

**Preparation, Characterisation and Toxicology  
of Cerium Oxide Nanoparticles**

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## Abstract

Ceria nanoparticles have a range of uses, including industrial polishing of silicon wafers and as a diesel fuel additive, and a proportion of these nanoparticles will be released into the environment. The size of nanoparticles provides the potential for interaction with living cells, as they are similar in size to natural organic matter and biological molecules which regularly interact with cells. *Pseudomonas putida* are bacteria which live in soil, and this work assessed the toxicity of ceria nanoparticles to these organisms.

Suspensions of ceria nanoparticles were prepared and characterised. A batch of nanoparticles was selected for toxicity testing alongside ceria nanoparticles from a commercial source. The bacteria were cultured in Minimal Davis Media and ceria nanoparticle concentrations between  $5\text{mgL}^{-1}$  and  $100\text{mgL}^{-1}$  were used in toxicity trials. The growth of the bacteria was measured over 24 hours, with and without added ceria nanoparticles, by monitoring the increase in turbidity at 595nm in a well plate reader. Four replicate suspensions were prepared for each set of test conditions. Inhibition of bacterial growth due the cerium oxide nanoparticles was evaluated. Commercial ceria inhibited the growth of the *P. putida* bacteria, but the lower concentrations of ceria were shown to be more toxic than the higher concentrations. Ceria nanoparticles prepared in this laboratory showed no toxicity, and appeared to enhance the growth of the bacteria.

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# 1. Introduction

## 1.1 Definition of nanomaterials

The debate about the definition of nanomaterials has ranged between definitions based on material properties (Auffan et al., 2009a) and those based solely on particle size. Nanomaterials have been defined by the European Commission as *"a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm – 100 nm"* (European Commission, 2011).

Some nanoparticles occur naturally in the environment, for example, inorganic particles are formed by weathering of minerals or from ash particles in plumes from volcanoes. Natural nanomaterials also include natural organic matter (NOM), which consists of complex organic macromolecules formed in groundwater and lakes as the decomposition products from plant and animal material (Ghosh et al., 2010).

Incidental nanoparticles arise anthropogenically (Navarro et al., 2008) but are not deliberately produced, occurring in diesel exhaust fumes, plumes from chimneys, and from wear processes, such as particles lost from vehicle tyres.

## 1.2 Manufactured nanoparticles

Manufactured nanoparticles are prepared specifically to make use of their unusual properties and an increasing number of these nanomaterials are used in the production of consumer goods (Limbach et al., 2008) or incorporated into products (Woodrow Wilson, 2011), and medicines (Sanvicens and Marco, 2008; Giljohann et al., 2010).

Woodrow Wilson (2011) includes a regularly updated inventory of consumer products



which contain nanomaterials and is intended to inform consumers and policymakers in the area of nanomaterials. The number of products has been rising steadily since the data collection began in 2005 and analyses of the product data are presented below, showing products by region (Table 1.1). While the USA remains the major producer of these goods, since 2010, the number of European products containing nanomaterials has increased to the extent that they now exceed those from Asia.

**Table 1.1. Number and percentage of consumer goods containing nanomaterials with region of origin, (derived from Woodrow Wilson, 2011)**

<b>Region</b>	<b>Number of products</b>	<b>% of products</b>
US	587	45.5
Europe	367	28.4
Asia	261	20.2
Other	73	5.7
non specific	2	0.2

Consumer products have also been classified by nanomaterial type (Woodrow Wilson, 2011) as shown in Table 1.2. The data was only available for 43% of products, but of these, silver is the most frequently used, often for its antimicrobial properties (Benn and Westerhoff, 2008; Rai et al., 2009). Carbon nanomaterials, used in electrode material, plastics and sports equipment (Understandingnano.com, 2007), and titania, used in paints and sunscreens as an absorber of ultraviolet light (Woodrow Wilson, 2011), are also used in a high proportion of the listed products.

**Table 1.2. Number and percentage of products containing specific nanomaterials, where identified, (derived from Woodrow Wilson, 2011)**

<b>Nanomaterial</b>	<b>Number of products</b>	<b>% of products</b>
Silver	313	55.4
Carbon	91	16.1
Titania/Titanium	59	10.4
Silica	43	7.6
Zinc	31	5.5
Gold	28	5.0

Data on the quantities of nanomaterials used in consumer products or manufacturing processes are not readily available. The quantity of metal oxides used in sun screens and cosmetics world-wide has been estimated at 1,000 tonnes per year for 2005-2010 by Klaine et al. (2008). Hendren et al. (2011) have estimated the production of five classes of nanoparticles (cerium oxide, silver, carbon nanotubes, fullerenes and titanium dioxide) as between 8,000 and 40,000 tons per year in the USA.

### **1.3 Environmental exposure**

It is inevitable that a proportion of the nanomaterials used will end up in the environment (Mueller and Nowack, 2008). Nanoparticles from clothes, cosmetics and sunscreens may enter the natural environment through abrasion in use, or enter the domestic waste water system (Benn et al., 2010; Gottschalk and Nowack, 2011) and can end up either in the effluent which flows into streams or in the sludge which is often spread on the land in Britain. Nanoparticles from outdoor paints have been found in runoff collected from the painted surfaces (Kaegi et al., 2008). Nanoparticles have also been deliberately introduced into the environment for remediation when there has been accidental release of toxic solvents (Benn and Westerhoff, 2008).

#### **1.4 Uptake and toxicity**

The size of nanoparticles provides the potential for interaction with living cells, (Niemeyer, 2001) as they are similar in size to NOM and biological molecules (including proteins and nucleic acids) which regularly interact with cells. The bioavailability of nanoparticles is an important factor in the toxicity of nanoparticles (Klaine et al., 2008), and coatings of NOM, present in natural waters, have been shown to break down agglomerates and stabilise the nanoparticle in suspension (Baalousha et al., 2006). Silver nanoparticles have antimicrobial applications in medical wound dressings and clothing, and silver is also known to be toxic to many aquatic organisms. The toxicity of nanoparticles in the environment has been shown to depend on size and shape of nanoparticles as well as on the type of material, (Kahru and Dubourguier, 2010). In addition, for organisms which can ingest nanoparticles, toxicity may arise from the solubility of particles within the organism (Limbach et al. , 2007).

#### **1.5 Uses of cerium oxide nanoparticles**

In this work, the focus is on cerium oxide nanoparticles. The variable oxidation state of ceria has applications in catalysis, and ceria nanoparticles are used as a diesel fuel additive for reducing the particulates by catalytic oxidation of the soot particles produced during combustion (Woodrow Wilson, 2011). Ceria nanoparticles are also used for polishing architectural glass (Nanophase Technologies Corporation, 2011) and the main industrial application of cerium oxide nanoparticles is in the chemical mechanical polishing (CMP) of silicon wafers (Rodea-Palomares et al., 2011).

## **1.6 Exposure to cerium oxide nanoparticles**

There is a potential for accidental release of ceria nanoparticles into the environment from its various industrial uses. It is known that nanoparticles are treated with surfactants in many of the industrial uses which may reduce particle agglomeration (Limbach et al., 2008) and up to 6% of the ceria nanoparticles in a model waste treatment plant passed into the exit stream from the plant (Rodea-Palomares et al., 2011). In addition to this type of release, ceria nanoparticles, as an additive in diesel fuel, will be released into the atmosphere, and find their way into soil and natural waters by sedimentation or during rainfall.

In 2008 cerium oxide was identified, along with thirteen other manufactured nanoparticles, in the 'Sponsorship Programme on the Testing of Manufactured Nanomaterials' for immediate testing to establish the environmental fate, environmental toxicity and mammalian toxicity, based on commercial use and volume of production (OECD, 2008). The OECD (Organisation for Economic Co-operation and Development) set up the sponsorship programme to help ensure environmental safety and human health, because of the limited data available for risk assessments in these areas. Work in this area has also continued independently of the OECD programme.

### 1.7 Toxicity of cerium oxide nanoparticles

Diverse effects of cerium oxide nanoparticles have been reported for toxicity studies in the published literature, and some publications have identified protective effects of ceria nanoparticles. A summary of the studies is set out in table 1.3.

**Table1.3. Toxicological effect of ceria nanoparticles on cells, showing the organisms tested and their response to the nanoparticles**

<b>Organism</b>	<b>Effect</b>	<b>References</b>
Human lung cells	No observed cytotoxicity	(Park et al., 2008a)
Rat lung cultures	No pro-inflammatory response No change in cell viability	(Fall et al., 2007)
Rat spinal cord neurons	Protection against hydrogen peroxide	(Das et al., 2007)
Mouse heart cells	Suppression of inflammatory processes, gene expression associated with stress inhibited	(Niu et al., 2007)
Human dermal fibroblasts	Cell toxicity DNA damage	(Auffan et al., 2009b)
Male Sprague-Dawley rats	Hepatotoxicity from intratracheal installation	(Nalabotu et al. 2011)
Human lung cells	No change in viability	(Raemy et al., 2011)
Human bronchial epithelial cells	Oxidative stress	(Park et al., 2008b)

Fall et al. (2007), found no pro-inflammatory response, or alteration in cell viability, when cultured slices of rat lung were exposed to cerium oxide nanoparticles in the diesel fuel additive, *Envirox*. Ceria nanoparticles have been suggested as a therapeutic agent for spinal injury (Das et al., 2007) and some measure of protection to the heart was observed in mice, with inhibition of genes associated with stress, and suppression

of inflammatory processes (Niu et al., 2007). Park et al. (2008a cited in Auffan et al., 2009b), reported *in vitro* toxicity testing of *Envirox*, and found that the NOEL (no-observed-effect-level) was three orders of magnitude below the anticipated environmental exposure levels. In contrast, Auffan et al. (2009b) reported DNA damage to human dermal fibroblasts, at ceria exposure levels between two and three orders of magnitude lower than the exposures causing cell toxicity. Liver toxicity was reported in rats whose lungs were exposed to ceria nanoparticles (Nalabotu et al. 2011), showing that transport was occurring through the body. Raemy et al. (2011) reported ceria nanoparticles were taken up by *in vitro* lung cells from the gas phase, and oxidative stress was recorded by Park et al. (2008b) in human bronchial epithelial cell cultures exposed to ceria nanoparticles.

The differences in toxicity may be due to differences in the nanoparticle surface area, shape, size, surface charge or coatings, as discussed by Auffan et al. (2009b), or may be caused by differences in the testing regimes for the particles. This highlights the need for well-characterised nanoparticles, and for consistent test regimes of nanoparticle toxicity testing, if comparable data is to be obtained. An understanding of the mechanisms of interaction between the cells and the nanoparticles will also play an important part in establishing a health and safety strategy for nanomaterials.

### **1.8 Environmental toxicity of cerium oxide nanoparticles**

The effect of particulates on mammals and humans has been studied over decades, and has formed the basis of human nanotoxicology (Stone et al., 2010). The number of studies of the effect of nanoparticles on environmental organisms has increased in recent years, as shown in reviews by Handy et al. (2008) and Ju-Nam and Lead

(2008), but the toxicity data for the effects of cerium nanoparticles in the environment is still limited, as shown in table 1.4, and no overall conclusions can be drawn.

**Table1.4. Toxicology of ceria nanoparticles on environmental organisms, showing the organisms tested, the response to the nanoparticles and the level of exposure producing the effect**

<b>Organism</b>	<b>Effect</b>	<b>Exposure producing effects mg L<sup>-1</sup></b>	<b>References</b>
<i>T. platiurus</i>	No effect	>1,000	(Hoecke et al., 2009)
<i>Daphnia magna</i>	Reproduction inhibition	11.8-42.7	(Hoecke et al., 2009)
<i>Pseudokirchneriella subcapitata</i>	Growth inhibition	7.6 - 28.8	(Hoecke et al., 2009)
<i>Danio rerio</i>	No effect	>200	(Hoecke et al., 2009)
<i>E.Coli</i>	Inhibition of the cell function	1 -230	(Thill et al., 2006)
<i>Pseudokirchneriella subcapitata</i>	Inhibition of the cell function	2.4-29.6	(Rodea-Palomares et al., 2011)
<i>Anabaena CPB4337</i>	Inhibition of the cell function	0.27-6.3	(Rodea-Palomares et al., 2011)
<i>E.Coli</i>	Growth inhibition	50-150	(Pelletier et al., 2010)
<i>B. subtilis,</i>	Growth inhibition	50-150	(Pelletier et al., 2010)
<i>S. oneidensis</i>	No effect	>150	(Pelletier et al., 2010)
<i>E.Coli</i> in normal saline	Reduction in viability	20-200	(He et al., 2012)
<i>E.Coli</i> in phosphate buffered saline	No effect	>200	(He et al., 2012)
<i>Caenorhabditis elegans</i>	Lifespan reduction	1.72 x 10 <sup>-4</sup> -1.72 x 10 <sup>-2</sup>	(Zhang et al., 2011)

Hoecke et al. (2009) investigated the effect of these nanoparticles on three different aquatic organisms: daphnia (*Daphnia magna*), green algae (*Pseudokirchneriella subcapitata*) and embryos of zebrafish (*Danio rerio*). Some level of growth reduction was seen with nanoparticle concentration, and this effect was seen to be related to the surface area of the nanoparticles, rather than to the mass used. This may imply that the mechanism of the cytotoxicity depends on the degree of surface contact between the nanoparticles and bacteria. The toxicity of ceria nanoparticles to *E. Coli* bacteria was evaluated by Thill et al. (2006). The ceria nanoparticles were found to be positively charged in the solutions used in this study with neutral pH, and were strongly attracted to the negatively charged bacteria, forming a surface coating. The toxicity of the nanoparticles to the bacteria was dependent on physical contact between the cell wall and the particle, and occurred within a short time of adsorption. Rodea-Palomares et al. (2011) tested the toxicity of ceria nanoparticles to green algae (*P. subcapitata*) and to *Anabaena CPB4337* bacteria. A 50% inhibition of the cell function ( $EC_{50}$ ) was found at levels of between 0.27 and 6.3 mg L<sup>-1</sup> of nanoparticles for the bacteria, and at the higher levels of 2.4-29.6 mg L<sup>-1</sup> for the green algae. No nanoparticle uptake was observed in either organism, but the contact with the particles appeared to cause damage to the cell walls, possibly by production of reactive oxygen species (ROS) at the nanoparticle surface. An unexpected feature was the coating of the negatively charged bacteria with nanoparticles with negative surface charge. Cerium oxide nanoparticles, tested on three different bacteria, inhibited the growth of *Escherichia Coli* (*E. Coli*) and *B. subtilis*, whereas *S. oneidensis* was not affected (Pelletier et al., 2010), showing that it is not possible to generalise the effect of the nanoparticles without considering toxicity mechanisms.



The authors He et al. (2012) carried out a study to establish the effect of different media on the surface charge of the ceria nanoparticles and the subsequent effect on the toxicity of the ceria, using *E. Coli* in normal saline (NS) and phosphate-buffered saline (PBS). The ceria nanoparticles were positively charged in NS and negatively charged in PBS. The effect of *E. Coli* in the presence of ceria nanoparticles was measured, and a significant reduction in viability was seen in NS, accompanied by increased ROS levels in the bacteria, but no changes were measured with ceria nanoparticles in PBS. TEM images showed contact between bacteria and particles in NS, whereas in PBS zones without particles were seen around the bacteria, indicative of electrostatic repulsion between particles and cells in PBS. A mechanism was proposed where the nanoparticle contact with the cell membrane increased the ROS in the cell with resultant cell wall damage, and reduced viability. The electrostatic charge on the nanoparticle surface would determine whether contact between the cells and particles could occur.

With the risk of increasing levels of ceria nanoparticles entering the environment, Zhang et al. (2011) investigated the effect of ceria nanoparticles at environmentally realistic concentrations on the nematode, *Caenorhabditis elegans*. Ceria nanoparticles have the potential to undergo redox cycling, generating ROS, and the nematode was selected because it is sensitive to oxidative stress. It was found that the lifespan of the nematode was reduced at all levels of ceria nanoparticles tested, producing a 12% reduction in lifespan at the lowest exposure levels, which rose with increasing exposure levels. The levels of oxidative degeneration indicated that ageing was accelerated by the presence of cerium oxide nanoparticles. The exposure levels in this work, reflecting potential environmental exposure, were three or more orders of

magnitude lower than other investigations of environmental exposure (See table 1.4), and showed significant effects on the organism under examination.

### **1.9 Preparation of cerium oxide nanoparticles**

A wide range of methods have been used to prepare ceria nanoparticles, including laser processing and milling to reduce particle size (Ju-Nam and Lead 2008), co-precipitation (Chen and Chang, 2004), sol-gel methods (Rossignol, et al., 1999), emulsion methods (Lee et al., 2005) spray pyrolysis (Kang et al., 2006), micro-emulsion methods (Zhang et al., 2001), and hydrothermal synthesis (Wu et al., 2008; Lu et al., 2009).

Hydrothermal synthesis, as defined by the *Royal Society of Chemistry (RSC Publishing, 2012)*, is a method of growing crystals from aqueous solution at high pressure and temperature. The reaction chamber is inside a sealed pressure vessel, and as the temperature is raised and water vapour is formed, the pressure rises inside the vessel. This has the effect of increasing the boiling point of the water inside the reaction chamber, allowing the temperature of the solution to be raised above 100°C (the boiling point for water at atmospheric pressure). The solubility of chemicals in water changes with temperature, and material not normally soluble in water may dissolve at the higher temperatures and pressures available with hydrothermal synthesis. Crystal formation occurs as the temperature of the solution is lowered and the solubility of the material decreases. Hydrothermal synthesis has been used to prepare ceria nanoparticles in a variety of shapes and sizes as shown in table 1.5 and, because of this versatility, it was selected as the synthesis method for this work.

**Table1.5. Ceria nanoparticles, produced by hydrothermal synthesis, showing typical size and shape, precursors and hydrolysis reagent**

Shape	Precursor	Hydrolysis	Typical Size /nm	Reference
Spheres	Nitrate	PVP	50	(Zhou et al., 2007)
Cubes	Nitrate	NH <sub>4</sub> OH	10	(Taniguchi et al., 2011)
Cubes	Nitrate	NaOH	30	(Wu et al., 2008)
Wires	Chloride	NaOH	500	(Wu et al., 2008)
Cubes	Nitrate	NaOH	36	(Mai et al., 2005)
Rods	Nitrate	NaOH	13	(Mai et al., 2005)
Cube	Nitrate	NaOH	55	(Yang et al., 2007)
Rods	Nitrate	NaOH	20	(Yang et al., 2007)
Cubes	Nitrate	NH <sub>4</sub> OH	12	(Wang et al., 2010)
Rods	Chloride	NH <sub>4</sub> OH	40	(Wang et al., 2010)
Triangular plates	Nitrate	Carbamide	400	(Guo et al. , 2006)
Polygonal	Nitrate	NaOH	20	(Tan et al., 2011)

### 1.10 Nanoparticle suspensions

Suspensions of nanoparticles are required for a number of the characterisation techniques for nanoparticles, and for toxicological evaluation (Handy et al., 2008), but the material produced by hydrothermal synthesis is frequently in the form of precipitated, agglomerated crystals.

Particles which have higher specific gravity than the surrounding liquid in a suspension have a tendency to form a sediment. Because of their small size, convection in the liquid and Brownian motion can provide sufficient energy for nanoparticles to overcome gravitational forces (Shaw, 1992 p 21). Nanoparticles, because of their small size and high surface area to volume ratio, have high surface

energy and, consequently, a strong tendency to agglomerate (Ju-Nam and Lead, 2008).

Van der Waals forces describe the electrostatic attraction between molecules or small particles with permanent or induced electric dipoles or permanent charges. For molecules or particles without a permanent dipole, London (1930) (cited in Shaw, 1992), described the intermolecular forces as arising from the spontaneous generation of short-lived charge separation. The London forces, described in equation 1.1 below, form almost all of the van der Waals forces between nanoparticles at small distances, unless the material is highly polar (Shaw, 1992 p 216).

$$V_A = -Aa/12H \quad \text{Equation 1.1}$$

where

$V_A$  = London dispersion interaction energy, *in vacuo*,

$a$  = radius of the particles,

$H$  = separation of the particles, and

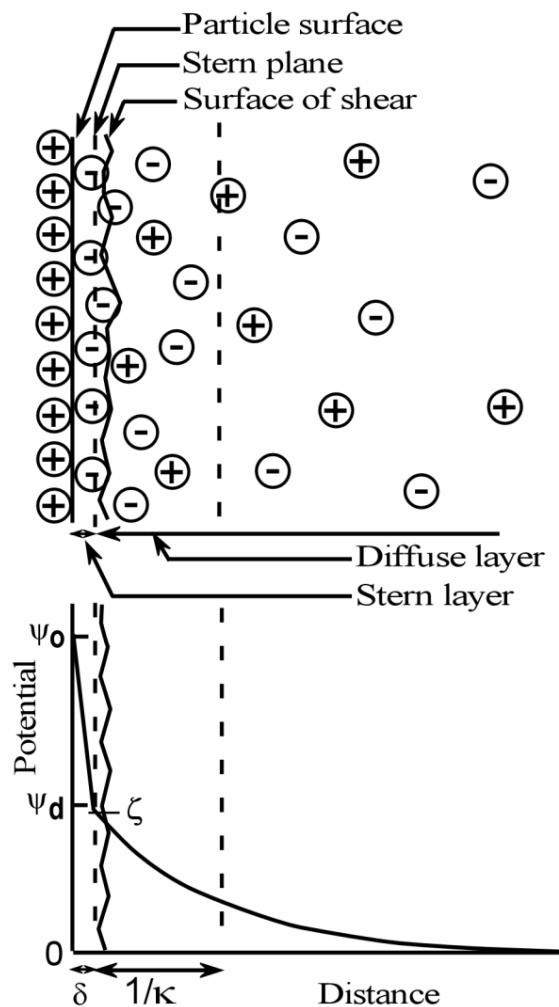
$A$  = Hamaker constant.

The London dispersion interaction energy,  $V_A$ , can be calculated using published values for the Hamaker constant for a range of particles and suspending fluids (Visser, 1972, and associated references). In the absence of additional stabilisation, the attractive forces described by London will cause particles to agglomerate. Coatings on particles, introducing surface charges and steric factors, have commonly been used to stabilise nanoparticle suspensions.

### 1.10.1 Charge stabilisation

The attractive forces between particles can be modified if a surface charge is built up on the particles. This can arise from ions or dipoles, formed at the particle surface or dissolved in the suspending medium. An electric double layer forms when ions of the opposite charge are attracted to the charges formed on the particle surface. This charged layer will prevent particles of the same charge from approaching the nanoparticle, by electrostatic repulsion (Shaw, 1992 p 178).

**Figure 1.1. Stern plane and electric double layer**



*Shaw (1992) p183, re-drawn*

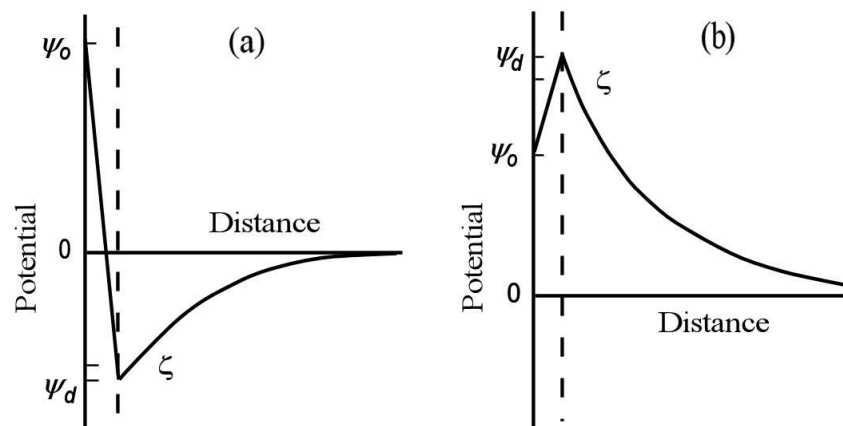
Stern (1924, cited in Shaw, 1992 p181) proposed a model for the electric double layer, shown in figure 1.1. The Stern plane, situated just beyond the radius of a solvated ion from the particle surface, defines the region of adsorbed ions which are firmly attracted to the particle and not disturbed by thermal vibrations. Beyond the Stern plane, the ions in the outer half of the double layer form a more diffuse, dynamic layer.

As seen in figure 1.2, with counter ions adsorbed on the surface, the potential at the surface,  $\Psi_0$ , changes rapidly through zero to give  $\Psi_d$ , the potential at the Stern plane, the opposite sign from  $\Psi_0$ . The potential then falls to zero as the distance from the surface increases. When ions of the same charge are adsorbed on the surface, the potential increases from  $\Psi_0$  to  $\Psi_d$ , then falls to zero as the distance from the Stern plane is increased.

**Figure 1.2. Potential changes with adsorption of ions on particle surface.**

**(a) Counter-ion adsorption causes charge reversal.**

**(b) Co-ion adsorption causes charge increase.**



The theory of colloidal suspension stability was developed independently by Verwey and Overbeek (1948, cited in Takeo, 1999) and Derjaguin and Landau (1941, cited in Takeo, 1999), and the two approaches are now combined in DLVO theory (Takeo, 1999 p 219). The balance between the electrostatic repulsive forces and the van der Waals forces of attraction results in the formation of a charge stabilised colloid.

Takeo (1999 p220) used DLVO theory to calculate the free energy of the interaction between two spherical particles,  $\Delta G$ , as shown in equation 1.2.

$$\Delta G_T = -Aa/12H + ((64n^0 k_B T) / \kappa^2) \pi a \gamma^2 e^{-\kappa H} \quad \text{Equation 1.2}$$

where  $\Delta G_T$  = total interaction free energy,

$n^0$  = number of ionic species/unit volume,

$k_B$  = Boltzmann constant,

$T$  = temperature in Kelvin,

$\kappa$ , = Debye-Hückel screening parameter, and

$\gamma$  is defined in equation 1.3

$$\gamma = \tanh(ze\Psi_0/4k_B T) . \quad \text{Equation 1.3}$$

where  $z$  = formal charge number for the ion,

$e$  = unit of electrical charge,

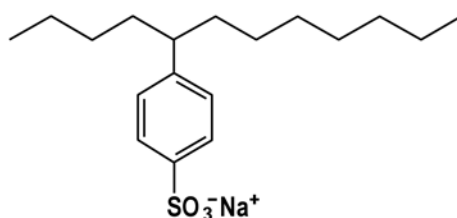
$\Psi_0$  = surface potential.

Zeta potential measurements more positive than +30mV or more negative than -30mV usually indicates charge stabilisation in nanoparticle suspensions (Malvern

Instruments, 2005). The difference in density between the particle and the suspending medium, however, will also have an influence on the sedimentation of the particles.

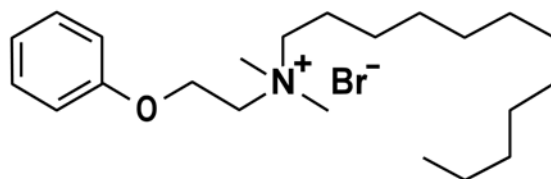
Charge stabilisation can be developed by ionic surfactants which have a hydrophilic component and a hydrophobic organic group. The surfactant is ionised when dissolved in water (Jódar-Reyes et al., 2006), and the hydrophobic part, with its associated charge, can be adsorbed onto particles to stabilise the suspension. Sodium dodecylbenzenesulfonate, (figure 1.3) is a commonly used anionic surfactant, and dodecyltrimethyl-2-phenoxyethyl ammonium bromide, (figure 1.4) is an example of a cationic surfactant. Charge stabilisation can also occur at high or low pH with  $\text{H}_3\text{O}^+$  or  $\text{OH}^-$  ions from the aqueous medium forming the electric double layer.

**Figure 1.3. Structure of sodium dodecylbenzenesulfonate.**



[http://upload.wikimedia.org/wikipedia/commons/f/fa/Sodium\\_dodecylbenzenesulfonate.png](http://upload.wikimedia.org/wikipedia/commons/f/fa/Sodium_dodecylbenzenesulfonate.png)

**Figure 1.4. Structure of dodecyltrimethyl-2-phenoxyethyl ammonium bromide.**



<http://www.chemblink.com/products/538-71-6.htm>



Nanoparticles are often prepared in the presence of capping agents which coat the nanoparticle as it forms and so prevent agglomeration. Sodium citrate is a commonly-used, charge-stabilising capping agent, which has been used extensively in this project. The citrate ion is shown in figure 1.5.

**Figure 1.5. The citrate ion.**



<http://en.wikipedia.org/wiki/File:Citrate-2D-skeletal.png>

Charge stabilisation is dependent on the charged species on the particle surface, which means that if the ionic strength or pH of the medium surrounding the particle is altered, the charge on the particle surface can be changed, and this can have a destabilising effect on the particle suspension (Handy et al., 2008).

### **1.10.2 Steric stabilisation**

Steric stabilisation occurs when molecules with long hydrophilic chains (Christian et al., 2008) such as manufactured polymers or natural organic matter present in environmental water (Petosa et al., 2011), coat nanoparticle surfaces and prevent nanoparticles from aggregating. Takeo (1999 p 226) explained that when long polymer chains are extended into the suspending medium, the chains are free to rotate with the Brownian motion of the solvent. Flory (1969, cited in Takeo,1999) , who developed the theory of polymers in solution, discussed this in terms of a radius of

gyration of the polymer chain. Two mechanisms contribute to the repulsive forces between the polymer chains. When the polymers approach each other, the solvent is displaced between the chains, and osmotic pressure is a driving force to restore solvent to this region (Takeo, 1999 p 227). In addition to this, the entanglement of polymer chains restricts the free rotation of the polymer, resulting in a loss of entropy in the system as the conformations the polymer can adopt are restricted. Because of the wide range of conformations that a long polymer chain can adopt, the entropy of the system is a large component in the free energy of the system, and restoration of this entropy is the driving force in steric repulsion.

Because there are no charges involved in this process, the sterically stabilised suspensions are usually insensitive to the presence of ions. Sterically stabilised nanoparticles are likely to have more stability in natural waters which contain dissolved salts.

### **1.10.3 Electrosteric stabilisation**

Electrosteric stabilisation can occur when long chain molecules also have a charge (El Badawy et al., 2010), and characteristics of both steric and charge stabilisation are present. Natural organic matter (NOM) consists of complex organic molecules, derived from the decomposition of flora and fauna (Ghosh et al., 2010). These molecules form coatings on material they come into contact with, and can be used to stabilise nanoparticles. In neutral conditions and at high pH, dissociation of the carboxylic acid groups occurs and the molecules develop a negative charge (Baalousha et al., 2006). This can produce electrosteric stabilisation of particles in suspension with NOM.

A range of different surfactants, with varying characteristics, were investigated in the preparation of stable suspensions from the particles produced for this work, and the results will be presented in a later section.

### **1.11 Nanoparticle characterisation prior to toxicity testing**

When considering the environmental impact of nanoparticles, the toxicity may be influenced by a range of different characteristics of the nanomaterial. Oberdörster et al. (2005), Handy et al. (2008) Domingos et al. (2009) and have all recognised the need for well-characterised materials and standardisation in test procedures for nanomaterials used in toxicity testing. Conditions close to predicted environmental exposure were also recommended (Stone et al., 2010).

Two papers characterising commercial ceria nanoparticles have been published by Baalousha et al. Part one described size measurements (Baalousha et al., 2012a) using TEM, AFM, DLS, BET (Brunauer, Emmett and Teller) surface area measurements and X-ray diffraction. The authors tabulated the characteristics of each measurement technique, and discussed the limitations of the data. The sample preparation methods were carefully selected to minimise artefacts. Good agreement was obtained for the primary particle size and agglomerate size between all these measurements, although TEM was the only technique where both the aggregate and primary particle size could be measured. Measurements of characteristics other than size were discussed in part 2 (Baalousha et al., 2012b), as shape, crystal structure, composition and surface charge may all influence nanoparticle toxicity. A range of techniques, with their advantages and limitations were discussed. The applicability of models used for zeta potential measurements was also detailed. Recommendations

were set out for the characterisation of nanoparticles prior to environmental toxicity evaluation. The different characterisation methods used in this work are described in the next chapter.

Roebben et al. (2011), published a comparison between laboratories of nanoparticle size and surface charge measurements of cerium oxide nanoparticles for characterisation before biological testing. Intensity measurements for particle size by DLS are very sensitive to polydispersity, with results heavily weighted towards a small number of large particles in suspension, and some laboratories reported significantly different results for the particle size. After sonication to break down aggregates, both DLS and zeta potential results were more variable. Differences between the ultrasonic baths were believed to be responsible for some of the observed variation.

### **1.12 Experimental work undertaken**

The limited work on environmental exposure to ceria nanoparticles and the variations in the observed effects mean that there is a need for studies with well-characterised nanoparticles on organisms in the environment, to develop a broader understanding of the nanoparticle properties which influence the environmental toxicity. The aim of this work is to prepare, disperse and characterise ceria nanoparticles for comparative toxicological studies on bacteria.

Cerium oxide nanoparticles were prepared by hydrothermal synthesis. The toxicity of these particles to bacteria were compared with that of commercially available cerium oxide nanoparticles, as characterised by Baalousha et al. (2012a; 2012b).

*Pseudomonas putida* bacteria were selected to evaluate the toxicity of ceria nanoparticles. These are gram-negative, rod-shaped bacteria with one or more polar flagella for motility (MicrobiologyBytes, 2012) which are commonly found in soil and watercourses. They feed on organic matter from dead organisms, but are non-pathogenic. These bacteria are of great interest for remediation work, as they can break down a wide range of toxic organic molecules in the soil (US Department of Energy, 2012).

### **1.13 Hypothesis**

The toxicity of ceria nanoparticles to bacteria is to be tested, as set out in the hypothesis below.

**1H<sub>0</sub> Cerium oxide nanoparticles are not toxic to *Pseudomonas putida* bacteria.**

**1H<sub>a</sub>. Cerium oxide nanoparticles are toxic to *Pseudomonas putida* bacteria.**

## 2. Methodology

### 2.1 Preparation methods

#### 2.1.1 Chemicals

The chemicals used to prepare nanoparticles, the purity and the supplier are listed in Table 2.1.

**Table 2.1. List of chemicals used in sample preparation, with purity and supplier**

<b>Chemical</b>	<b>Purity</b>	<b>Supplier</b>
Cerium(IV) oxide	Nanopowder <25nm particle size (BET)	Aldrich
Cerium(III) nitrate hexahydrate,	99%	Aldrich
Cerium(III) chloride heptahydrate,	99.9%	Aldrich
Ammonium hydroxide	Analytical reagent	Sigma Aldrich
Sodium hydroxide	Analytical reagent	Fisher Scientific
Ammonium chloride	99.5%	Fluka
Trisodium citrate dihydrate	> 99% < 0.001% heavy metal	Sigma-Aldrich
Polyvinyl pyrrolidone, 10K	11.5-12.8% nitrogen	Sigma Aldrich
Suwannee River Fulvic Acid	Standard material	IHSS
Sodium dodecyl sulphonate	> 99%	Fisher Scientific
Tween 80	A mixture of oleic and other organic acids	Sigma Aldrich
Dipotassium phosphate	ACS $\geq$ 98%	Sigma-Aldrich
Monopotassium phosphate	>99.0%	Sigma
Sodium citrate	>99.5%	Sigma
Magnesium sulphate	Analar	Normapur
Ammonium sulphate	$\geq$ 98%	Sigma-Aldrich
Glucose	Analytical reagent	Fisher Scientific

### 2.1.2 Cleaning of plastic and glassware

All plastic and glassware used in the preparation of nanoparticles was washed in water, and then soaked in 10% nitric acid for 16 hours to remove metal ions and organic contamination from the glass surface. The glass was then rinsed five times in water and dried in air before use.

### 2.1.3 Acid Digestion Vessel

**Figure 2.1. Structure of the 4748 Acid Digestion Vessel**



[www.parrinst.com](http://www.parrinst.com) Redrawn by M.S. Beevers

#### 2.1.3.1 Assembly

An Acid Digestion Vessel 4748, manufactured by the Parr Instrument Company, was used to prepare cerium oxide particles. This consists of a PTFE cup and lid with a metal jacket, as shown in figures 2.1 and 2.2. The PTFE cup containing the reaction

mixture, covered with the lid, was placed inside the metal jacket. A metal corrosion disc and a thicker rupture disc were placed over the PTFE lid before the pressure plate, spring and compression ring were added. The lid was screwed down tightly onto the jacket, and the locking screws were tightened, as described in the instruction manual (Parr Instrument Company 2011).

**Figure 2.2. Components of the disassembled Acid Digestion Vessel**



#### **2.1.3.2 Conditioning the PTFE cup**

To close the pores in the PTFE and reduce leakage in subsequent runs, the PTFE cup was conditioned by heating under pressure with pure water, before the first chemical reaction in the vessel. A charge of 50ml water was placed in the cup and the Acid Digestion Vessel was assembled as described above. The vessel was heated in the oven for one hour at 150°C, as suggested in the manufacturer's instructions, and then the temperature was raised to 180°C to check the functioning of the vessel and the operation of the oven at the cerium oxide processing temperature.

#### **2.1.3.3 Use of the Acid Digestion Vessel**

To ensure safe operation of the vessel, the maximum operating temperature of 250°C must not be exceeded. Overloading the vessel will cause excessive pressure, because

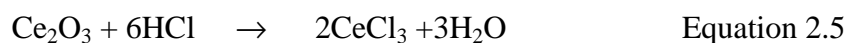
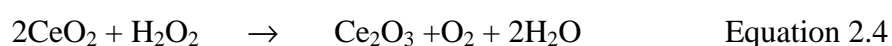
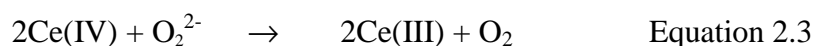


the volume of a liquid is increased by almost 25% from room temperature to 250°C, and it is essential that sufficient vapour space is left above the liquid for this expansion.

The PTFE cup has a capacity of 125ml, and the maximum fill volume is 83.75ml for water and inorganic materials where no gases are generated. Where organic material may be broken down, a minimum of 66% free space must be left, and a maximum of 0.5g of organic material should be used in the cup.

#### 2.1.3.4 Cleaning the PTFE cup

Cerium(IV) oxide is insoluble in acids and alkali. A reducing agent and an acid are necessary to dissolve the material as cerium(III) salts. A mixture of 10% hydrogen peroxide and 4M hydrochloric acid was stirred for three hours in the PTFE cup. A yellow colour was visible as the cerium(III) chloride was formed on the PTFE surface, and oxygen bubbles were observed. The yellow cerium (III) salt was dissolved in the acid solution.



The acid washed PTFE cup was washed five times with water, and soaked in for 24 hours before re-use.

#### **2.1.4 Oven**

A Carbolite LHT6/60 oven with a Carbolite 301 controller was used to heat the Acid Digestion Vessel for preparation of cerium oxide particles. The ramp rate, reaction temperature and hold time were controlled, and the cooling occurred naturally when the power to the elements was shut off.

#### **2.1.5 Digital thermometer**

A calibrated K-type thermocouple (for temperature measurements between -50°C and 750°C) with a Fisher Scientific digital readout was used to monitor the Carbolite oven temperatures, to ensure the accuracy of the oven controller. This was to prevent heating of the Acid Digestion Vessel beyond its safe working limits.

#### **2.1.6 Reaction process**

Cerium oxide nanoparticles were prepared by precipitation of the hydroxide from solutions of the cerium(III) salt. The hydroxide was converted to the oxide by heating at high temperature and pressure, (hydrothermal synthesis) with the formation of cerium (IV) from the cerium(III) occurring by oxidation in air. A typical reaction scheme is shown in the equations below.

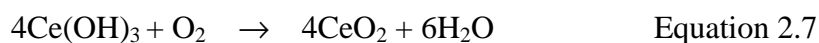
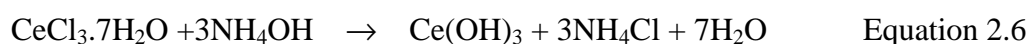


Table 2.2. Details of batches prepared by hydrothermal synthesis

Batch	Precursor	Reagent mM	Solvent for salt	Base	Wash	Solvent for heating	Temp °C Time hrs	Expected shape	References
1A	Ce(NO <sub>3</sub> ) <sub>3</sub> .6H <sub>2</sub> O	6.24	12.5ml H <sub>2</sub> O	NH <sub>4</sub> OH 6.25 ml	2 x 25ml H <sub>2</sub> O	25ml H <sub>2</sub> O	180 3	polygon	Wang et al., (2010)
2	Ce(NO <sub>3</sub> ) <sub>3</sub> .6H <sub>2</sub> O	6.24	12.5ml H <sub>2</sub> O	NH <sub>4</sub> OH 6.25 ml	2 x 25ml H <sub>2</sub> O	25ml H <sub>2</sub> O	180 8	polygon	Wang et al., (2010)
3	CeCl <sub>3</sub> .7H <sub>2</sub> O	6.24	12.5ml H <sub>2</sub> O	NH <sub>4</sub> OH 6.25 ml	2 x 25ml H <sub>2</sub> O	25ml H <sub>2</sub> O	180 8	Wires,	Yang et al. (2007)
4	Ce(NO <sub>3</sub> ) <sub>3</sub> .6H <sub>2</sub> O	2.30	14ml H <sub>2</sub> O	NaOH 10% 16ml	After heating	Added 10ml H <sub>2</sub> O	180 24	cubes	Mai et al., (2005)
5	CeCl <sub>3</sub> .7H <sub>2</sub> O	0.30	6ml H <sub>2</sub> O	NH <sub>4</sub> OH 3ml	2 x 25ml H <sub>2</sub> O	30ml 0.25mM sodium citrate	180 24	rods?	
6	CeCl <sub>3</sub> .7H <sub>2</sub> O	6.24	12.5ml 0.25mM NH <sub>4</sub> Cl	NH <sub>4</sub> OH 6.25 ml	2 x 25ml NH <sub>4</sub> Cl	25ml H <sub>2</sub> O	180 24	rods?	
7	CeCl <sub>3</sub> .7H <sub>2</sub> O	1.82	25ml H <sub>2</sub> O	9M NaOH 30ml	Not washed		140 48	rods	
8	CeCl <sub>3</sub> .7H <sub>2</sub> O	0.056	6ml H <sub>2</sub> O	NH <sub>4</sub> OH 3ml	2 x 25ml H <sub>2</sub> O	30ml 10gL <sup>-1</sup> PVP	180 18	rods?	
9	CeCl <sub>3</sub> .7H <sub>2</sub> O	0.30	6ml H <sub>2</sub> O	NH <sub>4</sub> OH 3ml	2 x 25ml H <sub>2</sub> O	30ml 0.25mM sodium citrate	180 18	rods?	
10	Ce(NO <sub>3</sub> ) <sub>3</sub> .6H <sub>2</sub> O	0.30	6ml H <sub>2</sub> O	NH <sub>4</sub> OH 3ml	2 x 25ml H <sub>2</sub> O	30ml 0.25mM sodium citrate	180 18	cubes?	
11	CeCl <sub>3</sub> .7H <sub>2</sub> O	6.24	12.5ml 0.25mM NH <sub>4</sub> Cl	NH <sub>4</sub> OH 6.25 ml	3 x 15ml NH <sub>4</sub> Cl	25ml H <sub>2</sub> O	180 8	rods?	
12	CeCl <sub>3</sub> .7H <sub>2</sub> O	6.24	12.5ml H <sub>2</sub> O	NH <sub>4</sub> OH 6.25 ml	2 x 25ml H <sub>2</sub> O	25ml H <sub>2</sub> O	180 8	Wires,	
13	CeCl <sub>3</sub> .7H <sub>2</sub> O	6.24	12.5ml 0.25mM NH <sub>4</sub> Cl	NH <sub>4</sub> OH 6.25 ml	3 x 15ml NH <sub>4</sub> Cl	25ml H <sub>2</sub> O	180 8	rods?	

A full list of batches prepared and reaction conditions is given in table 2.2. In a typical reaction procedure, the cerium was dissolved in water, a solution of the base was added and the resultant slurry was stirred for 30 minutes. To remove salts formed during the hydrolysis reaction and any excess alkali, the mixture was centrifuged at 2500rpm for 10 minutes. The supernatant was discarded and the precipitate was suspended in 25ml water to wash the precipitate. After centrifugation the supernatant was discarded and the washing was repeated.

The washed precipitate was transferred to the PTFE reaction vessel with 25ml water which was sealed inside the digester as described above. In some batches, a solution of a capping agent was used instead of water as the reaction medium. The digester was placed inside the Carbolite oven and the oven was programmed to heat at 10°C per minute to the hold temperature for a set dwell time, then cool down naturally. In addition to the oven display, the temperature was monitored by a certified thermocouple with a digital display for calibration, and to ensure the working temperature of the vessel was not exceeded. When cooled, the PTFE reaction vessel was removed from the digester, and the precipitated cerium oxide formed was transferred to a bottle with the minimum of rinsing water. The PTFE reaction vessel was cleaned as described above.

### **2.1.7 Centrifuges**

Three centrifuges were used in this work.

- 1) A Heraeus Biofuge Primo centrifuge which could take four 50ml tubes was used for removal of washings after hydrolysis of the cerium salt during nanoparticle preparation.

- 2) A Beckman L7-65 ultracentrifuge with a SWTi40 Rotor was used to prepare samples for atomic force microscopy. The rotor and detachable buckets are kept in a cold room at 4°C. The balanced sample tubes were loaded into the buckets, and the lids were screwed down firmly. The buckets were loaded onto the rotor, making sure the numbers on the buckets matched those on the rotor. The rotor was loaded into the ultracentrifuge, and chamber was evacuated before the centrifuge was run at 30,000 rpm, for one hour, at 10°C.
- 3) An Eppendorf Centrifuge 5804R, which can take four 50ml centrifuge tubes, was used during washing of suspensions of bacteria before toxicity testing.

## **2.2 Dispersion**

To prepare dispersions of the nanoparticles, solutions of various surfactants were prepared and small quantities of the cerium oxide particles were added. The range of surfactants used is presented in table 2.1. The suspensions were agitated for 30 minutes in a Grant Instruments XUB12 ultrasonic bath, delivering power at an average of ~16 watts per litre, to break up the agglomerates.

Particles in 0.05gL<sup>-1</sup> Suwannee River Fulvic Acid solution were dispersed ultrasonically as described above, and shaken on an IKA KS 130 Basic sample shaker at 320 revolutions per minute for one month prior to measurement.

## 2.3 Characterisation

### 2.3.1 Dynamic Light Scattering

#### 2.3.1.1 Theory

In a suspension of nanoparticles, the solvent molecules are moving due to thermal motion and the nanoparticles are moving with Brownian motion, due to collisions with the solvent molecules. The movement of the nanoparticles is detected using a laser beam. The nanoparticles, in a suspension within the measurement cell, scatter the laser beam. The intensity of scattered light reaching the detector varies as particles within the beam move with Brownian motion. If two measurements are made in quick succession, before the particle scattering light has moved significantly, the scattered light intensity will not show much change. As measurements continue to be taken, the correlation between the first and subsequent measurements will reduce as the position of the scattering particle is changed (Hasselov et al., 2008). The movement of a particle by Brownian motion depends on the size of the particle: smaller particles will be moved further and faster than large particles following a collision, and the correlation will change more rapidly.

The relationship between scattered light and the size of the scattering particle is given in equation 2.8 (Krishnaswamy, 2005).

$$I = I_0(2\pi/\lambda)^4(d/2)^6[(1 + \cos^2\theta)/2R^2][(n^2-1)/(n^2+2)]^2 \quad \text{Equation 2.8}$$

where  $I$  is the intensity of the scattered light

$I_0$  is the intensity of the incident light

$\theta$  is the scattering angle

$R$  is the distance to the particle

$\lambda$  is the wavelength of the light

$n$  is the refractive index of the particle

$d$  is the particle diameter

The autocorrelation function,  $g(\tau)$  is calculated from the intensity of the scattered light, as described by Hasselov et al. (2008), who set out the relationships shown in equations 2.9 to 2.12.

$$g(\tau) = \frac{|G(\tau) - \langle I \rangle^2}{\gamma^2} = Ae^{-2\Gamma\tau} \quad \text{Equation 2.9}$$

where  $G(\tau)$  is the field autocorrelation function,

$\gamma$  is the coherence factor,

$\langle I \rangle^2$  is the base line,

$\Gamma$  is the decay rate,

$A$  is a constant for the particular instrument and.

$\tau$  is the delay time.

The diffusion coefficient,  $D$ , can be calculated from the decay rate, as shown in equation 2.10.

$$D = \Gamma/q^2 \quad \text{Equation 2.10}$$

where  $q$ , the wave vector defined in equation 2.11.

$$q = 4\pi n_s \sin(\pi/4)/\lambda \quad \text{Equation 2.11}$$

where  $n_s$  is the refractive index of the sample.

The hydrodynamic radius of the particle, ( $R_h$ ), is calculated from the Stokes-Einstein equation, 2.12.

$$R_h = kT/(3\pi\eta D) \quad \text{Equation 2.12}$$

where  $R_h$  is the hydrodynamic radius of the particle,

$k$  is the Boltzmann constant

$T$  is temperature in degrees Kelvin, and

$\eta$  is the viscosity of the suspending medium.

The hydrodynamic size of a particle is not the size of the solid particle itself, but the size of the particle plus any coating on the particle which may include the capping agent and adsorbed ions and solvent molecules associated with the particle.

### 2.3.1.2 Instrumentation

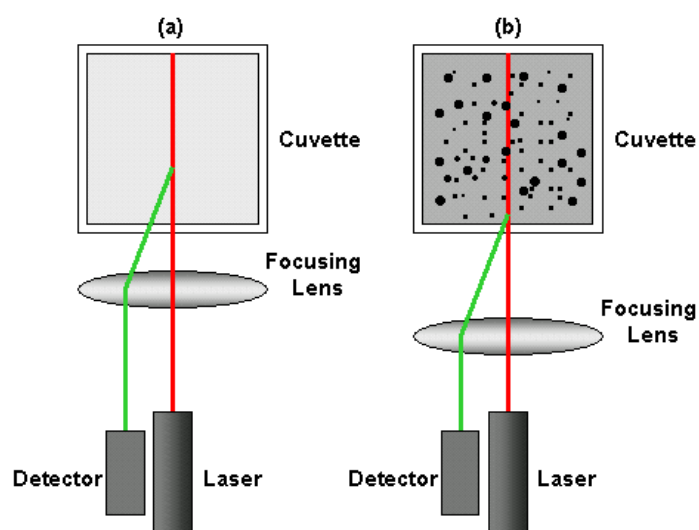
The intensity of the scattered light is monitored by a correlator which constructs a measure of the similarity between subsequent signals delayed by time  $\tau$ . The data from the correlator is analysed by the particle-sizer software, to generate the particle size information.

The laser beam in the Malvern Instruments Nanosizer ZS passes through a low-volume polystyrene cell containing the sample, and a detector is positioned at  $173^\circ$  from the transmitted light beam (Malvern Instruments technical note, 2012), to measure back-scattered light from the nanoparticles. If concentrated samples are used, the back-scattered light can be detected from the particles close to the surface of the



cell, and the laser beam does not need to pass through the whole sample for measurements to be made (Figure 2.3). With more dilute samples, scattered light from a greater thickness of sample can be used by adjusting the focusing lens.

**Figure 2.3 Schematic diagram of measurement position for (a) weakly scattering samples, (b) concentrated, opaque samples.**



*Malvern Instruments technical note (2012)*

It is assumed that the particles are far enough apart so that the light is scattered from only one particle, that the scattering is elastic (i.e. there is no energy absorbed by the particle in the light scattering process), and for shape independent scattering, the radius of the particle is less than one twentieth of the wavelength of the scattered light (Krishnaswamy, 2005).

Particle size measurements were carried out on the Malvern Instruments Nanosizer ZS. For determining particle size, five DLS measurements were recorded. When DLS

was used to determine particle stability in biological media over time, prior to toxicity testing, the number of measurements was reduced to three. This meant that the time difference between samples was reduced, giving data closer in time for the kinetic study of stability.

The particle size distribution is derived from the intensity of scattered light, which is proportional to the 6th power of the diameter of the particle, as shown in equation 2.8. When there is a broad or bimodal distribution, the larger particles will scatter more light, and the smaller particles may be masked by the large particles in the size distribution. Although the definition of a nanoparticle uses the number average (European Commission, 2011), it is not recommended that the number average is calculated from the intensity data, as demonstrated by Patty and Frisken (2006). Any slight errors in measurement of larger particle numbers in a polydispersed sample can cause large errors to arise in the calculated number distribution. It is recommended (Horiba Scientific, 2010) that this inter-conversion is only used when carrying out direct comparisons with particle size obtained by microscopy.

### **2.3.2 Zeta potential**

Particles in suspension can have charges on the surface due to adsorbed ions from the capping agent or the ions in the suspending medium. When an electric field is applied across the suspension, the charged particles move towards the electrode with the opposite charge, due to electrostatic attraction. The electrophoretic mobility of a particle,  $U_E$ , given in equation 2.13, is the observed rate of migration of the particle,  $v$ , divided by the electric field strength,  $E$ , (Ferard, 1994).

$$U_E = v/E \quad \text{Equation 2.13}$$

The zeta potential,  $Z$ , cannot be measured directly, but is derived from the measured electrophoretic mobility using the Henry equation (2.14).

$$U_E = [2 \varepsilon Z f(\kappa a)] / 3 \eta \quad \text{Equation 2.14}$$

Where  $U_E$  = electrophoretic mobility,

$\varepsilon$  = dielectric constant of the medium,

$Z$  = zeta potential,

$f(\kappa a)$  = Henry's function, and

$\eta$  = viscosity of the solution.

The zeta potential gives a measure of the surface charge on particles in the suspending medium used. This will vary as the pH of the medium and ions present in the suspension are altered. Suspensions of small charged particles are usually seen to be stable if the zeta potential value is greater than  $\pm 30\text{mV}$ . Between these values, when the zeta potential is closer to zero, the particles may agglomerate and form a precipitate.

The zeta potential values for nanoparticles were obtained using the Malvern Instruments Nanosizer ZS. An electric field was applied across the test sample and the movement of the particles in the electric field were measured, employing dynamic light scattering techniques similar to those used in particle size determination. A disposable folded capillary cell was used for these measurements, which was filled and emptied using a syringe. The cell was rinsed with ethanol, and then with water and flushed with 1.5ml of sample prior to filling the capillary.

### **2.3.3 Ultraviolet-visible spectroscopy**

The energy in visible and ultraviolet light is of the same order as the differences between electronic energy levels in atoms and ions. Outer electrons can be excited to higher energy levels by absorption of light, and the wavelength of the absorption is characteristic of the material under illumination.

Measurements of the absorption of visible and ultraviolet light by the cerium oxide nanoparticles were made using a Jenway 6800 scanning ultraviolet-visible spectrophotometer. The instrument is a double-beam spectrophotometer, and was set to scan through the wavelengths from 800nm to 220nm. The absorption was measured using polystyrene cuvettes with a path length of 10mm. A blank, with cuvettes containing water in both beams, was measured initially, and the cuvette in the sample beam was refilled with the nanoparticle sample to measure the absorption spectrum.

### **2.3.4 Fluorescence measurements**

The fluorescence spectrophotometer works on very similar principles to the ultraviolet-visible spectrophotometer. The incident beam excites electrons from the ground state in the test sample to a higher energy level. Some electrons fall back to the ground state and emit a photon of the same wavelength as the excitation wavelength, but some of the excited electrons fall down through a series of vibrational energy levels, for which the energy changes are less energetic than visible light. The electron finally falls back to the ground-state energy level, emitting a photon of lower energy than the excitation wavelength (Marose et al., 1998). The detector axis is at 90° to the direction of the incident light, and the cuvette used in the Varian Vary Eclipse 3D fluorescence spectrophotometer has four optical faces to permit

orthogonal measurements of the fluorescence. The fluorescence data is presented as a contour map of the measured intensity of the fluorescence, with the incident, excitation wavelength on the y-axis and the emitted, fluorescence wavelength on the x-axis. This allows the fluorescence of different organisms or materials which have similar emission wavelengths, but different excitation wavelengths, to be resolved.

### **2.3.5 Atomic Force Microscope**

#### **2.3.5.1 Theory and Instrumentation**

The atomic force microscope (AFM) does not use electromagnetic radiation or charged particles for imaging, as many microscopes do, but has a very sharp tip which is used to probe the surface of the substrate and any material deposited on the surface (Hasselov et al., 2008). As the tip scans across the substrate, it moves up and down over material deposited on the surface and the deflection of the tip gives an accurate measurement of the height of material on the substrate. A scanning electron microscope (SEM) image of a typical AFM tip is shown in figure 2.4.

**Figure 2.4. SEM image of an AFM tip**



The instrument used in this work is the Park Systems XE-100 Atomic Force Microscope. The tip, which is mounted on a cantilever, is lowered close to the surface of the substrate containing the sample to be measured. As the tip approaches the surface the van der Waals forces attracting the tip cause a deflection of the cantilever (Meyer, 1992). To measure this deflection, the system is set up with the cantilever centrally under the optical microscope on the AFM, and a laser beam is aligned visually with the back of the cantilever. The system software then monitors the laser as the x and y mirror position is adjusted, to optimise the reflection of the laser from the back of the cantilever onto the detector, as shown in figure 2.5. The reflected laser beam is detected with a position-sensitive photodiode (PSPD) ( Park Systems 2008a).

**Figure 2.5. Reflection of laser light to measure AFM tip deflection.**



<http://www3.physik.uni-greifswald.de/method/afm/eafm.htm> 16/6/12

The AFM was used in non-contact mode in this work, with the tip oscillating just above the sample surface. This reduces sample damage and prolongs the life of the measurement tips. When the tip approaches a nanoparticle on the substrate it is attracted to the particle and the laser beam, reflected from the back of the cantilever, moves away from the centre of the detector as the cantilever is deflected by the

proximity of the particle surface (Park Systems 2008b). A feedback loop is used to adjust the height of the tip above the sample surface and restore the laser beam to the centre of the detector. As the surface is scanned, the changes in position of the tip provide a topographical map of the sample, with the changes of height across the sample accurately recorded. The images produced give an idea of the shape and size of particles present but the height is the only dimension accurately measured by this technique (Hasselov et al., 2008).

### **2.3.5.2 Sample preparation**

Samples for AFM measurements were prepared in an ultracentrifuge. Squares of mica, approximately 6mm square, were cut from larger sheets to fit inside the ultracentrifuge tubes. The sheets were cleaved by inserting a scalpel blade into the corner of the mica to initiate the process, and the pieces were pulled apart with tweezers. The mica was placed onto a PTFE plug, held on a threaded rod, with the fresh mica surface pointing upwards. The PTFE plug was inserted into the ultracentrifuge tube, and lowered firmly into the base of the tube. The rod was removed and a suspension of the nanoparticles, at concentrations of 20ng per litre, was slowly introduced into the tube without disturbing the mica sheet. The filled tube was sealed with Parafilm, and ultracentrifuged at 30,000 rpm for 1 hour at 10°C.

After centrifugation the supernatant was removed carefully by pipette, without disturbing the mica. The threaded rod was re-introduced into the PTFE plug, and the plug was raised. The mica substrate was lifted from the plug with tweezers and washed twice by immersion into clean water for one minute, then left to dry in the

fume hood. The dry mica sheet, fresh surface upwards, was attached to a small steel disc using double-sided sticky-tape, and stored in a sealed tube.

### **2.3.5.3 AFM sample measurement procedure**

The measurement head was removed from the instrument and the sample was mounted onto the magnetic sample holder. The cantilever was mounted onto the measurement head, which was re-installed and the electronics plugged into the head. The laser was switched on, the cantilever was aligned optically, and the laser was set up as described earlier. The measurement settings were optimised by the software, and the cantilever was lowered until it was seen to be close to the substrate. The cantilever was then brought closer to the surface, using the optical microscope to monitor the position, and the final approach was controlled by the software.

The scan size and rate were set, and the data collection commenced. Further refinements to the parameters were made to optimise the images collected. After completion of the scan, new areas were selected and further images obtained. The topography images were used for particle measurements. At least 100 particles were counted from three or more areas for particle size analysis, and a histogram plot of the data was prepared to show the number versus size of the particles.

## **2.3.6 Transmission Electron Microscopy (TEM)**

### **2.3.6.1 TEM Instrumentation**

Electron microscopy uses electron beams to look at objects, and can record images with resolution approaching 0.1 nanometres (Tecnai, 2010). The electrons would be deflected by gas molecules, and so high vacuum conditions must be maintained



within the TEM. Under these conditions, the beam of electrons travels in the same way as a beam of light would, and has wave characteristics, where the wavelength of the electrons can be defined, as well as particle characteristics. Because of the negative charge on the electrons, the electrostatic repulsion causes spreading of the electron beam, and electric and magnetic fields can be used to collimate and focus the electron beams.

The TEM is formed of several sections (Tecnai, 2010).

**Figure 2.6. Transmission electron microscope schematic diagram**



A high-voltage electron source provides the electron beam. A system of magnetic lenses controls the path of the electron beams. The condenser lenses and associated apertures produce a parallel electron beam, and the objective lenses focus the beam which falls on the sample. For TEM, the sample needs to be thin enough to allow the beam to pass through. The image of the sample is projected below the sample onto a fluorescent screen which is illuminated when the electrons hit the screen.

In TEM measurements, the electrons cannot pass through the high density nanoparticles. Because measurements are made under high vacuum conditions, there is no hydrated coating on the particles, and low density organic coatings may not be visible. This means that the particle sizes measured by TEM are generally lower than those measured by dynamic light scattering which measures the size of the particle with its associated hydration sheath.

#### **2.3.6.2 Sample preparation for TEM**

Suspensions of nanoparticles were prepared, typically at concentrations of  $40\text{mgL}^{-1}$ . Agar carbon grids of about 3mm diameter were held horizontally in reverse-action tweezers. A drop of the suspension was placed on top of the grid and left for one hour for particles to settle on to the grid. Excess liquid was tipped from the grid, and the sample washed twice in separate containers of water, and dried in air under extraction.

#### **2.3.6.3 TEM measurements on samples**

Preliminary measurements were carried out on a JEOL 1200 microscope. The sample was introduced through a sample port. The sample chamber was evacuated, to remove the air surrounding the sample, before the sample was moved into the path of the

electron beam. The sample holder can take two sample grids and these can be interchanged within the evacuated chamber. Sample measurements were made at a range of magnifications according to the size of particles on the grid. At least one image was recorded at one hundred thousand times magnification for each sample examined, to allow direct visual comparison of images to be made. The scale bar for the image was generated by the software associated with the TEM, and was stored with the TEM image.

Measurements were also carried out at Oxford University Materials Science Department on a JEOL JEM-2010 TEM. The particles in the images from Oxford were measured using ImageJ, version 1.46r (Rasband, 2012). To measure a representative sample for particle size analysis, a minimum of 100 particles were required from three or more squares on the carbon grids.

### **2.3.7 pH meter**

A Fisherbrand Hydrus 300 pH meter was used to measure the pH of nanoparticle suspensions prepared in acid and alkaline solutions. Prior to use, a two-point calibration was carried out using pH 4 and pH 7 buffer solutions.

### **2.3.8 X-ray diffraction**

#### **2.3.8.1 Theory**

The regular array of atoms in crystal structures can reflect radiation of a suitable wavelength. Some of the reflections undergo destructive interference, but in other directions, constructive interference occurs and the intensity is built up by the reflections so that bright spots of reflected waves can be detected. The wavelength of the radiation in X-ray beams is of the same order as the spacing between crystal

planes, making it suitable for determining crystal structures. The condition for the occurrence of reflections from the crystal planes are described by Bragg's law (Schiels, 2010), in equation 2.15 below.

$$n\lambda = 2d\sin\theta \quad \text{Equation 2.15}$$

where  $n$  is an integer,

$\lambda$  is the wavelength of the X-ray radiation

$d$  is the spacing between the crystal planes and

$\theta$  is the incident angle of the X-ray beam hitting the crystal surface.

With radiation of a known wavelength, Bragg's law allows the spacing between the planes to be determined from the reflections.

Samples for X-ray crystallography can be single crystals or polycrystalline samples which have the crystal planes randomly aligned in a powdered sample. The sample is placed on the centre of the equipment and is irradiated with the X-rays. The incident angle of the radiation is scanned through  $\theta$  degrees, and the detector tracks the reflected radiation at  $2\theta$  degrees. The reflections can be used to calculate the positions of atoms within the crystal structure to determine structures of novel materials, or can be used to compare the angle and intensity of reflected spots with known samples, to identify compounds and the crystal form. In this work, the reflections were matched to published data.

### **2.3.8.2 Sample preparation for X-ray crystallography**

Aliquots of 3ml of the batches were centrifuged at 10°C for 15 minutes at 4,000rpm and the supernatant was discarded. For each batch, the residue was washed three times with 3ml water, and centrifuged as above between washings. The tubes containing the washed residues were covered with filters of 0.1µm porosity to allow the residues to dry while preventing any fibres from leaving the tubes. The resultant dry pellet was crushed using a mortar and pestle under extraction. A disc of the powder was enclosed in *Scotch* tape, before mounting in the instrument.

### **2.3.8.3 XRD measurements**

For polycrystalline cerium oxide samples, X-ray diffraction measurements were made on a Bruker AXS D8 instrument in transmission mode, with incident angles of between 20° and 90°. The X-ray radiation was Cu K<sub>α1</sub>, at a wavelength of 1.5406 Å. The diffraction patterns were matched using Eva, a programme for comparing experimental data with published patterns.

## **2.4 Biological testing**

### **2.4.1 Aseptic techniques**

Samples for biological testing were prepared using aseptic techniques to prevent bacterial contamination.

- Clean sample jars and lids, with autoclave tape attached, were autoclaved to reach 120°C at 1 atmosphere air pressure, for 15 minutes, and cooled. The dark lines on the tape showed that the correct temperature had been reached for sterilisation of the containers.
- The water used for making up solutions was sterilised.

- The jars of media had light excluded by a layer of aluminium foil. The media was stored in the fridge, but was pre-warmed to 30°C before use to prevent thermal shock.
- The surfaces inside the laminar flow cabinet were swabbed down with ethanol wipes.
- All the equipment required was placed inside the laminar flow cabinet. Ethanol wipes were used to swab down equipment before it was placed inside the cabinet. The instructions were placed close to the cabinet, to be visible while working inside the cabinet.
- Gloves were swabbed with ethanol, and once work commenced, the hands remained inside the laminar flow cabinet to avoid introducing bacteria.
- Before removing lids of sample jars, the lid was flamed in the edge of a blue Bunsen flame inside the cabinet. The lid was removed and held in the hand or placed open side down on the cleaned base of the cabinet to avoid bacteria falling into the lid. The neck of the jar was flamed before introducing any liquids.
- The tops and lids of media jars were flamed before the bottles were opened.
- When pipettes were removed from wrappers, care was taken to ensure that they did not touch the bench surface, or outside of jars, to avoid contamination.
- Pipette tips, wipes, etc. used in the laminar flow cabinet were disposed of in bags of biological waste for incineration.
- The cabinet was swabbed down with ethanol after use, and the door replaced.

When it was necessary to transfer solutions from the jars outside the laminar flow cabinet, for example to make measurements in other laboratories, techniques to minimise bacteria contamination were employed.

- The bench was swabbed down with ethanol.
- Jars were cracked open just far enough to allow the pipette to be introduced, and quickly re-sealed.

#### 2.4.2 Preparation of media

Minimal Davis media (MDM) was used for this work. The composition of MDM is listed in table 2.3. The prepared media was sterilised in a Boxer 200/40L autoclave at 121°C for 15 minutes to sterilise the media, and the cooled solution was wrapped in aluminium foil to exclude light, and stored at 4°C. After autoclaving, a solution of 0.5g dextrose in 2ml water was purified by filtration through a 0.2 micron filter, added to 0.5 litres of the cooled media. Both MDM and MDM + glucose were used in preliminary bacterial toxicity work.

**Table 2.3. Composition of Minimal Davis Media:**  
Weights of salts in gL<sup>-1</sup>

Material	gL <sup>-1</sup>
Di-potassium phosphate	7.0
Mono-potassium phosphate	2.0
Sodium citrate	0.5
Magnesium sulphate	0.1
Ammonium sulphate	1.0

### 2.4.3 Bacteria

The bacteria used in this work were *Pseudomonas putida*, (*P. putida*). Media and water used in this work were pre-heated to 35.6°C in a LMS bench-top incubator. The bacterial culture was stored on a Petri dish at 4°C, and a small portion of this was used to inoculate 50ml of MDM in a conical flask, under aseptic conditions. The flask was incubated in a Raven 2 incubator at 28.2°C, on a shaker circulating at 120rpm. After 24 hours, the bacterial culture was centrifuged at 5,000rpm for 5 minutes, and the supernatant discarded. The residue was re-suspended in 50ml MDM, re-centrifuged and the supernatant discarded, to remove any waste products from the bacteria. The washing process was repeated, and the bacteria were re-suspended in fresh MDM. This cleaned culture was used in subsequent toxicity testing.

### 2.4.4 Toxicity testing

The toxicity of the ceria nanoparticles to *Pseudomonas putida* was assessed in a 96-well plate, by measuring the growth of the bacteria over a 24 hour period without nanoparticles and with various concentrations of ceria nanoparticles. Each well, as shown in figure 2.7, holds 200µl of solution, and four replicates of each blank, control and test solution were measured into individual wells, under aseptic conditions. The contents of the individual wells under evaluation in the plate are set out in table 2.4 for Run 1 and in table 2.5 for Run 2.



**Figure 2.7. The well arrangement for the 96-well plate**

[http://www.core.uaf.edu/protocols/96\\_well\\_plate.html](http://www.core.uaf.edu/protocols/96_well_plate.html) 7/7/12

**Table 2.4. Components in each well for Run 1**

<b>Wells</b>	<b>Component 1, 100µl</b>	<b>Component 2, 100µl</b>	
A-D 1	Empty	100mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water 50mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water 20mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water 10mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water	
A-D 2	<i>P. putida</i> in 1:5 MDM		
A-D 3	<i>P. putida</i> in 1:10 MDM		
A-D 4	<i>P. putida</i> in 1:10 MDM		
A-D 5	<i>P. putida</i> in 1:10 MDM		
A-D 6	Empty		
A-D 7	Empty		
A-D 8	Empty		
A-D 9	<i>P. putida</i> in 1:1 MDM		<i>P. putida</i> in 1:1 MDM
A-D 10	<i>P. putida</i> in 1:10 MDM		<i>P. putida</i> in 1:10 MDM
A-D 11	<i>P. putida</i> in 1:10 MDM		1:10 MDM
A-D 12	<i>P. putida</i> in 1:1 MDM		<i>P. putida</i> in 1:1 MDM
E-H 1	Empty	100mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water 50mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water 20mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water 10mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water	
E-H 2	<i>P. putida</i> in 1:10 MDM + glucose		
E-H 3	<i>P. putida</i> in 1:10 MDM + glucose		
E-H 4	<i>P. putida</i> in 1:10 MDM + glucose		
E-H 5	<i>P. putida</i> in 1:10 MDM + glucose		
E-H 6	Empty		
E-H 7	Empty		
E-H 8	Empty		
E-H 9	Empty		
E-H 10	Empty		
E-H 11	Empty		
E-H 12	Empty		

The plate was installed in a 96-well plate reader, and the blanks, samples and controls were identified in the layout. The temperature of the plate was maintained at 30°C throughout the test period, and the absorbance of the sample in each well was monitored at a wavelength of 595nm for 24hours. As bacteria grow in the wells, the turbidity of the sample increases as the light is scattered by the bacteria, and the absorbance rises.

**Table 2.5. Components in each well for Run 2**

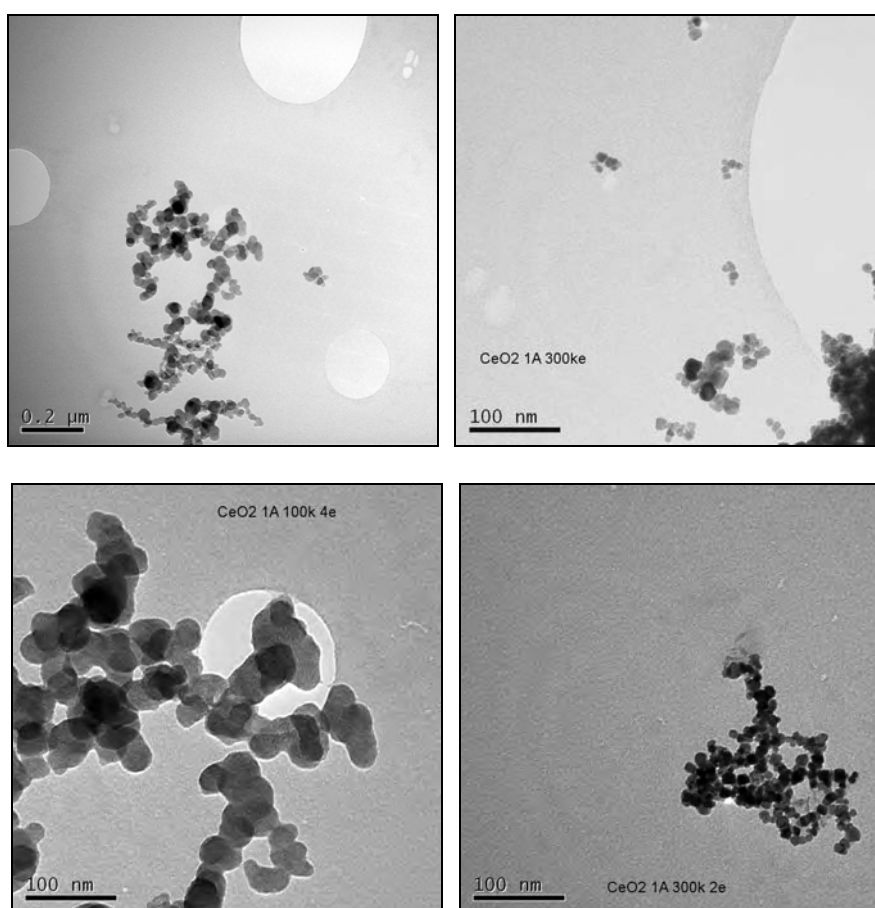
<b>Wells</b>	<b>Component 1, 100µl</b>	<b>Component 2, 100µl</b>
A-D 1	1:10 MDM	1:10 MDM
A-D 2	<i>P. putida</i> in 1:5 MDM	20mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water
A-D 3	<i>P. putida</i> in 1:5 MDM	40mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water
A-D 4	<i>P. putida</i> in 1:5 MDM	100mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water
A-D 5	<i>P. putida</i> in 1:5 MDM	200mgL <sup>-1</sup> Comm. CeO <sub>2</sub> in water
A-D 6	1:10 MDM	1:10 MDM
A-D 7	<i>P. putida</i> in 1:5MDM	20mgL <sup>-1</sup> B10 CeO <sub>2</sub> in sodium citrate
A-D 8	<i>P. putida</i> in 1:5 MDM	40mgL <sup>-1</sup> B10 CeO <sub>2</sub> in sodium citrate
A-D 9	<i>P. putida</i> in 1:5 MDM	100mgL <sup>-1</sup> B10 CeO <sub>2</sub> in sodium citrate
A-D 10	<i>P. putida</i> in 1:5 MDM	200mgL <sup>-1</sup> B10 CeO <sub>2</sub> in sodium citrate
A-D 11	<i>P. putida</i> in 1:5 MDM	Water
A-D 12	<i>P. putida</i> in 1:5 MDM	1g L <sup>-1</sup> sodium citrate filtered
E-H 1	Empty	
E-H 2	Empty	
E-H 3	Empty	
E-H 4	Empty	
E-H 5	Empty	
E-H 6	Empty	
E-H 7	Empty	
E-H 8	Empty	
E-H 9	Empty	
E-H 10	Empty	
E-H 11	Empty	
E-H 12	Empty	

### 3. Results

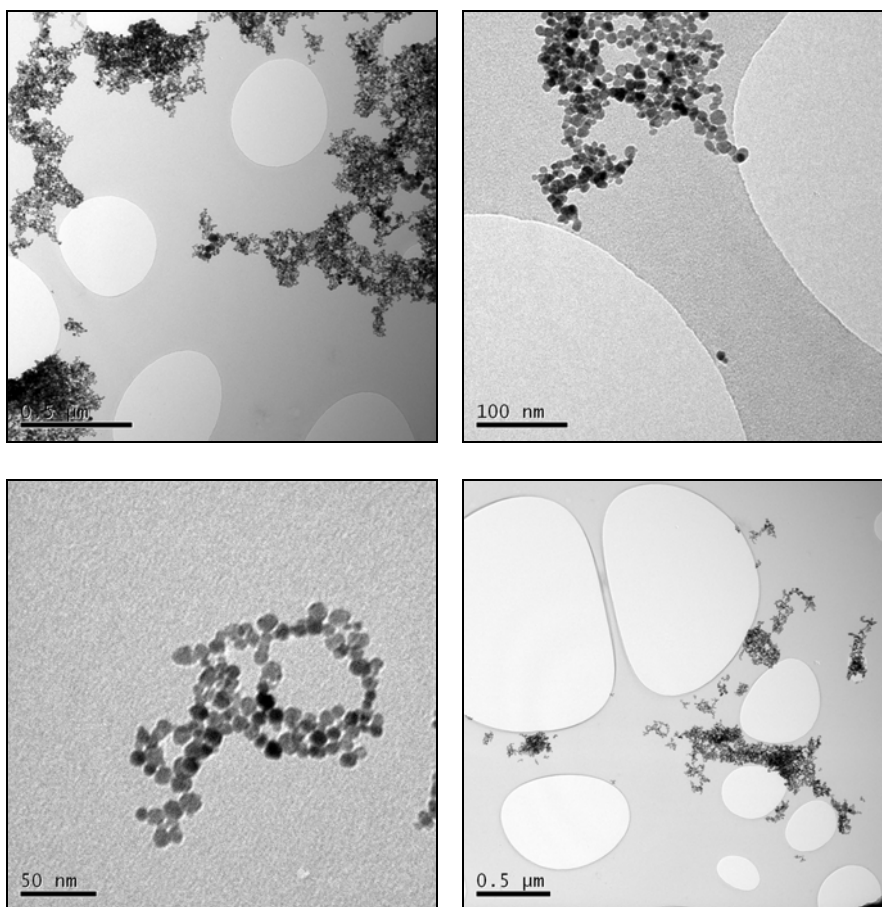
#### 3.1 As -prepared samples

Thirteen batches of cerium oxide nanoparticles were prepared as detailed in the methodology chapter. Precipitates were obtained which did not disperse readily in water. TEM images were obtained of the initial, as-prepared batches, and the particles were seen to be agglomerated. Typical images are presented in figure 3.1 to 3.4, with additional images in Appendix 1

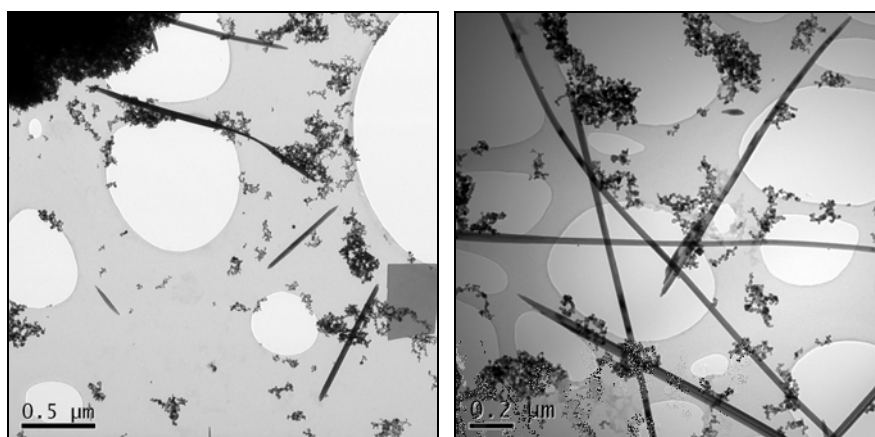
**Figure 3.1. TEM images of as-prepared Batch 1.**

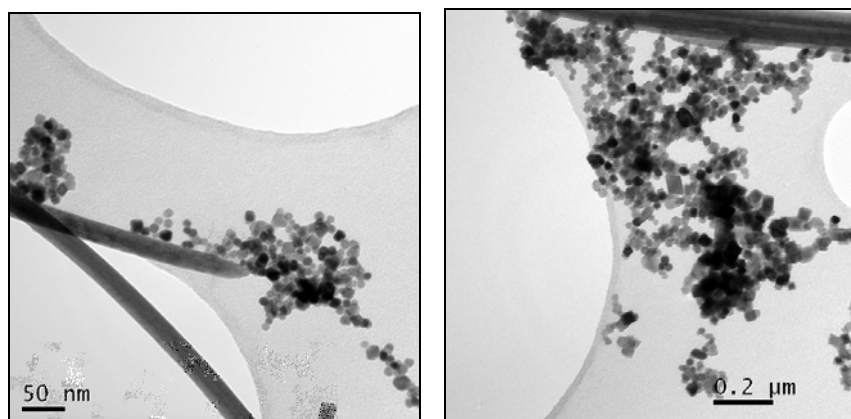


The aspect ratio of the primary particles in these images was close to 1 and the primary particle size was 22nm with a standard deviation of 14nm, based on 275 measurements using ImageJ (Rasbond, W., 2012).

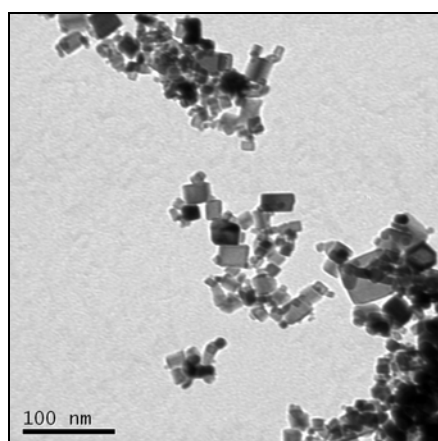
**Figure 3.2. TEM images of as-prepared Batch 2**

The primary particle size for batch 2 was found to be 10.6nm with a standard deviation of 4.7nm from a sample of 230 measurements. The aspect ratio of the particles was close to 1.

**Figure 3.3. TEM images of as-prepared Batch 3**

**Figure 3.3 continued. TEM images of as-prepared Batch 3**

Particles formed in batch 3 were of two forms: small rounded or cubic particles of primary particle size 20nm with a standard deviation of 12nm from 240 particles, and wires with width typically 29nm, with standard deviation of 12nm and length measured at 1160nm with a standard deviation of 940nm. The values for the wires was based on a sample of only 11 particles, and the value underestimates the average length of the wires, as only particles where both ends were visible were measured, and a number of the long wires which were visible in the images did not have both ends within the frame.

**Figure 3.4. TEM image of as-prepared Batch 4**

The primary particles in batch 4 were predominantly cubic with aspect ratios close to 1, and of primary particle size 15nm with a standard deviation of 8nm, based on 114 measurements.

The XRD data for Batches 2, 3, 4, 6, 11, 12 and 13 are given in Appendix 2. There was insufficient sample to obtain XRD traces from batches 9 and 10. The relative intensities of these files were compared with the batches measured, as shown in table 3.1.

**Table 3.1. Comparison of intensity of XRD reflections with published data for CeO<sub>2</sub>**

Batch	Intensities relative to 200 reflection				Intensities relative to 220 reflection			
	Peak 111 reflect 28.54 2θ	Peak 200 reflect 33.08 2θ	Peak 220 reflect 47.48 2θ	Peak 311 reflect 56.34 2θ	Peak 111 reflect 28.54 2θ	Peak 200 reflect 33.08 2θ	Peak 220 reflect 47.48 2θ	Peak 311 reflect 56.34 2θ
CeO <sub>2</sub> 04-013-4361	3.6	1.0	1.7	1.2	2.1	0.6	1.0	0.7
CeO <sub>2</sub> 01-078- 3280	3.5	1.0	1.6	1.2	2.1	0.6	1.0	0.7
B2	2.3	1.0	1.3	1.3	1.8	0.8	1.0	1.0
B3	2.9	1.0	1.6	1.4	1.8	0.6	1.0	0.8
B4	2.1	1.0	1.4	1.0	1.5	0.7	1.0	0.7
B6	3.6	1.0	1.8	1.1	2.0	0.5	1.0	0.6
B11	3.8	1.0	1.6	1.3	2.4	0.6	1.0	0.8
B12	3.8	1.0	1.6	1.4	2.3	0.6	1.0	0.9
B13	3.8	1.0	2.0	1.4	1.8	0.5	1.0	0.7

### 3.2 Dispersion of nanoparticles

Various methods of dispersing the particles were investigated. Batches 2 and 4 were selected as model batches for the dispersion, because they had aspect ratios close to 1, as seen by TEM, and so could be characterised by DLS. The effect of pH on particle size and zeta potential were investigated, and the data is presented in Table 3.2, and the DLS size and zeta potential graphs are shown in Appendices 3 and 4. The quality classifications of the results were based on the 'expert advice' generated by the Malvern Instruments Nanosizer ZS, for each set of particle size measurements. This allowed the conditions which produced stable nanoparticle suspensions to be identified. Dispersions with Z-average particle size below 200nm, and were seen

below pH 4 and above pH 9, but these pH ranges are outside environmentally realistic conditions and other dispersion methods were investigated. Table 3.3 presents data for Batch 2 with different surfactants. Sodium citrate appeared to offer the best stabilisation of ceria particles in suspension, with the DLS data classified as meeting quality requirements.

**Table 3.2. Zeta potential, particle size and quality of Batch 2 ceria suspensions with a range of pH**

Sample	Measured pH	Z-average particle size/nm	Data quality	Zeta potential /mV	Data quality
B2 nominal pH 4, 0.3g/litre	3.53	109.8	good	39.19	good
B2 nominal pH 5, 0.3g/litre	3.66	180.7	good	21.94	good
B2 nominal pH 6, 0.3g/litre	4.32	796.2	poor	7.31	poor
B2 nominal pH 7, 0.3g/litre	5.00	3110	poor	-3.67	poor
B2 nominal pH 9, 0.3g/litre	7.34	2560	poor	-1.32	poor
B2 nominal pH 10, 0.3g/litre	8.37	1068	poor	-0.263	poor
B2 nominal pH 11, 0.3g/litre	10.71	197.1	good	-45.72	good

**Table 3.3. Particle size and dispersion quality and dispersion conditions for Batch 2 ceria suspensions with various surfactants**

Surfactant	Particle conc /mgL <sup>-1</sup>	Surfactant /conc gL <sup>-1</sup>	Ultrasonic /mins	Z-average particle size/nm	Particle size quality	Solids present
PVP	2.9	5	20	88.5	poor	yes
SRFA	2.9	0.05	20	419.1	poor	yes
Tween 80	2.9	0.02	20	195.7	poor	yes
Sodium citrate	2.9	0.20	20	222.8	poor	Slight
Sodium citrate	0.3	0.02	Shake 3 days	237.4	good	no
Sodium citrate	2.9	2.00	Shake 3 days	182.8	good	no
Sodium citrate	20	1.00	30	118.2	good	no

A systematic study of particle and sodium citrate concentration was carried out on Batch 4 to establish the optimum working range for particle stabilisation, as shown in tables 3.4 and 3.5. Suspensions were prepared with 30 minutes ultrasonic dispersion.

Sodium citrate concentrations of 0.2 and 2gL<sup>-1</sup> gave good stability, as shown by the quality of the particle size measurements with particle concentrations above 1mgL<sup>-1</sup> ceria.

**Table 3.4. Z-average particle size, in nm, and quality of results for Batch 4 ceria suspensions at concentrations between 0.12mgL<sup>-1</sup> and 37.2mgL<sup>-1</sup> ceria, and sodium citrate concentrations between 0.02gL<sup>-1</sup> and 10gL<sup>-1</sup>**

Concentration	Batch 4	Batch 4 1.24mgL <sup>-1</sup>	Batch 4 12.4mgL <sup>-1</sup>	Batch 4 37.2mgL <sup>-1</sup>
Sodium citrate concentration 0.02gL <sup>-1</sup> Quality	382.2 poor	249.0 poor	477.4 poor	159.2 good
Sodium citrate concentration 0.2gL <sup>-1</sup> Quality	503.2 poor	180.0 good	130.7 good	203.7 poor
Sodium citrate concentration 2gL <sup>-1</sup> Quality	435.0 poor	327.4 good	182.8 good	192.1 good
Sodium citrate concentration 10gL <sup>-1</sup> Quality	481.6 poor	443.8 poor	550.7 poor	785.1 poor

**Table 3.5. Zeta potential, in mV, and quality of results for Batch 4 ceria suspensions, at concentrations between 0.12mgL<sup>-1</sup> and 37.2mgL<sup>-1</sup> ceria, and sodium citrate concentrations between 0.02gL<sup>-1</sup> and 10gL<sup>-1</sup>**

Concentration	Batch 4 0.12mgL <sup>-1</sup>	Batch 4 1.24mgL <sup>-1</sup>	Batch 4 12.4mgL <sup>-1</sup>	Batch 4 37.2mgL <sup>-1</sup>
Sodium citrate concentration 0.02gL <sup>-1</sup> Quality	-24.4 poor	-54.6 good	-51.5 good	-52.1 good
Sodium citrate concentration 0.2gL <sup>-1</sup> Quality	-5.8 poor	-14.7 poor	-46.8 good	-44.1 poor
Sodium citrate concentration 2gL <sup>-1</sup> Quality	-18.9 poor	-13.4 poor	-47.6 good	-49.0 good
Sodium citrate concentration 10gL <sup>-1</sup> Quality	-4.5 poor	-7.5 poor	-10.4 poor	-18.9 poor



As an alternative dispersion route, Suwannee River Fulvic Acid (SRFA) was also investigated. Suspensions of different batches of ceria nanoparticles were prepared in  $0.05\text{g L}^{-1}$  with 30 minutes ultrasonic dispersion, followed by shaking for 30 days, as shown in table 3.6.

**Table 3.6. Particle size and quality of measurement for ceria batches prepared by hydrothermal synthesis. Measured after 30 minutes ultrasonic dispersion in  $0.05\text{g L}^{-1}$  SRFA and 30 days on shaker.**

Batch	Conc ceria / $\text{mgL}^{-1}$	Z-average particle size/nm	Size quality
2	14.30	152.7	good
2	1.43	236.8	poor
3	14.30	219.7	good
3	1.43	155.8	good
4	0.83	160.6	good
4	0.08	191.2	poor
6	14.30	419.8	poor
6	1.43	492.3	poor

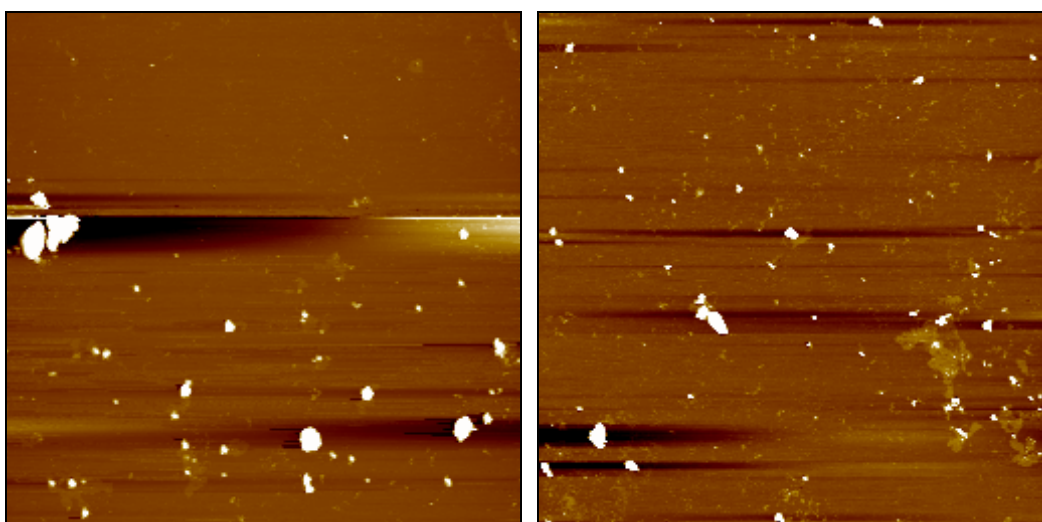
The particle sizes obtained were not significantly smaller with SRFA than with sodium citrate, but the quality was more variable and the process took longer. The decision was taken to use sodium citrate, at a concentration of  $1\text{g L}^{-1}$  for subsequent dispersions, with  $20\text{mg L}^{-1}$  identified as a useful working particle concentration. Table 3.7 shows the particle size data obtained for the various batches prepared by hydrothermal synthesis, after 30 minutes ultrasonic dispersion in  $1\text{g L}^{-1}$  sodium citrate solution.

**Table 3.7. Particle size, zeta potential and quality of measurements for ceria batches prepared by hydrothermal synthesis, after 30 minutes ultrasonic dispersion in  $1\text{gL}^{-1}$  sodium citrate solution.**

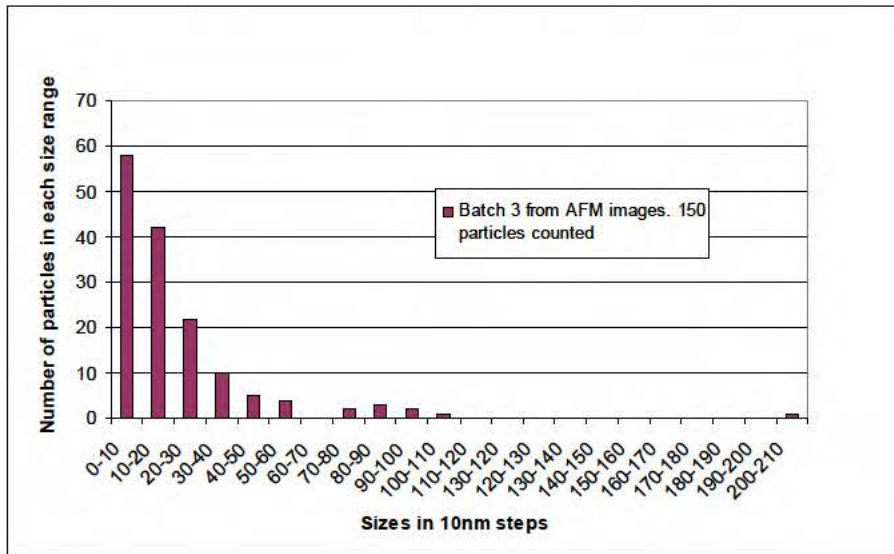
Batch	Z-average particle size/nm	Size quality	Zeta potential, /mV	Zeta potential quality
1	139.2	good	-48.6	good
2	118.2	good	-47.5	good
3	111.6	good	-52.2	poor
4	102.1	good	-51.5	good
6	221.6	good	-56.9	good
9	332.6	good	-50.2	good
10	140.1	good	-56.4	good
11	266.1	good	-56.0	ok
12	311.4	good	-56.2	good
13	400.1	ok	-57.4	ok

Samples were prepared from the batches listed in table 3.7 for examination on the AFM as described in the methodology chapter. Of these samples, only batches 3, 6 and 10, in  $1\text{g L}^{-1}$  sodium citrate solution gave good AFM images of particles and these are shown in figures 3.5, 3.7 and 3.9, and the particle size distributions are presented in figures 3.6, 3.8 and 3.10, and original data is in Appendix 5.

