



# EDGEWOOD

CHEMICAL BIOLOGICAL CENTER

U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

ECBC-TR-453

## DETERMINATION OF MS2 BACTERIOPHAGE STABILITY AT HIGH TEMPERATURES USING THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

Charles H. Wick  
Ilya Elashvili

RESEARCH AND TECHNOLOGY DIRECTORATE

Patrick E. McCubbin



OPTIMETRICS, INC.  
Bel Air, MD 21015-5203



Amnon Birenzvige  
GEO-CENTERS, INC. - GUNPOWDER BRANCH

December 2005

Approved for public release;  
distribution is unlimited.

20060302 172



ABERDEEN PROVING GROUND, MD 21010-5424

#### Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

# REPORT DOCUMENTATION PAGE

*Form Approved*  
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

<b>1. REPORT DATE (DD-MM-YYYY)</b> XX-12-2005			<b>2. REPORT TYPE</b> Final		<b>3. DATES COVERED (From - To)</b> Sep 2004-Jun 2005	
<b>4. TITLE AND SUBTITLE</b>  Determination of MS2 Bacteriophage Stability at High Temperatures Using the Integrated Virus Detection System (IVDS)					<b>5a. CONTRACT NUMBER</b>	
					<b>5b. GRANT NUMBER</b>	
					<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b>  Wick, Charles H.; Elashvili, Ilya (ECBC); McCubbin, Patrick E. (OPTIMETRICS); and Birenzvice, Amnon (GEO-CENTERS, INC.)					<b>5d. PROJECT NUMBER</b> None	
					<b>5e. TASK NUMBER</b>	
					<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  DIR, ECBC, ATTN: AMSRD-ECB-RT-DD, APG, MD 21010-5424 OPTIMETRICS, INC., 2107 Laurel Bush Road, Suite 209, Bel Air, MD 21015-5203 GEO-CENTERS, INC., Abingdon, MD 21009					<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>  ECBC-TR-453	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>					<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
					<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for public release; distribution is unlimited.						
<b>13. SUPPLEMENTARY NOTES</b>						
<b>14. ABSTRACT</b>  This is the third report in a series of reports that investigates the survivability of the MS2 virus in harsh environments. The first two reports study the effects of pH on the virus. In this report, we study the effect of elevated temperatures on the virus. The stability of the virus was studied by counting the number of virus particles using the Integrated Virus Detection System (IVDS). Test results show that the virus particles are relatively stable for a limited time (up to 0.50 hr) at temperatures as high as 63 °C. Longer exposure or exposure to higher temperatures, results in quick breakdown of the virus particles.						
<b>15. SUBJECT TERMS</b>						
Virus	Virus detection	Integrated Virus Detection System (IVDS )				
Purification	Detection	High temperature stability				
Bacteriophage	MS2	Separation				
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>		<b>18. NUMBER OF PAGES</b>	
<b>a. REPORT</b> U	<b>b. ABSTRACT</b> U	<b>c. THIS PAGE</b> U	UL		15	
<b>19a. NAME OF RESPONSIBLE PERSON</b> Sandra J. Johnson					<b>19b. TELEPHONE NUMBER (include area code)</b> (410) 436-2914	

Blank

## EXECUTIVE SUMMARY

This report characterizes the MS2 bacteriophage sample, MS2D, under conditions of elevated temperatures at various times. The sample was analyzed using the detection stage of the Integrated Virus Detector System (IVDS).

Blank

## PREFACE

This work was started in September 2004 and completed in June 2005.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release. Registered users should request additional copies from the Defense Technical Information Center; unregistered users should direct such requests to the National Technical Information Service.

### Acknowledgment

Special thanks to Dr. Deborah Kuzmanovic, National Institute of Standards and Technology, for supplying the initial stock solution of MS2 bacteriophage.

