Bacillus thuringiensis Detection and Characterization by Normal Raman and SERS at logaritmic and stationary growth phases

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Abstract

Spectroscopic techniques such as Normal Raman (NR) and Surface Enhanced Raman Spectroscopy (SERS) are considered fast, *in situ* alternative methods for identification for microorganisms. The techniques provide important information about the spectroscopic signatures of cellular components of *in vitro* or *in vivo* organisms. The techniques have significant benefits for Industrial Microbiology, Food Microbiology and biological warfare agents detection. The proposed method of this work is the use of vibrational Raman techniques as NR and SERS and to detect bioaerosol particles of *Bacillus thuringiensis (Bt)* employing a fast and simple synthesis of silver colloids based on reduction of silver nitrate with hydroxylamine hydrochloride and sodium citrate including pH changes to modified the surface charge of the nanoparticles (NP) to study the interaction of the NP and the bacteria.

State of the Art

Based on the current status of world wide antiterrorism efforts there is a need to develop effective standoff detection techniques for biological agents. Using spectroscopic techniques the target of this study, *Bt*, will provide a molecular identification of the strand. These gram-positive bacteria are recognized for their toxicity on larvae and are used commercially as insecticides. *B. thuringiensis* was chosen due to its similarity with *B. anthracis* which has a potential of being used during terrorist attacks. Both of these bacteria form spores which are able to tolerate extreme environments and make them suitable for transport before or during a biological attack.

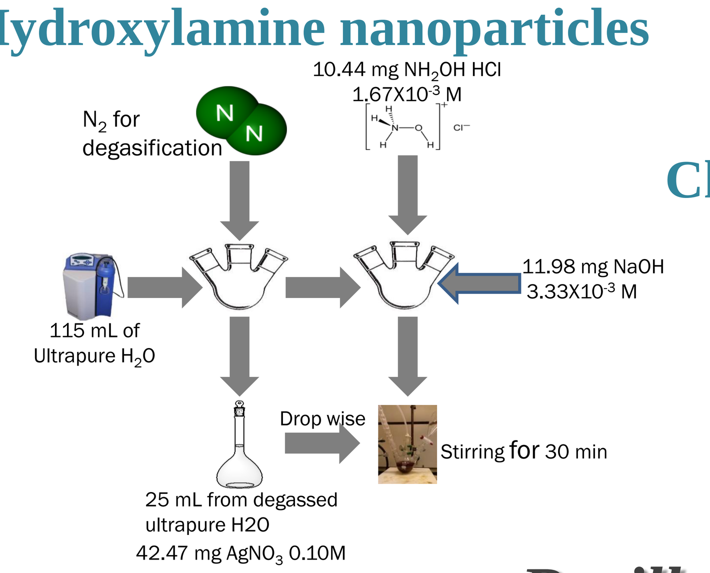
Challenges and Technology Transfer

Normal Raman Spectroscopy (NRS) and Surface Enhanced Raman Spectroscopy (SERS) can be used as quick methods for liquid bacterial detection in suspension and as bioaerosol particles with great interest on standoff detection.

Methodology

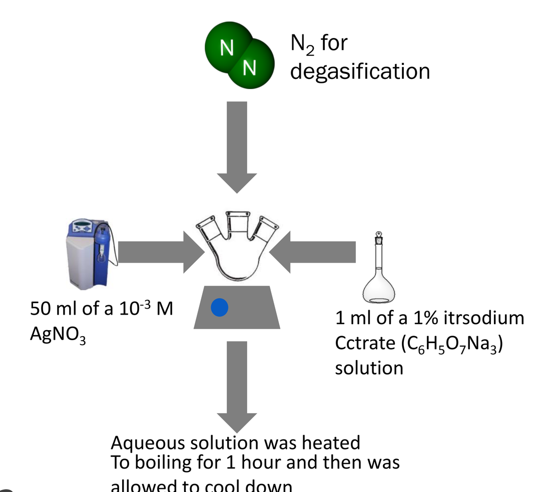
Synthesis of metallic nanoparticles for SERS experiments

