT would be included in the PLGA during preparation, however from IMS measurements a difference between the calculated and measured values was evident [5]. We believe that the TNT diffuses out of the PLGA microsphere as the microsphere loses solvent and cures into a solid particle. The TNT leaving the microspheres most likely remains dissolved in the 5 L water volume that constitutes the water bath. In order to corroborate this TNT loss, 1% and 5% PLGA-TNT microspheres were analyzed by GC-MS. GC-MS confirmed the loss of TNT from the microspheres [6]. We further confirmed this TNT loss using a mass fraction calculation for the PLGA and TNT developed from response curves generated by SEC/UV/Vis. Using SEC/UV/Vis we were able to simultaneously quantify both the PLGA and TNT in the polymer microspheres. The

method for calculating loss of TNT and the results are presented in the following.

Defining parameters:

MF_{TNT} is mass fraction of TNT in the calibration solution,

M_{TNT} is the measured mass of TNT,

M_{DCM} is mass of dichloromethane,

$MF_{TNTsphere}$ is mass fraction of TNT in the microsphere,

M_{sphere} is mass of sphere(s),

M_{PLGA} is the measured mass of PLGA,

MF_{sol} is mass fraction of TNT in the starting (stock) solution (used to make the microspheres), and

$M_{TNTStock}$ is the measured mass of TNT in the stock solution.

Equation (1) is used for calculating the mass fraction (MF_{TNT}) of TNT *in* the calibration curve. Equation (2) gives the mass fraction of TNT in the microspheres. Equation (3) provides a means to determine the mass of TNT in the starting or stock solution, initially used to make the microspheres. Equation (4) is used to calculate the loss of TNT in spheres from the starting solutions.

$$\text{Calibration solution}: MF_{TNT} = \frac{M_{TNT}}{M_{TNT} + M_{DCM}} \quad (1)$$

$$\text{Mass fraction of TNT in sphere}: MF_{TNTsphere} = \frac{M_{TNT}}{M_{sphere}} \quad (2)$$

$$\text{Mass TNT from solution}: M_{TNT} = \frac{MF_{sol}(M_{DCM} + M_{PLGA})}{(1 - MF_{sol})} \quad (3)$$

gives:

$$\text{TNT loss in sphere(s)}: \%Loss = \left[\frac{(M_{TNTStock} - M_{TNTspheres})}{M_{TNTStock}}\right] \times 100 \quad (4)$$

Using the equations above, the percent losses of TNT in the microspheres were calculated for a series of TNT microspheres (nominally 0.5% TNT microspheres, 0.3% TNT microspheres and 1% TNT microspheres) produced at different times and stored in different locations. The results of the calculated percentage loss of TNT are shown in Table 3 along with the date produced and the storage conditions. Results indicate that approximately 30% of the explosive

Table 3. Results of SEC-UV/Vis and GC-MS analysis for TNT-PLGA microspheres.

Sample	Storage conditions	Measured PLGA MPLGA [µg]	Measured TNT MTNT [µg]	Measured spheres Mspheres = MPLGA + MTNT [µg]	MTNT/ Mspheres
Fabricated stock 0.5% TNT in PLGA microspheres made 6/19/07	Bench top (25 °C)	25955 ± 1500	159 ± 2.5	26114 ± 1500	0.00609
19.6 mg Spheres from Fabricated stock 0.5% TNT in PLGA microspheres made 6/19/07	Bench top (25 °C)	18861 ± 1500	51 ± 2.5	18912 ± 1500	0.00269
Fabricated stock 0.3% TNT in PLGA microspheres made 11/8/11	Refrigerator (1–5 °C)	22789 ± 1500	80.3 ± 2.5	22869 ± 1500	0.00351
5.94 mg from Fabricated stock 0.3% TNT in PLGA microspheres made 11/8/11	Refrigerator (1–5 °C)	6021 ± 1500	14.9 ± 2.5	6036 ± 1500	0.00246
19.6 mg spheres from fabricated stock 0.3% TNT in PLGA microspheres made 11/8/11	Refrigerator (1–5 °C)	18844 ± 1500	47.1 ± 2.5	18891 ± 1500	0.00249
Fabricated stock 1% TNT in PLGA microspheres made 6/8/09	Refrigerator (1–5 °C)	22302 ± 1500	267 ± 2.5	22569 ± 1500	0.01438
18.53 mg spheres from Fabricated stock 1% TNT in PLGA microspheres made 6/8/09	Refrigerator (1–5 °C)	18374 ± 1500	160 ± 2.5	18534 ± 1500	0.00828