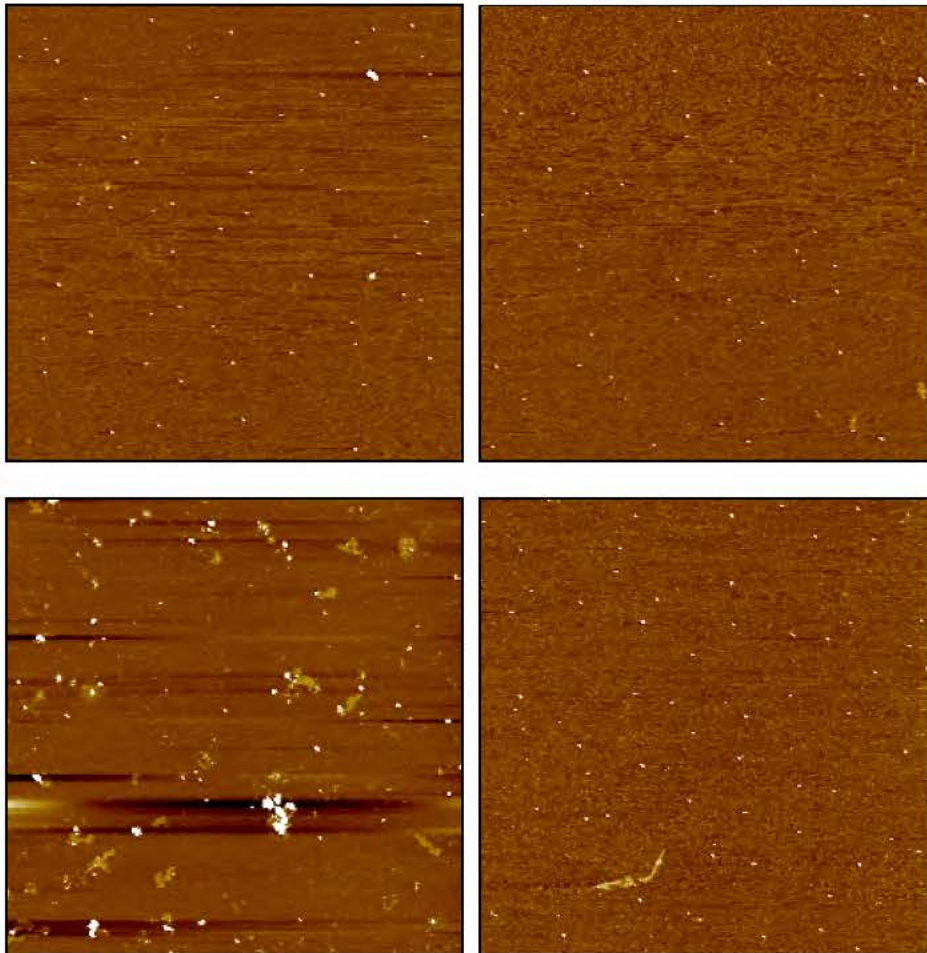
**Figure 3.5. AFM images of Batch 3 ceria (prepared 18/05/12):  $10\mu\text{m}$  squares**



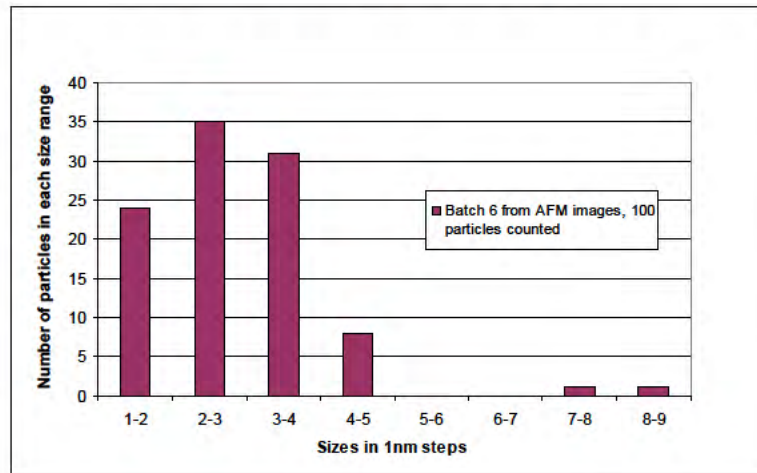
**Figure 3.6. Particle size distribution of Batch 3 ceria in  $1\text{gL}^{-1}$  sodium citrate solution (prepared 18/06/12), measured by AFM**



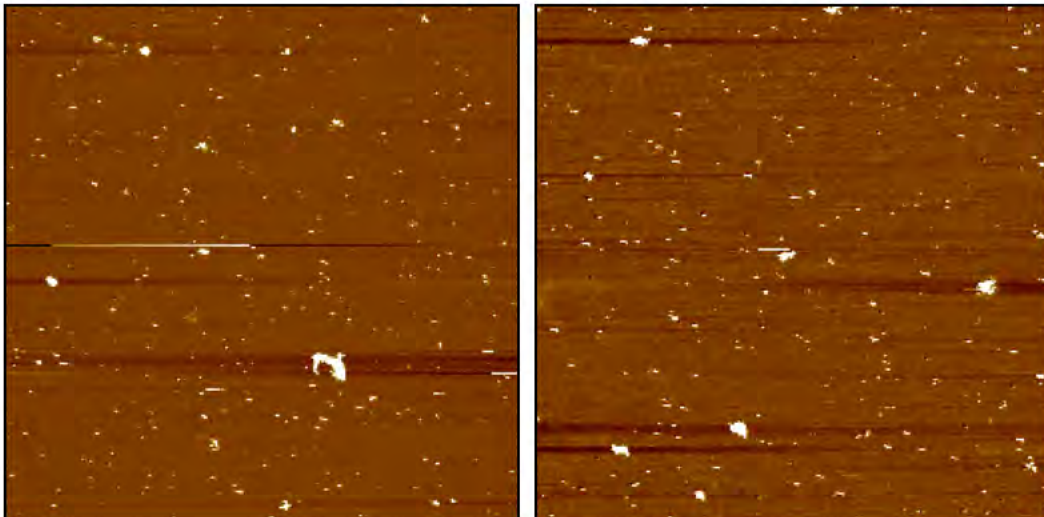
**Figure 3.7. AFM images of Batch 6 ceria (prepared 18/05/12):  $10\mu\text{m}$  squares**



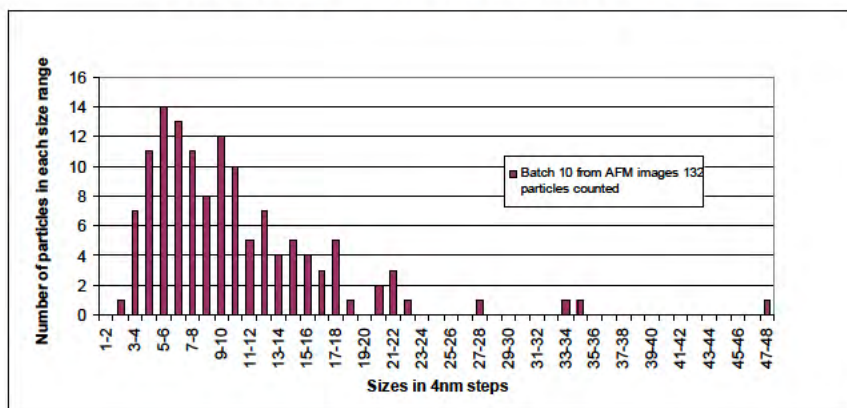
**Figure 3.8. Particle size of Batch 6 ceria in  $1\text{gL}^{-1}$  sodium citrate solution (prepared 18/05/12), measured by AFM**



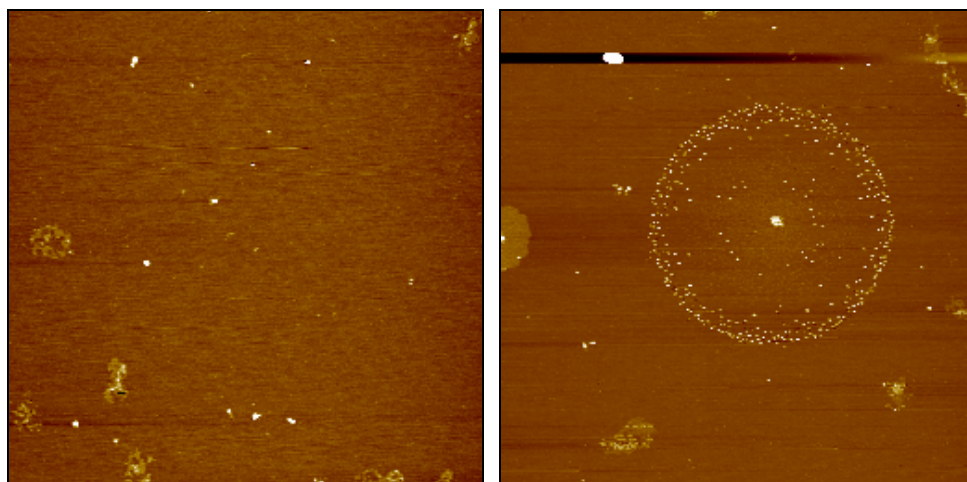
**Figure 3.9. AFM images of Batch 10 ceria (prepared 18/05/12):  $10\mu\text{m}$  squares**



**Figure 3.10. Particle size of Batch 10 ceria in  $1\text{gL}^{-1}$  sodium citrate solution, (prepared 18/05/12), measured by AFM**



**Figure 3.11. AFM images of Batch 9 ceria in  $1\text{gL}^{-1}$  sodium citrate solution, (prepared 18/05/12):  $10\mu\text{m}$  squares**



A comparison of particle size by DLS and AFM is made in table 3.8.

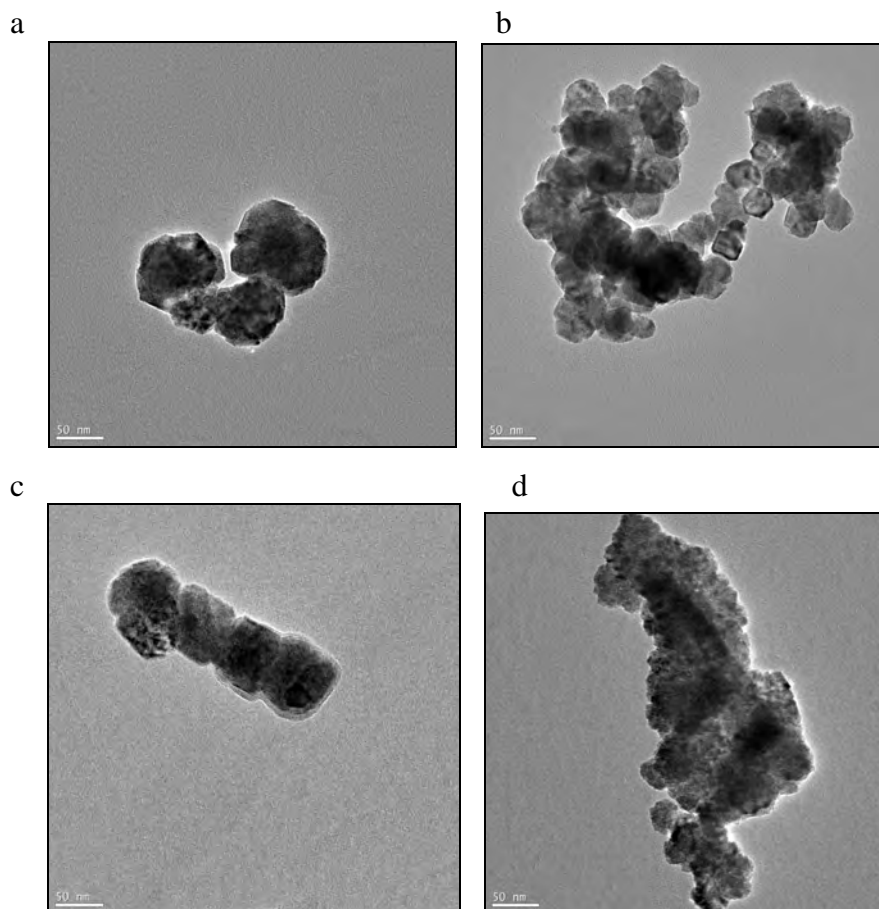
**Table 3.8. Comparison of particle size of ceria particles measured by DLS and AFM**

<b>Batch</b>	<b>DLS Z-average particle size/nm</b>	<b>AFM mean particle size/nm</b>
3	111.6	21.06
6	221.6	2.815
10	140.1	10.50

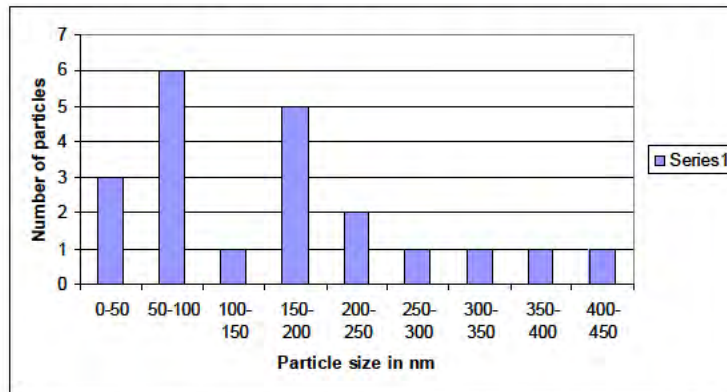
TEM images of dispersed particles, and the mean particle size were obtained for batches 6 and 10, (figures 3.12, to 3.15). The mean particle size for batch 6 based on 21 particles was 161.5nm, and for batch 10 the mean particle size was 38.1nm based on 4 particles.

**Figure 3.12 TEM images of Batch 6 ceria dispersed in  $1\text{gL}^{-1}$  sodium citrate solution**

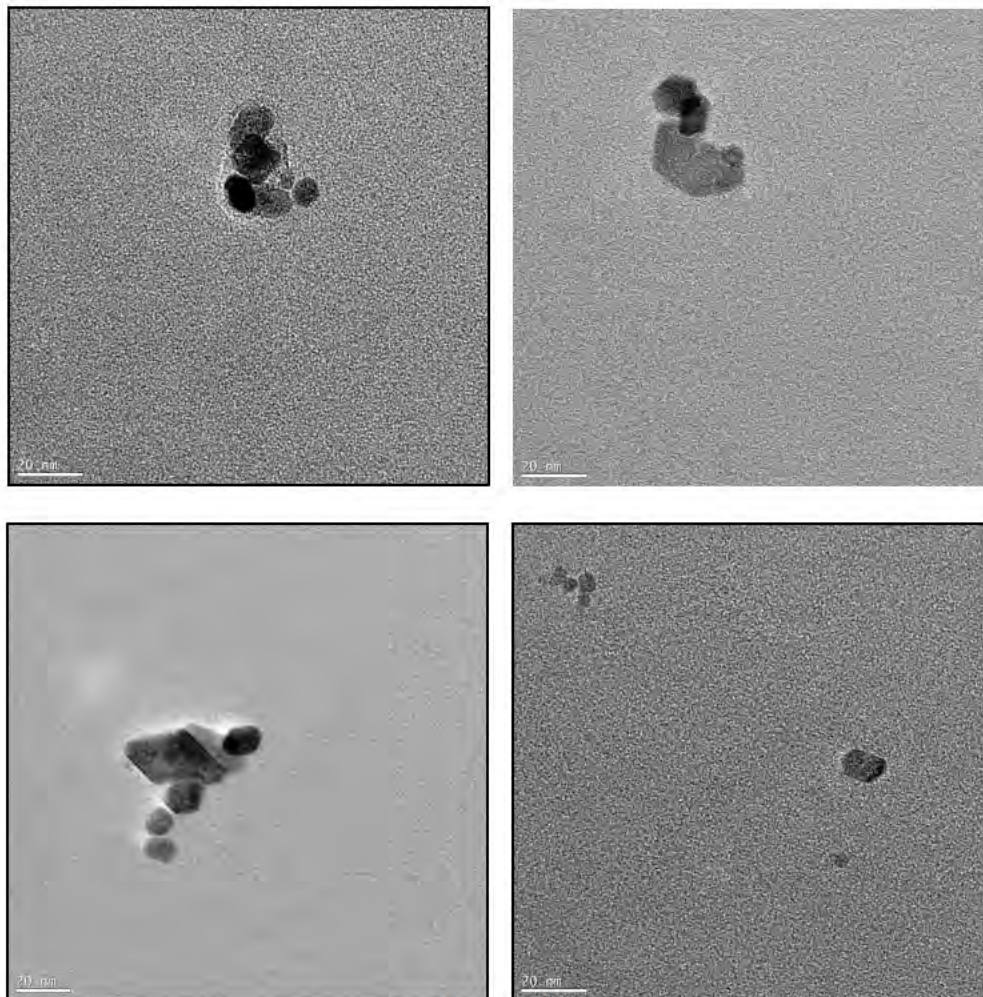
Additional images are presented in Appendix 6



**Figure 3.13 Batch 6 ceria dispersed in  $1\text{gL}^{-1}$  sodium citrate solution (diluted prior to deposition). Particle size from TEM, based on 21 particles**



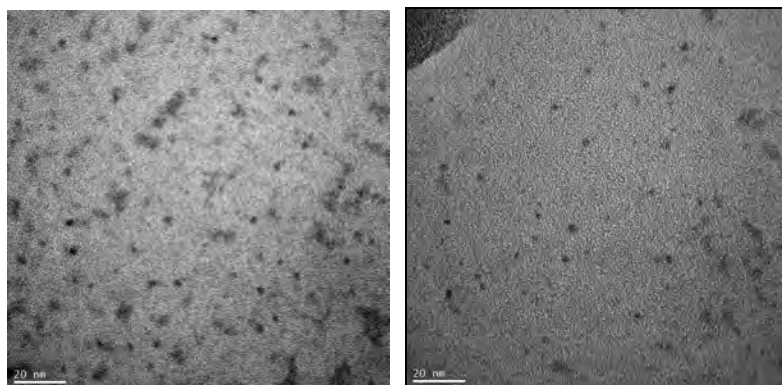
**Figure 3.14 TEM images of Batch 10 ceria dispersed in  $1\text{gL}^{-1}$  sodium citrate solution**



Scale bars 20nm

TEM images of other batches had diffuse, dark patches, (Figure3.15, Appendix 6).

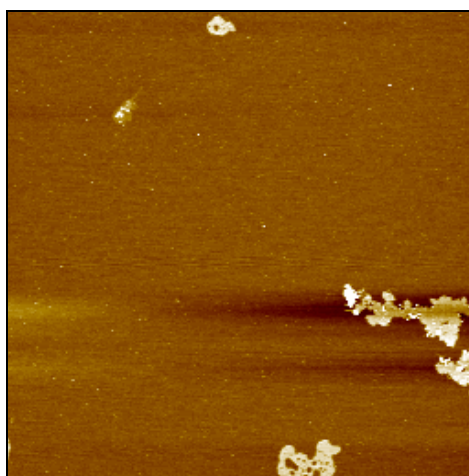
**Figure 3.15. TEM images of Batch 4 ceria dispersed in  $1\text{gL}^{-1}$  sodium citrate solution, washed twice**



Scale bars 20nm

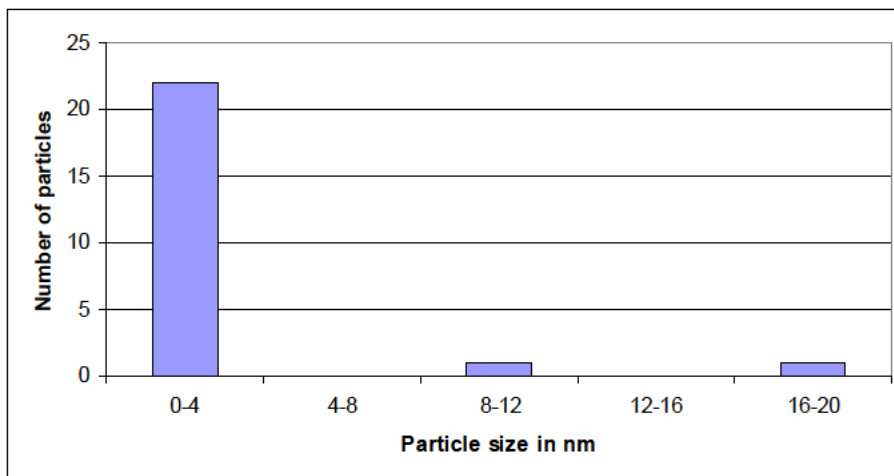
Because there appeared to be residual citrate on the substrates, freshly prepared mica substrates were dipped into  $1\text{gL}^{-1}$  sodium citrate solution without any ceria present. The mica was then washed twice in 25ml water, dried and examined by AFM to assess the quantity of sodium citrate remaining on the substrate. The AFM image presented in figure 3.16 shows some large regions of sodium citrate and small sodium citrate particles. The histogram showing the particle size distribution is presented in figure 3.17.

**Figure 3.16. AFM images of  $1\text{gL}^{-1}$  sodium citrate solution, substrate washed twice in water:  $10\mu\text{m}$  squares**





**Figure 3.17. Particle sizes by AFM from sodium citrate solution, washed twice on 25ml water**



Twenty-four particles were measured to give an indication of the height distribution.

The complete data for these particles is recorded in Appendix 5.9.

### **3.3 Biological toxicity methodology**

Preliminary 3D fluorescence measurements were carried out to assess the suitability of this technique for monitoring interaction of the nanoparticles with cells. Figures 3.18 and 3.19 show the fluorescence observed for cerium(III) and cerium(IV) ions in solution. No fluorescence was observed with sodium citrate solution alone, as shown in figure 3.20. Figures 3.21 and 3.22 show the fluorescence recorded for two different concentrations of cerium oxide nanoparticles in sodium citrate solution. Fluorescence is observed from the solid-state nanoparticles, which increases as the concentration of nanoparticles is increased, but it is at a much lower excitation wavelength than that observed for the solutions of the ions, and occurs over a broader emission range than for the ions.

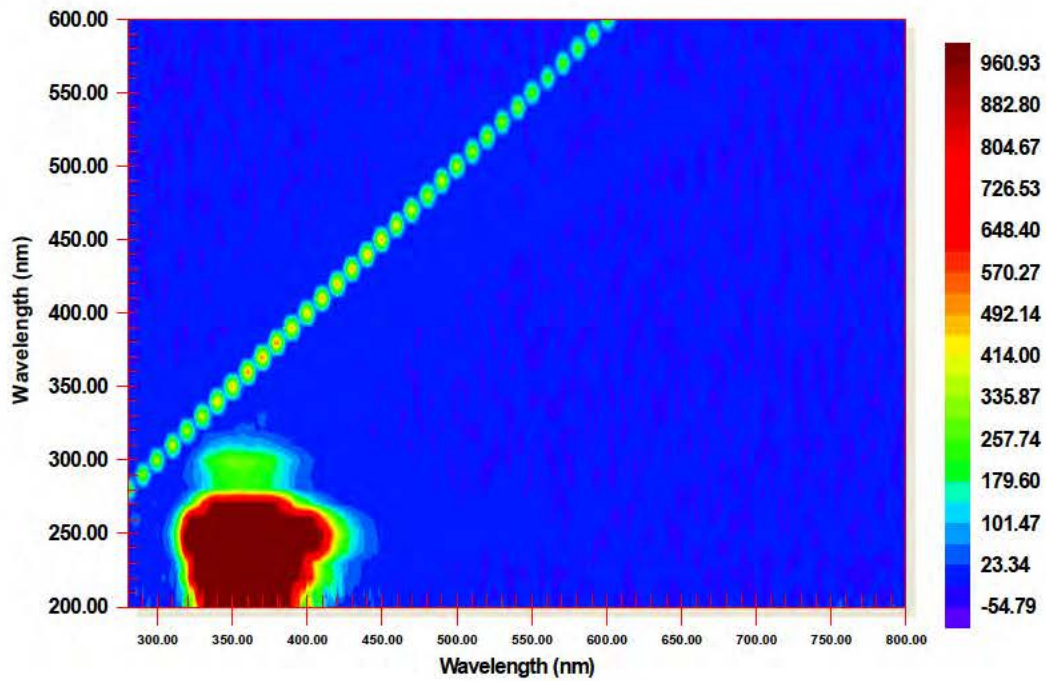
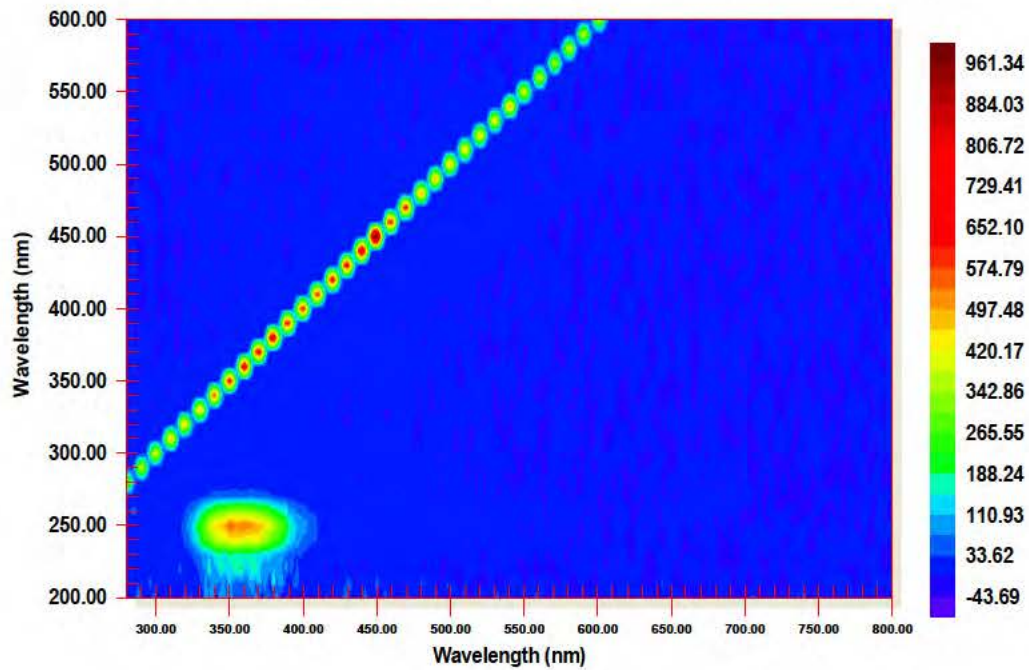
**Figure 3.18. Cerium(III) 0.025mM, 3D fluorescence measurements****Figure 3.19. Cerium(IV) 0.025mM, 3D fluorescence measurements**

Figure 3.20. Sodium citrate  $0.2\text{mgL}^{-1}$ , 3D fluorescence measurements

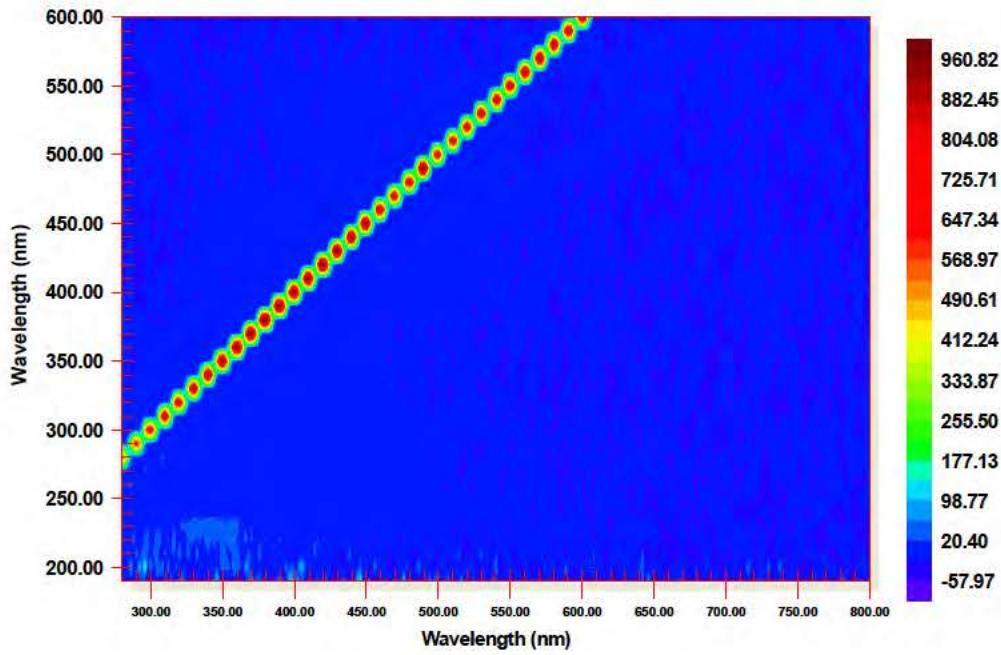
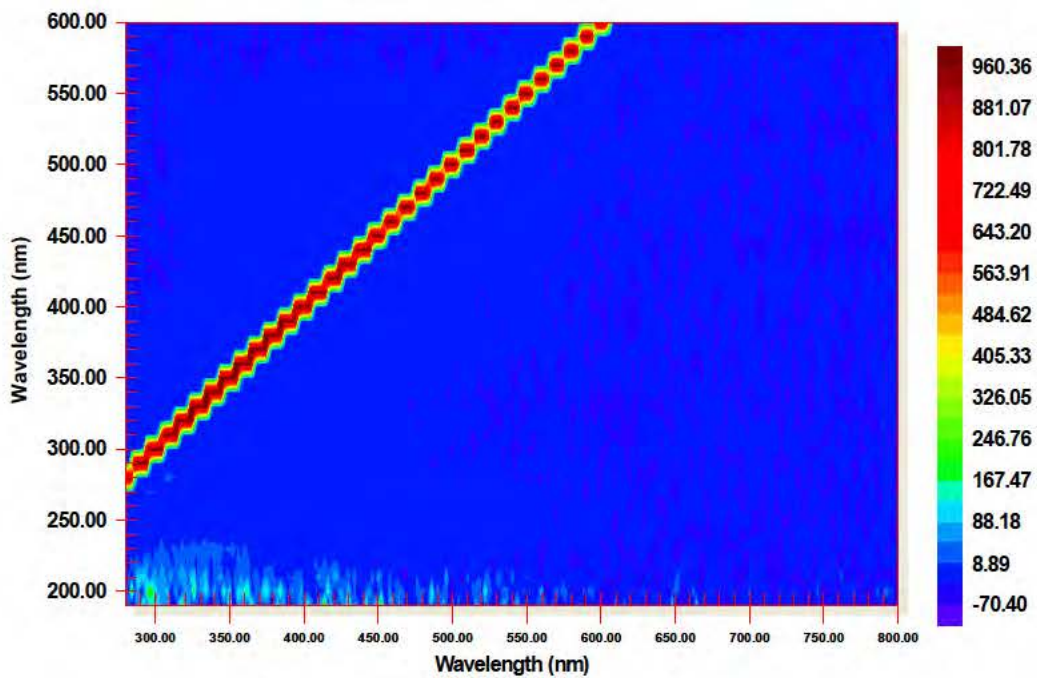
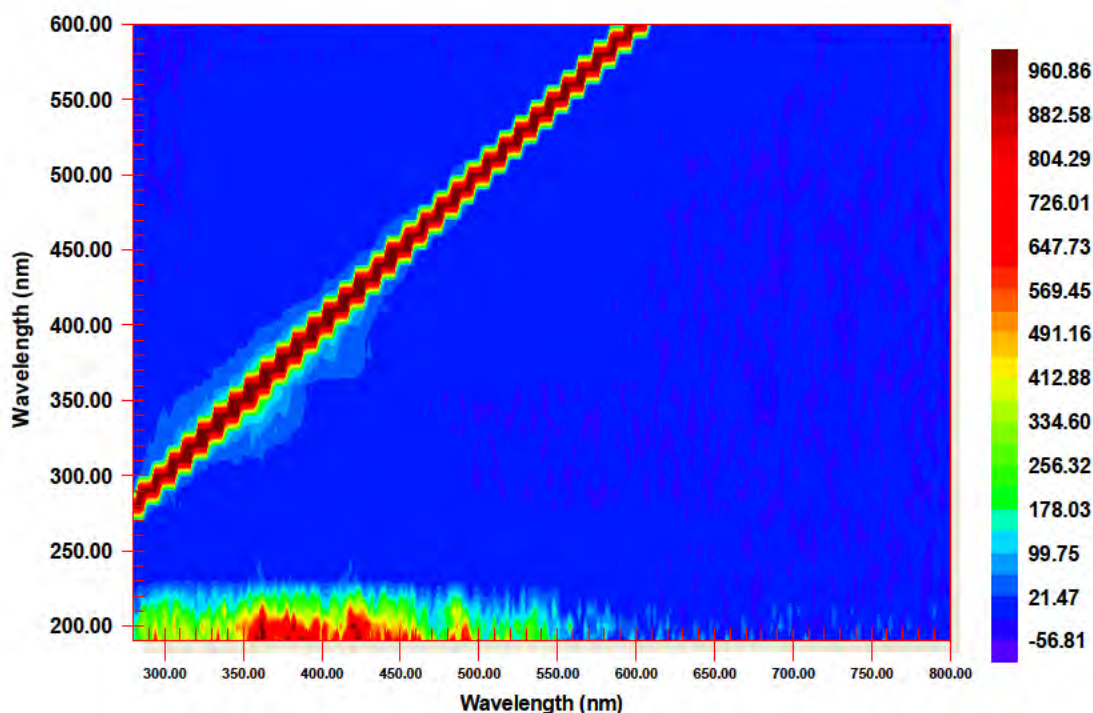


Figure 3.21. Batch 2 ceria  $2.86\text{mgL}^{-1}$  in  $0.2\text{mgL}^{-1}$  sodium citrate, 3D fluorescence measurements

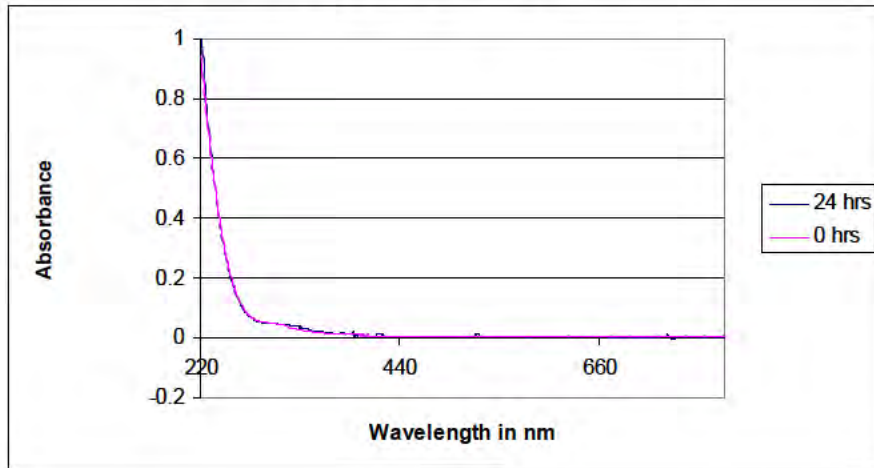


**Figure 3.22. Batch 2 ceria  $28.6\text{mgL}^{-1}$  in  $0.2\text{mgL}^{-1}$  sodium citrate,  
3D fluorescence measurements**

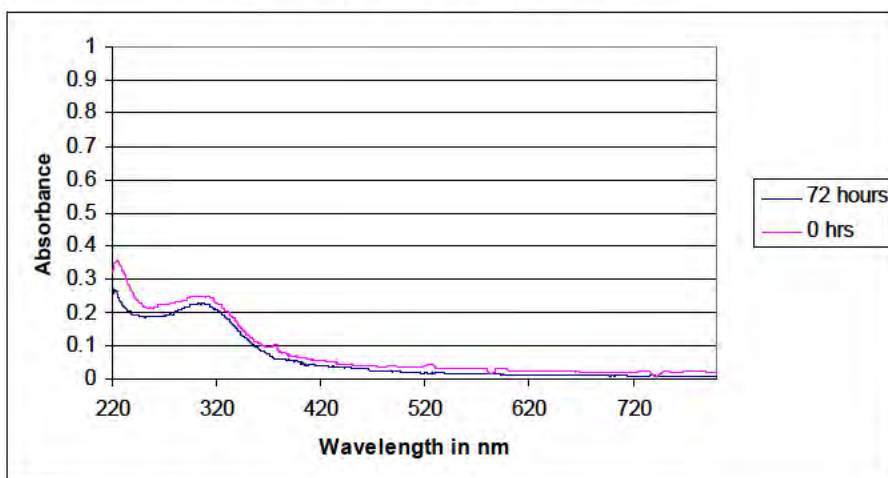


The decision was taken to monitor toxicity of the ceria to the bacterium *Pseudomonas putida* using a 96-well plate reader to monitor the growth of bacteria by the increase in turbidity in the suspension at a wavelength of 595nm. Batch 10 was selected for toxicity testing, for comparison with commercially available ceria nanoparticles, because preliminary AFM measurements had indicated that well-dispersed nanoparticles had been formed in batch 10 in sodium citrate solution. The UV-visible spectrum was measured for wavelengths between 800 and 220nm on mixing, and after a period in the medium to check that the absorbance is low at 595nm, the wavelength at which the bacteria are monitored, and that no changes are occurring in the spectrum due to interaction with the medium. These are shown in figures 3.23 and 3.24.

**Figure 3.23. UV-visible spectrum of Batch 10 ceria in 1:10MDM after 24 hours in the medium**



**Figure 3.24. UV-visible spectrum of commercial ceria in 1:10MDM after 72 hours in the medium**



### 3.4 Nanoparticle stability in media

#### 3.4.1 DLS and zeta potential in media

The particle size by DLS and Zeta potential were measured over time to assess the particle stability in various concentrations of the media. These results for the commercial ceria were presented in tables 3.9 to 3.11, and in figures 3.25 and 3.26.

The data for batch 10 are in table 3.12 and in figure 3.27. The MDM media, diluted

with water to 1 part MDM in 10 parts of water, was selected for biological studies as both particles were stable over time in this media. The stability of commercial ceria was evaluated in both MDM and MDM +1gL<sup>-1</sup> glucose (MDM + g). The particle size of the particles before and after exposure to this media was measured with the AFM, for commercial ceria and Batch 10. The AFM images and histograms showing particle size data are shown in figures 3.28 to 3.35. The data are summarised in table 3.13, with Appendices 5.4 to 5.8 listing the original particle size measurements.

**Table 3.9. Commercial ceria (5mg/litre).**

**Characterisation in biological media: particle size, and measurement quality**

<b>Commercial ceria</b>	<b>Time in days</b>	<b>Z-average particle size/nm</b>	<b>Size quality</b>
MDM 1 to 50	0	726.5	ok
MDM 1 to 10	0	260.6	ok
MDM 1 to 4	0	136.3	good
MDM 1 to 1	0	142.0	good
Water	0	417.0	poor
MDM control	0	403.7	poor
MDM 1 to 50	3	178.0	ok
MDM 1 to 10	3	151.3	good
MDM 1 to 4	3	412.9	good
MDM 1 to 1	3	859.1	ok
Water	3	1109.0	poor
MDM control	3	624.8	poor
MDM 1 to 50	5	206.0	poor
MDM 1 to 10	5	154.8	good
MDM 1 to 4	5	493.8	good
MDM 1 to 1	5	2516.0	poor
Water	5	1564.0	poor
MDM control	5	909.5	poor
MDM 1 to 50	7	226.0	poor
MDM 1 to 10	7	154.6	good
MDM 1 to 4	7	609.2	good
MDM 1 to 1	7	1560.0	poor
Water	7	1786.0	poor
MDM control	7	811.4	poor

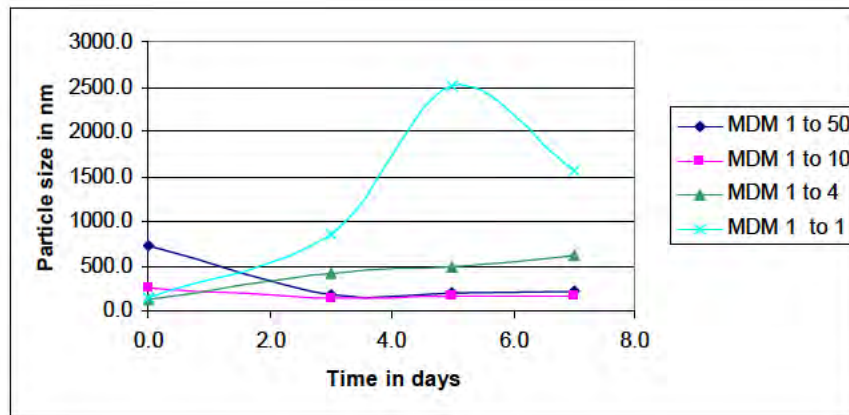
**Table 3.10. Commercial Ceria (10mg/litre). Characterisation in biological media after 7 days: zeta potential and measurement quality**

Commercial ceria	Zeta potential /mV	Zeta potential quality
MDM 1 to 50	-36.4	ok
MDM 1 to 10	-10.1	ok
MDM 1 to 4	-35.3	ok
MDM 1 to 1	-14.5	poor

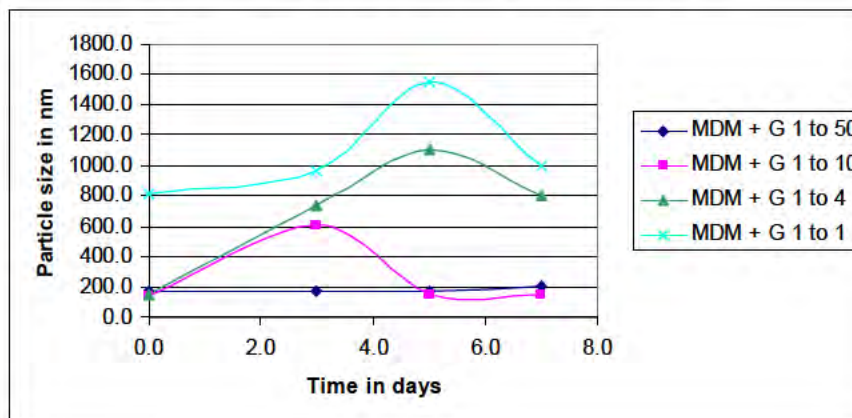
**Table 3.11. Commercial ceria (5mg/litre). Characterisation in biological media + glucose: particle size and measurement quality**

Commercial ceria	Time in days	Z-average particle size/nm	Size quality
MDM + G 1 to 50	0	171.0	ok
MDM + G 1 to 10	0	141.0	good
MDM + G 1 to 4	0	156.2	good
MDM + G 1 to 1	0	808.5	poor
Water	0	417.0	poor
MDM + glucose control	0	471.0	poor
MDM + G 1 to 50	3	174.0	poor
MDM + G 1 to 10	3	606.0	poor
MDM + G 1 to 4	3	742.0	poor
MDM + G 1 to 1	3	969.5	poor
Water	3	1109.0	poor
MDM + glucose control	3	767.5	poor
MDM + G 1 to 50	5	168.9	poor
MDM + G 1 to 10	5	156.1	good
MDM + G 1 to 4	5	1102.0	poor
MDM + G 1 to 1	5	1547.0	poor
Water	5	1564.0	poor
MDM + glucose control	5	601.0	poor
MDM + G 1 to 50	7	209.9	good
MDM + G 1 to 10	7	156.7	good
MDM + G 1 to 4	7	870.5	poor
MDM + G 1 to 1	7	1001.0	poor
Water	7	1786.0	poor
MDM + glucose control	7	no result	

**Figure 3.25. Stability of commercial ceria (5mg/litre)  
in Minimal Davis Medium (MDM)**



**Figure 3.26. Stability of commercial ceria (5mg/litre)  
in Minimal Davis Medium (MDM) + glucose.**



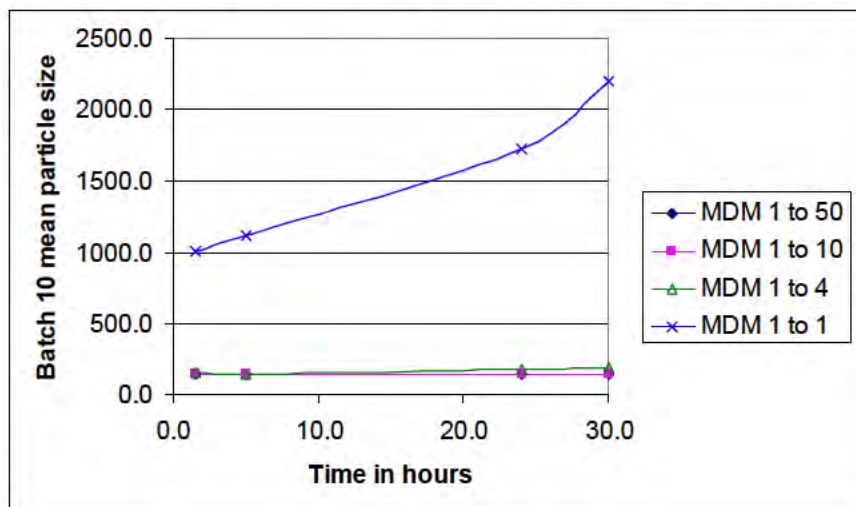


**Table 3.12. Batch 10 ceria (20mg/litre in 1g/litre sodium citrate solution).**

**Characterisation in Minimal Davis Medium (MDM) + glucose:  
particle size, zeta potential and measurement quality**

Batch 10 concentration of MDM + glucose	Time in hours	Z-average particle size/nm	Size quality	Zeta potential /mV	Zeta potential quality
1 to 50	1.5	145.4	good		
1 to 10	1.5	141.5	good		
1 to 4	1.5	153.5	poor		
1 to 1	1.5	1002.0	good		
1 to 50	5	138.9	good	-45.2	ok
1 to 10	5	143.1	good	-42.9	ok
1 to 4	5	147.2	good	-39.4	ok
1 to 1	5	1114.0	poor	-27.0	v. poor
1 to 50	24	143.9	ok	-37.7	poor
1 to 10	24	143.7	good	-38.7	poor
1 to 4	24	179.1	good	-33.6	ok
1 to 1	24	1727.0	poor	-26.3	poor
1 to 50	30	138.8	good		
1 to 10	30	138.8	good		
1 to 4	30	193.2	good		
1 to 1	30	2196.0	poor		

**Figure 3.27. Stability of Batch 10 ceria (20mg/litre, 1g/litre sodium citrate solution) in Minimal Davis Medium (MDM) + glucose**

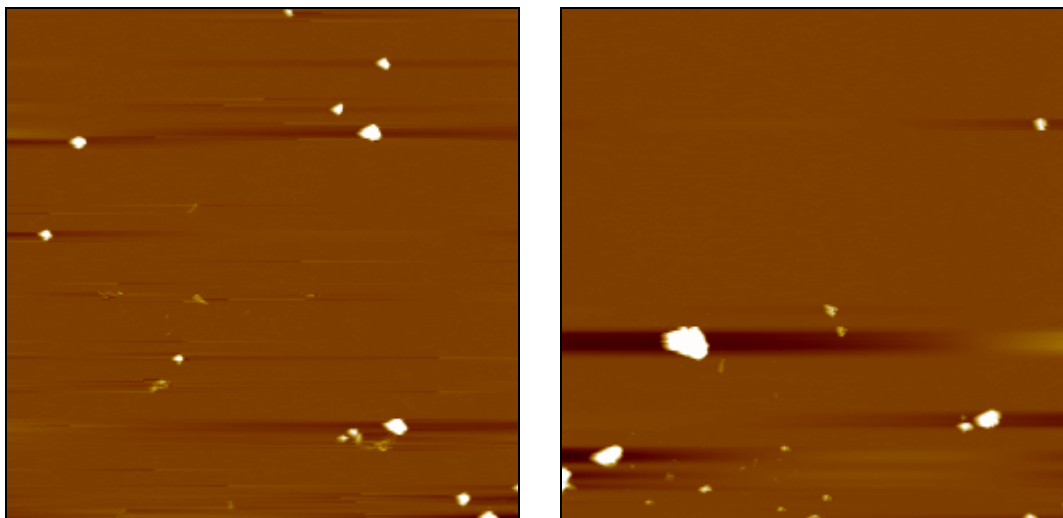


### 3.4.2 AFM measurements

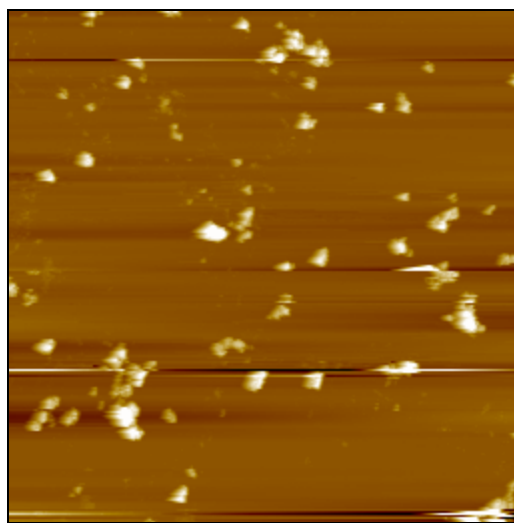
#### 3.4.2.1 Commercial ceria

Figures 3.28 and 3.29 show AFM images of commercially obtained ceria nanoparticles from dispersions in water at two different ceria concentrations. The effect of concentration on the particle size distribution is shown in figure 3.30.

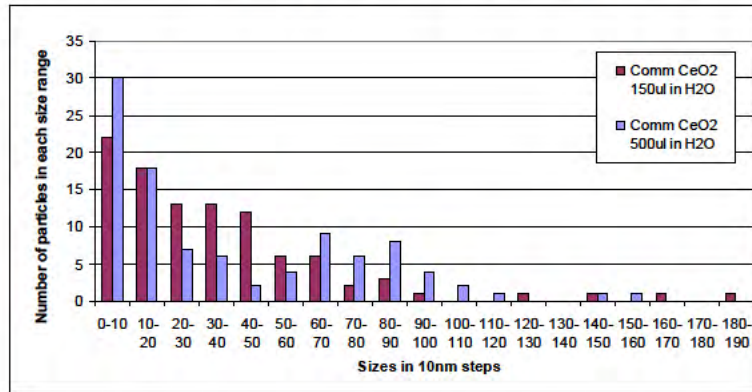
**Figure 3.28. AFM images of commercial ceria in H<sub>2</sub>O (batch 150 $\mu$ L 230712):  
10 $\mu$ m squares.**



**Figure 3.29. AFM image of commercial ceria in water, (batch 500 $\mu$ L 230712):  
10 $\mu$ m square**

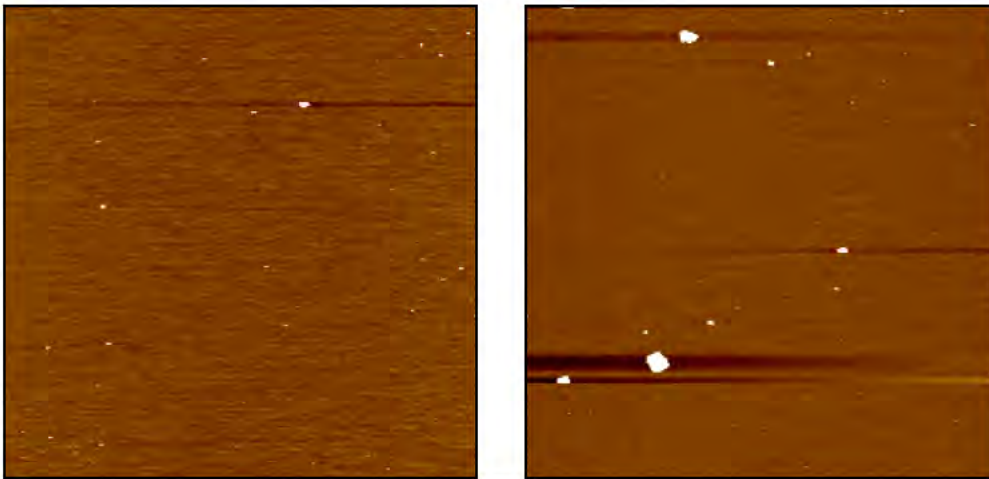


**Figure 3.30. Comparison of particle size for different concentrations of commercial ceria in water measured by AFM**

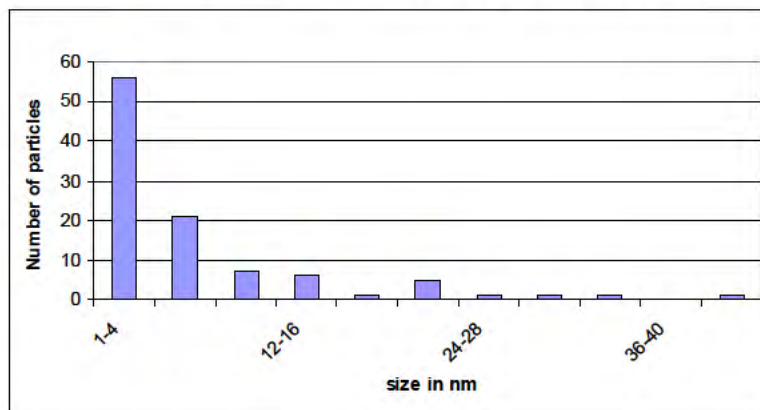


The particle size distribution of commercial ceria in 1:10 MDM was also examined. As shown in figures 3.31 and 3.32.

**Figure 3.31. AFM images of commercial ceria in 1:10 MDM, (batch conc. 290612): 10 $\mu$ m squares.**



**Figure 3.32. Particle size of commercial ceria in 1:10 MDM measured by AFM**

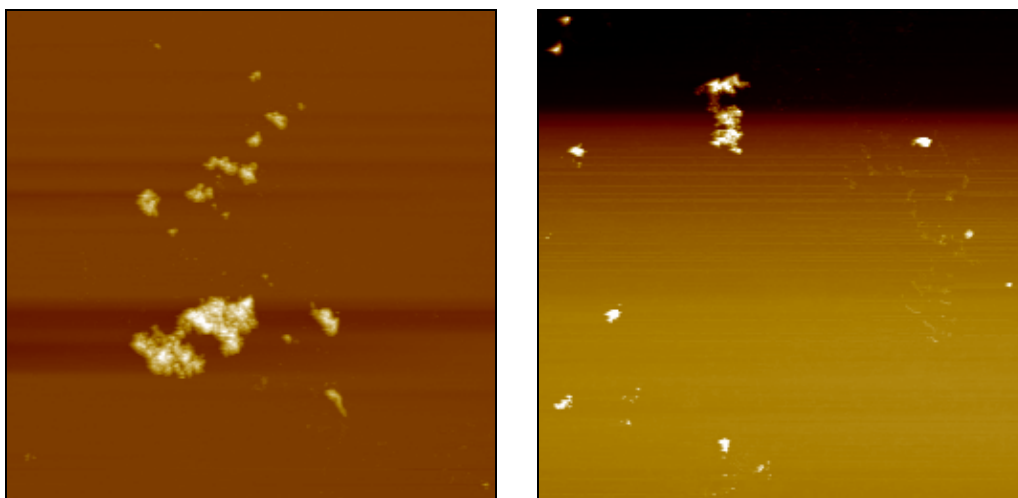


### 3.4.2.2 Batch 10

Changes in particle size of batch 10 dispersed in water and MDM were also examined by AFM as shown in figures 3.33 - 3.34. The histograms comparing particle sizes shown in figure 3.35 shows a higher number of particles below 10nm in MDM than in the water dispersion. Table 3.35 summarises the particle size data obtained by AFM for batch 10 and the commercial ceria.

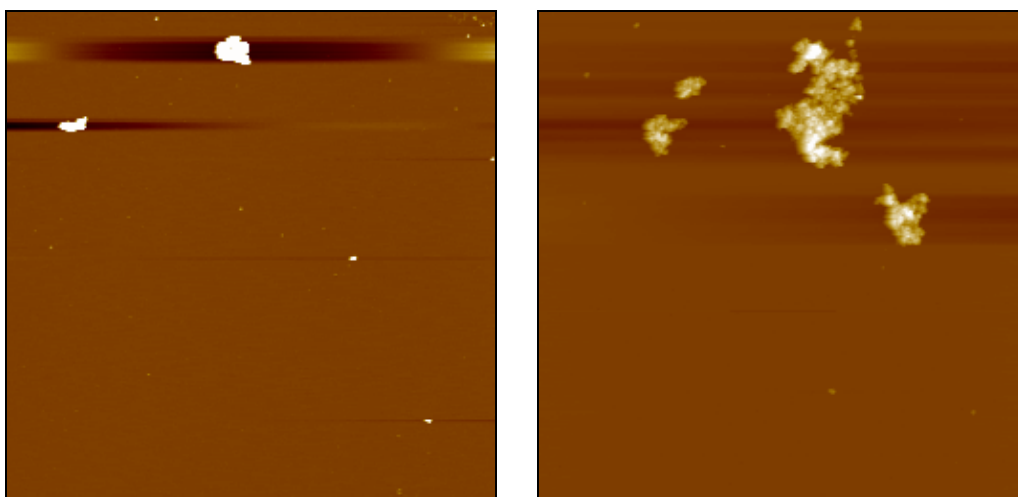
**Figure 3.33. AFM images of Batch 10 ceria in water (Batch 060712):**

**10 $\mu$ m squares**

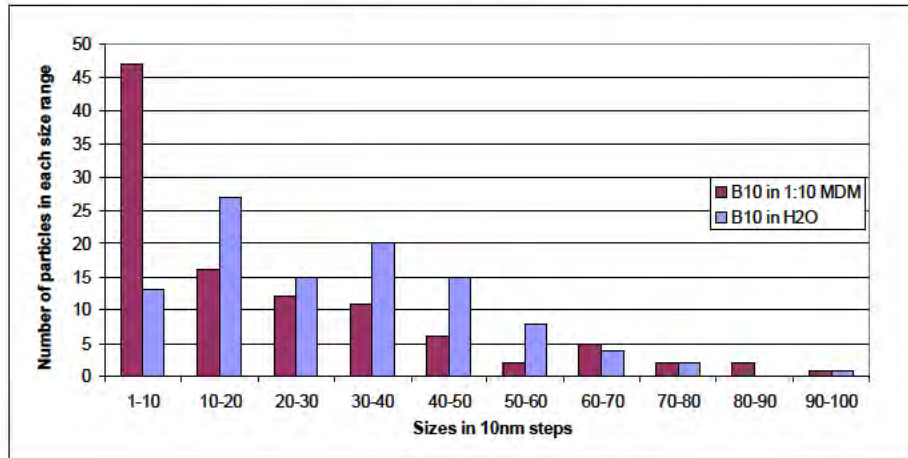


**Figure 3.34. AFM images of Batch 10 ceria in 1:10 MDM (Batch 230712):**

**10 $\mu$ m squares.**



**Figure 3.35. Comparison of particle size of Batch 10 ceria in water and 1:10 MDM measured by AFM**



**Table 3.13. Particle size data from AFM measurements for commercial ceria and batch 10 ceria**

Material	Batch	No of particles	Mean size/nm
Commercial CeO <sub>2</sub> in H <sub>2</sub> O	150μL, 230712	100	35.23
Commercial CeO <sub>2</sub> in H <sub>2</sub> O	500μL, 230712	100	39.06
Commercial CeO <sub>2</sub> in 1:10 MDM	Conc B, 290612	77	25.42
B10 CeO <sub>2</sub> in H <sub>2</sub> O	060712	106	32.79
B10 CeO <sub>2</sub> 1:10 MDM	230712	104	21.12

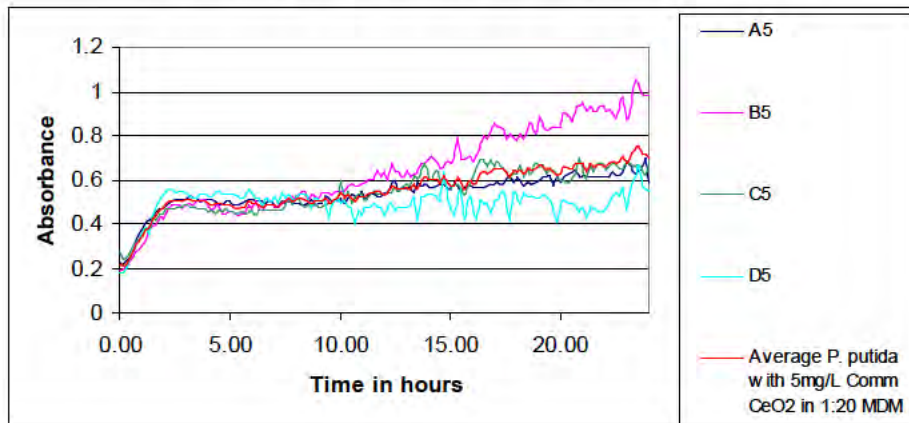
### 3.5 Toxicity data

Test runs 1 and 2 in the 96 well-plate are detailed in the methodology, chapter 2. Raw data are shown in Appendix 7.

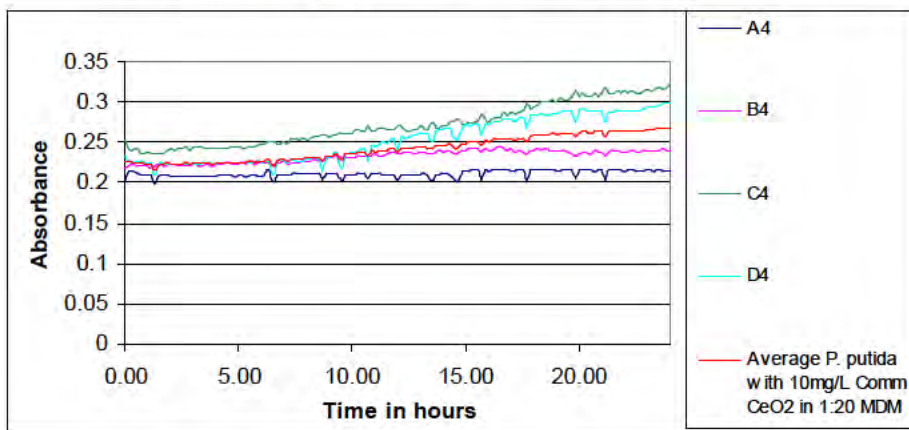
#### 3.5.1 Run 1 (26/6/12)

The original bacteria was grown in 1:10 MDM, with and without glucose. The final concentration of MDM in the wells was 1:20. Individual results from each well, and the average of the four replicates, are presented in figures 3.36 to 3.43.

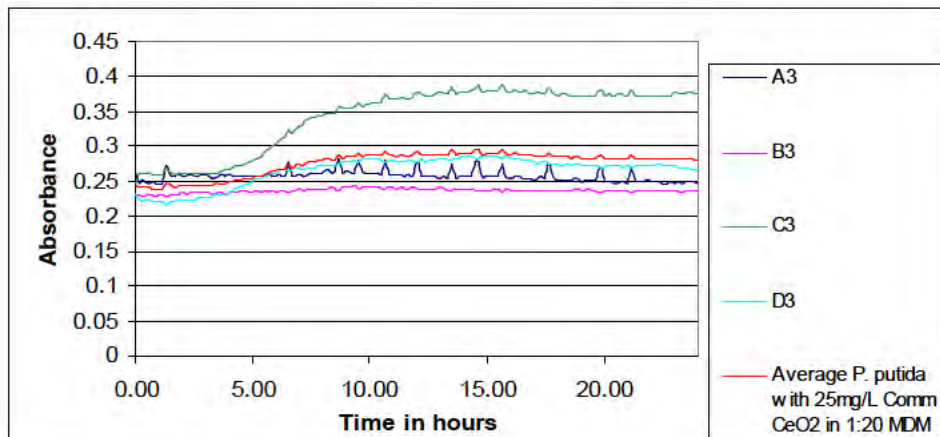
**Figure 3.36. Effect of commercial ceria nanoparticles, concentration  $5\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:20 MDM**



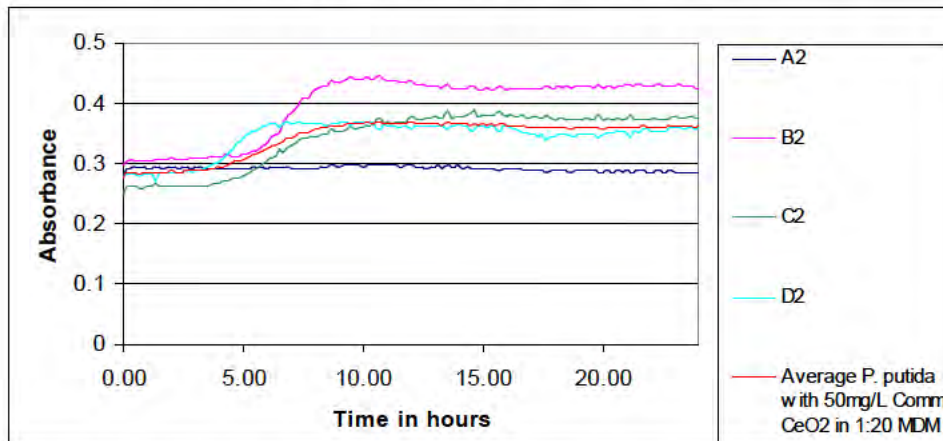
**Figure 3.37. Effect of commercial ceria nanoparticles, concentration  $10\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:20 MDM**



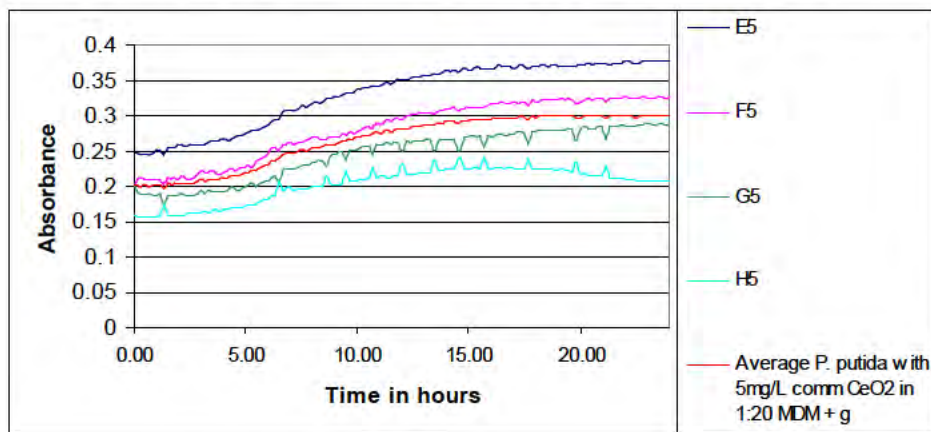
**Figure 3.38. Effect of commercial ceria nanoparticles, concentration  $25\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:20 MDM**



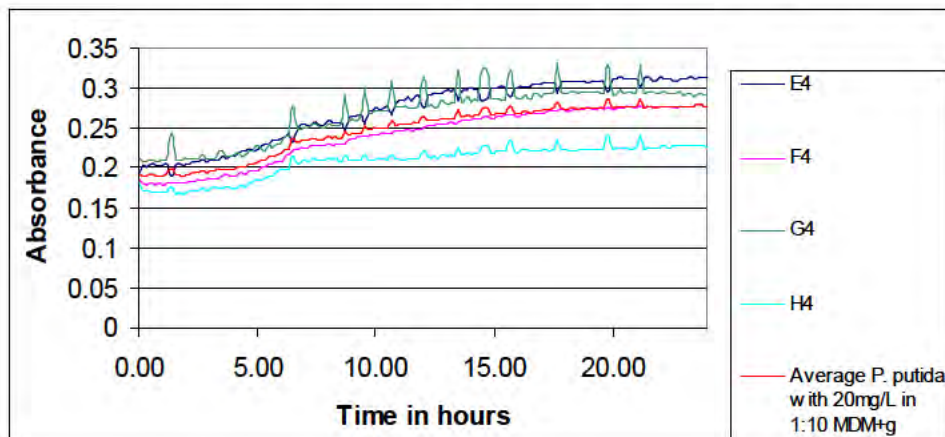
**Figure 3.39. Effect of commercial ceria nanoparticles, concentration  $50\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:20 MDM**



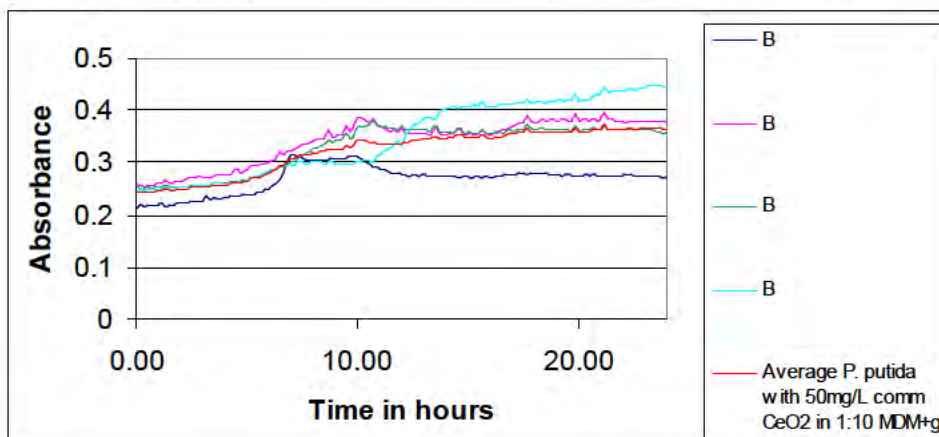
**Figure 3.40. Effect of commercial ceria nanoparticles, concentration  $5\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:20 MDM + glucose**



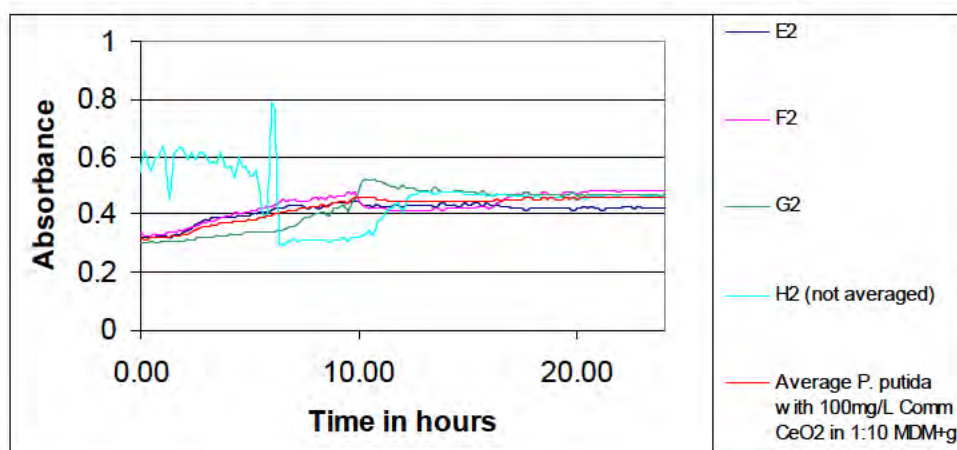
**Figure 3.41. Effect of commercial ceria nanoparticles, concentration  $10\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:20 MDM + glucose**



**Figure 3.42. Effect of commercial ceria nanoparticles, concentration  $25\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:20 MDM + glucose**



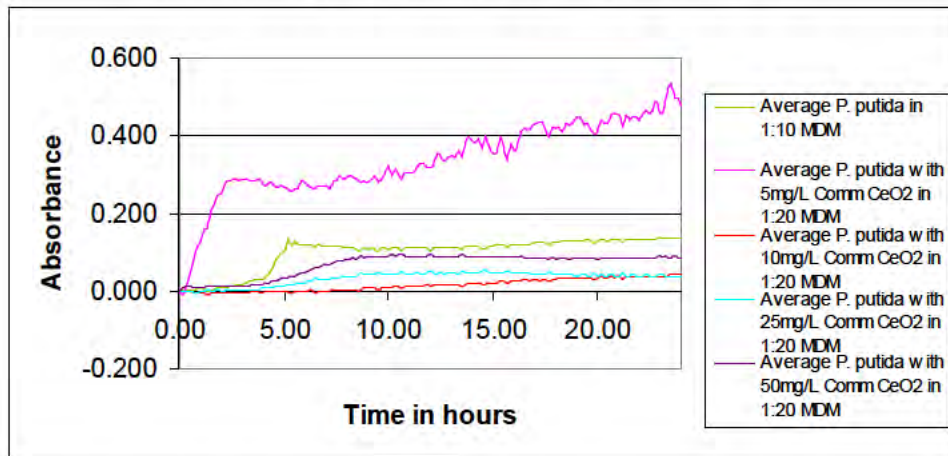
**Figure 3.43. Effect of commercial ceria nanoparticles, concentration  $50\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:20 MDM + glucose**



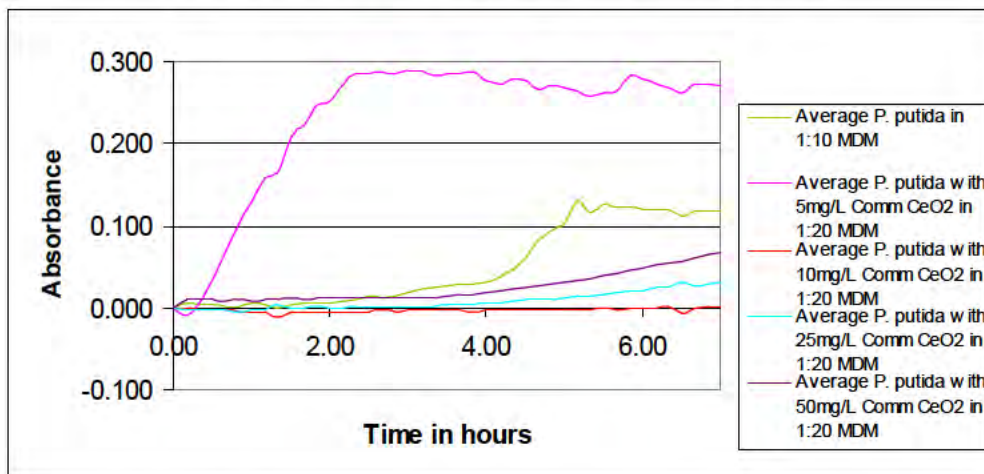
In figures 3.44 to 3.47, the average values for the different concentrations of ceria were compared to each other. The initial value of the absorbance was subtracted from all subsequent values for each trace, to normalise the data and allow the traces to be compared.



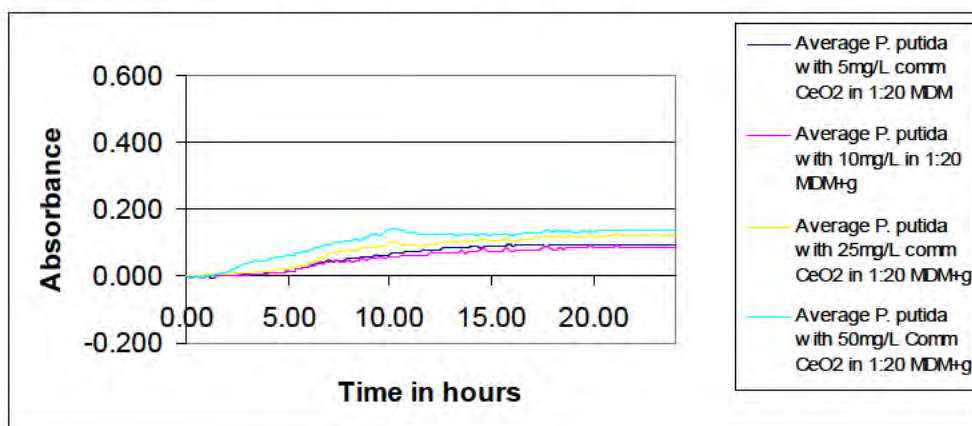
**Figure 3.44. Effect of commercial ceria nanoparticles on bacteria (*Pseudomonas putida*) in 1:20 MDM**



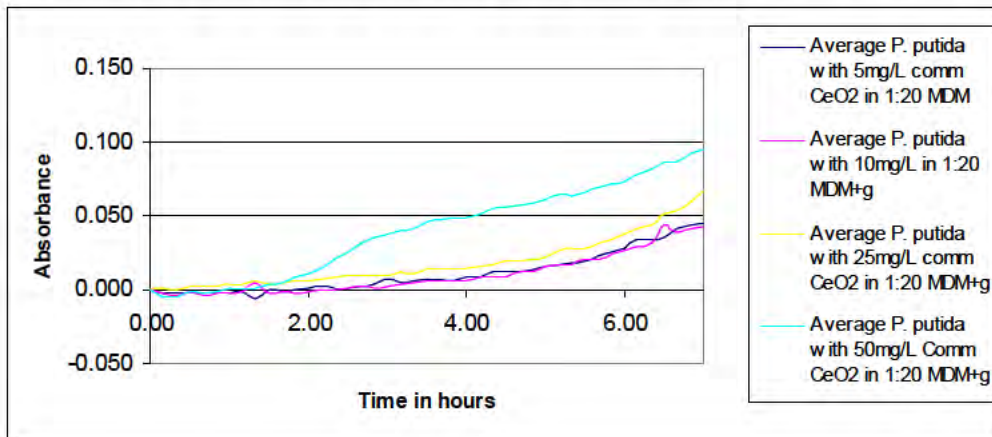
**Figure 3.45. Effect of commercial ceria nanoparticles on bacteria (*Pseudomonas putida*) in 1:20 MDM. Results from initial 7 hours**



**Figure 3.46. Effect of commercial ceria nanoparticles on bacteria (*Pseudomonas putida*) in 1:20 MDM + glucose, 26/6/12. Average of four runs**

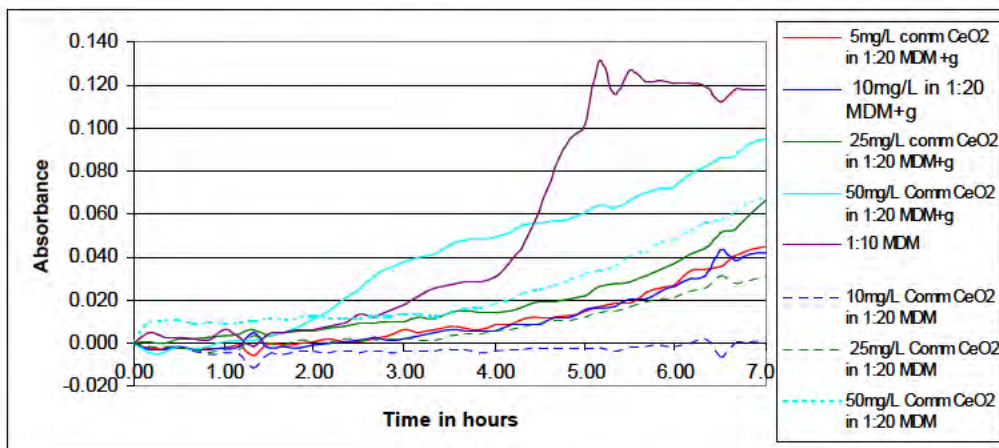


**Figure 3.47. Effect of commercial ceria nanoparticles on bacteria (*Pseudomonas putida*) in 1:20 MDM + glucose, 26/6/12 Results from initial 7 hours**



The data for the *P. putida* growth over the first seven hours with different levels of commercial ceria in MDM and MDM + glucose are presented in figure 3.48.

**Figure 3.48. Comparison of *P. putida* growth over seven hours in MDM and MDM + glucose with different levels of commercial ceria, (Data for 5mgL<sup>-1</sup> in MDM excluded for clarity).**



### 3.5.2 Run 2 (060712)

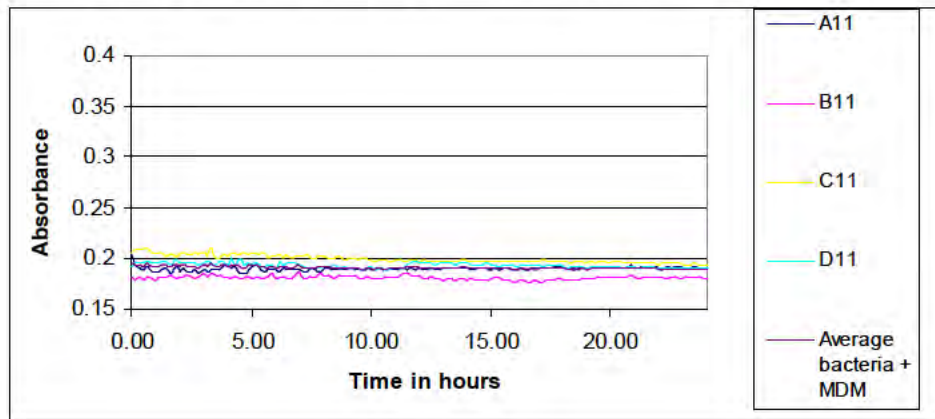
The original bacteria were grown in 1:1 MDM, without glucose. The final concentration of MDM in the wells was 1:10. Individual results from each well, and the average of the four replicates, are presented in figures 3.51-3.54 and 3.57 to 3.60.

The average data is compared in figures 3.55, 3.56, 3.61 and 3.62. The effect of ceria concentration was examined in figure 3.63, and the influence of sodium citrate in figure 3.64.

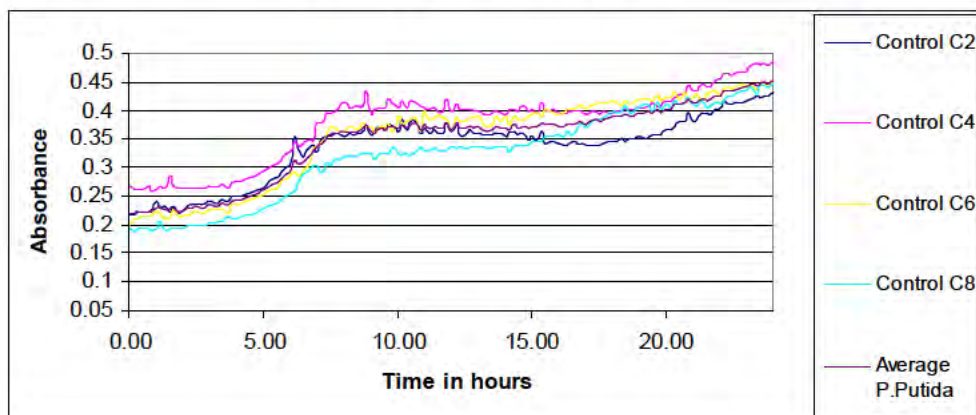
### 3.5.2.1 *P. putida* in 1:10 MDM

Four replicates of bacteria in 1:10 MDM, and 1:10 MDM with  $0.5 \text{ gL}^{-1}$  sodium citrate solution are presented in figures 3.49 and 3.50.

**Figure 3.49. Growth of *Pseudomonas putida* in 1:10 MDM**

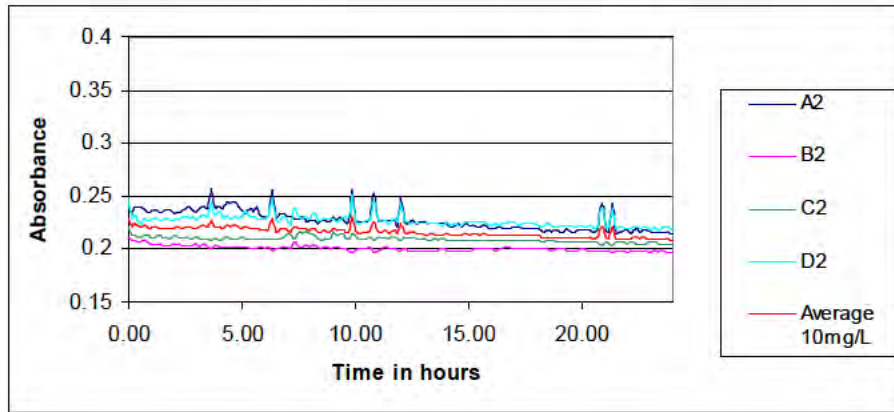


**Figure 3.50. Growth of *Pseudomonas putida* in 1:10 MDM and  $0.5 \text{ gL}^{-1}$  sodium citrate**

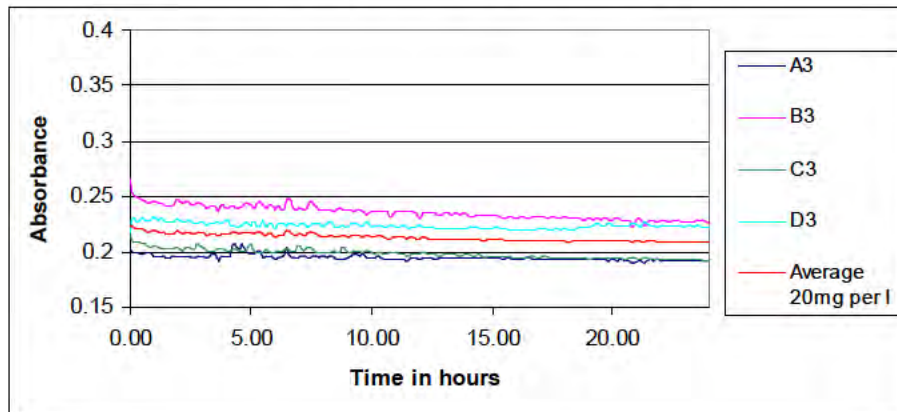


### 3.5.2.2 Commercial ceria in 1:10 MDM

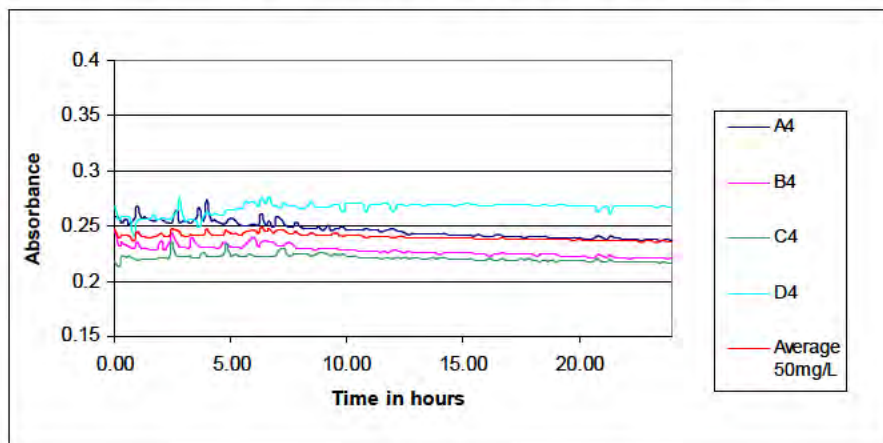
**Figure 3.51. Effect of commercial ceria nanoparticles, concentration  $10\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:10 MDM**



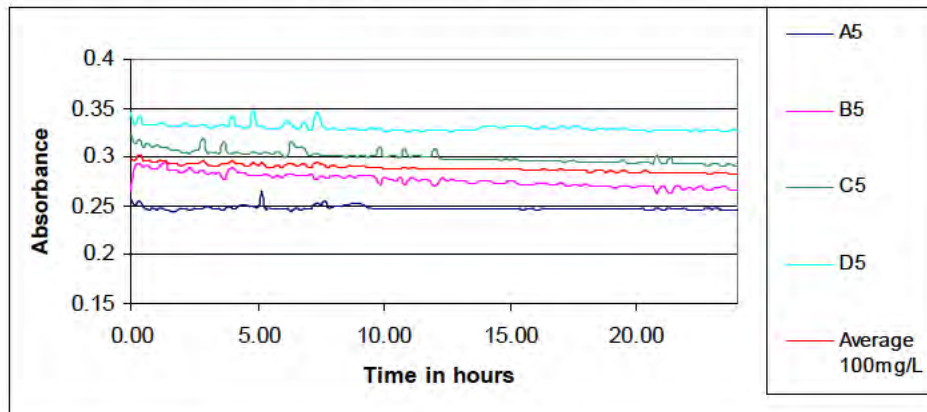
**Figure 3.52. Effect of commercial ceria nanoparticles, concentration  $20\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:10 MDM**



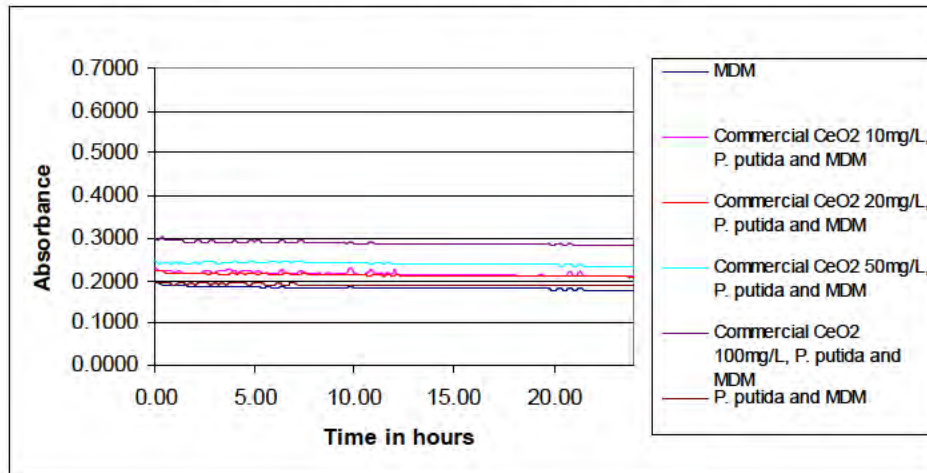
**Figure 3.53. Effect of commercial ceria nanoparticles, concentration  $50\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:10 MDM**



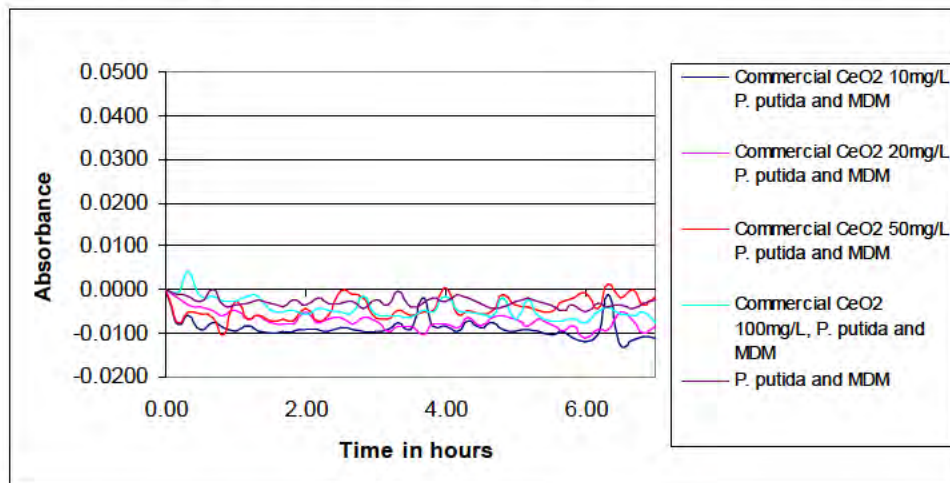
**Figure 3.54. Effect of commercial ceria nanoparticles, concentration  $100\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:10 MDM**



**Figure 3.55. Effect of commercial ceria nanoparticles on bacteria (*Pseudomonas putida*) in 1:10 MDM (6/7/12). Average of four runs**



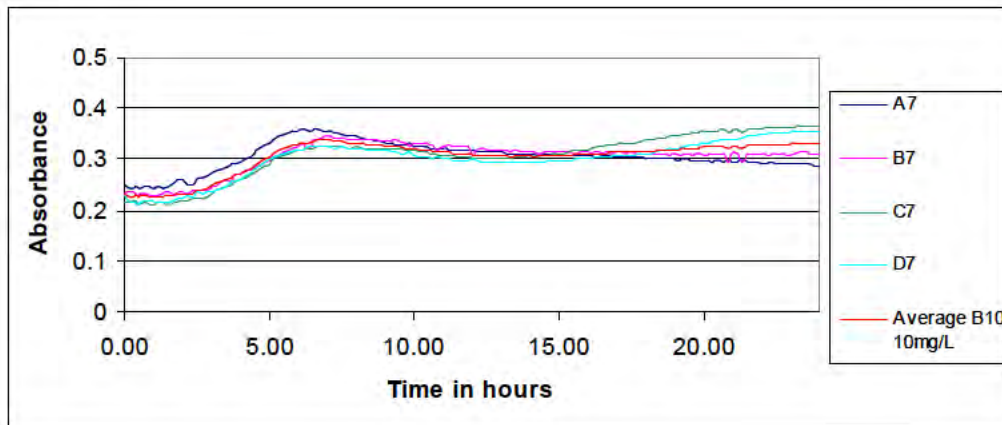
**Figure 3.56. Effect of commercial ceria nanoparticles on bacteria (*Pseudomonas putida*) in 1:10 MDM (6/7/12). Results from initial 7 hours**



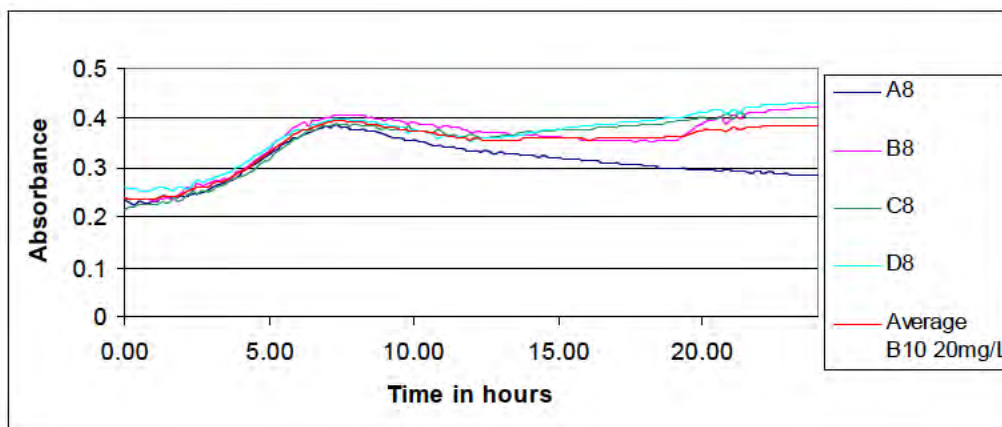
### 3.5.2.3 Batch 10 ceria in 1:10MDM

The data from individual wells and their averages are shown in figures 3.57 to 3.60, and plots of the averaged data are presented in figures 3.61 and 3.62.