Blank



## CONTENTS

1.	INTRODUCTION .....	9
2.	EXPERIMENTAL PROCEDURES .....	9
3.	RESULTS AND DISCUSSIONS .....	9
4.	CONCLUSIONS.....	10
	LITERATURE CITED .....	15

## FIGURES

1.	MS2 Initial Sample .....	11
2.	MS2 Initial Sample Showing Fragments at ~14 nm.....	11
3.	Stability of MS2 as a Function of Temperature.....	12
4.	Number of MS2 Particles as a Function of Time at 63 °C.....	12
5.	Survivability of MS2 at 70 °C.....	13
6.	Concentration of Breakdown Product at 63 °C.....	13
7.	Concentration of Breakdown Products at 70 °C .....	14

DETERMINATION OF MS2 BACTERIOPHAGE STABILITY  
AT HIGH TEMPERATURES  
USING THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

1. INTRODUCTION

The stability of viruses under different environmental condition had always been a problem for micro-biologists. Measuring this stability requires subjecting the virus to a harsh environment and monitoring the decay of the number concentration of virus particle with time. Up until recently measuring this stability had been exceedingly difficult. However, recently a new device – the Integrated Virus Detection System (IVDS), which can characterize and measure the number concentration of viruses, had been developed at the U.S. Army Edgewood Chemical & Biological Center (ECBC). The IVDS rely on measuring physical characteristics (size) of the virus instead of bacteriological means. This allows us to measure the number concentration of virus particles quickly. The IVDS is described in ECBC-TR-018. This report is the third in a series of reports that describe studies on the survivability of MS2 Bacteriophage under different environmental conditions. This report discusses the fate of MS2 at high temperatures. Two previous reports (ECBC-TR-472 and ECBC-TR-473) dealt with the fate of MS2 at high and low pHs.

2. EXPERIMENTAL PROCEDURES

A sample of MS2 stock solution (2 ml) was obtained from Dr. Deborah Kuzmanovic of the National Institute of Standards and Technology (NIST). The stock solution was diluted with 100 ml of distilled water and filtered by the Ultra Filtration (UF) subsystem of the IVDS using 100-K Dalton filters (for details on the IVDS and its different subsystems, the reader is referred to ECBC-TR-018<sup>1</sup> and ECBC-TR-468. The filtration system's function is to remove any material with molecular weight smaller than the filtration system is set for (100K Daltons in this case), such as growth media, salt molecules, and proteins from the solution and leave a concentrated virus solution. The concentrated MS2 solution was added to about 20 ml of 20 mM ammonium acetate solution. The ammonium acetate is needed to increase the conductivity of the solution to allow it to be injected into the test module of the IVDS. The MS2 solution was concentrated again by the UF subsystem to a total of 2.5 ml of clean solution. The bacteriophage solution was then subjected to elevated temperatures. Periodically samples were extracted, quenched in an ice bath, and scanned by the IVDS.

3. RESULTS AND DISCUSSIONS

Analysis result of the original MS2 stock solution (after purification and concentration) is shown in Figures 1 and 2. Figure 1 shows the counts of the MS2 at around 24 nm. Figure 2, which is an expended scale of Figure 1, shows a peak at around 14 nm, which is a break down protein of the virus.

Figure 3 shows the number of virus particles counted as a function of temperature. At each temperature the residence time was 10 min. We can see that the number of virus particles decline somewhat (by about 25%) at temperatures of 62 °C-63 °C after 10 min. Further increase of temperature to 64 °C causes a sharp decline in the number of surviving virus particles, and the number of surviving particles continue to decrease as the temperature increased. The unexpected increase in the number of virus particles at 70 °C is probably due to some error.

Figure 4 shows the number of particles counted as a function of time when the MS2 stock solution was exposed to 63 °C. There is some drop in the number of virus particles in the first 30 min. In the next 30 min, there is a sharp drop in the number of particles. This reduction in the number of virus particles continues, until after 2 hr, <10% of the virus particles survive.

Figure 5 shows the number of particles counted after exposure to 70 °C for different time. There is about a 50% decline in the number count after 1 min of exposure. Exposure of up to 7 min does not change the survival rate of the virus particles. However, longer exposure (10 min) results in a sharp reduction in the survival rate.

Figures 6 and 7 show the concentration of lower molecular weight particles respectively in four particle size bins. In both cases, initially, we see a mix of break down products in all four size bins. With time, the number of particles in all four bins diminishes, probably as a result of further breakdown to a molecule of smaller molecular weight.