Hydroxylamine nanoparticles

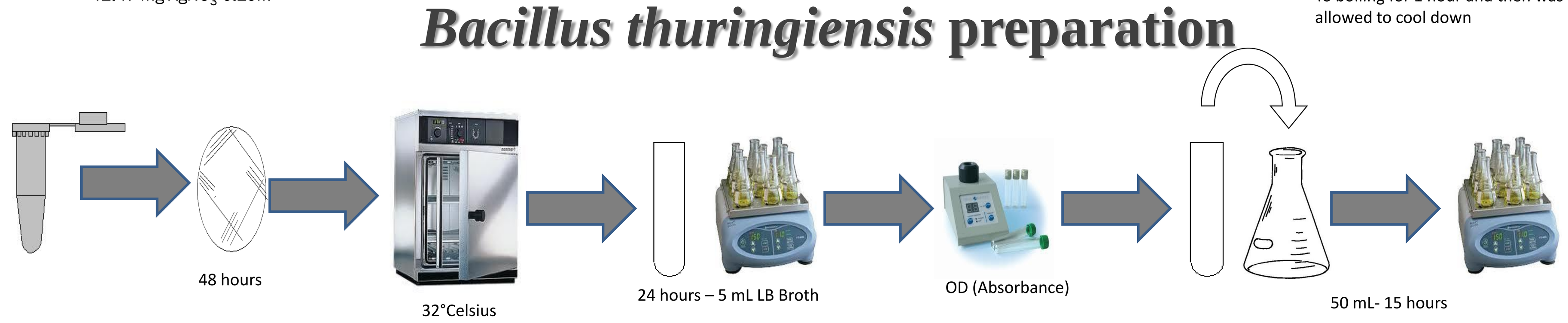


Changes of pH in colloidal nanoparticles

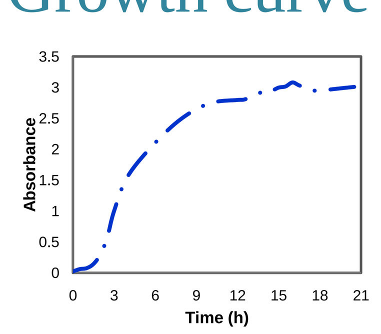
Citrate nanoparticles



Bacillus thuringiensis preparation

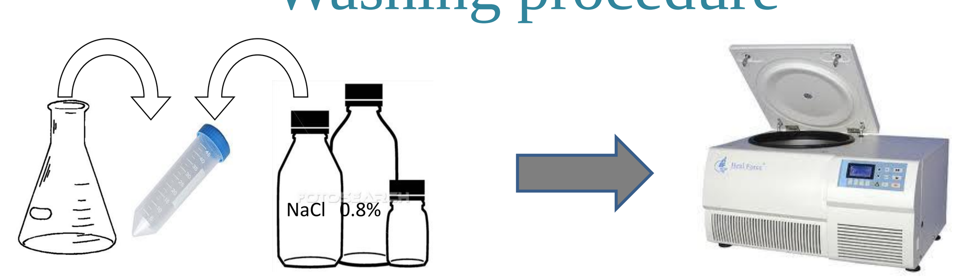


Growth curve



Growth curve of Bt

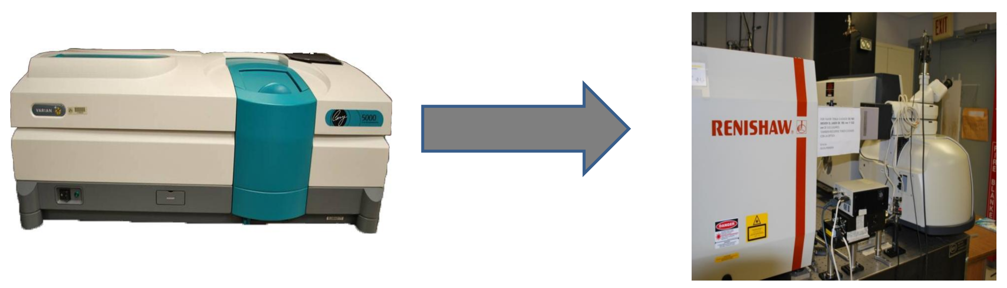
Washing procedure



One sample of 40-50 mL was centrifuged for 20 minutes at 6,000 rpm. A bacterial pellet was formed at the bottom of the microtube, while LB broth media formed the supernatant and was removed using a micropipette. In order to remove the remaining LB broth from the pellets, bacteria was washed with 5 mL of NaCl 0.80% w/v. Bacteria were centrifuged for 20 minutes at 6,000 rpm and the NaCl supernatant was removed with a micropipette. This procedure should be repeated twice. Then, the bacterial pellet is resuspended in NaCl 0.8% w/v to obtain a solution containing the bacterial endospore cells.