was not incorporated during the manufacturing of the microspheres and subsequent loss from the cured microspheres is minimal if kept in the refrigerator (1% and 0.3% TNT microspheres). Table 4 also shows results for the 1%

Table 4. The overall TNT loss from encapsulated PLGA microspheres following manufacturing.

Sample	Percentage TNT loss [%]
19.6 mg spheres from 0.5% TNT stock	56 ± 4.0
5.94 mg spheres from 0.3% TNT stock	30 ± 4.0
19.6 mg spheres from 0.3% TNT stock	29 ± 4.0
18.53 mg spheres from 1% TNT spheres	30 ± 4.0
1% TNT spheres GC-MS	30 ± 3.0

TNT microspheres that were independently verified at NIST using GC-MS [6]. Storage conditions may play a role in the explosive loss. Preliminary results indicate that if the microspheres are kept in the refrigerator then, aside from the initial 30% loss of TNT, no significant loss subsequently occurs. However, if the samples are stored on the benchtop (e.g., 0.5% TNT microspheres) at room temperature there may be loss of the TNT. We are reporting the results for the PLGA/TNT microspheres stored at normal room temperature for completeness of the study. We do not fully understand why this sample has lost more TNT than the refrigerated samples. It should be pointed out that the additional loss is approximately (26 ± 4)% since the microspheres appear to lose 30% of the TNT in the curing process. TNT is one of the more volatile military explosives with a vapor pressure of 7.7×10^{-4} Pa (5.8×10^{-6} Torr) at 25 °C. By comparison, RDX vapor pressure is 6.1×10^{-7} Pa (4.6×10^{-9} Torr) at the same temperature [8]. TNT has a measured sublimation rate of 7.5×10^{-3} ng s^{-1} (0.27 ng h^{-1}) at 25 °C [9]. The rate-limiting step of TNT loss in PLGA would be the diffusion of TNT through the polymer matrix. Once the TNT molecules reach the PLGA surface, they should sublime at

the rate reported above. On going quantification of the percent loss over the lifetime of the microspheres is currently underway.

Conclusions

We have developed an analytical technique using SEC/UV/Vis to quantify TNT in PLGA microspheres that simulate plastic explosives. This technique includes corrections for evaporative losses of the volatile solvent, DCM. Additionally, limits of detection were determined for TNT in the microspheres without and with evaporation loss to be 69.4 and 62.1 ng g^{-1} respectively. Using size exclusion chromatography, the percentage loss and thus TNT not incorporated in TNT-PLGA microspheres was quantified for three polymer microspheres samples manufactured at three different time frames and stored at two different storage conditions (bench top and refrigerator). Results for TNT loss in the microspheres were independently verified by GC-MS and in good agreement between SEC/UV/Vis and GC-MS.

Additional work is needed to quantify other explosives, e.g., RDX and PETN, that have been encapsulated in PLGA as well as to determine loss of explosives over time. We expect similar behavior for the other explosive – PLGA materials and similar approaches to quantifying these materials.

Disclaimer

*Certain commercial equipment, instruments, or materials are identified in this paper to adequately specify the experimental procedure. Such identification does not imply recommendation nor endorsement of the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Acknowledgments

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