#### 4. CONCLUSIONS

The Integrated Virus Detection System (IVDS) has proven itself as a useful tool to investigate the stability of virus particles in harsh environments. In this study, we have shown that MS2 can survive for a limited time (up to 30 min) at temperatures as high as 63 °C. Longer exposure or exposure to higher temperatures results in a quick drop in the survivability of the virus particles.

MS2D

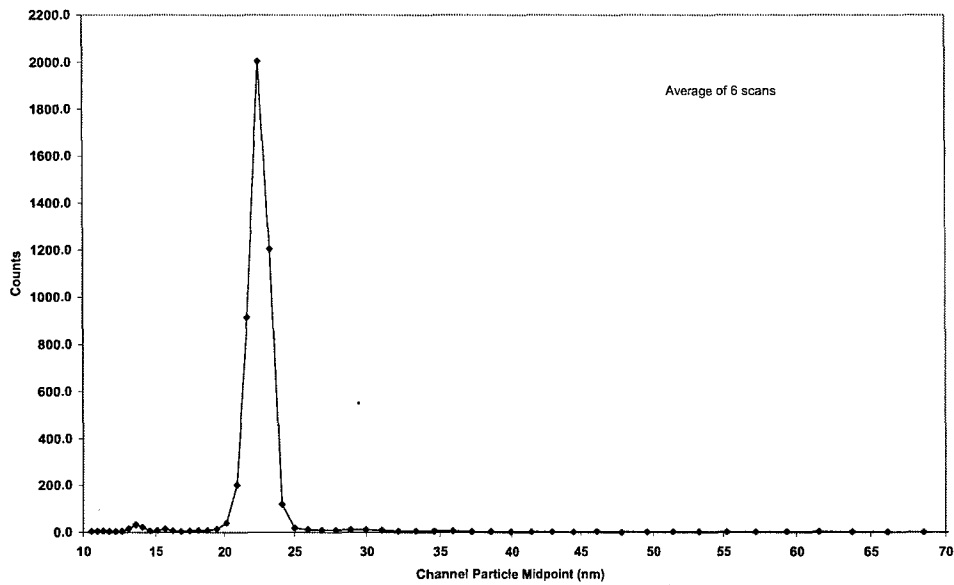


Figure 1. MS2 Initial Sample (6 Scan Average)

MS2D

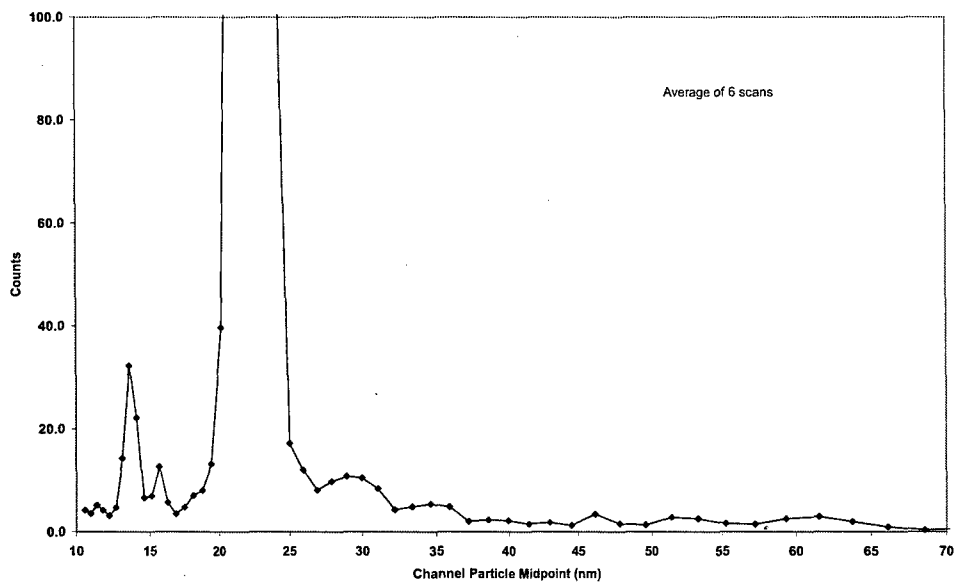


Figure 2. MS2 Initial Sample Showing Fragments at ~14 nm (6 Scan Average)