Biological sample preparation for Raman experiments

Characterization in UV-VIS and SERS



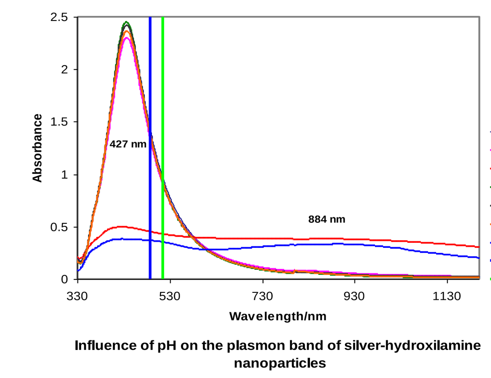
The SERS experiments will be performed in solution and on solid substrates. For experiments in solution sodium chloride will be used as a SERS active substrate. For SERS analysis, 200 µL of the silver colloids, 25uL of *Bacillus thuriengiesis* and 25uL of NaCl 0.8% to get a VR=0.02 to be analyzed will be combined in a vortex vial and experiments will be run at different times until 30-120 minutes

Results

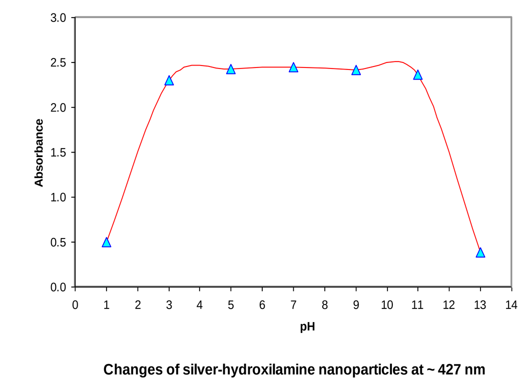
Synthesis of Ag-NP for SERS experiments

Hydroxylamine nanoparticles

Objective: Behavior of the surface hydroxylamine reduced Ag-NP at different pH
Results: Max absorbance: 3-11 pH: very stable Ag-NP



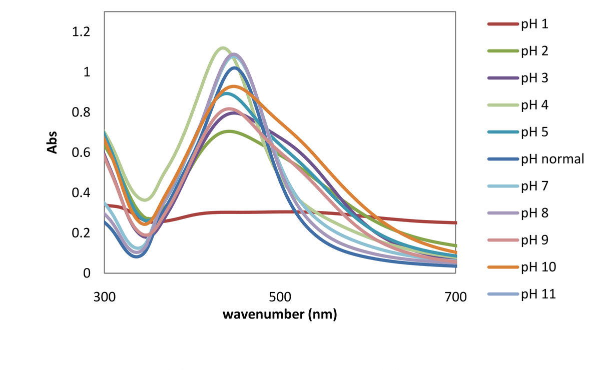
Influence of pH on the plasmon band of silver-hydroxilamine nanoparticles