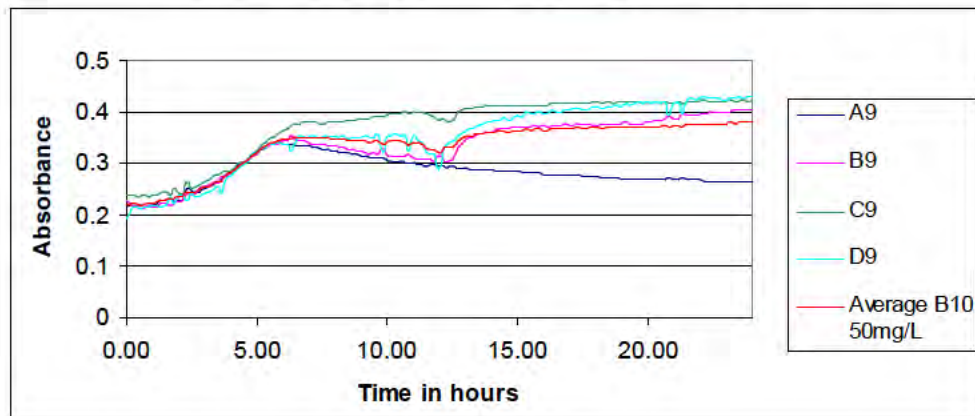
**Figure 3.57. Effect of Batch 10 ceria nanoparticles, concentration  $10\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:10 MDM**



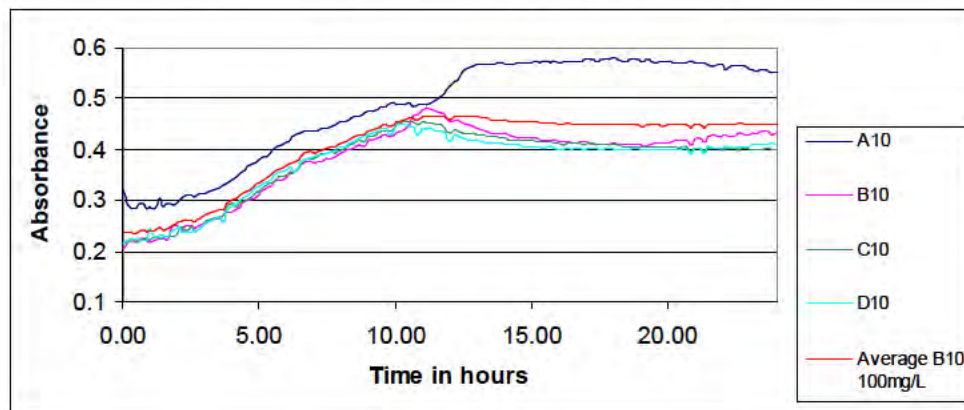
**Figure 3.58. Effect of Batch 10 ceria nanoparticles, concentration  $20\text{mgL}^{-1}$ , on bacteria (*Pseudomonas putida*) in 1:10 MDM**



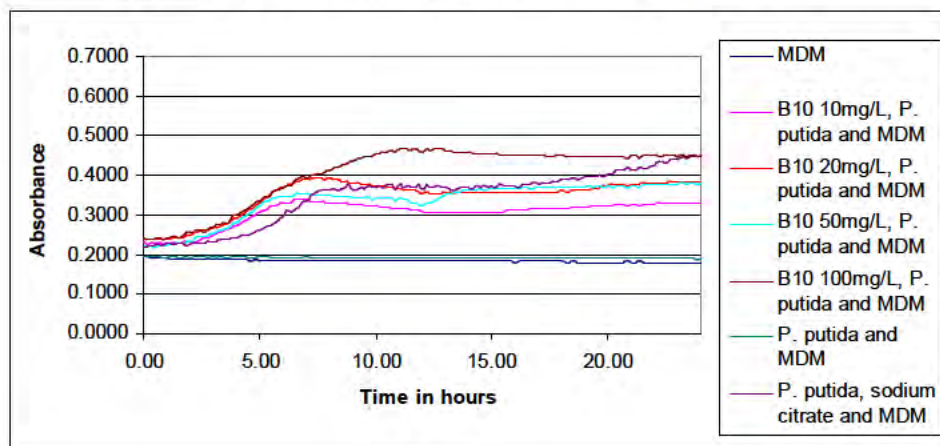
**Figure 3.59. Effect of Batch 10 ceria nanoparticles, concentration 50mgL<sup>-1</sup>, on bacteria (*Pseudomonas putida*) in 1:10 MDM**



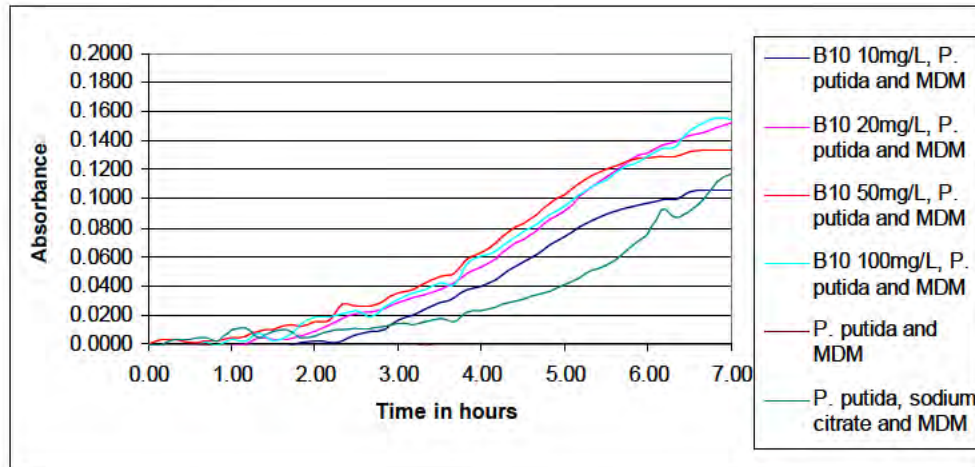
**Figure 3.60. Effect of Batch 10 ceria nanoparticles, concentration 100mgL<sup>-1</sup>, on bacteria (*Pseudomonas putida*) in 1:10 MDM**



**Figure 3.61. Effect of Batch 10 ceria nanoparticles on bacteria (*Pseudomonas putida*) in 1:10 MDM (6/7/12). Average of four runs**



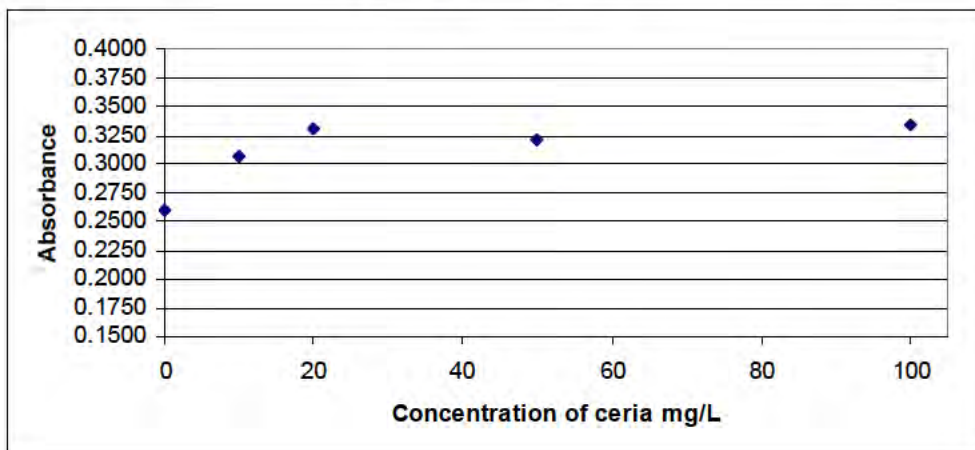
**Figure 3.62. Effect of Batch 10 ceria nanoparticles on bacteria (*Pseudomonas putida* in 1:10 MDM (6/7/12). Results from initial 7 hours**



The correlations of bacterial growth with ceria concentration and with sodium citrate concentration are plotted in figures 3.63 and 3.64.

**Figure 3.63. Influence of concentration of Batch 10 ceria nanoparticles on the growth of *Pseudomonas putida* in 1:10 MDM after 5 hours (6/7/12).**

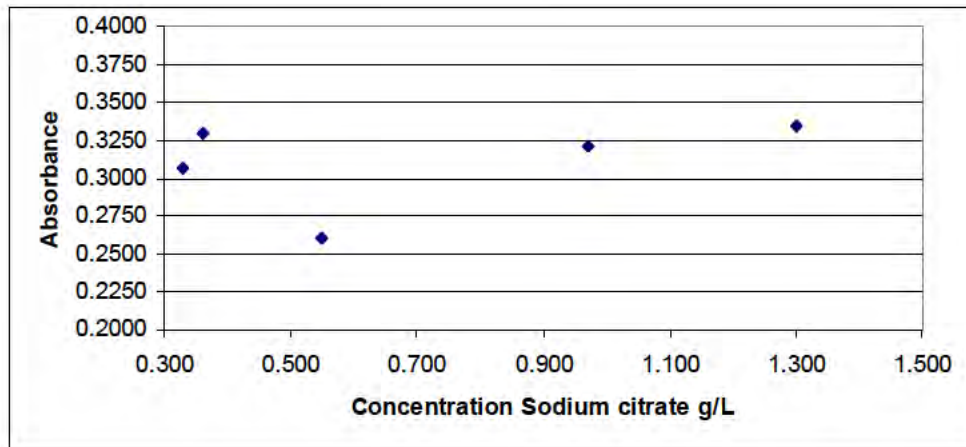
**Average of four runs**





**Figure 3.64. Influence of concentration of sodium citrate on the growth of *Pseudomonas putida* in 1:10 MDM after 5 hours (6/7/12).**

**Average of four runs**



## **4. Discussion**

### **4.1 Preparation of batches**

The batches of cerium oxide nanoparticles prepared are listed in the methodology, in Table 2.2. The initial aim was to prepare batches of nanoparticles with different aspect ratios and compare the toxicology. Batches 1 and 2 were prepared from the same initial batch, but an aliquot was removed after 3 hours to form batch 1, and the remainder was heated for an additional 5 hours to form batch 2.

Batch 7 was prepared with sodium hydroxide in the reaction vessel. It was intended to wash the nanoparticles using the ultracentrifuge, but the tubes were not suitable for use with material at high pH and the batch was not washed or characterised.

#### **4.1.1 Preparation of batches with capping agents**

Batches 5, 8, 9 and 10 were prepared with capping agents in the hydrothermal reaction vessel. Batch 8 contained PVP and was discarded immediately because the dark colour and the smell of caramelised sugar on opening the reaction vessel suggested that the PVP was not stable under the reaction conditions used. The other batches were prepared with sodium citrate, which appeared to be more thermally stable than PVP. Lower concentrations of ceria were prepared in these reactions than in the batches without surfactant, as the concentration of organic material from the surfactant had to be kept below 0.5 grams to avoid excessive pressure in the vessel during heating. Although some sedimentation occurred in these batches, it was a slower process than in batches without surfactant.

## 4.2 Characterisation of as-prepared batches by TEM and XRD

TEM images of the as-prepared batches 1 to 4 are presented in figures 3.1 to 3.4.

Batch 1 appeared to be formed of larger, less dense particles than batch 2, suggesting that the formation of the oxide was not complete after three hours.

Two general observations were made from the TEM work. Firstly, the nanoparticles were all agglomerated, which meant that it would be necessary to prepare stable dispersions of the particles before toxicity testing could be carried out. Secondly, the observed shape of these nanoparticles matched those predicted from the literature, as shown in Table 2.2, but the proportion of nanowires was lower than expected.

Whereas Wang et al. (2010) reported more than 80% nanowires, (based on estimates from TEM images), in this work at least 50% of the particles (by volume), were cubes in TEM images of batch 3. The change in shape between nanocubes and nanorods reported by Wang et al. (2010) occurred by changing between nitrate and chloride as precursors, and they found 2.1% chloride ions in nanowires by EDX, but very little chloride was measured in the nanoparticles. They concluded that adsorbed chloride ions promoted preferential growth of specific crystal faces, resulting in the formation of wires. The same conclusion was reached by Wu et al. (2008), who investigated replacement of surface chloride ions with nitrate in the cerium hydroxide precursor precipitated from chloride salts, and found that this resulted in the formation of nanocubes. To increase the proportion of nanowires in the product, batch 6, 11 and 13 were washed with ammonium chloride solution prior to heating.

The XRD reflection intensities for the batches were compared with published values as shown in table 3.1. The database from the International Centre for Diffraction Data used by Birmingham University Chemistry Department gave a good match for files

04-013-4361 ( $\text{CeO}_2$ ) and 01-078-3280 ( $\text{CeO}_{1.88}$ ) and the peak positions and relative intensities for these two materials were almost identical for the main reflection peaks observed. Wang et al. (2010) had reported enhanced reflections for the 111 plane (at  $2\theta = 28.52$  degrees) for batches containing nanowires. This was not the case for the batches prepared here, where none of the 111 reflections showed significantly higher relative intensity than the standard cerium(IV) oxide. This may indicate that none of the batches contained a significant proportion of nanowires or that their orientation on the substrate did not allow detection of the 111 plane.

### **4.3 Dispersion of nanoparticles from batches without surfactant**

For toxicity testing it is important to use well-characterised suspensions of particles, as the concentration of material in the suspension will alter if particles agglomerate and settle out of suspension, and the interaction with cells may depend on the particle size. The batches prepared without surfactants in this work formed dense precipitates and it was necessary to break down agglomerates and suspend the particles before characterisation for biological testing. Batches 2 and 4, which contained particles with aspect ratios close to one, were chosen to investigate formation of stable dispersions, because the underlying theory of DLS, (Baalousha et al. 2012a) makes the assumption that the particles are spherical, and this makes the data from rods and wires difficult to interpret.

#### **4.3.1 Dispersion of nanoparticles by changes in pH**

The effect of changing the pH of a suspension of batch 2, using nitric acid and sodium hydroxide solutions, is shown in table 3.2. At pH values between 4 and 8.5 the Z-average particle size of batch 2 as measured by DLS was above 700nm, showing values above 3 microns at pH 5. The data quality in this pH range is poor, as

indicated by the instrumental 'expert advice', because of aggregation and sedimentation of the particles. Below pH 4 and above pH 10.5 the particle size had fallen to below 200nm, due to the charges built up on the particle surfaces which reduced agglomeration and stabilised the suspensions. The quality of the data was reported as good, by the instrumental 'expert advice' and stable suspensions had formed giving reproducible particle size data over five replicates. The zeta potential data showed a corresponding trend, with good quality data obtained below pH 4, and values above 21mV. Between pH 4 and 8.5, the zeta potential was less than 10mV and not reproducible. When the pH was increased, there was a high negative zeta potential of -45mV for the particles, and good reproducibility between measurements. The reproducibility suggested that no dissolution of the nanoparticles occurred, and cerium oxide is generally insoluble under these conditions, although nanoparticles can show unusual behaviour. These results were encouraging, but not of practical use for biological tests as the pH values required for stabilisation were outside the range required for toxicity testing.

#### **4.3.2 Dispersion of nanoparticles using various surfactants**

The addition of various surfactants to batch 2 was investigated, as shown in table 3.3. Two characteristics of the suspension were used to evaluate the efficacy of the surfactant; particle size by DLS and the sedimentation from the suspension. Ultrasonic dispersion and agitation on a laboratory shaker were used to break down agglomerates. Although the lowest particle size was seen with PVP, the data quality was poor, with noticeable sedimentation occurring. The particle size was slightly higher in sodium citrate solutions than with PVP, but less sedimentation occurred. Sodium citrate was a material which was compatible with cells for toxicity testing, and was used for further dispersion trials.

### **4.3.3 Dispersion of nanoparticles using sodium citrate as a surfactant**

The suspensions were optimised for batch 4 by comparing properties of different concentrations of nanoparticles at a range of sodium citrate levels, as shown in table 3.4 and 3.5. All of the suspensions were prepared with 30 minutes ultrasonic dispersion to break down agglomerates. The particle size for the  $10\text{gL}^{-1}$  sodium citrate concentrations was high with aggregation occurring, due to the high concentration of the sodium citrate. At low particle concentrations the count rate was low, the size data was poor, and the zeta potential was between  $-5$  and  $-25\text{mV}$ : too low for stable suspensions. A concentration of  $20\text{mg L}^{-1}$  for the particles and  $1\text{gL}^{-1}$  sodium citrate concentration was selected as a suitable working concentration. Good quality particle size data, with particles below  $200\text{nm}$ , was obtained around this concentration, and the zeta potential measurements of between  $-40\text{mV}$  and  $-50\text{mV}$  indicated sufficient surface charge to stabilise suspensions. The particle sizes and zeta potential values for sodium citrate stabilised suspensions of most of the batches prepared are presented in table 3.7.

### **4.3.4 Dispersion of nanoparticles using SRFA as a surfactant**

Particle size data was obtained using SRFA with batches 2, 3, 4 and 6, after 30 minutes ultrasonic dispersion and 30 days shaking (Table 3.6). The data for batch 4 in SRFA and sodium citrate is compared in table 4.1, and shows a mean particle size below  $200\text{nm}$  for most sets of conditions. As the particle size was not significantly lower in SRFA, and the samples took much longer to prepare, further work focused on suspensions in sodium citrate solutions.

**Table 4.1. Comparison between particle size and quality of measurement for Batch 4 ceria dispersed in sodium citrate and SRFA solutions**

Surfactant and concentration	Conc. ceria /mgL <sup>-1</sup>	Z-average particle size/nm	Size quality
Sodium citrate 0.2gL <sup>-1</sup>	1.24mgL <sup>-1</sup>	180.0	good
Sodium citrate 0.2gL <sup>-1</sup>	12.4mgL <sup>-1</sup>	130.7	good
Sodium citrate 2gL <sup>-1</sup>	1.24mgL <sup>-1</sup>	327.4	good
Sodium citrate 2gL <sup>-1</sup>	12.4mgL <sup>-1</sup>	182.8	good
SRFA 0.05gL <sup>-1</sup>	0.83	160.6	good
SRFA 0.05gL <sup>-1</sup>	0.08	191.2	poor

#### 4.4 Particle Characterisation by AFM and TEM

The next stage of particle characterisation was to obtain additional particle size data by another method. The DLS provides a hydrodynamic particle size of the suspended particle which includes the solvated species on the surface of the particle as described by Stern (1924, cited in Shaw, 1992 p181). The AFM and TEM provide information about the dried particle and so are less influenced by solvation layers at the surface of the particle. A series of particles were prepared for AFM measurements on mica as described in the methodology. The size measurements by AFM are not the dimensions seen in the image, but are a measurement of the height of the particles seen in the image.

#### **4.4.1 Particle characterisation by AFM of ceria batches 3, 6 and 10**

The AFM images and histograms of the particle heights are presented in figures 3.5 to 3.10 for batches 3, 6 and 10. The particles were well distributed on the substrate, but when compared with the hydrodynamic particle size data in table 3.8, the mean particle size, (between 2.8nm and 21nm by AFM), was seen to be much smaller than anticipated. The factor of between 10 and 100 differences in mean particle size between the DLS and AFM measurements suggested that the particles seen on the mica substrate by AFM were too small to be ceria nanoparticles. In addition to this, the artefact seen on the substrate prepared from batch 9 (figure 3.11 which was circular in form appeared to be from drying, suggesting that significant levels of sodium citrate remained in solution on the substrate after washing.

#### **4.4.2 Sodium citrate coating on mica observed by AFM**

To establish the level of sodium citrate remaining on the mica substrate after washing, mica substrates were centrifuged in  $1\text{gL}^{-1}$  sodium citrate solution containing no ceria nanoparticles and then washed twice in 25ml water. Figure 3.16 shows the level of sodium citrate left on a mica substrate after drying. Areas of sodium citrate several microns in size were visible on the AFM images, which ranged from 1.6nm to 10.7nm in height. There were also many small nanoparticles visible on the surface, of similar size and shape to those observed in batch 10, figure 3.9.

#### **4.4.3 Sodium citrate coating observed by TEM**

TEM images of batches dispersed in sodium citrate solution, diluted prior to centrifugation and washed twice before drying, are shown in figures 3.12, and 3.14. The histogram for the batch 6 particle sizes (figure 3.13) is not biased to particle sizes



below 10nm as in previous batches, but there are some indications of sodium citrate crystals on the particle in figure 3.12d , and the particle from batch 10 in figure 3.14a appears to have a coating around it. TEM images of other batches prepared this way, as seen in Figure 3.15 and appendix 6 appear as dark diffuse patches, less than 10nm in diameter, on the TEM grid, and are probably due to sodium citrate remaining on the substrate.

#### **4.4.4 Minimisation of sodium citrate particles on samples prepared for AFM**

The presence of citrate on samples was addressed in two ways for subsequent AFM measurements: the suspensions under investigation were prepared and were diluted with water immediately prior to centrifugation to reduce sodium citrate concentration and the number of times the particles on the substrate were washed was increased from two to three.

There was still some doubt about the efficiency of the washing however, and a spreadsheet was set out to establish the number of washes required based on the sodium citrate concentration in the initial solution. Different volumes of water were pipetted onto a typical mica substrate, and it was established that 40 $\mu$ L of solution was required to fully coat the surface of the mica, and this volume was used as a measure of the solution remaining on the mica substrate after washing. The minimum particle size was set at a diameter of 1 nanometre and the number of citrate particles calculated on this basis. The threshold concentration for an acceptable number of citrate particles was set below 0.1 particles in a 10 micron square, to give less than one citrate particle in 100 ceria nanoparticles. The effect of the washing volume was

also investigated in the calculations. Table 4.2 sets out the change in particle number with the number of washes.

**Table 4.2. Washing required to remove 1nm diameter sodium citrate particles from an initial solution concentration of  $1\text{gL}^{-1}$  sodium citrate solution, using 25ml washing water.**

Washes	No of particles per substrate	No of particles per 10-micron square
Start	$4.34 \times 10^{16}$	$1.21 \times 10^{11}$
After 1 wash	$6.94 \times 10^{13}$	$1.93 \times 10^8$
After 2 washes	$1.11 \times 10^{11}$	$3.09 \times 10^5$
After 3 washes	$1.78 \times 10^8$	494
After 4 washes	$2.84 \times 10^5$	0.790
After 5 washes	455	$1.26 \times 10^{-3}$
After 6 washes	0.728	$2.02 \times 10^{-6}$

Changing the volume of the washing water to 15 or 40ml had no significant effect on the number of washes required, but reducing the sodium citrate concentration by a factor of 10 reduced the number of washes from 5 to 4. This is based on the assumption that after the 30 seconds in the washing water an equilibrium concentration is obtained, where the concentration of salts in the washings and in solution left on the mica is the same. With this limitation in mind, the volume of liquid remaining on the mica substrate was reduced between washings by touching the edge of the mica to a paper towel, to minimise solution transfer.

#### **4.5 Selection of batches for toxicity testing**

Characterisation of the dispersed batches by TEM and AFM did not identify any ceria rods. On the basis of AFM characterisations performed, batches 10 and a commercial

ceria were selected for use in toxicity testing, and these batches were characterised in water and 1:10 MDM solution. Data is presented in tables 3.9 to 3.12, and figures 3.25, 3.26 and 3.27 showing the particle size by DLS for these batches in a range of MDM concentrations. The data show (with the exception of an outlier in commercial ceria in 1:10 MDM + g) that the particles were stable over time in 1:10 MDM, and this concentration was used for subsequent bacterial tests.

#### 4.5.1 Levels of salts in MDM and the influence on AFM measurements

Batch 10 was prepared with sodium citrate as a surfactant, and MDM contains 10.6g of salts per litre, including 0.5g sodium citrate, so that even at concentrations of 1:10 MDM, the salt level is over  $1\text{g L}^{-1}$ . The AFM samples prepared for this work were evaluated for citrate particle content, using the spreadsheet described above and the results are shown in table 4.3.

**Table 4.3. Calculation of number of citrate particles remaining on AFM substrate after washing**

<b>Batches measured</b>	<b>Concentration of salts in solution /g L<sup>-1</sup></b>	<b>Number of washes</b>	<b>Wash volume /cm<sup>3</sup></b>	<b>Citrate particles in analysed data</b>
Commercial CeO <sub>2</sub> , H <sub>2</sub> O	0	2	25	none
Commercial CeO <sub>2</sub> , MDM	0.012	3	35	18
B10, H <sub>2</sub> O	0.02	3	25	3
B10, MDM	0.475	5	40	None

When the particle size distributions from AFM data for the commercial ceria in water and in MDM were compared, the batches have the highest number of particles in fraction between 1 and 10nm. The average particle size was smaller in MDM, with a higher number of particles below 10nm, which probably indicates that there was a

significant proportion of sodium citrate remaining on the substrate. Only 77 particles were measured in this batch, so the number of particles of citrate may represent more than 20% of the total.

**Table 4.4. Comparison of particle size for commercial ceria and Batch 10 in MDM from DLS and AFM measurements.**

Batches measured	Particle size by DLS/nm	Particle size by AFM/nm	Source
Commercial CeO <sub>2</sub> , 1:10 MDM	153.5	25.42	This work
Commercial CeO <sub>2</sub> , H <sub>2</sub> O		37.15	This work
Commercial CeO <sub>2</sub> , H <sub>2</sub> O	1162	14.9	Baalousa et al. (2012)
B10, 1:10 MDM	141.8	21.12	This work
B10, 1:10 H <sub>2</sub> O		32.8	This work

The mean particle sizes, at 37 and 25 nm are much smaller than the values measured by DLS, as shown in table 4.4. As Batch 10 ceria was washed 5 times in 40ml of water, and had a low initial concentration of salts, it is very unlikely that the particle size difference is due to the presence of residual salts on the surface. Baalousha et al. (2012a) have characterised the commercial ceria in water and published size comparisons by AFM, TEM and DLS. They also found that there was a significant discrepancy between particle size measurements by different methods, with an average size of 14.9nm by AFM, and over 1,000nm by DLS. The data from this work showed values for the mean particle size by AFM of 37.2nm and 25.4nm in water and 1:10 MDM respectively, and the Z-average particle size by DLS was 153nm in MDM. A comparison between measurement techniques by Domingos et al. (2009) concluded that particle size measurements by DLS produced consistently higher

values than other techniques, and additionally, were higher than anticipated from other characteristics of the material.

#### **4.6 Monitoring toxicity of ceria nanoparticles**

As shown in figures 3.18 and 3.19, the cerium(III) and cerium(IV) ions exhibit fluorescence when irradiated at wavelengths of around 250nm. Sodium citrate solution did not show fluorescence (figure 3.20), and very little fluorescence was observed in  $2.8\text{mgL}^{-1}$  ceria suspension (figure 3.21). The suspension of  $28\text{mgL}^{-1}$  ceria nanoparticles (figure 3.22) exhibited some fluorescence, over a broader emission wavelength range than the cerium ions. The peak excitation wavelength for the ceria nanoparticles appeared to be lower than 190nm, the lower limit of excitation on the instrument, and no fluorescence was observed at the wavelengths where the cerium(III) and cerium(IV) ions exhibited fluorescence. Because of the low excitation wavelength required by the ceria nanoparticles the technique was not suitable for monitoring nanoparticle adhesion on cell walls or uptake by cells, and the method was not pursued. Subsequent toxicity studies were performed using a 96-well plate, where the change in turbidity of the suspensions in the wells was used to monitor bacterial growth.

#### **4.7 Toxicity of commercial ceria nanoparticles measured in Run 1**

An image of a 96-well plate is shown in figure 2.7 and the layout of the wells is shown in figure 2.8. Each well held  $200\mu\text{L}$  of solution. The plate was used for preliminary toxicity testing, with the experimental set-up shown in table 2.4, Run 1. There were four replicates of each experiment on the plate. This run used commercial

ceria with *P. putida* 1:10 MDM and 1:10 MDM + g, in figures 3.36 to 3.39 and 3.40 to 3.43.

#### **4.7.1 Data quality**

When examining the graphs for each individual run, there are some noise spikes which appear to be associated with the instrument, as they are relatively uniform in size, occur at regular intervals and are observed on more than one trace at the same time interval. These spikes can be clearly seen on traces for wells G and H in figure 3.40, but are also present on each of the other figures containing data from individual wells from this run. The data from the individual runs was averaged and are presented in figures 3.44 and 3.46, and it can be seen that the noise spikes are averaged out to some extent. In figure 3.43, the data from well H2 was very erratic initially, and data from this well has not been included in the average.

#### **4.7.2 Effect of commercial ceria nanoparticles on bacterial growth**

The data in figure 3.44 shows two different phases. The initial phase showed the growth of the bacteria, lasting between 2 and 7 hours, and the absorbance rose as the concentration of bacteria increased. The second phase had a plateau region, with no further increase in bacterial growth. The one exception to this is the  $5\text{mgL}^{-1}$  commercial ceria nanoparticles in 1:20 MDM, where the growth rate fell after 2.3 hours, then began to rise again after 5 hours.

The initial phase is the region which can tell us most about the processes occurring in the wells. The bacteria grow and reproduce in the medium, and as the only differences between the data traces in figure 3.44 are the concentrations of ceria nanoparticles.

The bacteria in MDM grew slowly initially, and after 5 hours there was a significantly higher concentration of bacteria present. Addition of 10 to 50mgL<sup>-1</sup> of commercial ceria reduced the growth of the bacteria, but the trend was not in the expected direction as 10mgL<sup>-1</sup> concentration showed almost no growth at all over this period, whereas the 25 and 50mgL<sup>-1</sup> wells showed more growth over time with increasing the ceria content. The same trend is also observed in MDM + glucose, where the overall growth levels are higher, due to glucose providing an additional food source for the bacteria. During the first seven hours in MDM + glucose, the growth rates of bacteria with 5 and 10mgL<sup>-1</sup> are very close, but the growth rate increases as the ceria particle concentration is increased, as shown in figure 3.48.

The higher commercial ceria concentrations of 25mgL<sup>-1</sup> and 50mgL<sup>-1</sup> in MDM and MDM + glucose appear to be increasingly less toxic to the bacteria, compared with 10mgL<sup>-1</sup>. One explanation for this may be that agglomeration occurs at higher commercial ceria concentrations, which could have the effect of reducing the particle number, and if sedimentation of aggregates occurs, the concentration of ceria nanoparticles could be reduced. The particle size data by DLS is not available for the individual concentrations of nanoparticles in MDM to test this hypothesis.

In contrast to the data for other wells, after 20 minutes, the absorbance due to the bacteria with 5mgL<sup>-1</sup> commercial ceria in MDM rose steadily. Over the first two hours the bacteria showed more than 30 times the growth exhibited by bacteria without any ceria present, as measured by the change in absorbance. The same trend was seen in all the wells with 5mgL<sup>-1</sup> commercial ceria, (figure 3.36) although there was noticeable variation between the individual wells. This growth rate was not

reproduced in the bacteria grown in MDM + glucose and the only possible explanations would seem to be contamination with a solution containing higher levels of bacteria with a food source for the bacteria, although it is not possible to envisage how this could have occurred.

#### **4.8 Toxicity of commercial ceria nanoparticles and Batch 10 ceria nanoparticles measured in Run 2**

This run was carried out in 1:10 MDM media without glucose. Sodium citrate was used as a surfactant for Batch 10 ceria dispersions, and control wells containing sodium citrate solution with bacteria were also measured.

##### **4.8.1 Data quality**

The noise spikes seen in Run 1 are again in evidence in figures 3.59, 3.51, 3.53, 3.54, although the average data shown in figure 3.61 only shows slight traces of the noise.

##### **4.8.2 Effect of commercial ceria nanoparticles on bacterial growth**

The results for commercial ceria in Run 2 are significantly different from Run 1. In Run 2, there was no growth observed for any of the bacteria with commercial ceria or in the MDM with bacteria and no ceria added, as shown in figures 3.49 and 3.51 to 3.54. The average data shows no change in absorbance over 24 hours (figures 3.55 and 3.56).

##### **4.8.3 Influence of sodium citrate on bacterial growth**

In contrast to wells with *P. putida* in MDM, the bacteria in MDM with added sodium citrate (figure 3.50) showed an increase in the absorbance over the initial 7 hours and



a plateau region which rises again slightly after 17 hours. The data for the effect of batch 10, which was prepared with sodium citrate capping agent, also shows bacterial growth, as seen in figures 3.57 to 3.60. The normalised, averaged data, presented in figures 3.61 and 3.62, show that the addition of batch 10 nanoparticles caused an increase in the growth of *P. putida* in the medium, with the lowest growth rates seen in the medium plus citrate without batch 10 added.

#### **4.8.4 Differences in samples from Runs 1 and 2**

There were some differences between the suspensions of bacteria and commercial ceria in MDM, under test in Run 1 and Run 2. The concentration of commercial ceria and MDM were doubled from Runs 1 to 2, to bring them in line with the characterisation carried out in media at the start of the toxicology work. In addition to this, the bacteria were cultured prior to Run 1 in 1:10 MDM, and before use at 1:20 MDM concentration, whereas the bacteria were cultured in 1:1 MDM and used at 1:10 MDM concentration in Run 2. The data clearly show that the bacteria grew better in the presence of sodium citrate, and the reduction in the citrate level when the bacteria were transferred from 1:1 MDM to 1:10 MDM may have been too much of a shock for the bacterial cultures, in the absence of added sodium citrate, and this may explain the complete lack of growth without seen in figures 3.55 and 3.56.

#### **4.8.5 Effect of Batch 10 ceria nanoparticles on bacterial growth**

The increase in growth with increasing batch 10 concentration was a surprising feature of figures 3.61 and 3.62. This indicates that the toxicity of batch 10 ceria

nanoparticles is low. As sodium citrate was the capping agent for these nanoparticles, the presence of the particles also introduces additional citrate. Because of the way the solutions were prepared from different stock solutions, the concentration of citrate was not proportional to the concentration of ceria in the solutions. The influence of both ceria and sodium citrate were examined in figures 3.63 and 3.64. There does not appear to be any clear correlation with the sodium citrate concentration seen in figure 3.64. In figure 3.63, the absorbance rises from 0 to  $20\text{mgL}^{-1}$  ceria nanoparticles and then is almost constant throughout the concentration range measured, irrespective of sodium citrate concentration. This would appear to suggest that a level of ceria nanoparticles is beneficial to bacterial growth, but is not dose dependent above  $20\text{mgL}^{-1}$ . More detailed work with controlled sodium citrate levels would be needed to confirm this finding.

Because *P putida* has a negative surface charge, and the batch 10 nanoparticles are also negatively charged, there may be very little interaction between the nanoparticles and the bacteria. This could minimise the toxicity of the nanoparticles, but this would not account for the enhanced growth observed.

## 5 Conclusions

### 5.1. Shapes of nanoparticles

The initial TEM measurements on as-prepared batches showed the anticipated nanoparticle shapes, with **cubes from the nitrate and a mixture of cubes and wires from the chloride**. The XRD patterns from a wide range of batches prepared from both chloride and nitrate precursors matched those for cerium(IV) oxide, and did not show the enhanced reflections from the 111 plane associated with preferential crystal growth reported by Wang et al. (2010).

Although ceria wires were observed in the initial TEM images of batch 3, none of the TEM or AFM images of dispersed batches showed particles with high aspect ratios. The concentration of ceria particles in the images was often low, as the presence of the citrate initially gave the impression of a higher particle count. Batch 6 was prepared with chloride precursor and nanowires were expected from this batch, but the particles measured only showed one small particle with an aspect ratio higher than two. **The lack of wires in the TEM and AFM images from chloride precursor batches may suggest that the dispersion techniques were breaking down the long particles or no nanowires were formed in subsequent batches.**

### 5.2. Nanoparticle dispersions

Charge stabilisation of the suspensions occurred at high and low pH but the pH of these suspensions was outside the range that was suitable for biological testing. SRFA produced stable suspensions after a month of shaking. **The concentration range of sodium citrate and cerium oxide nanoparticles was explored, and a region of stable suspensions was identified.**

Ultrasonic dispersion was used to prepare the particle suspensions and, for consistency, the same ultrasonic bath was used throughout the work. The difference in power between ultrasonic baths is striking, and in addition to that, the power transfer may be affected by the temperature of the bath, which increases over time in use. Unlike most other aspects of nanoparticle characterisation, the power of the ultrasonic treatment is not normally considered as a process which could introduce variability into the work. This has been noted in an inter-lab comparison of particle size and zeta potential for gold, silica, polystyrene and ceria nanoparticles carried out by Roebbens et al. (2011), who found that reproducibility declined when sonication was carried out prior to zeta potential runs. The difference between the sonication equipment and power distribution within the containers were thought to be a possible cause of variability. **If sonication is used in preparation or characterisation of nanoparticles, some laboratory standardisation would be beneficial.**

### **5.3. The effect of washing AFM substrates**

Citrate particles formed on AFM substrates after washing produced unexpectedly low particle size results, and dark patches observed on TEM grids were probably due to sodium citrate. **Calculations of the number of washes required before AFM imaging produced significant information, not only for the ceria nanoparticles suspended in sodium citrate, but for all nanoparticles characterised in MDM medium.** Toxicity testing with nanoparticles is regularly carried out in 1:10 MDM, where the final salt concentration is  $1.06\text{gL}^{-1}$ , and at least five washes in 20ml of washing water are required to reduce the level of particles to below two citrate particles in a 10 micron square of the mica surface or TEM grid before characterisation.

**When thorough washing was carried out, the particle size by DLS was four times the size by AFM.** This is in broad agreement with observations in publications by Domingos et al. (2009) and Baalousha et al. (2012a) who found that DLS measurements gave consistently larger particle sizes than AFM or TEM measurements. This is probably because the dynamic light scattering measures the hydrodynamic diameter of the particles, which is the size of the particle and the associated surface ions and solvent molecules, whereas TEM and AFM measure the particle size of the washed and dried particle without the hydration sheath surrounding the particle.

#### **5.4. Toxicity testing**

Despite the fact that the cerium(III) and cerium(IV) ions showed fluorescence with an excitation wavelength centred around 250nm, the peak fluorescence excitation wavelength for ceria nanoparticles was below 190nm: too far into the ultraviolet for this to be a useful technique for monitoring the biological interaction of the nanoparticles.

**When considering the original hypothesis of this work, there was insufficient data collected to establish conclusively the toxicity of cerium oxide nanoparticles to *P. putida*.**

**An unexpected increase in toxicity was observed with decreasing ceria content for commercial ceria in MDM and MDM + glucose.** The growth was completely inhibited with 10mgL<sup>-1</sup> commercial ceria in MDM, but there was some residual growth measured at all ceria concentrations with added glucose. The reason for this is

unclear, but agglomeration of nanoparticles at higher particle concentrations is a potential mechanism.

**Sodium citrate added to the bacterial suspension promoted the growth of the *P. putida*.** Although enhanced bacterial growth was observed in wells with additional sodium citrate, either deliberately added or as the surfactant in batch 10 ceria nanoparticles, the results show no direct correlation between sodium citrate concentration and bacterial growth.

When examining the growth of *P. putida* in the presence of batch 10 ceria nanoparticles after five hours exposure, **a linear rise in bacterial growth with increasing ceria content between 0 and 20mgL<sup>-1</sup> batch 10 ceria nanoparticles was observed.** Addition of batch 10 ceria nanoparticles seemed to promote growth of the bacteria up to 20mgL<sup>-1</sup> ceria. No further change in growth was observed between 20mgL<sup>-1</sup> and 100mgL<sup>-1</sup> of batch 10. The implication of these results is that a certain level of ceria nanoparticles may be beneficial to the bacteria, with no further benefit seen above 20mgL<sup>-1</sup>. There is a clear need for further measurements in solutions with the same level of sodium citrate throughout the wells. This experiment was carried out, but the noise spikes observed in the first two runs were so extreme in the third run that it was impossible to extract the data from the noise.

## References

Auffan, M., Rose, J., Bottero, J., et al. (2009a) Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. **Nature Nanotechnology**, 4 (10): 634-641.

Auffan, M., Rose, J., Orsiere, T., et al. (2009b) CeO<sub>2</sub> nanoparticles induce DNA damage towards human dermal fibroblasts in vitro. **Nanotoxicology**, 3 (2): 161-171.

Baalousha, M., Ju-Nam, Y., Cole, P.A., et al. (2012a) Characterization of cerium oxide nanoparticles-Part 1: Size measurements. **Environmental Toxicology and Chemistry**, 31 (5): 983-993.

Baalousha, M., Ju-Nam, Y., Cole, P.A., et al. (2012b) Characterization of cerium oxide nanoparticles-Part 2: Nonsize measurements. **Environmental Toxicology and Chemistry**, 31 (5): 994-1003.

Baalousha, M., Motelica-Heino, M. and Coustumer, P.L. (2006) Conformation and size of humic substances: Effects of major cation concentration and type, pH, salinity, and residence time. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, 272 (1-2): 48-55.

Benn, T., Cavanagh, B., Hristovski, K., et al. (2010) The Release of Nanosilver from Consumer Products Used in the Home. **Journal of Environmental Quality**, 39 (6): 1875-1882.

Benn, T.M. and Westerhoff, P. (2008) Nanoparticle silver released into water from commercially available sock fabrics. **Environmental Science & Technology**, 42 (18): 7025-7026.

Chen, H. and Chang, H. (2004) Homogeneous precipitation of cerium dioxide nanoparticles in alcohol/water mixed solvents. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, 242 (1–3): 61-69.

Christian, P., Von der Kammer, F., Baalouusha, M. et al. (2008) Nanoparticles: structure, properties, preparation and behaviour in environmental media. **Ecotoxicology**, 17:326-343.

Derjaguin, B.V. and Landau, L.D. (1941) **Acta Physicochim.**, 14: 633.

Das, M., Patil, S., Bhargava, N., et al. (2007) Auto-catalytic ceria nanoparticles offer neuroprotection to adult rat spinal cord neurons. **Biomaterials**, 28 (10): 1918-1925.

Domingos, R.F., Baalouusha, M.A., Ju-Nam, Y., et al. (2009) Characterizing Manufactured Nanoparticles in the Environment: Multimethod Determination of Particle Sizes. **Environmental Science & Technology**, 43 (19): 7277-7284.

El Badawy, A.M., Luxton, T.P., Silva, R.G., et al. (2010) Impact of Environmental Conditions (pH, Ionic Strength, and Electrolyte Type) on the Surface Charge and Aggregation of Silver Nanoparticles Suspensions. **Environmental Science & Technology**, 44 (4): 1260-1266.



European commission, (2011) (<http://ec.europa.eu/environment/chemicals/nanotech/index.htm>) 27/11/11.

Fall, M., Guerbet, M., Park, B. et al. (2007) Evaluation of cerium oxide and cerium oxide based fuel additive safety on organotypic cultures of lung slices. **Nanotoxicology**, 1(3): 227-234.

Ferard, G., (1994) Quantities and units for electrophoresis in the clinical laboratory (IUPAC Recommendations 1994) **Pure & Applied Chemistry**, 66 (4): 891-896.

Flory, P.J., (1969) **Statistical Mechanics of Chain Molecules**. Wiley, New York.

Ghosh, S., Mashayekhi, H., Bhowmik, P., et al. (2010) Colloidal Stability of Al<sub>2</sub>O<sub>3</sub> Nanoparticles as Affected by Coating of Structurally Different Humic Acids. **Langmuir**, 26 (2): 873-879.

Giljohann, D., Seferos, D., Daniel, W., et al. (2010) Gold Nanoparticles for Biology and Medicine. **Angewandte Chemie International Edition**, 49 (19): 3280-3294.

Gottschalk, F. and Nowack, B. (2011) The release of engineered nanomaterials to the environment. **Journal of Environmental Monitoring**, 13 (5): 1145-1155.

Guo, Z., Du, F., Li, G. et al. (2006) Synthesis and Characterization of Single-Crystal Ce(OH)CO<sub>3</sub> and CeO<sub>2</sub> Triangular Microplates. **Inorganic Chemistry**, 45: 4167-4169.

Handy, R., von der Kammer, F., Lead, J., et al. (2008) The ecotoxicology and chemistry of manufactured nanoparticles. **Ecotoxicology**, 17 (4): 287-314.

Hasselov, M., Readman, J., Ranville, J., et al. (2008) Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. **Ecotoxicology**, 17 (5): 344-361.

He, X., Kuang, Y., Li, Y., et al. (2012) Changing exposure media can reverse the cytotoxicity of ceria nanoparticles for Escherichia coli. **Nanotoxicology**, 6 (3): 233-240.

Hendren, C.O., Mesnard, X., Droge, J., et al. (2011) Estimating Production Data for Five Engineered Nanomaterials as a Basis for Exposure Assessment. **Environmental Science & Technology**, 45 (7): 2562-2569.

Hoecke, K.V., Quik, J.T.K., Mankiewicz-Boczek, J., et al. (2009) Fate and Effects of CeO<sub>2</sub> Nanoparticles in Aquatic Ecotoxicity Tests. **Environmental Science & Technology**, 43 (12): 4537-4546.

Horiba Scientific, (2010) [http://www.horiba.com/fileadmin/uploads/Scientific/Documents/PSA/PSA\\_Guidebook.pdf](http://www.horiba.com/fileadmin/uploads/Scientific/Documents/PSA/PSA_Guidebook.pdf) 20/6/12.

Jódar-Reyes, A.B., Martín-Rodríguez, A. and Ortega-Vinuesa, J.L. (2006) Effect of the ionic surfactant concentration on the stabilization/destabilization of polystyrene colloidal particles. **Journal of Colloid and Interface Science**, 298 (1): 248-257.

Ju-Nam, Y. and Lead, J.R. (2008) Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications. **Science of the Total Environment**, 400 (1-3): 396-414.

Kaegi, R., Ulrich, A., Sinnet, B., et al. (2008) Synthetic TiO<sub>2</sub> nanoparticle emission from exterior facades into the aquatic environment **Environmental Pollution**, 156 (2): 233-239.

Kahru, A. and Dubourguier, H. (2010) From ecotoxicology to nanoecotoxicology. **Toxicology**, 269 (2-3): 105-119.

Kang, H.S., Kang, Y.C., Koo, H.Y., et al. (2006) Nano-sized ceria particles prepared by spray pyrolysis using polymeric precursor solution. **Materials Science and Engineering: B**, 127 (2-3): 99-104.

Klaine, S.J., Alvarez, P.J.J., Batley, G.E., et al. (2008) Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. **Environmental Toxicology and Chemistry**, 27 (9): 1825-1851.

Krishnaswamy, A., (2005) BioSpec: A Biophysically-Based Spectral Model of Light Interaction with Human Skin, Thesis for Master of Mathematics in Computer Science. Waterloo, Ontario, Canada.

Lee, J., Lee, J. and Choi, S. (2005) Synthesis of nano-sized ceria powders by two-emulsion method using sodium hydroxide. **Materials Letters**, 59 (2–3): 395-398.

Limbach, L.K., Bereiter, R., Muller, E., et al. (2008) Removal of Oxide Nanoparticles in a Model Wastewater Treatment Plant: Influence of Agglomeration and Surfactants on Clearing Efficiency. **Environmental Science & Technology**, 42 (15): 5828-5833.

Limbach, L.K., Wick, P., Manser, P. et al. (2007) Exposure of Engineered Nanoparticles to Human Lung Epithelial Cells: Influence of Chemical Composition and Catalytic Activity on Oxidative Stress. **Environmental Science & Technology**, 41(11): 4158–4163.

London, F. (1930) On the Theory and Systematic of Molecular Forces. **Zeitschrift fur Physik**, 63 (3-4 ): 245-279.

Lu, X., Li, X., Chen, F., et al. (2009) Hydrothermal synthesis of prism-like mesocrystal CeO<sub>2</sub>. **Journal of Alloys and Compounds**, 476 (1–2): 958-962.

Mai, H., Sun, L., Zhang, Y., et al. (2005) Shape-Selective Synthesis and Oxygen Storage Behavior of Ceria Nanopolyhedra, Nanorods, and Nanocubes. **The Journal of Physical Chemistry B**, 109 (51): 24380-24385.

Malvern Instruments (2005) Zeta Potential: An Introduction in 30 Minutes. **Zetasizer Nano Series Technical Note MRK654-01**.

Malvern Instruments Technical Note, (2012) <http://www.malvern.com/common/downloads/campaign/MRK656-01.pdf> 2/4/12.

Marose, S. Lindemann, C. and Scheper, P. (1998) Two-Dimensional Fluorescence Spectroscopy: A New Tool for On-Line Bioprocess Monitoring. **Biotechnol. Prog.**, 14: 63-74.

Meyer, E. (1992) Atomic force microscopy. **Progress in Surface Science**, 41 (1): 3-49.

MicrobiologyBytes, (2012) <http://www.microbiologybytes.com/video/Pputida.html> 7/7/12.

Mueller, N.C. and Nowack, B. (2008) Exposure Modeling of Engineered Nanoparticles in the Environment. **Environmental Science & Technology**, 42 (12): 4447-4453.

Nalabotu, S.K., Kolli, M.B., Triest, W.E., et al. (2011) Intratracheal instillation of cerium oxide nanoparticles induces hepatic toxicity in male Sprague-Dawley rats. **International Journal of Nanomedicine**, 6: 2327–2335.

Nanophase Technologies Corporation (2011) [www.Nanophase.com](http://www.Nanophase.com) 12/10/11.

Navarro, E., Baun, A., Behra, R., Hartmann, N.B. et al., (2008) Environmental Behavior and Ecotoxicity of Engineered Nanoparticles to Algae, Plants, and Fungi. **Ecotoxicology**, 17(5):372-386.

Niemeyer, C.M. (2001) Nanoparticles, Proteins, and Nucleic Acids: Biotechnology Meets Materials Science. **Angewandte Chemie International Edition**, 40 (22): 4128-4158.

Niu, J., Azfer, A., Rogers, L.M., et al. (2007) Cardioprotective effects of cerium oxide nanoparticles in a transgenic murine model of cardiomyopathy. **Cardiovascular Research**, 73 (3): 549-559.

Oberdörster, G., Maynard, A., Donaldson, K. et al., (2005) Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. **Particle and Fibre Toxicology**, 2(8): 1-35.

OECD, (2008) [http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=ENV/JM/MONO\(2008\)13/REV&doclanguage=en](http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=ENV/JM/MONO(2008)13/REV&doclanguage=en) 14/7/12.

Park Systems, (2008a) [http://www.parkafm.com/AFM\\_guide/how\\_afm\\_works.php](http://www.parkafm.com/AFM_guide/how_afm_works.php) 14/6/12.

Park Systems, (2008b) [http://www.parkafm.com/AFM\\_guide/spm\\_modes\\_1.php?id=117514/6/12](http://www.parkafm.com/AFM_guide/spm_modes_1.php?id=117514/6/12) 14/6/12.

Park, B., Donaldson, K., Duffin, R., et al. (2008a) Hazard and Risk Assessment of a Nanoparticulate Cerium Oxide-Based Diesel Fuel Additive—A Case Study. **Inhalation Toxicology**, 20 (6): 547-566.

Park, E., Choi, J., Park, Y., et al. (2008b) Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. **Toxicology**, 245 (1–2): 90-100.

Parr Instrument Company (2011) [Acid digestion vessels 4744-49 www.parrinst.com](http://www.parrinst.com) 4/11/11.

Patty, P.J. and Frisken, B.J. (2006) Direct determination of the number-weighted mean radius and polydispersity from dynamic light-scattering data. **Applied Optics**, 45 (10): 2209-2216.

Pelletier, D.A., Suresh, A.K., Holton, G.A. et al. (2010) Effects of Engineered Cerium Oxide Nanoparticles on Bacterial Growth and Viability. **Applied and Environmental Microbiology**, (24):7981–7989.

Petosa, A.R., Jaisi, D.P., Quevedo, I.R. et al. (2010) Aggregation and Deposition of Engineered nanomaterials in Aquatic Environments: Role of Physicochemical Interactions. **Environmental Science & Technology**, 44: 6532-6549.

Raemy, D.O., Limbach, L.K., Rothen-Rutishauser, B., et al. (2011) Cerium oxide nanoparticle uptake kinetics from the gas-phase into lung cells in vitro is transport limited. **European Journal of Pharmaceutics and Biopharmaceutics**, 77 (3): 368-375.

Rai, M., Yadav, A. and Gade, A. (2009) Silver nanoparticles as a new generation of antimicrobials. **Biotechnology Advances**, 27 (1): 76-83.

Rasbond, W. (2012) <http://imagej.nih.gov/ij> 1/6/12.

Rodea-Palomares, I., Boltes, K., Fernandez-Pinas, F. et al., (2011) Physicochemical Characterization and Ecotoxicological Assessment of CeO<sub>2</sub> Nanoparticles Using Two Aquatic Microorganisms. **Toxicological Sciences**, 119(1):135–145.

Roebben, G., Ramirez-Garcia, S., Hackley, V. A. et al. (2011) Interlaboratory comparison of size and surface charge measurements on nanoparticles prior to biological impact assessment. **Journal of Nanoparticle Research**, 13:2675–2687.

Rossignol, S., Gerard, F. and Duprez, D. (1999), Effect of the preparation method on the properties of zirconia-ceria materials. **Journal of Materials Chemistry**, 9 (7):1615-1620.

RSC Publishing, (2012) <http://www.rsc.org/publishing/journals/prospect/ontology.asp?id=CMO:0001377&MSID=b923755k> 16/7/12.



Sanvicens, N. and Marco, M.P. (2008) Multifunctional nanoparticles – properties and prospects for their use in human medicine. **Trends in Biotechnology**, 26 (8): 425-433.

Schiels, P.J., (2010) <http://www.eserc.stonybrook.edu/projectjava/bragg/> 17/6/12.

Shaw, D.J. (1992) **Introduction to Colloid and Surface Chemistry**. 4<sup>th</sup> ed. Oxford: Elsevier Science Ltd.

Stern, O. (1924) **Z. Elektrochem.**, 30: 508.

Stone, V., Nowack, B., Baun, A., et al. (2010) Nanomaterials for environmental studies: Classification, reference material issues, and strategies for physico-chemical characterisation. **Science of the Total Environment**, 408 (7): 1745-1754.

Takeo, M. (1999) **Disperse Systems**, Weinheim, Wiley-VCH.

Tan, J.P.Y., Tan, H.R., Boothroyd, C., et al. (2011) Three-Dimensional Structure of CeO<sub>2</sub> Nanocrystals. **Journal of Physical Chemistry C**, 115 (9): 3544-3551.

Taniguchi, T., Katsumata, K., Omata, S., et al. (2011) Tuning Growth Modes of Ceria-Based Nanocubes by a Hydrothermal Method. **Crystal Growth & Design**, 11(9): 3754-3760.

Tecnai, (2010) [http://www.fei.com/uploadedFiles/Documents/Content/Introduction to EM booklet July 10.pdf](http://www.fei.com/uploadedFiles/Documents/Content/Introduction_to_EM_booklet_July_10.pdf) 15/6/12.

Thill, A., Zeyons, O., Spalla, O., et al. (2006) Cytotoxicity of CeO<sub>2</sub> Nanoparticles for Escherichia coli. Physico-Chemical Insight of the Cytotoxicity Mechanism. **Environmental Science & Technology**, 40 (19): 6151-6156.

Understandingnano.com, (2007) <http://www.understandingnano.com/nanotubes-carbon.html> 2/7/12.

US Department of Energy, (2012) <http://genome.jgi-psf.org/psepu/psepu.home.html> 7/7/12.

Verwey, E.W. and Overbeek, J. T. G. (1948) **Theory of Stability of Lyophobic Colloids**, Elsevier, Amsterdam.

Visser, J. (1972) On Hamaker constants: A comparison between Hamaker constants and Lifshitz-van der Waals constants. **Advances in Colloid and Interface Science**, 3 (4): 331-363.

Wang, W., Howe, J.Y., Li, Y., et al. (2010) A surfactant and template-free route for synthesizing ceria nanocrystals with tunable morphologies. **Journal of Materials Chemistry**, 20 (36): 7776-7781.

Woodrow Wilson International Centre for Scholars, (2011) [Project on Emerging Technologies](http://www.nanotechproject.org/inventories/consumer/updates/), <http://www.nanotechproject.org/inventories/consumer/updates/> 1/7/12.

Wu, Q., Zhang, F., Xiao, P., et al. (2008) Great Influence of Anions for Controllable Synthesis of CeO<sub>2</sub> Nanostructures: From Nanorods to Nanocubes. **Journal of Physical Chemistry C**, 112 (44): 17076-17080.

Yang, Z., Zhou<sup>1</sup>, K., Liu, X., et al., (2007) Single-crystalline ceria nanocubes: size-controlled synthesis, characterization and redox property. **Nanotechnology**, 18:185606.

Zhang, H., He, X., Zhang, Z., et al. (2011) Nano-CeO<sub>2</sub> Exhibits Adverse Effects at Environmental Relevant Concentrations. **Environmental Science & Technology**, 45 (8): 3725-3730.

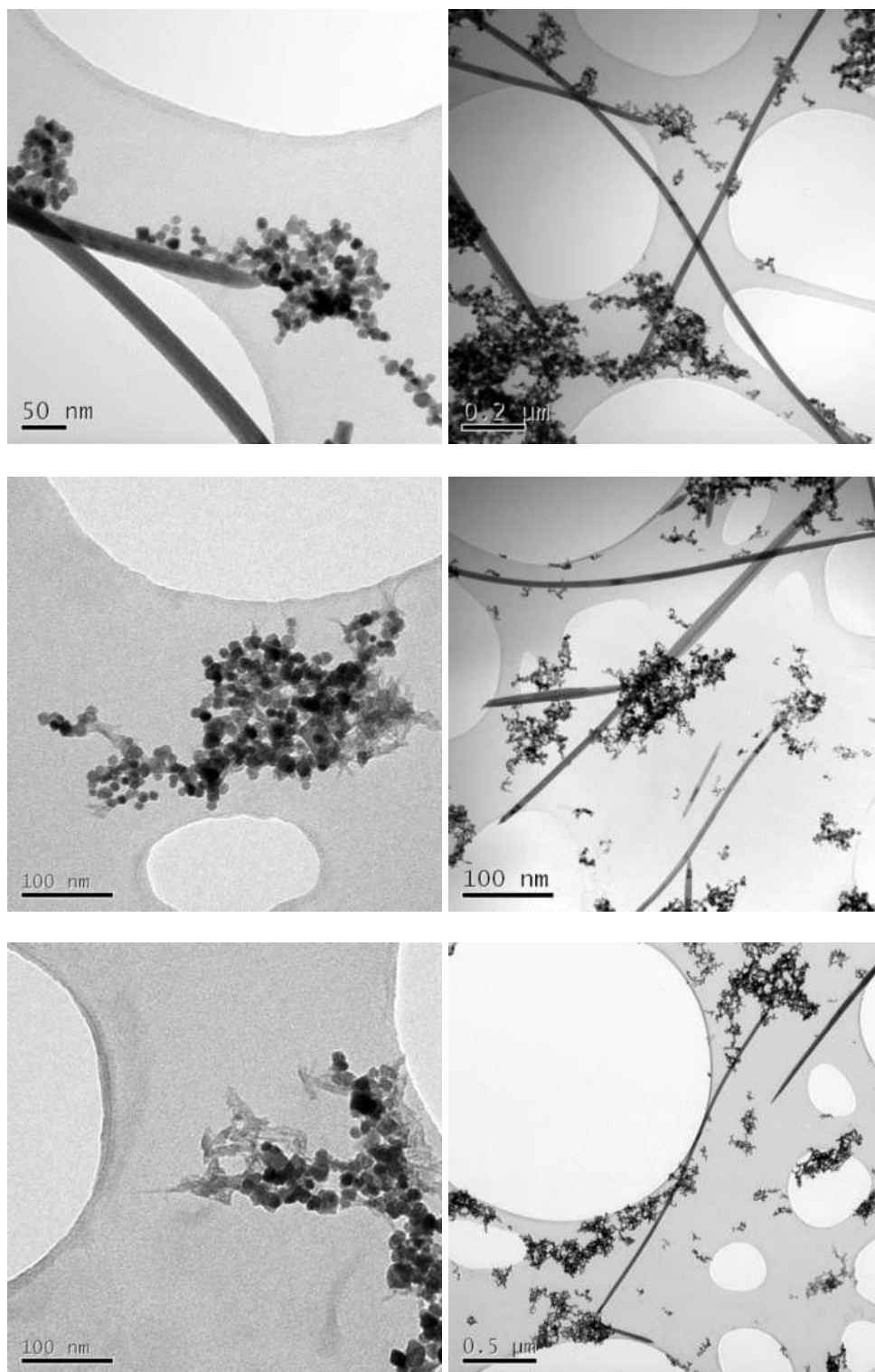
Zhang, J., Ju, X., Wu, Z.Y., et al. (2001) Structural Characteristics of Cerium Oxide Nanocrystals Prepared by the Microemulsion Method. **Chemistry of Materials**, 13 (11): 4192-4197.

Zhou, F., Zhao, X., Xu, H., et al. (2007) CeO<sub>2</sub> Spherical Crystallites: Synthesis, Formation Mechanism, Size Control, and Electrochemical Property Study. **Journal of Physical Chemistry C**, 111 (4): 1651-1657.

**APPENDICES**

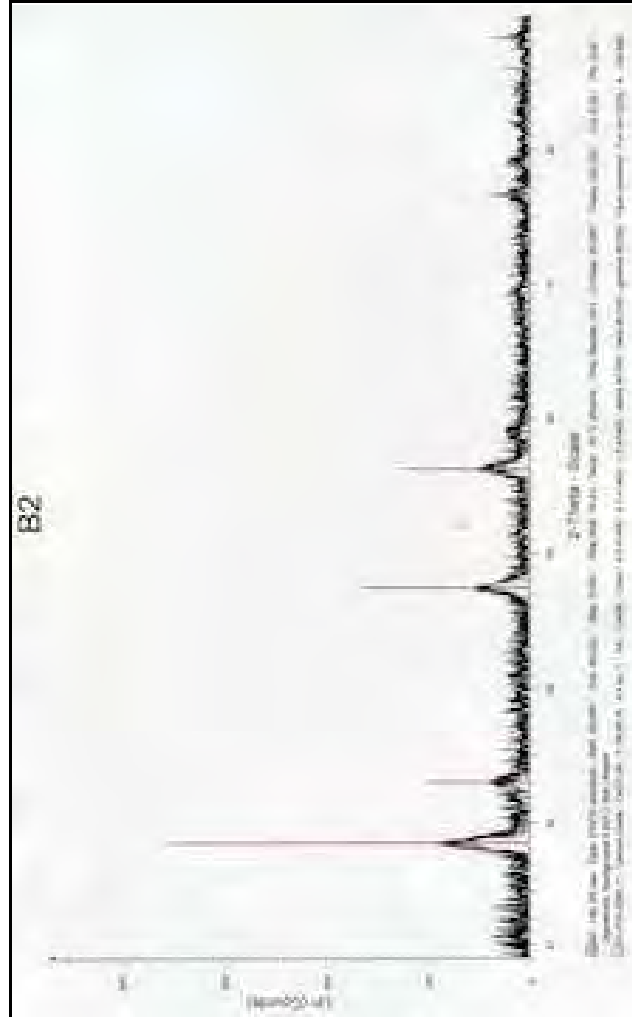
## Appendix 1

TEM images, as-prepared of batch 3



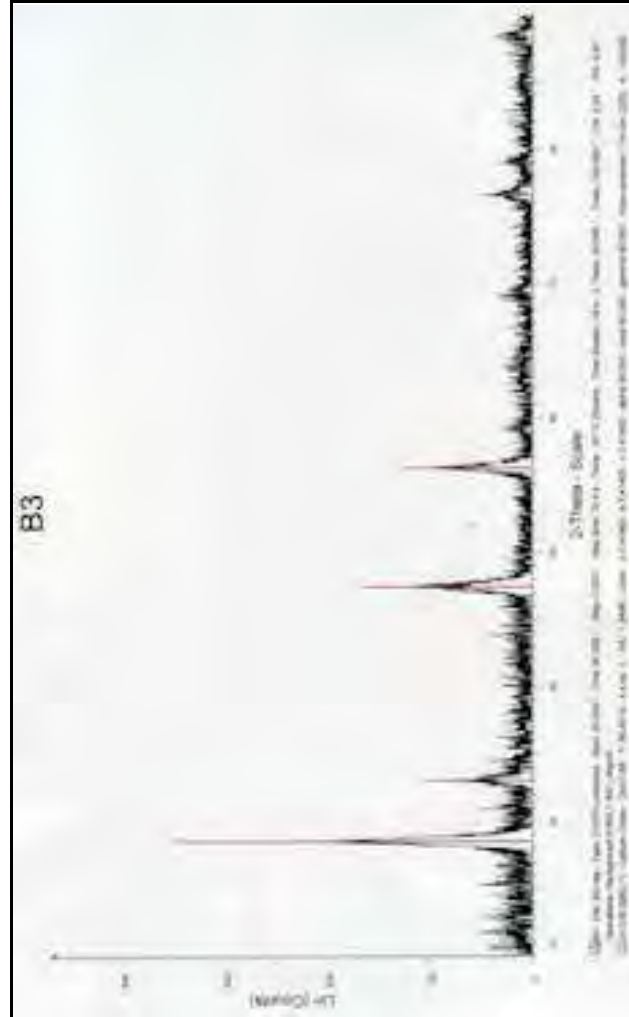
## Appendix 2.1

XRD of batch 2



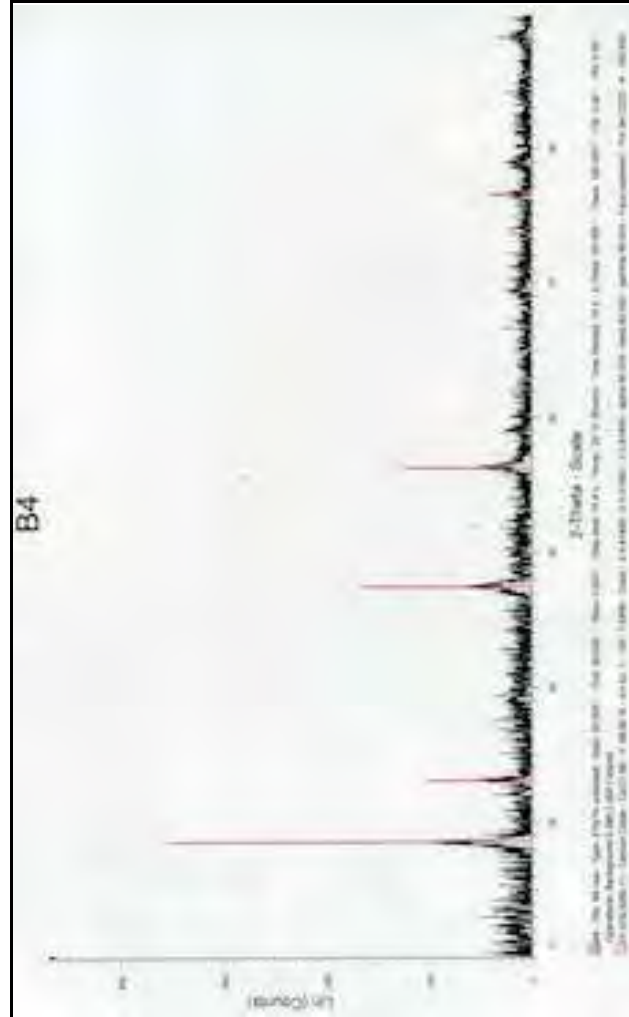
## Appendix 2.2

XRD of batch 3



### Appendix 2.3

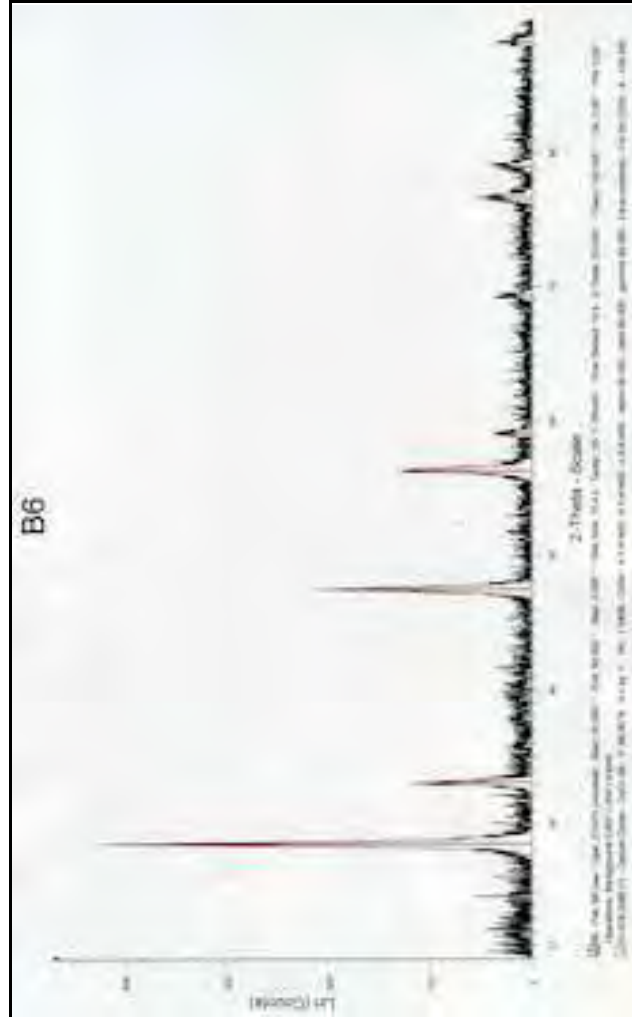
XRD of batch 4





## Appendix 2.4

XRD of batch 6





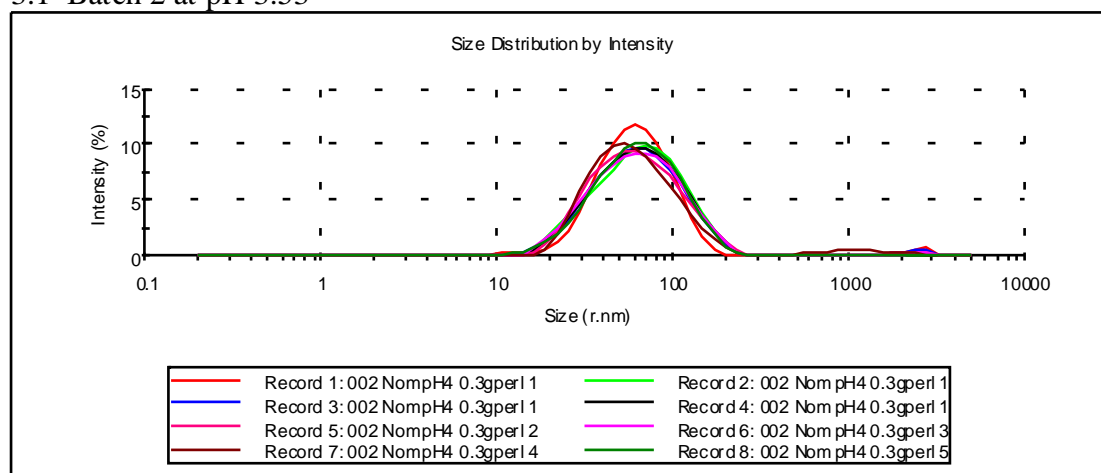




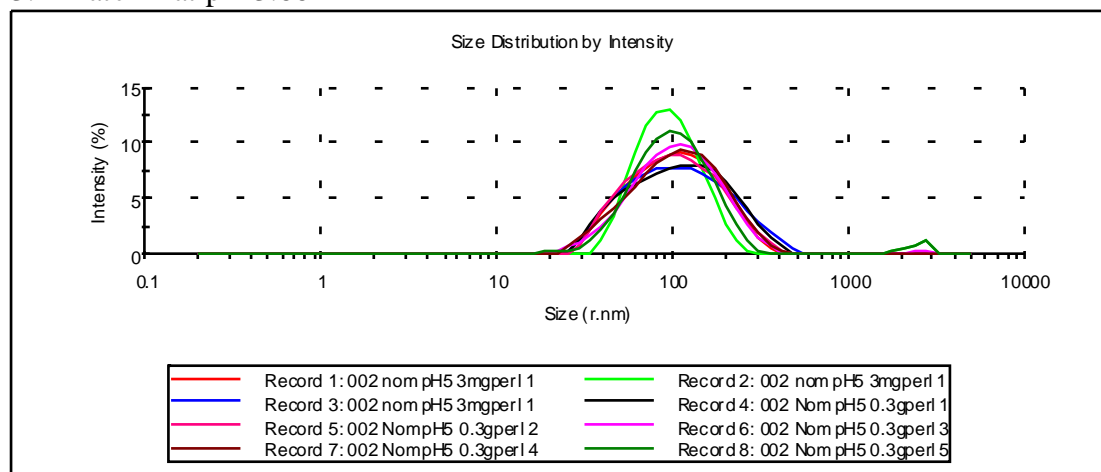
## Appendix 3

### DLS particle size graphs

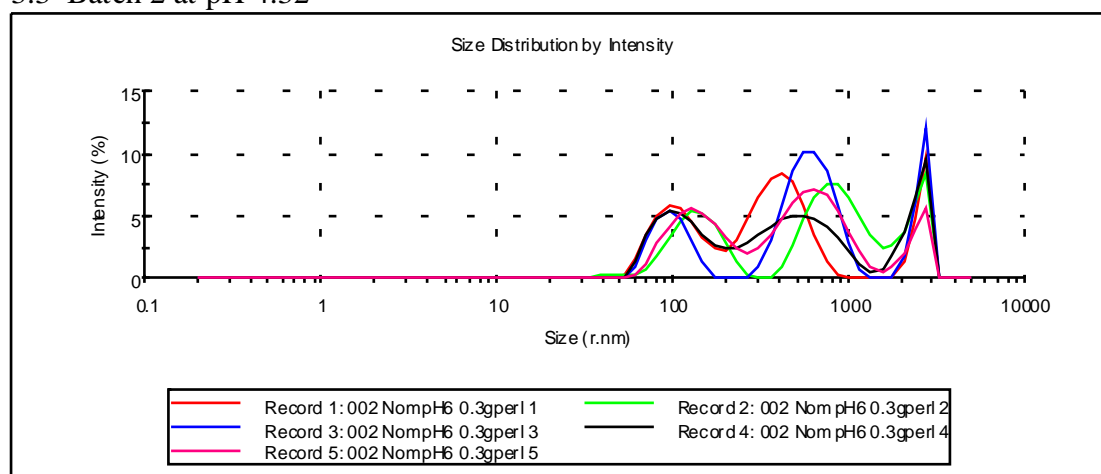
#### 3.1 Batch 2 at pH 3.53



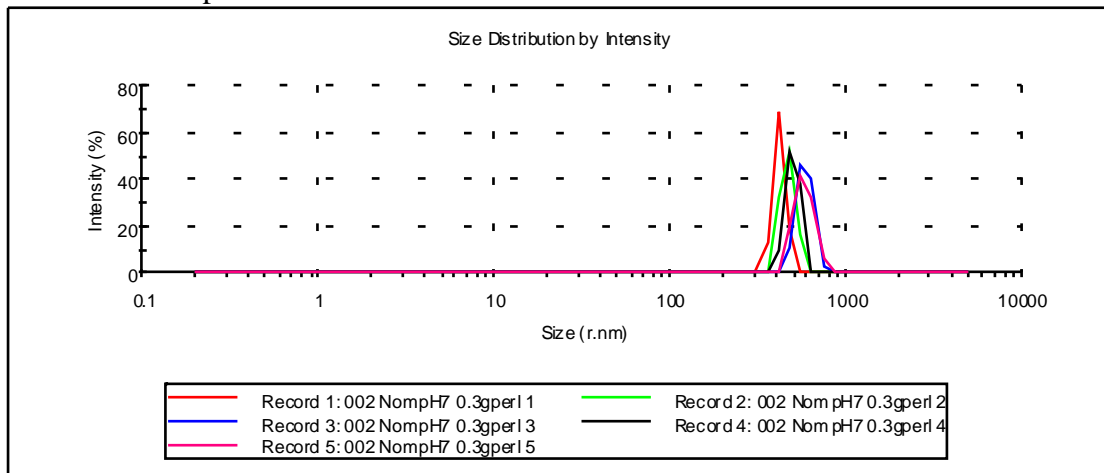
#### 3.2 Batch 2 at pH 3.66



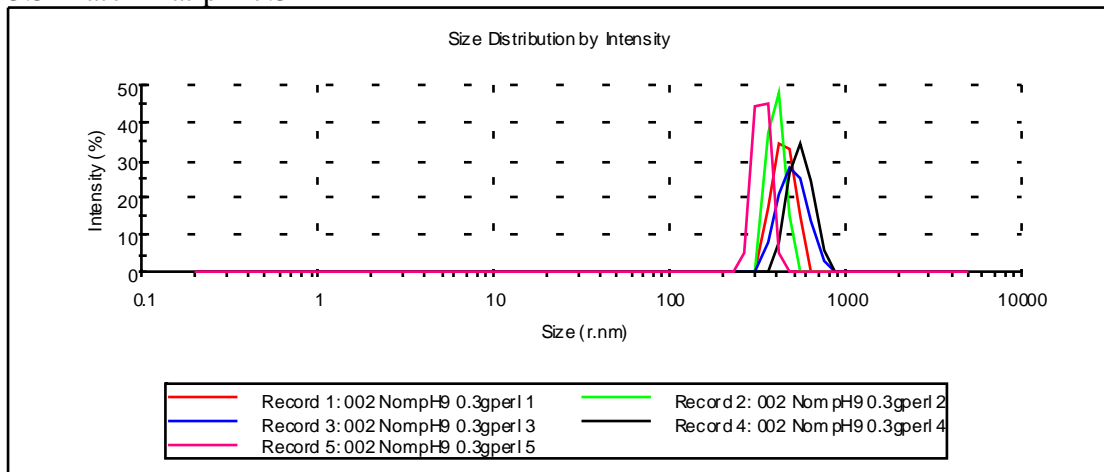
#### 3.3 Batch 2 at pH 4.32



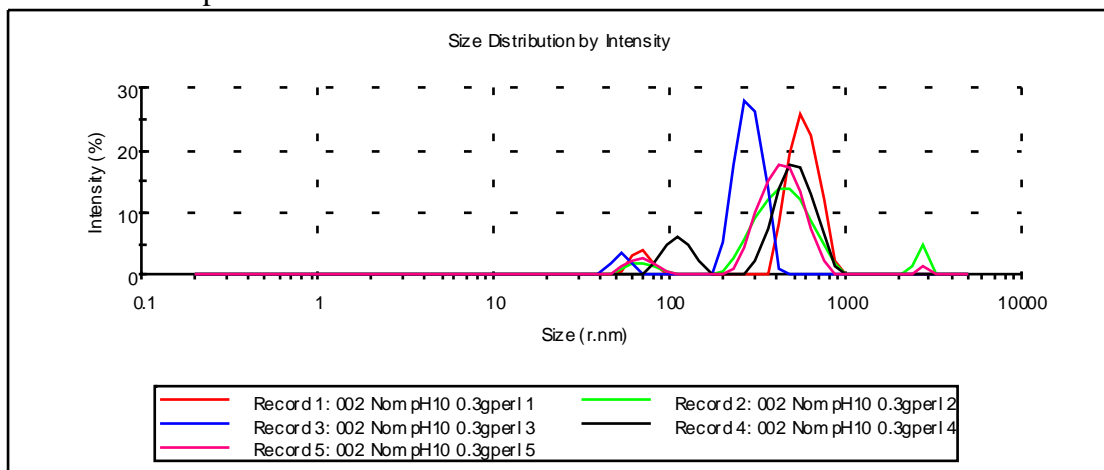
## 3.4 Batch 2 at pH 5.00



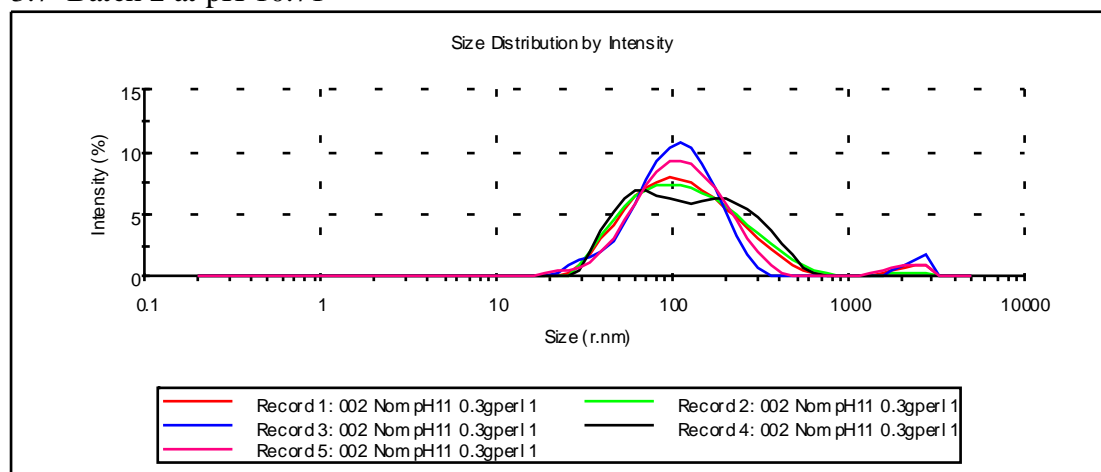
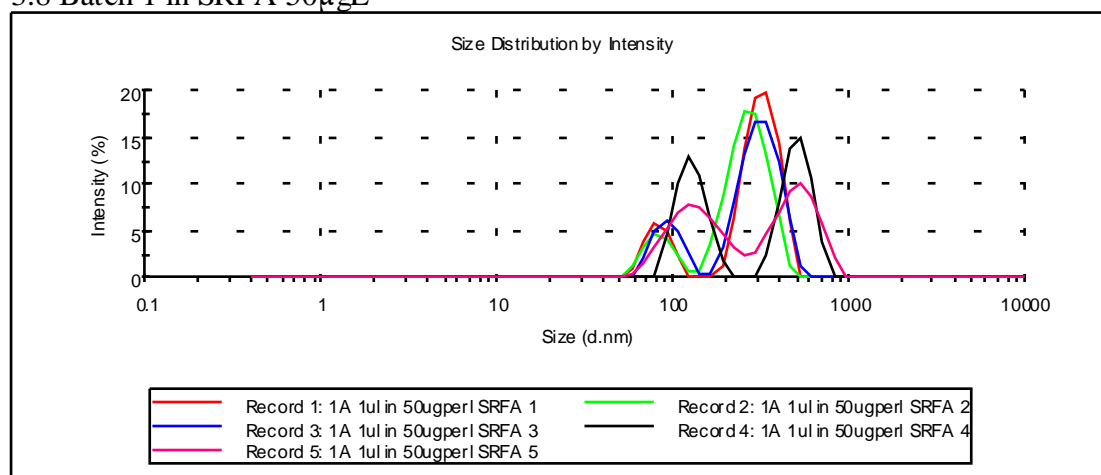
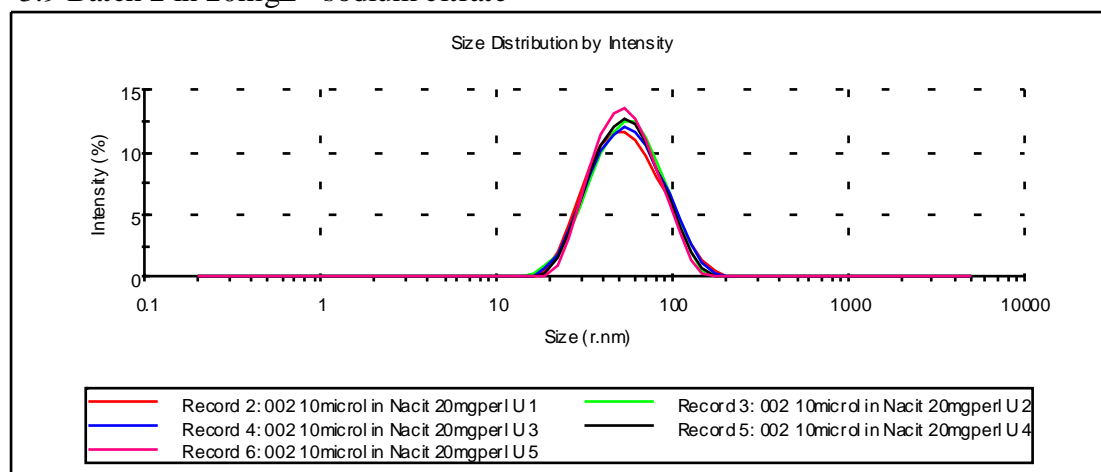
## 3.5 Batch 2 at pH 7.34

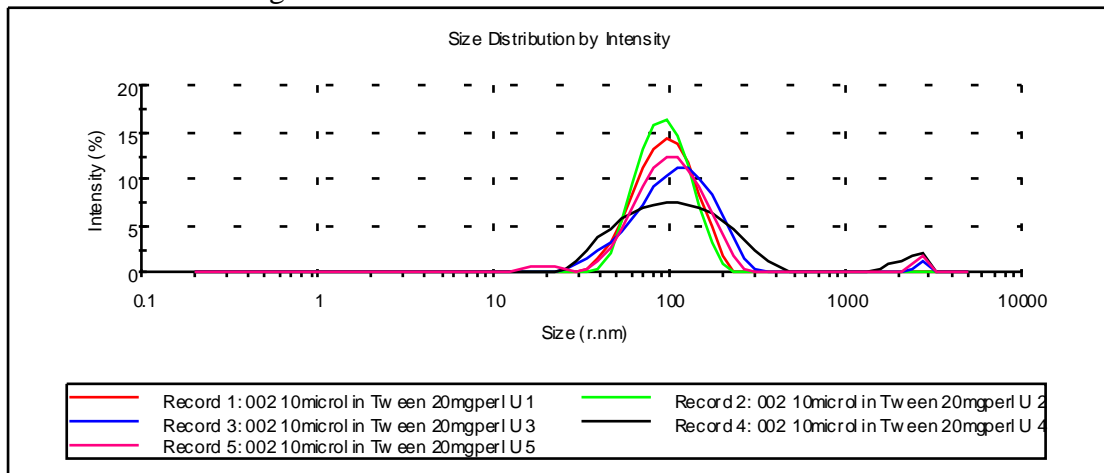
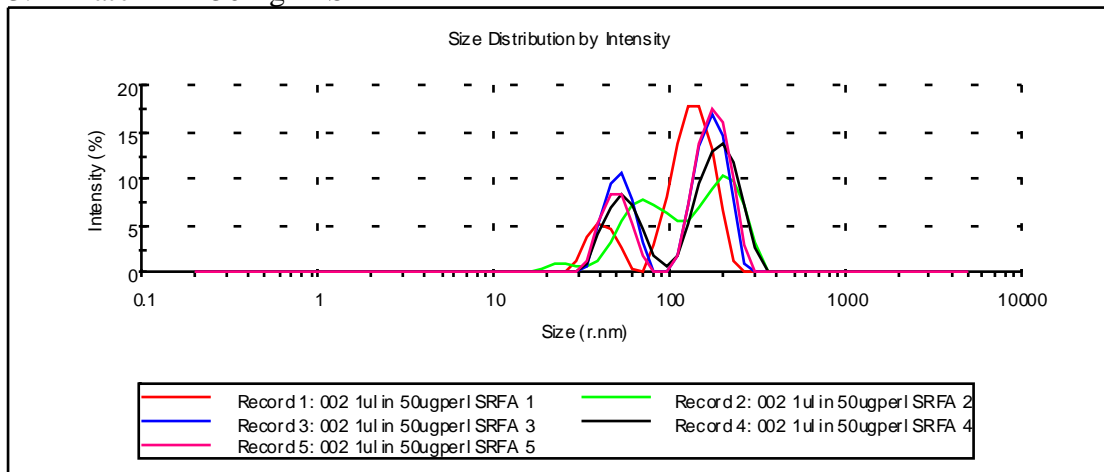
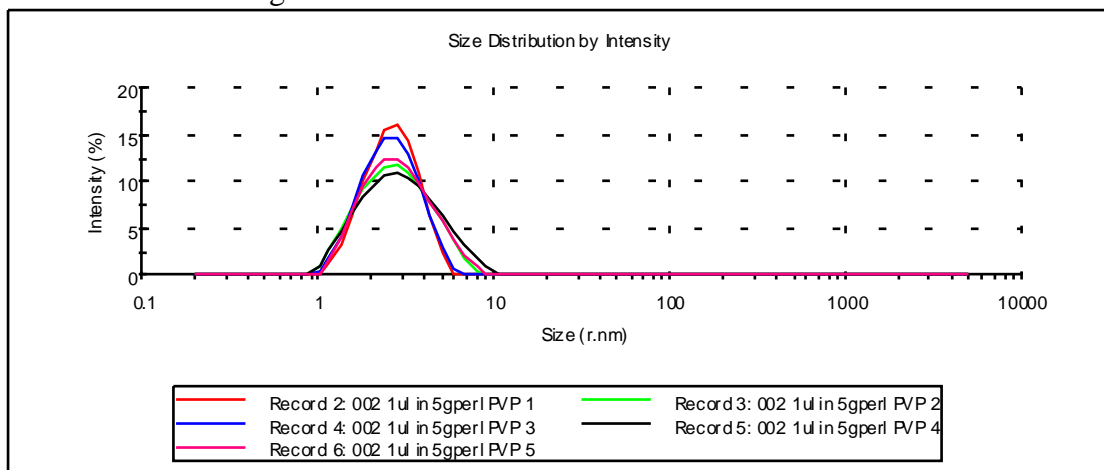


## 3.6 Batch 2 at pH 8.43



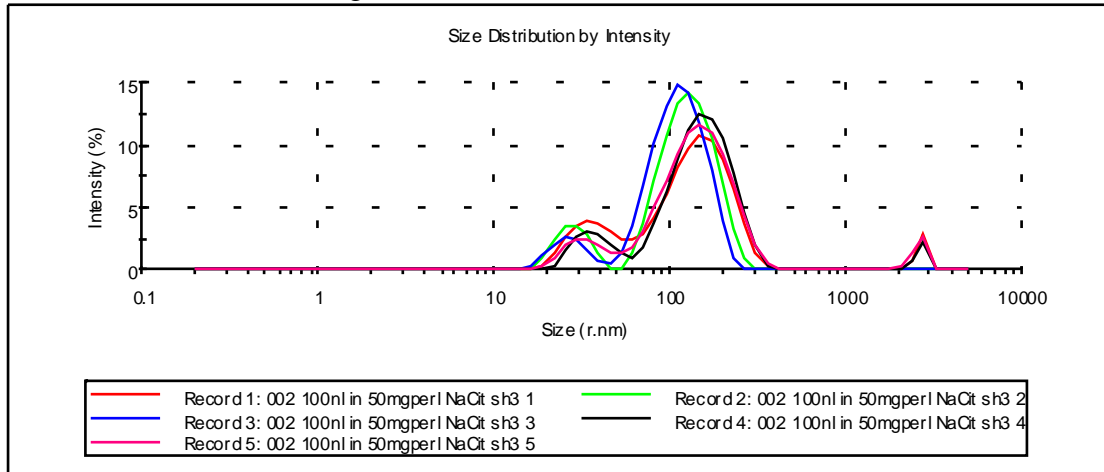
## 3.7 Batch 2 at pH 10.71

3.8 Batch 1 in SRFA 50 $\mu\text{gL}^{-1}$ 3.9 Batch 2 in 20 $\text{mgL}^{-1}$  sodium citrate

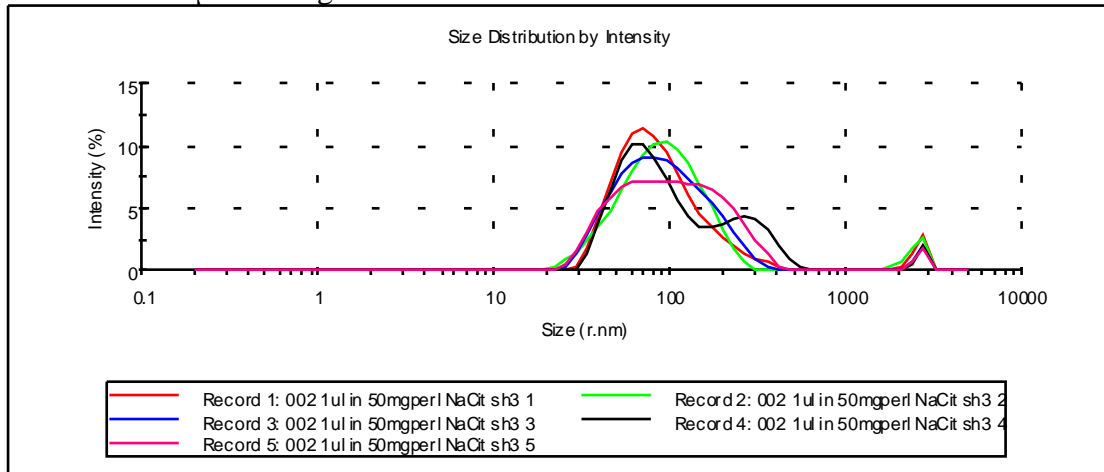
3.10 Batch 2 in 20mg L<sup>-1</sup> Tween 803.11 Batch 2 in 50mg L<sup>-1</sup> SRFA3.12 Batch 2 in 50mg L<sup>-1</sup> PVP



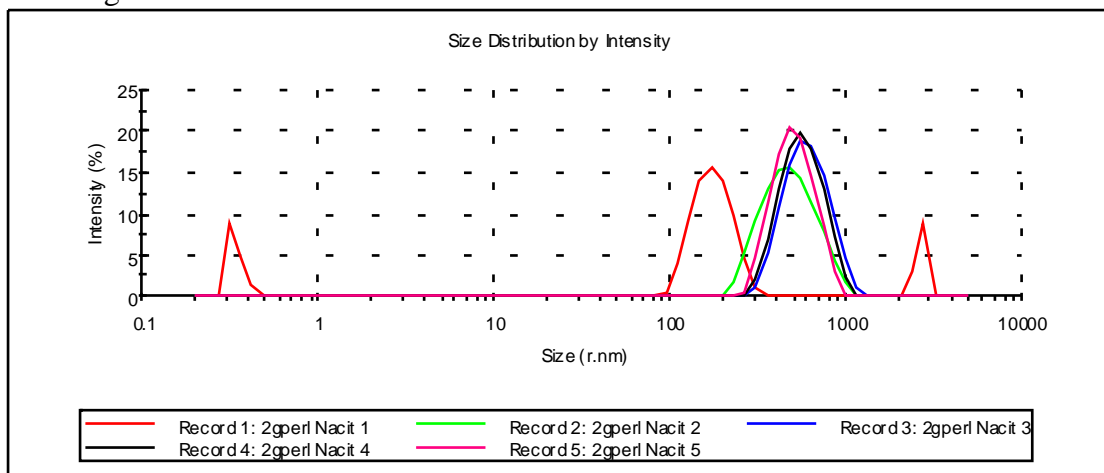
3.13 Batch 2 100nl in 50mgL<sup>-1</sup> sodium citrate

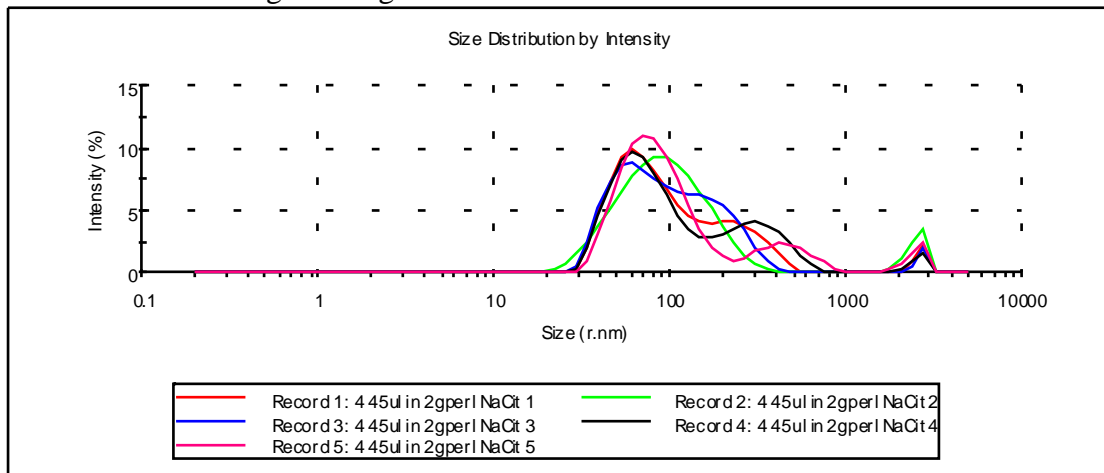
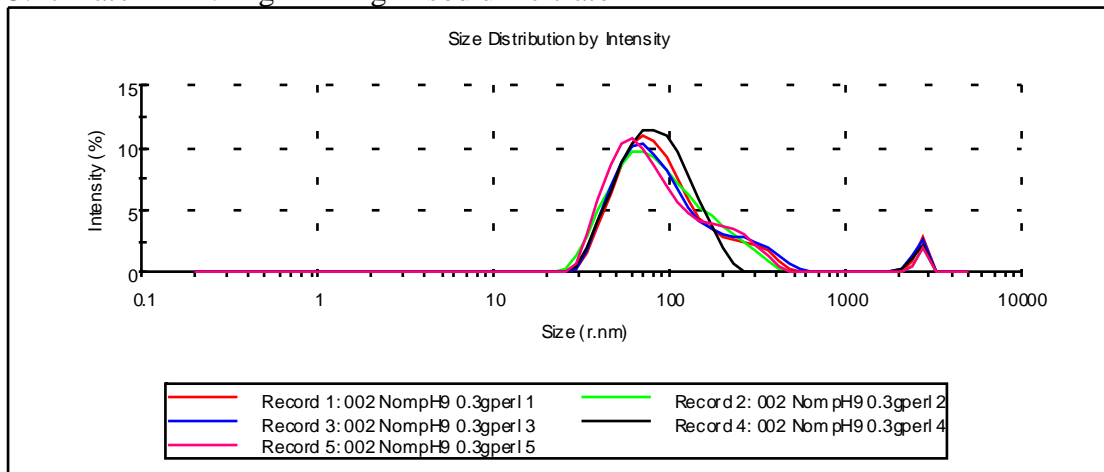
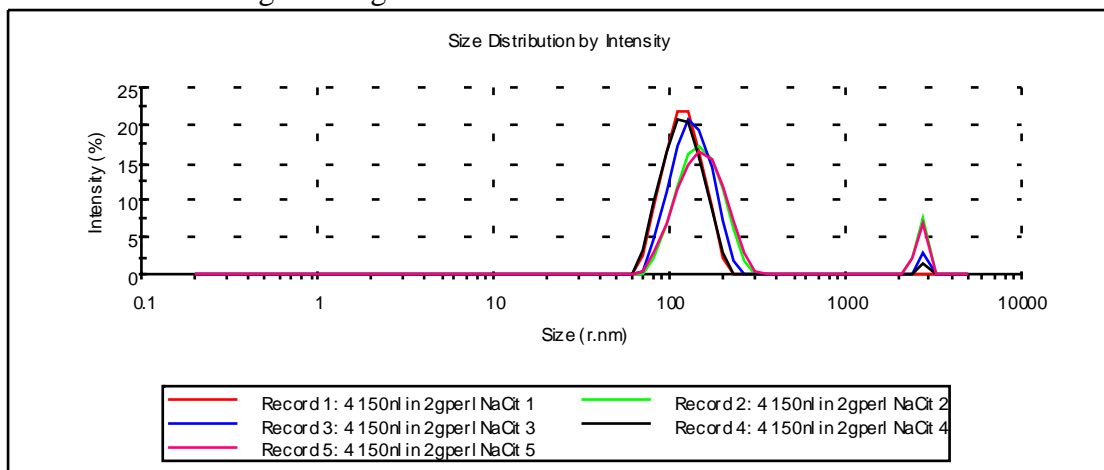