Stability of MS-2 as a function of temperature

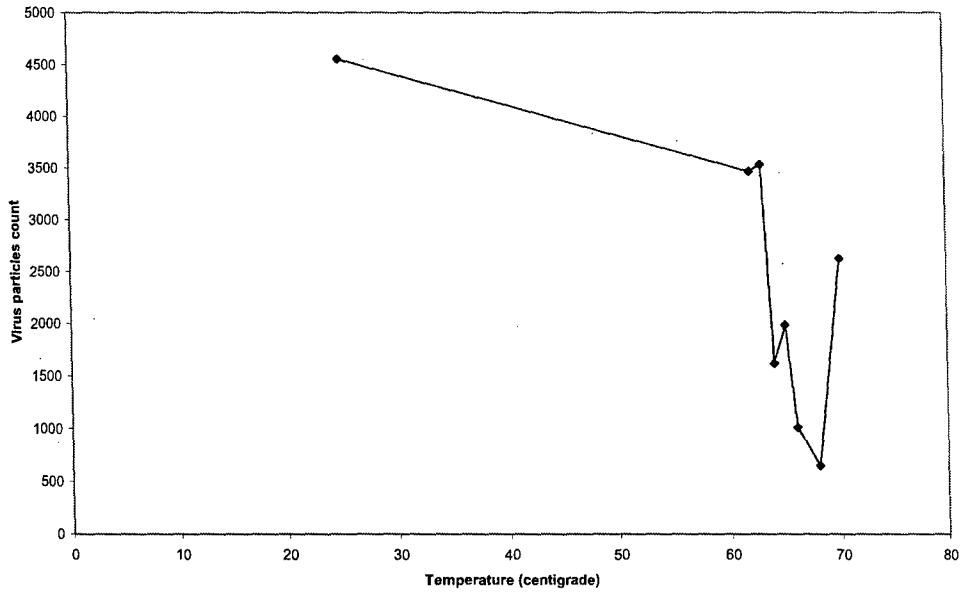


Figure 3. Stability of MS2 as a Function of Temperature

Survivability of MS-2 at 63 degrees (centigrades)