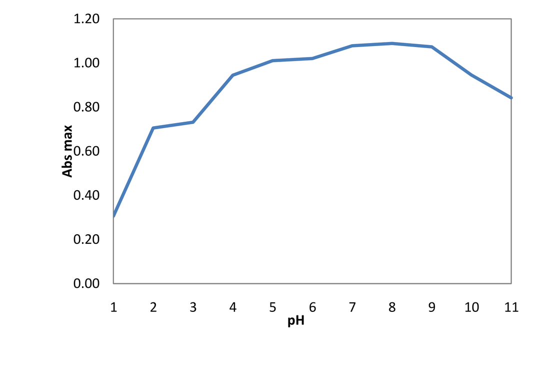
Changes of silver-hydroxilamine nanoparticles at ~427 nm

Citrate nanoparticles

Objective: Behavior of the surface citrate reduced Ag-NP at different pH
Result: Max absorbance: 7-9 pH



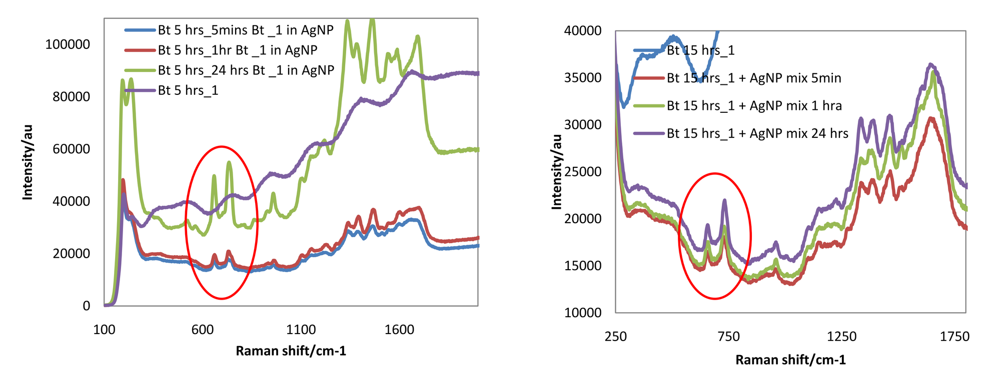
UV-Vis spectra of the citrate nanoparticles at different pH



Maximum absorbance of the citrate nanoparticles at 1-11 pH range

Raman experiments

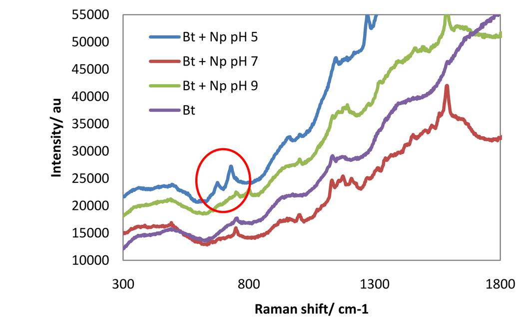
Hydroxylamine reduced Ag-NP



Raman spectra of Bt @ 5 and 15 h using hydroxylamine reduced Ag NP

Assignment	SERS pH 5.82	SERS pH 7.0	SERS pH 9
C-O-C ring deformation guanine (nucleic acid)	730		
	958		
C=C deformation	1146	1149	1149
Amide 3	1220		
amide 2°	1331	1350	1351
=C-O-C= unsaturated fatty acids in lipids	1383	1388	1390
CH_2 deformation	1459	1457	1434
ring stretching (adenine, guanine)	1504	1508	1508
Amide 1	1652	1641	1645

Band assignments of Bt experiments using hydroxylamine reduced Ag NP at different pH