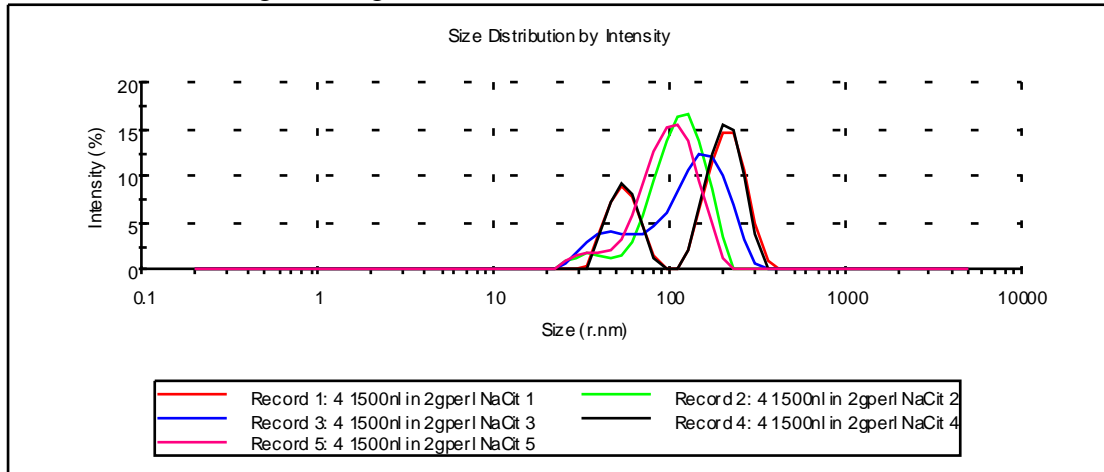
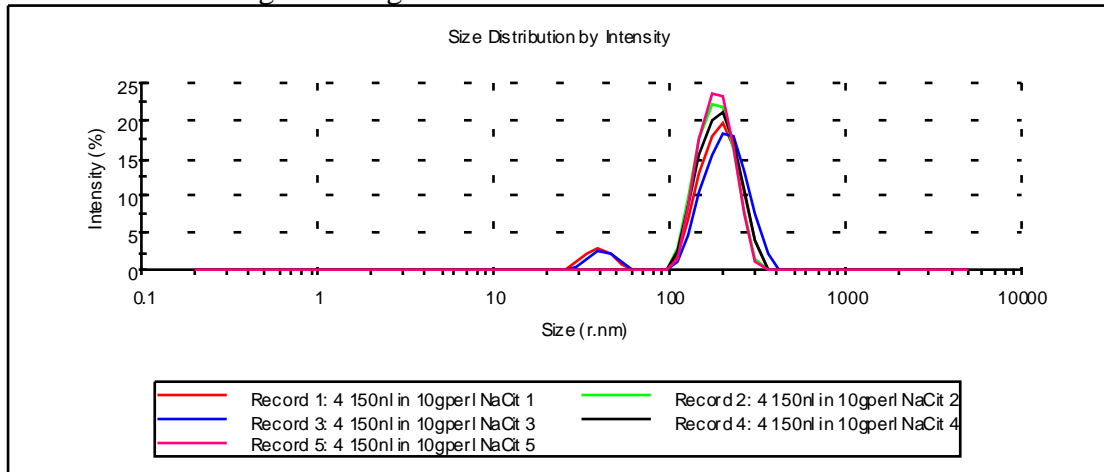
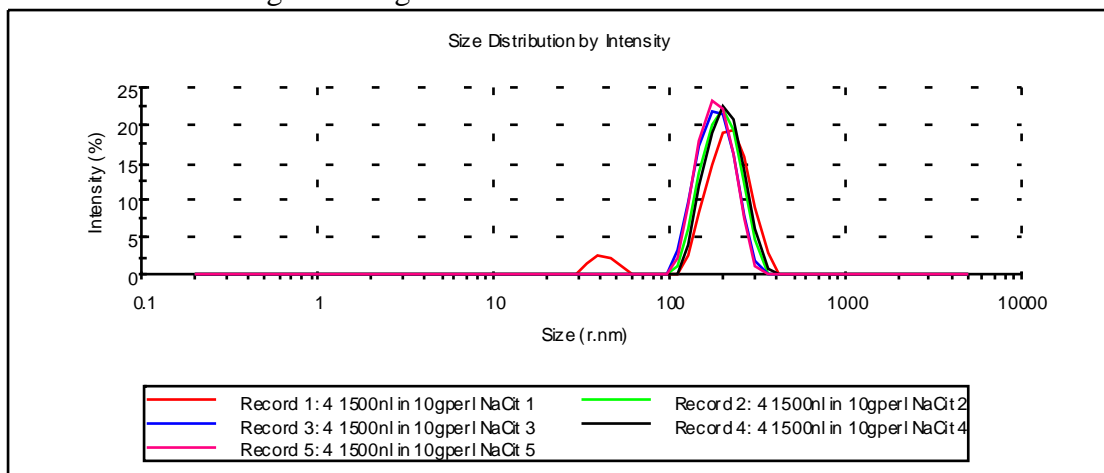
3.14 Batch 2 1µl in 50mgL<sup>-1</sup> sodium citrate

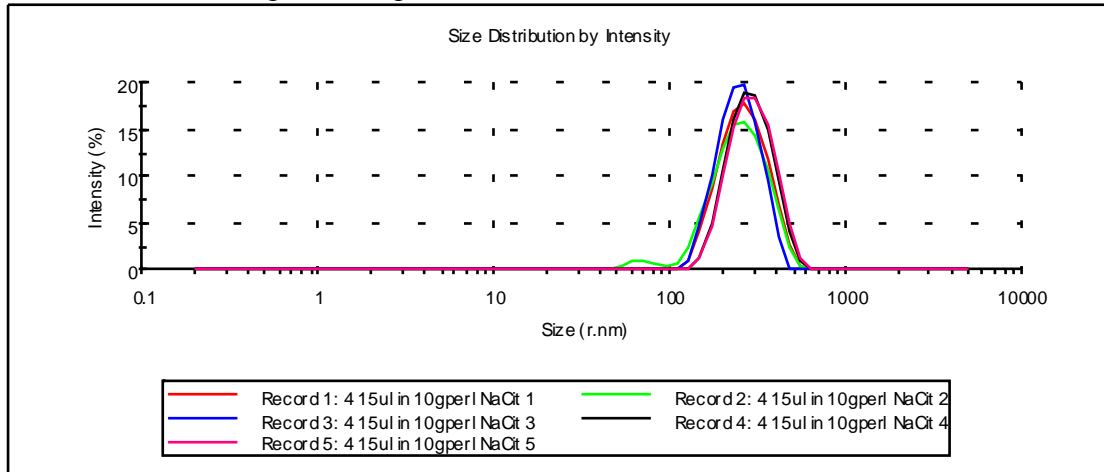
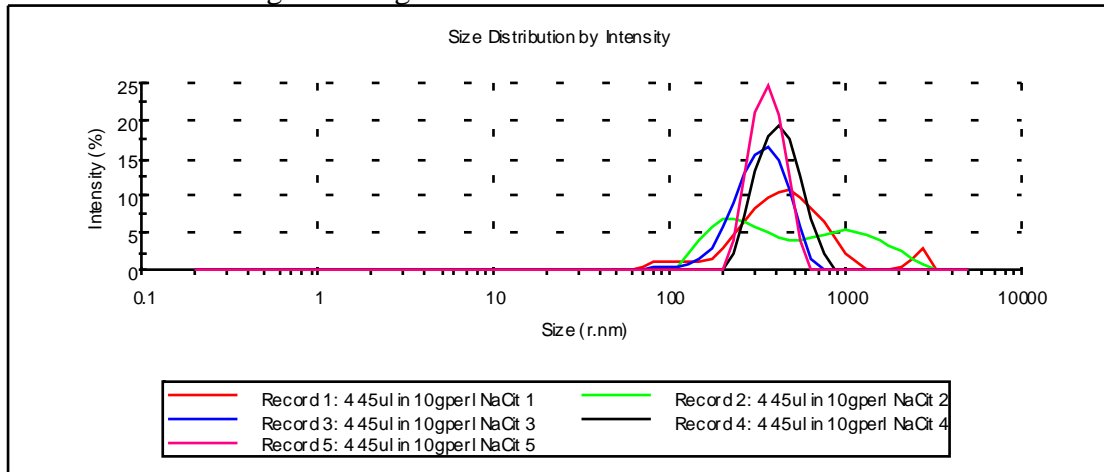
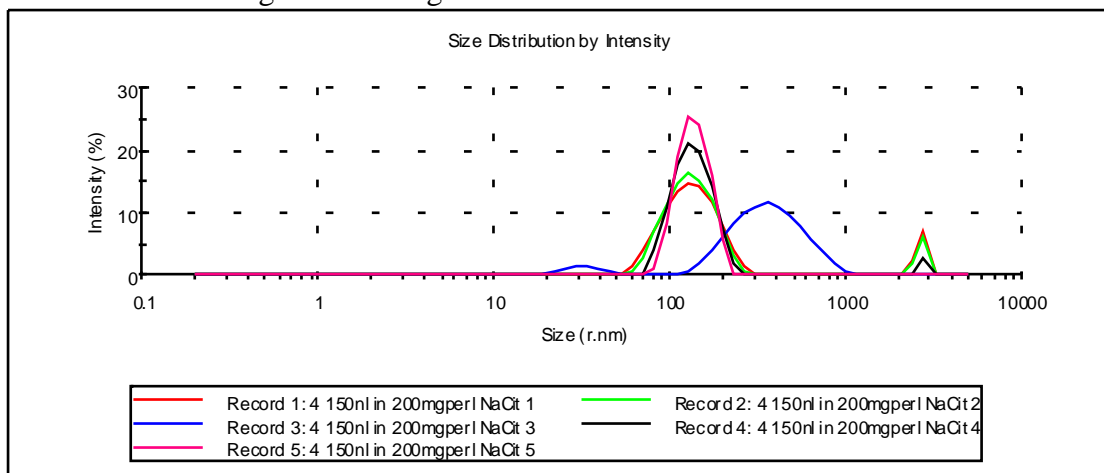


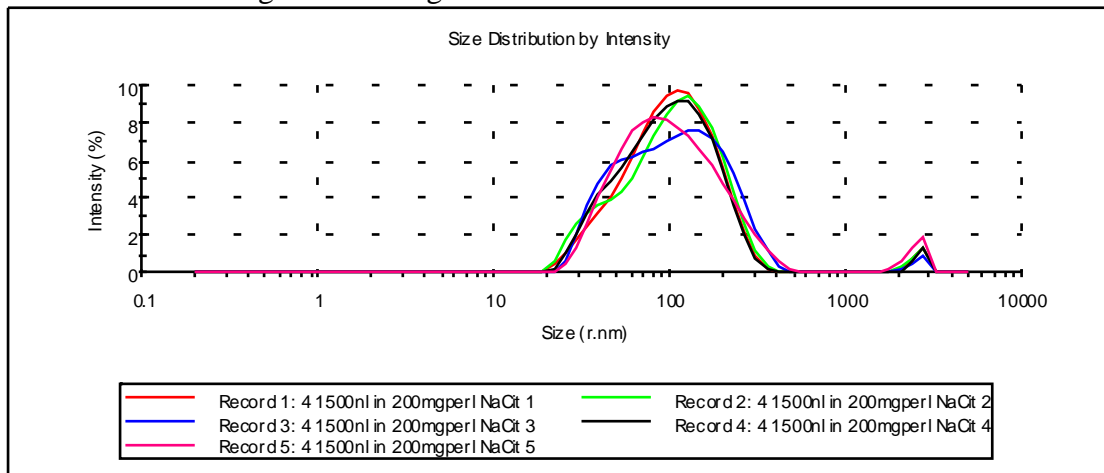
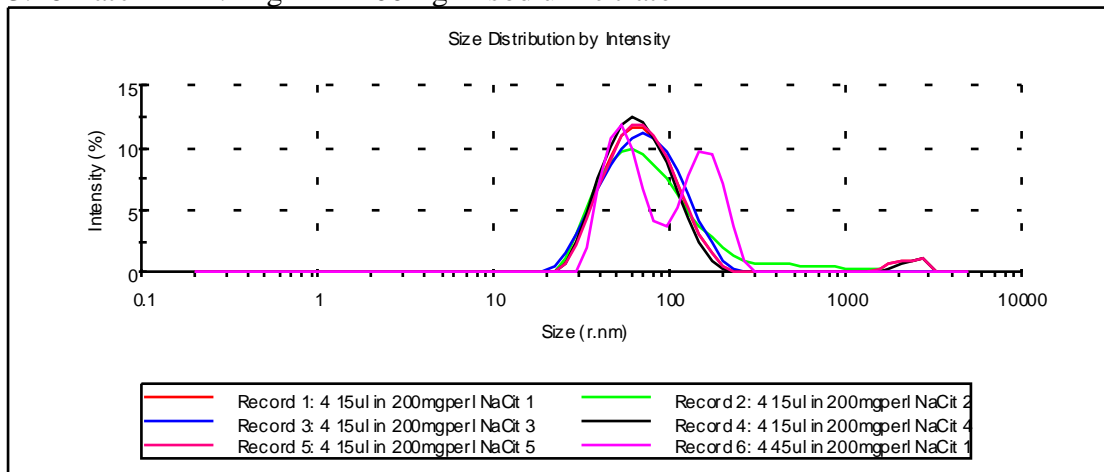
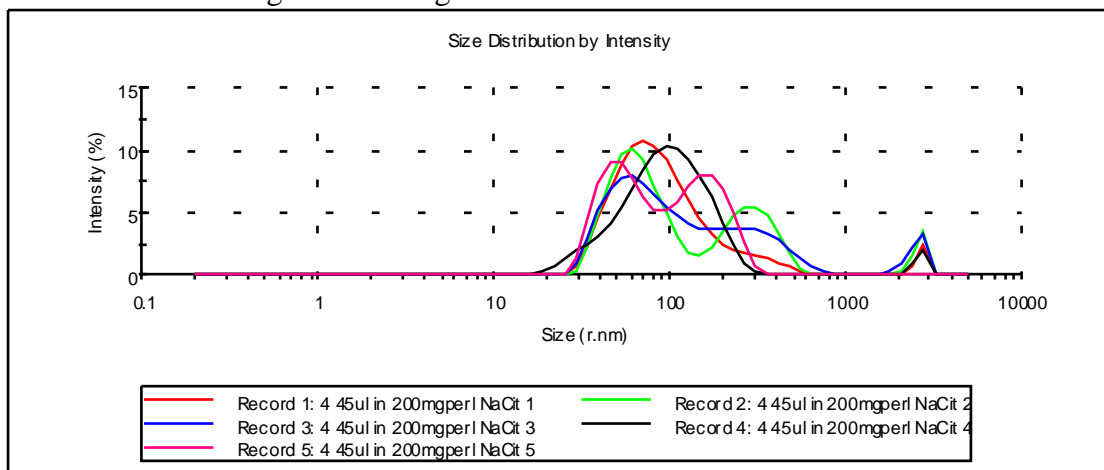
3.15 2gL<sup>-1</sup> sodium citrate

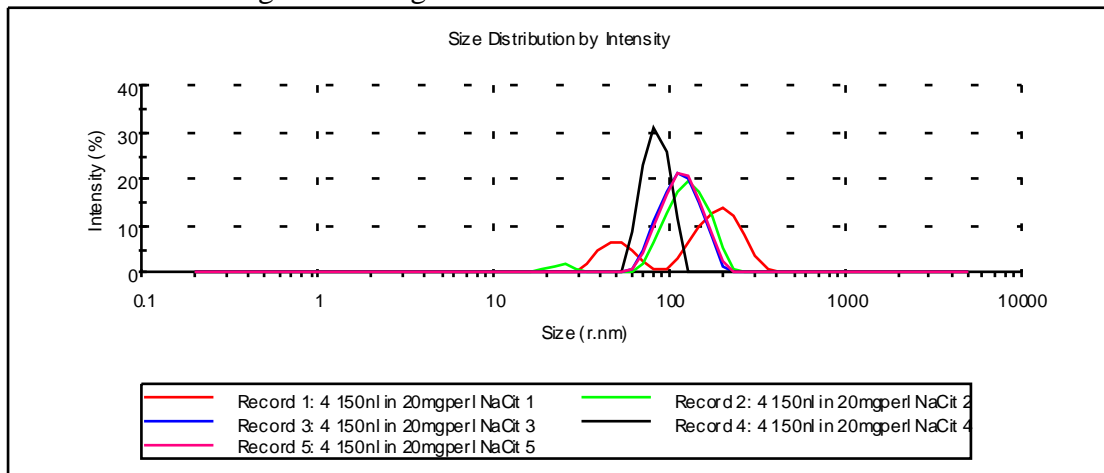
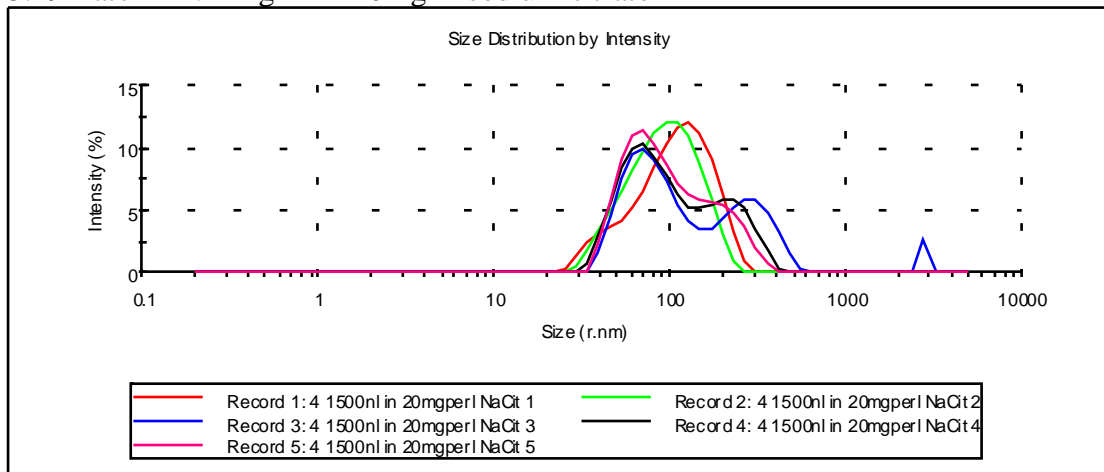
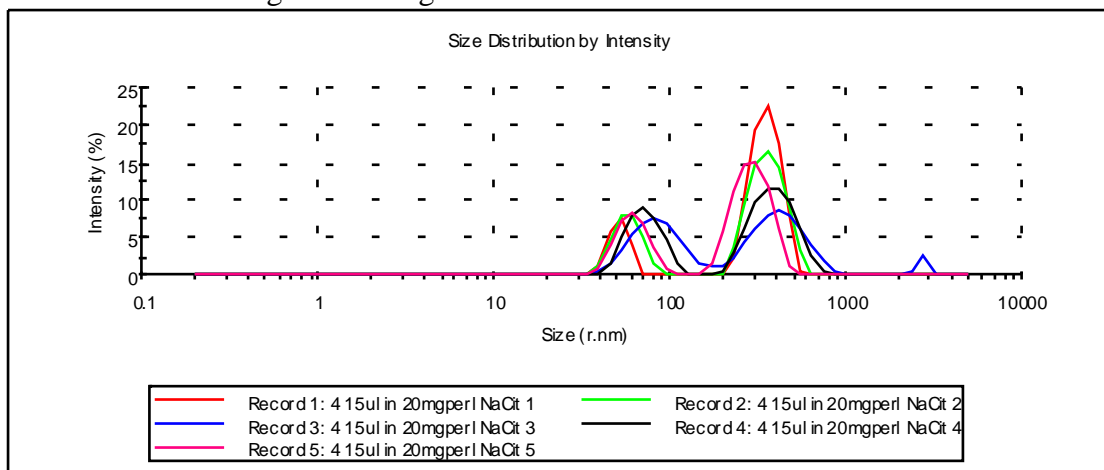


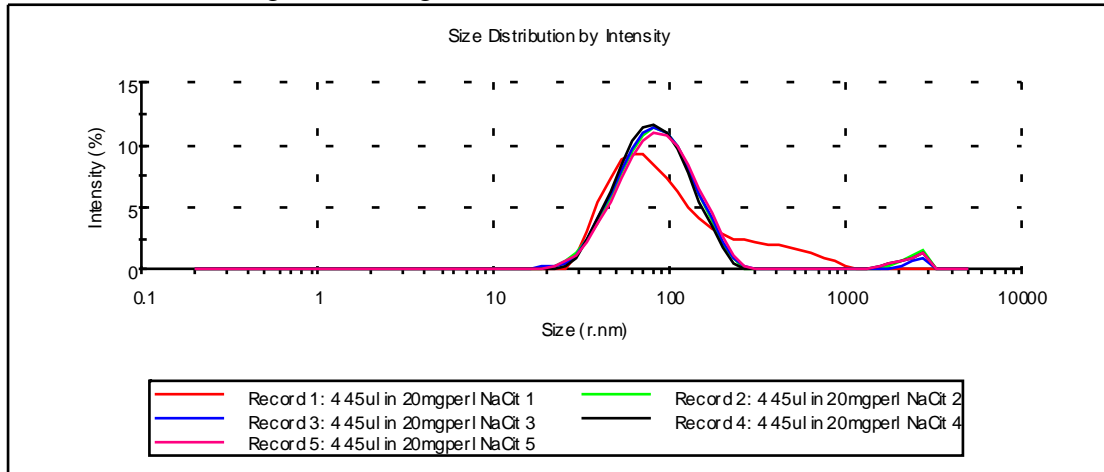
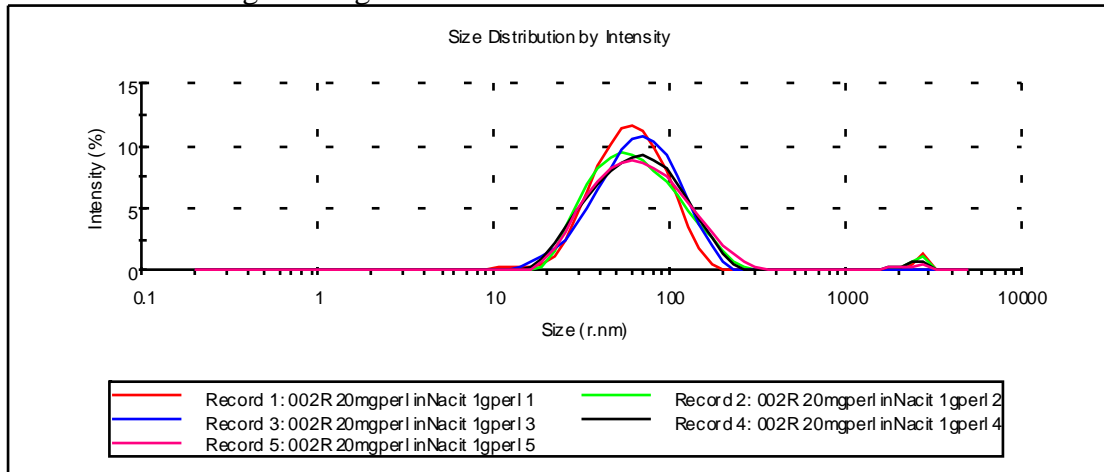
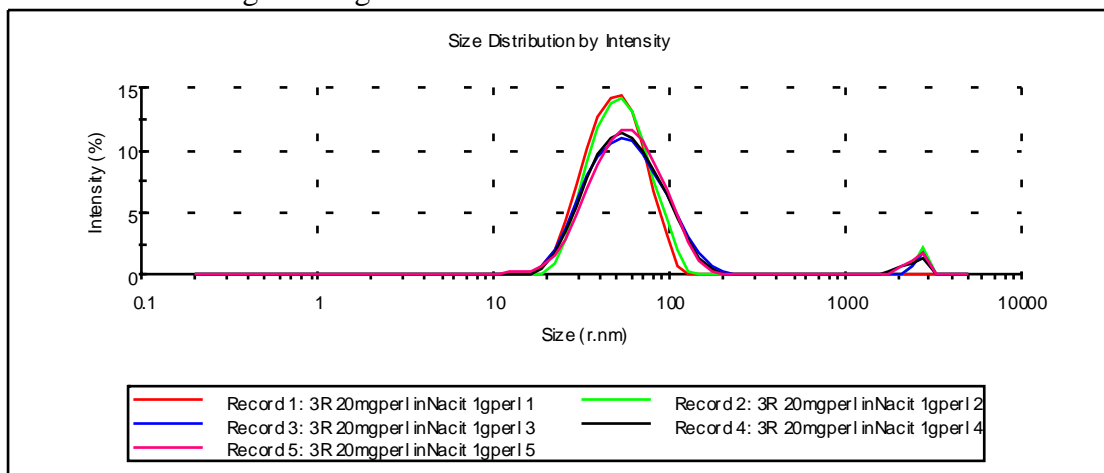
3.16 Batch 4  $37.2\text{mgL}^{-1}$  in  $2\text{gL}^{-1}$  sodium citrate3.17 Batch 4  $12.4\text{mgL}^{-1}$  in  $2\text{gL}^{-1}$  sodium citrate3.18 Batch 4  $0.12\text{mgL}^{-1}$  in  $2\text{gL}^{-1}$  sodium citrate

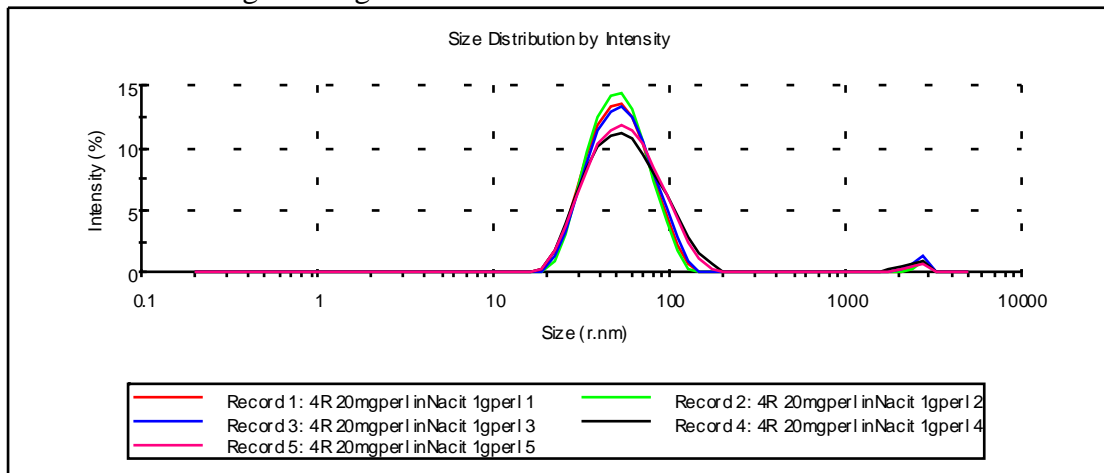
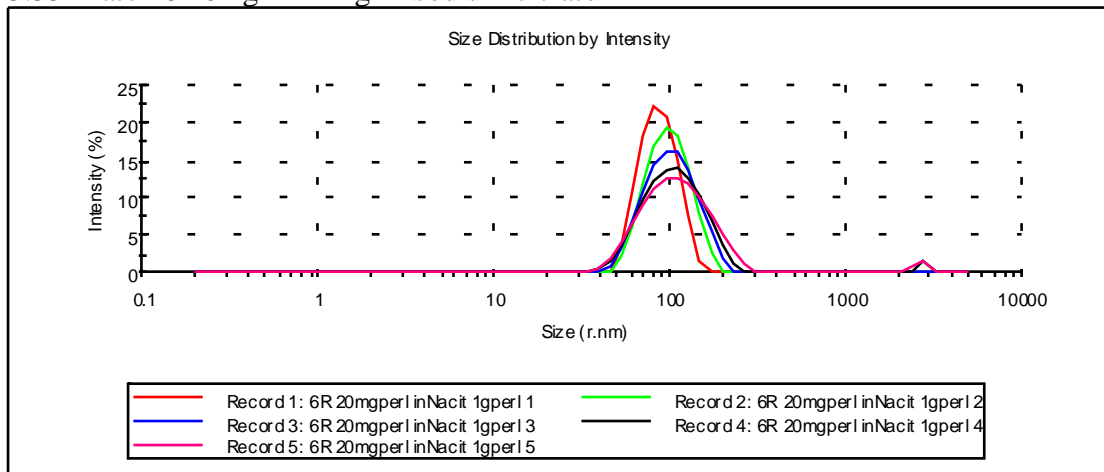
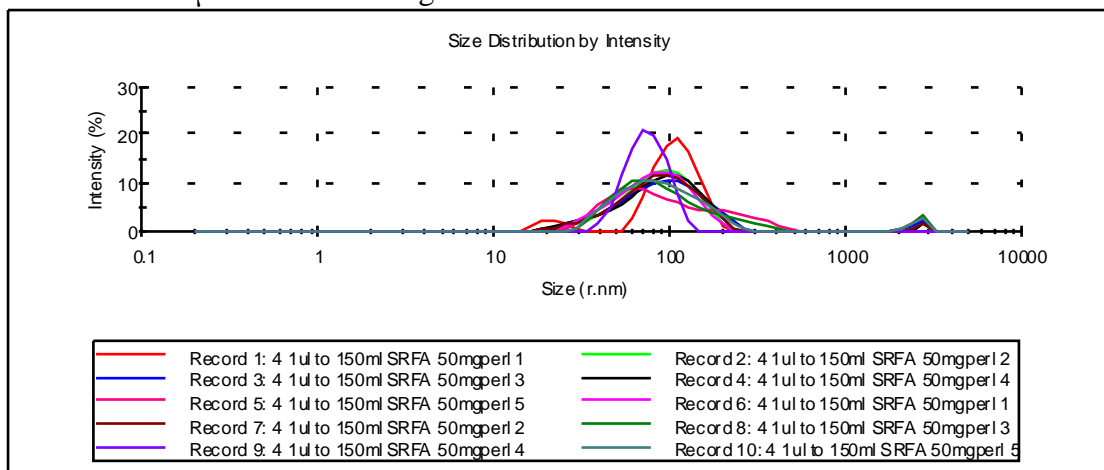
3.19 Batch 4  $1.24\text{mgL}^{-1}$  in  $2\text{gL}^{-1}$  sodium citrate3.20 Batch 4  $0.12\text{mgL}^{-1}$  in  $10\text{gL}^{-1}$  sodium citrate3.21 Batch 4  $1.24\text{mgL}^{-1}$  in  $10\text{gL}^{-1}$  sodium citrate

3.22 Batch 4  $12.4\text{mgL}^{-1}$  in  $10\text{gL}^{-1}$  sodium citrate3.23 Batch 4  $37.2\text{mgL}^{-1}$  in  $10\text{gL}^{-1}$  sodium citrate3.24 Batch 4  $0.12\text{mgL}^{-1}$  in  $200\text{mgL}^{-1}$  sodium citrate

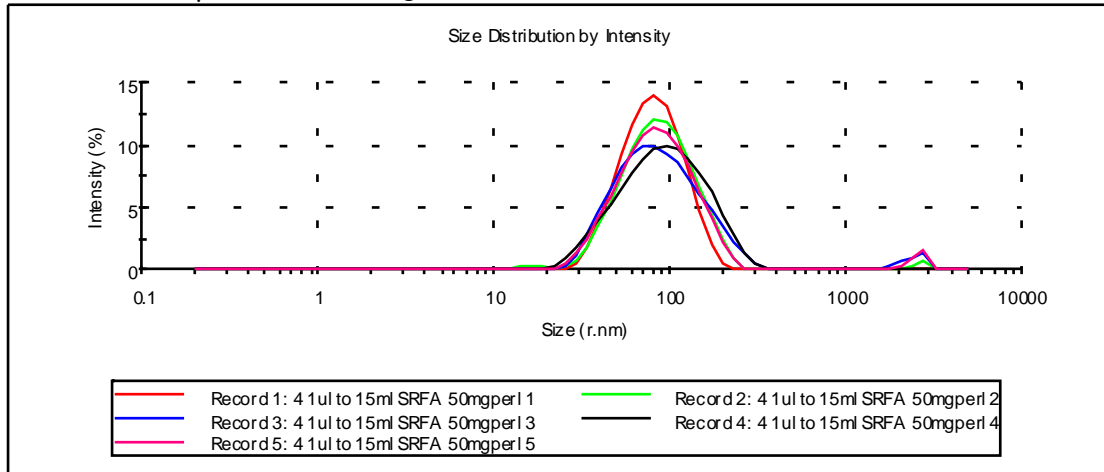
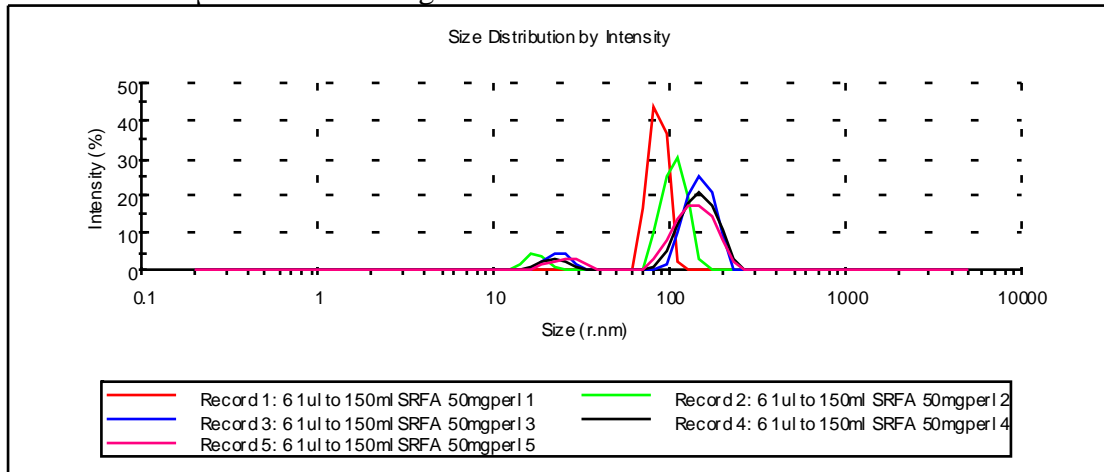
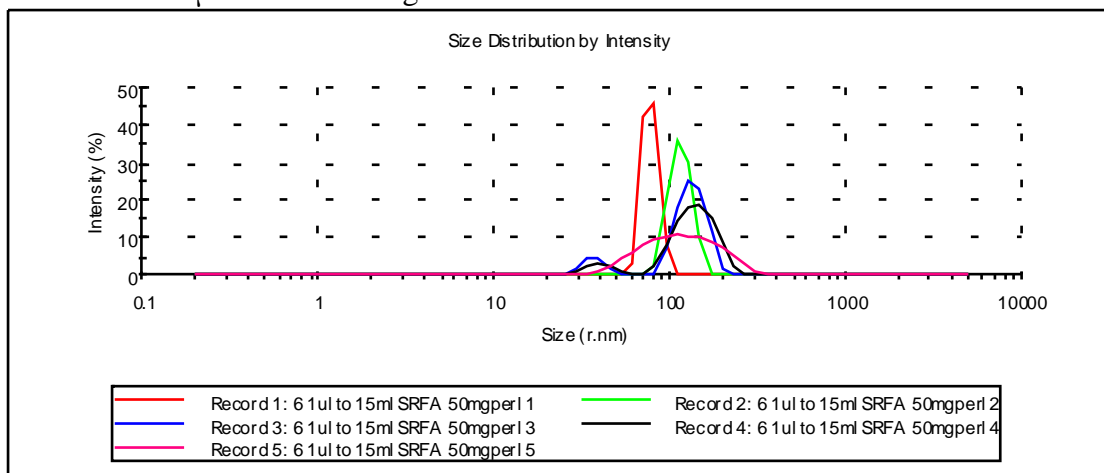
3.25 Batch 4  $1.24\text{mgL}^{-1}$  in  $200\text{mgL}^{-1}$  sodium citrate3.26 Batch 4  $12.4\text{mgL}^{-1}$  in  $200\text{mgL}^{-1}$  sodium citrate3.27 Batch 4  $37.2\text{mgL}^{-1}$  in  $200\text{mgL}^{-1}$  sodium citrate

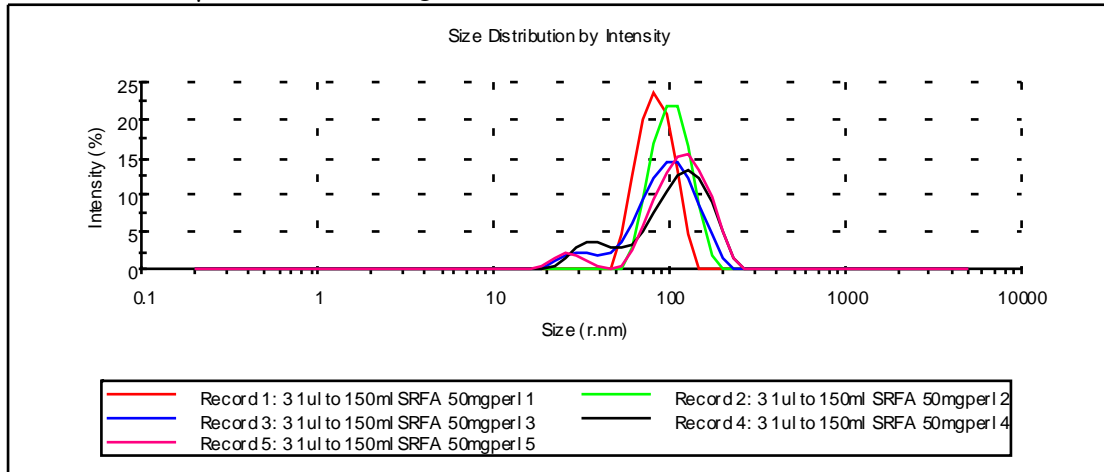
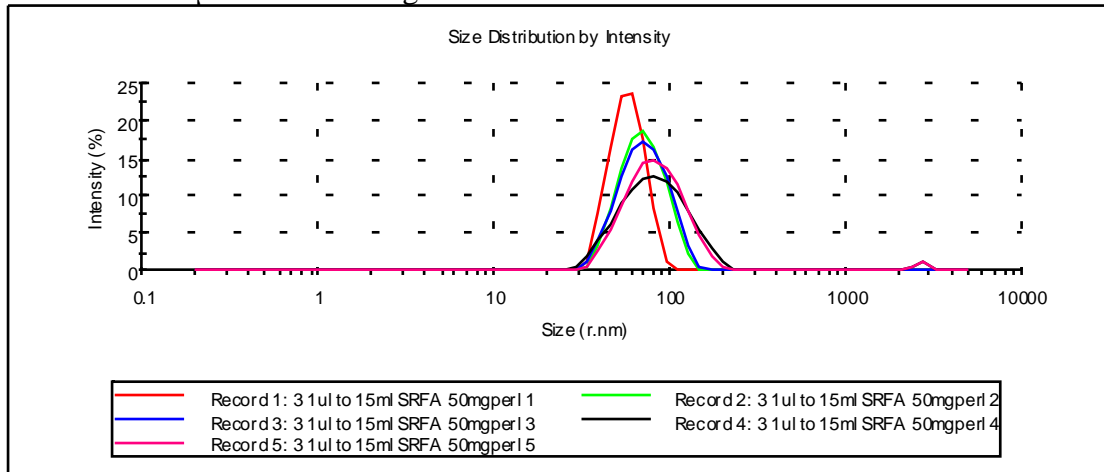
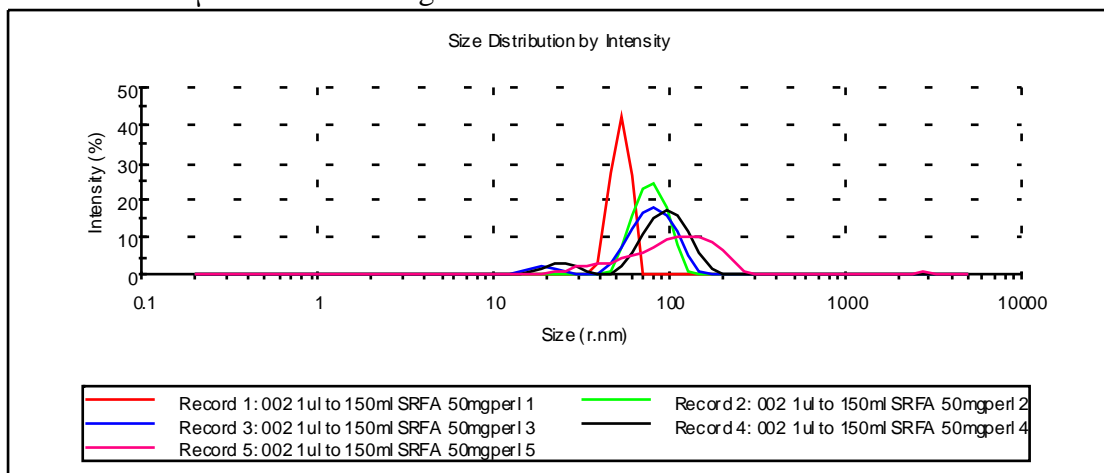
3.28 Batch 4  $0.12\text{mgL}^{-1}$  in  $20\text{mgL}^{-1}$  sodium citrate3.29 Batch 4  $1.24\text{mgL}^{-1}$  in  $20\text{mgL}^{-1}$  sodium citrate3.30 Batch 4  $12.4\text{mgL}^{-1}$  in  $20\text{mgL}^{-1}$  sodium citrate

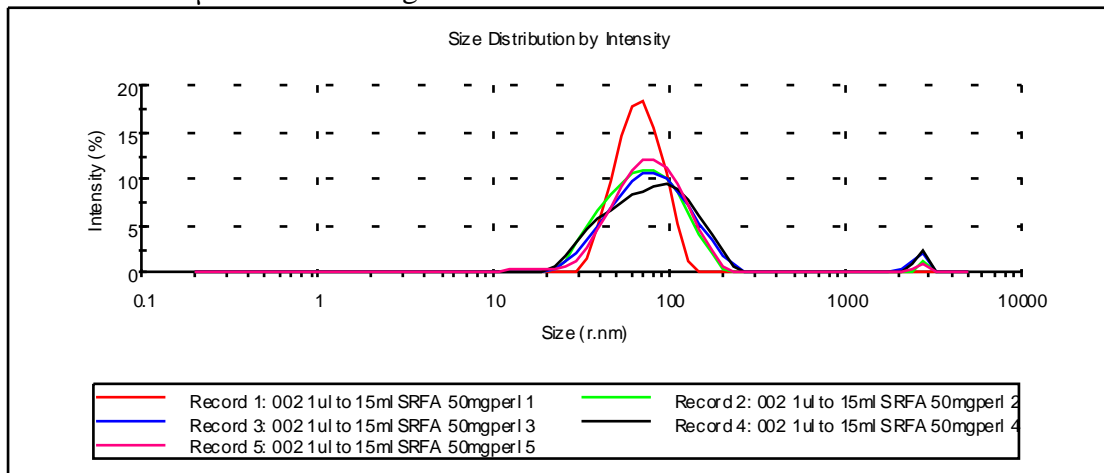
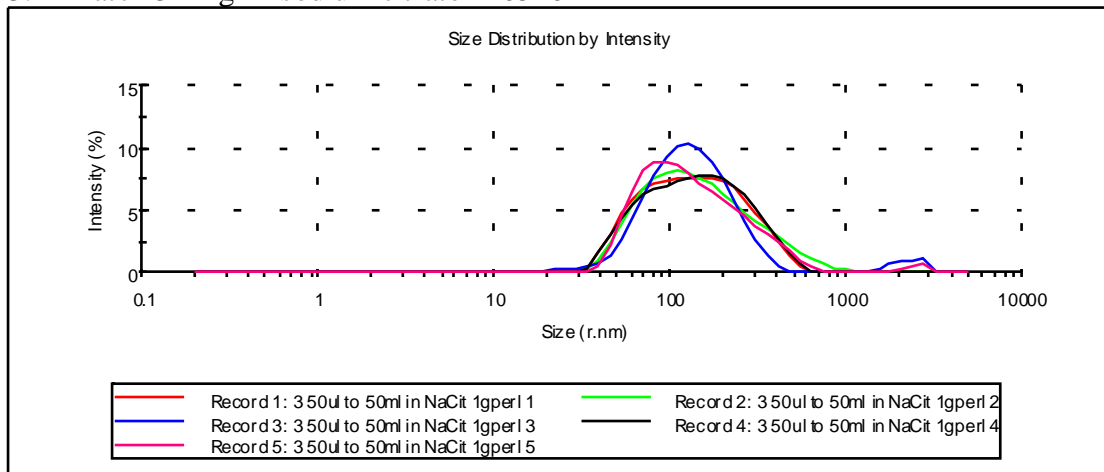
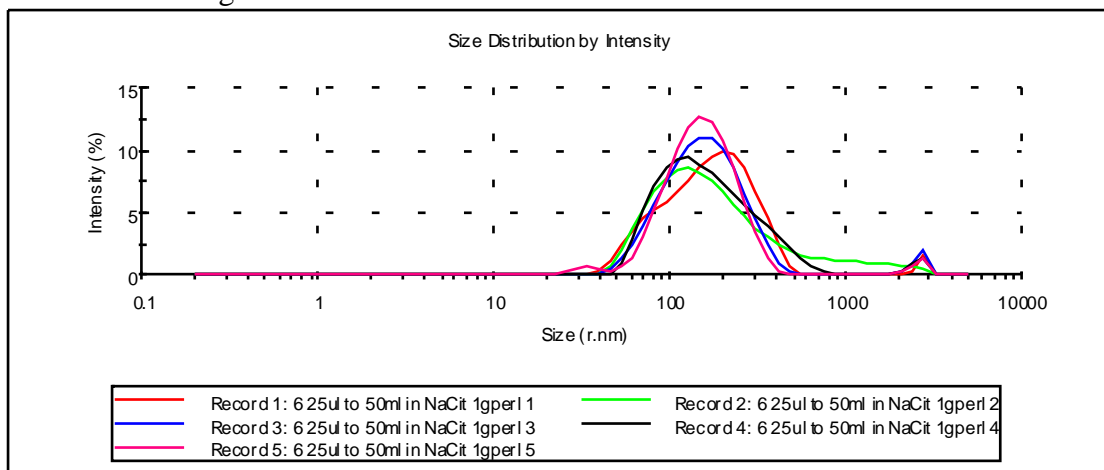
3.31 Batch 4  $37.2\text{mgL}^{-1}$  in  $20\text{mgL}^{-1}$  sodium citrate3.32 Batch 2  $20\text{mgL}^{-1}$  in  $1\text{gL}^{-1}$  sodium citrate3.33 Batch 3  $20\text{mgL}^{-1}$  in  $1\text{gL}^{-1}$  sodium citrate

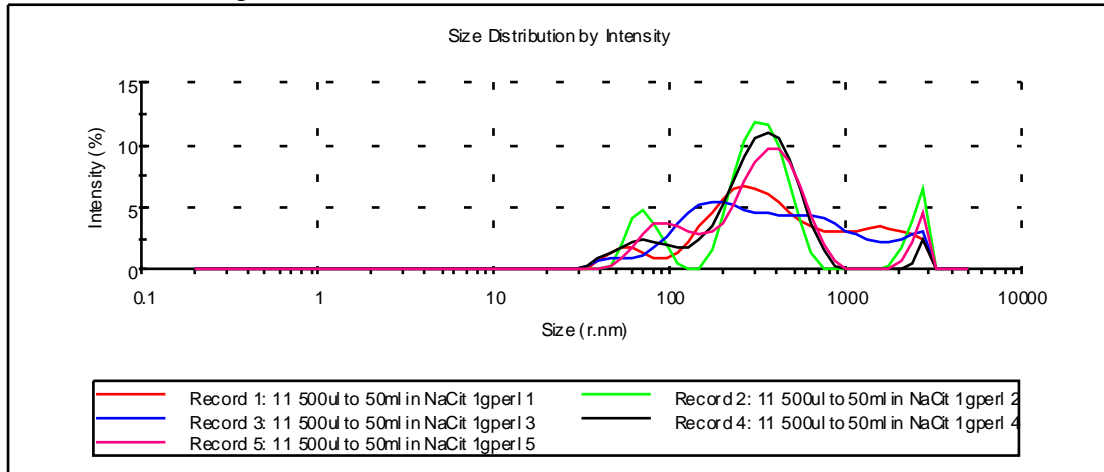
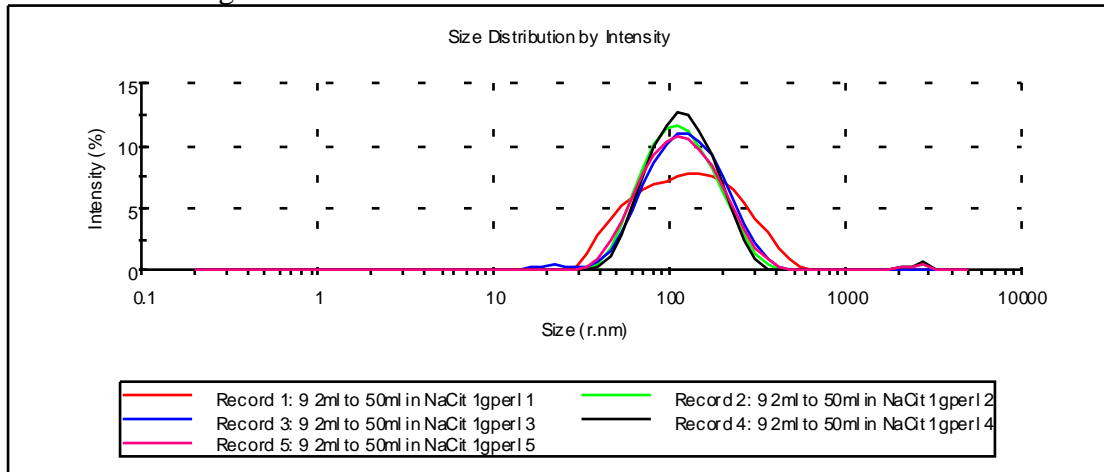
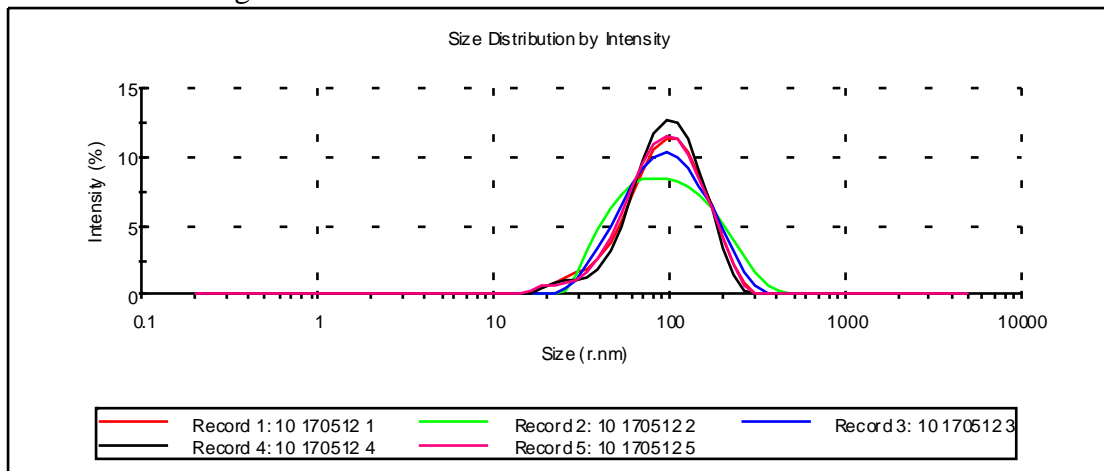
3.34 Batch 4 20mgL<sup>-1</sup> in 1gL<sup>-1</sup> sodium citrate3.35 Batch 6 20mgL<sup>-1</sup> in 1gL<sup>-1</sup> sodium citrate3.36 Batch 4 1μL in 150ml 50mgL<sup>-1</sup> SRFA



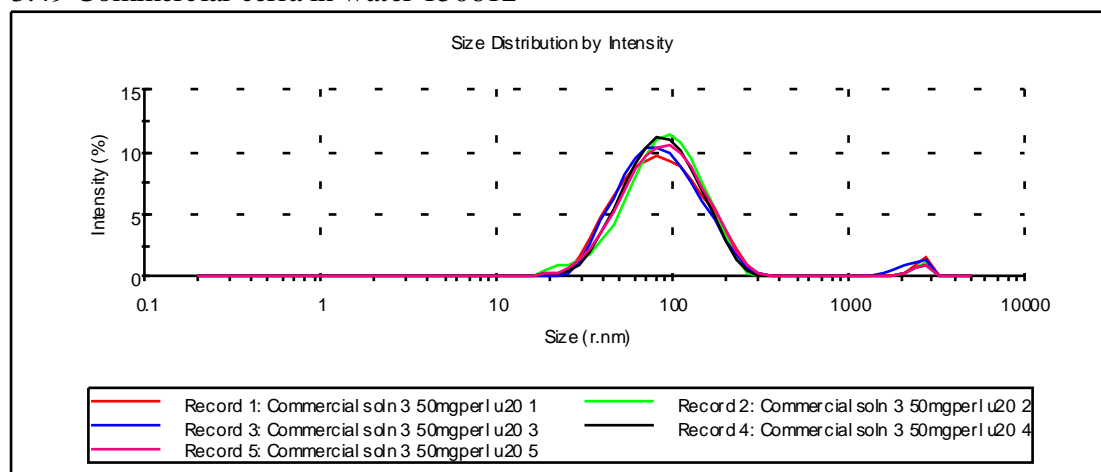
3.37 Batch 4 1 $\mu$ L in 15ml 50mgL<sup>-1</sup> SRFA3.38 Batch 6 1 $\mu$ L in 150ml 50mgL<sup>-1</sup> SRFA3.39 Batch 6 1 $\mu$ L in 15ml 50mgL<sup>-1</sup> SRFA

3.40 Batch 3 1 $\mu$ L in 150ml 50mgL<sup>-1</sup> SRFA3.41 Batch 3 1 $\mu$ L in 15ml 50mgL<sup>-1</sup> SRFA3.42 Batch 2 1 $\mu$ L in 150ml 50mgL<sup>-1</sup> SRFA

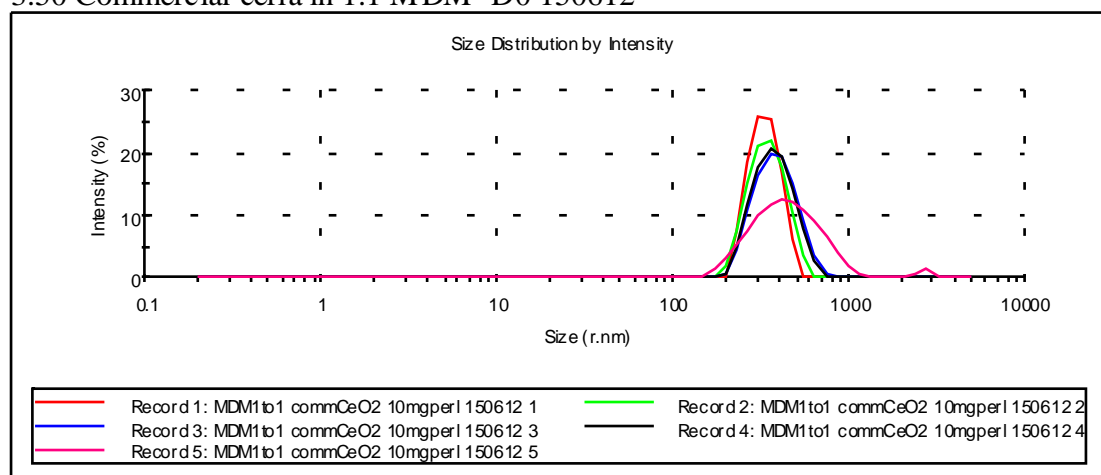
3.43 Batch 2 1 $\mu$ L in 15ml 50mgL<sup>-1</sup> SRFA3.44 Batch 3 in 1gL<sup>-1</sup> sodium citrate 240520123.45 Batch 6 in 1gL<sup>-1</sup> sodium citrate 24052012

3.46 Batch 11 in  $1\text{gL}^{-1}$  sodium citrate 240520123.47 Batch 9 in  $1\text{gL}^{-1}$  sodium citrate 240520123.48 Batch 10 in  $1\text{gL}^{-1}$  sodium citrate 24052012

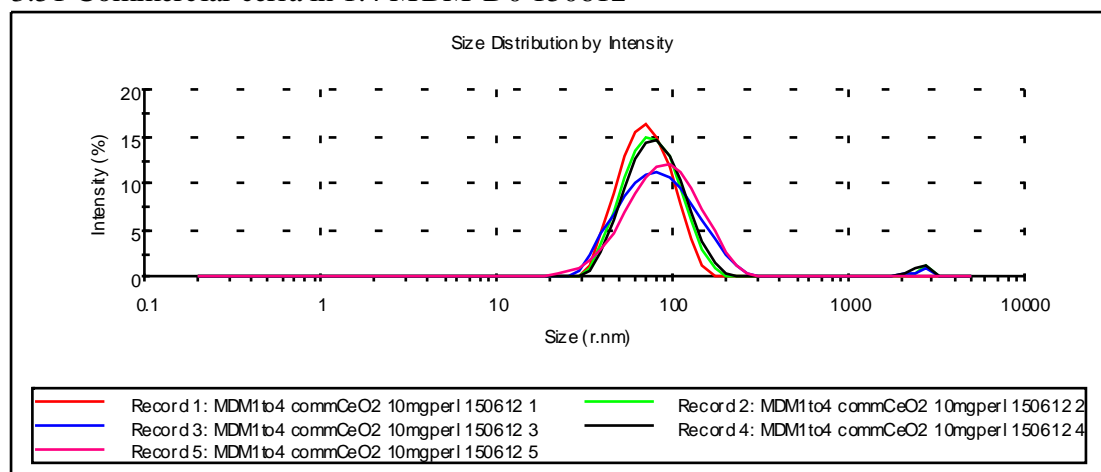
## 3.49 Commercial ceria in water 150612



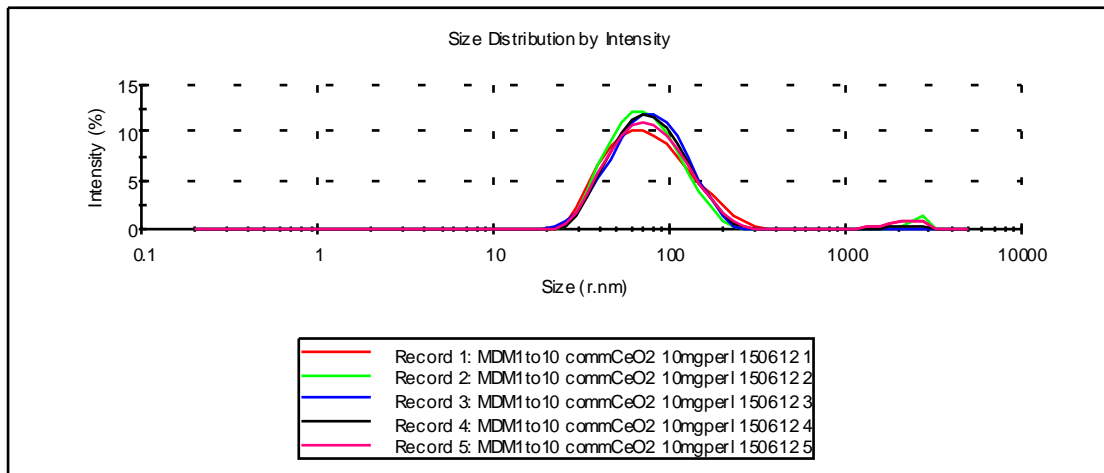
## 3.50 Commercial ceria in 1:1 MDM D0 150612



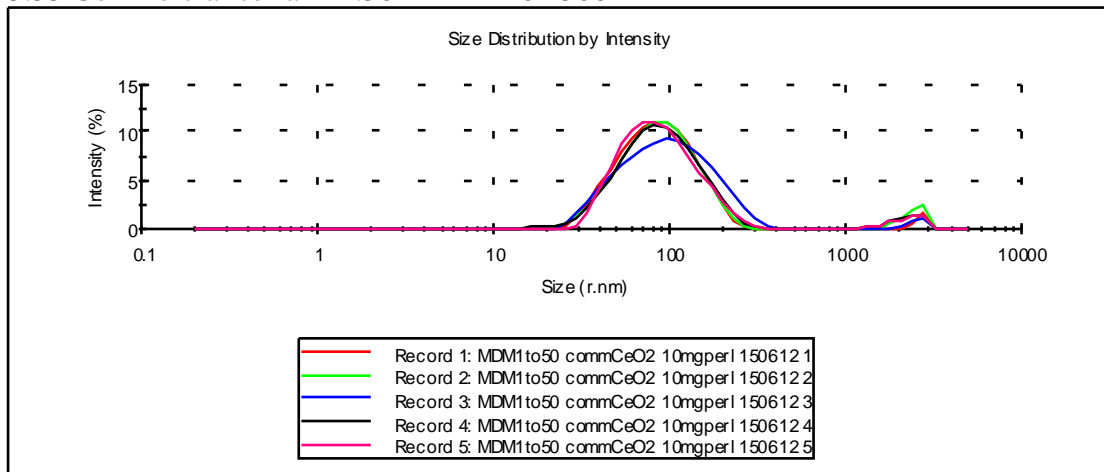
## 3.51 Commercial ceria in 1:4 MDM D0 150612



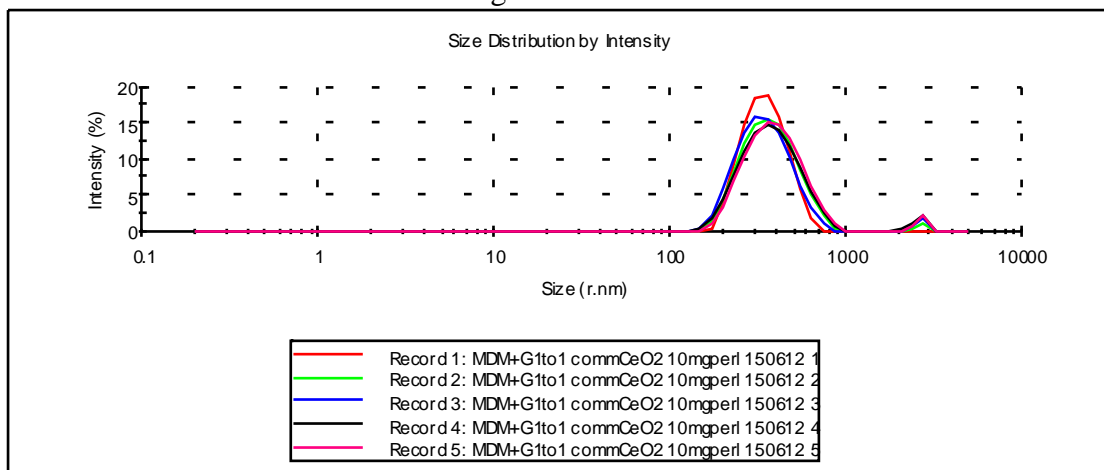
## 3.52 Commercial ceria in 1:10 MDM D0 150612



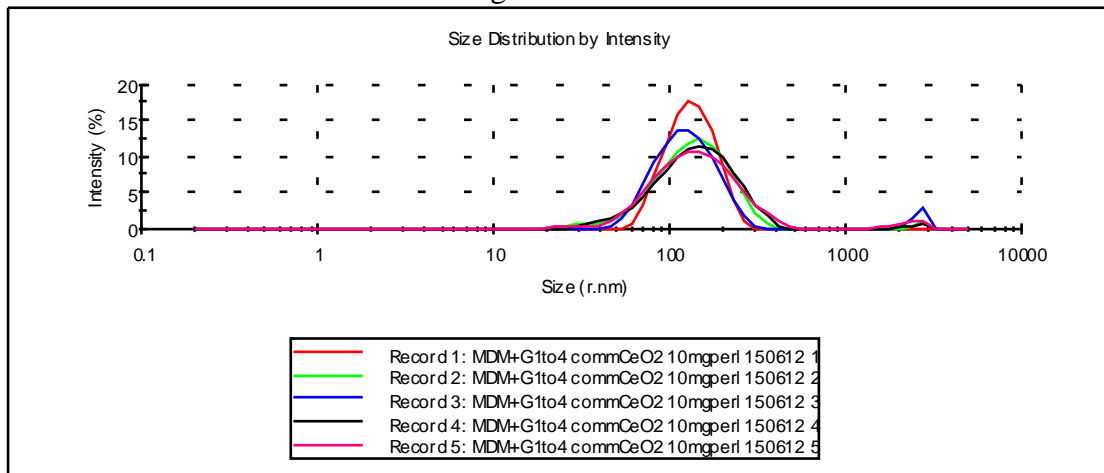
## 3.53 Commercial ceria in 1:50 MDM D0 150612



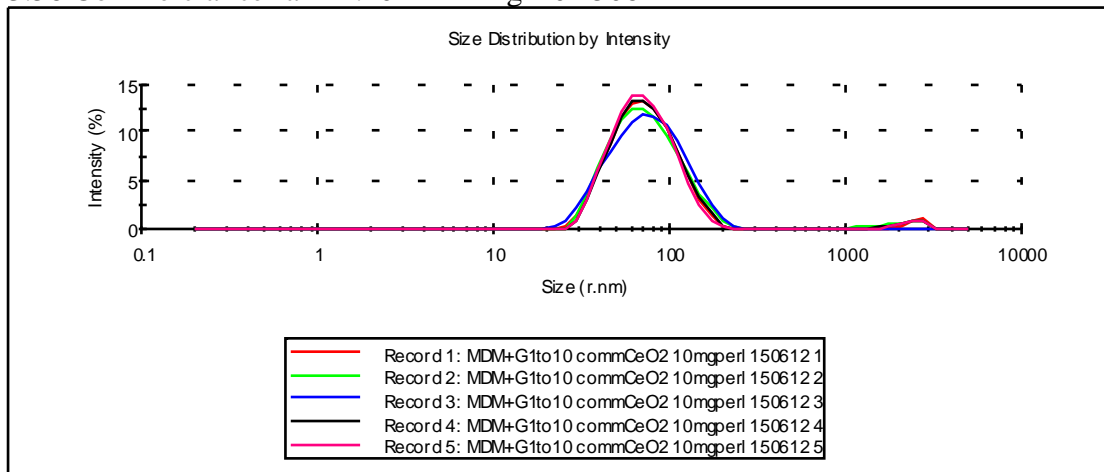
## 3.54 Commercial ceria in 1:1 MDM+g D0 150612



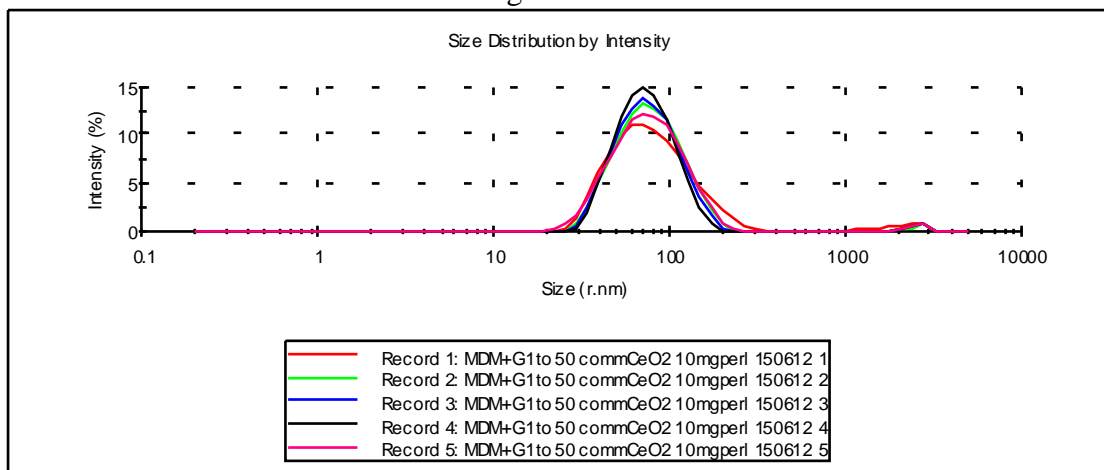
## 3.55 Commercial ceria in 1:4 MDM+g D0 150612



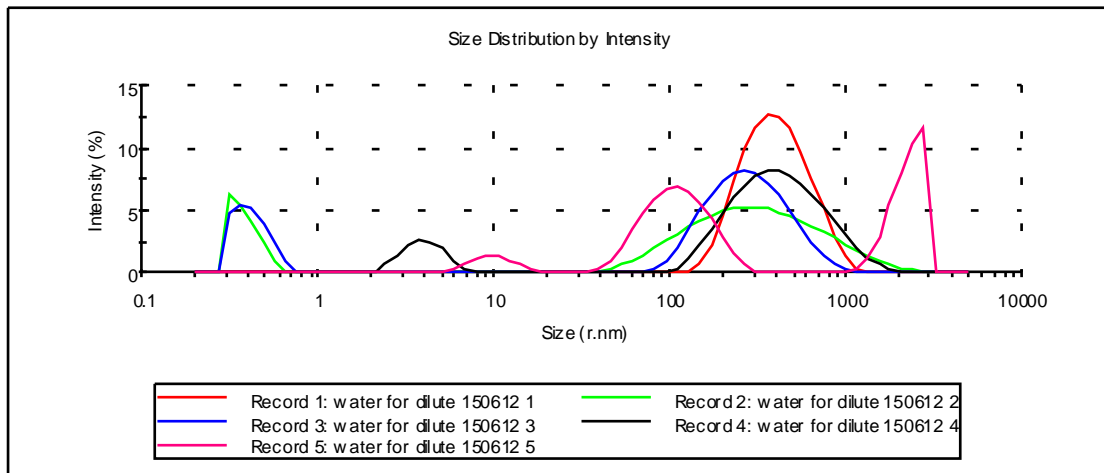
## 3.56 Commercial ceria in 1:10 MDM+g D0 150612



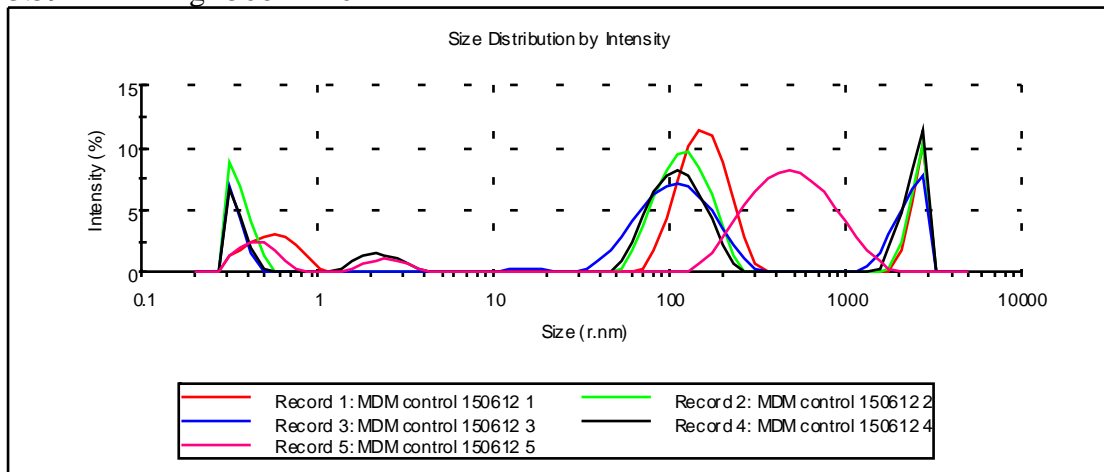
## 3.57 Commercial ceria in 1:50 MDM+g D0 150612



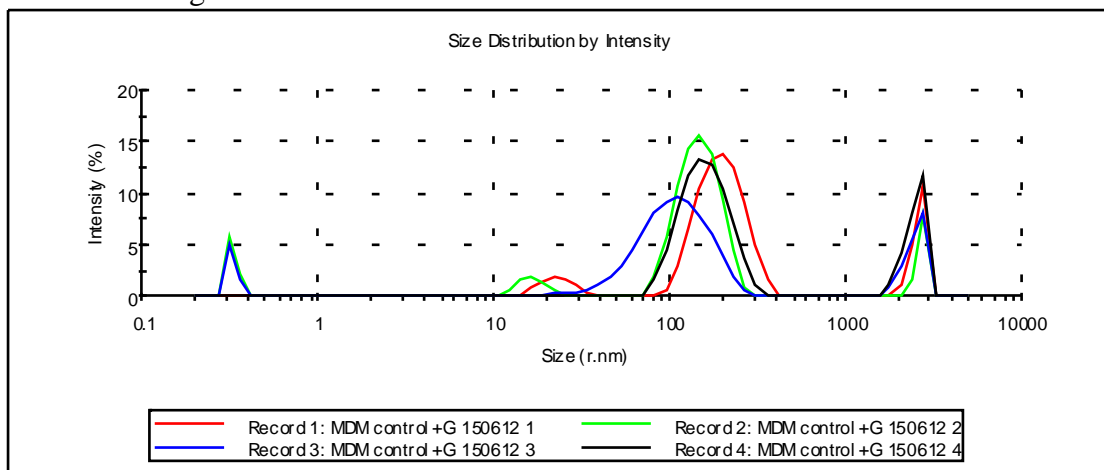
## 3.58 Water 150612 D0



## 3.59 MDM + g 150612 D0

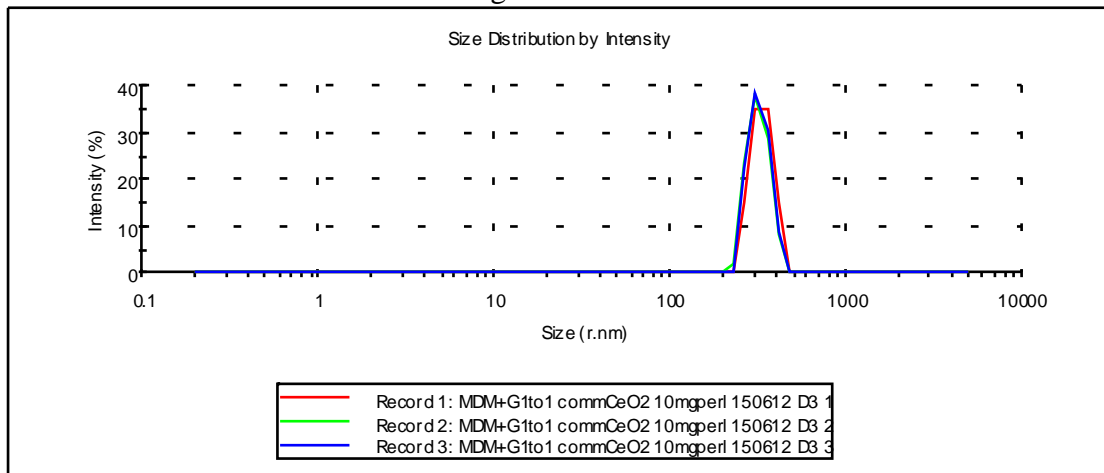


## 3.60 MDM + g 150612 D0



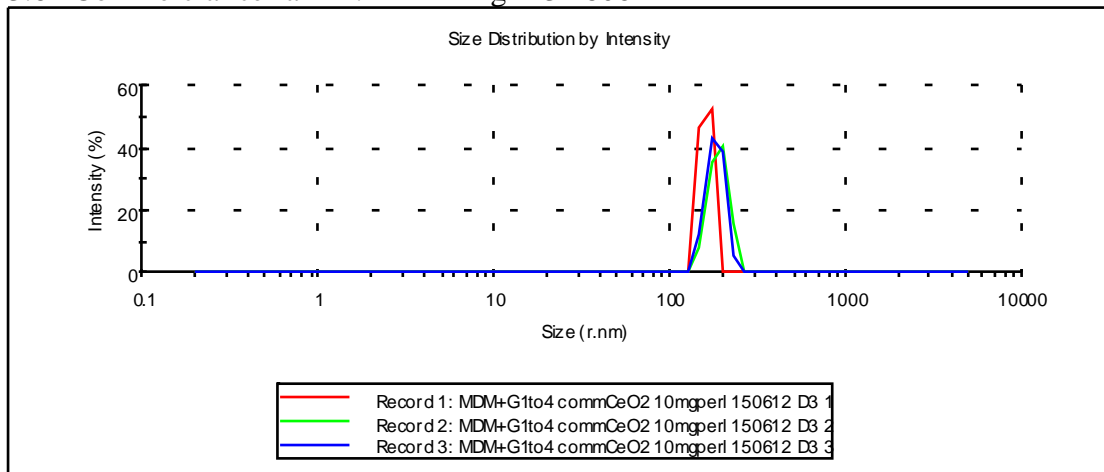


## 3.61 Commercial ceria in 1:1 MDM+g D3 180612

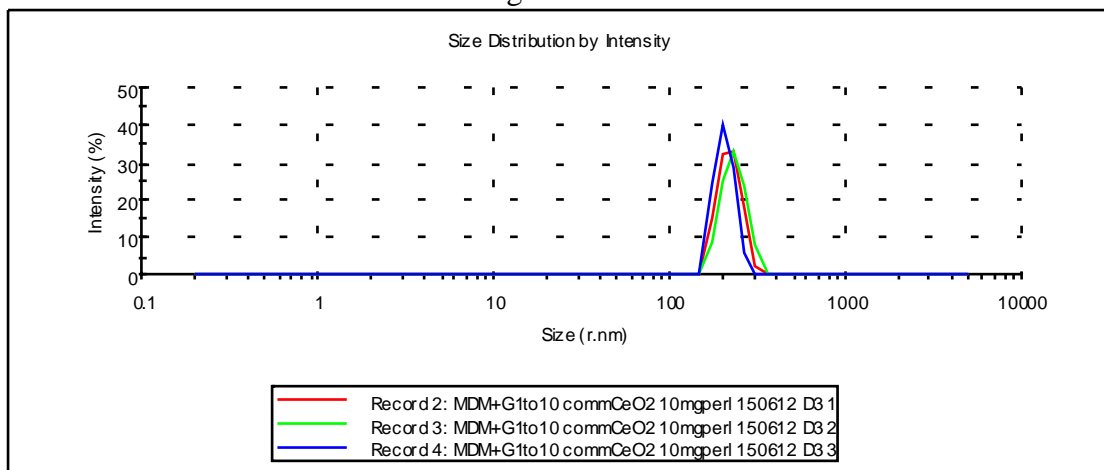


18062012

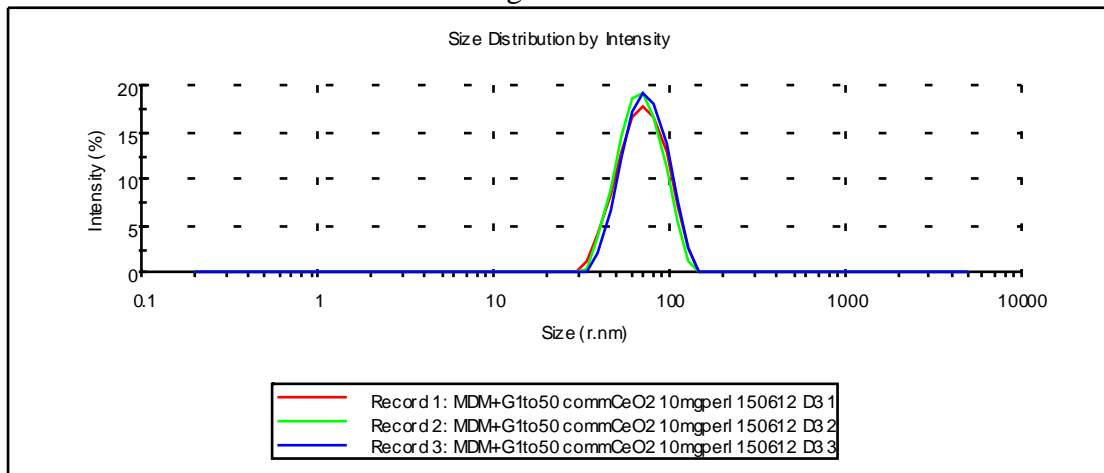
## 3.62 Commercial ceria in 1:4 MDM+g D3 180612



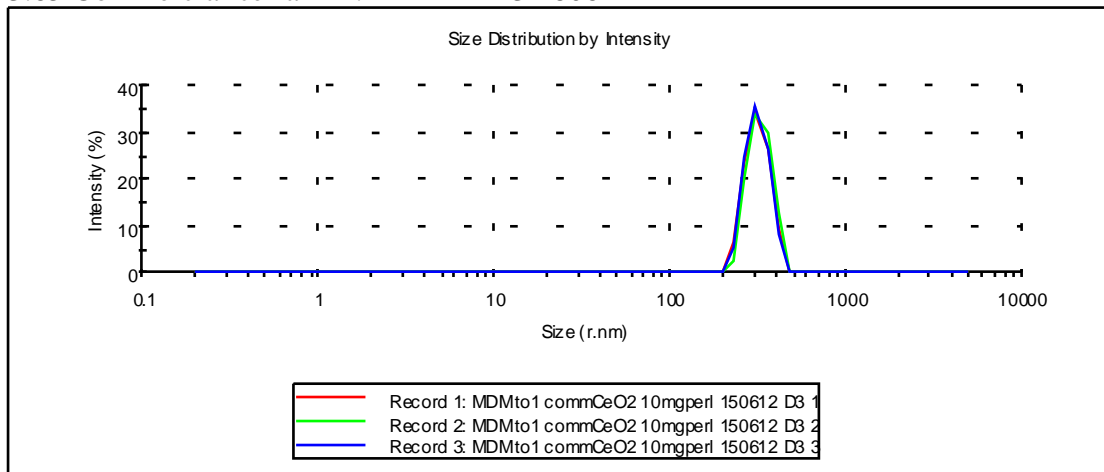
## 3.63 Commercial ceria in 1:10 MDM+g D3 180612



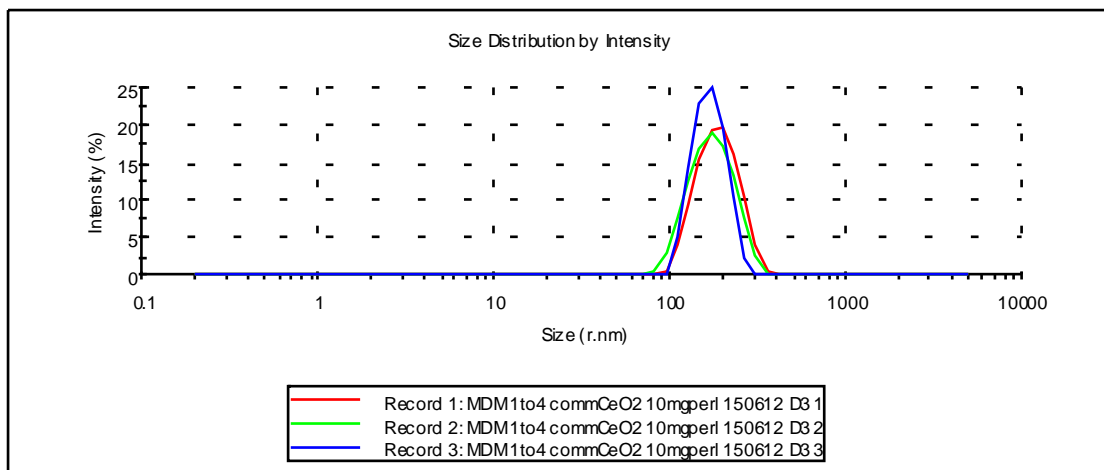
## 3.64 Commercial ceria in 1:50 MDM+g D3 180612



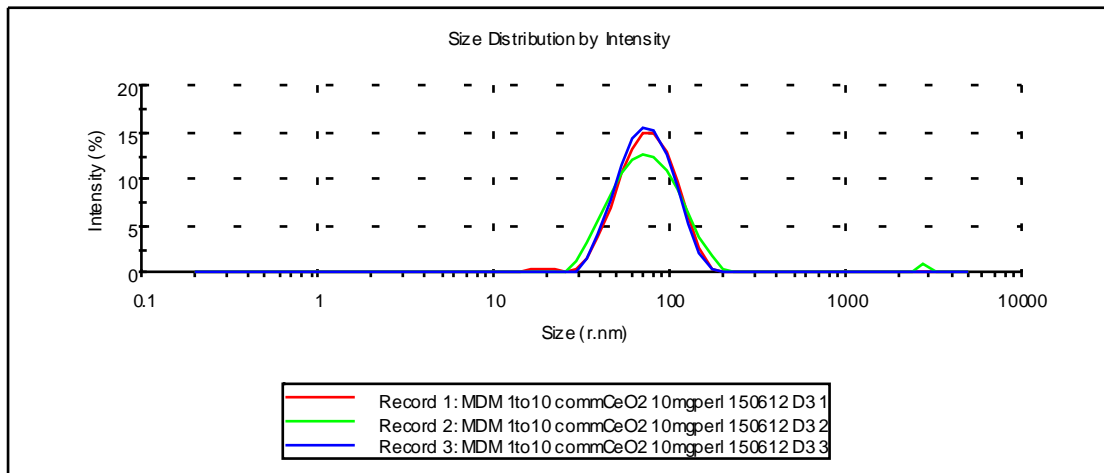
## 3.65 Commercial ceria in 1:1 MDM D3 180612



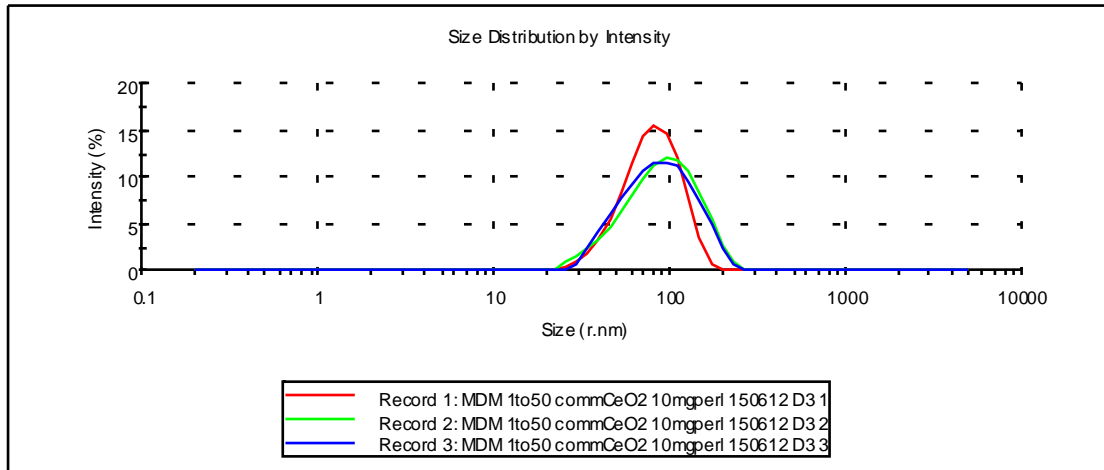
## 3.66 Commercial ceria in 1:4 MDM D3 180612



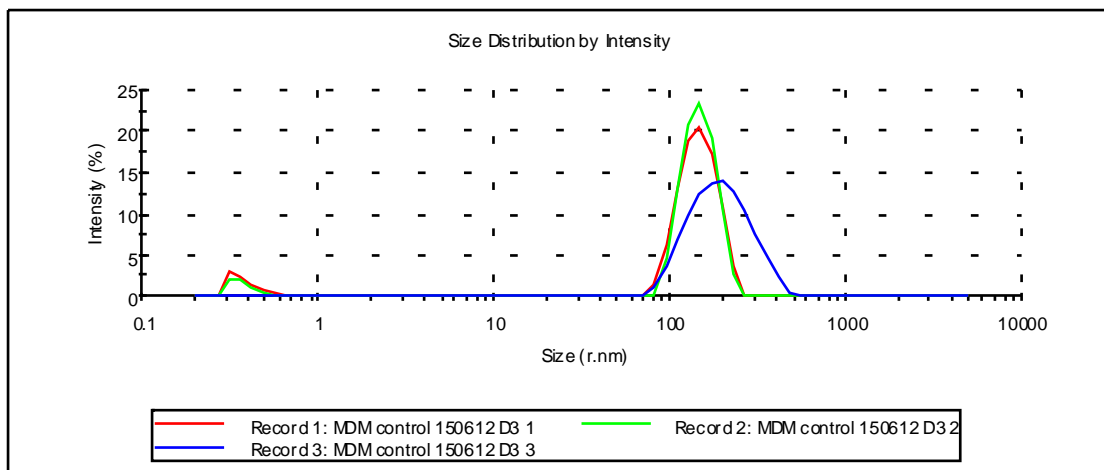
## 3.67 Commercial ceria in 1:10 MDM D3 180612



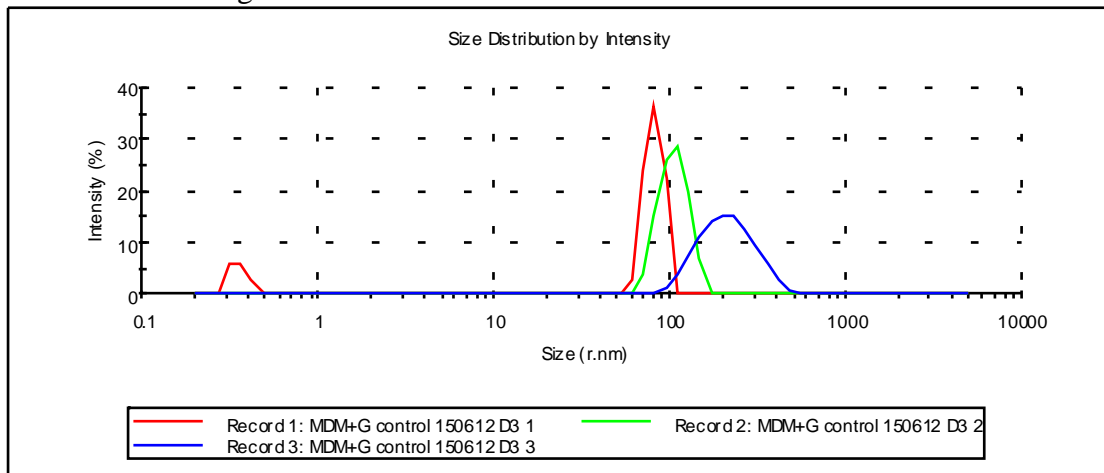
## 3.68 Commercial ceria in 1:50 MDM D3 180612



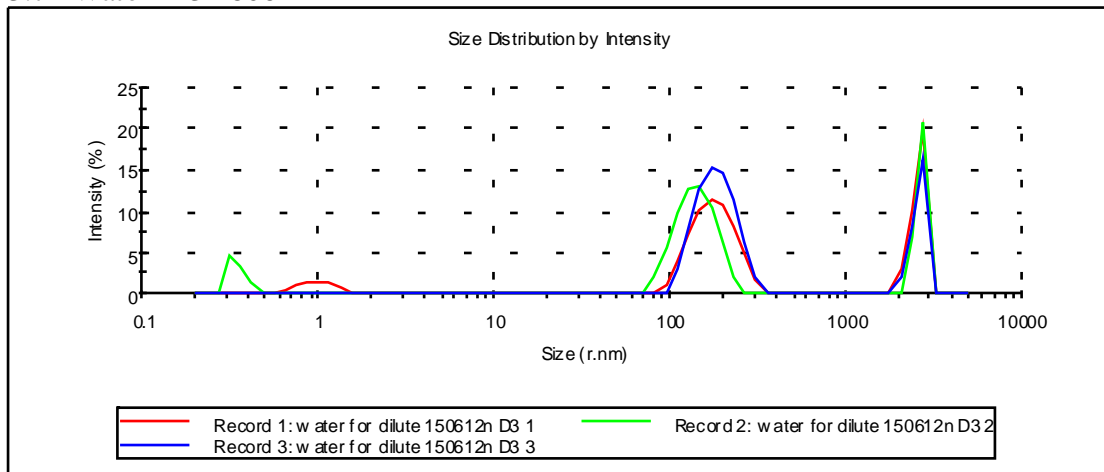
## 3.69 MDM D3 180612



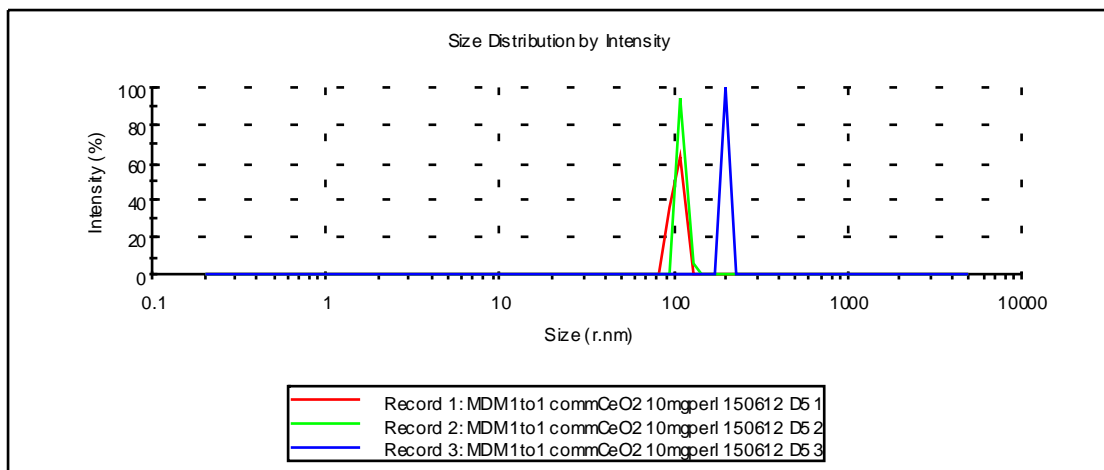
## 3.70 1:1 MDM + g D3 180612



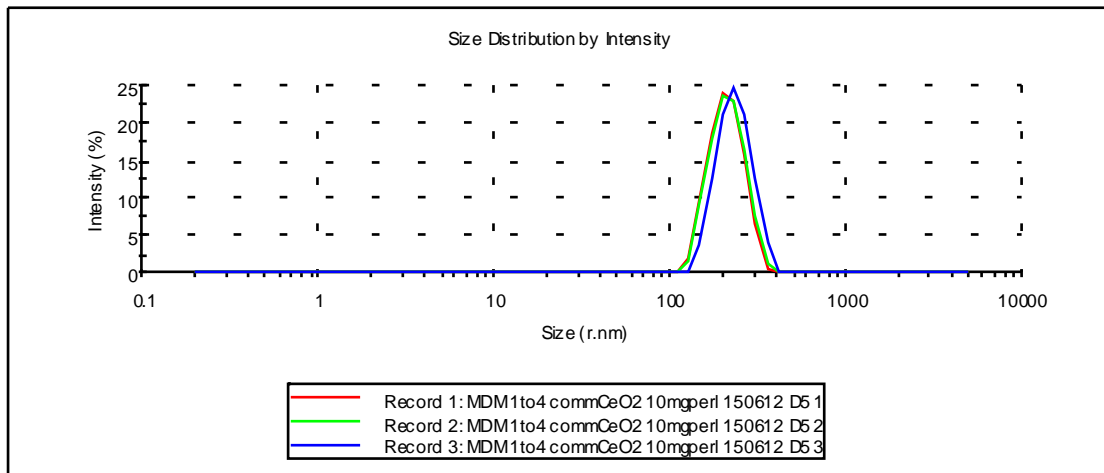
## 3.71 Water D3 180612



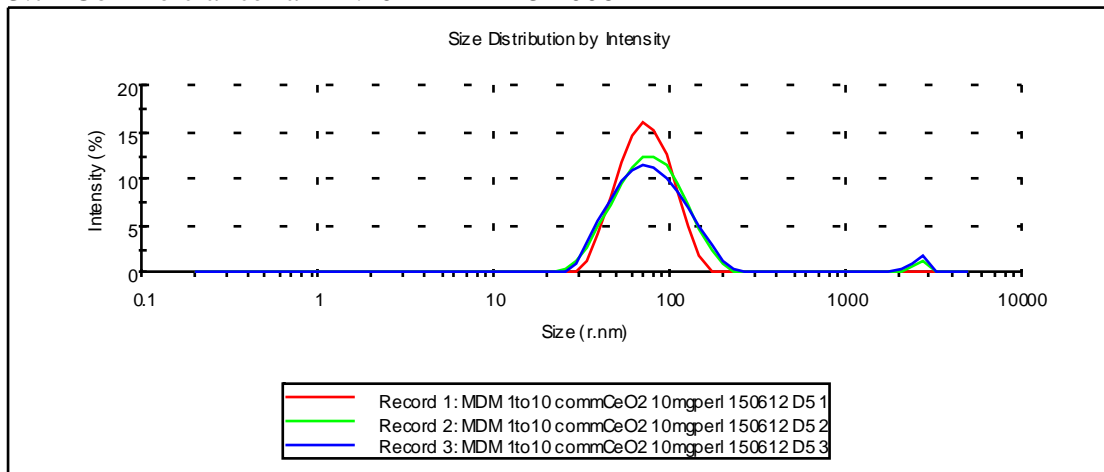
## 3.72 Commercial ceria in 1:1 MDM D5 200612