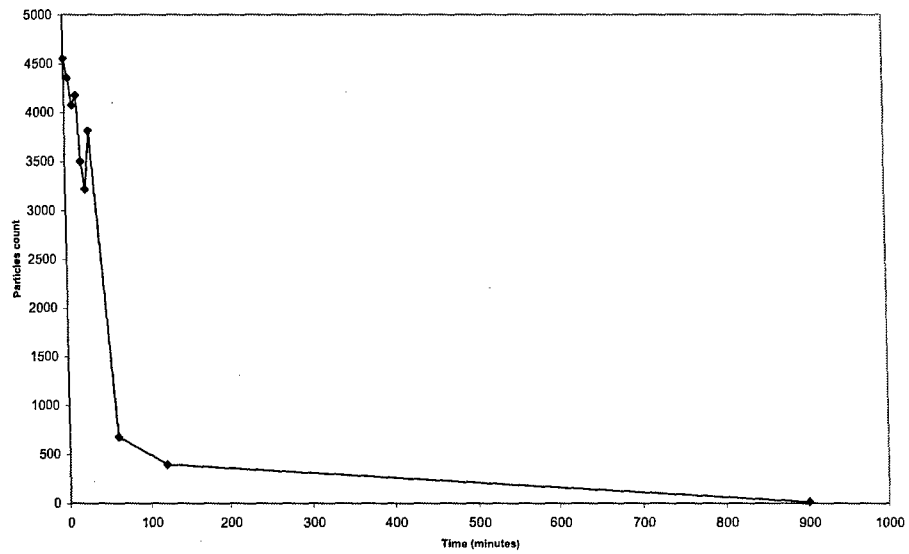


Figure 4. Number of MS2 Particles as a Function of Time at 63 °C

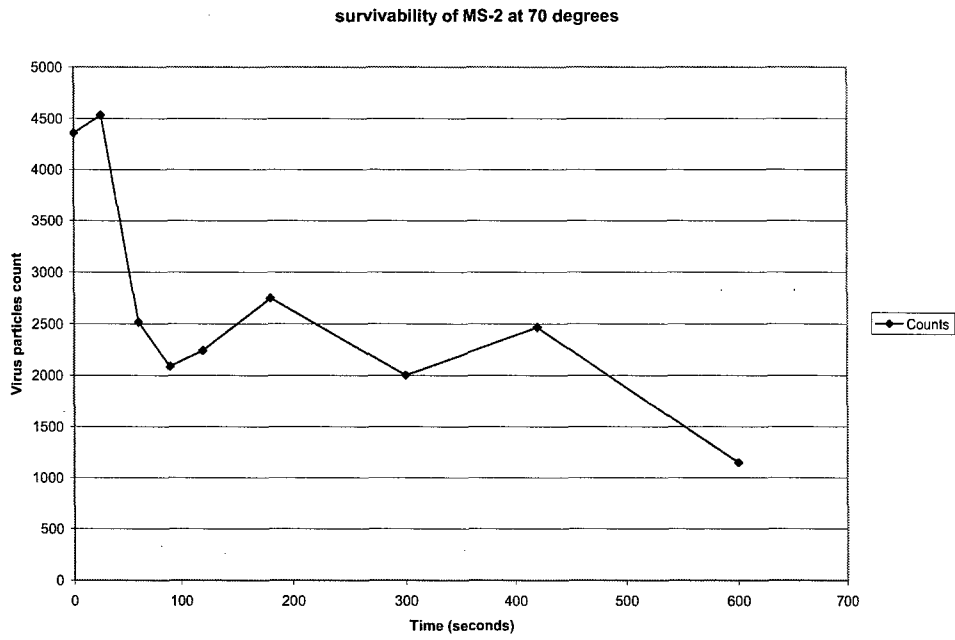


Figure 5. Survivability of MS2 at 70 °C

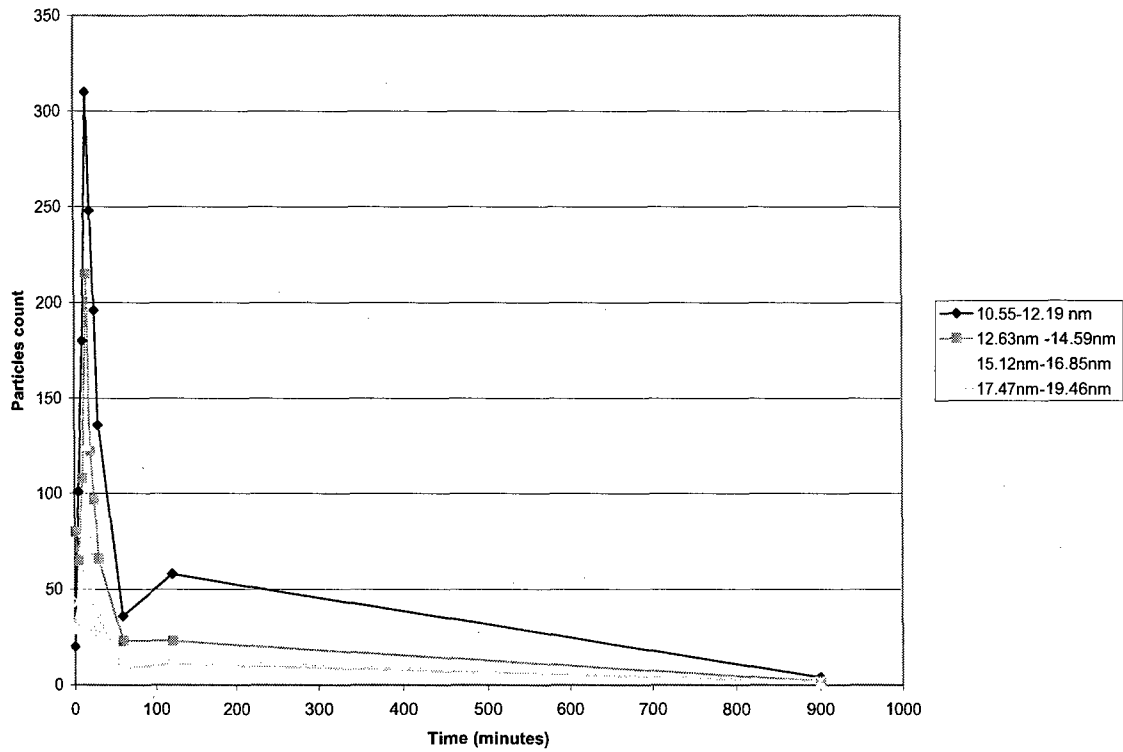


Figure 6. Concentration of Breakdown Product at 63 °C

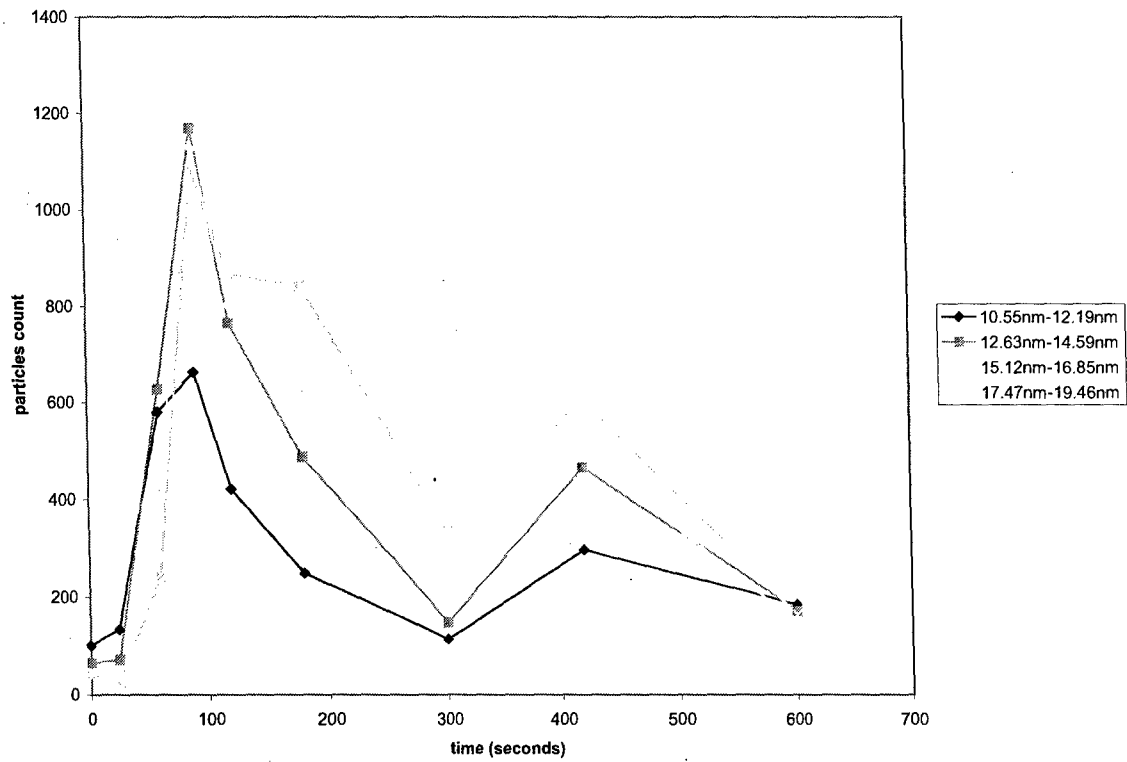


Figure 7. Concentration of Breakdown Products at 70 °C



## LITERATURE CITED

1. Wick, C.H.; Anderson, D.M.; McCubbin, P.E. *Characterization of the Integrated Virus Detection System (IVDS) Using MS2 Bacteriophage*; ECBC-TR-018; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 1999; UNCLASSIFIED Report (AD-364 117).
2. Wick, C.H.; Elashvili, I.; McCubbin, P.E.; Birenzvige, A. *Determination of MS2 Bacteriophage Stability at High pH Using the IVDS*; ECBC-TR-472; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2005; UNCLASSIFIED Report.
3. Wick, C.H.; Elashvili, I.; McCubbin, P.E.; Birenzvige, A. *Determination of MS2 Bacteriophage Stability at Low pH Using the IVDS*; ECBC-TR-473; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2005; UNCLASSIFIED Report.
4. Wick, C.H.; McCubbin, P.E.; Birenzvige, A. *Detection and Identification of Viruses Using the Integrated Virus Detection System (IVDS)*; ECBC-TR-463; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2005; UNCLASSIFIED Report.