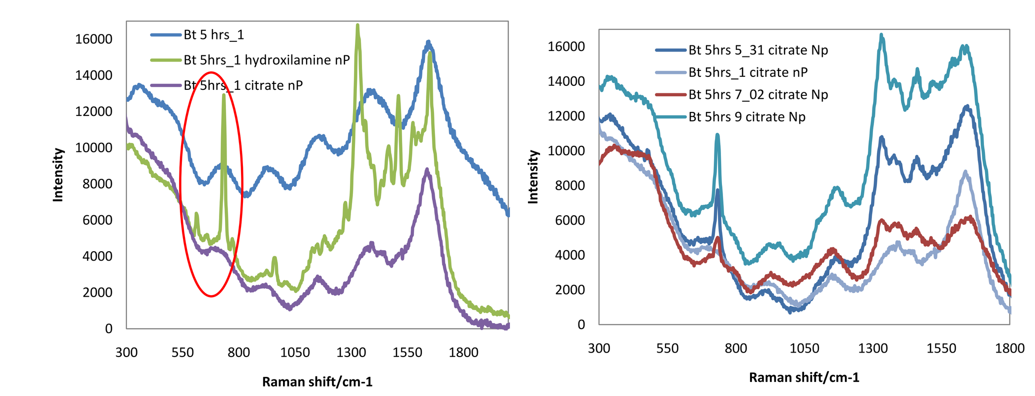
Raman spectra of Bt @ 15 hrs using 5, 7 and 9 pH 's of hydroxylamine Nps

VR=0.02
532 nm
(60-80mW, 10x, 3adq t)

Assignment	NR Bt	SERS 5 mins	SERS 1 hra	SERS 24 hrs
SS stretch cysteine		513		518
C-S methionine		656	657	656
C-O-C ring deformation guanine (nucleic acid)	731	730	731	732
	921	959	959	960
C=C deformation	1131	1146	1144	1145
Amide 3		1219		1222
amide 2°		1336	1330	1332
=C-O-C= unsaturated fatty acids in lipids	1389	1389	1386	1389
CH_2 deformation	1473	1461	1462	1461
ring stretching (adenine, guanine)		1508	1512	1506
Amide 1	1641	1657	1655	

Band assignments (Raman shifts) of the Bt experiments ~15 hrs growth using hydroxylamine nanoparticles at different hours

Citrate reduced Ag-NP



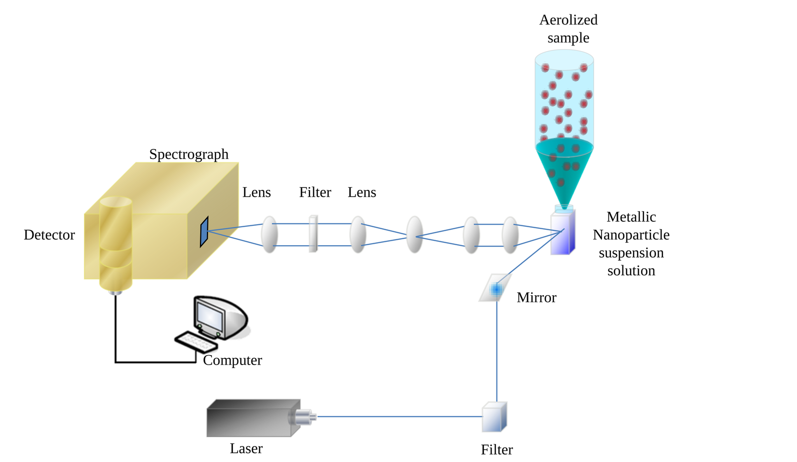
Raman spectra of Bt @ 15 hrs using 5, 7 and 9 pH 's of citrate Nps

Conclusions

Best results:
- Hydroxylamine reduced Ag NP
- 24 h after mix Bt and NP: best signal
- 532nm, 10s, 3 acq, 60-80 mW
- VR = 0.02

Future experiments

Experimental Raman set up for bioaerosol detection



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