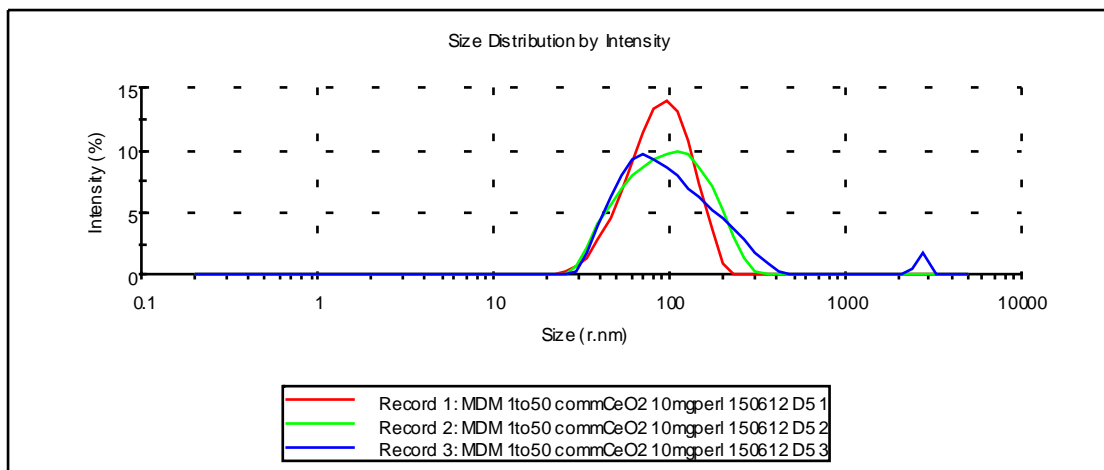
## 3.73 Commercial ceria in 1:4 MDM D5 200612



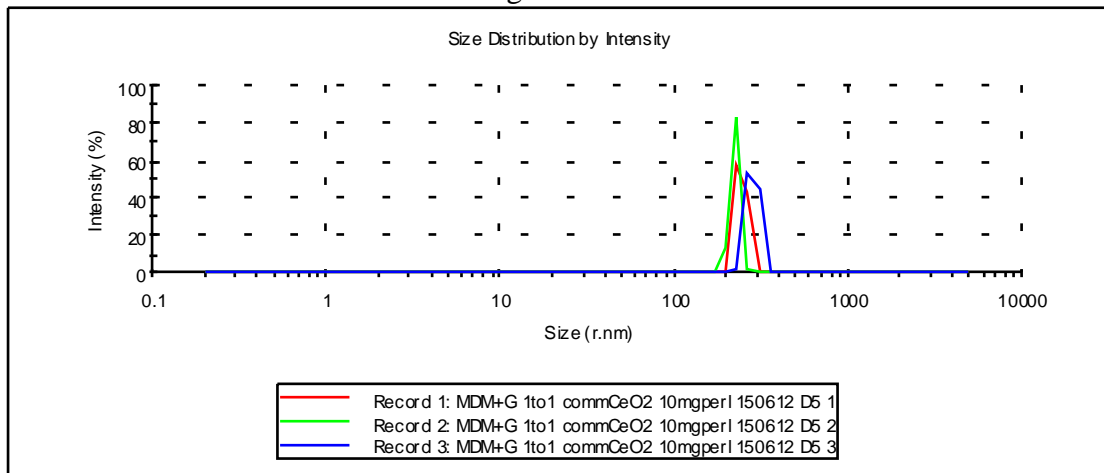
## 3.74 Commercial ceria in 1:10 MDM D5 200612



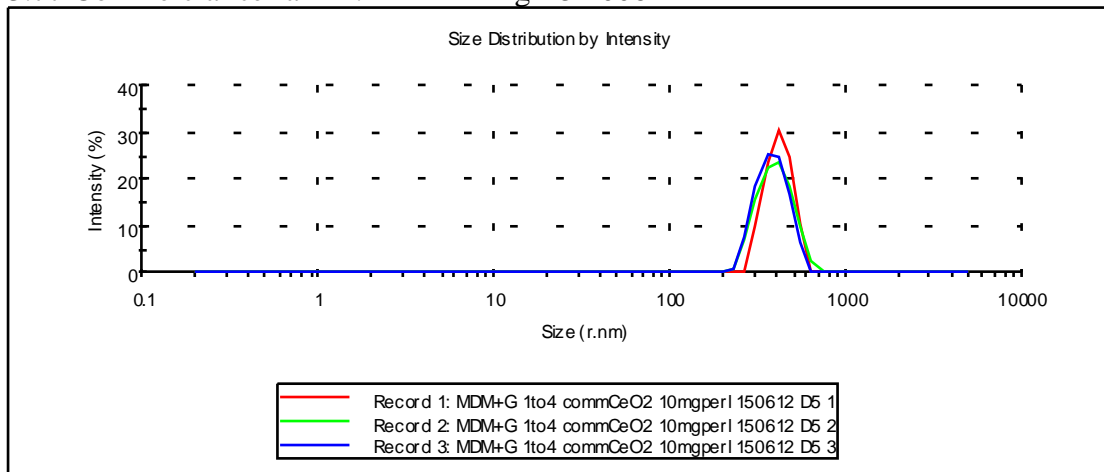
## 3.75 Commercial ceria in 1:50 MDM D5 200612



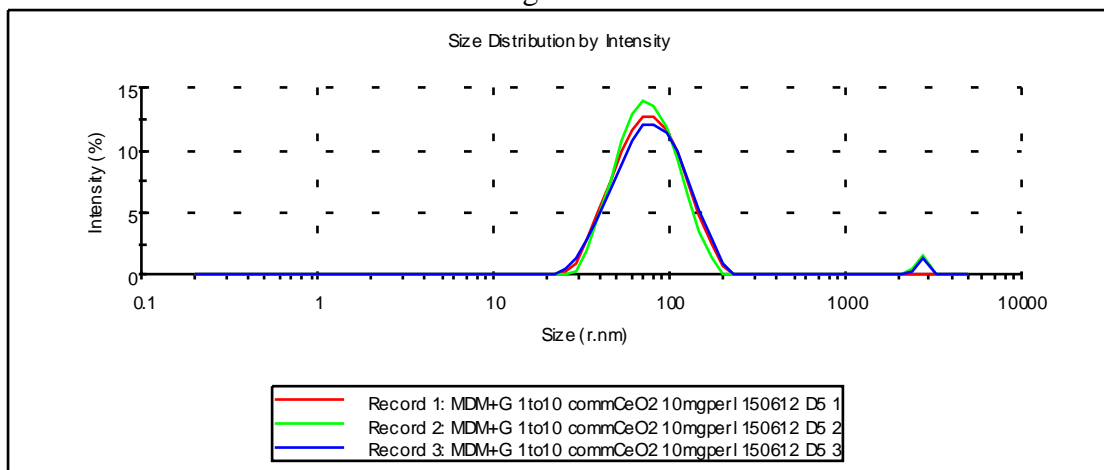
## 3.76 Commercial ceria in 1:1 MDM + g D5 200612



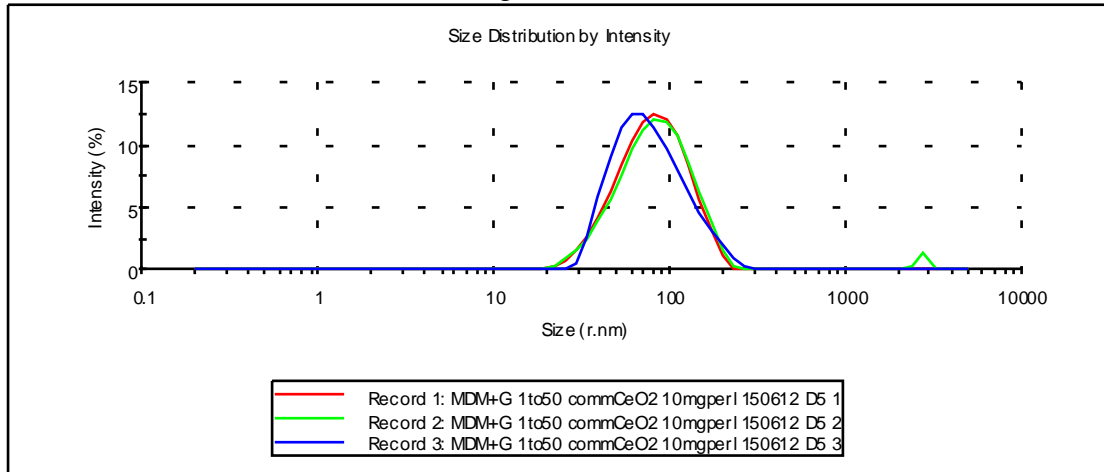
## 3.77 Commercial ceria in 1:4 MDM + g D5 200612



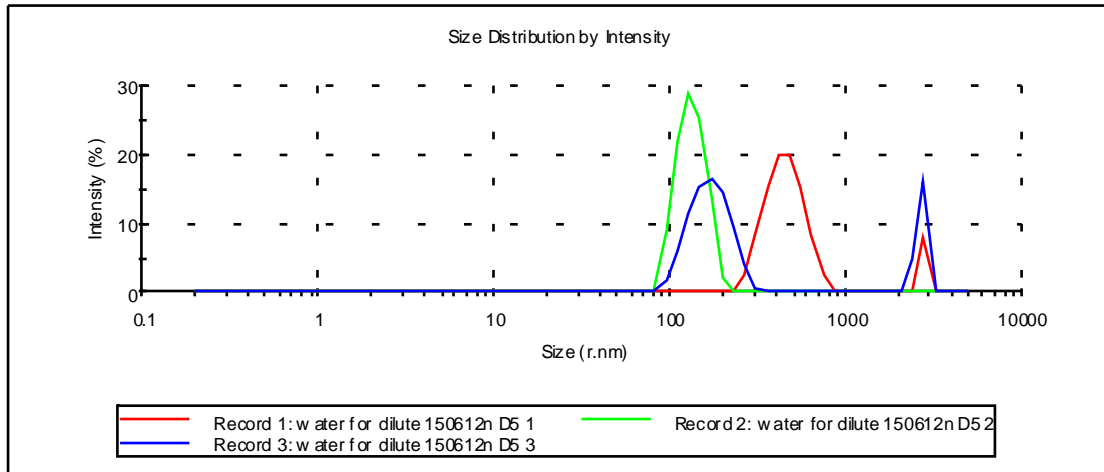
## 3.78 Commercial ceria in 1:10 MDM + g D5 200612



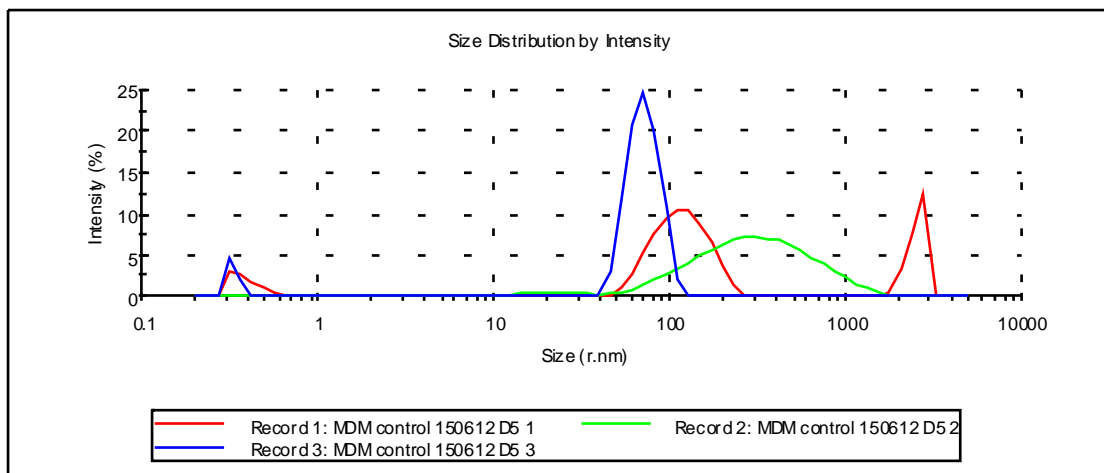
3.79 Commercial ceria in 1:1 MDM +g D5 200612



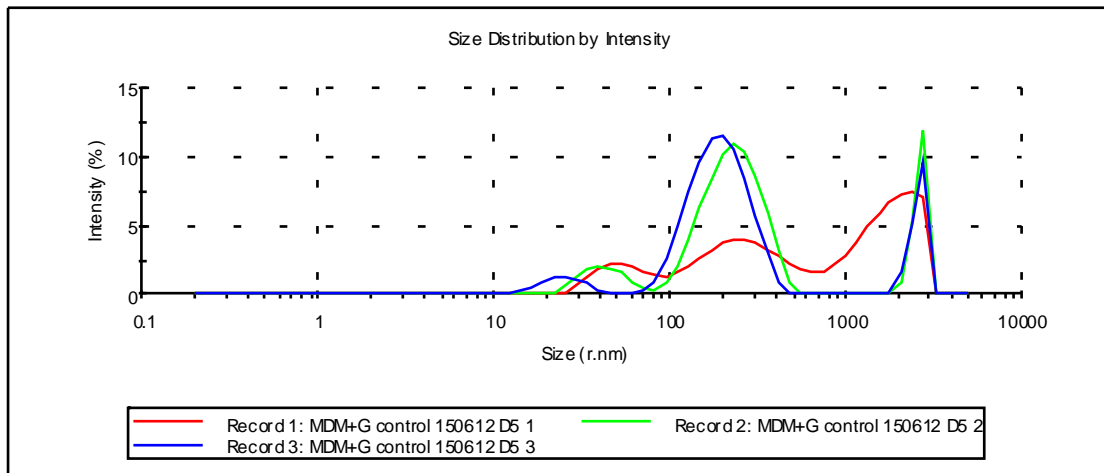
3.80 Water D5 200612



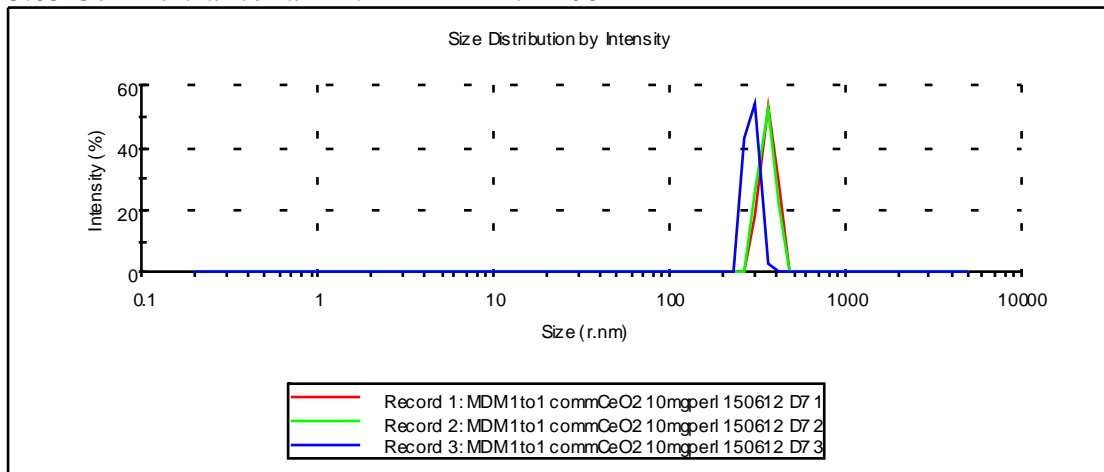
3.81 MDM D5 200612



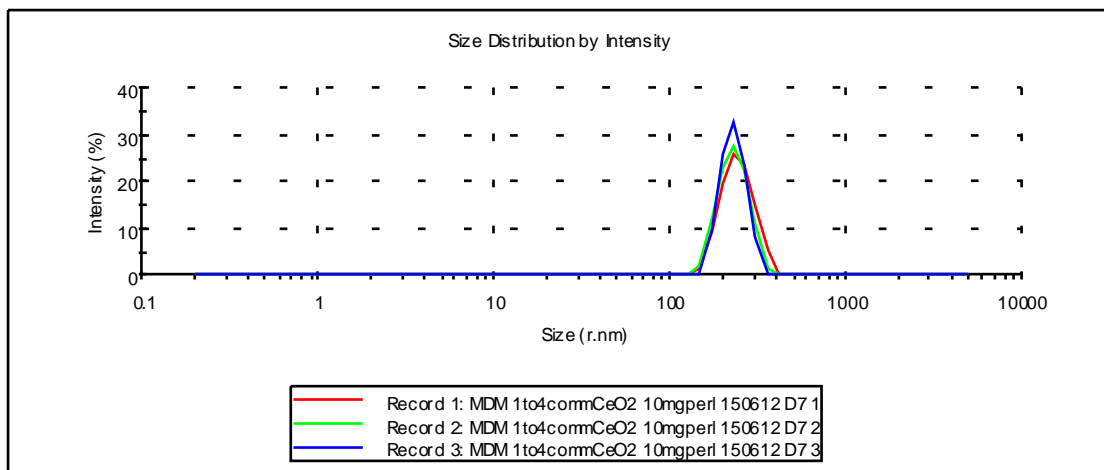
## 3.82 MDM D5 200612



## 3.83 Commercial ceria in 1:1 MDM D7 220612

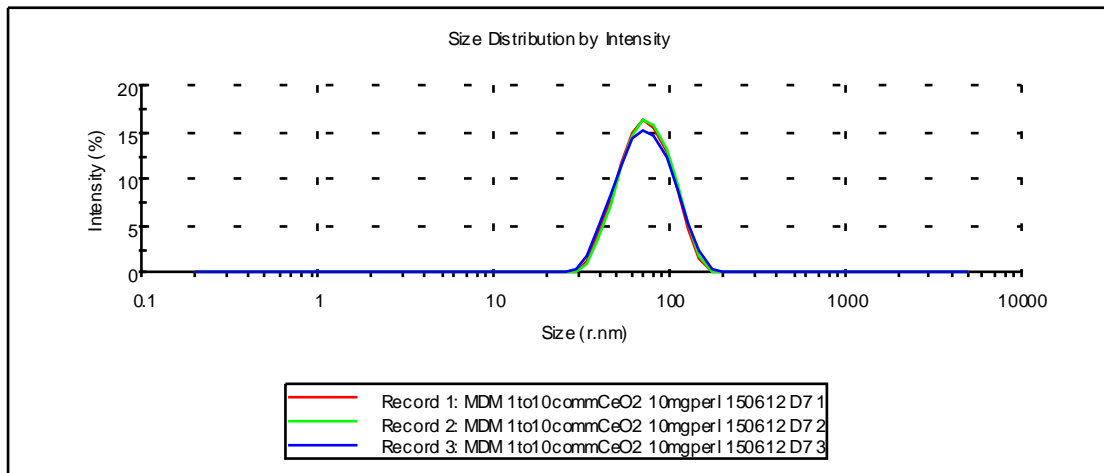


## 3.84 Commercial ceria in 1:4 MDM D7 220612

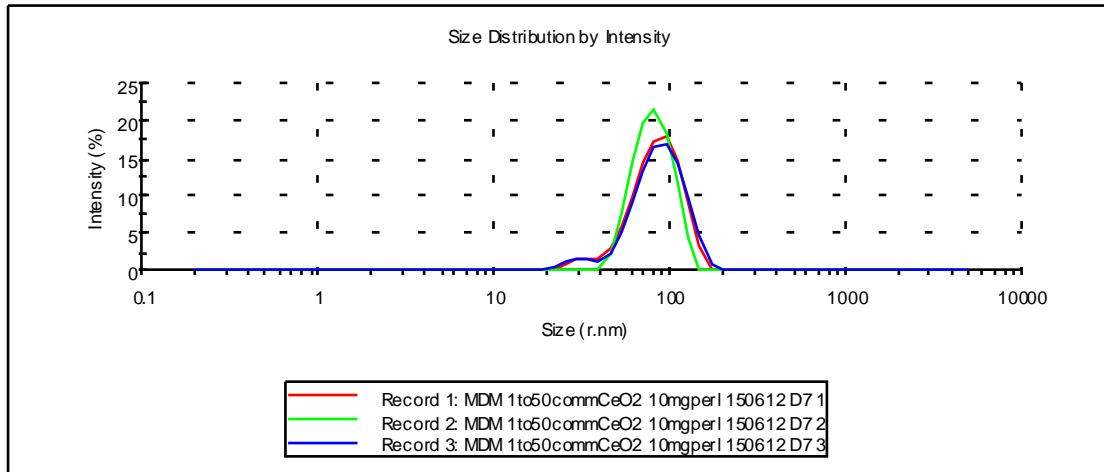




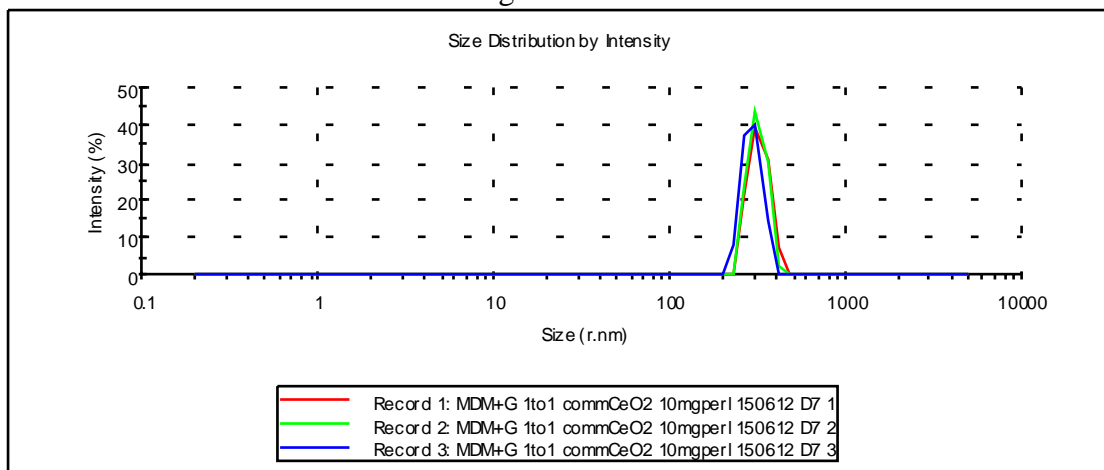
## 3.85 Commercial ceria in 1:10 MDM D7 220612



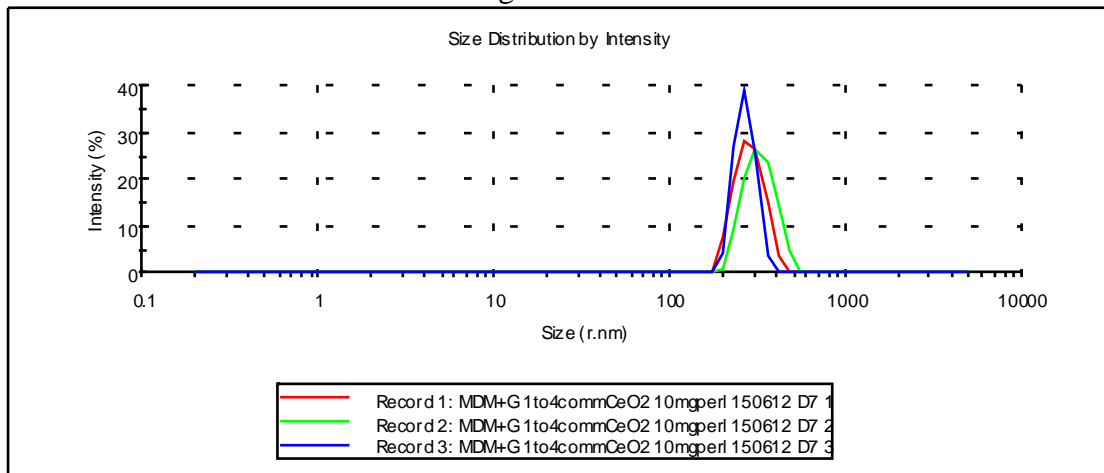
## 3.86 Commercial ceria in 1:50 MDM D7 220612



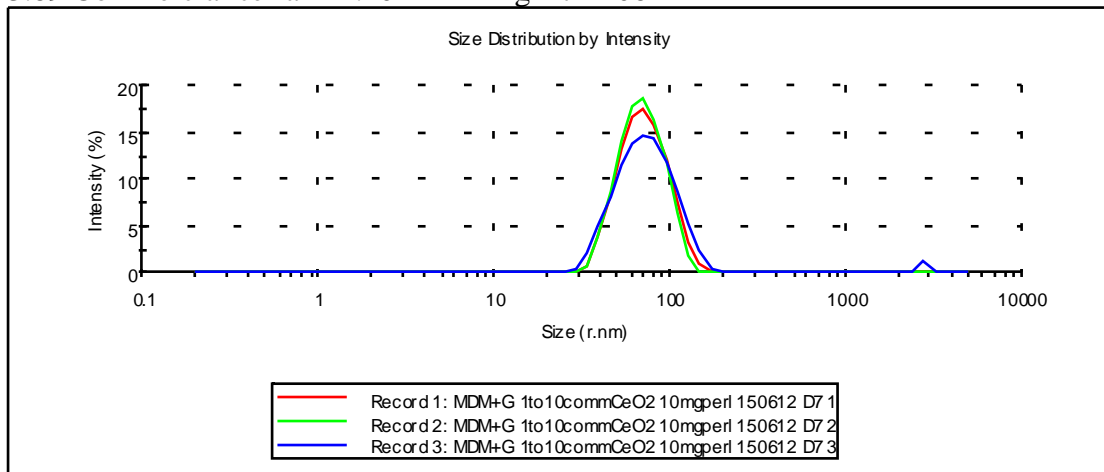
## 3.87 Commercial ceria in 1:1 MDM +g D7 220612



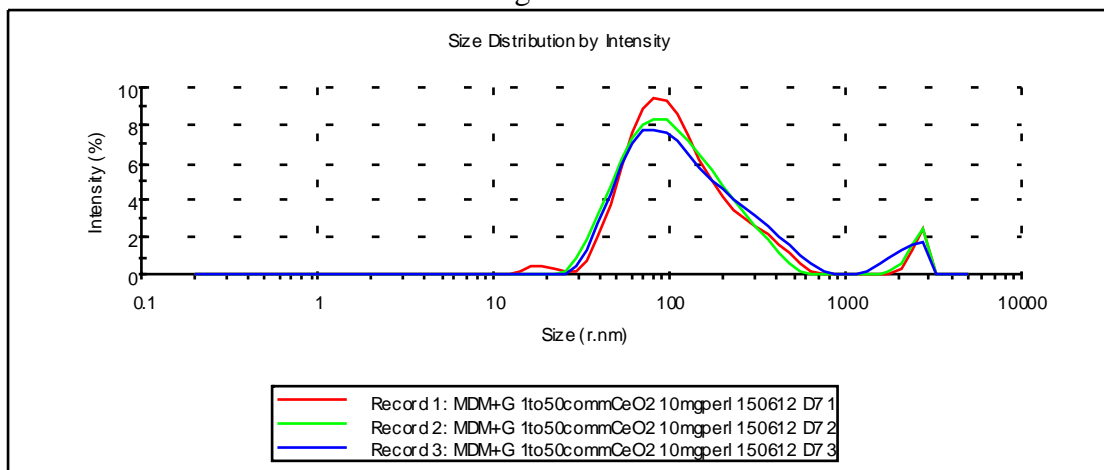
## 3.88 Commercial ceria in 1:4 MDM +g D7 220612



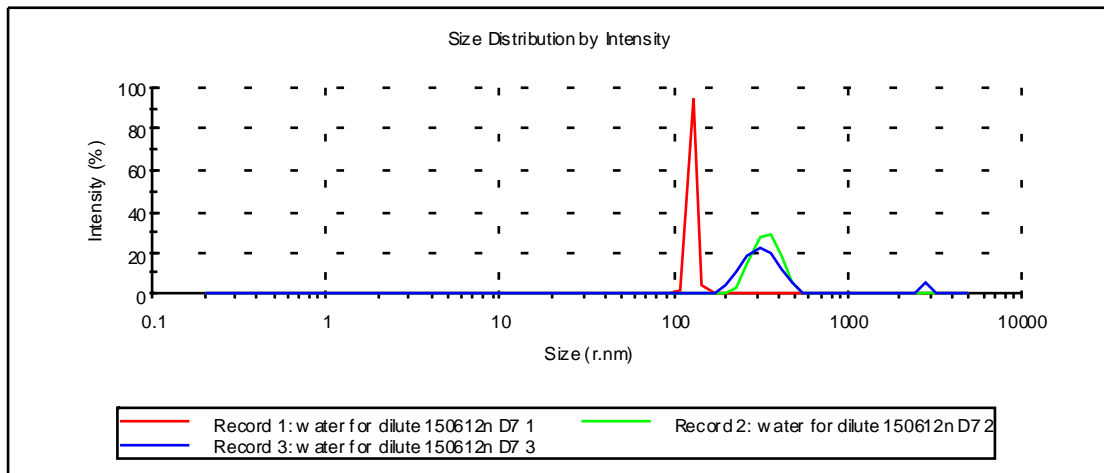
## 3.89 Commercial ceria in 1:10 MDM +g D7 220612



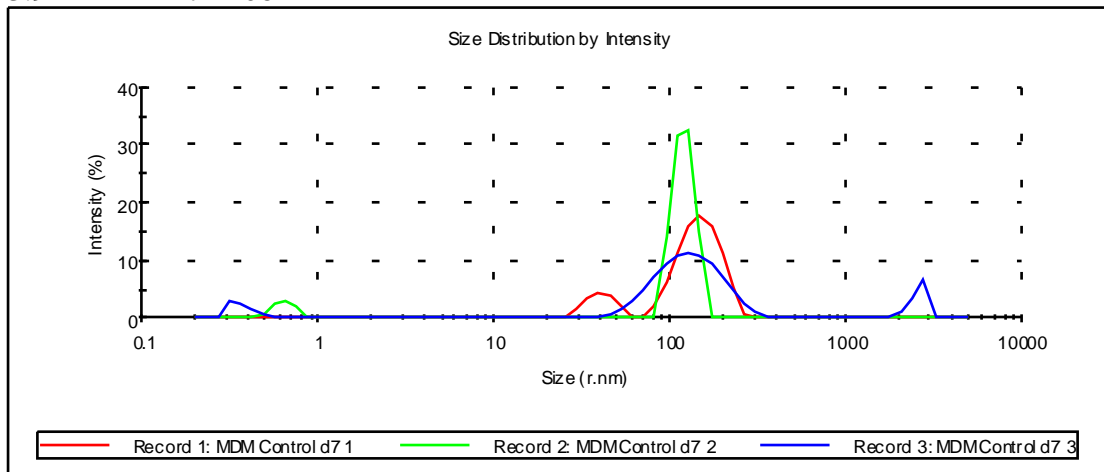
## 3.90 Commercial ceria in 1:50 MDM +g D7 220612



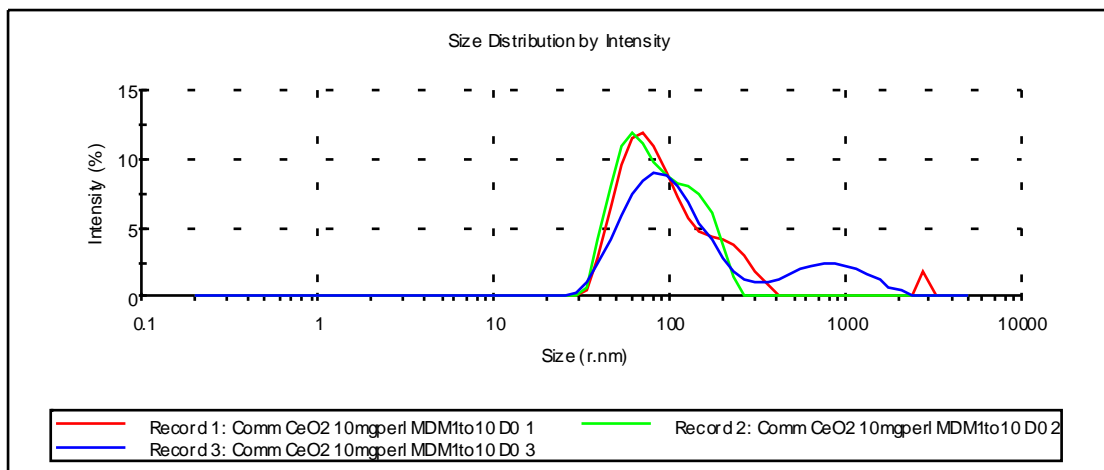
## 3.91 Water D7 220612

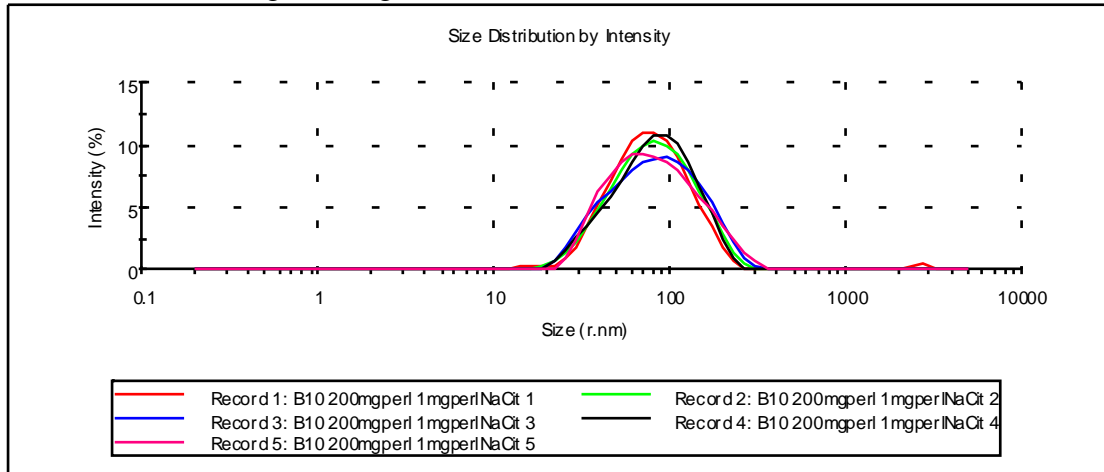
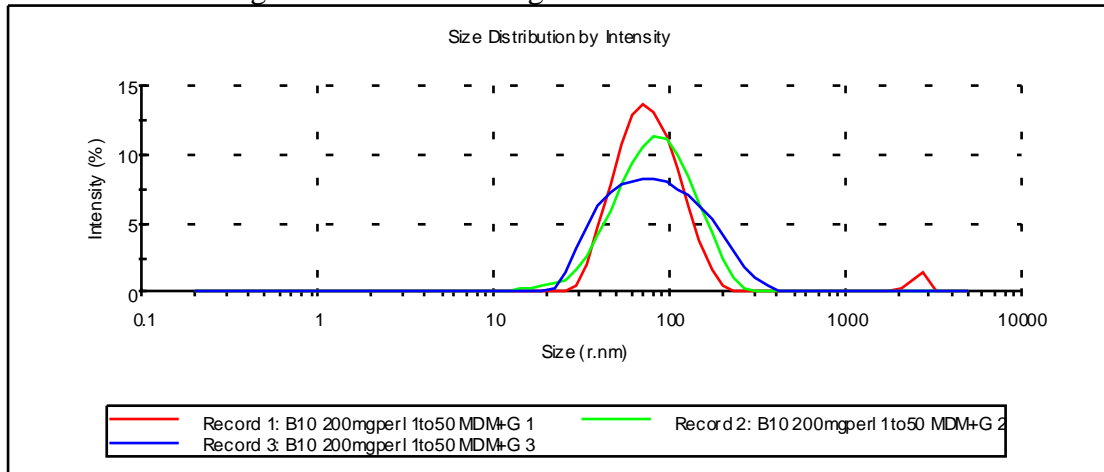
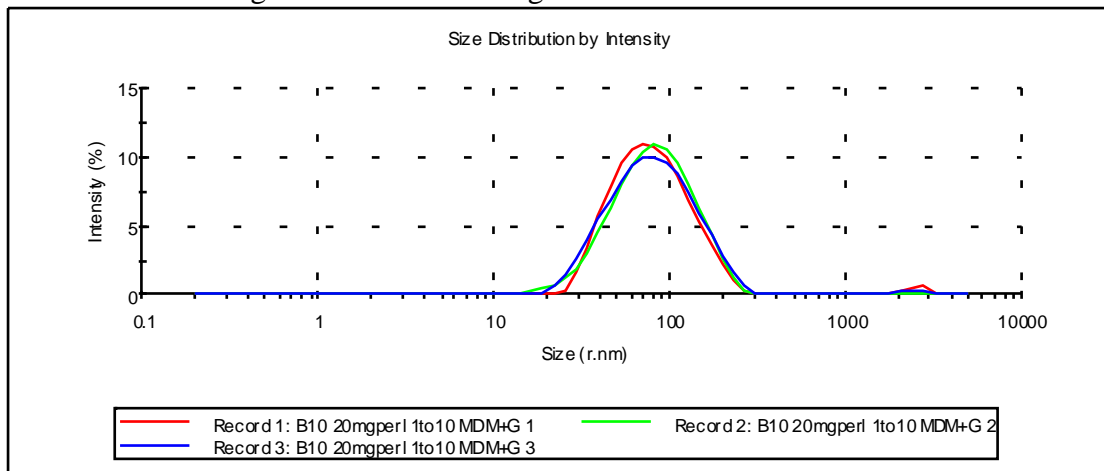


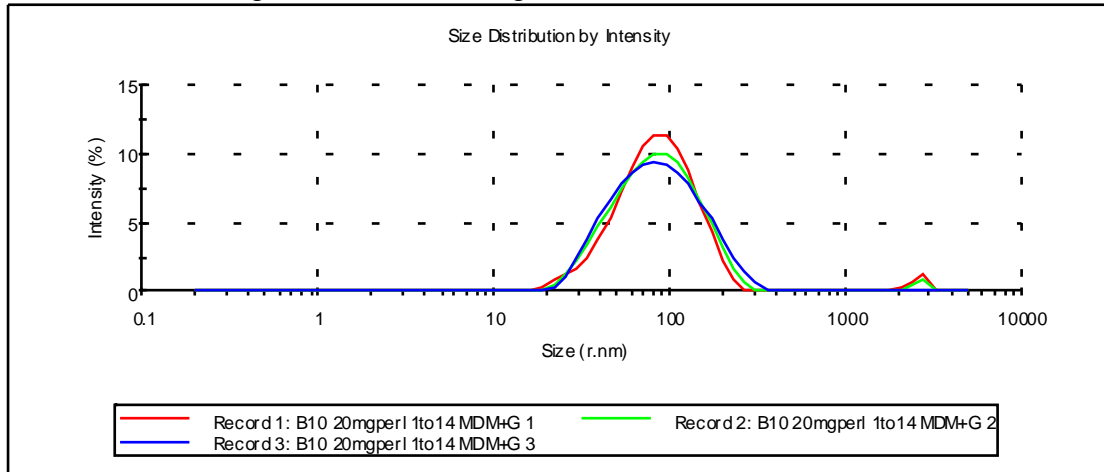
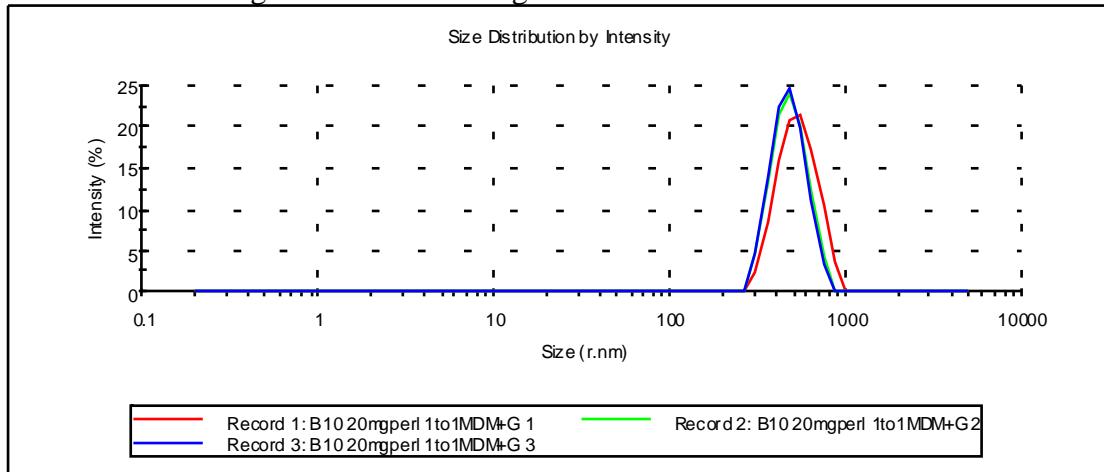
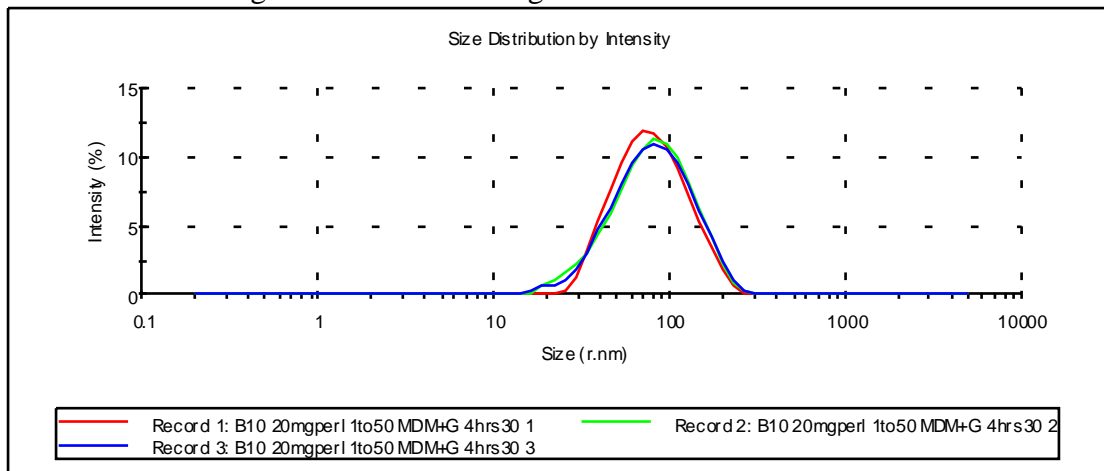
## 3.92 MDM D7 220612

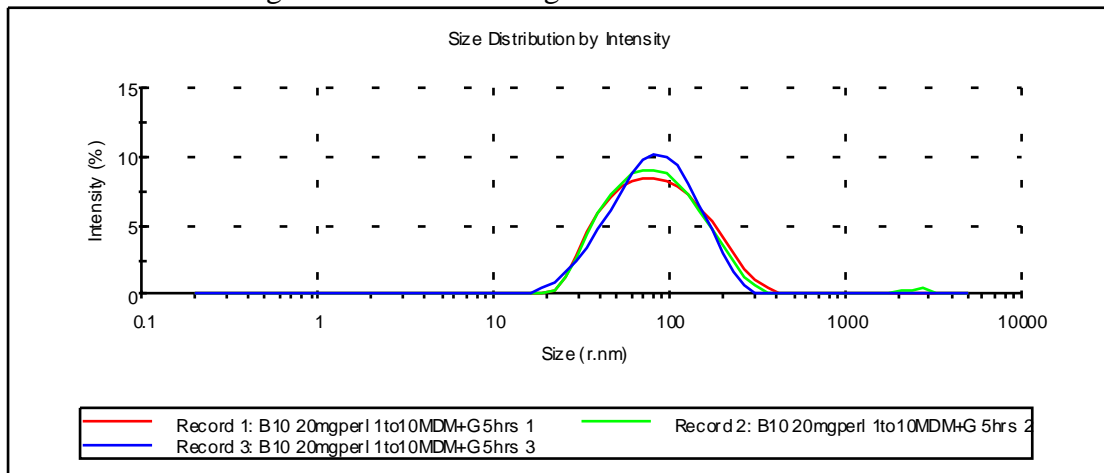
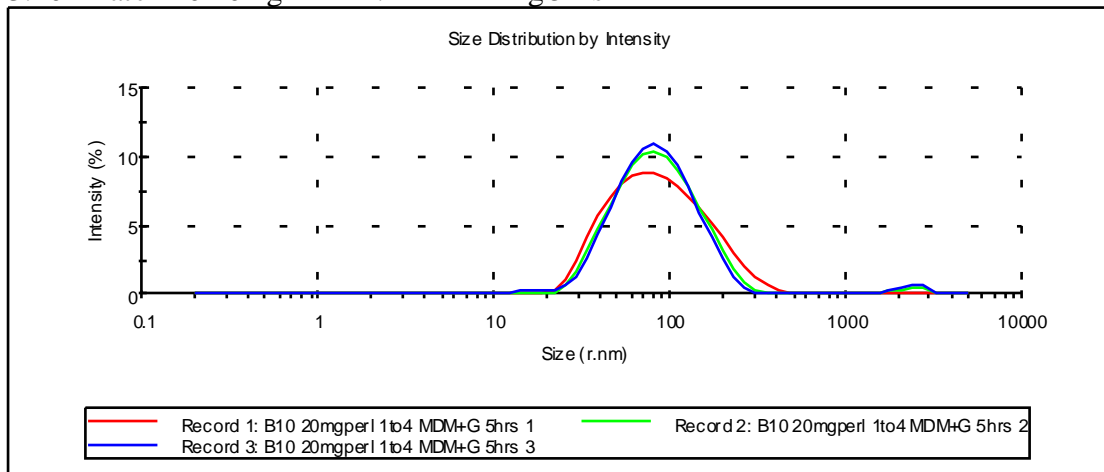
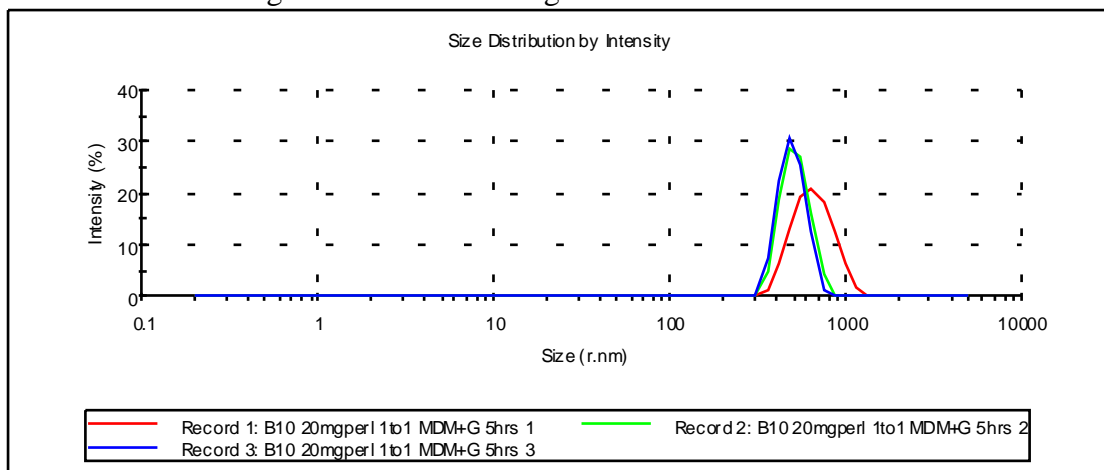


## 3.93 Commercial ceria in 1:10 MDM D7 050712

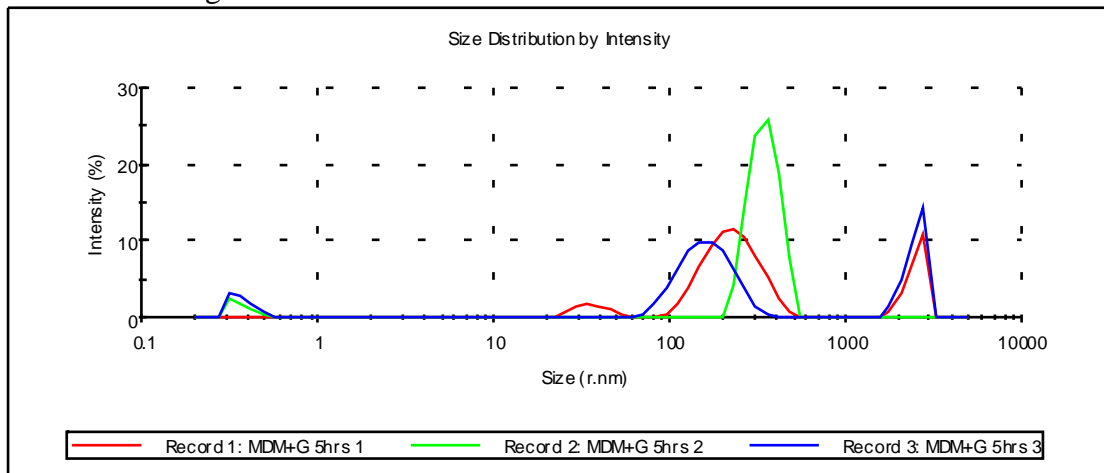
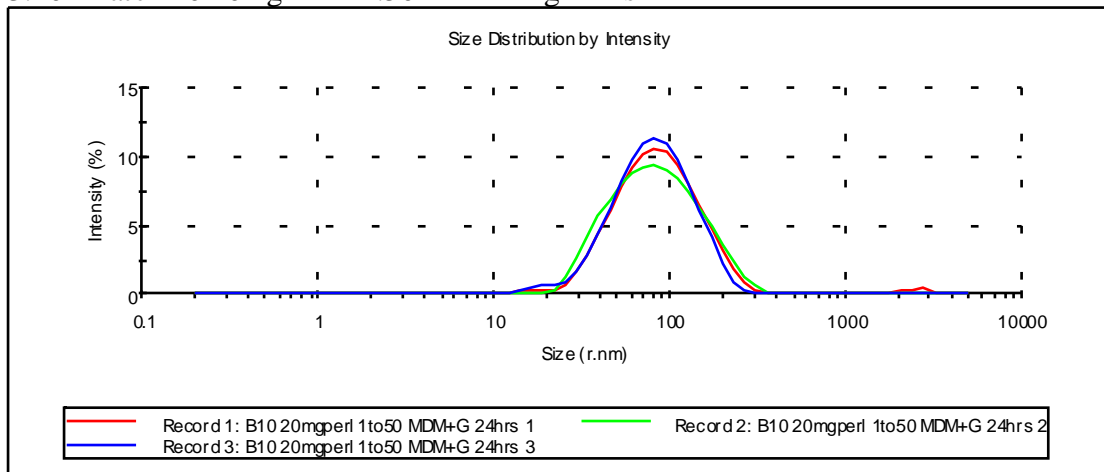
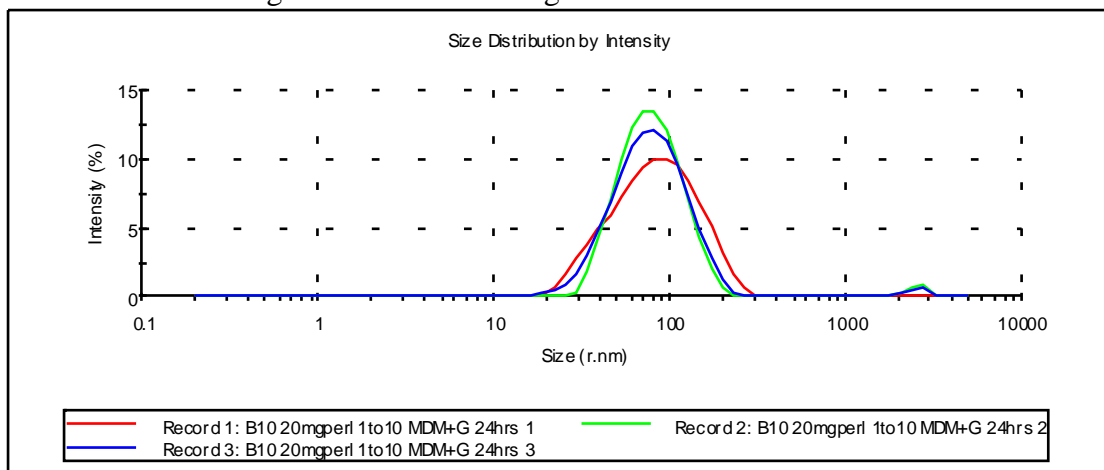


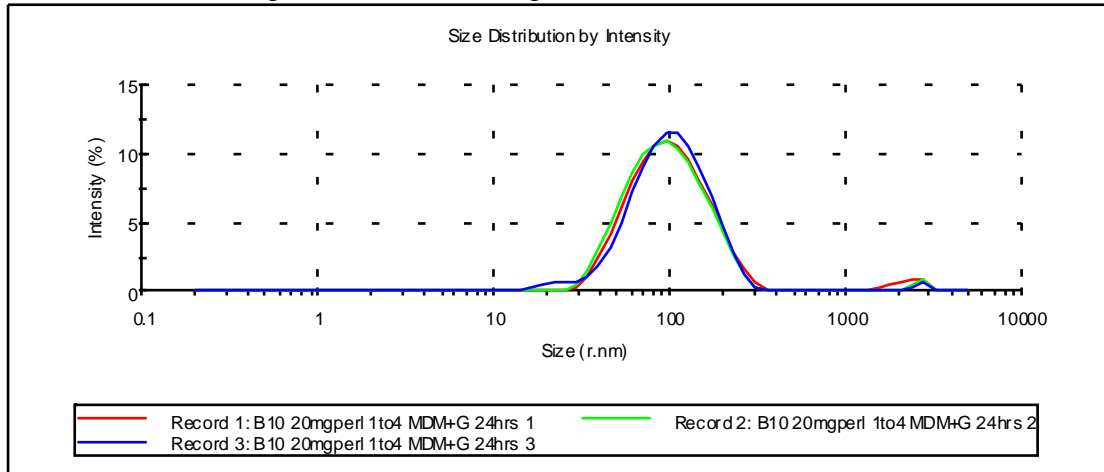
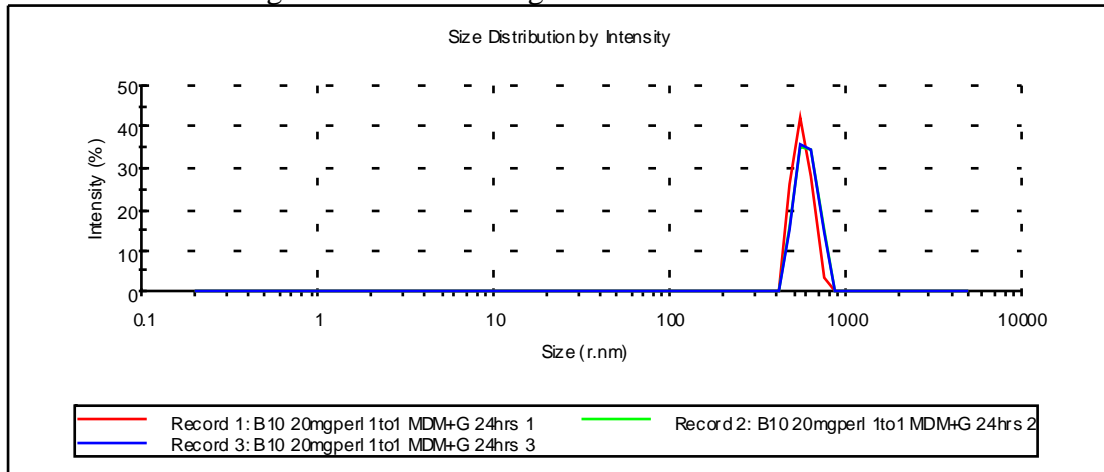
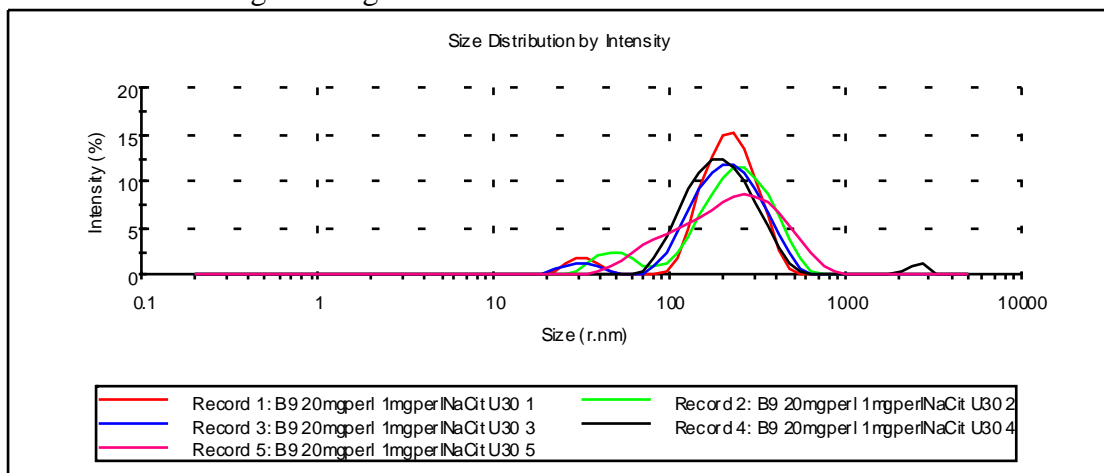
3.94 Batch 10  $200\text{mgL}^{-1}$  in  $1\text{g L}^{-1}$  sodium citrate3.95 Batch 10  $20\text{mgL}^{-1}$  in 1:50 MDM + g 1.5hrs3.96 Batch 10  $20\text{mgL}^{-1}$  in 1:10 MDM + g 1.5hrs

3.97 Batch 10 20mgL<sup>-1</sup> in 1:4 MDM + g 1.5hrs3.98 Batch 10 20mgL<sup>-1</sup> in 1:1 MDM + g 1.5hrs3.99 Batch 10 20mgL<sup>-1</sup> in 1:50 MDM + g 5hrs

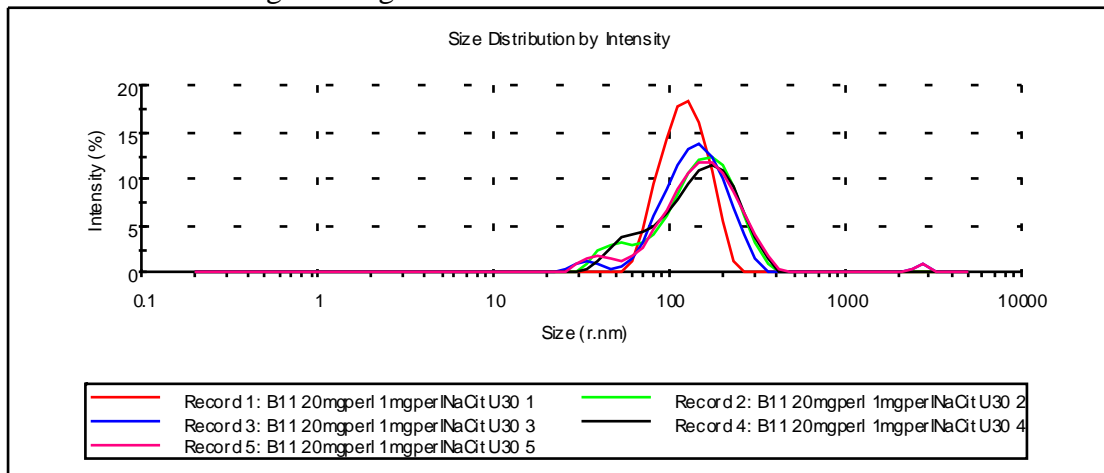
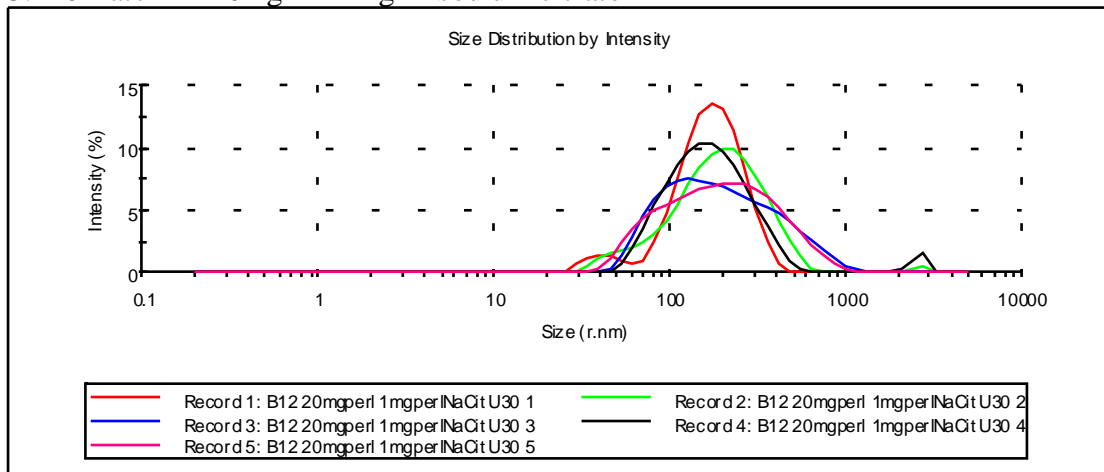
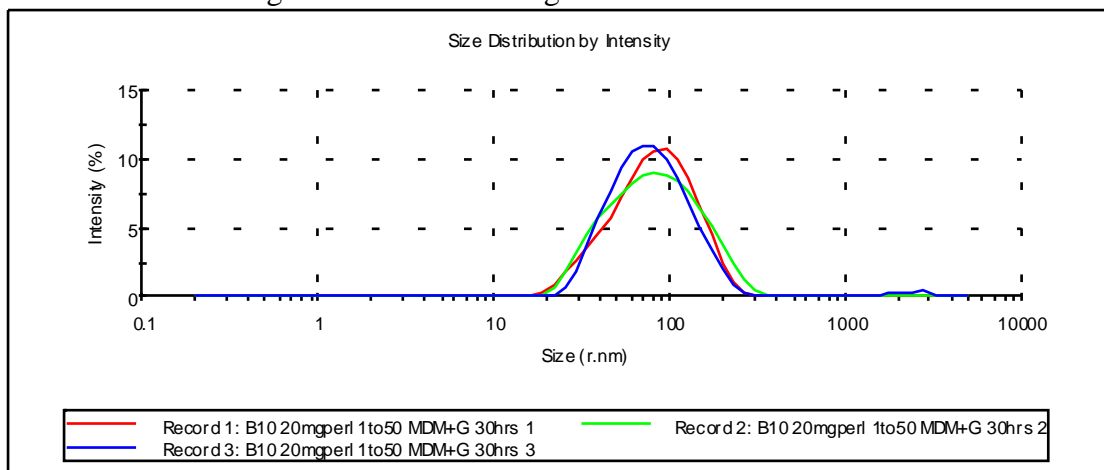
3.100 Batch 10 20mgL<sup>-1</sup> in 1:10 MDM + g 5hrs3.101 Batch 10 20mgL<sup>-1</sup> in 1:4 MDM + g 5hrs3.102 Batch 10 20mgL<sup>-1</sup> in 1:10 MDM + g 5hrs

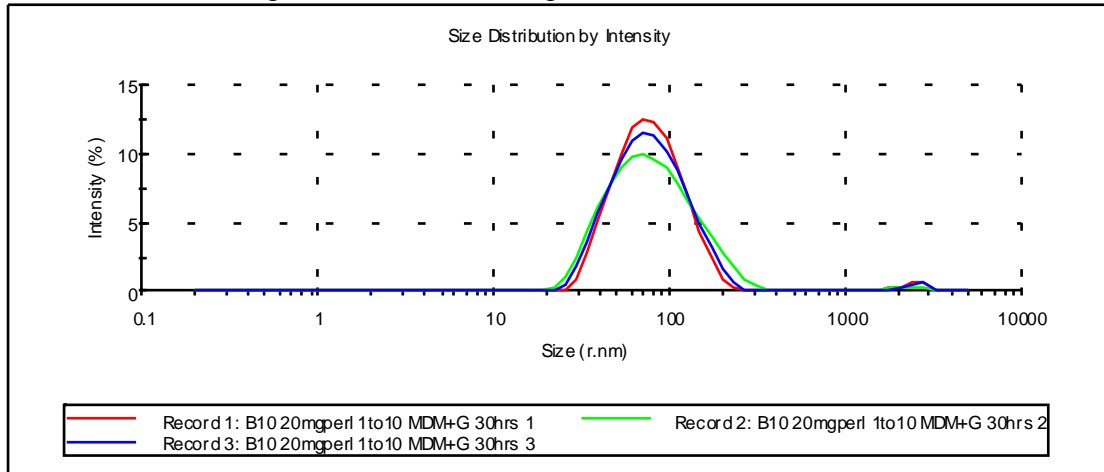
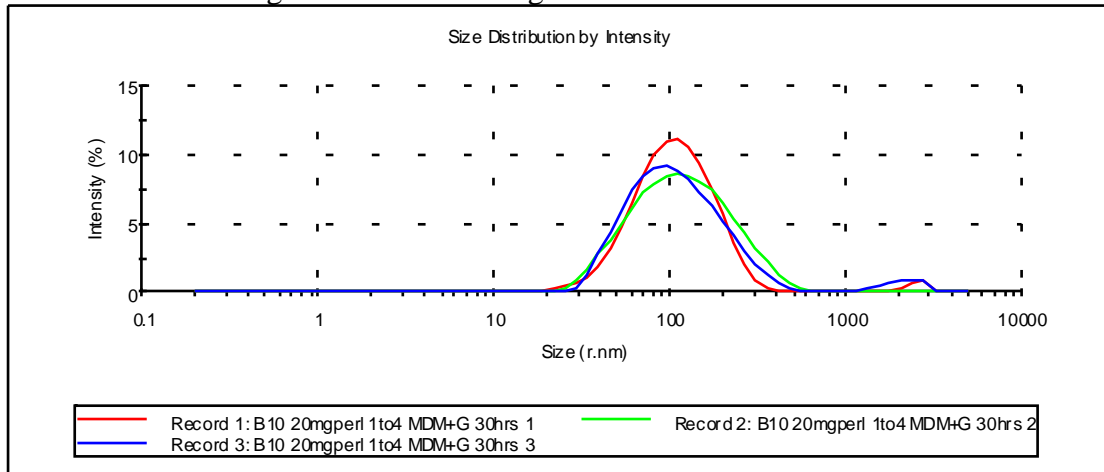
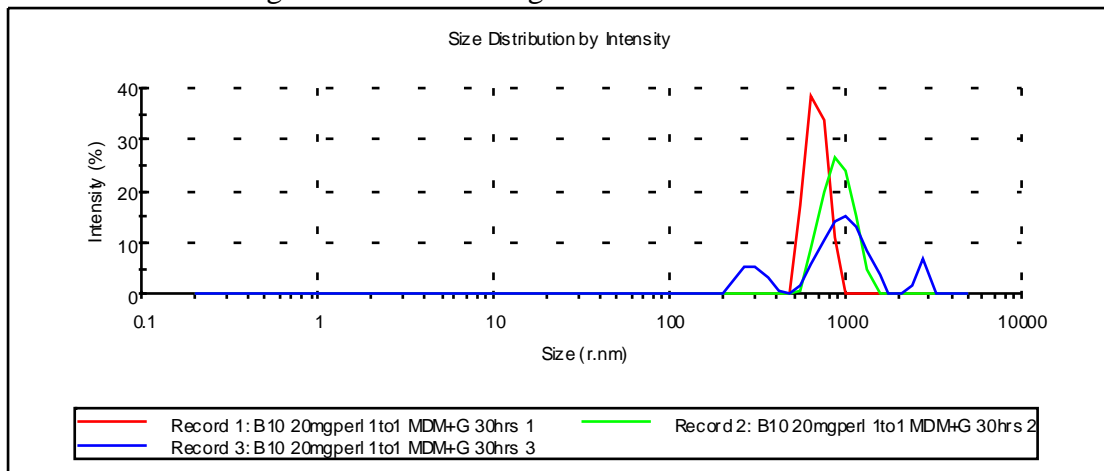
## 3.103 MDM + g 5hrs

3.104 Batch 10 20mgL<sup>-1</sup> in 1:50 MDM + g 24hrs3.105 Batch 10 20mgL<sup>-1</sup> in 1:10 MDM + g 24hrs

3.106 Batch 10 20mgL<sup>-1</sup> in 1:4 MDM + g 24hrs3.107 Batch 10 20mgL<sup>-1</sup> in 1:1 MDM + g 24hrs3.108 Batch 9 20mgL<sup>-1</sup> in 1gL<sup>-1</sup> sodium citrate



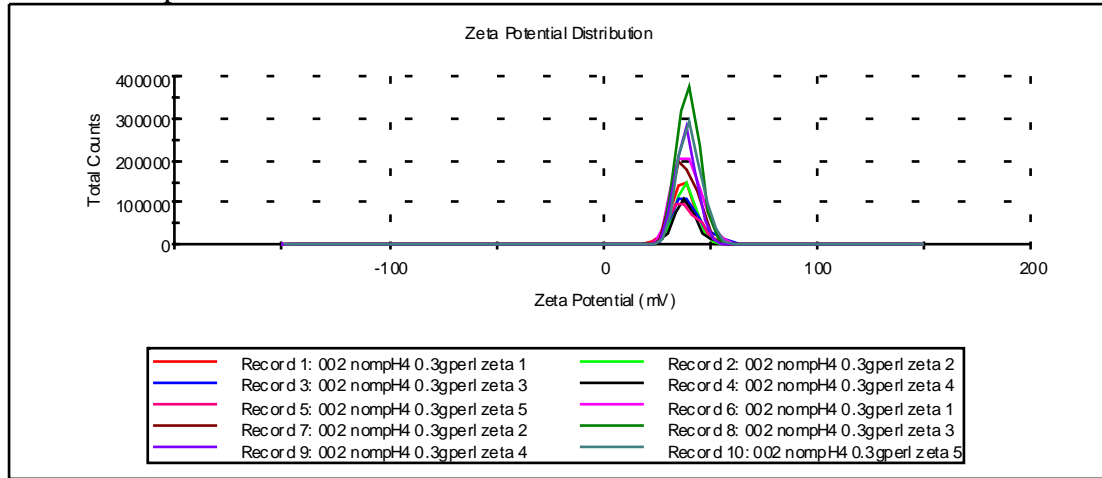
3.109 Batch 11  $20\text{mgL}^{-1}$  in  $1\text{gL}^{-1}$  sodium citrate3.110 Batch 12  $20\text{mgL}^{-1}$  in  $1\text{gL}^{-1}$  sodium citrate3.111 Batch 10  $20\text{mgL}^{-1}$  in 1:50 MDM + g 30hrs

3.112 Batch 10 20mgL<sup>-1</sup> in 1:10 MDM + g 30hrs3.113 Batch 10 20mgL<sup>-1</sup> in 1:4 MDM + g 30hrs3.114 Batch 10 20mgL<sup>-1</sup> in 1:1 MDM + g 30hrs

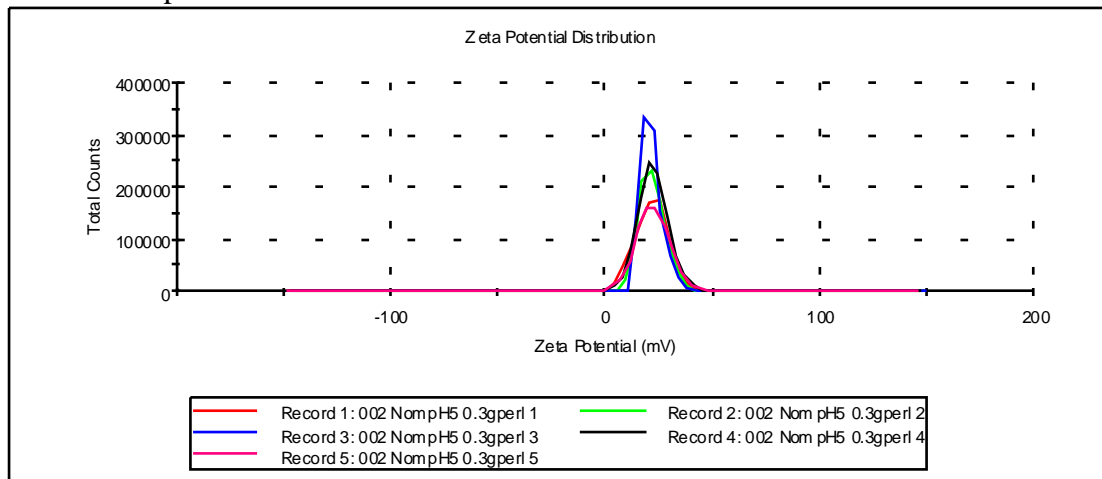
## APPENDIX 4

### Zeta potential measurements

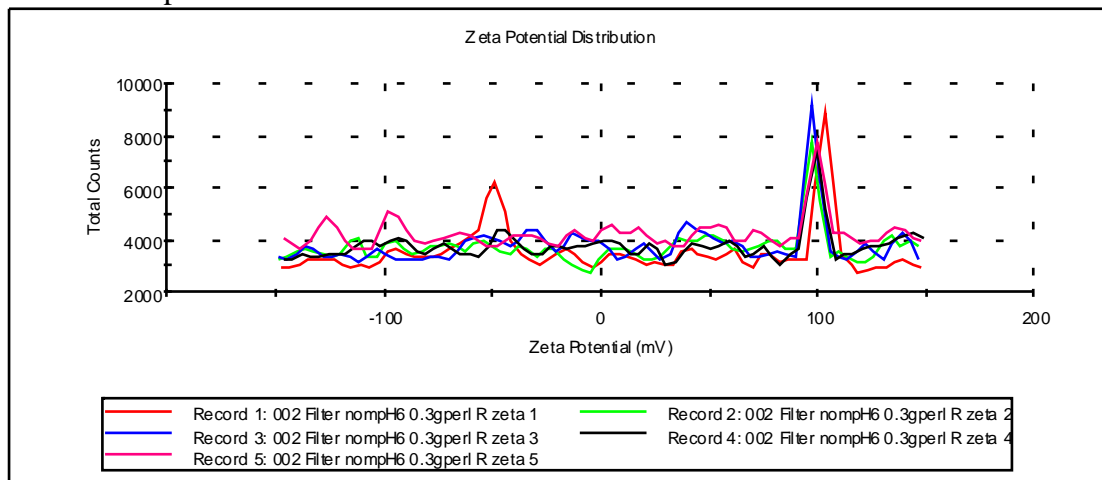
#### 4.1 Batch 2 pH 3.53



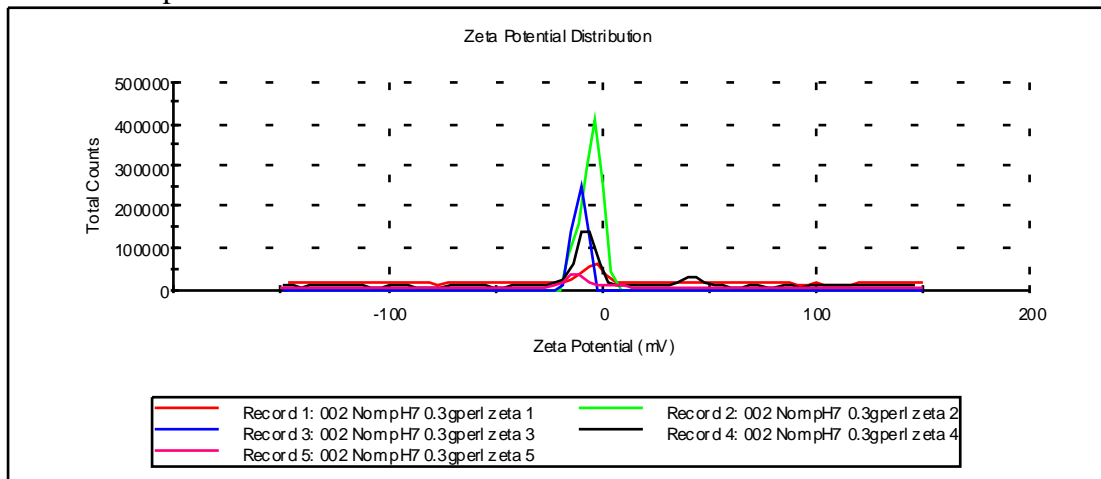
#### 4.2 Batch 2 pH 3.66



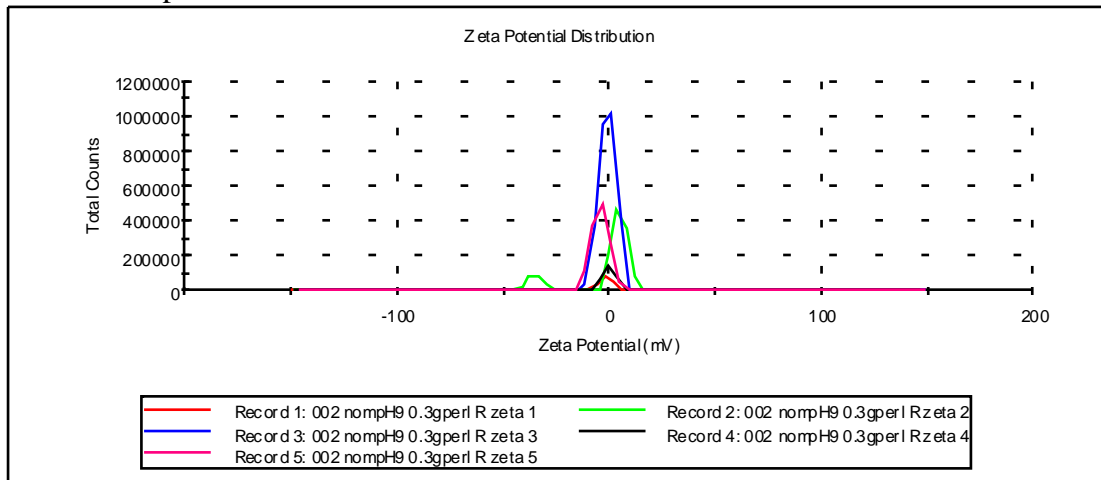
#### 4.3 Batch 2 pH 4.32



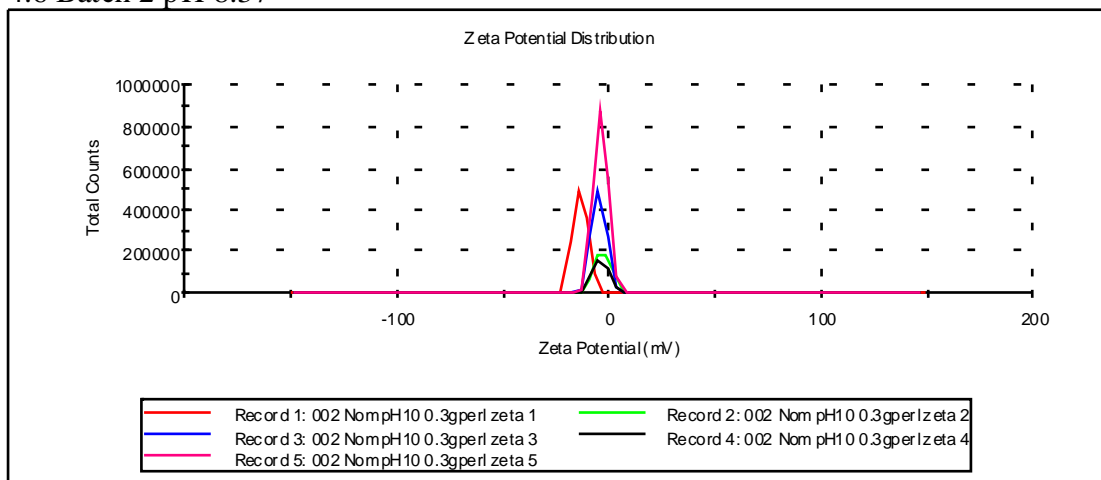
## 4.4 Batch 2 pH 5.00



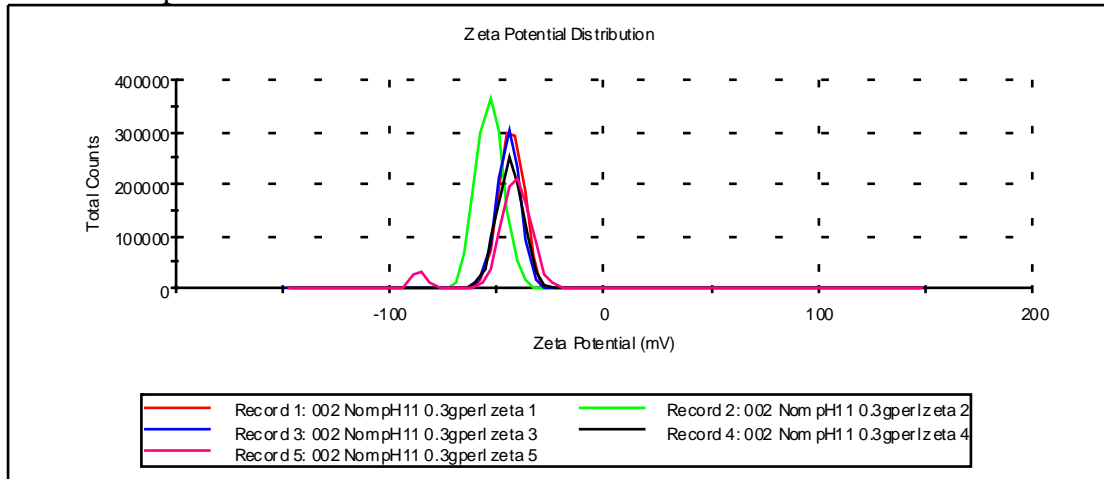
## 4.5 Batch 2 pH 7.43



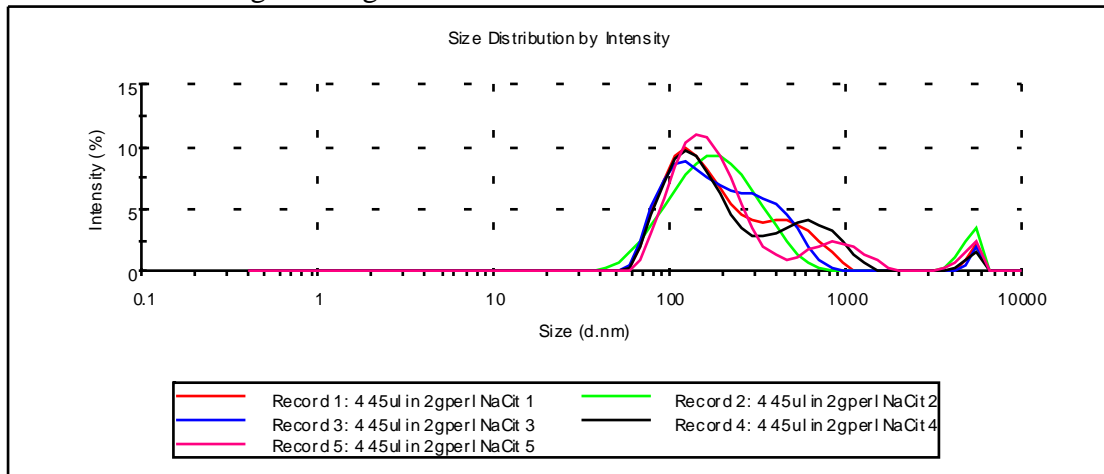
## 4.6 Batch 2 pH 8.37



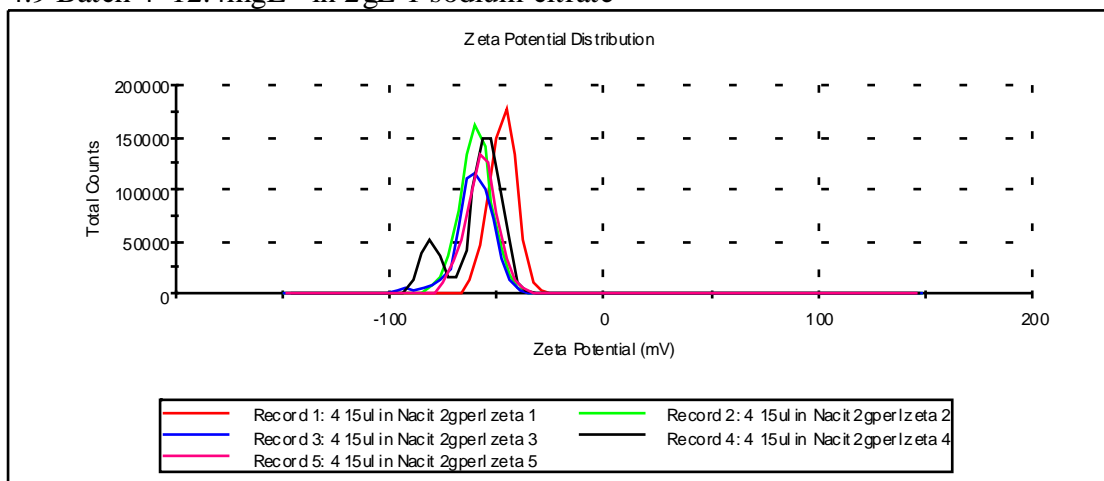
4.7 Batch 2 pH 10.71

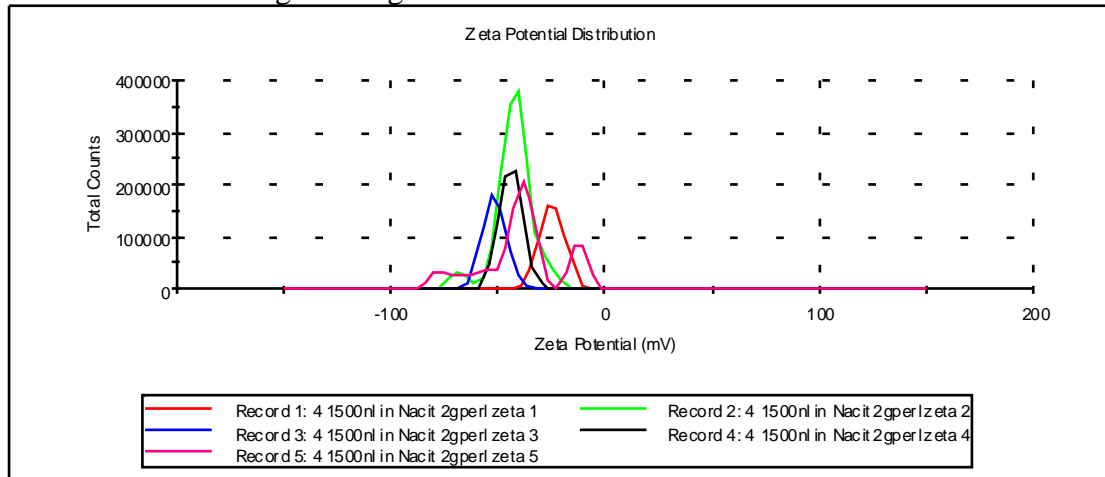
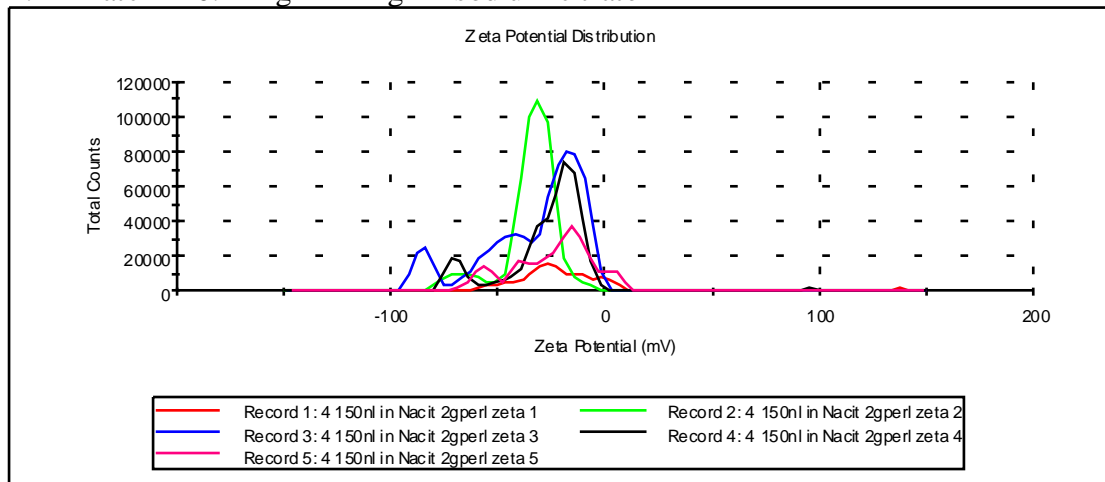
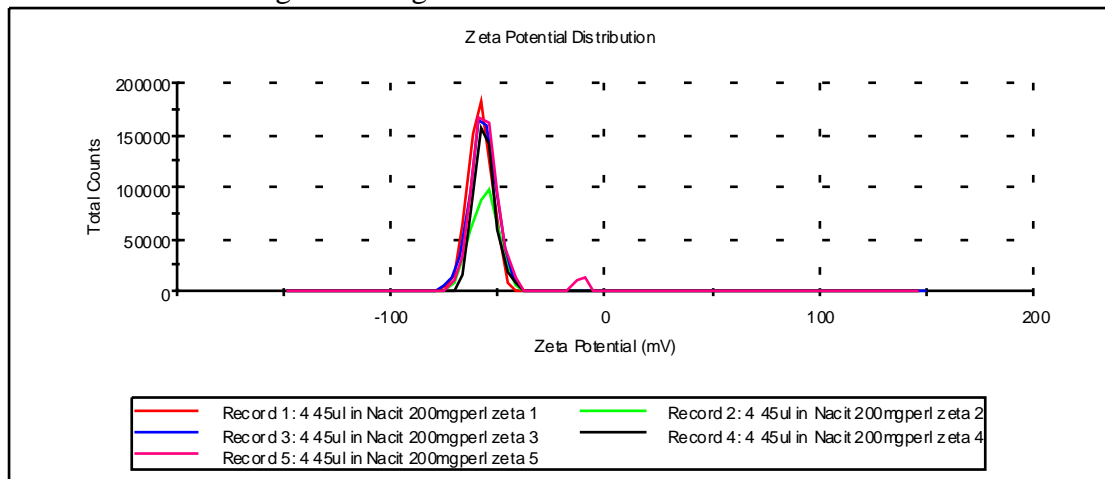


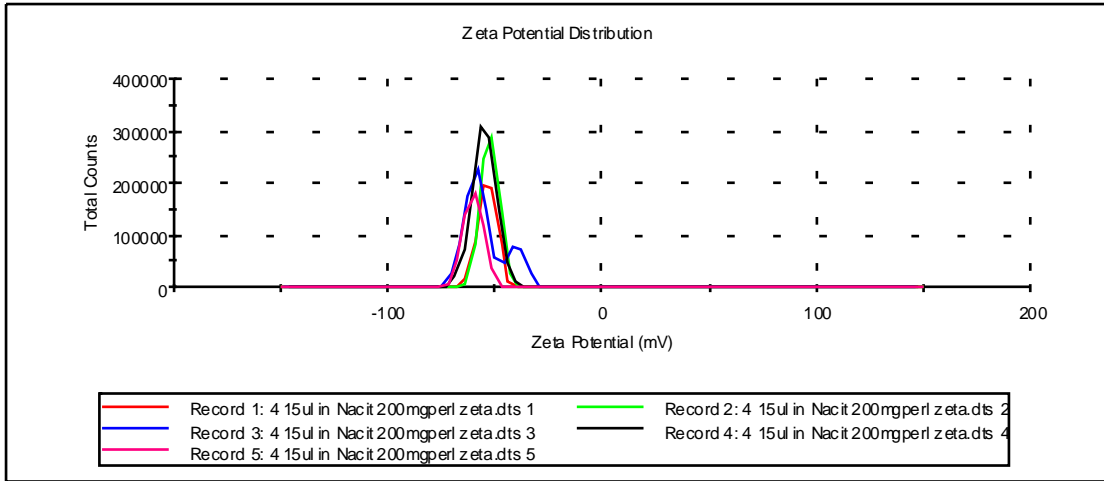
4.8 Batch 4 37.2mgL<sup>-1</sup> in 2gL<sup>-1</sup> sodium citrate



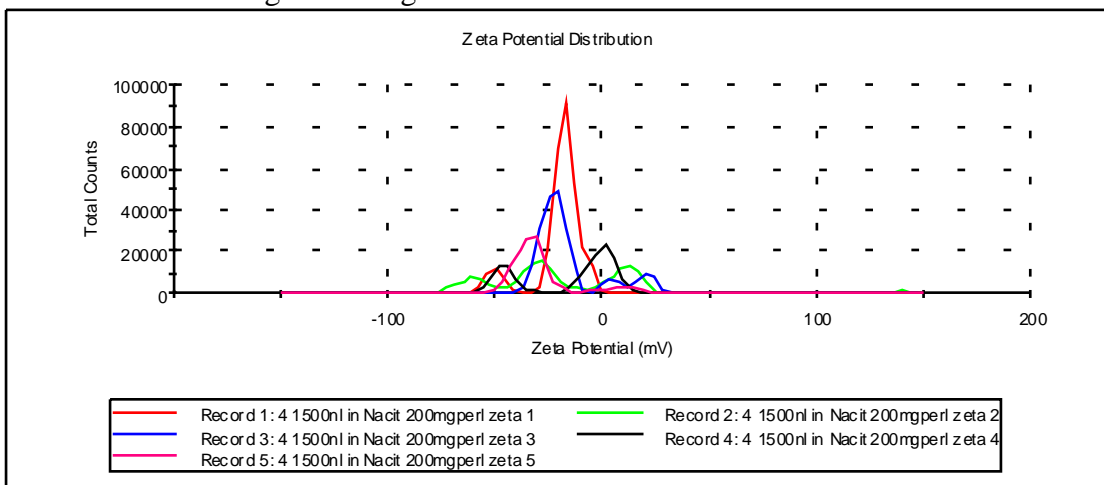
4.9 Batch 4 12.4mgL<sup>-1</sup> in 2gL<sup>-1</sup> sodium citrate



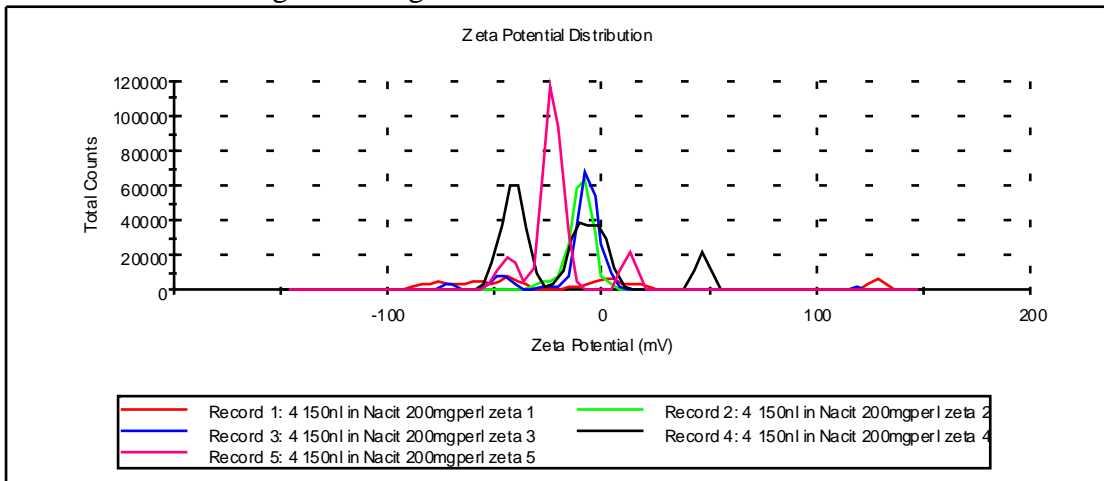
4.10 Batch 4  $1.24\text{mgL}^{-1}$  in  $2\text{gL}^{-1}$  sodium citrate4.11 Batch 4  $0.12\text{mgL}^{-1}$  in  $2\text{gL}^{-1}$  sodium citrate4.12 Batch 4  $37.2\text{mgL}^{-1}$  in  $0.2\text{gL}^{-1}$  sodium citrate4.13 Batch 4  $12.4\text{mgL}^{-1}$  in  $0.2\text{gL}^{-1}$  sodium citrate



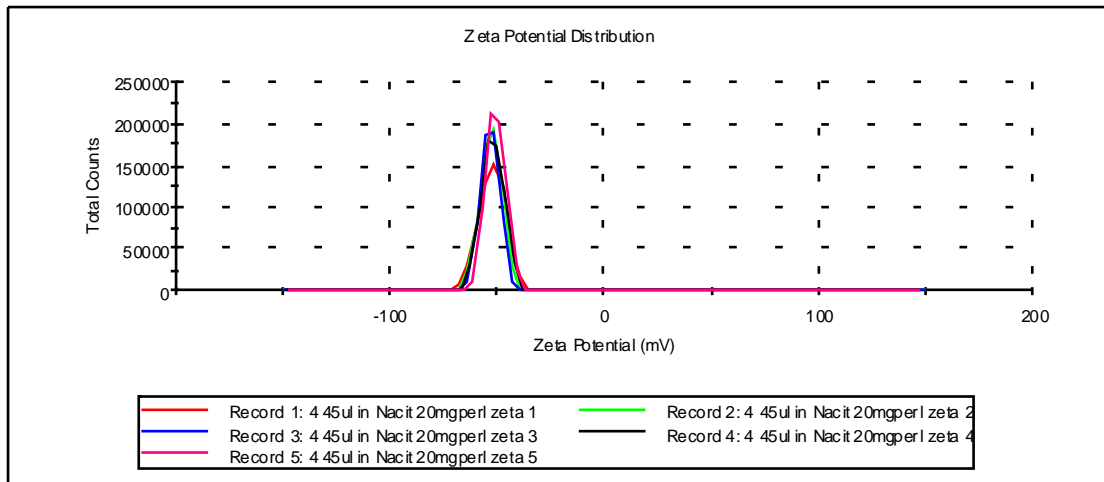
4.14 Batch 4  $1.24\text{mgL}^{-1}$  in  $0.2\text{gL}^{-1}$  sodium citrate



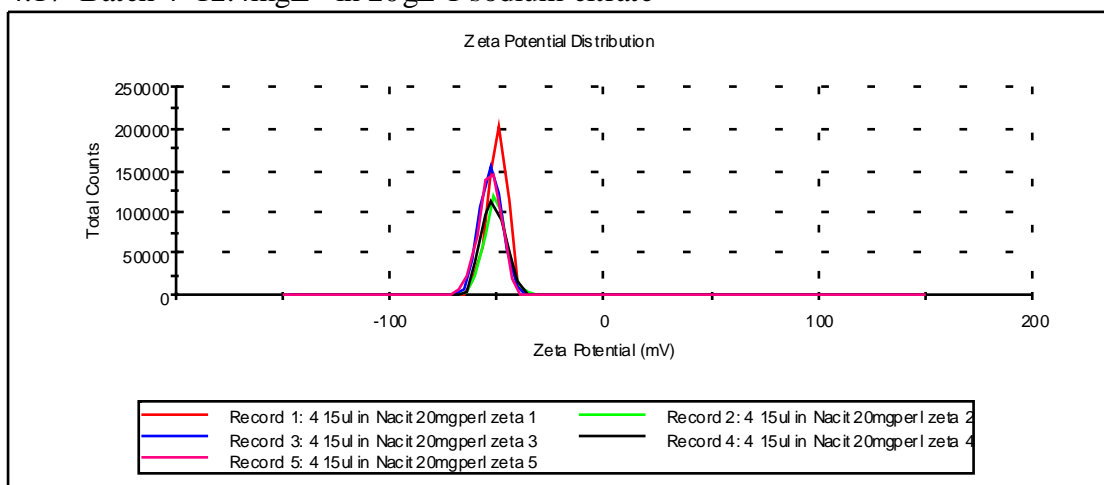
4.15 Batch 4  $0.12\text{mgL}^{-1}$  in  $0.2\text{gL}^{-1}$  sodium citrate



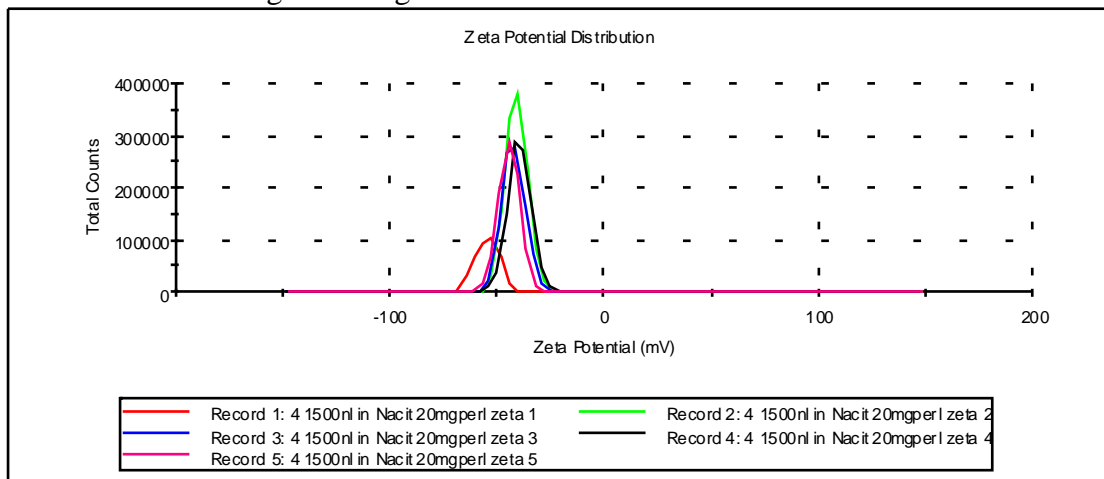
4.16 Batch 4  $37.2\text{mgL}^{-1}$  in  $20\text{gL}^{-1}$  sodium citrate



#### 4.17 Batch 4 $12.4\text{mgL}^{-1}$ in $20\text{gL}^{-1}$ sodium citrate

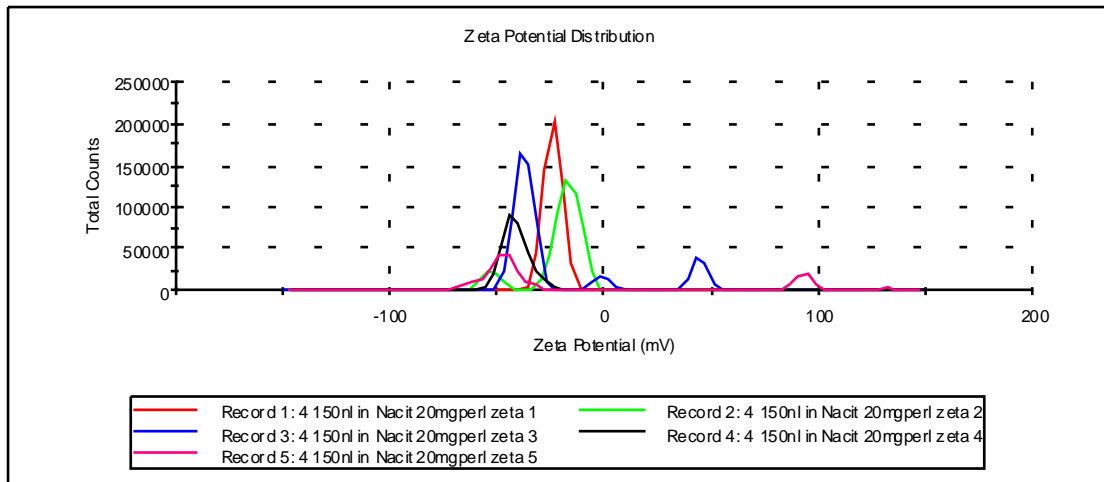


#### 4.18 Batch 4 $1.24\text{mgL}^{-1}$ in $20\text{gL}^{-1}$ sodium citrate

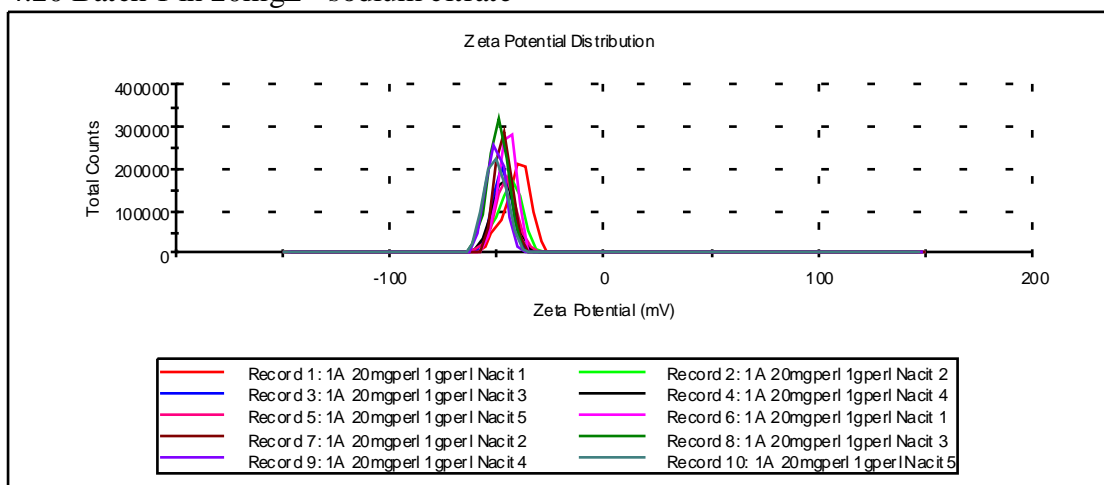


#### 4.19 Batch 4 $0.12\text{mgL}^{-1}$ in $20\text{gL}^{-1}$ sodium citrate

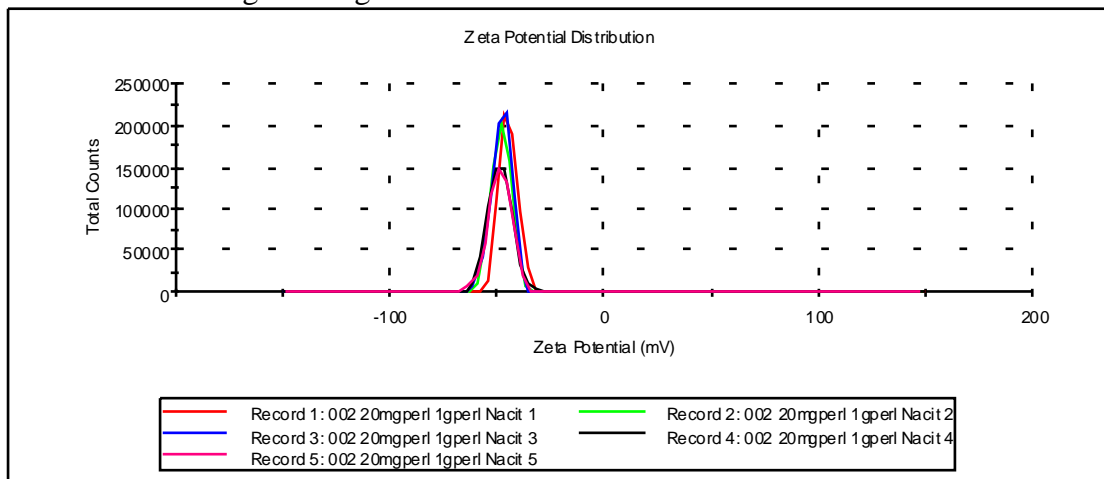




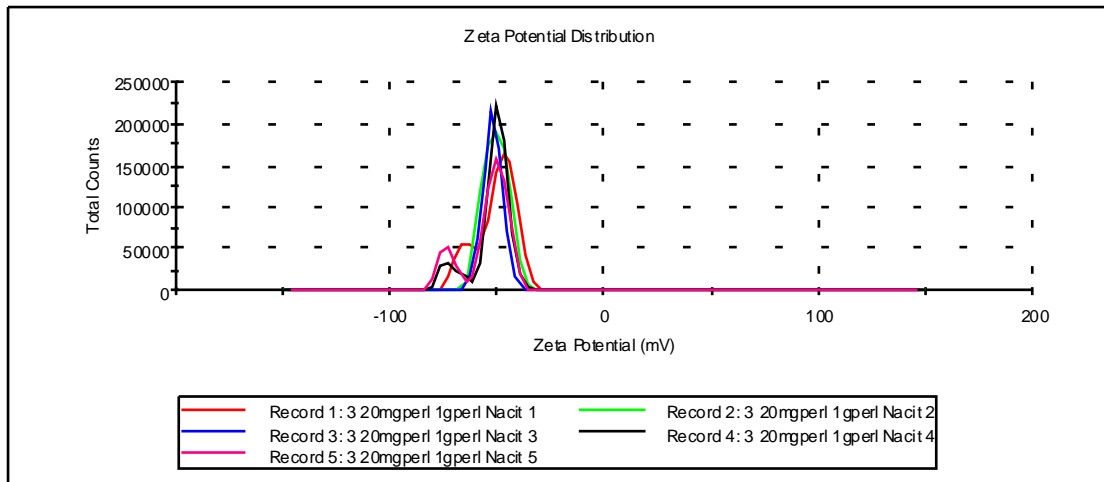
#### 4.20 Batch 1 in 20mgL<sup>-1</sup> sodium citrate



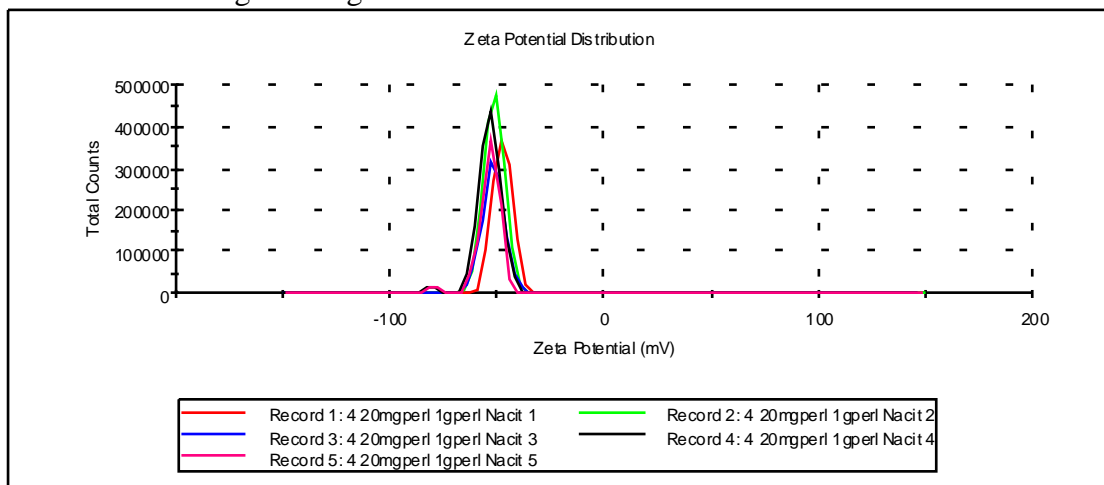
#### 4.21 Batch 2 20mgL<sup>-1</sup> in 1gL<sup>-1</sup> sodium citrate



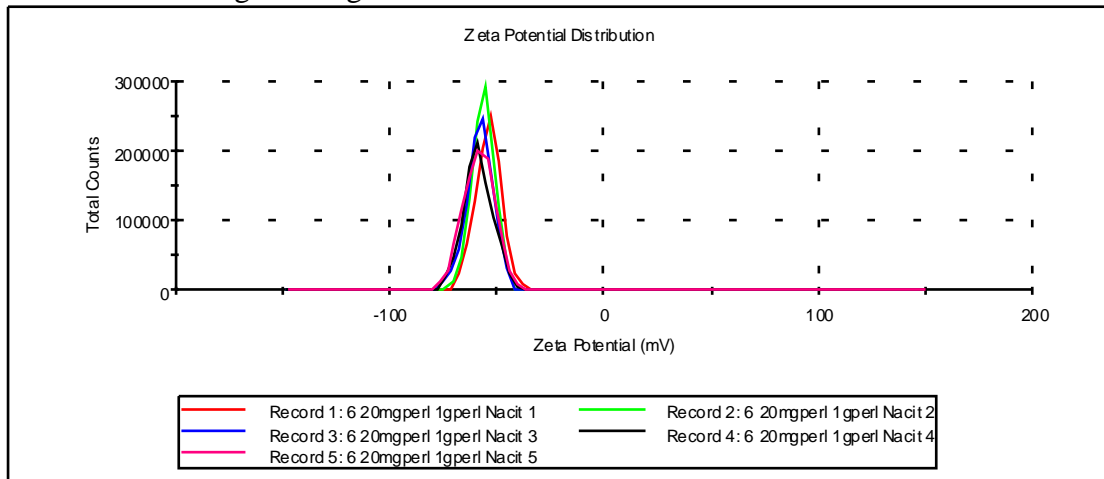
#### 4.22 Batch 3 20mgL<sup>-1</sup> in 1gL<sup>-1</sup> sodium citrate



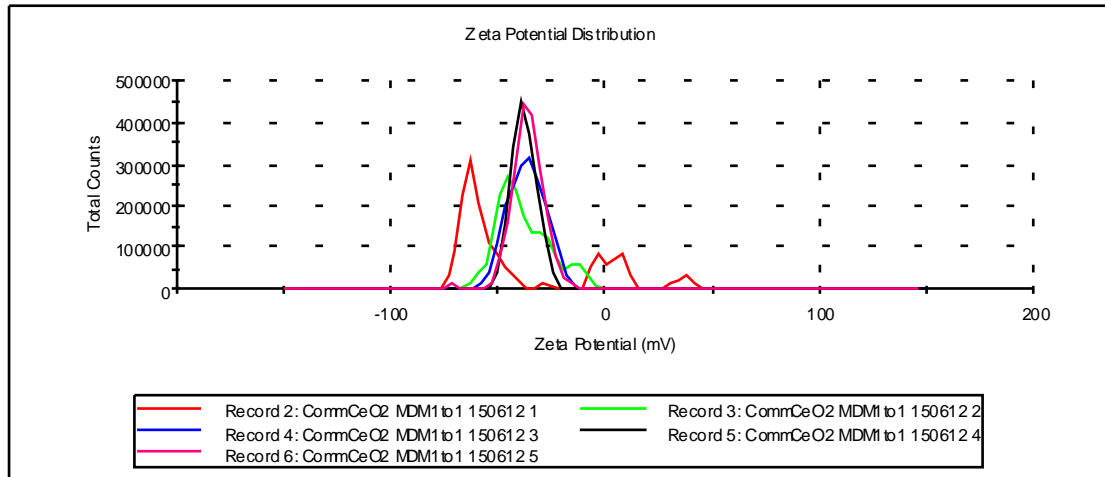
#### 4.23 Batch 4 20mgL<sup>-1</sup> in 1gL<sup>-1</sup> sodium citrate



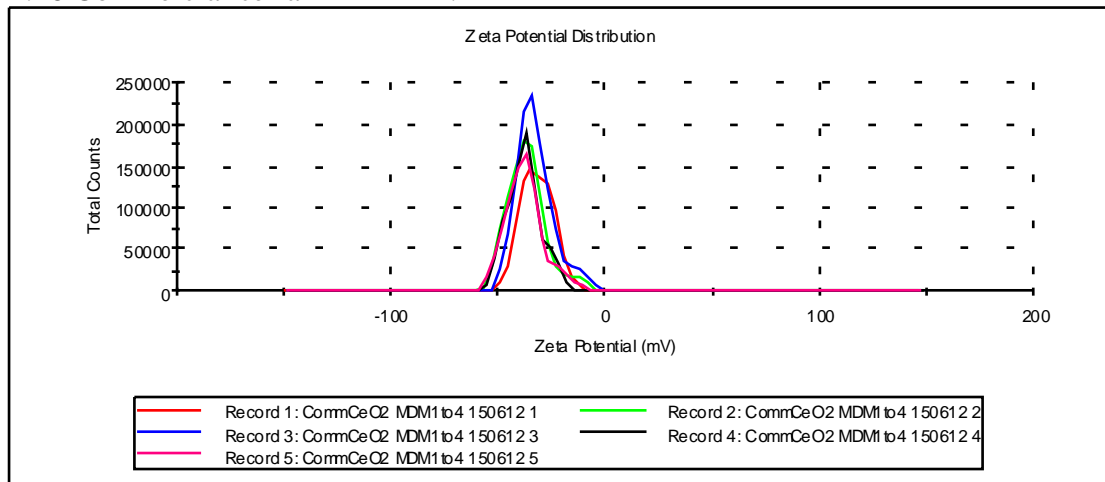
#### 4.24 Batch 6 20mgL<sup>-1</sup> in 1gL<sup>-1</sup> sodium citrate



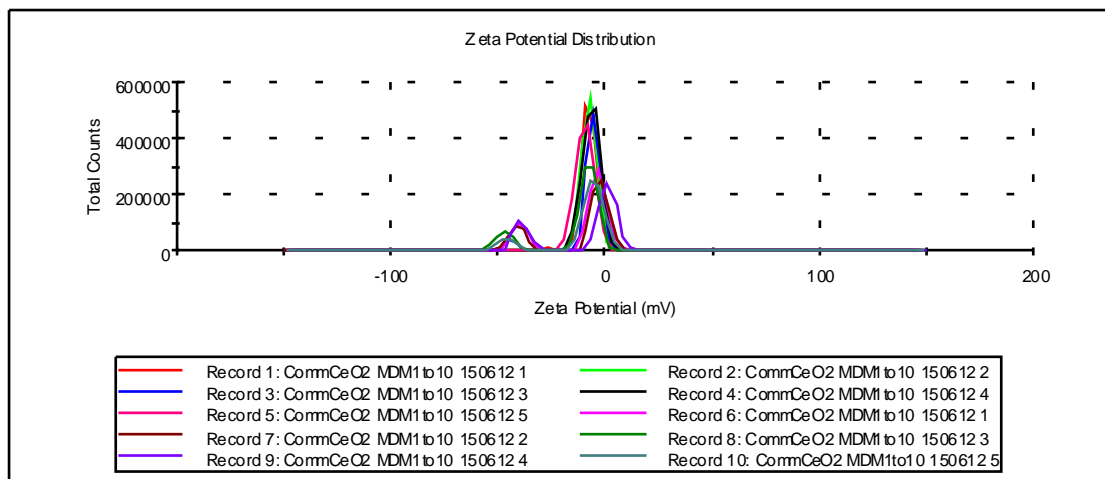
## 4.25 Commercial ceria in MDM 1:1

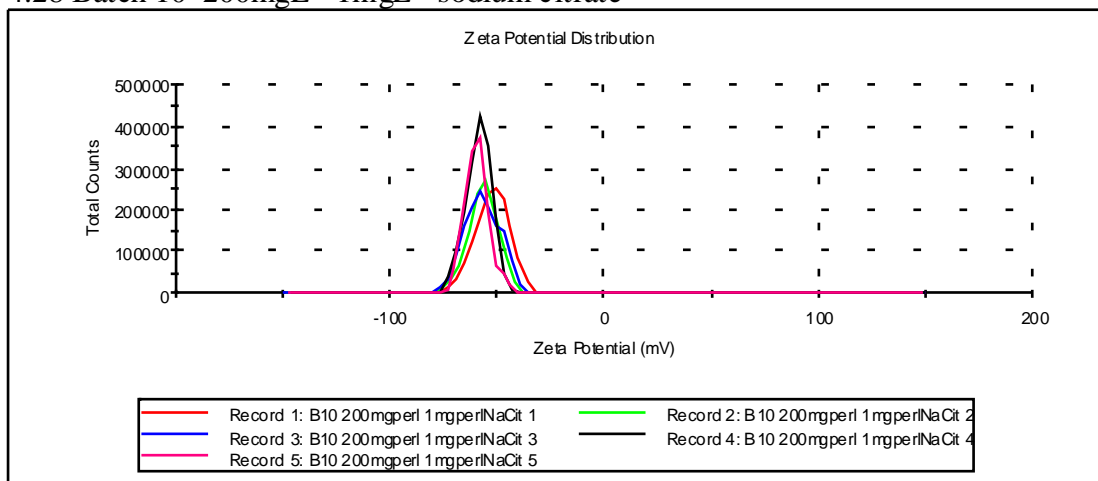
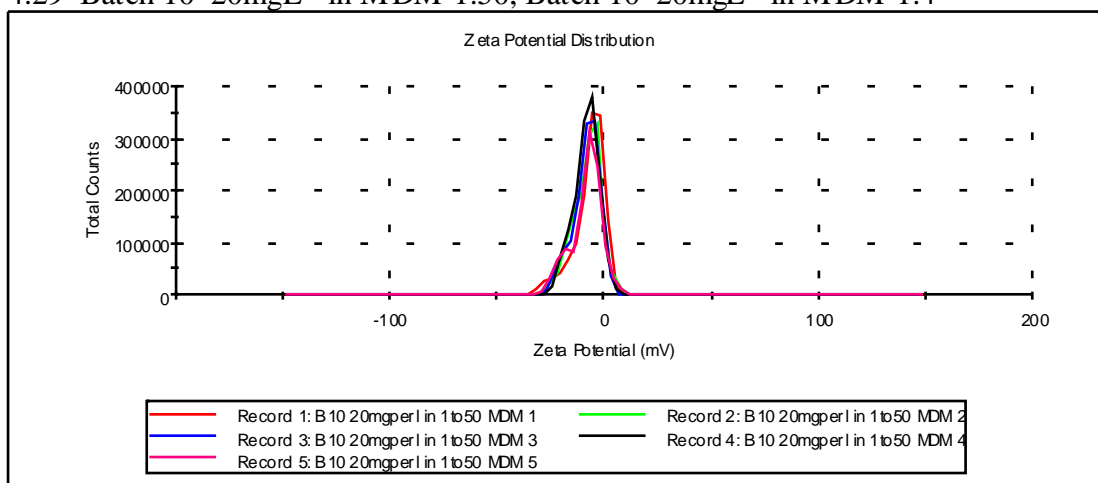
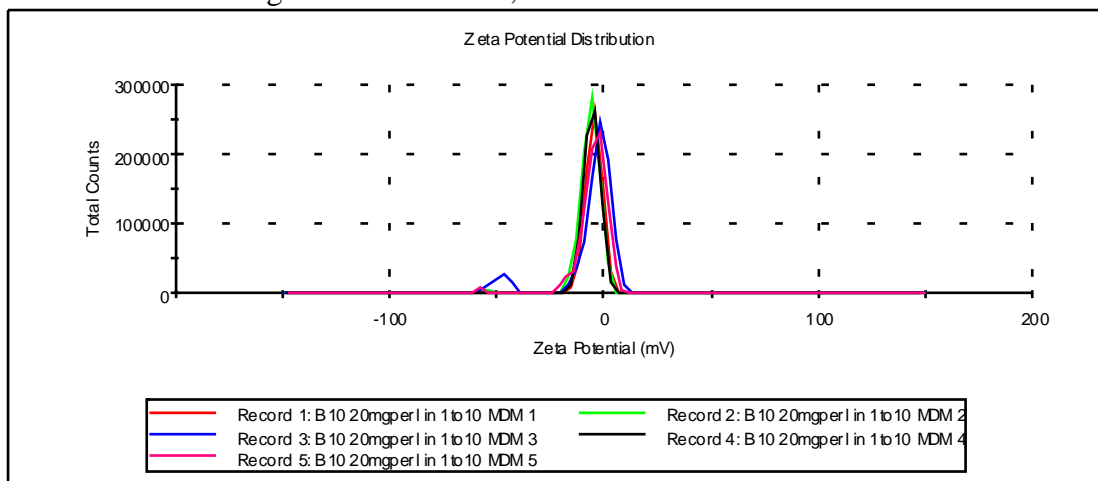


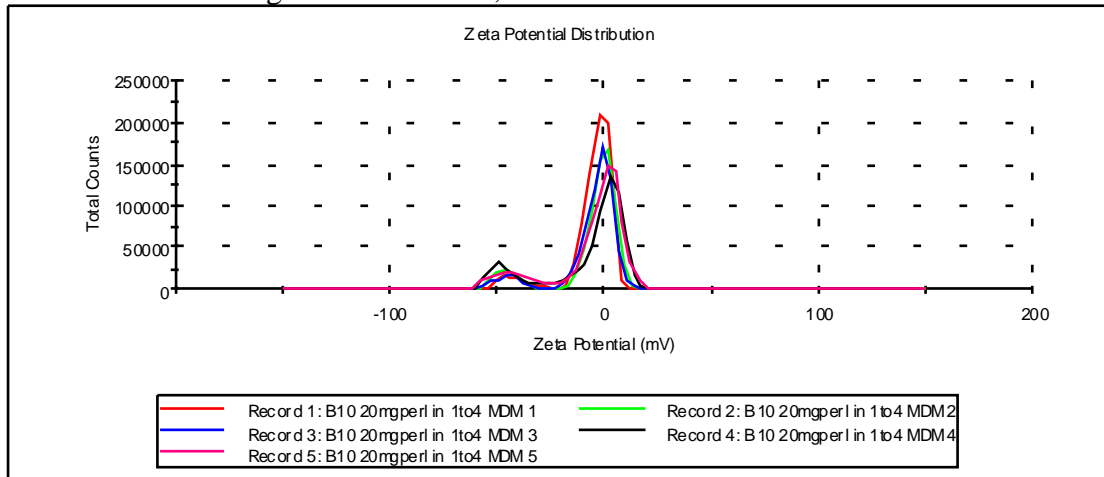
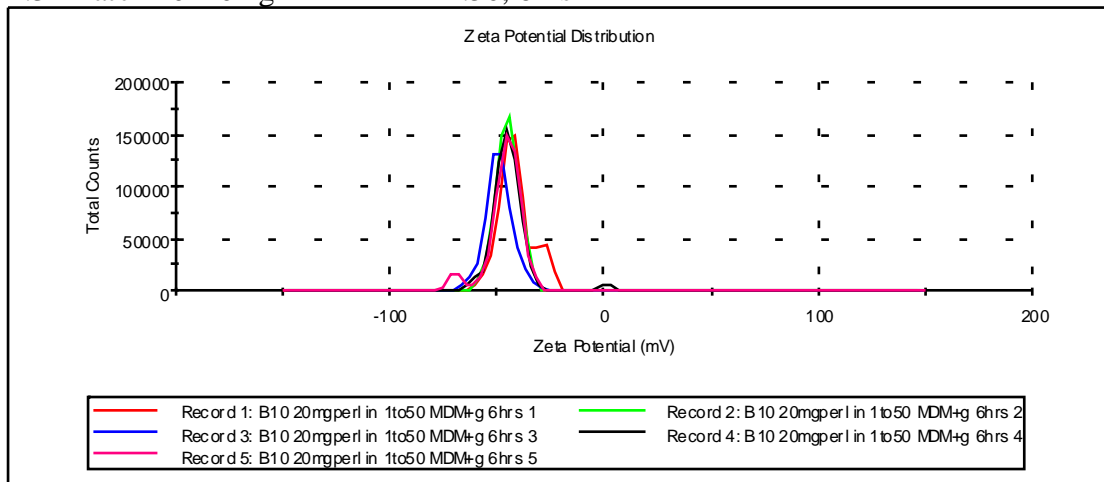
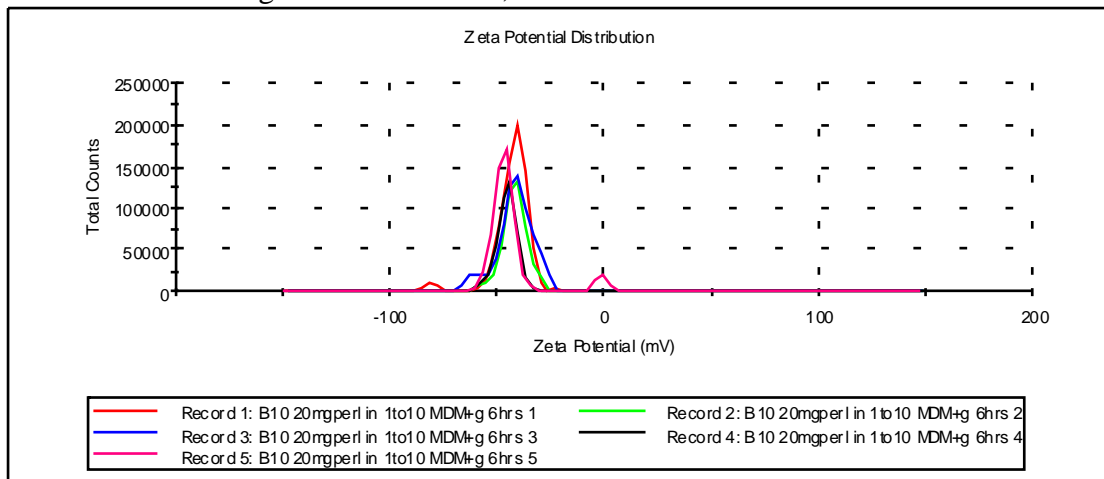
## 4.26 Commercial ceria in MDM 1:4

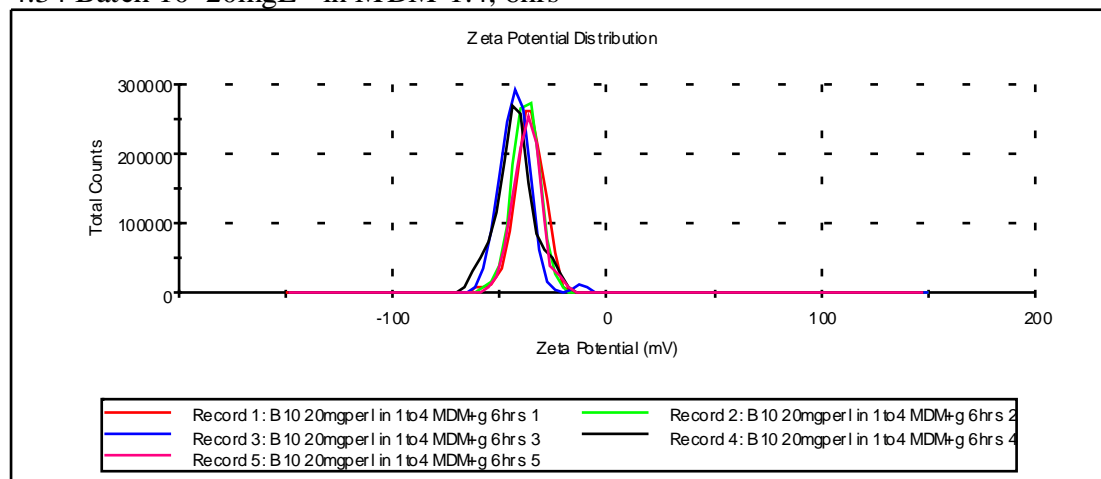
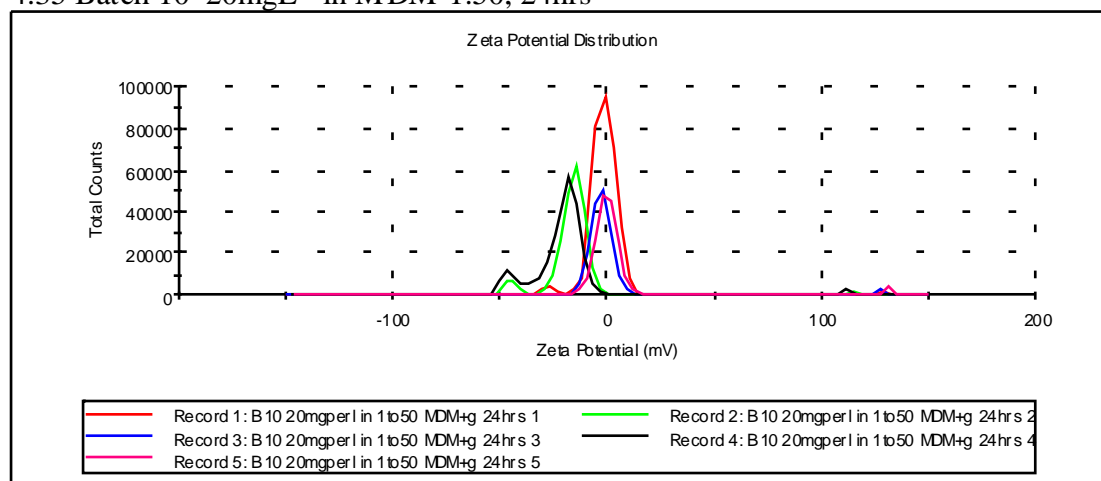
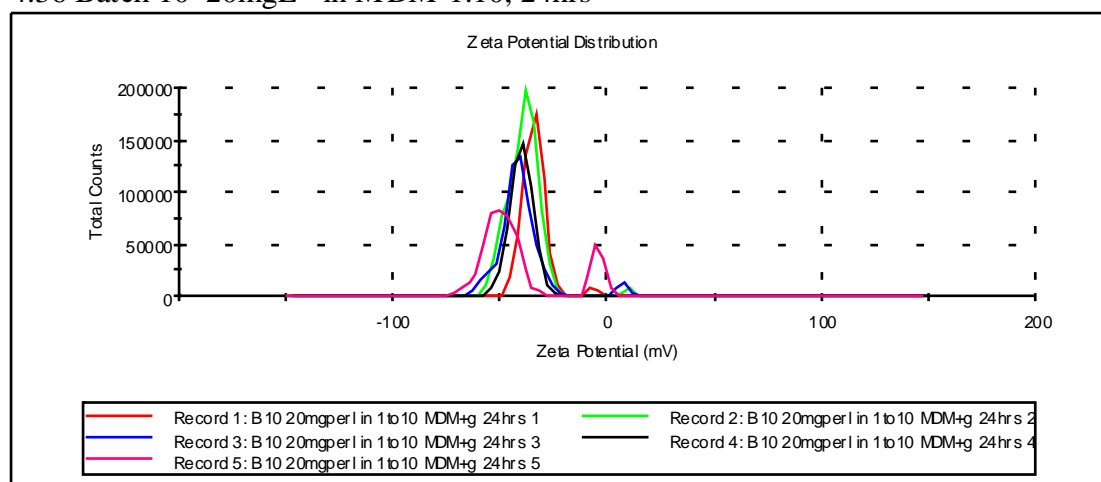


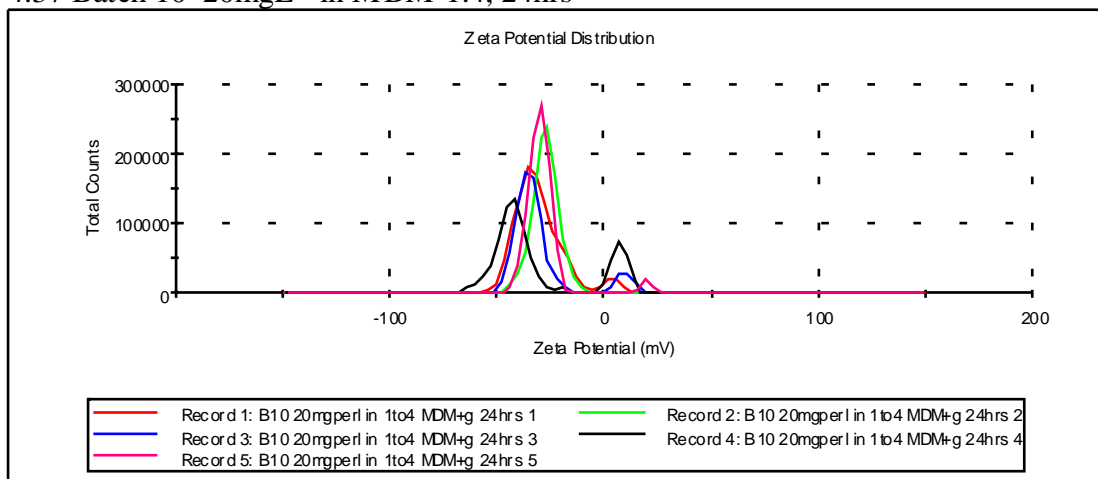
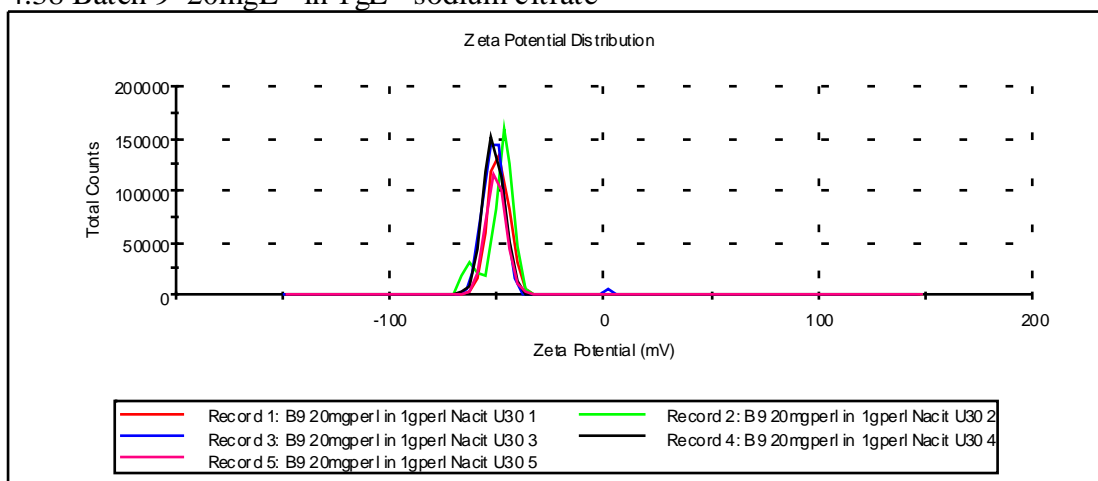
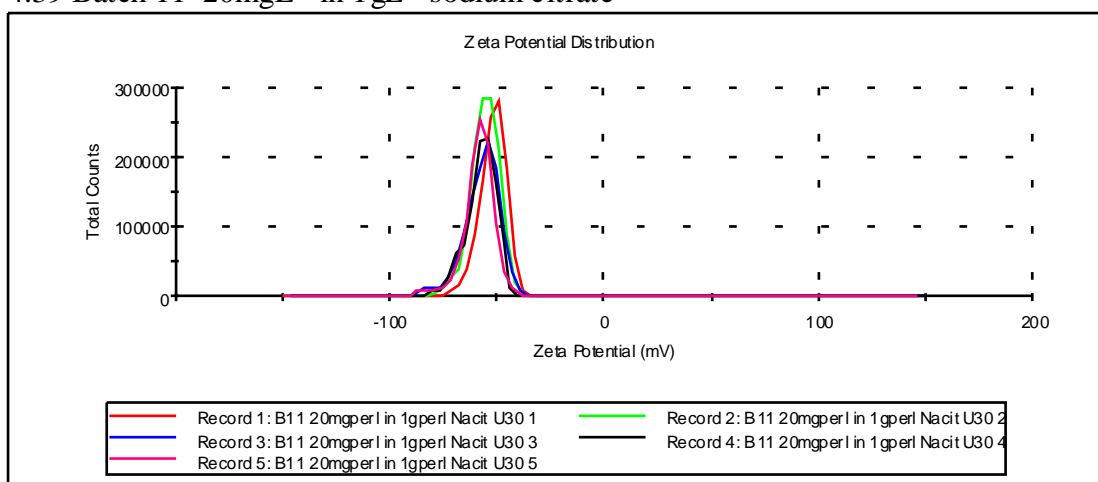
## 4.27 Commercial ceria in MDM 1:10

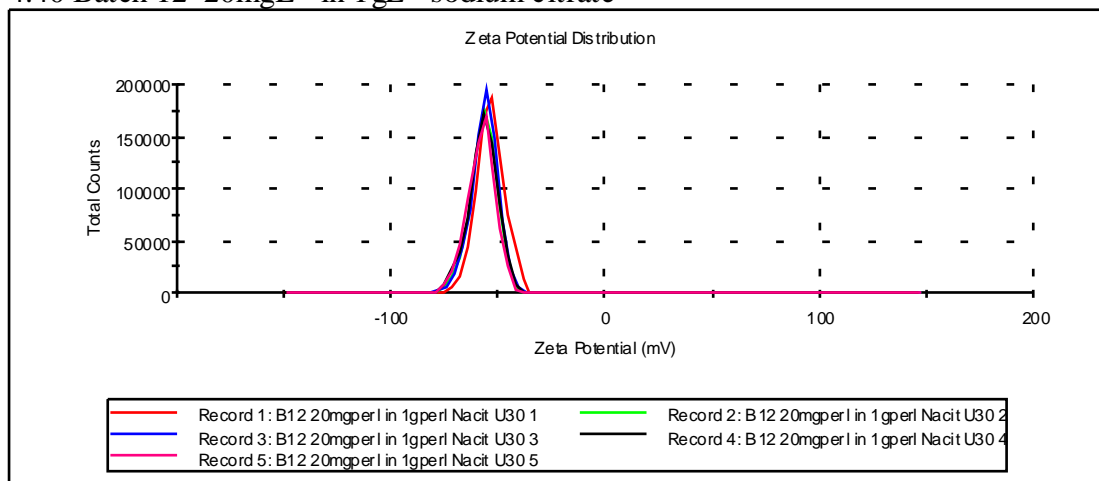
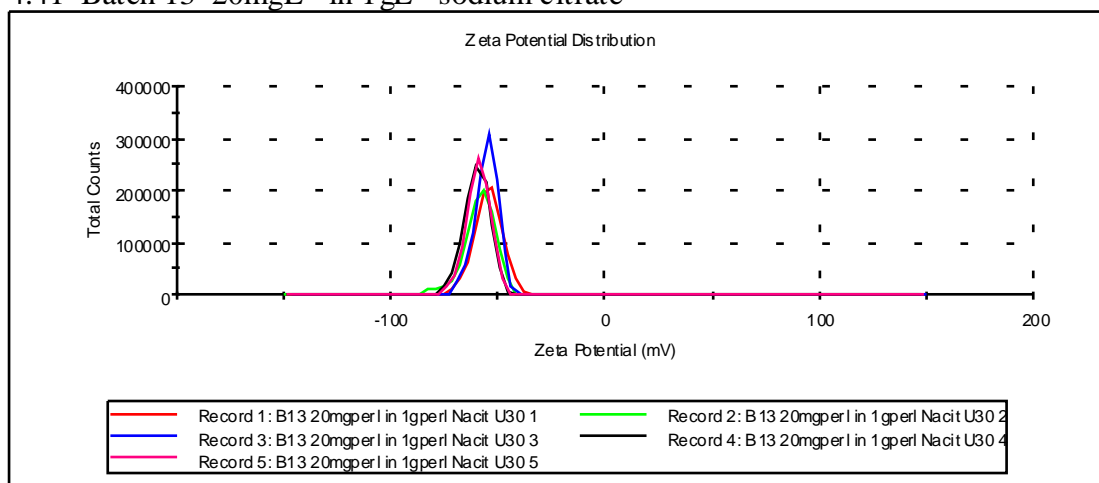


4.28 Batch 10  $200\text{mgL}^{-1}$   $1\text{mgL}^{-1}$  sodium citrate4.29 Batch 10  $20\text{mgL}^{-1}$  in MDM 1:50, Batch 10  $20\text{mgL}^{-1}$  in MDM 1:44.30 Batch 10  $20\text{mgL}^{-1}$  in MDM 1:10, 1.5hrs

4.31 Batch 10  $20\text{mgL}^{-1}$  in MDM 1:4, 1.5hrs4.32 Batch 10  $20\text{mgL}^{-1}$  in MDM 1:50, 6hrs4.33 Batch 10  $20\text{mgL}^{-1}$  in MDM 1:10, 6hrs

4.34 Batch 10  $20\text{mgL}^{-1}$  in MDM 1:4, 6hrs4.35 Batch 10  $20\text{mgL}^{-1}$  in MDM 1:50, 24hrs4.36 Batch 10  $20\text{mgL}^{-1}$  in MDM 1:10, 24hrs

4.37 Batch 10 20mgL<sup>-1</sup> in MDM 1:4, 24hrs4.38 Batch 9 20mgL<sup>-1</sup> in 1gL<sup>-1</sup> sodium citrate4.39 Batch 11 20mgL<sup>-1</sup> in 1gL<sup>-1</sup> sodium citrate

4.40 Batch 12  $20\text{mgL}^{-1}$  in  $1\text{gL}^{-1}$  sodium citrate4.41 Batch 13  $20\text{mgL}^{-1}$  in  $1\text{gL}^{-1}$  sodium citrate



**Appendix 5.1**B3 CeO<sub>2</sub>, in 1gL<sup>-1</sup> sodium citrate prepared 18/05/12Washed 2 times in 25ml H<sub>2</sub>O

Measured 21/05/12

No.	Size	No.	Size	No.	Size	No.	Size	No.	Size
1	11.274	31	27.596	61	7.749	91	12.054	121	20.136
2	10.161	32	21.11	62	9.71	92	14.078	122	3.189
3	18.034	33	16.273	63	11.13	93	21.243	123	31.787
4	10.284	34	15.971	64	20.291	94	8.995	124	17.176
5	14.305	35	18.774	65	4.972	95	7.587	125	33.565
6	84.477	36	34.674	66	2.79	96	59.135	126	19.462
7	208.75	37	20.845	67	11.78	97	3.081	127	26.17
8	77.248	38	19.148	68	8.379	98	18.756	128	34.068
9	24.413	39	12.182	69	6.577	99	28.023	129	21.897
10	9.205	40	3.135	70	4.771	100	4.418	130	6.858
11	8.966	41	2.424	71	10.171	101	5.208	131	9.134
12	17.974	42	14.669	72	3.177	102	28.407	132	11.813
13	9.636	43	32.389	73	6.897	103	4.316	133	19.192
14	13.997	44	24.942	74	6.655	104	3.353	134	2.323
15	11.483	45	18.405	75	48.197	105	2.54	135	3.033
16	40.252	46	2.283	76	13.984	106	14.814	136	9.159
17	5.985	47	7.532	77	6.679	107	9.9	137	16.718
18	34.131	48	2.187	78	7.675	108	17.027	138	6.351
19	11.451	49	14.221	79	15.739	109	19.992	139	4.523
20	20.371	50	1.885	80	81.19	110	5.479	140	5.217
21	11.633	51	2.664	81	26.452	111	5.906	141	88.373
22	46.747	52	18.167	82	56.525	112	9.85	142	12.219
23	51.897	53	41.608	83	22.943	113	24.662	143	97.108
24	18.265	54	2.927	84	42.305	114	70.334	144	39.601
25	13.032	55	6.768	85	8.378	115	20.644	145	5.144
26	20.3	56	24.302	86	52.034	116	26.285	146	13.518
27	31.865	57	4.781	87	8.74	117	20.033	147	35.741
28	108.102	58	6.711	88	31.455	118	5.706	148	10.681
29	97.727	59	6.078	89	9.044	119	6.946	149	4.317
30	20.186	60	3.604	90	3.67	120	16.519	150	14.358

**Appendix 5.2**B6 CeO<sub>2</sub>, in 1gL<sup>-1</sup> sodium citrate prepared 18/05/12Washed 2 times in 25ml H<sub>2</sub>O

Measured 21/05/12

No	Size	No	Size	No	Size	No	Size
1	4.004	26	3.597	51	2.878	76	2.677
2	2.447	27	2.805	52	1.087	77	3.453
3	3.106	28	1.047	53	1.827	78	3.023
4	3.226	29	3.624	54	1.287	79	2.959
5	7.312	30	3.340	55	1.775	80	1.847
6	2.633	31	3.410	56	3.165	81	2.686
7	3.124	32	3.373	57	2.038	82	3.827
8	1.676	33	2.000	58	3.193	83	3.685
9	1.964	34	2.025	59	3.093	84	4.210
10	2.222	35	2.447	60	3.811	85	2.180
11	2.397	36	1.359	61	2.246	86	4.188
12	2.823	37	2.737	62	1.467	87	4.907
13	2.462	38	1.642	63	2.124	88	3.988
14	2.536	39	1.663	64	3.735	89	3.924
15	1.470	40	2.421	65	1.054	90	4.284
16	1.766	41	2.583	66	3.182	91	3.544
17	1.582	42	2.230	67	3.632	92	2.321
18	1.788	43	8.516	68	4.964	93	4.215
19	4.420	44	1.774	69	2.552	94	3.332
20	2.524	45	1.919	70	2.494	95	3.320
21	2.228	46	3.589	71	2.962	96	1.687
22	1.616	47	3.640	72	2.209	97	2.534
23	2.667	48	3.269	73	2.388	98	1.022
24	3.710	49	3.212	74	1.825	99	3.714
25	1.494	50	2.080	75	2.293	100	3.235

**Appendix 5.3**B10 CeO<sub>2</sub>, in 1gL<sup>-1</sup> prepared 18/05/12Washed 2 times in 25ml H<sub>2</sub>O

Measured 28/05/12

No.	Size	No.	Size	No.	Size	No.	Size
1	6.440	34	17.865	67	16.007	100	5.876
2	8.084	35	9.326	68	3.985	101	9.581
3	10.241	36	27.081	69	6.108	102	9.215
4	20.277	37	5.245	70	10.039	103	3.859
5	11.196	38	5.124	71	4.685	104	6.040
6	9.183	39	5.167	72	8.383	105	15.108
7	20.520	40	6.669	73	6.093	106	4.995
8	10.597	41	5.613	74	15.865	107	8.250
9	7.003	42	11.861	75	6.745	108	13.642
10	9.558	43	4.897	76	11.504	109	3.423
11	7.415	44	7.349	77	11.921	110	9.871
12	4.716	45	22.626	78	2.660	111	17.475
13	12.162	46	4.465	79	13.227	112	8.093
14	8.063	47	6.640	80	6.871	113	47.384
15	3.005	48	8.512	81	17.344	114	18.777
16	10.199	49	9.431	82	12.932	115	5.176
17	4.929	50	4.058	83	4.899	116	5.480
18	11.213	51	7.334	84	4.353	117	33.733
19	34.770	52	7.226	85	10.161	118	14.246
20	9.348	53	8.901	86	7.714	119	12.514
21	7.147	54	6.167	87	6.812	120	7.555
22	3.456	55	5.796	88	21.796	121	10.101
23	5.316	56	6.926	89	17.934	122	8.767
24	9.070	57	12.619	90	17.978	123	6.048
25	15.007	58	3.046	91	16.522	124	5.605
26	7.873	59	5.651	92	10.704	125	4.764
27	21.391	60	12.050	93	13.464	126	14.656
28	16.662	61	6.622	94	13.502	127	10.375
29	12.500	62	7.873	95	5.130	128	21.761
30	9.533	63	10.034	96	14.849	129	20.889
31	7.660	64	15.359	97	9.411	130	5.761
32	14.578	65	5.864	98	3.188	131	4.491
33	14.400	66	10.826	99	9.334	132	12.763

**Appendix 5.4**Commercial CeO<sub>2</sub>, 500µl of 10mgL<sup>-1</sup> in H<sub>2</sub>O, prepared 23/07/12Washed 2 times in 40ml H<sub>2</sub>O

Measured 24/07/12

No	Size	No	Size	No	Size	No	Size
1	21.927	26	9.380	51	16.604	76	10.466
2	85.338	27	8.859	52	67.391	77	26.513
3	59.379	28	36.746	53	34.878	78	10.540
4	8.911	29	60.146	54	59.303	79	7.623
5	6.716	30	80.437	55	143.960	80	112.021
6	67.619	31	7.399	56	18.457	81	97.100
7	17.330	32	15.263	57	9.218	82	90.009
8	6.297	33	72.503	58	84.221	83	89.533
9	4.486	34	4.645	59	19.210	84	17.029
10	85.016	35	5.258	60	91.107	85	27.874
11	72.554	36	5.746	61	63.000	86	11.319
12	4.944	37	30.715	62	89.826	87	5.261
13	31.233	38	4.850	63	70.281	88	7.104
14	51.139	39	3.062	64	65.831	89	72.115
15	5.690	40	12.593	65	42.584	90	22.999
16	4.379	41	10.156	66	7.435	91	24.674
17	61.252	42	89.829	67	53.268	92	11.072
18	26.176	43	9.983	68	102.415	93	12.137
19	76.964	44	21.034	69	7.238	94	90.327
20	63.440	45	49.676	70	101.068	95	92.047
21	9.013	46	14.686	71	68.065	96	152.861
22	8.335	47	84.367	72	10.233	97	69.787
23	6.207	48	15.121	73	70.815	98	3.639
24	32.559	49	33.278	74	10.780	99	3.380
25	13.554	50	4.108	75	6.318	100	4.773

**Appendix 5.5**Commercial CeO<sub>2</sub>, 150µl of 10mgL<sup>-1</sup> in H<sub>2</sub>O, prepared 23/07/12Washed 2 times in 40ml H<sub>2</sub>O

Measured 24/07/12

No	Size	No	Size	No	Size	No	Size
1	165.970	26	16.420	51	55.145	76	17.915
2	145.178	27	35.468	52	38.337	77	47.522
3	40.134	28	3.765	53	7.457	78	15.590
4	7.927	29	8.163	54	35.697	79	16.276
5	48.156	30	65.436	55	82.998	80	5.528
6	25.013	31	11.254	56	82.641	81	43.575
7	32.070	32	182.728	57	10.381	82	10.339
8	16.795	33	44.750	58	7.170	83	23.911
9	96.513	34	76.298	59	17.452	84	63.519
10	66.208	35	11.946	60	40.008	85	34.861
11	39.016	36	5.423	61	22.417	86	38.542
12	14.677	37	26.456	62	56.478	87	6.046
13	30.419	38	16.866	63	6.376	88	22.482
14	17.995	39	2.119	64	32.356	89	23.653
15	5.192	40	33.742	65	56.731	90	36.433
16	4.891	41	9.028	66	32.874	91	23.060
17	20.367	42	11.232	67	41.460	92	49.190
18	22.100	43	3.111	68	44.088	93	41.390
19	67.285	44	21.734	69	26.120	94	84.079
20	40.722	45	5.725	70	61.205	95	63.830
21	23.964	46	6.763	71	58.625	96	55.875
22	7.796	47	55.763	72	48.535	97	10.619
23	6.002	48	11.308	73	6.923	98	3.675
24	4.969	49	24.997	74	10.038	99	36.604
25	9.598	50	126.398	75	76.166	100	14.674

**Appendix 5.6**Commercial CeO<sub>2</sub>, in 1:10 MDM, prepared 2/07/12Washed 3 times in 25ml H<sub>2</sub>O

Measured 4/07/12

No	Size	No	Size	No	Size	No	Size
1	7.489	21	10.436	41	11.784	61	27.406
2	13.013	22	23.204	42	10.318	62	341.078
3	45.04	23	9.508	43	15.292	63	19.094
4	11.389	24	31.208	44	6.624	64	3.09
5	33.81	25	16.789	45	28.497	65	32.29
6	5.546	26	24.019	46	22.105	66	7.037
7	19.804	27	23.014	47	16.78	67	2.968
8	23.867	28	31.719	48	40.159	68	4.23
9	3.176	29	18.551	49	44.83	69	3.885
10	28.509	30	36.867	50	5.84	70	5.787
11	33.444	31	31.28	51	32.306	71	3.947
12	28.779	32	6.411	52	2.053	72	3.118
13	16.384	33	8.291	53	30.092	73	6.446
14	18.896	34	24.544	54	3.559	74	15.43
15	188.879	35	34.301	55	14.927	75	9.607
16	28.368	36	15.02	56	6.821	76	3.914
17	27.877	37	31.024	57	14.6	77	67.935
18	32.711	38	39.16	58	48.531		
19	18.073	39	5.166	59	8.496		
20	9.651	40	15.7	60	5.739		

**Appendix 5.7**B10 CeO<sub>2</sub>, in H<sub>2</sub>O prepared 6/07/12Washed 3 times in 25ml H<sub>2</sub>O

Measured 9/07/12

No	Size	No	Size	No	Size	No	Size
1	14.501	28	3.19	55	13.843	82	341.143
2	31.281	29	8.107	56	32.032	83	33.763
3	18.215	30	9.299	57	14.862	84	29.65
4	40.243	31	10.823	58	25.192	85	24.375
5	46.82	32	10.446	59	15030	86	13.597
6	44.215	33	34.69	60	31.868	87	10.724
7	54.764	34	41.35	61	41.162	88	23.889
8	43.796	35	51.254	62	58.763	89	8.401
9	33.044	36	21.449	63	10.216	90	46.035
10	46.55	37	39.691	64	67.546	91	35.302
11	16.032	38	27.322	65	19.117	92	51.555
12	28.245	39	36.139	66	19.917	93	41.194
13	2.536	40	17.116	67	35.611	94	40.223
14	5.384	41	20.405	68	41.249	95	32.801
15	7.221	42	13.831	69	12.866	96	18.783
16	12.003	43	92.291	70	11.661	97	12.057
17	76.315	44	26.358	71	41.448	98	30.379
18	62.712	45	37.005	72	34.415	99	5.443
19	28.777	46	42.817	73	11.687	100	16.791
20	68.184	47	19.72	74	38.289	101	15.881
21	8.53	48	34.245	75	71.471	102	26.229
22	5.25	49	19.309	76	52.921	103	67.008
23	34.398	50	30.103	77	39.144	104	12.06
24	3.984	51	14.823	78	25.637	105	54.498
25	5.707	52	38.52	79	59.45	106	40.775
26	24.459	53	59.094	80	42.742		
27	1.546	54	28.274	81	22.863		

**Appendix 5.8**B10 CeO<sub>2</sub>, in 1:10 MDM prepared 23/07/12Washed 5 times in 40ml H<sub>2</sub>O

Measured 24/07/12

No	Size	No	Size	No	Size	No	Size
1	3.887	27	7.827	53	15.914	79	36.345
2	12.352	28	4.706	54	14.773	80	34.707
3	35.963	29	6.582	55	34.189	81	44.823
4	1.551	30	5.184	56	1.703	82	40.927
5	2.145	31	65.556	57	39.958	83	25.746
6	22.569	32	49.727	58	6.407	84	17.626
7	1.552	33	10.600	59	28.150	85	21.731
8	34.754	34	1.569	60	5.800	86	36.901
9	5.461	35	14.757	61	4.751	87	11.026
10	8.247	36	2.383	62	5.341	88	3.966
11	63.347	37	17.579	63	3.000	89	1.634
12	32.517	38	20.298	64	3.222	90	3.207
13	2.341	39	38.106	65	64.340	91	1.722
14	5.292	40	41.824	66	13.639	92	3.541
15	2.439	41	17.664	67	11.321	93	2.657
16	16.402	42	99.412	68	5.563	94	3.896
17	10.785	43	61.967	69	5.211	95	3.970
18	2.254	44	7.459	70	56.196	96	2.934
19	1.338	45	76.455	71	23.555	97	62.781
20	2.327	46	27.537	72	14.677	98	21.602
21	1.739	47	5.036	73	20.346	99	2.463
22	3.391	48	33.965	74	6.369	100	80.229
23	40.589	49	4.247	75	11.649	101	34.277
24	2.904	50	81.915	76	24.589	102	46.541
25	72.003	51	7.645	77	14.874	103	4.703
26	5.239	52	20.333	78	28.929	104	57.941



**Appendix 5.9**AFM data for mica with  $1\text{gL}^{-1}$  sodium citrate solution, washed twice in 25ml water

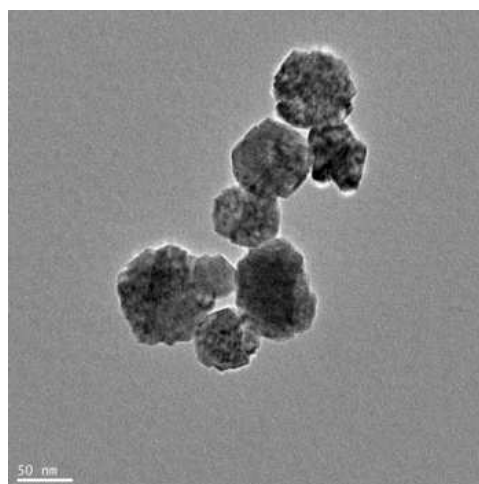
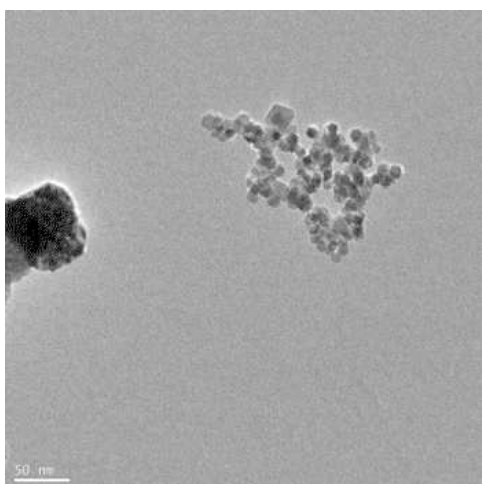
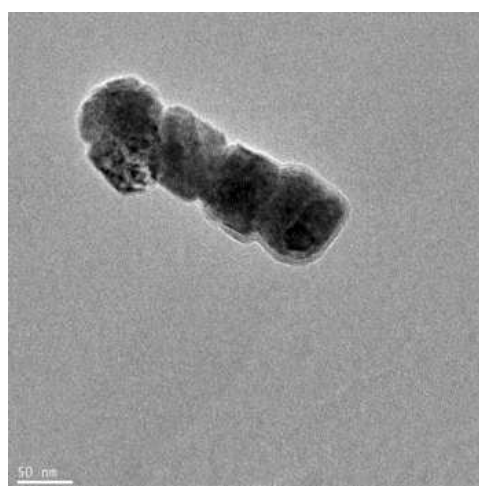
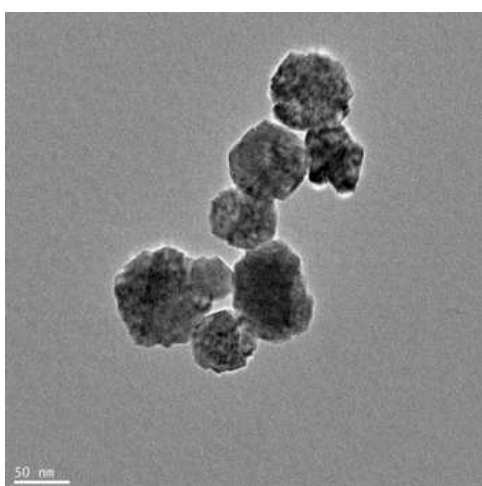
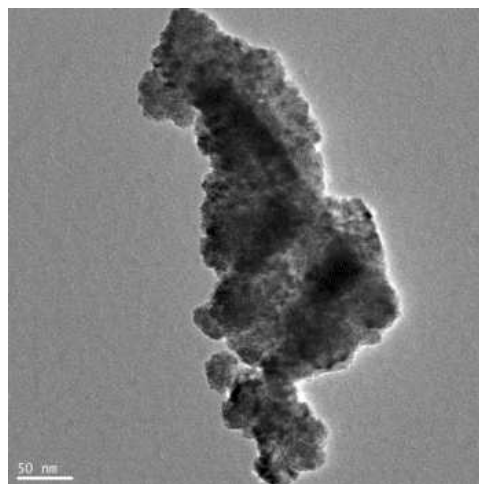
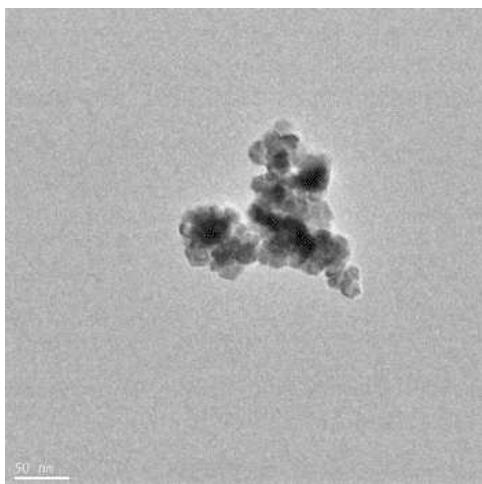
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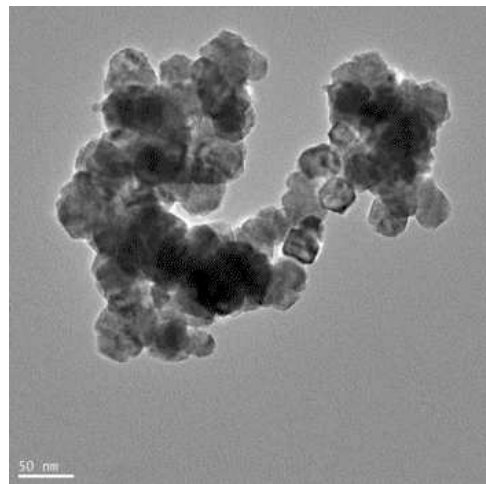
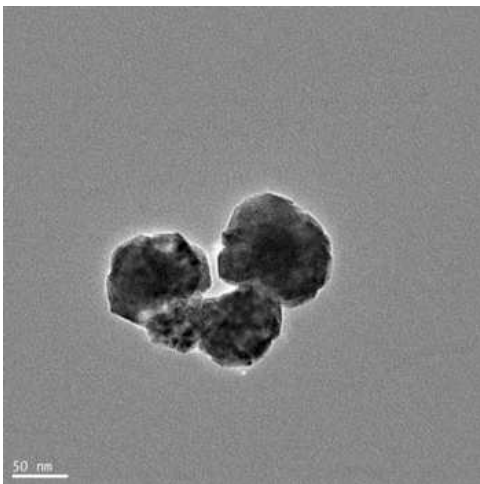
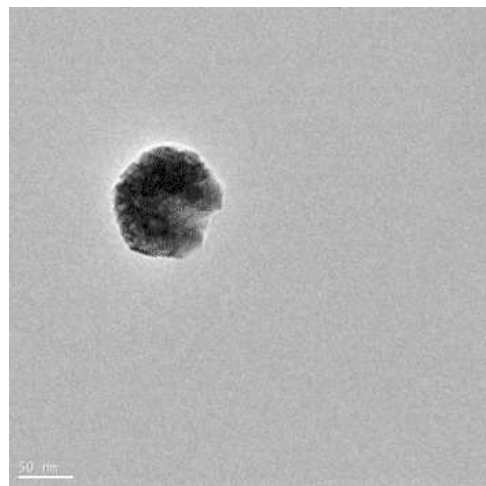
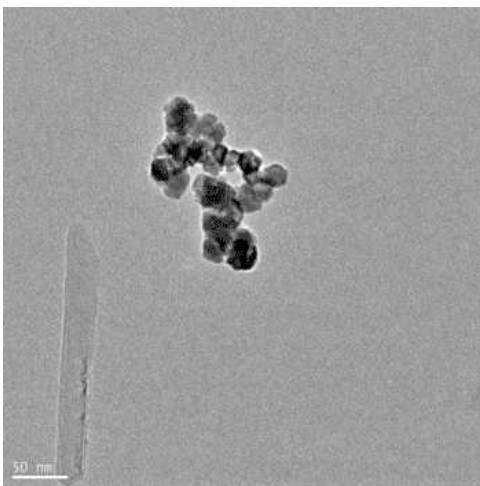
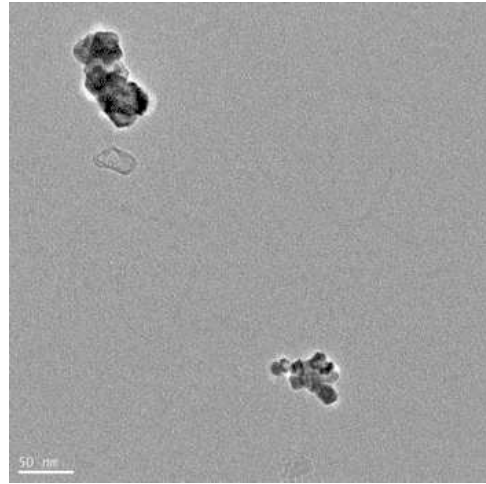
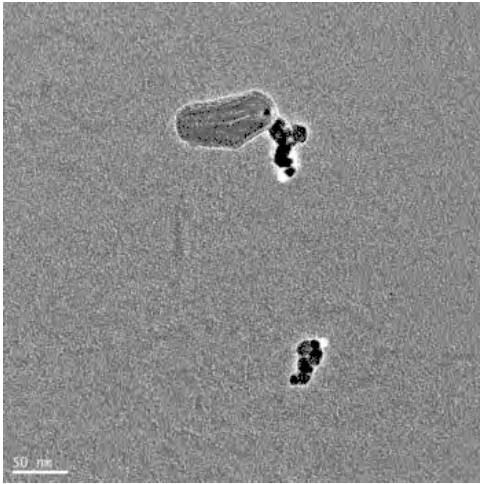
**Appendix 6.1**

TEM images of batch 6 dispersed in  $1\text{gL}^{-1}$  sodium citrate solution.

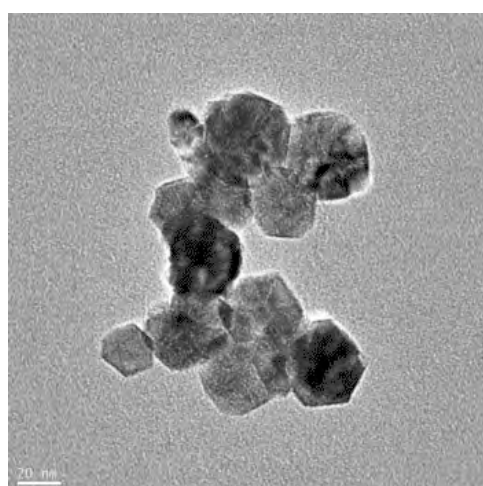
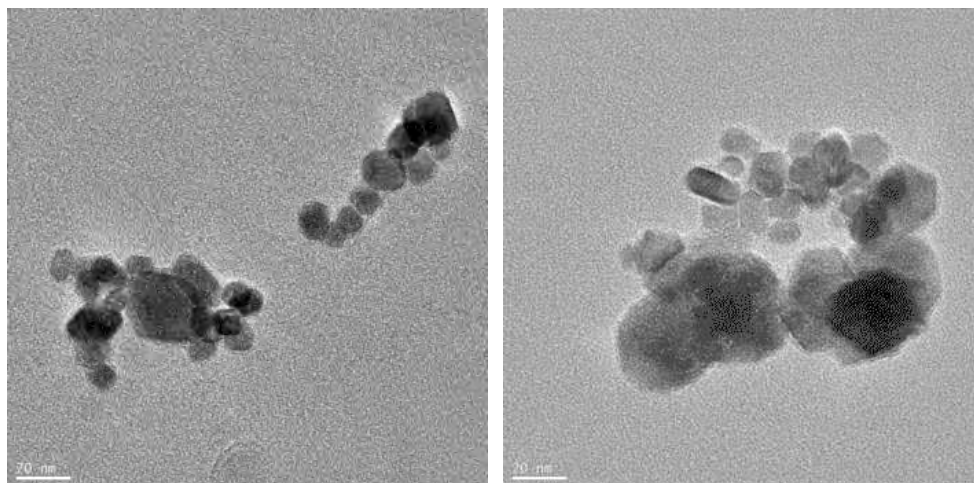
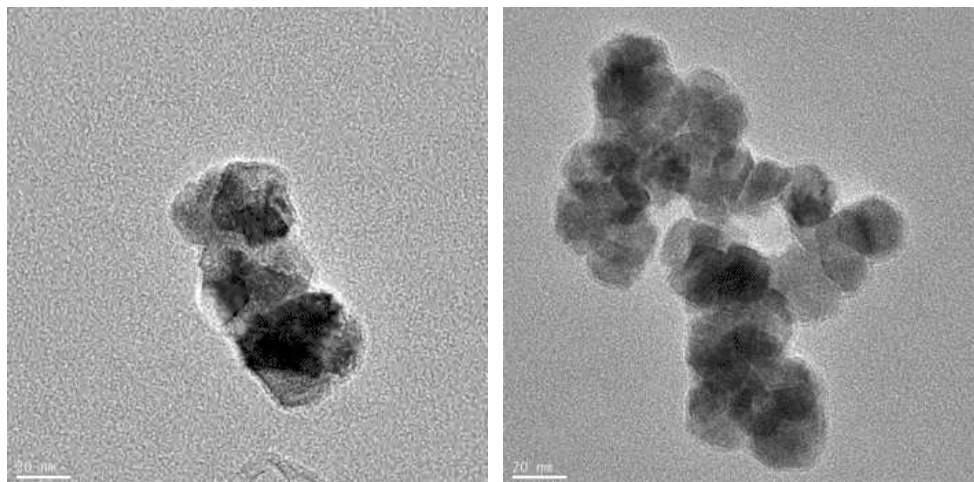
Sample prepared by drop method,  $40\text{mgL}^{-1}$ .

a) Scale bars = 50nm

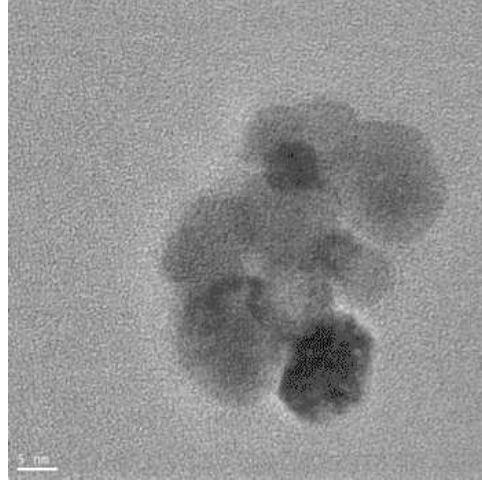
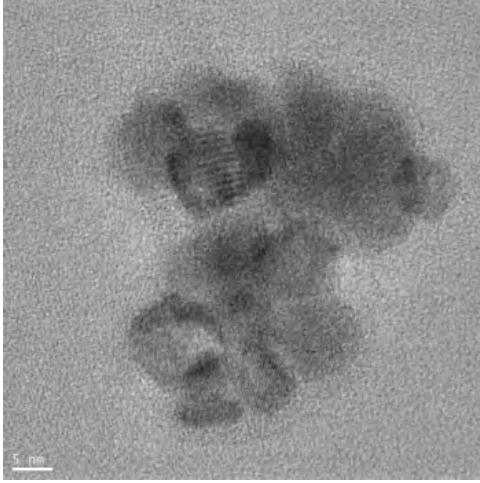




b) Scale bars = 20nm



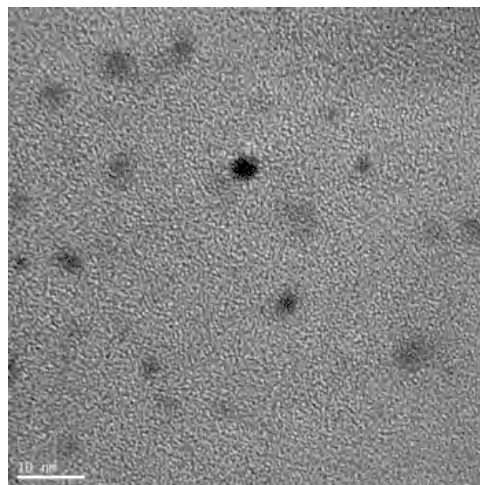
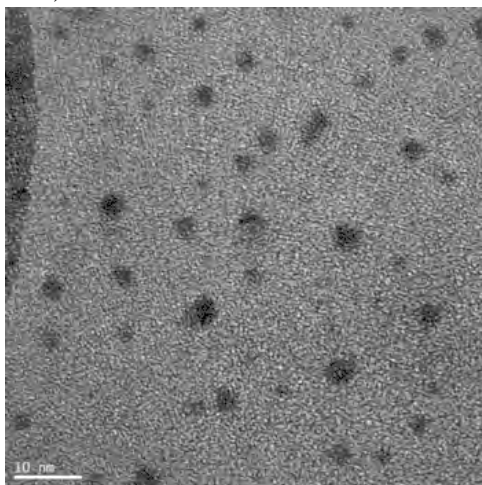
c) Scale bars = 5nm



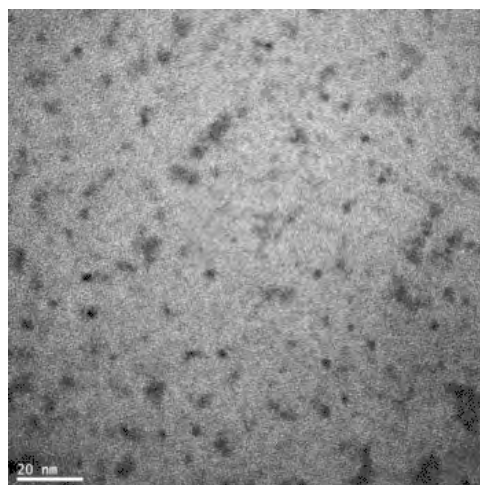
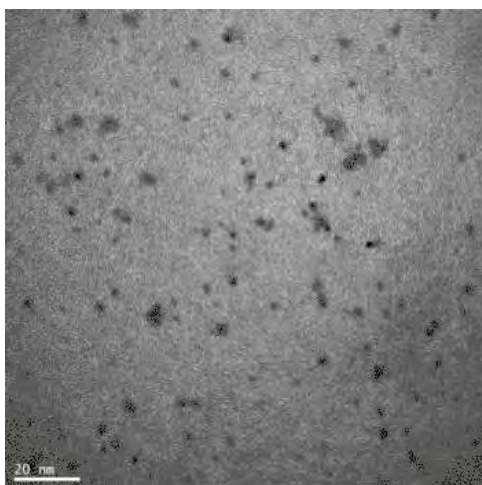
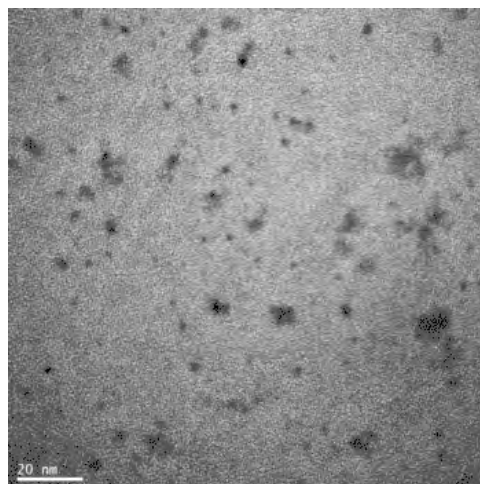
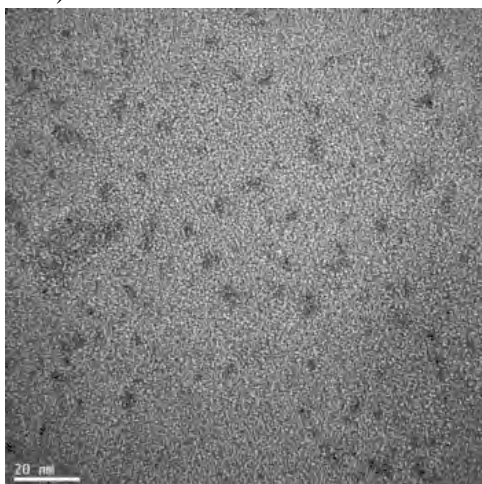
**Appendix 6.2**

TEM images for batch 4 dispersed in  $1\text{gL}^{-1}$  sodium citrate solution.  
Sample diluted with water, and prepared by ultracentrifuge method.

a) Scale bars 10nm



b) Scale bar 20nm

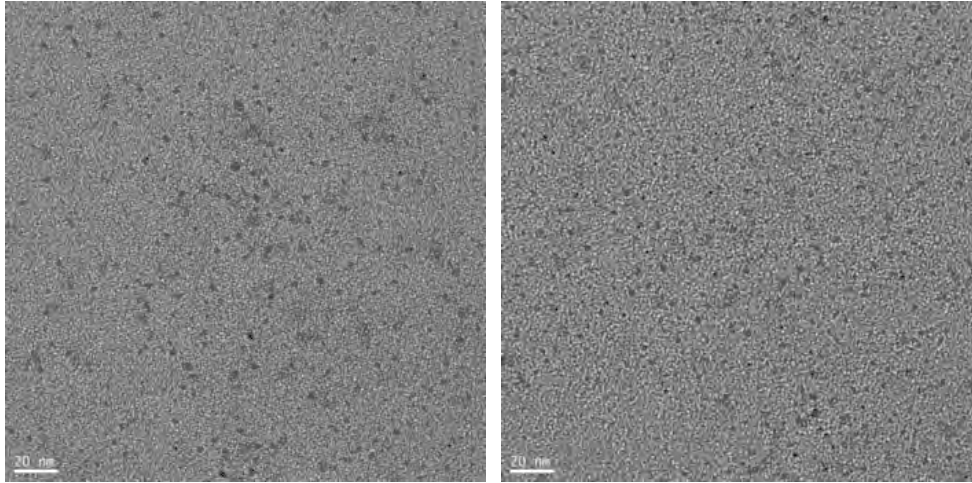


**Appendix 6.3**

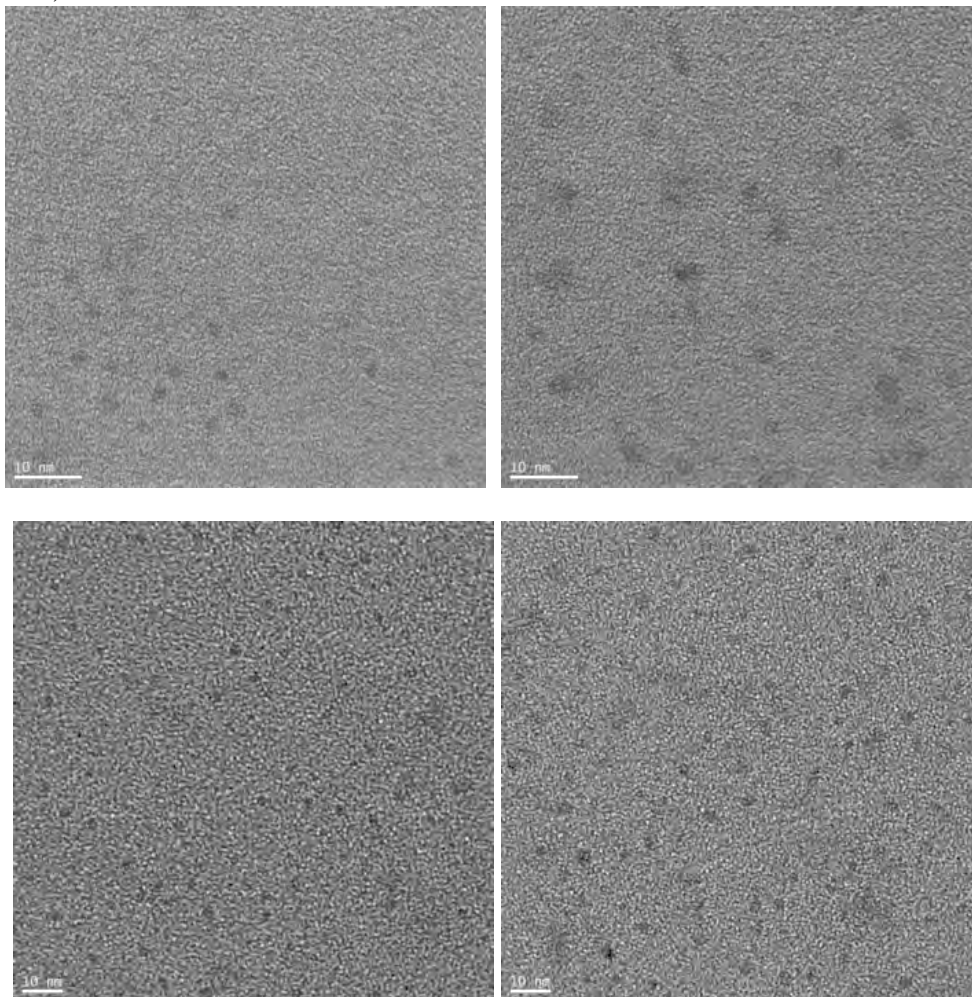
TEM images for batch 10 dispersed in  $1\text{gL}^{-1}$  sodium citrate solution.

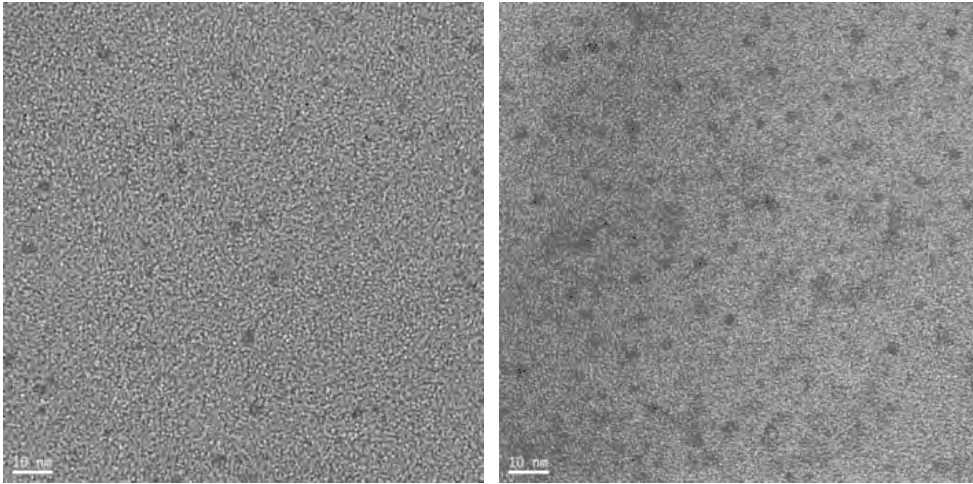
Sample diluted with water, and prepared by ultracentrifuge method.

a) Scale bars 20nm



b) Scale bars 10nm

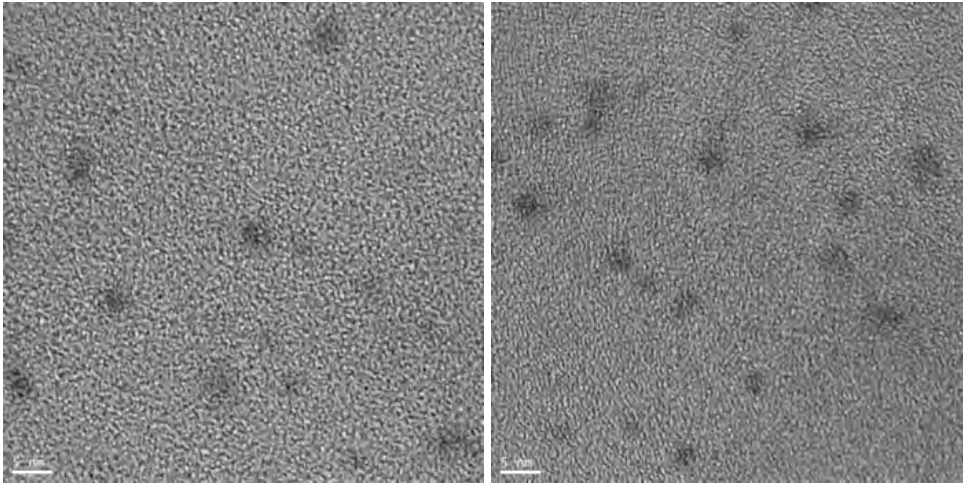




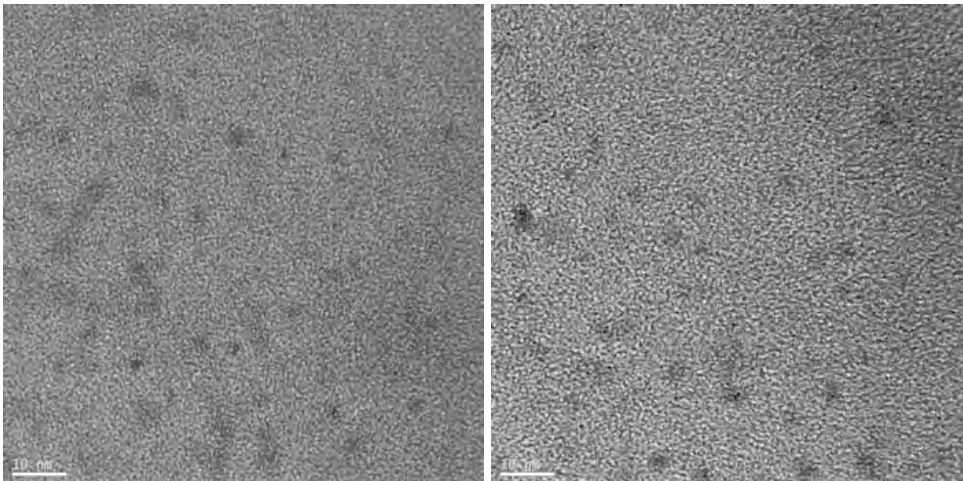


**Appendix 6.4 TEM images for batch 6 dispersed in  $1\text{gL}^{-1}$  sodium citrate solution.  
Sample diluted with water prior to ultracentrifugation.**

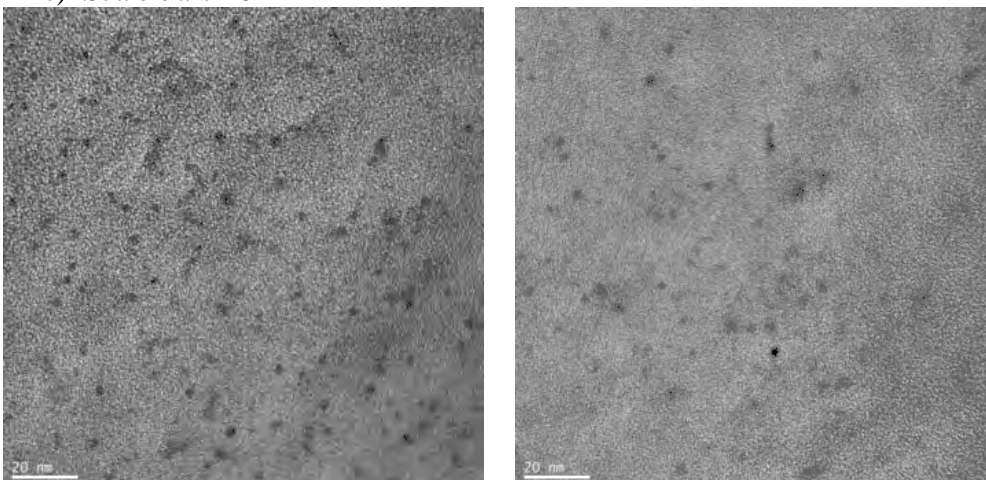
a) Scale bars 5nm

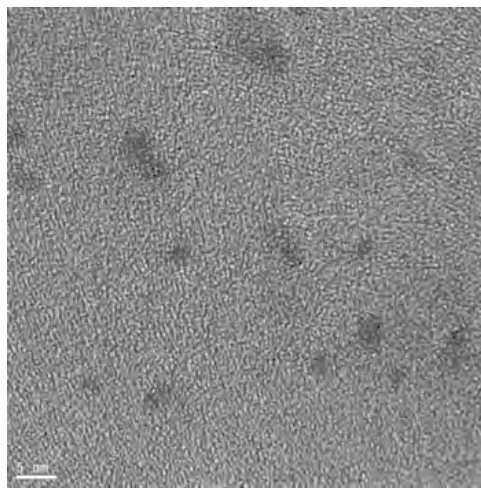
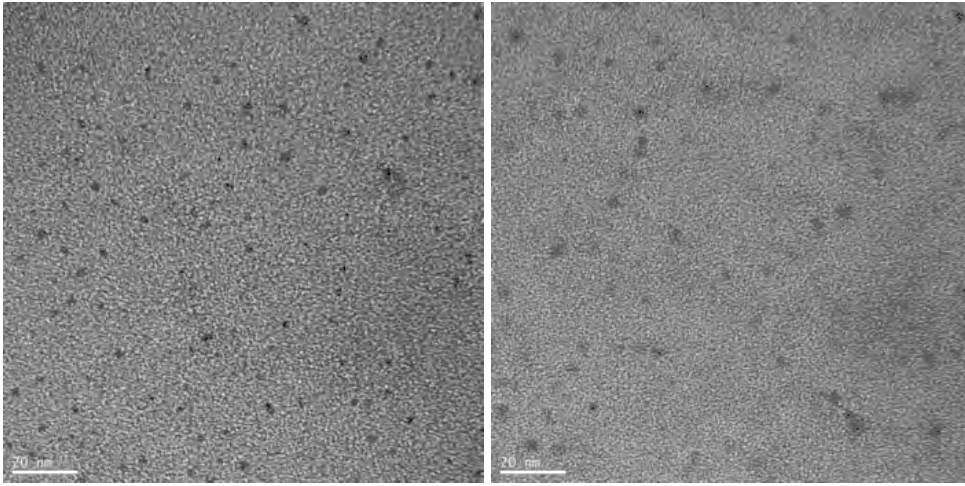


b) Scale bars 10nm



c) Scale bars 20nm





## Appendix 7.1

User: USER  
 Test Name: Pseudomonas  
 Absorbance

Path: C:\Program Files\BMG\Omega\User\Data\  
 Absorbance values are displayed as OD

Date: 26/06/2012  
 Time: 16:13:08

Well Row	Well Col	Content 1	Content 2	Raw Data (595) 1 - 0 h	Raw Data (595) 2 - 0 h 10 min	Raw Data (595) 3 - 0 h 20 min	Raw Data (595) 4 - 0 h 30 min	Raw Data (595) 5 - 0 h 40 min	Raw Data (595) 6 - 0 h 50 min	Raw Data (595) 7 - 1 h	Raw Data (595) 8 - 1 h 10 min
Time, Hours				0.00	0.17	0.33	0.50	0.67	0.83	1.00	1.17
A 1		Empty	Empty	0.1611	0.1717	0.1711	0.1706	0.1692	0.1696	0.1699	0.1694
A 2		comm CeO2 100mg/L	Bact +MDM 1:10	0.2792	0.2928	0.2927	0.2943	0.2933	0.2938	0.2924	0.2921
A 3		comm CeO2 50mg/L	Bact +MDM 1:10	0.2612	0.2478	0.2512	0.2481	0.2489	0.2465	0.2474	0.248
A 4		comm CeO2 20mg/L	Bact +MDM 1:10	0.2039	0.2144	0.2129	0.2126	0.2106	0.2096	0.2096	0.2099
A 5		comm CeO2 10mg/L	Bact +MDM 1:10	0.2298	0.2222	0.233	0.2502	0.2845	0.3491	0.3729	0.4093
A 6		Empty	Empty	0.1738	0.1854	0.1856	0.1859	0.1852	0.1842	0.1863	0.1858
A 7		Empty	Empty	0.21	0.2403	0.2405	0.2414	0.2395	0.2391	0.2411	0.2402
A 8		Empty	Empty	0.2023	0.2082	0.2091	0.21	0.2093	0.2089	0.2096	0.2096
A 9		Bact + MDM 1:1	Bact + MDM 1:1	0.3383	0.3543	0.2898	0.2899	0.2911	0.2941	0.2977	0.3001
A 10		Bact + MDM 1:10	Bact + MDM 1:10	0.2035	0.1766	0.1766	0.1743	0.171	0.1709	0.1864	0.1712
A 11		Bact + MDM 1:1	Bact + MDM 1:1	1.3418	0.7781	0.7516	0.7415	0.743	0.7237	0.6809	0.7297
A 12		MDM 1:1	MDM 1:1	0.1641	0.1698	0.1704	0.1701	0.1697	0.1694	0.1675	0.1684
B 1		Empty	Empty	0.1888	0.1854	0.1877	0.1863	0.1842	0.1859	0.1842	0.1883
B 2		comm CeO2 100mg/L	Bact +MDM 1:10	0.2976	0.3024	0.3054	0.305	0.3028	0.3037	0.3027	0.3042
B 3		comm CeO2 50mg/L	Bact +MDM 1:10	0.2322	0.2302	0.2299	0.2304	0.2311	0.2296	0.2307	0.2311
B 4		comm CeO2 20mg/L	Bact +MDM 1:10	0.2185	0.221	0.223	0.2212	0.2208	0.2194	0.2198	0.2191
B 5		comm CeO2 10mg/L	Bact +MDM 1:10	0.1875	0.1942	0.2106	0.2333	0.2616	0.2847	0.3029	0.326
B 6		Empty	Empty	0.2109	0.2018	0.2006	0.2	0.1989	0.1984	0.1997	0.1991
B 7		Empty	Empty	0.2191	0.2279	0.231	0.2296	0.2283	0.2275	0.2239	0.2265
B 8		Empty	Empty	0.2244	0.2293	0.2258	0.228	0.2276	0.2253	0.2288	0.226
B 9		Bact + MDM 1:1	Bact + MDM 1:1	0.2864	0.3366	0.2788	0.279	0.2809	0.2844	0.2896	0.2884
B 10		Bact + MDM 1:10	Bact + MDM 1:10	0.1948	0.224	0.2173	0.2184	0.2209	0.2191	0.2229	0.2227
B 11		Bact + MDM 1:1	Bact + MDM 1:1	0.1833	0.1769	0.1748	0.176	0.1763	0.1743	0.1754	0.173
B 12		MDM 1:1	MDM 1:1	0.1931	0.2283	0.225	0.2278	0.2304	0.2249	0.2301	0.2266

Appendix 7.1

Well Row	Well Col	Content	Group	Raw Data (595) 1 - 0 h	Raw Data (595) 2 - 0 h 10 min	Raw Data (595) 3 - 0 h 20 min	Raw Data (595) 4 - 0 h 30 min	Raw Data (595) 5 - 0 h 40 min	Raw Data (595) 6 - 0 h 50 min	Raw Data (595) 7 - 1 h	Raw Data (595) 8 - 1 h 10 min
Time, Hours				0.00	0.17	0.33	0.50	0.67	0.83	1.00	1.17
C 1		Empty	Empty	0.1655	0.1712	0.1722	0.171	0.1703	0.1684	0.1701	0.1701
C 2		comm CeO2 100mg/L	Bact +MDM 1:10	0.3428	0.3258	0.3247	0.3253	0.322	0.3214	0.3209	0.3208
C 3		comm CeO2 50mg/L	Bact +MDM 1:10	0.2467	0.2625	0.2625	0.2623	0.2594	0.2591	0.2609	0.2605
C 4		comm CeO2 20mg/L	Bact +MDM 1:10	0.2529	0.2421	0.2401	0.2414	0.2365	0.236	0.2374	0.2358
C 5		comm CeO2 10mg/L	Bact +MDM 1:10	0.2736	0.2398	0.2484	0.275	0.3029	0.3302	0.3489	0.3806
C 6		Empty	Empty	0.1791	0.1793	0.1769	0.1776	0.1758	0.1751	0.1758	0.176
C 7		Empty	Empty	0.2031	0.2092	0.209	0.2102	0.2072	0.2085	0.207	0.2084
C 8		Empty	Empty	0.1911	0.196	0.1957	0.1964	0.194	0.1948	0.1934	0.1937
C 9		Bact + MDM 1:1	Bact + MDM 1:1	0.3418	0.3685	0.3346	0.3366	0.356	0.341	0.348	0.3494
C 10		Bact + MDM 1:10	Bact + MDM 1:10	0.2498	0.26	0.2615	0.2618	0.2604	0.2605	0.2605	0.261
C 11		Bact + MDM 1:1	Bact + MDM 1:1	0.3798	0.2767	0.2613	0.2646	0.2577	0.2591	0.2742	0.2687
C 12		MDM 1:1	MDM 1:1	0.2284	0.2138	0.2114	0.2116	0.2112	0.2086	0.2121	0.2091
D 1		Empty	Empty	0.2356	0.2274	0.226	0.2256	0.224	0.2224	0.2231	0.2217
D 2		comm CeO2 100mg/L	Bact +MDM 1:10	0.2788	0.2817	0.2823	0.2831	0.2802	0.2834	0.2816	0.2828
D 3		comm CeO2 50mg/L	Bact +MDM 1:10	0.2325	0.225	0.222	0.2228	0.2204	0.2203	0.2209	0.2208
D 4		comm CeO2 20mg/L	Bact +MDM 1:10	0.2341	0.2271	0.2249	0.2273	0.2273	0.2253	0.2257	0.2251
D 5		comm CeO2 10mg/L	Bact +MDM 1:10	0.1774	0.1793	0.2017	0.2542	0.292	0.319	0.3569	0.382
D 6		Empty	Empty	0.2177	0.2176	0.214	0.2152	0.215	0.2133	0.2108	0.2107
D 7		Empty	Empty	0.2248	0.2238	0.2219	0.2217	0.2201	0.2201	0.2218	0.221
D 8		Empty	Empty	0.2298	0.227	0.226	0.2242	0.2229	0.2246	0.2251	0.2238
D 9		Bact + MDM 1:1	Bact + MDM 1:1	0.2771	0.2659	0.2711	0.2731	0.2761	0.2799	0.2833	0.2843
D 10		Bact + MDM 1:10	Bact + MDM 1:10	0.2033	0.2107	0.2081	0.2076	0.2064	0.2069	0.2071	0.2078
D 11		Bact + MDM 1:1	Bact + MDM 1:1	0.2334	0.2317	0.232	0.2323	0.2309	0.2303	0.2309	0.23
D 12		MDM 1:1	MDM 1:1	0.2164	0.1939	0.1919	0.1918	0.1919	0.1898	0.1906	0.1881

Appendix 7.1

Well Row	Well Col	Content	Group	Raw Data (595) 1 - 0 h	Raw Data (595) 2 - 0 h 10 min	Raw Data (595) 3 - 0 h 20 min	Raw Data (595) 4 - 0 h 30 min	Raw Data (595) 5 - 0 h 40 min	Raw Data (595) 6 - 0 h 50 min	Raw Data (595) 7 - 1 h	Raw Data (595) 8 - 1 h 10 min
Time, Hours				0.00	0.17	0.33	0.50	0.67	0.83	1.00	1.17
E 1		Empty	Empty	0.1886	0.1918	0.1903	0.1904	0.1892	0.1891	0.1889	0.1886
E 2		comm CeO2 100mg/L	Bact +MDM 1:10 + g	0.3185	0.3181	0.318	0.3236	0.3213	0.3241	0.3266	0.3278
E 3		comm CeO2 50mg/L	Bact +MDM 1:10 + g	0.2117	0.2193	0.2166	0.2206	0.2194	0.2204	0.22	0.2211
E 4		comm CeO2 20mg/L	Bact +MDM 1:10 + g	0.194	0.2043	0.2014	0.2051	0.2033	0.2031	0.2037	0.2066
E 5		comm CeO2 10mg/L	Bact +MDM 1:10 + g	0.2504	0.2465	0.2439	0.2454	0.2457	0.2486	0.2522	0.2533
E 6		Empty	Empty	0.2007	0.1992	0.2003	0.1989	0.1985	0.1979	0.1985	0.1971
F 1		Empty	Empty	0.1989	0.1997	0.1996	0.1998	0.1985	0.1974	0.1973	0.1979
F 2		comm CeO2 100mg/L	Bact +MDM 1:10 + g	0.3425	0.3292	0.3277	0.3298	0.3315	0.3314	0.3338	0.3338
F 3		comm CeO2 50mg/L	Bact +MDM 1:10 + g	0.2547	0.2554	0.255	0.2572	0.2585	0.2582	0.2604	0.2604
F 4		comm CeO2 20mg/L	Bact +MDM 1:10 + g	0.1853	0.1801	0.1791	0.1811	0.1788	0.1804	0.1795	0.18
F 5		comm CeO2 10mg/L	Bact +MDM 1:10 + g	0.1995	0.2102	0.2115	0.2112	0.2102	0.2104	0.21	0.2091
F 6		Empty	Empty	0.2431	0.2437	0.2452	0.2444	0.2428	0.2422	0.241	0.2413
G 1		Empty	Empty	0.2921	0.2972	0.2984	0.2982	0.2968	0.2997	0.3015	0.3021
G 2		comm CeO2 100mg/L	Bact +MDM 1:10 + g	0.3026	0.3024	0.3048	0.3054	0.3029	0.304	0.3051	0.3067
G 3		comm CeO2 50mg/L	Bact +MDM 1:10 + g	0.2608	0.2488	0.2504	0.252	0.2505	0.2522	0.2526	0.2537
G 4		comm CeO2 20mg/L	Bact +MDM 1:10 + g	0.2129	0.2068	0.2076	0.2094	0.2075	0.2077	0.2104	0.2099
G 5		comm CeO2 10mg/L	Bact +MDM 1:10 + g	0.2045	0.1917	0.1906	0.1903	0.1892	0.1884	0.1872	0.1893
G 6		Empty	Empty	0.1784	0.1789	0.1792	0.1805	0.1785	0.1791	0.1799	0.18
H 1		Empty	Empty	0.1928	0.2015	0.2016	0.2035	0.2016	0.2008	0.2036	0.2035
H 2		comm CeO2 100mg/L	Bact +MDM 1:10 + g	0.5483	0.6158	0.581	0.552	0.5987	0.5971	0.6383	0.5951
H 3		comm CeO2 50mg/L	Bact +MDM 1:10 + g	0.2422	0.2488	0.2479	0.2483	0.2485	0.2486	0.2508	0.2506
H 4		comm CeO2 20mg/L	Bact +MDM 1:10 + g	0.1797	0.1705	0.171	0.1701	0.1697	0.1694	0.1695	0.1689
H 5		comm CeO2 10mg/L	Bact +MDM 1:10 + g	0.16	0.1578	0.157	0.1586	0.1576	0.1577	0.1571	0.1591
H 6		Empty	Empty	0.1884	0.1979	0.1979	0.1969	0.1951	0.1953	0.1964	0.1962

## Appendix 7.1

User: USER

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 9 - 1 h 19 min	Raw Data (595) 10 - 1 h 30 min	Raw Data (595) 11 - 1 h 40 min	Raw Data (595) 12 - 1 h 50 min	Raw Data (595) 13 - 2 h	Raw Data (595) 14 - 2 h 9 min	Raw Data (595) 15 - 2 h 20 min	Raw Data (595) 16 - 2 h 30 min	Raw Data (595) 17 - 2 h 39 min	Raw Data (595) 18 - 2 h 50 min	Raw Data (595) 19 - 3 h	Raw Data (595) 20 - 3 h 9 min
Time, Hours		1.33	1.50	1.67	1.83	2.00	2.17	2.33	2.50	2.67	2.83	3.00	3.17
A 1		0.1677	0.1703	0.1691	0.1701	0.1685	0.169	0.169	0.1695	0.169	0.1685	0.1699	0.1686
A 2		0.2955	0.2941	0.2923	0.294	0.2938	0.2932	0.2929	0.2937	0.2937	0.293	0.2938	0.2933
A 3		0.2732	0.2569	0.2569	0.2604	0.2574	0.2573	0.2598	0.2582	0.2572	0.2586	0.2585	0.2565
A 4		0.1989	0.2087	0.2088	0.2093	0.2076	0.2084	0.208	0.2076	0.2069	0.2077	0.2081	0.2075
A 5		0.4196	0.4337	0.4573	0.4698	0.474	0.4879	0.4999	0.5075	0.5087	0.5112	0.5108	0.5236
A 6		0.188	0.1856	0.1857	0.1856	0.1844	0.1839	0.1847	0.1845	0.1845	0.1835	0.1845	0.1838
A 7		0.2394	0.2401	0.2404	0.2406	0.2417	0.2462	0.247	0.2481	0.2479	0.2485	0.2488	0.2482
A 8		0.2157	0.2095	0.2101	0.2107	0.2093	0.2101	0.2103	0.2103	0.2099	0.21	0.2096	0.2093
A 9		0.304	0.3059	0.3073	0.3117	0.3146	0.323	0.3348	0.342	0.3515	0.3398	0.3715	0.3768
A 10		0.1614	0.1717	0.1718	0.1722	0.1744	0.1758	0.1768	0.1914	0.1806	0.1841	0.1879	0.1902
A 11		0.896	0.7038	0.7116	0.6864	0.7072	0.7052	0.6962	0.7065	0.6755	0.6958	0.6618	0.6794
A 12		0.1786	0.1697	0.1684	0.1671	0.167	0.1677	0.1682	0.1667	0.1665	0.1676	0.1673	0.1668
B 1		0.1987	0.1906	0.1892	0.1892	0.189	0.1889	0.1909	0.1899	0.1914	0.1936	0.1915	0.1921
B 2		0.3076	0.3073	0.307	0.3071	0.3088	0.3077	0.3072	0.3073	0.3077	0.3089	0.3088	0.3081
B 3		0.2296	0.2318	0.2313	0.2327	0.2332	0.2318	0.2334	0.2332	0.2334	0.234	0.233	0.2334
B 4		0.2204	0.2216	0.2207	0.2224	0.2216	0.2218	0.2213	0.2207	0.2211	0.2211	0.2215	0.2215
B 5		0.3742	0.3926	0.4029	0.4314	0.4239	0.4572	0.4848	0.4892	0.4895	0.4737	0.4898	0.4946
B 6		0.1874	0.1987	0.2011	0.1991	0.2002	0.1987	0.1994	0.1995	0.2019	0.201	0.2007	0.2006
B 7		0.2419	0.2296	0.2276	0.2296	0.2269	0.2286	0.2288	0.2282	0.225	0.2283	0.2266	0.2277
B 8		0.2134	0.2256	0.2259	0.2276	0.2245	0.2247	0.2253	0.2248	0.2266	0.2258	0.2265	0.2244
B 9		0.2849	0.2976	0.3016	0.3063	0.3083	0.315	0.3225	0.3292	0.3443	0.3518	0.3723	0.3942
B 10		0.2277	0.2261	0.2262	0.2287	0.2273	0.229	0.2307	0.2298	0.2353	0.2337	0.2397	0.2428
B 11		0.1739	0.1742	0.173	0.1749	0.1733	0.1716	0.1728	0.1714	0.1737	0.1724	0.1736	0.1726
B 12		0.2061	0.2266	0.2288	0.2357	0.2306	0.2319	0.2334	0.2319	0.2361	0.2322	0.2343	0.233

Appendix 7.1

Well Row	Well Col	Raw Data (595) 9 - 1 h 19 min	Raw Data (595) 10 - 1 h 30 min	Raw Data (595) 11 - 1 h 40 min	Raw Data (595) 12 - 1 h 50 min	Raw Data (595) 13 - 2 h	Raw Data (595) 14 - 2 h 9 min	Raw Data (595) 15 - 2 h 20 min	Raw Data (595) 16 - 2 h 30 min	Raw Data (595) 17 - 2 h 39 min	Raw Data (595) 18 - 2 h 50 min	Raw Data (595) 19 - 3 h	Raw Data (595) 20 - 3 h 9 min
Time, Hours		1.33	1.50	1.67	1.83	2.00	2.17	2.33	2.50	2.67	2.83	3.00	3.17
C 1		0.18	0.1703	0.1694	0.1704	0.1685	0.1696	0.1693	0.1698	0.1703	0.17	0.1705	0.1695
C 2		0.324	0.3225	0.3224	0.3229	0.3233	0.3232	0.3249	0.3245	0.3278	0.3312	0.335	0.3395
C 3		0.2664	0.2621	0.2605	0.2619	0.2612	0.2617	0.262	0.2612	0.2619	0.2626	0.2626	0.2623
C 4		0.2357	0.2347	0.2365	0.2393	0.242	0.2397	0.2419	0.2411	0.2445	0.2406	0.244	0.2432
C 5		0.3785	0.4352	0.4186	0.448	0.4439	0.4671	0.4714	0.4715	0.4764	0.4753	0.4833	0.4817
C 6		0.1788	0.1757	0.1764	0.1757	0.175	0.1744	0.1753	0.175	0.1754	0.1759	0.1754	0.1753
C 7		0.1983	0.2084	0.2094	0.2078	0.2072	0.208	0.2085	0.2074	0.2068	0.2082	0.2068	0.2067
C 8		0.1908	0.1945	0.1949	0.1945	0.1941	0.1947	0.1941	0.1936	0.1931	0.1941	0.1939	0.1932
C 9		0.3543	0.3632	0.3616	0.3642	0.3692	0.3759	0.3799	0.3838	0.3906	0.4091	0.4093	0.4116
C 10		0.2545	0.2624	0.2634	0.2642	0.2644	0.2651	0.2667	0.2693	0.2671	0.2729	0.2748	0.2803
C 11		0.2935	0.2656	0.2623	0.268	0.2641	0.2539	0.2567	0.2546	0.2674	0.2544	0.2721	0.2609
C 12		0.2264	0.2093	0.2113	0.2097	0.206	0.2072	0.2079	0.2063	0.2074	0.2044	0.2099	0.2072
D 1		0.2208	0.2217	0.2207	0.2205	0.2204	0.2203	0.22	0.2192	0.2202	0.2193	0.2189	0.2178
D 2		0.272	0.285	0.2847	0.285	0.2859	0.285	0.2856	0.2864	0.2888	0.2869	0.2885	0.289
D 3		0.2167	0.2219	0.2223	0.2238	0.2225	0.223	0.2249	0.2244	0.2261	0.2256	0.2261	0.2269
D 4		0.2099	0.2241	0.2236	0.2235	0.2225	0.2223	0.2228	0.2227	0.223	0.2226	0.2223	0.2229
D 5		0.3522	0.4407	0.4789	0.5087	0.5326	0.5496	0.5546	0.5449	0.546	0.55	0.5415	0.5281
D 6		0.2394	0.2111	0.2131	0.2104	0.2101	0.2114	0.2091	0.2114	0.2082	0.2083	0.2115	0.2077
D 7		0.2014	0.2202	0.2203	0.2205	0.2193	0.2187	0.2194	0.2196	0.2206	0.2184	0.2186	0.217
D 8		0.2059	0.2247	0.2254	0.2258	0.2241	0.2245	0.2236	0.2235	0.2239	0.2229	0.2242	0.2236
D 9		0.2895	0.293	0.2928	0.2943	0.2972	0.3048	0.3086	0.3164	0.3169	0.3303	0.3403	0.3532
D 10		0.2025	0.2086	0.2105	0.2117	0.2114	0.2138	0.2146	0.2164	0.2177	0.2202	0.2203	0.2254
D 11		0.2252	0.23	0.2309	0.2294	0.2296	0.2295	0.2339	0.2292	0.2305	0.229	0.2306	0.2289
D 12		0.1939	0.188	0.1899	0.1881	0.1875	0.1887	0.1879	0.1881	0.1872	0.1868	0.1871	0.1862

Appendix 7.1

Well Row	Well Col	Raw Data (595) 9 - 1 h 19 min	Raw Data (595) 10 - 1 h 30 min	Raw Data (595) 11 - 1 h 40 min	Raw Data (595) 12 - 1 h 50 min	Raw Data (595) 13 - 2 h	Raw Data (595) 14 - 2 h 9 min	Raw Data (595) 15 - 2 h 20 min	Raw Data (595) 16 - 2 h 30 min	Raw Data (595) 17 - 2 h 39 min	Raw Data (595) 18 - 2 h 50 min	Raw Data (595) 19 - 3 h	Raw Data (595) 20 - 3 h 9 min
Time, Hours		1.33	1.50	1.67	1.83	2.00	2.17	2.33	2.50	2.67	2.83	3.00	3.17
E 1		0.1895	0.188	0.1885	0.1883	0.188	0.1883	0.1882	0.1887	0.1888	0.1884	0.1871	0.1872
E 2		0.321	0.3285	0.332	0.3338	0.339	0.3453	0.3551	0.3646	0.3728	0.3775	0.3835	0.3849
E 3		0.2171	0.2197	0.22	0.2218	0.2213	0.2229	0.2232	0.2261	0.2272	0.2264	0.2268	0.2357
E 4		0.1906	0.2035	0.2049	0.2027	0.2055	0.2061	0.2067	0.2087	0.2092	0.208	0.2102	0.2107
E 5		0.244	0.2543	0.2544	0.2556	0.2588	0.2588	0.2576	0.2592	0.2608	0.2598	0.2599	0.2603
E 6		0.2124	0.1996	0.1985	0.1988	0.1974	0.1978	0.1985	0.1971	0.1976	0.1981	0.1979	0.1966
F 1		0.196	0.1981	0.1981	0.1987	0.199	0.1993	0.1993	0.1984	0.1997	0.199	0.1988	0.1984
F 2		0.3378	0.3385	0.3406	0.3454	0.3489	0.351	0.3558	0.3591	0.366	0.3703	0.3736	0.3771
F 3		0.2681	0.2607	0.2637	0.265	0.2654	0.2671	0.2695	0.2705	0.271	0.2734	0.2705	0.2712
F 4		0.1805	0.1817	0.1813	0.1813	0.1816	0.1818	0.1825	0.1842	0.1845	0.186	0.1863	0.1874
F 5		0.2016	0.2123	0.2101	0.2115	0.2099	0.2143	0.2117	0.2105	0.2133	0.2169	0.2229	0.2198
F 6		0.2252	0.243	0.2402	0.2409	0.2401	0.2416	0.2409	0.2401	0.2403	0.2414	0.2396	0.2405
G 1		0.3014	0.303	0.3032	0.3042	0.3047	0.3046	0.3052	0.3056	0.305	0.305	0.3055	0.3069
G 2		0.3085	0.3068	0.3057	0.3086	0.3102	0.3123	0.3144	0.3159	0.319	0.3202	0.3207	0.3218
G 3		0.2575	0.2526	0.2536	0.2543	0.2542	0.2546	0.2549	0.2569	0.2541	0.2551	0.2553	0.2562
G 4		0.2436	0.2106	0.2108	0.2103	0.2122	0.2118	0.2128	0.2115	0.216	0.2112	0.2116	0.2117
G 5		0.1727	0.1884	0.1874	0.1884	0.1879	0.1897	0.1873	0.1873	0.1883	0.1889	0.195	0.1903
G 6		0.1934	0.1791	0.1789	0.1785	0.1798	0.1791	0.1795	0.1781	0.1796	0.1788	0.1791	0.1784
H 1		0.2004	0.2031	0.204	0.2039	0.2039	0.2033	0.2055	0.2054	0.2062	0.2071	0.2064	0.2051
H 2		0.4504	0.6044	0.6207	0.6321	0.6171	0.5926	0.6141	0.5916	0.6099	0.6152	0.6036	0.5769
H 3		0.2527	0.2515	0.251	0.2521	0.2526	0.2536	0.2545	0.2537	0.2552	0.2554	0.2558	0.2556
H 4		0.1765	0.1675	0.1695	0.1681	0.1695	0.1709	0.172	0.1723	0.1728	0.1726	0.1733	0.1759
H 5		0.1738	0.1603	0.1593	0.1598	0.1604	0.1597	0.1612	0.1613	0.1619	0.1631	0.1637	0.1638
H 6		0.1893	0.1978	0.1973	0.1965	0.1957	0.1966	0.1975	0.1978	0.1985	0.1982	0.1994	0.1968



## Appendix 7.1

User: USER

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 21 - 3 h 20 min	Raw Data (595) 22 - 3 h 30 min	Raw Data (595) 23 - 3 h 39 min	Raw Data (595) 24 - 3 h 50 min	Raw Data (595) 25 - 4 h	Raw Data (595) 26 - 4 h 10 min	Raw Data (595) 27 - 4 h 19 min	Raw Data (595) 28 - 4 h 30 min	Raw Data (595) 29 - 4 h 40 min	Raw Data (595) 30 - 4 h 49 min	Raw Data (595) 31 - 5 h	Raw Data (595) 32 - 5 h 10 min
Time, Hours		3.33	3.50	3.67	3.83	4.00	4.17	4.33	4.50	4.67	4.83	5.00	5.17
A 1		0.1682	0.1682	0.1692	0.1675	0.1673	0.1697	0.1685	0.1696	0.1695	0.168	0.1685	0.1694
A 2		0.2918	0.2908	0.2919	0.2903	0.2905	0.2915	0.2916	0.292	0.2927	0.2916	0.2908	0.292
A 3		0.2555	0.258	0.2585	0.2568	0.2596	0.2574	0.2573	0.2577	0.2584	0.2567	0.2569	0.2577
A 4		0.2071	0.2084	0.2081	0.2068	0.208	0.2095	0.2085	0.2093	0.2096	0.2083	0.2094	0.2093
A 5		0.5164	0.5166	0.5052	0.5089	0.5134	0.5077	0.5056	0.4878	0.4893	0.5009	0.499	0.4833
A 6		0.1833	0.1841	0.1845	0.1836	0.183	0.1832	0.1835	0.184	0.1837	0.183	0.183	0.1841
A 7		0.2494	0.2486	0.2502	0.2489	0.2484	0.2537	0.2552	0.2562	0.2565	0.2559	0.2557	0.2564
A 8		0.2099	0.2088	0.2092	0.2086	0.2087	0.2098	0.2095	0.2095	0.2093	0.2097	0.2094	0.209
A 9		0.3593	0.3605	0.3609	0.3575	0.3571	0.3531	0.3528	0.351	0.3501	0.3477	0.3475	0.3465
A 10		0.1941	0.1917	0.1945	0.1953	0.1969	0.2074	0.2156	0.2282	0.2531	0.2626	0.2428	0.2871
A 11		0.6618	0.7092	0.6832	0.6548	0.7328	0.6663	0.6817	0.6525	0.6681	0.6358	0.6654	0.6593
A 12		0.1658	0.1674	0.1678	0.1672	0.1683	0.1659	0.1667	0.167	0.1662	0.1663	0.1672	0.1678
B 1		0.1924	0.1921	0.1921	0.1916	0.1926	0.1921	0.1939	0.1937	0.1924	0.1921	0.1931	0.1949
B 2		0.3083	0.3108	0.312	0.3111	0.312	0.312	0.3143	0.3139	0.313	0.3136	0.3157	0.3165
B 3		0.2326	0.2338	0.2334	0.2327	0.2345	0.2346	0.2343	0.2362	0.2357	0.2336	0.2347	0.2359
B 4		0.2212	0.2227	0.223	0.2214	0.2222	0.223	0.2228	0.2247	0.223	0.2234	0.2235	0.2238
B 5		0.4875	0.4997	0.4996	0.5124	0.4785	0.4664	0.49	0.4762	0.4495	0.4489	0.4575	0.4538
B 6		0.2011	0.2033	0.2019	0.2021	0.2026	0.2047	0.2019	0.2027	0.2038	0.2011	0.2014	0.2033
B 7		0.2283	0.2264	0.2284	0.2254	0.2267	0.2257	0.2281	0.2281	0.2246	0.2263	0.2291	0.2275
B 8		0.2237	0.2255	0.2242	0.2244	0.2244	0.2256	0.2237	0.2246	0.2269	0.2234	0.2237	0.2261
B 9		0.3573	0.3568	0.3568	0.3577	0.3529	0.3556	0.3533	0.3513	0.3538	0.3481	0.35	0.3497
B 10		0.2448	0.2464	0.2486	0.2496	0.2507	0.2541	0.2577	0.268	0.2996	0.3027	0.3119	0.3528
B 11		0.1735	0.177	0.1767	0.1764	0.1764	0.1793	0.1779	0.1822	0.185	0.1843	0.1867	0.1896
B 12		0.2335	0.2395	0.2327	0.2329	0.236	0.2364	0.2322	0.2379	0.2372	0.2301	0.2331	0.2299

Appendix 7.1

Well Row	Well Col	Raw Data (595) 21 - 3 h 20 min	Raw Data (595) 22 - 3 h 30 min	Raw Data (595) 23 - 3 h 39 min	Raw Data (595) 24 - 3 h 50 min	Raw Data (595) 25 - 4 h	Raw Data (595) 26 - 4 h 10 min	Raw Data (595) 27 - 4 h 19 min	Raw Data (595) 28 - 4 h 30 min	Raw Data (595) 29 - 4 h 40 min	Raw Data (595) 30 - 4 h 49 min	Raw Data (595) 31 - 5 h	Raw Data (595) 32 - 5 h 10 min
Time, Hours		3.33	3.50	3.67	3.83	4.00	4.17	4.33	4.50	4.67	4.83	5.00	5.17
C 1		0.1701	0.1696	0.1705	0.1683	0.1699	0.1692	0.1706	0.1711	0.1702	0.1706	0.1714	0.1717
C 2		0.3443	0.3499	0.3545	0.359	0.3642	0.3675	0.37	0.3729	0.3731	0.3734	0.375	0.3737
C 3		0.2616	0.263	0.2656	0.2669	0.267	0.2698	0.2714	0.2735	0.2763	0.2775	0.2805	0.2824
C 4		0.2449	0.2415	0.2419	0.2414	0.2415	0.2421	0.2419	0.2424	0.2441	0.2452	0.2431	0.2429
C 5		0.4806	0.4737	0.4688	0.4673	0.4587	0.4596	0.4494	0.4606	0.4568	0.4673	0.4571	0.4547
C 6		0.1749	0.1758	0.1748	0.1739	0.1745	0.1741	0.175	0.1748	0.1745	0.1741	0.1741	0.174
C 7		0.2067	0.2087	0.2086	0.2069	0.2073	0.2079	0.2076	0.2059	0.2075	0.206	0.2062	0.207
C 8		0.1929	0.1945	0.1948	0.1921	0.1926	0.1942	0.1941	0.1933	0.193	0.1921	0.193	0.1934
C 9		0.4141	0.4184	0.4171	0.4135	0.4126	0.4113	0.4119	0.4135	0.4104	0.4098	0.408	0.4088
C 10		0.2867	0.2872	0.2892	0.287	0.288	0.2977	0.3103	0.3295	0.3428	0.3618	0.373	0.399
C 11		0.2658	0.2605	0.2604	0.2588	0.2552	0.2589	0.2567	0.2618	0.2626	0.263	0.2621	0.2629
C 12		0.2075	0.2046	0.2075	0.2056	0.2023	0.2038	0.2027	0.211	0.2094	0.2122	0.2135	0.2154
D 1		0.2183	0.2182	0.2184	0.2164	0.217	0.218	0.2165	0.2166	0.2181	0.218	0.2179	0.2162
D 2		0.2904	0.2947	0.2965	0.2993	0.3034	0.3102	0.3155	0.3219	0.3274	0.3364	0.3428	0.347
D 3		0.2279	0.2309	0.2324	0.2327	0.2342	0.2374	0.2406	0.2429	0.2456	0.2475	0.2503	0.254
D 4		0.223	0.2242	0.2239	0.2232	0.2236	0.2241	0.2238	0.2251	0.2249	0.2253	0.2253	0.2253
D 5		0.5162	0.5212	0.5366	0.5322	0.5257	0.5216	0.5425	0.5517	0.5341	0.5353	0.5309	0.5327
D 6		0.2067	0.2122	0.2114	0.2086	0.2064	0.2096	0.2072	0.2095	0.2068	0.2068	0.207	0.2092
D 7		0.2189	0.2197	0.2201	0.2181	0.2193	0.2192	0.2187	0.2182	0.2184	0.2193	0.2199	0.2183
D 8		0.2236	0.224	0.2245	0.222	0.2255	0.2251	0.2259	0.2261	0.2269	0.2269	0.2283	0.2275
D 9		0.3535	0.3451	0.3459	0.3414	0.3435	0.3411	0.3413	0.3397	0.339	0.3366	0.3378	0.3375
D 10		0.2287	0.2335	0.2344	0.2329	0.2384	0.2444	0.2538	0.2701	0.2817	0.3034	0.3322	0.3371
D 11		0.2288	0.2303	0.2315	0.2307	0.2313	0.2336	0.2367	0.2386	0.2391	0.2406	0.2434	0.2447
D 12		0.1858	0.1871	0.1881	0.1853	0.1861	0.1876	0.1854	0.1877	0.1852	0.1857	0.1857	0.1865

## Appendix 7.1

Well Row	Well Col	Raw Data (595) 21 - 3 h 20 min	Raw Data (595) 22 - 3 h 30 min	Raw Data (595) 23 - 3 h 39 min	Raw Data (595) 24 - 3 h 50 min	Raw Data (595) 25 - 4 h	Raw Data (595) 26 - 4 h 10 min	Raw Data (595) 27 - 4 h 19 min	Raw Data (595) 28 - 4 h 30 min	Raw Data (595) 29 - 4 h 40 min	Raw Data (595) 30 - 4 h 49 min	Raw Data (595) 31 - 5 h	Raw Data (595) 32 - 5 h 10 min
Time, Hours		3.33	3.50	3.67	3.83	4.00	4.17	4.33	4.50	4.67	4.83	5.00	5.17
E 1		0.1876	0.1887	0.1884	0.1866	0.1875	0.1876	0.187	0.1873	0.1872	0.1869	0.1881	0.1878
E 2		0.3871	0.3916	0.39	0.3904	0.39	0.392	0.3915	0.3948	0.3946	0.395	0.399	0.3996
E 3		0.2299	0.2315	0.2309	0.2305	0.2318	0.2331	0.2354	0.2363	0.2362	0.2388	0.2383	0.2412
E 4		0.2109	0.214	0.2131	0.2142	0.2156	0.22	0.2204	0.2202	0.2238	0.2204	0.224	0.2279
E 5		0.2615	0.2646	0.2649	0.2644	0.2678	0.2644	0.2673	0.271	0.2724	0.2719	0.2747	0.2769
E 6		0.1957	0.1968	0.198	0.1965	0.1946	0.1979	0.1973	0.1964	0.1957	0.1969	0.1972	0.1969
F 1		0.1982	0.1979	0.1989	0.1977	0.1974	0.1997	0.1976	0.1977	0.1989	0.198	0.1983	0.1973
F 2		0.3798	0.3848	0.3895	0.3913	0.3939	0.3947	0.406	0.4015	0.4041	0.4059	0.4089	0.4174
F 3		0.2709	0.2739	0.2763	0.2777	0.277	0.2784	0.2786	0.2861	0.2824	0.2855	0.2865	0.2948
F 4		0.1865	0.1893	0.1922	0.1903	0.1894	0.192	0.1934	0.193	0.1956	0.1956	0.1973	0.2012
F 5		0.2202	0.2232	0.2194	0.2187	0.2211	0.2234	0.2257	0.2237	0.2257	0.2276	0.2286	0.23
F 6		0.2397	0.2389	0.2393	0.2375	0.2385	0.2397	0.2398	0.2375	0.2398	0.2376	0.2379	0.2378
G 1		0.3046	0.3063	0.3062	0.3058	0.3043	0.3073	0.3085	0.3076	0.3084	0.308	0.3084	0.3098
G 2		0.3215	0.3248	0.327	0.3275	0.3275	0.3313	0.3316	0.3348	0.3364	0.3373	0.3381	0.3389
G 3		0.2575	0.2604	0.2604	0.2596	0.2597	0.2604	0.2632	0.2647	0.2641	0.2657	0.2675	0.2698
G 4		0.2209	0.2208	0.2139	0.2139	0.2165	0.2184	0.2145	0.2166	0.2208	0.2218	0.228	0.224
G 5		0.194	0.1913	0.1923	0.1917	0.1918	0.1945	0.199	0.1969	0.1954	0.1967	0.1993	0.2017
G 6		0.1777	0.1794	0.1785	0.1786	0.1774	0.1785	0.1769	0.1779	0.1788	0.1776	0.1789	0.1784
H 1		0.2064	0.2083	0.2079	0.2077	0.2071	0.2082	0.2068	0.2092	0.2094	0.2087	0.2097	0.2091
H 2		0.5827	0.5815	0.6146	0.5717	0.5574	0.568	0.5255	0.5976	0.5655	0.5629	0.5331	0.5356
H 3		0.2561	0.2597	0.2615	0.2586	0.2579	0.2586	0.2597	0.2605	0.263	0.2635	0.2661	0.2699
H 4		0.1736	0.1738	0.1762	0.1755	0.1744	0.1752	0.1776	0.177	0.1804	0.1826	0.1855	0.185
H 5		0.1635	0.1658	0.1667	0.1656	0.1667	0.1673	0.1692	0.1702	0.1694	0.1698	0.173	0.1751
H 6		0.1976	0.1993	0.1984	0.1985	0.1984	0.2003	0.1989	0.2011	0.2004	0.1999	0.2007	0.2014

## Appendix 7.1

User: USER

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 33 - 5 h 19 min	Raw Data (595) 34 - 5 h 30 min	Raw Data (595) 35 - 5 h 40 min	Raw Data (595) 36 - 5 h 49 min	Raw Data (595) 37 - 6 h	Raw Data (595) 38 - 6 h 10 min	Raw Data (595) 39 - 6 h 19 min	Raw Data (595) 40 - 6 h 30 min	Raw Data (595) 41 - 6 h 40 min	Raw Data (595) 42 - 6 h 49 min	Raw Data (595) 43 - 7 h	Raw Data (595) 44 - 7 h 10 min
Time, Hours		5.33	5.50	5.67	5.83	6.00	6.17	6.33	6.50	6.67	6.83	7.00	7.17
A 1		0.1692	0.1699	0.1687	0.1703	0.1699	0.1709	0.1697	0.1704	0.1691	0.1712	0.1697	0.1698
A 2		0.2913	0.2932	0.2948	0.2946	0.2924	0.2943	0.2945	0.2948	0.2935	0.2934	0.2929	0.2925
A 3		0.2569	0.2572	0.2587	0.2584	0.2576	0.2586	0.2578	0.2766	0.2584	0.258	0.261	0.2574
A 4		0.2082	0.2087	0.2093	0.208	0.2077	0.2099	0.2165	0.2014	0.2104	0.2106	0.2106	0.2089
A 5		0.4839	0.4974	0.5051	0.5072	0.5062	0.5008	0.4983	0.5045	0.494	0.5012	0.4781	0.4751
A 6		0.1824	0.1835	0.1828	0.1833	0.1836	0.1834	0.184	0.1853	0.1823	0.1834	0.1832	0.1806
A 7		0.2542	0.2573	0.256	0.2574	0.2568	0.2572	0.2569	0.2563	0.2564	0.2574	0.2574	0.255
A 8		0.2082	0.2096	0.2085	0.2087	0.2079	0.2085	0.2089	0.2144	0.2086	0.2082	0.2091	0.2074
A 9		0.3444	0.3444	0.3441	0.343	0.3412	0.3421	0.342	0.3433	0.3394	0.3387	0.3396	0.3371
A 10		0.2981	0.3016	0.2931	0.2945	0.291	0.2895	0.2877	0.274	0.2848	0.284	0.2827	0.2801
A 11		0.6602	0.6414	0.6681	0.6431	0.6484	0.6709	0.6522	0.7987	0.6731	0.6407	0.6793	0.6785
A 12		0.1669	0.1649	0.1673	0.1658	0.1658	0.1663	0.1659	0.1767	0.1656	0.164	0.1659	0.1637
B 1		0.1941	0.1948	0.1948	0.196	0.1947	0.1956	0.1964	0.2009	0.1933	0.1963	0.1944	0.1939
B 2		0.3182	0.3212	0.3233	0.33	0.3347	0.3419	0.3489	0.3526	0.3649	0.3763	0.3841	0.3898
B 3		0.234	0.2355	0.2351	0.2359	0.235	0.2362	0.2364	0.2351	0.2369	0.2377	0.2379	0.2368
B 4		0.2224	0.2249	0.2235	0.2256	0.2244	0.225	0.2249	0.2231	0.2239	0.2241	0.2258	0.2222
B 5		0.438	0.4466	0.4465	0.4836	0.473	0.5042	0.4648	0.4637	0.4826	0.4881	0.4808	0.4785
B 6		0.2033	0.2024	0.2027	0.2033	0.2023	0.2027	0.2025	0.189	0.2049	0.2037	0.2033	0.203
B 7		0.2254	0.228	0.2254	0.2272	0.225	0.2282	0.227	0.237	0.2236	0.2262	0.2255	0.2213
B 8		0.2239	0.2239	0.2256	0.2237	0.2234	0.2227	0.2241	0.2088	0.2256	0.2242	0.2251	0.2229
B 9		0.3495	0.3466	0.3471	0.3453	0.3449	0.3439	0.3431	0.336	0.3451	0.3446	0.3443	0.3399
B 10		0.3355	0.3431	0.3348	0.3434	0.3401	0.3394	0.3369	0.3395	0.3376	0.3366	0.3396	0.336
B 11		0.1925	0.1941	0.2005	0.2038	0.2099	0.2177	0.2296	0.2496	0.2563	0.281	0.2814	0.2976
B 12		0.2336	0.2381	0.2367	0.2332	0.231	0.2309	0.2347	0.2042	0.2395	0.2356	0.239	0.2322

Appendix 7.1

Well Row	Well Col	Raw Data (595) 33 - 5 h 19 min	Raw Data (595) 34 - 5 h 30 min	Raw Data (595) 35 - 5 h 40 min	Raw Data (595) 36 - 5 h 49 min	Raw Data (595) 37 - 6 h	Raw Data (595) 38 - 6 h 10 min	Raw Data (595) 39 - 6 h 19 min	Raw Data (595) 40 - 6 h 30 min	Raw Data (595) 41 - 6 h 40 min	Raw Data (595) 42 - 6 h 49 min	Raw Data (595) 43 - 7 h	Raw Data (595) 44 - 7 h 10 min
Time, Hours		5.33	5.50	5.67	5.83	6.00	6.17	6.33	6.50	6.67	6.83	7.00	7.17
C 1		0.171	0.1709	0.1719	0.1716	0.1719	0.1725	0.1732	0.1797	0.1716	0.173	0.1732	0.1702
C 2		0.3736	0.3735	0.3741	0.3756	0.3736	0.3743	0.3744	0.379	0.3723	0.3731	0.3723	0.3704
C 3		0.2858	0.292	0.2956	0.3003	0.3035	0.3094	0.3137	0.3234	0.3197	0.3244	0.3289	0.3322
C 4		0.241	0.2449	0.2446	0.247	0.2474	0.2475	0.2512	0.2498	0.2494	0.2494	0.251	0.2488
C 5		0.4473	0.4555	0.4483	0.4559	0.4603	0.4379	0.4627	0.4644	0.4645	0.4665	0.4616	0.4625
C 6		0.1741	0.1745	0.1749	0.1751	0.1736	0.1746	0.1746	0.1776	0.1733	0.1738	0.1736	0.1731
C 7		0.207	0.2068	0.2062	0.2065	0.2067	0.2062	0.206	0.1972	0.2065	0.2062	0.2077	0.2061
C 8		0.1935	0.1935	0.1936	0.1943	0.1932	0.1937	0.1932	0.1892	0.1942	0.1937	0.1936	0.1935
C 9		0.4071	0.4071	0.4065	0.406	0.4068	0.4063	0.405	0.4039	0.404	0.4033	0.4019	0.4021
C 10		0.3644	0.3842	0.3812	0.3769	0.3766	0.3784	0.3771	0.371	0.3743	0.3749	0.3744	0.3754
C 11		0.2608	0.267	0.2727	0.2767	0.2876	0.2999	0.3152	0.3398	0.3189	0.3313	0.3383	0.3392
C 12		0.2117	0.2129	0.2128	0.2155	0.2168	0.2176	0.2171	0.2325	0.2158	0.2192	0.2149	0.2149
D 1		0.216	0.2162	0.216	0.2164	0.2168	0.2163	0.2167	0.2134	0.2156	0.218	0.2174	0.2174
D 2		0.3502	0.3552	0.3584	0.3629	0.3633	0.3665	0.3673	0.361	0.3673	0.3691	0.367	0.367
D 3		0.2546	0.2577	0.2596	0.2616	0.2621	0.2655	0.2666	0.2637	0.2678	0.2694	0.2696	0.268
D 4		0.2239	0.2252	0.225	0.2259	0.2247	0.2266	0.2263	0.2103	0.2267	0.228	0.2263	0.2254
D 5		0.5269	0.5176	0.524	0.5551	0.5408	0.5184	0.5155	0.4815	0.5179	0.5009	0.526	0.4948
D 6		0.2082	0.2105	0.2088	0.2112	0.2071	0.2097	0.2088	0.2347	0.2092	0.2095	0.2118	0.2093
D 7		0.219	0.2181	0.2186	0.219	0.2184	0.2198	0.2182	0.2011	0.2169	0.2189	0.2176	0.2168
D 8		0.2261	0.2262	0.2279	0.2272	0.227	0.2287	0.2281	0.2086	0.2275	0.2274	0.2257	0.2248
D 9		0.3352	0.3349	0.3349	0.3346	0.3328	0.3332	0.3313	0.332	0.3298	0.3314	0.3298	0.328
D 10		0.317	0.3301	0.329	0.3251	0.3252	0.3272	0.3273	0.3154	0.3264	0.3266	0.3256	0.3237
D 11		0.2471	0.2502	0.2546	0.2583	0.2666	0.2769	0.2843	0.2913	0.3086	0.327	0.3282	0.3305
D 12		0.1856	0.1859	0.1852	0.1856	0.1841	0.1862	0.1856	0.187	0.185	0.1863	0.1848	0.1837

Appendix 7.1

Well Row	Well Col	Raw Data (595) 33 - 5 h 19 min	Raw Data (595) 34 - 5 h 30 min	Raw Data (595) 35 - 5 h 40 min	Raw Data (595) 36 - 5 h 49 min	Raw Data (595) 37 - 6 h	Raw Data (595) 38 - 6 h 10 min	Raw Data (595) 39 - 6 h 19 min	Raw Data (595) 40 - 6 h 30 min	Raw Data (595) 41 - 6 h 40 min	Raw Data (595) 42 - 6 h 49 min	Raw Data (595) 43 - 7 h	Raw Data (595) 44 - 7 h 10 min
Time, Hours		5.33	5.50	5.67	5.83	6.00	6.17	6.33	6.50	6.67	6.83	7.00	7.17
E 1		0.187	0.187	0.1866	0.188	0.1861	0.1881	0.1877	0.1879	0.1861	0.1872	0.1871	0.1868
E 2		0.4017	0.4058	0.4077	0.4091	0.4128	0.4211	0.4213	0.4209	0.4255	0.4332	0.4337	0.4334
E 3		0.241	0.2429	0.2445	0.2484	0.2499	0.2555	0.2596	0.2666	0.2801	0.2999	0.3141	0.3136
E 4		0.2295	0.2304	0.2341	0.2348	0.238	0.2399	0.2429	0.2332	0.2489	0.255	0.2541	0.2533
E 5		0.2797	0.2794	0.2827	0.2844	0.2876	0.292	0.2939	0.2952	0.3039	0.3067	0.3084	0.3073
E 6		0.1949	0.1956	0.1949	0.1968	0.1955	0.1976	0.1966	0.2098	0.1969	0.1949	0.1949	0.1932
F 1		0.1983	0.1977	0.1975	0.1989	0.1978	0.1984	0.1989	0.1959	0.1978	0.2	0.202	0.2005
F 2		0.4139	0.4187	0.4235	0.4288	0.4305	0.4358	0.4422	0.4527	0.449	0.4522	0.4538	0.4484
F 3		0.293	0.2936	0.2993	0.2973	0.3022	0.306	0.3096	0.3227	0.3165	0.3209	0.3226	0.3265
F 4		0.2002	0.2052	0.2061	0.2069	0.208	0.2139	0.2172	0.2209	0.2231	0.2247	0.2254	0.2252
F 5		0.2278	0.2346	0.239	0.243	0.2472	0.2561	0.2544	0.2506	0.2589	0.2611	0.2615	0.2609
F 6		0.2371	0.2376	0.2385	0.2383	0.2375	0.238	0.2376	0.2221	0.2369	0.2366	0.2372	0.2355
G 1		0.3085	0.3086	0.316	0.3154	0.3154	0.3155	0.3174	0.32	0.3164	0.3174	0.3164	0.3153
G 2		0.3375	0.3384	0.3418	0.342	0.3395	0.342	0.346	0.3487	0.3498	0.3577	0.3614	0.3676
G 3		0.2714	0.2718	0.2744	0.2798	0.2829	0.2869	0.2884	0.2916	0.2916	0.2945	0.3005	0.303
G 4		0.2222	0.23	0.2261	0.2304	0.2328	0.2389	0.2391	0.2778	0.2487	0.2491	0.2512	0.2507
G 5		0.2051	0.2002	0.2052	0.2075	0.2073	0.2118	0.2151	0.2046	0.2215	0.2249	0.2248	0.2248
G 6		0.1777	0.1788	0.1786	0.1786	0.1782	0.1795	0.1785	0.1947	0.1778	0.1785	0.1799	0.1778
H 1		0.2104	0.2113	0.2125	0.2107	0.2104	0.2134	0.2145	0.2113	0.2151	0.2146	0.2158	0.2129
H 2		0.5546	0.4457	0.3909	0.4114	0.7849	0.7547	0.3013	0.2979	0.3041	0.3084	0.3123	0.3112
H 3		0.275	0.2744	0.2768	0.2802	0.2839	0.2877	0.2888	0.2949	0.294	0.2955	0.2982	0.2956
H 4		0.1877	0.1891	0.1892	0.1967	0.198	0.1984	0.1993	0.2141	0.2058	0.2077	0.2099	0.2078
H 5		0.1749	0.1759	0.1805	0.1818	0.1823	0.1887	0.1886	0.2075	0.1943	0.1969	0.1995	0.1957
H 6		0.1998	0.2013	0.2006	0.2022	0.1991	0.2005	0.2029	0.193	0.2029	0.202	0.2017	0.2008

## Appendix 7.1

User: USER

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 45 - 7 h 19 min	Raw Data (595) 46 - 7 h 30 min	Raw Data (595) 47 - 7 h 40 min	Raw Data (595) 48 - 7 h 49 min	Raw Data (595) 49 - 8 h	Raw Data (595) 50 - 8 h 9 min	Raw Data (595) 51 - 8 h 20 min	Raw Data (595) 52 - 8 h 30 min	Raw Data (595) 53 - 8 h 39 min	Raw Data (595) 54 - 8 h 50 min	Raw Data (595) 55 - 9 h	Raw Data (595) 56 - 9 h 9 min
Time, Hours		7.33	7.50	7.67	7.83	8.00	8.17	8.33	8.50	8.67	8.83	9.00	9.17
A 1		0.1716	0.1719	0.171	0.1709	0.1728	0.1735	0.1738	0.1722	0.1741	0.1738	0.1741	0.1734
A 2		0.293	0.2929	0.2914	0.2913	0.2919	0.2913	0.2951	0.2942	0.2965	0.2965	0.296	0.2965
A 3		0.2606	0.2618	0.2623	0.2619	0.2628	0.2678	0.263	0.2637	0.2818	0.2632	0.2639	0.2622
A 4		0.2099	0.2116	0.2109	0.2113	0.2119	0.2115	0.2124	0.2109	0.2037	0.2125	0.2113	0.2108
A 5		0.499	0.4964	0.4991	0.495	0.4968	0.4915	0.4885	0.4939	0.5063	0.4871	0.4918	0.4961
A 6		0.1825	0.1823	0.1809	0.1812	0.1823	0.182	0.1828	0.1809	0.1846	0.1824	0.1818	0.1821
A 7		0.2571	0.2573	0.2555	0.2563	0.2574	0.2578	0.2582	0.2562	0.2565	0.2584	0.2586	0.2592
A 8		0.2088	0.2082	0.2069	0.2077	0.2079	0.2077	0.2084	0.2062	0.2136	0.2083	0.2075	0.2081
A 9		0.3391	0.3381	0.3368	0.3371	0.3368	0.3361	0.3364	0.3357	0.3382	0.3362	0.3361	0.3359
A 10		0.28	0.2772	0.2742	0.2717	0.2714	0.2698	0.2693	0.2667	0.2557	0.2668	0.2661	0.2657
A 11		0.7129	0.729	0.723	0.7291	0.7464	0.7137	0.709	0.7305	0.8286	0.7068	0.7121	0.7112
A 12		0.1658	0.1657	0.1646	0.1651	0.1669	0.1638	0.1635	0.1648	0.1787	0.1653	0.1669	0.1645
B 1		0.196	0.1964	0.195	0.1949	0.1961	0.1966	0.1967	0.1961	0.2019	0.1973	0.1964	0.1999
B 2		0.3989	0.4064	0.4084	0.4155	0.4212	0.425	0.4274	0.4282	0.4363	0.4351	0.4347	0.437
B 3		0.2387	0.239	0.2383	0.24	0.24	0.24	0.2414	0.239	0.2375	0.2412	0.2416	0.2425
B 4		0.2253	0.2249	0.2245	0.227	0.2275	0.227	0.228	0.2268	0.2266	0.2293	0.2303	0.2317
B 5		0.4903	0.5058	0.5176	0.5254	0.5139	0.5312	0.5456	0.5111	0.5351	0.523	0.5164	0.5403
B 6		0.2029	0.2044	0.2042	0.2031	0.2038	0.2036	0.2028	0.2028	0.1887	0.2027	0.2047	0.202
B 7		0.2256	0.225	0.2215	0.2243	0.2254	0.2233	0.2252	0.2216	0.2371	0.2269	0.2226	0.2245
B 8		0.2233	0.2239	0.2235	0.2233	0.2242	0.2214	0.222	0.2222	0.2084	0.2222	0.2247	0.2227
B 9		0.341	0.341	0.3399	0.3403	0.3413	0.3384	0.3386	0.3382	0.3317	0.3378	0.3403	0.3385
B 10		0.3369	0.3353	0.3359	0.3343	0.3347	0.332	0.3317	0.3288	0.3331	0.332	0.33	0.3319
B 11		0.3205	0.3125	0.2964	0.3104	0.3135	0.3176	0.3186	0.3115	0.3125	0.3125	0.3118	0.3113
B 12		0.2322	0.2299	0.2377	0.2394	0.2329	0.2307	0.2347	0.2296	0.2022	0.231	0.2292	0.2326

Appendix 7.1

Well Row	Well Col	Raw Data (595) 45 - 7 h 19 min	Raw Data (595) 46 - 7 h 30 min	Raw Data (595) 47 - 7 h 40 min	Raw Data (595) 48 - 7 h 49 min	Raw Data (595) 49 - 8 h	Raw Data (595) 50 - 8 h 9 min	Raw Data (595) 51 - 8 h 20 min	Raw Data (595) 52 - 8 h 30 min	Raw Data (595) 53 - 8 h 39 min	Raw Data (595) 54 - 8 h 50 min	Raw Data (595) 55 - 9 h	Raw Data (595) 56 - 9 h 9 min
Time, Hours		7.33	7.50	7.67	7.83	8.00	8.17	8.33	8.50	8.67	8.83	9.00	9.17
C 1		0.1723	0.172	0.1701	0.1706	0.175	0.171	0.1712	0.1698	0.176	0.1732	0.1706	0.1735
C 2		0.373	0.3728	0.3715	0.3711	0.3718	0.3731	0.3735	0.3723	0.3791	0.3759	0.3771	0.3787
C 3		0.3388	0.3423	0.3422	0.3441	0.3458	0.3465	0.3481	0.3483	0.3575	0.3537	0.3555	0.3546
C 4		0.2542	0.2545	0.2536	0.2542	0.2533	0.2558	0.2572	0.2572	0.2583	0.2592	0.2581	0.2598
C 5		0.4636	0.4797	0.517	0.4872	0.5265	0.5112	0.4772	0.4841	0.478	0.4835	0.4778	0.4775
C 6		0.175	0.1748	0.1734	0.1726	0.1742	0.1733	0.1732	0.1728	0.1774	0.1734	0.1733	0.1739
C 7		0.2057	0.2065	0.2057	0.2053	0.2059	0.2054	0.2061	0.2044	0.1948	0.2059	0.2057	0.2048
C 8		0.1924	0.1943	0.1929	0.1922	0.1935	0.1925	0.1928	0.1921	0.1884	0.1931	0.1936	0.1923
C 9		0.4034	0.4024	0.4015	0.4024	0.403	0.4045	0.4034	0.4021	0.4031	0.403	0.4045	0.4036
C 10		0.3761	0.3754	0.3736	0.3706	0.3722	0.3701	0.3692	0.367	0.3638	0.3702	0.3689	0.369
C 11		0.3841	0.386	0.3716	0.3765	0.3746	0.3707	0.3726	0.3674	0.3739	0.3681	0.3655	0.3651
C 12		0.2201	0.2167	0.2141	0.2152	0.2147	0.2192	0.2161	0.2167	0.2366	0.2177	0.2188	0.2195
D 1		0.2223	0.2199	0.217	0.2184	0.2192	0.2203	0.219	0.2199	0.214	0.2199	0.2179	0.2178
D 2		0.3689	0.3671	0.3651	0.3661	0.3664	0.3654	0.365	0.364	0.3653	0.3671	0.3671	0.368
D 3		0.27	0.2698	0.2702	0.2717	0.2723	0.2724	0.2733	0.2737	0.2739	0.2776	0.2774	0.2784
D 4		0.2285	0.2276	0.2263	0.2276	0.2284	0.2298	0.2301	0.2288	0.2147	0.2318	0.2318	0.2329
D 5		0.5384	0.5168	0.5307	0.5127	0.5097	0.5317	0.5345	0.5255	0.472	0.5158	0.5105	0.5025
D 6		0.2085	0.2078	0.2092	0.2098	0.2093	0.2067	0.2057	0.2066	0.2321	0.2082	0.2091	0.2057
D 7		0.2201	0.2203	0.2173	0.2162	0.2179	0.219	0.2183	0.2173	0.2018	0.2179	0.2171	0.2177
D 8		0.2285	0.2286	0.2257	0.2256	0.2282	0.2288	0.229	0.2262	0.2093	0.2283	0.2276	0.2286
D 9		0.3304	0.3295	0.3282	0.3272	0.328	0.3279	0.3273	0.3266	0.3284	0.3275	0.3262	0.327
D 10		0.3261	0.3255	0.3231	0.3225	0.3224	0.3193	0.3205	0.318	0.3079	0.3197	0.319	0.322
D 11		0.3404	0.3469	0.3696	0.38	0.3772	0.3709	0.375	0.3718	0.3628	0.3739	0.3702	0.3735
D 12		0.1843	0.1833	0.1828	0.1836	0.1839	0.1828	0.1834	0.1818	0.1808	0.1837	0.1842	0.1826



Appendix 7.1

Well Row	Well Col	Raw Data (595) 45 - 7 h 19 min	Raw Data (595) 46 - 7 h 30 min	Raw Data (595) 47 - 7 h 40 min	Raw Data (595) 48 - 7 h 49 min	Raw Data (595) 49 - 8 h	Raw Data (595) 50 - 8 h 9 min	Raw Data (595) 51 - 8 h 20 min	Raw Data (595) 52 - 8 h 30 min	Raw Data (595) 53 - 8 h 39 min	Raw Data (595) 54 - 8 h 50 min	Raw Data (595) 55 - 9 h	Raw Data (595) 56 - 9 h 9 min
Time, Hours		7.33	7.50	7.67	7.83	8.00	8.17	8.33	8.50	8.67	8.83	9.00	9.17
E 1		0.1882	0.1869	0.1853	0.1867	0.187	0.1863	0.1867	0.1851	0.1864	0.187	0.1863	0.1855
E 2		0.4339	0.4283	0.424	0.4259	0.4269	0.4244	0.4302	0.4267	0.4319	0.4373	0.4373	0.4391
E 3		0.3133	0.3098	0.3053	0.3035	0.3047	0.3032	0.3036	0.3047	0.3031	0.3077	0.3082	0.3096
E 4		0.2555	0.2578	0.2535	0.2554	0.2591	0.2564	0.2574	0.2582	0.2454	0.2624	0.2647	0.2653
E 5		0.3089	0.3141	0.3119	0.3148	0.318	0.3188	0.3181	0.3201	0.3246	0.3252	0.3265	0.328
E 6		0.1954	0.1944	0.1938	0.1937	0.1951	0.1952	0.1954	0.1931	0.2079	0.195	0.1939	0.1939
F 1		0.2015	0.203	0.202	0.2018	0.2036	0.2031	0.2031	0.2023	0.1979	0.2042	0.2033	0.2037
F 2		0.4488	0.4473	0.4485	0.453	0.4568	0.453	0.4578	0.4541	0.4668	0.4609	0.4634	0.467
F 3		0.3272	0.3341	0.3346	0.3396	0.342	0.3443	0.3462	0.3455	0.3629	0.3509	0.3509	0.3539
F 4		0.2271	0.2279	0.2274	0.2282	0.2293	0.2289	0.2305	0.2297	0.2313	0.2348	0.2363	0.2376
F 5		0.2625	0.2637	0.2643	0.2667	0.2697	0.27	0.2669	0.2682	0.2665	0.2709	0.2701	0.2702
F 6		0.2369	0.2375	0.2364	0.2373	0.2407	0.2397	0.2403	0.2394	0.2246	0.2405	0.2395	0.2403
G 1		0.3157	0.3185	0.3156	0.3165	0.32	0.3186	0.318	0.3189	0.3232	0.3196	0.3202	0.3201
G 2		0.378	0.3868	0.3908	0.3957	0.3996	0.403	0.4064	0.4049	0.3943	0.4197	0.4213	0.4289
G 3		0.3083	0.3141	0.3181	0.3234	0.3271	0.3291	0.3319	0.3346	0.3414	0.3393	0.3413	0.3427
G 4		0.2557	0.2542	0.2507	0.2513	0.2552	0.2537	0.2535	0.2555	0.2912	0.2604	0.2619	0.2601
G 5		0.2279	0.2302	0.2306	0.2307	0.234	0.2351	0.2379	0.2386	0.2293	0.2451	0.2458	0.2489
G 6		0.1795	0.1794	0.1782	0.1786	0.1801	0.1796	0.1805	0.1798	0.1944	0.1793	0.1801	0.1788
H 1		0.2147	0.216	0.2148	0.2143	0.2144	0.2136	0.2156	0.2151	0.2107	0.2156	0.2151	0.2158
H 2		0.3158	0.3169	0.3152	0.3154	0.3155	0.3142	0.3148	0.3126	0.3049	0.3166	0.3159	0.3169
H 3		0.3002	0.3004	0.2996	0.3005	0.301	0.3013	0.2999	0.2989	0.2999	0.2999	0.2986	0.298
H 4		0.2127	0.2096	0.2098	0.2104	0.2126	0.2111	0.2123	0.2084	0.2166	0.21	0.2107	0.2099
H 5		0.1972	0.1978	0.1967	0.1965	0.1997	0.1997	0.2003	0.1992	0.2161	0.2022	0.202	0.2037
H 6		0.2024	0.2019	0.2014	0.2014	0.2016	0.1992	0.2009	0.2018	0.194	0.2035	0.2042	0.2015

## Appendix 7.1

User: USER

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 57 - 9 h 20 min	Raw Data (595) 58 - 9 h 30 min	Raw Data (595) 59 - 9 h 39 min	Raw Data (595) 60 - 9 h 50 min	Raw Data (595) 61 - 10 h	Raw Data (595) 62 - 10 h 9 min	Raw Data (595) 63 - 10 h 20 min	Raw Data (595) 64 - 10 h 30 min	Raw Data (595) 65 - 10 h 39 min	Raw Data (595) 66 - 10 h 50 min	Raw Data (595) 67 - 11 h	Raw Data (595) 68 - 11 h 9 min
Time, Hours		9.33	9.50	9.67	9.83	10.00	10.17	10.33	10.50	10.67	10.83	11.00	11.17
A 1		0.1744	0.1763	0.1735	0.1739	0.1724	0.1745	0.1742	0.1758	0.1758	0.1766	0.1773	0.1767
A 2		0.297	0.2986	0.2965	0.2969	0.2957	0.2976	0.298	0.2985	0.2965	0.298	0.298	0.2969
A 3		0.2633	0.2814	0.2649	0.2624	0.2622	0.2616	0.2629	0.2602	0.2796	0.2598	0.259	0.2598
A 4		0.2119	0.2029	0.2101	0.2106	0.2094	0.2102	0.2118	0.2117	0.205	0.2127	0.2112	0.2116
A 5		0.5284	0.5195	0.5019	0.5009	0.5207	0.4953	0.5193	0.5345	0.526	0.517	0.549	0.5198
A 6		0.1817	0.1847	0.1817	0.1819	0.1804	0.1822	0.1824	0.1825	0.1843	0.1817	0.183	0.1818
A 7		0.2593	0.2581	0.2592	0.2599	0.258	0.2594	0.26	0.2597	0.2578	0.2598	0.2605	0.2606
A 8		0.2086	0.2143	0.2086	0.2095	0.2082	0.2074	0.2089	0.2079	0.2139	0.2084	0.2087	0.2082
A 9		0.3386	0.3417	0.3382	0.3403	0.3384	0.3382	0.3388	0.3385	0.3414	0.3387	0.3378	0.3363
A 10		0.2658	0.2539	0.2644	0.2645	0.2629	0.2646	0.2645	0.2627	0.2506	0.2623	0.2628	0.2611
A 11		0.7021	0.806	0.6781	0.6785	0.6933	0.6738	0.655	0.6579	0.7559	0.6244	0.5957	0.5949
A 12		0.1667	0.1792	0.1656	0.1667	0.168	0.1669	0.1673	0.1659	0.1809	0.1663	0.1648	0.1654
B 1		0.1988	0.2031	0.1976	0.1991	0.1968	0.197	0.1965	0.1983	0.2023	0.1981	0.1994	0.1989
B 2		0.4405	0.444	0.4396	0.441	0.4406	0.4429	0.4404	0.4398	0.4453	0.4395	0.4373	0.4361
B 3		0.2435	0.2399	0.2416	0.2416	0.2411	0.2416	0.2411	0.2403	0.2394	0.2411	0.2419	0.2402
B 4		0.2327	0.2304	0.2321	0.2321	0.2319	0.2321	0.2329	0.2336	0.233	0.2339	0.2348	0.2343
B 5		0.539	0.5418	0.5368	0.5494	0.5528	0.5508	0.5735	0.579	0.5711	0.5724	0.5771	0.5896
B 6		0.2027	0.1892	0.2029	0.2027	0.2035	0.2022	0.2031	0.2024	0.1917	0.2041	0.2028	0.2023
B 7		0.2265	0.2366	0.2241	0.2255	0.2222	0.2261	0.2241	0.2272	0.2359	0.2244	0.2248	0.2255
B 8		0.2222	0.2074	0.223	0.2222	0.2235	0.2212	0.2238	0.2212	0.209	0.223	0.2209	0.222
B 9		0.3363	0.3301	0.3384	0.3368	0.3385	0.3358	0.3383	0.3362	0.3289	0.3363	0.3359	0.3355
B 10		0.3292	0.3314	0.331	0.3305	0.3308	0.3279	0.3312	0.3304	0.333	0.3326	0.3336	0.3304
B 11		0.3125	0.31	0.3158	0.3146	0.3133	0.3125	0.315	0.3117	0.3078	0.3127	0.3116	0.3126
B 12		0.2274	0.204	0.2339	0.233	0.2358	0.2298	0.2364	0.232	0.2041	0.2293	0.2327	0.2347

Appendix 7.1

Well Row	Well Col	Raw Data (595) 57 - 9 h 20 min	Raw Data (595) 58 - 9 h 30 min	Raw Data (595) 59 - 9 h 39 min	Raw Data (595) 60 - 9 h 50 min	Raw Data (595) 61 - 10 h	Raw Data (595) 62 - 10 h 9 min	Raw Data (595) 63 - 10 h 20 min	Raw Data (595) 64 - 10 h 30 min	Raw Data (595) 65 - 10 h 39 min	Raw Data (595) 66 - 10 h 50 min	Raw Data (595) 67 - 11 h	Raw Data (595) 68 - 11 h 9 min
Time, Hours		9.33	9.50	9.67	9.83	10.00	10.17	10.33	10.50	10.67	10.83	11.00	11.17
C 1		0.1723	0.1767	0.1718	0.1721	0.1727	0.1731	0.1731	0.1734	0.175	0.1751	0.1735	0.1738
C 2		0.3788	0.3826	0.3794	0.3802	0.3795	0.3795	0.3798	0.3793	0.3822	0.3802	0.38	0.382
C 3		0.357	0.3622	0.358	0.3611	0.3602	0.3625	0.3626	0.3655	0.3743	0.3672	0.3678	0.3674
C 4		0.2603	0.2623	0.2616	0.2621	0.2624	0.2649	0.265	0.2634	0.269	0.264	0.2629	0.2683
C 5		0.487	0.4961	0.4972	0.5154	0.5972	0.5067	0.5508	0.4968	0.5192	0.5339	0.5303	0.5134
C 6		0.1733	0.1768	0.1734	0.1742	0.1739	0.1742	0.1743	0.1746	0.1767	0.1745	0.1737	0.1739
C 7		0.2061	0.1955	0.2053	0.2052	0.2052	0.2057	0.2045	0.2053	0.1973	0.206	0.2063	0.2051
C 8		0.1936	0.1894	0.1933	0.1938	0.193	0.1931	0.1931	0.1927	0.1895	0.1933	0.1934	0.1937
C 9		0.4034	0.4039	0.4032	0.4038	0.4039	0.4028	0.4052	0.404	0.4044	0.4038	0.4045	0.404
C 10		0.3689	0.365	0.3704	0.3718	0.3715	0.373	0.3749	0.3754	0.3692	0.3758	0.3768	0.3755
C 11		0.3641	0.3659	0.3625	0.3623	0.3596	0.36	0.3604	0.3591	0.3602	0.3602	0.3592	0.3621
C 12		0.2183	0.2381	0.2186	0.2201	0.2204	0.221	0.2217	0.2208	0.2368	0.2212	0.2188	0.2235
D 1		0.2194	0.2134	0.2197	0.2203	0.2179	0.2179	0.2195	0.2201	0.2127	0.2203	0.2191	0.2213
D 2		0.3677	0.3632	0.368	0.3689	0.369	0.3683	0.3684	0.3661	0.3585	0.3649	0.3624	0.362
D 3		0.2796	0.2788	0.2817	0.2829	0.284	0.2829	0.2829	0.281	0.2781	0.28	0.2795	0.2786
D 4		0.2369	0.2192	0.2352	0.2361	0.2375	0.2382	0.2404	0.2411	0.2278	0.246	0.2452	0.2463
D 5		0.506	0.4253	0.4864	0.5122	0.4968	0.5037	0.4815	0.4683	0.3999	0.463	0.4431	0.4771
D 6		0.211	0.2304	0.2076	0.209	0.2111	0.2092	0.2065	0.21	0.23	0.2078	0.2063	0.2039
D 7		0.2185	0.2014	0.218	0.2189	0.2178	0.2187	0.2194	0.2187	0.2026	0.2194	0.2198	0.2189
D 8		0.2293	0.2079	0.2283	0.2284	0.231	0.2308	0.2333	0.2329	0.2095	0.2339	0.2325	0.2331
D 9		0.3268	0.3264	0.326	0.3262	0.3246	0.3264	0.3259	0.3263	0.3262	0.3248	0.3248	0.3236
D 10		0.322	0.3098	0.3228	0.3228	0.3224	0.3231	0.3241	0.3239	0.3116	0.326	0.3271	0.3243
D 11		0.3731	0.3605	0.3749	0.376	0.3725	0.3724	0.3718	0.3722	0.3549	0.3701	0.3705	0.3691
D 12		0.1839	0.1839	0.1829	0.1847	0.1827	0.1828	0.184	0.184	0.1808	0.1833	0.1831	0.1827

Appendix 7.1

Well Row	Well Col	Raw Data (595) 57 - 9 h 20 min	Raw Data (595) 58 - 9 h 30 min	Raw Data (595) 59 - 9 h 39 min	Raw Data (595) 60 - 9 h 50 min	Raw Data (595) 61 - 10 h	Raw Data (595) 62 - 10 h 9 min	Raw Data (595) 63 - 10 h 20 min	Raw Data (595) 64 - 10 h 30 min	Raw Data (595) 65 - 10 h 39 min	Raw Data (595) 66 - 10 h 50 min	Raw Data (595) 67 - 11 h	Raw Data (595) 68 - 11 h 9 min
Time, Hours		9.33	9.50	9.67	9.83	10.00	10.17	10.33	10.50	10.67	10.83	11.00	11.17
E	1	0.1863	0.1862	0.1867	0.1861	0.1867	0.1864	0.1867	0.1867	0.1883	0.1881	0.1879	0.1882
E	2	0.4405	0.4375	0.4451	0.4445	0.4437	0.4359	0.4357	0.4315	0.4247	0.4313	0.4303	0.4292
E	3	0.3095	0.3087	0.3132	0.3131	0.3132	0.3074	0.3048	0.2995	0.2924	0.2911	0.289	0.287
E	4	0.2674	0.2548	0.2711	0.2711	0.275	0.2726	0.2756	0.2769	0.2661	0.2808	0.2841	0.2848
E	5	0.33	0.3326	0.3322	0.3328	0.3364	0.3367	0.3405	0.34	0.3435	0.3433	0.3436	0.3442
E	6	0.1949	0.207	0.1939	0.1959	0.1943	0.1939	0.1955	0.1951	0.2064	0.1968	0.1951	0.1939
F	1	0.2036	0.1987	0.2036	0.2038	0.2022	0.2041	0.2039	0.2041	0.1983	0.2052	0.2044	0.204
F	2	0.466	0.4782	0.473	0.4749	0.4443	0.4319	0.4255	0.4215	0.4238	0.4189	0.419	0.4183
F	3	0.3532	0.3714	0.3609	0.3649	0.3865	0.3885	0.3841	0.3784	0.3831	0.3727	0.3705	0.3662
F	4	0.2383	0.2403	0.2403	0.2419	0.2422	0.2422	0.2429	0.244	0.2429	0.2463	0.2469	0.2468
F	5	0.274	0.2714	0.2764	0.2759	0.2775	0.2795	0.2816	0.2844	0.2836	0.2911	0.2863	0.2896
F	6	0.2418	0.228	0.2448	0.2527	0.2504	0.2524	0.2532	0.2535	0.2336	0.2549	0.2542	0.2539
G	1	0.3207	0.325	0.3209	0.322	0.3195	0.3215	0.3204	0.3213	0.3251	0.3227	0.3223	0.321
G	2	0.4331	0.4089	0.4393	0.4503	0.4843	0.5151	0.5244	0.5216	0.525	0.5145	0.5139	0.5063
G	3	0.3444	0.3549	0.3466	0.3508	0.3649	0.3706	0.3714	0.3722	0.379	0.3716	0.3713	0.3696
G	4	0.2632	0.3005	0.2683	0.2689	0.2703	0.2714	0.2702	0.2719	0.309	0.2767	0.2767	0.2781
G	5	0.2488	0.2377	0.2522	0.2516	0.2536	0.2561	0.2582	0.2577	0.2446	0.2595	0.26	0.2602
G	6	0.18	0.1971	0.1808	0.1787	0.1786	0.18	0.1792	0.1806	0.1965	0.1807	0.1812	0.1802
H	1	0.2157	0.2109	0.2159	0.2153	0.2158	0.2151	0.2152	0.2146	0.212	0.2166	0.2159	0.2173
H	2	0.3191	0.3075	0.3197	0.3213	0.3222	0.3277	0.3326	0.3428	0.3274	0.3741	0.3864	0.3986
H	3	0.2992	0.2976	0.2991	0.2993	0.3006	0.3017	0.3038	0.3062	0.3019	0.3132	0.3164	0.3216
H	4	0.2105	0.2176	0.2102	0.2107	0.2106	0.2122	0.2124	0.2102	0.2163	0.2116	0.2122	0.2106
H	5	0.2045	0.2221	0.2066	0.2077	0.2077	0.2104	0.211	0.2122	0.2264	0.2127	0.214	0.2141
H	6	0.2025	0.1929	0.2037	0.2042	0.2027	0.202	0.2027	0.2012	0.1942	0.2029	0.2018	0.2036

## Appendix 7.1

User: USER  
 Test Name: Pseudomonas  
 Absorbance

Well Row	Well Col	Raw Data (595) 69 - 11 h 20 min	Raw Data (595) 70 - 11 h 30 min	Raw Data (595) 71 - 11 h 39 min	Raw Data (595) 72 - 11 h 50 min	Raw Data (595) 73 - 12 h	Raw Data (595) 74 - 12 h 9 min	Raw Data (595) 75 - 12 h 20 min	Raw Data (595) 76 - 12 h 30 min	Raw Data (595) 77 - 12 h 39 min	Raw Data (595) 78 - 12 h 50 min	Raw Data (595) 79 - 13 h	Raw Data (595) 80 - 13 h 9 min
Time, Hours		11.33	11.50	11.67	11.83	12.00	12.17	12.33	12.50	12.67	12.83	13.00	13.17
A 1		0.1777	0.1785	0.1782	0.1783	0.1794	0.1815	0.1797	0.1804	0.1795	0.1804	0.1802	0.1811
A 2		0.297	0.2958	0.2961	0.2962	0.2962	0.2948	0.2949	0.2948	0.2931	0.2946	0.2946	0.2973
A 3		0.2608	0.2581	0.2589	0.2578	0.2783	0.2576	0.2577	0.2583	0.2569	0.256	0.2555	0.2576
A 4		0.2102	0.2089	0.2101	0.2101	0.2026	0.2101	0.2093	0.209	0.2089	0.2099	0.2101	0.2114
A 5		0.5338	0.5343	0.5272	0.526	0.5412	0.538	0.58	0.5966	0.5571	0.5658	0.553	0.5635
A 6		0.1814	0.1796	0.1815	0.1814	0.1843	0.1824	0.1812	0.1809	0.181	0.1813	0.1815	0.1809
A 7		0.2603	0.2588	0.2602	0.2601	0.2596	0.2607	0.261	0.2599	0.2592	0.2606	0.2602	0.2607
A 8		0.2086	0.2073	0.2076	0.2082	0.2134	0.208	0.2075	0.2069	0.2067	0.2078	0.2072	0.2076
A 9		0.3389	0.3354	0.3371	0.337	0.3407	0.3387	0.3382	0.3378	0.3379	0.3388	0.3384	0.3387
A 10		0.262	0.2585	0.2602	0.2592	0.2485	0.2601	0.2594	0.2579	0.2573	0.2589	0.2579	0.2583
A 11		0.6035	0.5884	0.5839	0.5532	0.6353	0.5327	0.5332	0.5062	0.5029	0.4746	0.4752	0.453
A 12		0.1665	0.164	0.166	0.1652	0.1821	0.1647	0.1659	0.1652	0.1657	0.1645	0.1657	0.1655
B 1		0.1999	0.1975	0.1977	0.1986	0.2043	0.1991	0.1985	0.1997	0.1983	0.2008	0.2013	0.199
B 2		0.4373	0.4335	0.4352	0.4344	0.4385	0.4323	0.4313	0.4325	0.4293	0.4288	0.4296	0.4271
B 3		0.2408	0.239	0.2399	0.2406	0.2392	0.2397	0.2397	0.239	0.2383	0.2391	0.2408	0.2389
B 4		0.234	0.2346	0.2368	0.2367	0.2358	0.2362	0.2368	0.237	0.2354	0.2356	0.2377	0.2369
B 5		0.6025	0.6027	0.6439	0.6089	0.6326	0.5998	0.6732	0.6369	0.6354	0.6089	0.6404	0.6153
B 6		0.203	0.2006	0.2031	0.2044	0.1922	0.2035	0.2021	0.2021	0.2024	0.2028	0.2024	0.2032
B 7		0.226	0.2237	0.2239	0.2218	0.2337	0.2246	0.2254	0.2253	0.223	0.2256	0.2248	0.2228
B 8		0.2227	0.2207	0.2215	0.2224	0.2105	0.2216	0.22	0.2212	0.2217	0.2191	0.2199	0.2217
B 9		0.3339	0.3333	0.3343	0.3349	0.3264	0.3337	0.3314	0.3326	0.3338	0.3309	0.3313	0.3333
B 10		0.3307	0.3302	0.3297	0.3311	0.3334	0.3295	0.3306	0.3312	0.3321	0.3334	0.3323	0.3352
B 11		0.3129	0.3118	0.3134	0.3141	0.3121	0.3134	0.3133	0.3164	0.317	0.3184	0.3173	0.3215
B 12		0.229	0.2312	0.2308	0.234	0.1988	0.2303	0.2275	0.2305	0.2375	0.2266	0.2299	0.234

Appendix 7.1

Well Row	Well Col	Raw Data (595) 69 - 11 h 20 min	Raw Data (595) 70 - 11 h 30 min	Raw Data (595) 71 - 11 h 39 min	Raw Data (595) 72 - 11 h 50 min	Raw Data (595) 73 - 12 h	Raw Data (595) 74 - 12 h 9 min	Raw Data (595) 75 - 12 h 20 min	Raw Data (595) 76 - 12 h 30 min	Raw Data (595) 77 - 12 h 39 min	Raw Data (595) 78 - 12 h 50 min	Raw Data (595) 79 - 13 h	Raw Data (595) 80 - 13 h 9 min
Time, Hours		11.33	11.50	11.67	11.83	12.00	12.17	12.33	12.50	12.67	12.83	13.00	13.17
C 1		0.1742	0.1733	0.1731	0.1747	0.1769	0.1746	0.1728	0.1735	0.1729	0.1736	0.1733	0.1729
C 2		0.3818	0.3811	0.382	0.3825	0.3861	0.3848	0.3824	0.3824	0.3828	0.3847	0.3848	0.3845
C 3		0.37	0.3681	0.3689	0.3703	0.378	0.3753	0.3729	0.3734	0.3736	0.375	0.3778	0.376
C 4		0.2674	0.2651	0.2666	0.2664	0.2705	0.269	0.2647	0.2632	0.2656	0.2666	0.2695	0.2674
C 5		0.5331	0.5455	0.5328	0.5347	0.5309	0.5428	0.5412	0.5658	0.5528	0.5713	0.5818	0.5752
C 6		0.1739	0.1725	0.1742	0.1743	0.1773	0.1739	0.1729	0.1728	0.1732	0.1736	0.1736	0.1726
C 7		0.2047	0.2034	0.205	0.2043	0.1954	0.2045	0.2051	0.2068	0.2051	0.2064	0.2059	0.206
C 8		0.1943	0.1947	0.1943	0.1949	0.1925	0.1948	0.1949	0.1965	0.1951	0.1962	0.1945	0.1947
C 9		0.4032	0.4012	0.4028	0.4023	0.4015	0.4018	0.4015	0.3999	0.3991	0.4002	0.4005	0.3991
C 10		0.3749	0.376	0.3783	0.3765	0.3722	0.3809	0.3786	0.3801	0.3791	0.3813	0.3807	0.3827
C 11		0.36	0.3603	0.3641	0.3629	0.3675	0.3651	0.3604	0.3611	0.3637	0.3648	0.3682	0.3684
C 12		0.2208	0.2176	0.2239	0.2221	0.2381	0.2234	0.2177	0.2151	0.2191	0.2168	0.2219	0.2209
D 1		0.2192	0.2179	0.2214	0.2205	0.213	0.2196	0.2179	0.2192	0.217	0.2186	0.2191	0.2198
D 2		0.3623	0.361	0.3635	0.3638	0.3583	0.3636	0.3621	0.3641	0.3632	0.3636	0.3618	0.3637
D 3		0.2788	0.2776	0.2791	0.2814	0.2774	0.282	0.2786	0.2792	0.2806	0.2812	0.2823	0.2838
D 4		0.25	0.2492	0.252	0.2545	0.2396	0.2566	0.2551	0.257	0.2585	0.2602	0.2617	0.2615
D 5		0.4814	0.4944	0.4767	0.4822	0.4428	0.4803	0.4913	0.4631	0.4751	0.4778	0.4818	0.4863
D 6		0.207	0.2034	0.2078	0.2076	0.2307	0.2047	0.2088	0.2065	0.2092	0.2067	0.2073	0.2079
D 7		0.2183	0.219	0.2203	0.2197	0.2029	0.2198	0.2183	0.2192	0.2197	0.2203	0.2214	0.2212
D 8		0.234	0.2337	0.2342	0.2347	0.2112	0.2341	0.2312	0.2329	0.2315	0.2342	0.2325	0.2325
D 9		0.3242	0.3227	0.3245	0.324	0.3249	0.3237	0.322	0.3223	0.322	0.3214	0.3211	0.3208
D 10		0.3257	0.3255	0.3271	0.3261	0.3137	0.3281	0.3269	0.3274	0.3255	0.3285	0.3282	0.3285
D 11		0.37	0.3715	0.3723	0.371	0.3558	0.3738	0.3724	0.3744	0.3735	0.3777	0.3744	0.3769
D 12		0.1825	0.1808	0.1827	0.1819	0.1811	0.1833	0.1816	0.1821	0.181	0.1819	0.1817	0.1819

Appendix 7.1

Well Row	Well Col	Raw Data (595) 69 - 11 h 20 min	Raw Data (595) 70 - 11 h 30 min	Raw Data (595) 71 - 11 h 39 min	Raw Data (595) 72 - 11 h 50 min	Raw Data (595) 73 - 12 h	Raw Data (595) 74 - 12 h 9 min	Raw Data (595) 75 - 12 h 20 min	Raw Data (595) 76 - 12 h 30 min	Raw Data (595) 77 - 12 h 39 min	Raw Data (595) 78 - 12 h 50 min	Raw Data (595) 79 - 13 h	Raw Data (595) 80 - 13 h 9 min
Time, Hours		11.33	11.50	11.67	11.83	12.00	12.17	12.33	12.50	12.67	12.83	13.00	13.17
E 1		0.1885	0.1874	0.1887	0.1879	0.189	0.1891	0.1878	0.1884	0.1879	0.1884	0.1886	0.1873
E 2		0.4302	0.4292	0.4314	0.433	0.4254	0.4331	0.432	0.4326	0.4342	0.4335	0.4339	0.4353
E 3		0.2842	0.2818	0.281	0.2798	0.2768	0.2784	0.275	0.2766	0.276	0.2748	0.2759	0.2746
E 4		0.2882	0.2845	0.2895	0.2908	0.2761	0.2919	0.2903	0.2935	0.2935	0.2926	0.297	0.2963
E 5		0.3485	0.3458	0.3477	0.3513	0.3517	0.3523	0.3526	0.3546	0.3554	0.3548	0.3578	0.3579
E 6		0.1944	0.1928	0.195	0.1951	0.2073	0.194	0.1945	0.1941	0.1935	0.1945	0.1934	0.1941
F 1		0.2036	0.202	0.2037	0.2037	0.1989	0.2042	0.2041	0.2033	0.2032	0.2049	0.2049	0.2039
F 2		0.415	0.4149	0.4154	0.4147	0.4178	0.4168	0.4155	0.4158	0.4172	0.4166	0.4173	0.4172
F 3		0.3633	0.3603	0.3619	0.3592	0.3669	0.3562	0.3571	0.3565	0.3549	0.3575	0.3556	0.3566
F 4		0.2474	0.2467	0.2481	0.2492	0.2501	0.2518	0.2525	0.2533	0.2541	0.2561	0.2559	0.2565
F 5		0.2944	0.2913	0.2947	0.2972	0.2954	0.2996	0.3011	0.3017	0.3031	0.305	0.3059	0.3054
F 6		0.2561	0.2552	0.2552	0.2555	0.2357	0.2579	0.2574	0.2563	0.2568	0.2603	0.2592	0.257
G 1		0.3214	0.3198	0.3207	0.3205	0.3251	0.3206	0.3208	0.3205	0.3205	0.3211	0.3217	0.3201
G 2		0.5017	0.4982	0.4941	0.4913	0.5005	0.4906	0.4895	0.4893	0.4848	0.4871	0.4839	0.4829
G 3		0.3669	0.3659	0.3652	0.3635	0.3711	0.3644	0.3627	0.3622	0.3621	0.3636	0.3618	0.3618
G 4		0.2754	0.2752	0.2756	0.2767	0.3132	0.2785	0.2799	0.2764	0.2769	0.2845	0.2809	0.2815
G 5		0.2623	0.2602	0.2627	0.2633	0.2497	0.2641	0.2632	0.2648	0.2653	0.2657	0.266	0.2665
G 6		0.1803	0.1784	0.1789	0.179	0.1956	0.1805	0.1798	0.1788	0.1783	0.1803	0.1796	0.1794
H 1		0.2155	0.2152	0.2157	0.2158	0.2121	0.2151	0.2157	0.2168	0.215	0.2153	0.216	0.2168
H 2		0.4156	0.4212	0.434	0.4432	0.4102	0.4495	0.4632	0.4701	0.4697	0.4751	0.4763	0.4779
H 3		0.3256	0.3296	0.3353	0.3437	0.3323	0.3523	0.3612	0.3689	0.3733	0.377	0.3835	0.3878
H 4		0.2119	0.212	0.213	0.2131	0.2213	0.2145	0.2131	0.2143	0.2132	0.2162	0.2152	0.2159
H 5		0.2133	0.2132	0.2147	0.2146	0.2324	0.2168	0.2168	0.2178	0.2171	0.2193	0.2191	0.2197
H 6		0.202	0.2017	0.2029	0.2021	0.1932	0.2011	0.2019	0.2037	0.2024	0.2018	0.2017	0.2026

## Appendix 7.1

User: USER

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 81 - 13 h 20 min	Raw Data (595) 82 - 13 h 30 min	Raw Data (595) 83 - 13 h 39 min	Raw Data (595) 84 - 13 h 50 min	Raw Data (595) 85 - 14 h	Raw Data (595) 86 - 14 h 9 min	Raw Data (595) 87 - 14 h 20 min	Raw Data (595) 88 - 14 h 30 min	Raw Data (595) 89 - 14 h 39 min	Raw Data (595) 90 - 14 h 50 min	Raw Data (595) 91 - 15 h	Raw Data (595) 92 - 15 h 9 min
Time, Hours		13.33	13.50	13.67	13.83	14.00	14.17	14.33	14.50	14.67	14.83	15.00	15.17
A 1		0.1806	0.1818	0.1816	0.1815	0.1831	0.1835	0.1827	0.1836	0.1836	0.1821	0.1831	0.1832
A 2		0.2931	0.2963	0.2936	0.2937	0.2974	0.293	0.2936	0.2906	0.2908	0.2908	0.2927	0.2907
A 3		0.2546	0.275	0.2555	0.2583	0.2576	0.2561	0.2562	0.278	0.2776	0.2563	0.258	0.2562
A 4		0.2094	0.2041	0.2105	0.2106	0.2107	0.2109	0.2106	0.2033	0.2018	0.2136	0.2134	0.2142
A 5		0.5409	0.5912	0.574	0.5807	0.5706	0.5655	0.5739	0.5835	0.5827	0.5644	0.5604	0.5896
A 6		0.1804	0.1839	0.1817	0.1812	0.1815	0.1818	0.1815	0.1832	0.1829	0.1808	0.1815	0.1808
A 7		0.2592	0.2606	0.2607	0.2611	0.2612	0.2618	0.2618	0.2593	0.2588	0.2606	0.2614	0.2622
A 8		0.2062	0.2153	0.2074	0.2081	0.2078	0.2077	0.2075	0.2122	0.2131	0.2071	0.2066	0.2071
A 9		0.337	0.3413	0.3374	0.3383	0.3378	0.3376	0.337	0.3393	0.34	0.3354	0.337	0.3358
A 10		0.2565	0.2465	0.2572	0.2575	0.2573	0.2564	0.2561	0.244	0.2435	0.2539	0.2553	0.2547
A 11		0.44	0.4646	0.4213	0.416	0.4052	0.4054	0.4024	0.4258	0.425	0.3927	0.3933	0.3866
A 12		0.1654	0.1818	0.1651	0.1654	0.166	0.1655	0.1659	0.1818	0.1818	0.1656	0.1653	0.1637
B 1		0.1995	0.207	0.2006	0.2015	0.2008	0.2024	0.2014	0.2058	0.2073	0.2009	0.201	0.2014
B 2		0.426	0.4317	0.4258	0.4254	0.4251	0.4254	0.4248	0.4278	0.429	0.4228	0.4235	0.4243
B 3		0.237	0.2367	0.2385	0.2394	0.239	0.2391	0.2393	0.2364	0.2358	0.2363	0.2367	0.2378
B 4		0.2345	0.2363	0.2384	0.2392	0.2381	0.2402	0.2394	0.2365	0.2378	0.2395	0.2403	0.2418
B 5		0.6281	0.6324	0.6739	0.6749	0.6895	0.7052	0.6861	0.6537	0.6611	0.6842	0.6767	0.6953
B 6		0.2036	0.1906	0.2025	0.2029	0.2054	0.2042	0.2023	0.1904	0.1919	0.2033	0.2037	0.2037
B 7		0.2212	0.2365	0.224	0.2237	0.2216	0.2235	0.2248	0.2343	0.2349	0.2221	0.223	0.2234
B 8		0.221	0.2072	0.2213	0.2215	0.2234	0.2204	0.2208	0.2076	0.2082	0.2214	0.2209	0.2209
B 9		0.3326	0.3243	0.3302	0.331	0.3344	0.331	0.3295	0.3229	0.3249	0.3318	0.3326	0.3323
B 10		0.3314	0.3352	0.3338	0.3344	0.3349	0.3336	0.3345	0.3378	0.3369	0.3384	0.3404	0.3398
B 11		0.3197	0.3193	0.3224	0.3249	0.3269	0.3259	0.3275	0.3244	0.3279	0.3337	0.3356	0.3372
B 12		0.2263	0.1979	0.2308	0.2336	0.2328	0.2255	0.2308	0.2003	0.2051	0.2347	0.2356	0.2359



Appendix 7.1

Well Row	Well Col	Raw Data (595) 81 - 13 h 20 min	Raw Data (595) 82 - 13 h 30 min	Raw Data (595) 83 - 13 h 39 min	Raw Data (595) 84 - 13 h 50 min	Raw Data (595) 85 - 14 h	Raw Data (595) 86 - 14 h 9 min	Raw Data (595) 87 - 14 h 20 min	Raw Data (595) 88 - 14 h 30 min	Raw Data (595) 89 - 14 h 39 min	Raw Data (595) 90 - 14 h 50 min	Raw Data (595) 91 - 15 h	Raw Data (595) 92 - 15 h 9 min
Time, Hours		13.33	13.50	13.67	13.83	14.00	14.17	14.33	14.50	14.67	14.83	15.00	15.17
C 1		0.1714	0.1761	0.1735	0.1736	0.1745	0.1746	0.1746	0.1751	0.1752	0.1735	0.1738	0.1732
C 2		0.3831	0.3872	0.3856	0.385	0.3857	0.3865	0.3891	0.3864	0.3876	0.3852	0.3857	0.3856
C 3		0.3751	0.3865	0.3764	0.3772	0.3777	0.3789	0.3808	0.3841	0.3887	0.3776	0.38	0.3798
C 4		0.266	0.2749	0.2684	0.2684	0.2711	0.2705	0.2734	0.2776	0.2787	0.2724	0.2731	0.2743
C 5		0.6209	0.6003	0.6252	0.6733	0.6237	0.597	0.6434	0.5973	0.5924	0.5962	0.567	0.5802
C 6		0.1731	0.1772	0.1734	0.1735	0.174	0.1738	0.1742	0.1758	0.1765	0.1733	0.1738	0.1732
C 7		0.2055	0.1968	0.2069	0.2082	0.207	0.2071	0.2075	0.1967	0.1973	0.2065	0.2067	0.2069
C 8		0.1948	0.1932	0.1956	0.1965	0.1967	0.1955	0.1961	0.1913	0.1934	0.1953	0.1953	0.196
C 9		0.3978	0.4005	0.3987	0.3988	0.3988	0.399	0.3988	0.3976	0.3985	0.3974	0.3975	0.398
C 10		0.383	0.3816	0.3855	0.3866	0.3887	0.3886	0.3906	0.3859	0.387	0.3911	0.3924	0.3936
C 11		0.367	0.3771	0.3713	0.3712	0.3726	0.3753	0.3776	0.3816	0.3786	0.3774	0.3783	0.3805
C 12		0.218	0.2383	0.2171	0.2178	0.2186	0.2198	0.2196	0.2362	0.2367	0.2176	0.2175	0.218
D 1		0.2173	0.2142	0.2187	0.2183	0.2187	0.22	0.2181	0.2116	0.2123	0.2176	0.2179	0.2163
D 2		0.3622	0.3566	0.3626	0.3627	0.3647	0.3643	0.3638	0.3554	0.3557	0.3628	0.3622	0.3605
D 3		0.2813	0.2814	0.283	0.2834	0.2855	0.2854	0.2861	0.2822	0.2831	0.2851	0.2862	0.2857
D 4		0.2608	0.2503	0.2645	0.266	0.2659	0.2676	0.2684	0.2543	0.2543	0.268	0.2712	0.2691
D 5		0.5239	0.4188	0.5265	0.5236	0.5317	0.5362	0.5277	0.5018	0.6334	0.5237	0.48	0.4375
D 6		0.2055	0.2325	0.2072	0.2094	0.2089	0.2075	0.2075	0.2287	0.2307	0.2077	0.2082	0.2082
D 7		0.2198	0.2045	0.2222	0.2219	0.2219	0.2219	0.2226	0.205	0.2049	0.2212	0.2232	0.2239
D 8		0.2315	0.2108	0.2351	0.2338	0.2351	0.2332	0.2353	0.2119	0.2138	0.237	0.2362	0.2361
D 9		0.3196	0.3218	0.3212	0.3212	0.3207	0.3205	0.3199	0.32	0.3198	0.3182	0.3191	0.3194
D 10		0.3286	0.3142	0.3316	0.3322	0.3307	0.3332	0.3317	0.3165	0.317	0.3333	0.3332	0.3357
D 11		0.3773	0.361	0.3818	0.3825	0.3828	0.3842	0.3823	0.3678	0.3665	0.3853	0.3868	0.3878
D 12		0.1816	0.1805	0.182	0.1823	0.1812	0.1829	0.1818	0.1783	0.1816	0.1813	0.1814	0.1822

Appendix 7.1

Well Row	Well Col	Raw Data (595) 81 - 13 h 20 min	Raw Data (595) 82 - 13 h 30 min	Raw Data (595) 83 - 13 h 39 min	Raw Data (595) 84 - 13 h 50 min	Raw Data (595) 85 - 14 h	Raw Data (595) 86 - 14 h 9 min	Raw Data (595) 87 - 14 h 20 min	Raw Data (595) 88 - 14 h 30 min	Raw Data (595) 89 - 14 h 39 min	Raw Data (595) 90 - 14 h 50 min	Raw Data (595) 91 - 15 h	Raw Data (595) 92 - 15 h 9 min
Time, Hours		13.33	13.50	13.67	13.83	14.00	14.17	14.33	14.50	14.67	14.83	15.00	15.17
E 1		0.1872	0.1904	0.1871	0.1875	0.1886	0.1886	0.1889	0.1885	0.1887	0.1874	0.1879	0.1883
E 2		0.4352	0.4299	0.4352	0.4375	0.4367	0.435	0.4364	0.4277	0.429	0.4376	0.435	0.4353
E 3		0.275	0.2742	0.2744	0.2742	0.274	0.2729	0.2749	0.2708	0.2705	0.2727	0.2735	0.2722
E 4		0.2982	0.2839	0.2953	0.2972	0.2993	0.2978	0.2987	0.2856	0.2876	0.3001	0.3	0.2993
E 5		0.3574	0.3603	0.3606	0.3616	0.3638	0.363	0.3644	0.3631	0.3649	0.367	0.3651	0.3659
E 6		0.1917	0.2075	0.1948	0.1942	0.1942	0.1965	0.1946	0.2067	0.2061	0.194	0.1937	0.1931
F 1		0.203	0.1987	0.2039	0.2042	0.2046	0.2056	0.2051	0.1978	0.1981	0.2029	0.2039	0.2056
F 2		0.4171	0.4207	0.419	0.4178	0.4193	0.4191	0.4195	0.4209	0.4241	0.4199	0.4228	0.4233
F 3		0.3543	0.3648	0.3547	0.3533	0.3537	0.3561	0.3553	0.3641	0.3641	0.3543	0.3564	0.3557
F 4		0.256	0.2579	0.2587	0.2587	0.26	0.261	0.2618	0.2617	0.2637	0.2624	0.263	0.2646
F 5		0.305	0.3029	0.3074	0.3082	0.3093	0.3112	0.3114	0.307	0.3091	0.311	0.3123	0.3121
F 6		0.2582	0.2373	0.2565	0.2584	0.2598	0.2605	0.2597	0.2355	0.2367	0.2586	0.2599	0.2616
G 1		0.3198	0.3254	0.3218	0.3209	0.3216	0.3222	0.3208	0.3248	0.3259	0.3207	0.3199	0.3214
G 2		0.4798	0.494	0.4821	0.4811	0.479	0.4799	0.4781	0.4874	0.4862	0.4768	0.4749	0.4781
G 3		0.3602	0.3697	0.3582	0.3589	0.3584	0.3587	0.3572	0.3675	0.3658	0.3571	0.358	0.3573
G 4		0.2822	0.3218	0.2812	0.2887	0.2827	0.2866	0.2849	0.3199	0.3239	0.2857	0.2865	0.2864
G 5		0.2647	0.2509	0.2671	0.2667	0.2684	0.2665	0.2677	0.2522	0.2534	0.2702	0.2704	0.2714
G 6		0.1803	0.1986	0.1803	0.1812	0.1794	0.1803	0.1804	0.1976	0.1963	0.1794	0.1807	0.18
H 1		0.2161	0.2125	0.2164	0.2153	0.2166	0.2164	0.2159	0.2113	0.2128	0.2152	0.2167	0.2162
H 2		0.4774	0.4696	0.4808	0.4808	0.48	0.4793	0.478	0.4789	0.4795	0.4745	0.4721	0.4735
H 3		0.3883	0.3833	0.398	0.4002	0.4039	0.4028	0.4061	0.4053	0.4079	0.4067	0.4083	0.4088
H 4		0.2151	0.2253	0.2172	0.2186	0.2173	0.2182	0.22	0.2269	0.2284	0.2185	0.2189	0.2204
H 5		0.2198	0.2381	0.2229	0.2223	0.2243	0.2248	0.2243	0.2386	0.2396	0.2249	0.2245	0.2259
H 6		0.2016	0.1953	0.2023	0.2015	0.2052	0.2034	0.2014	0.1937	0.1968	0.2018	0.2046	0.2026

## Appendix 7.1

User: USER

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 93 - 15 h 20 min	Raw Data (595) 94 - 15 h 30 min	Raw Data (595) 95 - 15 h 39 min	Raw Data (595) 96 - 15 h 50 min	Raw Data (595) 97 - 16 h	Raw Data (595) 98 - 16 h 10 min	Raw Data (595) 99 - 16 h 19 min	Raw Data (595) 100 - 16 h 30 min	Raw Data (595) 101 - 16 h 40 min	Raw Data (595) 102 - 16 h 49 min	Raw Data (595) 103 - 17 h	Raw Data (595) 104 - 17 h 10 min
Time, Hours		15.33	15.50	15.67	15.83	16.00	16.17	16.33	16.50	16.67	16.83	17.00	17.17
A 1		0.1831	0.1827	0.1833	0.1836	0.1825	0.184	0.1833	0.1836	0.1833	0.1832	0.1823	0.1828
A 2		0.2914	0.2912	0.2901	0.2915	0.2904	0.2911	0.2904	0.291	0.2899	0.2895	0.2886	0.2883
A 3		0.2562	0.2565	0.2754	0.2565	0.2551	0.2579	0.2562	0.2548	0.2548	0.2556	0.2521	0.2543
A 4		0.2155	0.2165	0.2041	0.217	0.2145	0.2158	0.2162	0.2158	0.2154	0.2157	0.2144	0.2144
A 5		0.5701	0.5503	0.5648	0.5695	0.5616	0.5613	0.5638	0.5654	0.5746	0.5836	0.5807	0.5858
A 6		0.1811	0.1812	0.1831	0.1816	0.1802	0.1812	0.1815	0.1821	0.1809	0.1822	0.1814	0.1825
A 7		0.2621	0.2618	0.2605	0.2629	0.2615	0.2627	0.2626	0.2624	0.2621	0.2627	0.2614	0.2626
A 8		0.2069	0.2069	0.2136	0.2082	0.2071	0.2078	0.2084	0.2076	0.2072	0.2076	0.2063	0.2077
A 9		0.3349	0.3363	0.3391	0.3357	0.334	0.3376	0.3363	0.3359	0.3353	0.3364	0.3359	0.3363
A 10		0.2536	0.2534	0.2426	0.2542	0.2511	0.2532	0.2525	0.2523	0.252	0.2501	0.2499	0.2504
A 11		0.379	0.39	0.4084	0.3785	0.3777	0.3807	0.3744	0.3745	0.3723	0.3787	0.374	0.3713
A 12		0.1633	0.1657	0.1806	0.1647	0.1642	0.1643	0.1635	0.1633	0.1627	0.1647	0.1626	0.1636
B 1		0.2015	0.2023	0.2101	0.2029	0.2027	0.2031	0.2025	0.2021	0.2024	0.2035	0.2021	0.2044
B 2		0.4241	0.4232	0.4275	0.4246	0.4234	0.4242	0.4254	0.4248	0.4252	0.4255	0.425	0.4257
B 3		0.2385	0.2381	0.2341	0.2381	0.2371	0.2367	0.237	0.2373	0.2374	0.2353	0.2374	0.2373
B 4		0.2422	0.2417	0.2386	0.2419	0.2388	0.2405	0.2421	0.2433	0.2416	0.2402	0.2422	0.2387
B 5		0.789	0.6866	0.6919	0.708	0.6823	0.7123	0.7459	0.7964	0.7871	0.8149	0.8543	0.8394
B 6		0.2036	0.2036	0.1904	0.2034	0.2021	0.2044	0.204	0.2047	0.2031	0.2027	0.2021	0.2031
B 7		0.2238	0.2233	0.2338	0.2247	0.2242	0.2216	0.2212	0.2204	0.2223	0.2242	0.2234	0.2231
B 8		0.221	0.2221	0.2062	0.2214	0.2181	0.2214	0.2209	0.2226	0.2209	0.2203	0.2191	0.2188
B 9		0.3294	0.3322	0.3218	0.3308	0.327	0.3293	0.3295	0.3309	0.3282	0.3273	0.3266	0.3277
B 10		0.3408	0.3416	0.3401	0.3437	0.3404	0.3414	0.343	0.3452	0.3447	0.3426	0.3472	0.348
B 11		0.3392	0.3388	0.3364	0.3432	0.3424	0.346	0.3479	0.3496	0.3502	0.3514	0.3536	0.355
B 12		0.2351	0.2342	0.1974	0.2363	0.224	0.2282	0.2305	0.2314	0.2301	0.226	0.2318	0.2285

Appendix 7.1

Well Row	Well Col	Raw Data (595) 93 - 15 h 20 min	Raw Data (595) 94 - 15 h 30 min	Raw Data (595) 95 - 15 h 39 min	Raw Data (595) 96 - 15 h 50 min	Raw Data (595) 97 - 16 h	Raw Data (595) 98 - 16 h 10 min	Raw Data (595) 99 - 16 h 19 min	Raw Data (595) 100 - 16 h 30 min	Raw Data (595) 101 - 16 h 40 min	Raw Data (595) 102 - 16 h 49 min	Raw Data (595) 103 - 17 h	Raw Data (595) 104 - 17 h 10 min
Time, Hours		15.33	15.50	15.67	15.83	16.00	16.17	16.33	16.50	16.67	16.83	17.00	17.17
C 1		0.1736	0.1739	0.1747	0.1738	0.1728	0.1726	0.1741	0.1741	0.174	0.1739	0.1731	0.1736
C 2		0.3869	0.3852	0.3852	0.3861	0.3842	0.3852	0.3873	0.3846	0.3839	0.3827	0.3846	0.3837
C 3		0.3817	0.3809	0.388	0.3801	0.3787	0.3796	0.3792	0.3781	0.3798	0.3758	0.3762	0.3769
C 4		0.2743	0.2761	0.2852	0.2818	0.2754	0.2781	0.2815	0.2819	0.2869	0.2822	0.2878	0.2847
C 5		0.5811	0.5605	0.5304	0.5778	0.6229	0.6569	0.6882	0.6884	0.6592	0.6925	0.6555	0.6796
C 6		0.1736	0.1734	0.1771	0.1731	0.1724	0.1742	0.1738	0.1735	0.1735	0.1729	0.1729	0.1724
C 7		0.2073	0.2073	0.1977	0.2078	0.2075	0.2082	0.2072	0.2086	0.2089	0.2131	0.2104	0.2143
C 8		0.1966	0.1978	0.194	0.1973	0.1982	0.1993	0.1987	0.1986	0.1985	0.2008	0.1984	0.2003
C 9		0.3979	0.3965	0.3972	0.3973	0.3941	0.3968	0.3963	0.3951	0.3947	0.3948	0.3943	0.3937
C 10		0.3946	0.3953	0.3938	0.3981	0.3965	0.3996	0.3998	0.4002	0.4023	0.4036	0.4034	0.4035
C 11		0.3805	0.3835	0.3885	0.3887	0.3841	0.3873	0.3886	0.39	0.392	0.391	0.3937	0.392
C 12		0.2175	0.2192	0.2347	0.2174	0.2131	0.2177	0.2175	0.217	0.2201	0.2166	0.2175	0.2151
D 1		0.2182	0.2161	0.2115	0.2177	0.2148	0.217	0.2169	0.2151	0.2166	0.2159	0.2151	0.2162
D 2		0.364	0.3628	0.354	0.3635	0.3617	0.3612	0.3586	0.3534	0.3525	0.3509	0.3481	0.3468
D 3		0.2857	0.2851	0.2816	0.2848	0.2832	0.2834	0.2823	0.2814	0.2804	0.2803	0.2771	0.2757
D 4		0.2717	0.2713	0.2591	0.2732	0.2723	0.2746	0.2757	0.2769	0.2781	0.2787	0.278	0.2772
D 5		0.5152	0.5596	0.4262	0.526	0.4532	0.427	0.5084	0.4868	0.4962	0.4466	0.499	0.5092
D 6		0.2088	0.2123	0.2345	0.2088	0.2117	0.2134	0.2115	0.2119	0.2105	0.2119	0.2101	0.2127
D 7		0.2227	0.222	0.2052	0.2243	0.2221	0.2242	0.2237	0.223	0.2235	0.2235	0.2227	0.2244
D 8		0.2364	0.2377	0.2129	0.238	0.2375	0.24	0.2394	0.2371	0.2394	0.2384	0.2376	0.2369
D 9		0.3189	0.3189	0.3189	0.3185	0.3172	0.3188	0.3199	0.3176	0.3186	0.3179	0.3169	0.318
D 10		0.3357	0.3358	0.3206	0.338	0.336	0.3365	0.3403	0.3393	0.3393	0.3403	0.3402	0.3424
D 11		0.388	0.388	0.3707	0.3944	0.3918	0.3942	0.396	0.3942	0.397	0.3957	0.3984	0.3997
D 12		0.1811	0.1817	0.1778	0.1814	0.1803	0.1807	0.1822	0.181	0.1815	0.1817	0.1801	0.182

Appendix 7.1

Well Row	Well Col	Raw Data (595) 93 - 15 h 20 min	Raw Data (595) 94 - 15 h 30 min	Raw Data (595) 95 - 15 h 39 min	Raw Data (595) 96 - 15 h 50 min	Raw Data (595) 97 - 16 h	Raw Data (595) 98 - 16 h 10 min	Raw Data (595) 99 - 16 h 19 min	Raw Data (595) 100 - 16 h 30 min	Raw Data (595) 101 - 16 h 40 min	Raw Data (595) 102 - 16 h 49 min	Raw Data (595) 103 - 17 h	Raw Data (595) 104 - 17 h 10 min
Time, Hours		15.33	15.50	15.67	15.83	16.00	16.17	16.33	16.50	16.67	16.83	17.00	17.17
E	1	0.1892	0.189	0.1889	0.1882	0.187	0.1873	0.1882	0.1884	0.1871	0.1883	0.1872	0.1882
E	2	0.4392	0.4376	0.4289	0.4388	0.4364	0.4369	0.4365	0.4291	0.4291	0.4286	0.4224	0.4238
E	3	0.2744	0.2735	0.2705	0.2728	0.2705	0.272	0.275	0.2747	0.2757	0.2771	0.2762	0.2781
E	4	0.302	0.3013	0.2881	0.3011	0.3013	0.3022	0.3037	0.3021	0.3051	0.3045	0.3038	0.3073
E	5	0.3701	0.367	0.3667	0.3685	0.3679	0.3684	0.3717	0.3699	0.3688	0.3698	0.3679	0.3706
E	6	0.1937	0.1937	0.2052	0.1939	0.1937	0.1947	0.1947	0.196	0.194	0.1953	0.1949	0.1955
F	1	0.2051	0.2032	0.2	0.2053	0.2041	0.2041	0.2039	0.2058	0.2038	0.2048	0.2034	0.2066
F	2	0.4225	0.425	0.4267	0.4263	0.4239	0.4305	0.4395	0.4464	0.453	0.4607	0.4627	0.4662
F	3	0.3544	0.3552	0.3646	0.3555	0.3526	0.3547	0.3575	0.3616	0.3636	0.3672	0.3698	0.3723
F	4	0.2653	0.2657	0.2663	0.266	0.2649	0.2672	0.2667	0.2688	0.2685	0.2685	0.2682	0.2696
F	5	0.3137	0.3134	0.3115	0.3151	0.3167	0.3168	0.317	0.3194	0.3181	0.3184	0.3194	0.3186
F	6	0.2625	0.2623	0.2422	0.2629	0.2619	0.262	0.2645	0.2654	0.2642	0.2642	0.2633	0.2652
G	1	0.3205	0.3209	0.3254	0.3204	0.3203	0.3213	0.3219	0.32	0.321	0.3214	0.3202	0.3222
G	2	0.4775	0.4722	0.4826	0.4743	0.4716	0.4674	0.4735	0.4694	0.4693	0.4716	0.4732	0.4729
G	3	0.3556	0.3556	0.3641	0.3568	0.3549	0.3545	0.3581	0.3576	0.3598	0.3627	0.3607	0.3631
G	4	0.2841	0.2834	0.324	0.2909	0.2877	0.2863	0.2916	0.2869	0.2927	0.2933	0.29	0.2925
G	5	0.2703	0.273	0.2552	0.2723	0.2696	0.2723	0.2743	0.274	0.2727	0.2745	0.2749	0.278
G	6	0.18	0.1791	0.1974	0.1813	0.1803	0.1799	0.18	0.1796	0.1804	0.1806	0.179	0.1806
H	1	0.2165	0.2187	0.2118	0.2171	0.2167	0.2155	0.2159	0.2169	0.2151	0.2155	0.2154	0.2142
H	2	0.4737	0.4686	0.4739	0.468	0.466	0.465	0.469	0.4709	0.4693	0.4677	0.4669	0.4652
H	3	0.4105	0.4089	0.4166	0.409	0.407	0.4085	0.4085	0.4101	0.4109	0.4121	0.4116	0.4153
H	4	0.2204	0.2204	0.2342	0.2213	0.2199	0.2225	0.2225	0.2208	0.2214	0.2213	0.2219	0.2234
H	5	0.2273	0.226	0.2435	0.227	0.2251	0.2278	0.2269	0.2263	0.226	0.2263	0.225	0.2259
H	6	0.204	0.205	0.1947	0.2047	0.205	0.2037	0.2057	0.2043	0.2031	0.2049	0.2052	0.2041

## Appendix 7.1

User: USER

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 105 - 17 h 19 min	Raw Data (595) 106 - 17 h 30 min	Raw Data (595) 107 - 17 h 40 min	Raw Data (595) 108 - 17 h 49 min	Raw Data (595) 109 - 18 h	Raw Data (595) 110 - 18 h 10 min	Raw Data (595) 111 - 18 h 19 min	Raw Data (595) 112 - 18 h 30 min	Raw Data (595) 113 - 18 h 40 min	Raw Data (595) 114 - 18 h 49 min	Raw Data (595) 115 - 19 h	Raw Data (595) 116 - 19 h 10 min
Time, Hours		17.33	17.50	17.67	17.83	18.00	18.17	18.33	18.50	18.67	18.83	19.00	19.17
A 1		0.1836	0.1835	0.1838	0.1839	0.1837	0.1833	0.1846	0.1848	0.1834	0.1836	0.1839	0.1835
A 2		0.2893	0.2896	0.286	0.2897	0.2896	0.2884	0.2891	0.2893	0.2872	0.29	0.2891	0.2885
A 3		0.256	0.2538	0.2739	0.2539	0.2532	0.2532	0.253	0.2523	0.252	0.2535	0.2501	0.2515
A 4		0.2157	0.2151	0.2017	0.2153	0.2164	0.2155	0.2169	0.2157	0.2158	0.2157	0.2143	0.2145
A 5		0.5795	0.6025	0.616	0.5869	0.6083	0.5958	0.5687	0.5849	0.5808	0.5899	0.5993	0.6109
A 6		0.1819	0.183	0.1871	0.1843	0.1835	0.1846	0.184	0.1836	0.1841	0.1832	0.1837	0.1836
A 7		0.2619	0.2625	0.2602	0.2633	0.2628	0.2628	0.2623	0.2632	0.2621	0.2628	0.2627	0.2634
A 8		0.2063	0.2081	0.2126	0.2082	0.2075	0.2078	0.2077	0.2077	0.2071	0.207	0.2072	0.2072
A 9		0.3368	0.3354	0.3395	0.3367	0.3355	0.3362	0.3347	0.3346	0.3344	0.3352	0.3344	0.3338
A 10		0.2505	0.2498	0.2378	0.2497	0.2481	0.2492	0.248	0.249	0.2466	0.248	0.2479	0.2469
A 11		0.3802	0.3766	0.4203	0.3954	0.411	0.4397	0.4985	0.5108	0.5364	0.5464	0.546	0.5386
A 12		0.1643	0.1622	0.1817	0.1644	0.1646	0.1628	0.1642	0.1637	0.163	0.1633	0.1628	0.1624
B 1		0.203	0.2034	0.2102	0.202	0.2042	0.2041	0.2019	0.203	0.2041	0.2023	0.2032	0.2036
B 2		0.4256	0.4248	0.4282	0.4269	0.4261	0.4282	0.4267	0.4273	0.4254	0.4292	0.4271	0.4277
B 3		0.2377	0.2362	0.2355	0.2365	0.2378	0.2378	0.2367	0.2376	0.2369	0.2379	0.236	0.2369
B 4		0.2406	0.238	0.2364	0.2408	0.239	0.2413	0.2398	0.2411	0.239	0.2407	0.239	0.2391
B 5		0.8343	0.7861	0.811	0.7949	0.7765	0.8114	0.7878	0.8273	0.8517	0.8072	0.8895	0.8375
B 6		0.2033	0.2038	0.1912	0.2054	0.2042	0.2036	0.2051	0.2037	0.203	0.2049	0.2038	0.203
B 7		0.2236	0.2215	0.2338	0.2204	0.2224	0.2236	0.2212	0.223	0.223	0.2225	0.2231	0.2233
B 8		0.2217	0.2199	0.2076	0.2215	0.2199	0.2189	0.2211	0.221	0.2176	0.2211	0.2182	0.2195
B 9		0.3291	0.3274	0.3198	0.3286	0.3268	0.3265	0.3277	0.3293	0.3242	0.3274	0.3258	0.3264
B 10		0.3494	0.3481	0.3488	0.3516	0.352	0.3533	0.3539	0.3575	0.3539	0.3588	0.3579	0.3593
B 11		0.3567	0.357	0.3556	0.3622	0.3637	0.3649	0.3648	0.3684	0.3663	0.3704	0.3707	0.3728
B 12		0.2335	0.2268	0.2042	0.2302	0.2245	0.2333	0.2269	0.2302	0.2244	0.2355	0.2258	0.2276

Appendix 7.1

Well Row	Well Col	Raw Data (595) 105 - 17 h 19 min	Raw Data (595) 106 - 17 h 30 min	Raw Data (595) 107 - 17 h 40 min	Raw Data (595) 108 - 17 h 49 min	Raw Data (595) 109 - 18 h	Raw Data (595) 110 - 18 h 10 min	Raw Data (595) 111 - 18 h 19 min	Raw Data (595) 112 - 18 h 30 min	Raw Data (595) 113 - 18 h 40 min	Raw Data (595) 114 - 18 h 49 min	Raw Data (595) 115 - 19 h	Raw Data (595) 116 - 19 h 10 min
Time, Hours		17.33	17.50	17.67	17.83	18.00	18.17	18.33	18.50	18.67	18.83	19.00	19.17
C 1		0.1736	0.1732	0.1742	0.1728	0.1736	0.1738	0.1736	0.1735	0.1728	0.1736	0.1728	0.1723
C 2		0.3838	0.3826	0.3832	0.3844	0.3821	0.3859	0.3835	0.3854	0.3834	0.3864	0.3862	0.3863
C 3		0.3751	0.3757	0.3821	0.3752	0.373	0.3747	0.3742	0.3733	0.3732	0.3732	0.3721	0.3725
C 4		0.2877	0.289	0.2991	0.2922	0.294	0.2957	0.2994	0.2999	0.2994	0.3019	0.3007	0.3041
C 5		0.6535	0.632	0.6054	0.635	0.63	0.6637	0.6106	0.6529	0.6288	0.6765	0.6585	0.6635
C 6		0.1736	0.1733	0.1758	0.1733	0.1742	0.1735	0.1742	0.1739	0.1727	0.1732	0.1727	0.1739
C 7		0.2132	0.2145	0.2037	0.2158	0.2153	0.2165	0.2153	0.2142	0.2162	0.2148	0.2152	0.2151
C 8		0.2001	0.2006	0.1975	0.2013	0.2016	0.2026	0.2018	0.2011	0.2017	0.2014	0.2007	0.2026
C 9		0.3935	0.3933	0.3952	0.3927	0.3939	0.3932	0.3922	0.3925	0.393	0.3925	0.3922	0.3935
C 10		0.4057	0.4059	0.4051	0.4093	0.4108	0.4115	0.4111	0.4131	0.4126	0.4139	0.4146	0.4182
C 11		0.3945	0.394	0.4011	0.3966	0.3973	0.3962	0.3996	0.401	0.3966	0.403	0.4	0.4028
C 12		0.2189	0.2156	0.2349	0.2148	0.215	0.2153	0.217	0.2196	0.2136	0.2162	0.2151	0.2165
D 1		0.2168	0.2173	0.2116	0.2168	0.2159	0.2151	0.2171	0.2154	0.2155	0.2159	0.2153	0.2147
D 2		0.3463	0.3471	0.3385	0.3464	0.346	0.3475	0.3471	0.347	0.3459	0.3459	0.3473	0.3469
D 3		0.2767	0.2775	0.2757	0.2743	0.2744	0.2737	0.2742	0.274	0.2728	0.2741	0.2728	0.2729
D 4		0.2791	0.2815	0.2669	0.2827	0.2838	0.2837	0.2845	0.284	0.284	0.2859	0.2855	0.2867
D 5		0.5487	0.5053	0.4156	0.5463	0.5279	0.4959	0.5401	0.5284	0.523	0.5201	0.519	0.5343
D 6		0.2131	0.2138	0.2378	0.2141	0.2131	0.2174	0.2163	0.2194	0.2158	0.2181	0.2176	0.2134
D 7		0.2232	0.2243	0.207	0.2231	0.224	0.2238	0.225	0.2243	0.2248	0.2239	0.2245	0.2248
D 8		0.2382	0.238	0.2131	0.2369	0.2397	0.2382	0.237	0.2382	0.2384	0.2373	0.2378	0.2396
D 9		0.317	0.3168	0.3166	0.3167	0.3165	0.3164	0.3161	0.3162	0.3166	0.3168	0.3163	0.3165
D 10		0.3408	0.3423	0.3259	0.3418	0.3441	0.3432	0.3461	0.3458	0.3469	0.3472	0.3481	0.3492
D 11		0.3977	0.4015	0.3823	0.4	0.4018	0.4039	0.4032	0.4045	0.4057	0.4055	0.4083	0.4097
D 12		0.182	0.1802	0.1756	0.1813	0.1816	0.1804	0.1811	0.1811	0.18	0.1814	0.1804	0.1814

Appendix 7.1

Well Row	Well Col	Raw Data (595) 105 - 17 h 19 min	Raw Data (595) 106 - 17 h 30 min	Raw Data (595) 107 - 17 h 40 min	Raw Data (595) 108 - 17 h 49 min	Raw Data (595) 109 - 18 h	Raw Data (595) 110 - 18 h 10 min	Raw Data (595) 111 - 18 h 19 min	Raw Data (595) 112 - 18 h 30 min	Raw Data (595) 113 - 18 h 40 min	Raw Data (595) 114 - 18 h 49 min	Raw Data (595) 115 - 19 h	Raw Data (595) 116 - 19 h 10 min
Time, Hours		17.33	17.50	17.67	17.83	18.00	18.17	18.33	18.50	18.67	18.83	19.00	19.17
E	1	0.1884	0.1878	0.1894	0.1884	0.1877	0.1878	0.1888	0.1887	0.1873	0.1874	0.1879	0.1872
E	2	0.4234	0.4219	0.4154	0.4235	0.4216	0.4215	0.4222	0.423	0.4213	0.422	0.42	0.42
E	3	0.2792	0.2792	0.2789	0.2799	0.2793	0.2796	0.2793	0.2794	0.2773	0.278	0.2764	0.2757
E	4	0.3078	0.3072	0.2938	0.3084	0.3088	0.3095	0.3103	0.3089	0.3087	0.3101	0.3078	0.309
E	5	0.3719	0.3692	0.3666	0.3706	0.3699	0.3705	0.3716	0.3696	0.3719	0.3715	0.3702	0.3709
E	6	0.195	0.1957	0.208	0.1961	0.1952	0.1973	0.1961	0.1963	0.1965	0.1964	0.1987	0.1977
F	1	0.2057	0.2067	0.2004	0.2071	0.2077	0.2072	0.2081	0.2069	0.2075	0.2084	0.2094	0.2084
F	2	0.4701	0.4694	0.4756	0.4731	0.4707	0.4722	0.4715	0.475	0.4733	0.4739	0.4723	0.4728
F	3	0.3762	0.377	0.39	0.3809	0.3768	0.3778	0.3819	0.3805	0.3815	0.3834	0.3816	0.3801
F	4	0.2705	0.2704	0.2718	0.2729	0.2722	0.272	0.2722	0.2727	0.2728	0.274	0.2744	0.2742
F	5	0.3207	0.3209	0.3142	0.3216	0.321	0.3206	0.323	0.3228	0.3225	0.322	0.3234	0.3247
F	6	0.2642	0.2652	0.2437	0.2662	0.2663	0.2657	0.2655	0.2667	0.2668	0.2657	0.2663	0.2698
G	1	0.321	0.3223	0.3268	0.3213	0.3217	0.3223	0.3216	0.3216	0.3208	0.3218	0.3216	0.3224
G	2	0.472	0.4721	0.4791	0.4732	0.4714	0.4731	0.4718	0.4713	0.4705	0.4721	0.4713	0.473
G	3	0.364	0.3647	0.372	0.3655	0.3644	0.3623	0.365	0.3625	0.364	0.3637	0.3635	0.362
G	4	0.2928	0.2969	0.3313	0.2954	0.2934	0.2928	0.296	0.2929	0.2969	0.2965	0.2954	0.2964
G	5	0.2778	0.2762	0.2593	0.2767	0.2778	0.2793	0.2793	0.2804	0.2798	0.279	0.281	0.2812
G	6	0.1801	0.1805	0.1962	0.1812	0.1795	0.1807	0.1812	0.1805	0.1802	0.182	0.1807	0.1802
H	1	0.216	0.2167	0.2122	0.2172	0.2161	0.2171	0.2186	0.2168	0.2165	0.2172	0.2155	0.2163
H	2	0.4629	0.4645	0.4664	0.4618	0.46	0.4625	0.4613	0.4601	0.4601	0.4599	0.4607	0.4616
H	3	0.4142	0.4143	0.4225	0.4151	0.4159	0.4169	0.4175	0.4154	0.4167	0.4188	0.4181	0.4212
H	4	0.2221	0.2243	0.2344	0.2232	0.223	0.2219	0.2219	0.2219	0.2231	0.223	0.2229	0.2235
H	5	0.2259	0.226	0.2404	0.2262	0.2257	0.2259	0.2246	0.2245	0.2242	0.2232	0.2219	0.2238
H	6	0.206	0.207	0.1994	0.2088	0.2064	0.2102	0.2093	0.2092	0.2069	0.208	0.2081	0.2076



## Appendix 7.1

User: USER

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 117 - 19 h 19 min	Raw Data (595) 118 - 19 h 30 min	Raw Data (595) 119 - 19 h 40 min	Raw Data (595) 120 - 19 h 49 min	Raw Data (595) 121 - 20 h	Raw Data (595) 122 - 20 h 10 min	Raw Data (595) 123 - 20 h 19 min	Raw Data (595) 124 - 20 h 30 min	Raw Data (595) 125 - 20 h 40 min	Raw Data (595) 126 - 20 h 49 min	Raw Data (595) 127 - 21 h	Raw Data (595) 128 - 21 h 10 min
Time, Hours		19.33	19.50	19.67	19.83	20.00	20.17	20.33	20.50	20.67	20.83	21.00	21.17
A 1		0.1839	0.1842	0.1835	0.1838	0.183	0.1846	0.1841	0.1834	0.1845	0.1836	0.1842	0.1843
A 2		0.2876	0.2877	0.2889	0.2843	0.2864	0.2886	0.287	0.2871	0.2885	0.2869	0.2887	0.2849
A 3		0.2508	0.2512	0.2518	0.2705	0.2502	0.25	0.2501	0.2477	0.2502	0.2487	0.2475	0.2691
A 4		0.2161	0.2156	0.2157	0.2044	0.2159	0.2162	0.2157	0.2158	0.2149	0.2162	0.2161	0.2036
A 5		0.6117	0.6166	0.5691	0.6166	0.5873	0.6107	0.6313	0.6316	0.6448	0.6108	0.6206	0.6148
A 6		0.1842	0.1843	0.1836	0.187	0.1828	0.185	0.1833	0.184	0.1852	0.1839	0.1848	0.1878
A 7		0.2633	0.2628	0.2627	0.2613	0.2617	0.264	0.2633	0.263	0.2645	0.2636	0.2644	0.2629
A 8		0.2076	0.2065	0.2063	0.2127	0.2061	0.2077	0.2076	0.2066	0.2074	0.2068	0.2078	0.2133
A 9		0.335	0.3338	0.3334	0.3363	0.3331	0.3335	0.3339	0.3321	0.332	0.3332	0.3333	0.3356
A 10		0.2466	0.2458	0.2455	0.2337	0.2449	0.2459	0.2453	0.2441	0.2445	0.2444	0.2443	0.2315
A 11		0.5622	0.5602	0.5739	0.6479	0.5844	0.558	0.5691	0.5775	0.5675	0.5911	0.5564	0.6762
A 12		0.1624	0.1626	0.162	0.1802	0.1628	0.1621	0.1637	0.1627	0.1629	0.1647	0.1623	0.1797
B 1		0.2044	0.2013	0.2008	0.211	0.2029	0.2036	0.2038	0.2045	0.2042	0.2037	0.2048	0.2112
B 2		0.428	0.4272	0.426	0.4308	0.4267	0.428	0.4283	0.427	0.4283	0.4286	0.4306	0.4315
B 3		0.2387	0.2363	0.2358	0.2336	0.2359	0.2373	0.2367	0.2358	0.2365	0.2366	0.2365	0.2333
B 4		0.2392	0.2378	0.2358	0.2341	0.2352	0.2379	0.2371	0.2365	0.2388	0.2383	0.238	0.2344
B 5		0.8221	0.8329	0.8371	0.8428	0.8428	0.902	0.8829	0.8652	0.9253	0.9334	0.9479	0.9098
B 6		0.2036	0.2042	0.2052	0.1896	0.2031	0.2051	0.2054	0.2033	0.2026	0.2042	0.204	0.1901
B 7		0.2248	0.2188	0.217	0.2341	0.2205	0.2227	0.2211	0.222	0.2244	0.221	0.2224	0.2342
B 8		0.2179	0.2205	0.2241	0.2061	0.2189	0.2206	0.2215	0.2195	0.2197	0.22	0.2199	0.2043
B 9		0.324	0.3272	0.3288	0.3164	0.324	0.3259	0.3256	0.3235	0.324	0.3254	0.3243	0.3151
B 10		0.358	0.3628	0.3619	0.359	0.3584	0.3647	0.3642	0.363	0.3666	0.3657	0.3676	0.3632
B 11		0.3684	0.3755	0.3755	0.3708	0.3729	0.3777	0.3785	0.3781	0.381	0.3819	0.3833	0.3781
B 12		0.2262	0.2342	0.2365	0.196	0.2254	0.2322	0.2266	0.2276	0.2312	0.23	0.2345	0.1956

Appendix 7.1

Well Row	Well Col	Raw Data (595) 117 - 19 h 19 min	Raw Data (595) 118 - 19 h 30 min	Raw Data (595) 119 - 19 h 40 min	Raw Data (595) 120 - 19 h 49 min	Raw Data (595) 121 - 20 h	Raw Data (595) 122 - 20 h 10 min	Raw Data (595) 123 - 20 h 19 min	Raw Data (595) 124 - 20 h 30 min	Raw Data (595) 125 - 20 h 40 min	Raw Data (595) 126 - 20 h 49 min	Raw Data (595) 127 - 21 h	Raw Data (595) 128 - 21 h 10 min
Time, Hours		19.33	19.50	19.67	19.83	20.00	20.17	20.33	20.50	20.67	20.83	21.00	21.17
C 1		0.173	0.172	0.1718	0.1723	0.1717	0.1736	0.1722	0.1727	0.1726	0.1735	0.1733	0.1727
C 2		0.3866	0.3868	0.3872	0.3856	0.3856	0.39	0.3875	0.3886	0.3901	0.3886	0.3889	0.3867
C 3		0.3738	0.3721	0.3725	0.3803	0.3719	0.3745	0.3727	0.3721	0.3749	0.3726	0.374	0.3813
C 4		0.3031	0.302	0.3058	0.3124	0.3066	0.3102	0.3074	0.3073	0.3116	0.3076	0.311	0.3168
C 5		0.6265	0.6408	0.6455	0.6219	0.5935	0.5975	0.5906	0.6099	0.6371	0.6999	0.6387	0.6078
C 6		0.1734	0.1724	0.1721	0.1753	0.1724	0.1745	0.1736	0.1723	0.1742	0.1733	0.174	0.1764
C 7		0.216	0.2162	0.2166	0.2049	0.2164	0.2153	0.2174	0.218	0.2168	0.2178	0.2182	0.2051
C 8		0.2027	0.2018	0.2015	0.1967	0.2024	0.2027	0.2048	0.2052	0.2056	0.206	0.2063	0.2003
C 9		0.3931	0.3911	0.3913	0.3907	0.3916	0.3918	0.392	0.3901	0.3914	0.3908	0.3904	0.3894
C 10		0.4157	0.4178	0.4187	0.4173	0.418	0.4222	0.4199	0.4204	0.4237	0.4224	0.4237	0.4217
C 11		0.4008	0.4007	0.4036	0.4086	0.402	0.4077	0.4031	0.4025	0.4065	0.4029	0.4059	0.4176
C 12		0.218	0.2127	0.2137	0.2352	0.2164	0.2193	0.2156	0.2151	0.2169	0.2128	0.2168	0.2359
D 1		0.2162	0.2157	0.2147	0.2093	0.2135	0.2159	0.2145	0.2144	0.2154	0.214	0.2133	0.2093
D 2		0.347	0.3485	0.3484	0.3415	0.3475	0.3515	0.3517	0.3514	0.3528	0.3527	0.3519	0.3436
D 3		0.2727	0.2721	0.2718	0.271	0.2696	0.2724	0.2718	0.2713	0.2716	0.2707	0.2724	0.271
D 4		0.2899	0.2902	0.2896	0.2745	0.2887	0.2917	0.29	0.2888	0.2892	0.289	0.2903	0.2739
D 5		0.5237	0.5096	0.4743	0.4055	0.4861	0.5129	0.4947	0.4906	0.4752	0.4561	0.4773	0.4219
D 6		0.2193	0.216	0.2151	0.2415	0.2179	0.2176	0.2203	0.2184	0.2182	0.219	0.2187	0.245
D 7		0.2245	0.2241	0.2247	0.2082	0.225	0.2272	0.2263	0.2263	0.2273	0.2267	0.2264	0.2078
D 8		0.2389	0.2384	0.2393	0.2143	0.2381	0.2415	0.2417	0.2399	0.2398	0.2412	0.2401	0.2152
D 9		0.3159	0.3154	0.3158	0.3146	0.3142	0.3165	0.3163	0.3139	0.3145	0.3144	0.3152	0.3145
D 10		0.3471	0.3493	0.3485	0.3337	0.3452	0.3504	0.3477	0.3466	0.3501	0.3478	0.3488	0.3335
D 11		0.4074	0.4122	0.4103	0.394	0.4091	0.4143	0.4122	0.4123	0.4158	0.4147	0.4178	0.3965
D 12		0.1808	0.18	0.1801	0.1738	0.1792	0.1817	0.1803	0.1789	0.1807	0.1797	0.1803	0.176

Appendix 7.1

Well Row	Well Col	Raw Data (595) 117 - 19 h 19 min	Raw Data (595) 118 - 19 h 30 min	Raw Data (595) 119 - 19 h 40 min	Raw Data (595) 120 - 19 h 49 min	Raw Data (595) 121 - 20 h	Raw Data (595) 122 - 20 h 10 min	Raw Data (595) 123 - 20 h 19 min	Raw Data (595) 124 - 20 h 30 min	Raw Data (595) 125 - 20 h 40 min	Raw Data (595) 126 - 20 h 49 min	Raw Data (595) 127 - 21 h	Raw Data (595) 128 - 21 h 10 min
Time, Hours		19.33	19.50	19.67	19.83	20.00	20.17	20.33	20.50	20.67	20.83	21.00	21.17
E 1		0.1878	0.1874	0.1871	0.1877	0.1872	0.1892	0.1887	0.1877	0.1886	0.188	0.1881	0.1894
E 2		0.4198	0.4221	0.4201	0.4144	0.4225	0.4235	0.4259	0.4239	0.4247	0.4236	0.4244	0.4156
E 3		0.2754	0.276	0.2745	0.2745	0.274	0.2754	0.2765	0.275	0.2759	0.2746	0.2749	0.2753
E 4		0.3085	0.3093	0.3076	0.2963	0.3112	0.3115	0.3132	0.3125	0.3117	0.3126	0.3113	0.3004
E 5		0.3701	0.3709	0.3711	0.3704	0.3713	0.3724	0.3747	0.3732	0.3746	0.3747	0.3751	0.374
E 6		0.1984	0.1984	0.1968	0.2107	0.196	0.1985	0.1966	0.1955	0.1982	0.1979	0.1976	0.2103
F 1		0.2082	0.2099	0.2075	0.2026	0.2084	0.2092	0.2101	0.2083	0.2091	0.2089	0.2085	0.2038
F 2		0.472	0.4751	0.4758	0.4804	0.4728	0.4797	0.479	0.4806	0.483	0.4814	0.4815	0.4854
F 3		0.3835	0.381	0.38	0.3944	0.3804	0.3827	0.382	0.3814	0.3842	0.3807	0.3826	0.3957
F 4		0.274	0.2747	0.2742	0.2754	0.2739	0.2761	0.2754	0.2761	0.2767	0.2767	0.2762	0.2769
F 5		0.3236	0.3238	0.323	0.3175	0.3212	0.3227	0.3255	0.3234	0.325	0.3239	0.3243	0.3188
F 6		0.2685	0.2688	0.2672	0.2421	0.2657	0.2688	0.2687	0.268	0.2681	0.2678	0.2689	0.246
G 1		0.3225	0.3211	0.3215	0.3276	0.3207	0.3213	0.3238	0.3218	0.3228	0.3234	0.3226	0.3287
G 2		0.4667	0.4723	0.4701	0.475	0.466	0.4711	0.4679	0.4699	0.4738	0.4715	0.4741	0.473
G 3		0.362	0.3618	0.3607	0.3683	0.359	0.3616	0.3633	0.3636	0.3656	0.3632	0.3624	0.371
G 4		0.296	0.2941	0.2944	0.3302	0.2924	0.2927	0.2991	0.2941	0.2967	0.2967	0.2965	0.3289
G 5		0.2812	0.2826	0.2828	0.2649	0.2815	0.2845	0.2826	0.2823	0.2836	0.2839	0.2847	0.2671
G 6		0.1817	0.18	0.1794	0.1955	0.1801	0.1808	0.181	0.1798	0.1809	0.1817	0.1805	0.1971
H 1		0.2175	0.2171	0.2167	0.2113	0.2171	0.2176	0.2171	0.2179	0.2177	0.2181	0.2187	0.2132
H 2		0.4566	0.4619	0.4587	0.4604	0.4522	0.4573	0.4539	0.4579	0.4631	0.4632	0.4671	0.4684
H 3		0.4194	0.4216	0.422	0.4321	0.4195	0.4213	0.4197	0.4222	0.4264	0.4299	0.4328	0.4461
H 4		0.2237	0.2235	0.2252	0.2422	0.2227	0.2262	0.2259	0.2247	0.2259	0.2225	0.2247	0.2411
H 5		0.2216	0.2222	0.2211	0.234	0.218	0.2189	0.2161	0.2162	0.2159	0.2145	0.2153	0.2289
H 6		0.2092	0.2083	0.2083	0.1983	0.2084	0.2085	0.2088	0.2085	0.2096	0.2103	0.211	0.1994

## Appendix 7.1

User: USER

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 129 - 21 h 19 min	Raw Data (595) 130 - 21 h 30 min	Raw Data (595) 131 - 21 h 40 min	Raw Data (595) 132 - 21 h 49 min	Raw Data (595) 133 - 22 h	Raw Data (595) 134 - 22 h 10 min	Raw Data (595) 135 - 22 h 19 min	Raw Data (595) 136 - 22 h 30 min	Raw Data (595) 137 - 22 h 40 min	Raw Data (595) 138 - 22 h 49 min	Raw Data (595) 139 - 23 h	Raw Data (595) 140 - 23 h 10 min
Time, Hours		21.33	21.50	21.67	21.83	22.00	22.17	22.33	22.50	22.67	22.83	23.00	23.17
A 1		0.1843	0.1844	0.1841	0.1843	0.1847	0.1848	0.1846	0.1843	0.1836	0.1852	0.1848	0.1853
A 2		0.2867	0.2886	0.2882	0.2865	0.2868	0.2872	0.2873	0.2869	0.286	0.2881	0.2872	0.2872
A 3		0.2494	0.2501	0.2502	0.2508	0.2484	0.2489	0.2505	0.2481	0.2465	0.2484	0.2472	0.2487
A 4		0.2155	0.2155	0.215	0.215	0.2137	0.215	0.2159	0.2147	0.214	0.2139	0.215	0.2151
A 5		0.6117	0.6108	0.6149	0.611	0.6116	0.6155	0.6375	0.6194	0.6193	0.636	0.6831	0.6639
A 6		0.1834	0.1845	0.1841	0.1846	0.184	0.1838	0.1844	0.1831	0.184	0.1838	0.1839	0.1845
A 7		0.2641	0.2654	0.2651	0.2653	0.265	0.266	0.2647	0.2651	0.2644	0.2664	0.2662	0.2663
A 8		0.207	0.2077	0.2081	0.2069	0.207	0.2077	0.2074	0.2064	0.2064	0.207	0.2073	0.2075
A 9		0.3317	0.3313	0.332	0.3317	0.3312	0.3327	0.3329	0.3309	0.3307	0.3307	0.3306	0.3304
A 10		0.2433	0.2434	0.2432	0.2425	0.2421	0.2429	0.2421	0.241	0.2404	0.2406	0.2409	0.2401
A 11		0.5869	0.5793	0.5845	0.5919	0.5693	0.5789	0.5882	0.5691	0.5779	0.5727	0.5623	0.5782
A 12		0.1619	0.1639	0.1631	0.162	0.1619	0.1625	0.1635	0.1614	0.1618	0.1628	0.1624	0.162
B 1		0.2013	0.2053	0.203	0.2022	0.2049	0.2033	0.204	0.2025	0.2033	0.2034	0.2043	0.2048
B 2		0.429	0.4304	0.4297	0.4289	0.4284	0.428	0.4307	0.4293	0.4294	0.4294	0.4297	0.4309
B 3		0.2353	0.2371	0.2369	0.2354	0.2364	0.2355	0.2359	0.2354	0.2355	0.2356	0.2357	0.2353
B 4		0.2384	0.239	0.2389	0.2386	0.2381	0.2382	0.2393	0.2387	0.2379	0.239	0.2401	0.2396
B 5		0.9297	0.9067	0.9137	0.9306	0.9076	0.9195	0.9009	0.8766	0.9362	0.9714	0.8673	0.8981
B 6		0.204	0.2041	0.2043	0.2041	0.2029	0.2047	0.2023	0.2018	0.2031	0.2022	0.2034	0.2027
B 7		0.2202	0.2247	0.2216	0.22	0.2228	0.2202	0.2225	0.2205	0.22	0.2224	0.2225	0.2225
B 8		0.2208	0.2197	0.2204	0.221	0.2194	0.221	0.2201	0.2182	0.2196	0.2191	0.22	0.2185
B 9		0.3268	0.3239	0.3248	0.3265	0.3219	0.3244	0.3232	0.3228	0.3234	0.3215	0.3225	0.3211
B 10		0.369	0.3679	0.3702	0.3701	0.3683	0.3707	0.3712	0.3706	0.3705	0.3721	0.3738	0.3737
B 11		0.3852	0.3839	0.386	0.3881	0.3864	0.3883	0.3866	0.3882	0.3889	0.3878	0.3894	0.3895
B 12		0.2352	0.2277	0.2335	0.2385	0.2274	0.2323	0.2328	0.2322	0.2273	0.2302	0.2281	0.227

Appendix 7.1

Well Row	Well Col	Raw Data (595) 129 - 21 h 19 min	Raw Data (595) 130 - 21 h 30 min	Raw Data (595) 131 - 21 h 40 min	Raw Data (595) 132 - 21 h 49 min	Raw Data (595) 133 - 22 h	Raw Data (595) 134 - 22 h 10 min	Raw Data (595) 135 - 22 h 19 min	Raw Data (595) 136 - 22 h 30 min	Raw Data (595) 137 - 22 h 40 min	Raw Data (595) 138 - 22 h 49 min	Raw Data (595) 139 - 23 h	Raw Data (595) 140 - 23 h 10 min
Time, Hours		21.33	21.50	21.67	21.83	22.00	22.17	22.33	22.50	22.67	22.83	23.00	23.17
C 1		0.1722	0.1729	0.1732	0.1725	0.1723	0.1727	0.1728	0.1714	0.1719	0.1722	0.1727	0.1723
C 2		0.387	0.3886	0.3895	0.3891	0.3884	0.3887	0.3884	0.3877	0.3878	0.387	0.39	0.3902
C 3		0.3729	0.3727	0.3732	0.3726	0.3735	0.3737	0.3728	0.3723	0.3727	0.3739	0.3776	0.3758
C 4		0.3112	0.3102	0.3126	0.3111	0.3134	0.3113	0.3129	0.3114	0.3112	0.3132	0.3154	0.3141
C 5		0.6693	0.6385	0.6692	0.6737	0.6401	0.6625	0.6754	0.6656	0.649	0.6504	0.6743	0.671
C 6		0.1728	0.1734	0.1747	0.1735	0.1745	0.1741	0.1749	0.1749	0.1754	0.176	0.1774	0.1764
C 7		0.2185	0.2193	0.217	0.2189	0.2171	0.2189	0.2185	0.2192	0.2182	0.2194	0.219	0.2201
C 8		0.206	0.2074	0.2062	0.2071	0.2063	0.2068	0.2075	0.2065	0.2069	0.2072	0.2076	0.2074
C 9		0.3904	0.3896	0.3902	0.3896	0.3893	0.3893	0.3891	0.3882	0.3868	0.3886	0.3887	0.3889
C 10		0.4241	0.424	0.4251	0.4257	0.4257	0.4249	0.4261	0.4255	0.4273	0.4287	0.4299	0.4292
C 11		0.4055	0.405	0.4079	0.4068	0.4071	0.4077	0.4071	0.4077	0.4065	0.4077	0.4124	0.4104
C 12		0.2138	0.2147	0.2171	0.2135	0.2164	0.2162	0.2142	0.213	0.2125	0.2144	0.2166	0.2142
D 1		0.2139	0.2131	0.2134	0.2141	0.2141	0.2146	0.2131	0.2138	0.212	0.2134	0.2129	0.2128
D 2		0.353	0.3522	0.3558	0.3559	0.3541	0.3556	0.3551	0.3554	0.3556	0.359	0.36	0.3609
D 3		0.2714	0.272	0.2733	0.273	0.2723	0.273	0.2741	0.2734	0.2717	0.2715	0.2721	0.271
D 4		0.2883	0.2881	0.2886	0.2886	0.2889	0.2886	0.2897	0.2897	0.2895	0.2915	0.293	0.2932
D 5		0.4764	0.4674	0.4647	0.4522	0.4708	0.4952	0.5241	0.5376	0.5261	0.5647	0.4646	0.545
D 6		0.219	0.2212	0.2209	0.2192	0.2232	0.2215	0.223	0.221	0.2225	0.2194	0.2267	0.2275
D 7		0.227	0.2273	0.2293	0.2293	0.2283	0.2307	0.2293	0.2304	0.2296	0.2352	0.2322	0.2325
D 8		0.2389	0.2384	0.2418	0.2409	0.2417	0.242	0.2413	0.2413	0.2414	0.2436	0.2426	0.2408
D 9		0.3132	0.3138	0.3143	0.3146	0.3126	0.3138	0.3135	0.3124	0.312	0.3131	0.3124	0.3128
D 10		0.3488	0.3476	0.3483	0.3506	0.3479	0.349	0.3485	0.3473	0.3484	0.3493	0.3506	0.351
D 11		0.4164	0.4123	0.4162	0.4165	0.4152	0.4156	0.4158	0.4158	0.4157	0.42	0.4211	0.4188
D 12		0.1802	0.1798	0.1789	0.1798	0.1794	0.1792	0.1798	0.1782	0.1785	0.1791	0.1795	0.1785

Appendix 7.1

Well Row	Well Col	Raw Data (595) 129 - 21 h 19 min	Raw Data (595) 130 - 21 h 30 min	Raw Data (595) 131 - 21 h 40 min	Raw Data (595) 132 - 21 h 49 min	Raw Data (595) 133 - 22 h	Raw Data (595) 134 - 22 h 10 min	Raw Data (595) 135 - 22 h 19 min	Raw Data (595) 136 - 22 h 30 min	Raw Data (595) 137 - 22 h 40 min	Raw Data (595) 138 - 22 h 49 min	Raw Data (595) 139 - 23 h	Raw Data (595) 140 - 23 h 10 min
Time, Hours		21.33	21.50	21.67	21.83	22.00	22.17	22.33	22.50	22.67	22.83	23.00	23.17
E 1		0.1876	0.1885	0.1883	0.1878	0.1883	0.1878	0.1885	0.188	0.187	0.1886	0.188	0.1887
E 2		0.4218	0.4231	0.4245	0.422	0.4239	0.4247	0.425	0.4251	0.422	0.425	0.4229	0.4224
E 3		0.2739	0.275	0.2752	0.2743	0.2752	0.2757	0.2759	0.2746	0.2738	0.2743	0.2752	0.2741
E 4		0.3112	0.314	0.312	0.3097	0.3134	0.3118	0.3119	0.3111	0.3104	0.312	0.3121	0.3122
E 5		0.3734	0.3761	0.3758	0.3744	0.3763	0.3763	0.376	0.3751	0.3751	0.3776	0.3775	0.3768
E 6		0.1984	0.197	0.1983	0.199	0.1964	0.199	0.1976	0.1971	0.1985	0.1973	0.1987	0.1983
F 1		0.2093	0.2107	0.2097	0.2092	0.2091	0.2101	0.2097	0.2092	0.2085	0.2096	0.2094	0.2115
F 2		0.4817	0.4823	0.4823	0.4803	0.479	0.4825	0.4824	0.4828	0.4823	0.4841	0.4835	0.4833
F 3		0.3822	0.3823	0.38	0.3814	0.3809	0.3776	0.3804	0.3801	0.3796	0.3804	0.3817	0.3812
F 4		0.2765	0.2779	0.2771	0.2778	0.2778	0.2776	0.278	0.2778	0.2763	0.2784	0.2787	0.2785
F 5		0.3248	0.3246	0.3253	0.3251	0.3266	0.3258	0.3254	0.3252	0.3256	0.3276	0.3259	0.326
F 6		0.2672	0.2702	0.2675	0.2688	0.2686	0.2688	0.2687	0.2675	0.2677	0.2687	0.2695	0.269
G 1		0.3224	0.3234	0.3224	0.3219	0.322	0.3236	0.3229	0.3231	0.3231	0.3233	0.3226	0.3227
G 2		0.4716	0.4701	0.4714	0.4718	0.4697	0.4703	0.4702	0.4705	0.4696	0.4709	0.4723	0.4733
G 3		0.364	0.3637	0.3617	0.3622	0.3621	0.362	0.3623	0.3624	0.3621	0.3641	0.3622	0.3619
G 4		0.2939	0.2952	0.2952	0.2925	0.2931	0.2963	0.2933	0.2931	0.2936	0.2956	0.2915	0.2931
G 5		0.2843	0.2861	0.2849	0.2869	0.2854	0.2853	0.2861	0.2861	0.2868	0.2879	0.2892	0.2888
G 6		0.1807	0.1821	0.1807	0.1802	0.1804	0.1832	0.1808	0.1811	0.1811	0.1809	0.1806	0.1812
H 1		0.2178	0.2184	0.2166	0.2176	0.2173	0.2175	0.2174	0.2172	0.2161	0.2171	0.2173	0.218
H 2		0.4615	0.4621	0.4607	0.4646	0.4591	0.4596	0.462	0.4592	0.4613	0.4606	0.4637	0.4634
H 3		0.4339	0.4364	0.437	0.4379	0.4393	0.4376	0.4406	0.4398	0.4415	0.4421	0.4454	0.447
H 4		0.2232	0.2263	0.2262	0.226	0.2253	0.2273	0.2249	0.2258	0.2272	0.2284	0.2284	0.228
H 5		0.2133	0.2129	0.2116	0.2119	0.2099	0.2094	0.2098	0.2081	0.2079	0.207	0.2085	0.2082
H 6		0.2102	0.2092	0.2092	0.2091	0.2096	0.2084	0.2113	0.2082	0.2092	0.2103	0.2097	0.2094

## Appendix 7.1

User: USER

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 141 - 23 h 19 min	Raw Data (595) 142 - 23 h 30 min	Raw Data (595) 143 - 23 h 40 min	Raw Data (595) 144 - 23 h 49 min	Raw Data (595) 145 - 24 h
Time, Hours		23.33	23.50	23.67	23.83	24.00
A 1		0.1844	0.1843	0.1853	0.1838	0.1846
A 2		0.2864	0.2864	0.2872	0.2871	0.2864
A 3		0.2481	0.2482	0.2484	0.2494	0.2479
A 4		0.2149	0.2154	0.2149	0.2149	0.2137
A 5		0.624	0.6465	0.6449	0.6972	0.5908
A 6		0.1834	0.1842	0.1841	0.1832	0.1835
A 7		0.2649	0.2662	0.2667	0.265	0.2655
A 8		0.2066	0.2072	0.2072	0.2073	0.2067
A 9		0.33	0.3305	0.3304	0.3308	0.329
A 10		0.2406	0.2403	0.241	0.2395	0.2398
A 11		0.592	0.5855	0.5887	0.6126	0.579
A 12		0.1618	0.1628	0.1625	0.163	0.1612
B 1		0.2032	0.2049	0.2046	0.2031	0.2039
B 2		0.4288	0.4289	0.4294	0.4254	0.4258
B 3		0.2348	0.2366	0.2367	0.2363	0.2355
B 4		0.2396	0.2401	0.2417	0.2401	0.2394
B 5		1.0533	1.0307	0.9852	0.9828	0.9786
B 6		0.2028	0.2028	0.202	0.2026	0.2015
B 7		0.2214	0.2234	0.2236	0.2209	0.2226
B 8		0.2192	0.2195	0.2189	0.2193	0.2192
B 9		0.3234	0.3227	0.3221	0.3217	0.3205
B 10		0.3727	0.3747	0.377	0.3744	0.3751
B 11		0.3894	0.3927	0.3931	0.3927	0.3927
B 12		0.2321	0.2326	0.2323	0.2316	0.2289

Appendix 7.1

Well Row	Well Col	Raw Data (595) 141 - 23 h 19 min	Raw Data (595) 142 - 23 h 30 min	Raw Data (595) 143 - 23 h 40 min	Raw Data (595) 144 - 23 h 49 min	Raw Data (595) 145 - 24 h
Time, Hours		23.33	23.50	23.67	23.83	24.00
C 1		0.1719	0.1726	0.1726	0.1716	0.1717
C 2		0.3877	0.3879	0.3891	0.3863	0.3874
C 3		0.3758	0.3758	0.3768	0.3742	0.3758
C 4		0.3163	0.3179	0.3162	0.318	0.3212
C 5		0.6621	0.6646	0.6525	0.6192	0.6713
C 6		0.1755	0.1762	0.1758	0.1765	0.1765
C 7		0.2189	0.2197	0.2209	0.2198	0.219
C 8		0.2065	0.2082	0.2077	0.207	0.207
C 9		0.3885	0.3885	0.3878	0.3873	0.3875
C 10		0.4292	0.4313	0.4328	0.4323	0.4337
C 11		0.4082	0.4095	0.411	0.4101	0.4131
C 12		0.2128	0.2147	0.2139	0.2137	0.2158
D 1		0.2132	0.2125	0.2141	0.2131	0.212
D 2		0.3582	0.3586	0.3603	0.3583	0.3607
D 3		0.2696	0.269	0.2685	0.2662	0.2653
D 4		0.2945	0.2966	0.2985	0.2971	0.2979
D 5		0.6135	0.6631	0.575	0.5602	0.5491
D 6		0.2239	0.2287	0.2302	0.2283	0.2319
D 7		0.234	0.2324	0.2334	0.2349	0.2336
D 8		0.2431	0.2415	0.2411	0.2407	0.2412
D 9		0.3119	0.3126	0.3119	0.3106	0.311
D 10		0.35	0.3505	0.3508	0.3497	0.3507
D 11		0.4195	0.4195	0.4199	0.4179	0.4152
D 12		0.1786	0.1795	0.1793	0.1788	0.1776



Appendix 7.1

Well Row	Well Col	Raw Data (595) 141 - 23 h 19 min	Raw Data (595) 142 - 23 h 30 min	Raw Data (595) 143 - 23 h 40 min	Raw Data (595) 144 - 23 h 49 min	Raw Data (595) 145 - 24 h
Time, Hours		23.33	23.50	23.67	23.83	24.00
E	1	0.1881	0.1886	0.1892	0.188	0.1885
E	2	0.4241	0.4216	0.4226	0.4224	0.4233
E	3	0.2737	0.2734	0.2731	0.2719	0.2731
E	4	0.3128	0.3123	0.3139	0.3131	0.313
E	5	0.3767	0.3767	0.378	0.3771	0.3776
E	6	0.1971	0.1991	0.1998	0.1975	0.198
F	1	0.2092	0.2108	0.212	0.2104	0.2091
F	2	0.4843	0.4834	0.484	0.4816	0.4845
F	3	0.3799	0.3796	0.3788	0.3788	0.3778
F	4	0.2786	0.2783	0.2791	0.2776	0.2785
F	5	0.326	0.3267	0.3269	0.3249	0.3267
F	6	0.2691	0.2699	0.2704	0.2683	0.2679
G	1	0.3231	0.3221	0.3239	0.3224	0.3243
G	2	0.4706	0.4704	0.472	0.4682	0.4713
G	3	0.362	0.3588	0.3608	0.3575	0.3593
G	4	0.2936	0.2899	0.2942	0.2917	0.2909
G	5	0.2883	0.2906	0.29	0.2887	0.2889
G	6	0.1818	0.1818	0.1821	0.1808	0.1816
H	1	0.2185	0.219	0.218	0.2186	0.2182
H	2	0.4631	0.4631	0.4626	0.4589	0.4626
H	3	0.4474	0.4474	0.4476	0.4459	0.4451
H	4	0.229	0.2273	0.229	0.2282	0.2264
H	5	0.2064	0.2077	0.2082	0.2073	0.2064
H	6	0.2102	0.2109	0.2094	0.2094	0.2112

## Appendix 7.2

Path: C:\Program Files\BMG\Omega\User\Data\

Test Name: Pseudomonas

Date: 06/07/2012

Absorbance

Absorbance values are displayed as OD

Well Row	Well Col	Content 1	Content 2	Raw Data (595) 1 - 0 h	Raw Data (595) 2 - 0 h 10 min	Raw Data (595) 3 - 0 h 20 min	Raw Data (595) 4 - 0 h 30 min	Raw Data (595) 5 - 0 h 40 min	Raw Data (595) 6 - 0 h 50 min
Time, hours				0.00	0.17	0.33	0.50	0.67	0.83
A 1		1:10 MDM	1:10 MDM	0.1845	0.1788	0.1786	0.179	0.1791	0.1774
A 2		P. putida in 1:1 MDM	20mgL <sup>-1</sup> Comm. CeO2 in water	0.2378	0.2346	0.2394	0.2396	0.2385	0.2354
A 3		P. putida in 1:1 MDM	40mgL <sup>-1</sup> Comm. CeO2 in water	0.2019	0.1992	0.1998	0.1971	0.1989	0.1988
A 4		P. putida in 1:1 MDM	500mgL <sup>-1</sup> Comm. CeO2 in water	0.2582	0.2565	0.2527	0.2554	0.2522	0.2561
A 5		P. putida in 1:1 MDM	100mgL <sup>-1</sup> Comm. CeO2 in water	0.257	0.2518	0.2556	0.2502	0.2468	0.2479
A 6		1:10 MDM	1:10 MDM	0.2329	0.2118	0.2088	0.2085	0.209	0.2062
A 7		P. putida in 1:1MDM	20mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2496	0.2432	0.2452	0.2429	0.2452	0.2479
A 8		P. putida in 1:1 MDM	40mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2378	0.2278	0.2262	0.233	0.2274	0.2294
A 9		P. putida in 1:1 MDM	100mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2157	0.2192	0.2139	0.2171	0.2161	0.2145
A 10		P. putida in 1:1 MDM	200mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.3215	0.2899	0.2855	0.284	0.2927	0.2845
A 11		P. putida in 1:1 MDM	Water	0.2037	0.1937	0.1898	0.189	0.1921	0.188
A 12		P. putida in 1:1 MDM	1g L <sup>-1</sup> sodium citrate filtered	0.217	0.2178	0.2232	0.2228	0.2225	0.2248
B 1		1:10 MDM	1:10 MDM	0.1682	0.1659	0.1628	0.1618	0.1618	0.1589
B 2		P. putida in 1:1 MDM	20mgL <sup>-1</sup> Comm. CeO2 in water	0.2113	0.2087	0.2091	0.2077	0.2086	0.2071
B 3		P. putida in 1:1 MDM	40mgL <sup>-1</sup> Comm. CeO2 in water	0.2657	0.2526	0.2491	0.2478	0.2459	0.2434
B 4		P. putida in 1:1 MDM	100mgL <sup>-1</sup> Comm. CeO2 in water	0.2457	0.2323	0.235	0.232	0.2308	0.2302
B 5		P. putida in 1:1 MDM	200mgL <sup>-1</sup> Comm. CeO <sup>2</sup> in water	0.2655	0.2904	0.2927	0.2903	0.2923	0.2887
B 6		1:10 MDM	1:10 MDM	0.1585	0.1632	0.1636	0.1622	0.163	0.1608
B 7		P. putida in 1:1MDM	20mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2313	0.2353	0.2327	0.2305	0.2334	0.2283
B 8		P. putida in 1:1 MDM	40mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2393	0.2375	0.2374	0.2355	0.2376	0.2353
B 9		P. putida in 1:1 MDM	100mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2278	0.2169	0.2185	0.2117	0.2125	0.2171
B 10		P. putida in 1:1 MDM	200mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.206	0.2189	0.2215	0.2177	0.2241	0.2207
B 11		P. putida in 1:1 MDM	Water	0.1813	0.1789	0.1816	0.1789	0.1823	0.1802
B 12		P. putida in 1:1 MDM	1g L <sup>-1</sup> sodium citrate filtered	0.2687	0.2602	0.2617	0.2622	0.2678	0.2594

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Content 1	Content 2	Raw Data (595) 1 - 0 h	Raw Data (595) 2 - 0 h 10 min	Raw Data (595) 3 - 0 h 20 min	Raw Data (595) 4 - 0 h 30 min	Raw Data (595) 5 - 0 h 40 min	Raw Data (595) 6 - 0 h 50 min
Time, hours				0.00	0.17	0.33	0.50	0.67	0.83
C 1		1:10 MDM	1:10 MDM	0.2004	0.2071	0.2063	0.2048	0.2058	0.2036
C 2		P. putida in 1:1 MDM	20mgL <sup>-1</sup> Comm. CeO2 in water	0.221	0.2136	0.2139	0.212	0.2126	0.2126
C 3		P. putida in 1:1 MDM	40mgL <sup>-1</sup> Comm. CeO2 in water	0.2147	0.2084	0.2079	0.2075	0.2066	0.204
C 4		P. putida in 1:1 MDM	100mgL <sup>-1</sup> Comm. CeO <sup>2</sup> in water	0.2167	0.2138	0.2224	0.2215	0.2227	0.2195
C 5		P. putida in 1:1 MDM	200mgL <sup>-1</sup> Comm. CeO <sup>2</sup> in water	0.3227	0.314	0.3183	0.3112	0.3138	0.3113
C 6		1:10 MDM	1:10 MDM	0.2177	0.23	0.228	0.2277	0.2273	0.2248
C 7		P. putida in 1:1MDM	20mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2173	0.216	0.2183	0.2135	0.2158	0.2126
C 8		P. putida in 1:1 MDM	40mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2141	0.2234	0.2224	0.2245	0.2243	0.2238
C 9		P. putida in 1:1 MDM	100mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2381	0.2387	0.2388	0.2356	0.2378	0.2363
C 10		P. putida in 1:1 MDM	200mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2145	0.2169	0.2222	0.2197	0.2216	0.224
C 11		P. putida in 1:1 MDM	Water	0.2053	0.2097	0.2095	0.2086	0.2111	0.2055
C 12		P. putida in 1:1 MDM	1g L <sup>-1</sup> sodium citrate filtered	0.199	0.2104	0.2124	0.2138	0.2132	0.2138
D 1		1:10 MDM	1:10 MDM	0.2016	0.1866	0.1871	0.1834	0.1852	0.1835
D 2		P. putida in 1:1 MDM	20mgL <sup>-1</sup> Comm. CeO2 in water	0.2486	0.2294	0.2323	0.2236	0.2296	0.2283
D 3		P. putida in 1:1 MDM	40mgL <sup>-1</sup> Comm. CeO2 in water	0.2159	0.2301	0.2267	0.2295	0.2281	0.2284
D 4		P. putida in 1:1 MDM	100mgL <sup>-1</sup> Comm. CeO2 in water	0.2683	0.2563	0.2585	0.258	0.2581	0.2412
D 5		P. putida in 1:1 MDM	200mgL <sup>-1</sup> Comm. CeO <sup>2</sup> in water	0.3464	0.3332	0.3418	0.3336	0.3329	0.3333
D 6		1:10 MDM	1:10 MDM	0.2064	0.2021	0.2015	0.1991	0.2001	0.1987
D 7		P. putida in 1:1MDM	20mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2294	0.2149	0.2167	0.214	0.2195	0.219
D 8		P. putida in 1:1 MDM	40mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2614	0.2567	0.2566	0.2526	0.2544	0.2544
D 9		P. putida in 1:1 MDM	100mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.1914	0.2115	0.2139	0.2134	0.2139	0.2139
D 10		P. putida in 1:1 MDM	200mgL <sup>-1</sup> B10 CeO2 in sodium citrate	0.2147	0.2219	0.2236	0.2235	0.223	0.2222
D 11		P. putida in 1:1 MDM	Water	0.1921	0.1957	0.1959	0.195	0.1974	0.195
D 12		P. putida in 1:1 MDM	1g L <sup>-1</sup> sodium citrate filtered	0.1932	0.1882	0.194	0.1927	0.1926	0.1887

Appendix 7.2

Test ID: 19

Test Name: Pseudomonas

Time: 17:18:52

Absorbance

Well Row	Well Col	Raw Data (595) 7 - 1 h	Raw Data (595) 8 - 1 h 10 min	Raw Data (595) 9 - 1 h 19 min	Raw Data (595) 10 - 1 h 30 min	Raw Data (595) 11 - 1 h 40 min	Raw Data (595) 12 - 1 h 50 min	Raw Data (595) 13 - 2 h	Raw Data (595) 14 - 2 h 9 min	Raw Data (595) 15 - 2 h 20 min	Raw Data (595) 16 - 2 h 30 min	Raw Data (595) 17 - 2 h 39 min	Raw Data (595) 18 - 2 h 50 min
Time, hours		1.00	1.17	1.33	1.50	1.67	1.83	2.00	2.17	2.33	2.50	2.67	2.83
A	1	0.1778	0.1769	0.1763	0.1771	0.1766	0.1761	0.1769	0.1758	0.1755	0.1762	0.1754	0.1751
A	2	0.2356	0.2376	0.2347	0.2356	0.2369	0.2371	0.2343	0.2355	0.2359	0.2384	0.2397	0.2367
A	3	0.1969	0.1962	0.1969	0.1954	0.1955	0.1959	0.196	0.1961	0.1956	0.1968	0.1952	0.1969
A	4	0.2688	0.2568	0.2579	0.2555	0.254	0.2552	0.2559	0.2543	0.2535	0.2537	0.2646	0.2525
A	5	0.2463	0.2477	0.247	0.2469	0.2452	0.2463	0.2472	0.2477	0.2466	0.2471	0.2478	0.2477
A	6	0.2054	0.2026	0.2035	0.2035	0.2038	0.2046	0.2022	0.2003	0.202	0.2029	0.2019	0.2014
A	7	0.2434	0.2459	0.2444	0.2473	0.2526	0.2588	0.2589	0.2506	0.2509	0.2599	0.2652	0.2684
A	8	0.2307	0.2318	0.242	0.24	0.2385	0.2391	0.2416	0.2434	0.2463	0.2482	0.2515	0.2547
A	9	0.2176	0.2218	0.2296	0.2274	0.2314	0.2262	0.2291	0.2287	0.2518	0.247	0.2464	0.2462
A	10	0.2866	0.2851	0.3035	0.2864	0.2946	0.2916	0.2968	0.3042	0.3104	0.3106	0.3059	0.3148
A	11	0.1865	0.1866	0.191	0.1884	0.186	0.1914	0.1869	0.1907	0.1871	0.188	0.1893	0.1857
A	12	0.2408	0.2282	0.2314	0.2227	0.232	0.221	0.2262	0.2318	0.2344	0.2357	0.2354	0.2366
B	1	0.1585	0.1594	0.1589	0.1583	0.1567	0.1575	0.1571	0.1568	0.1585	0.1565	0.1561	0.1558
B	2	0.2054	0.206	0.2048	0.2036	0.2038	0.2041	0.2053	0.2049	0.2044	0.2037	0.2033	0.2034
B	3	0.2459	0.2439	0.2443	0.2417	0.2418	0.2424	0.2471	0.2435	0.2452	0.2434	0.2428	0.2434
B	4	0.2353	0.2291	0.2299	0.2286	0.2289	0.2287	0.2363	0.2281	0.2339	0.243	0.235	0.2306
B	5	0.2878	0.2909	0.2955	0.2862	0.2868	0.2859	0.2851	0.2856	0.2901	0.2843	0.285	0.2859
B	6	0.1602	0.1613	0.1617	0.1607	0.1601	0.16	0.1601	0.1597	0.1609	0.1595	0.1593	0.1591
B	7	0.2294	0.2303	0.233	0.2367	0.231	0.2344	0.2361	0.234	0.2414	0.2388	0.2397	0.2404
B	8	0.2331	0.2357	0.2357	0.2392	0.2367	0.2412	0.2511	0.2609	0.2551	0.2667	0.2656	0.2665
B	9	0.2147	0.2168	0.2208	0.2209	0.2198	0.2214	0.2252	0.2288	0.2405	0.2389	0.2421	0.2511
B	10	0.2185	0.221	0.224	0.2243	0.2266	0.252	0.2479	0.242	0.2525	0.2499	0.2457	0.2511
B	11	0.1795	0.1824	0.182	0.182	0.1831	0.1808	0.1819	0.1827	0.1842	0.1814	0.181	0.1816
B	12	0.2621	0.2668	0.2631	0.2845	0.2662	0.2635	0.2634	0.2631	0.2643	0.2649	0.2651	0.266

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 7 - 1 h	Raw Data (595) 8 - 1 h 10 min	Raw Data (595) 9 - 1 h 19 min	Raw Data (595) 10 - 1 h 30 min	Raw Data (595) 11 - 1 h 40 min	Raw Data (595) 12 - 1 h 50 min	Raw Data (595) 13 - 2 h	Raw Data (595) 14 - 2 h 9 min	Raw Data (595) 15 - 2 h 20 min	Raw Data (595) 16 - 2 h 30 min	Raw Data (595) 17 - 2 h 39 min	Raw Data (595) 18 - 2 h 50 min
Time, hours		1.00	1.17	1.33	1.50	1.67	1.83	2.00	2.17	2.33	2.50	2.67	2.83
C	1	0.2031	0.2027	0.2025	0.203	0.2015	0.2012	0.2011	0.2011	0.2007	0.2003	0.2001	0.1997
C	2	0.2121	0.2121	0.2126	0.2122	0.211	0.2105	0.2136	0.2116	0.2114	0.2115	0.2107	0.2109
C	3	0.2054	0.2036	0.2027	0.203	0.2031	0.2023	0.2044	0.2015	0.2035	0.2046	0.2018	0.2069
C	4	0.219	0.2203	0.2206	0.2195	0.2195	0.2205	0.2216	0.2211	0.2209	0.2349	0.2227	0.2225
C	5	0.3127	0.3112	0.3102	0.309	0.3067	0.3081	0.3049	0.3054	0.3045	0.3072	0.3066	0.3194
C	6	0.2255	0.2246	0.2241	0.2241	0.223	0.2225	0.2236	0.2224	0.2225	0.2216	0.2215	0.2215
C	7	0.214	0.2155	0.2156	0.2138	0.2157	0.216	0.2186	0.2193	0.2211	0.2226	0.2241	0.2253
C	8	0.2268	0.2252	0.2335	0.2304	0.2379	0.2325	0.2353	0.2462	0.2519	0.2458	0.2528	0.251
C	9	0.2382	0.2381	0.2425	0.2453	0.2394	0.2516	0.2422	0.2425	0.2645	0.2521	0.254	0.2591
C	10	0.2228	0.228	0.2259	0.2267	0.2269	0.2282	0.2373	0.2487	0.2412	0.2493	0.2385	0.2458
C	11	0.2064	0.2058	0.2038	0.2031	0.2044	0.2028	0.204	0.2054	0.204	0.2046	0.2059	0.204
C	12	0.2226	0.2211	0.2118	0.215	0.2252	0.2149	0.2163	0.2169	0.2195	0.2216	0.2208	0.2231
D	1	0.1829	0.1819	0.1813	0.1808	0.1807	0.1801	0.1802	0.1795	0.1802	0.1797	0.179	0.1789
D	2	0.2272	0.2297	0.2283	0.2281	0.2287	0.2283	0.2282	0.2297	0.2296	0.2301	0.2287	0.2303
D	3	0.2318	0.228	0.2299	0.2279	0.2265	0.2266	0.2303	0.2273	0.2272	0.2282	0.2259	0.2258
D	4	0.2546	0.2554	0.2561	0.256	0.2597	0.2562	0.2576	0.2567	0.2564	0.2566	0.2624	0.2755
D	5	0.3333	0.3353	0.3333	0.3312	0.3319	0.3322	0.3323	0.3353	0.331	0.332	0.3313	0.3333
D	6	0.2001	0.1993	0.1992	0.1981	0.1988	0.1991	0.198	0.198	0.1983	0.1984	0.1978	0.1984
D	7	0.2144	0.2141	0.2173	0.2173	0.2185	0.2215	0.2219	0.2297	0.2248	0.235	0.2332	0.2349
D	8	0.2559	0.2592	0.2597	0.2555	0.2538	0.2615	0.2605	0.2566	0.2691	0.2746	0.2723	0.2756
D	9	0.2189	0.2181	0.2165	0.2188	0.2341	0.2226	0.2375	0.2396	0.2284	0.2417	0.2361	0.2356
D	10	0.2441	0.2299	0.2332	0.23	0.2298	0.24	0.2478	0.2381	0.2374	0.2398	0.2426	0.245
D	11	0.1962	0.1957	0.1965	0.1959	0.1937	0.1983	0.195	0.1956	0.1949	0.1963	0.196	0.194
D	12	0.1929	0.2057	0.1912	0.1928	0.1945	0.1943	0.194	0.2002	0.1991	0.199	0.1991	0.1999

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 19 - 3 h	Raw Data (595) 20 - 3 h 9 min	Raw Data (595) 21 - 3 h 20 min	Raw Data (595) 22 - 3 h 30 min	Raw Data (595) 23 - 3 h 39 min	Raw Data (595) 24 - 3 h 50 min	Raw Data (595) 25 - 4 h	Raw Data (595) 26 - 4 h 10 min	Raw Data (595) 27 - 4 h 19 min	Raw Data (595) 28 - 4 h 30 min	Raw Data (595) 29 - 4 h 40 min	Raw Data (595) 30 - 4 h 49 min
Time, hours		3.00	3.17	3.33	3.50	3.67	3.83	4.00	4.17	4.33	4.50	4.67	4.83
A	1	0.1747	0.1746	0.174	0.1738	0.1764	0.1743	0.1739	0.1736	0.1737	0.1736	0.173	0.1729
A	2	0.2355	0.2391	0.2409	0.2389	0.2579	0.2399	0.2394	0.2391	0.2448	0.2428	0.2456	0.2403
A	3	0.1969	0.1947	0.1964	0.1985	0.1922	0.1963	0.196	0.1961	0.2068	0.2018	0.2071	0.2004
A	4	0.2542	0.2532	0.2536	0.2568	0.2672	0.2544	0.2738	0.2545	0.2558	0.2537	0.2512	0.2527
A	5	0.2498	0.2474	0.2477	0.2476	0.2468	0.2493	0.2484	0.2485	0.2507	0.2507	0.2495	0.2494
A	6	0.2006	0.2013	0.1992	0.1984	0.2024	0.2005	0.201	0.2007	0.2008	0.199	0.1985	0.1989
A	7	0.2747	0.2765	0.2816	0.2854	0.2898	0.2925	0.296	0.3013	0.3059	0.313	0.3215	0.3276
A	8	0.2606	0.2635	0.2667	0.2703	0.274	0.2799	0.2866	0.2945	0.3017	0.3063	0.3143	0.3203
A	9	0.2528	0.2541	0.2593	0.2638	0.2694	0.2776	0.2828	0.2888	0.2971	0.3028	0.3096	0.3176
A	10	0.3145	0.318	0.3202	0.3249	0.3303	0.3339	0.3414	0.3471	0.3533	0.3599	0.3681	0.3729
A	11	0.1882	0.187	0.1883	0.1884	0.1886	0.1917	0.1935	0.1899	0.1926	0.186	0.1859	0.1854
A	12	0.2408	0.2389	0.2422	0.2451	0.2413	0.2496	0.2484	0.2505	0.2523	0.257	0.2599	0.262
B	1	0.1557	0.1555	0.155	0.155	0.15	0.1558	0.1544	0.1553	0.1549	0.1541	0.1543	0.1542
B	2	0.2056	0.2027	0.2058	0.2023	0.2	0.2033	0.2024	0.2025	0.2024	0.2019	0.2018	0.2023
B	3	0.2425	0.2402	0.2411	0.2408	0.2365	0.2428	0.2402	0.2412	0.2403	0.2401	0.2406	0.2448
B	4	0.2313	0.2302	0.2397	0.2333	0.2306	0.231	0.2308	0.2307	0.2309	0.23	0.2345	0.2336
B	5	0.283	0.2843	0.2831	0.284	0.2771	0.2845	0.2903	0.2865	0.2838	0.2846	0.2814	0.2819
B	6	0.1588	0.1591	0.1583	0.1583	0.1567	0.159	0.1573	0.159	0.1572	0.1581	0.157	0.1577
B	7	0.2471	0.2482	0.2563	0.2612	0.2577	0.2701	0.2707	0.2757	0.2794	0.2827	0.2875	0.294
B	8	0.2716	0.2733	0.2751	0.2769	0.2785	0.2891	0.2938	0.2998	0.3057	0.3128	0.3201	0.3288
B	9	0.2561	0.2573	0.2602	0.2622	0.2732	0.2762	0.2811	0.2876	0.2931	0.2997	0.3071	0.313
B	10	0.2539	0.2593	0.2608	0.2638	0.2583	0.277	0.2773	0.2857	0.2885	0.2997	0.3012	0.304
B	11	0.1847	0.1814	0.1858	0.183	0.1831	0.1828	0.1816	0.1811	0.1824	0.181	0.1805	0.1819
B	12	0.2677	0.2667	0.2686	0.2691	0.2658	0.2743	0.2755	0.2771	0.2807	0.2815	0.2859	0.2893

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 19 - 3 h	Raw Data (595) 20 - 3 h 9 min	Raw Data (595) 21 - 3 h 20 min	Raw Data (595) 22 - 3 h 30 min	Raw Data (595) 23 - 3 h 39 min	Raw Data (595) 24 - 3 h 50 min	Raw Data (595) 25 - 4 h	Raw Data (595) 26 - 4 h 10 min	Raw Data (595) 27 - 4 h 19 min	Raw Data (595) 28 - 4 h 30 min	Raw Data (595) 29 - 4 h 40 min	Raw Data (595) 30 - 4 h 49 min
Time, hours		3.00	3.17	3.33	3.50	3.67	3.83	4.00	4.17	4.33	4.50	4.67	4.83
C	1	0.1998	0.1993	0.1989	0.1989	0.1974	0.1989	0.1978	0.1982	0.1979	0.1977	0.198	0.1984
C	2	0.2118	0.2098	0.2104	0.2109	0.2087	0.2101	0.2092	0.21	0.2114	0.2101	0.2102	0.2106
C	3	0.2043	0.2011	0.2011	0.2005	0.2023	0.202	0.2005	0.2008	0.2017	0.2002	0.201	0.201
C	4	0.2222	0.2226	0.2212	0.2219	0.2216	0.226	0.2229	0.2225	0.2233	0.2224	0.2236	0.2339
C	5	0.305	0.3059	0.3049	0.3042	0.3159	0.3056	0.3043	0.3052	0.3042	0.3033	0.3042	0.3057
C	6	0.2218	0.221	0.2201	0.2202	0.2235	0.2202	0.2192	0.219	0.2191	0.2178	0.218	0.2184
C	7	0.2343	0.2413	0.2444	0.248	0.251	0.2569	0.2597	0.2628	0.2698	0.2753	0.2814	0.287
C	8	0.2546	0.2599	0.2632	0.2666	0.2771	0.2768	0.2821	0.2847	0.2919	0.2964	0.305	0.3128
C	9	0.264	0.2689	0.2733	0.2774	0.2807	0.2832	0.2854	0.2907	0.2997	0.3028	0.3111	0.3158
C	10	0.258	0.2626	0.2645	0.2687	0.2714	0.2866	0.2927	0.2869	0.2958	0.2977	0.3052	0.3152
C	11	0.2051	0.2024	0.2113	0.2014	0.2015	0.2037	0.2021	0.2064	0.2056	0.204	0.2049	0.2048
C	12	0.2258	0.2258	0.226	0.2273	0.2195	0.2336	0.236	0.2367	0.2413	0.245	0.2476	0.2493
D	1	0.1788	0.1782	0.178	0.1772	0.1772	0.1777	0.177	0.1765	0.1767	0.1766	0.1762	0.1765
D	2	0.2278	0.2313	0.2316	0.2303	0.2442	0.232	0.2348	0.2292	0.2321	0.2293	0.2305	0.2294
D	3	0.2266	0.2246	0.2259	0.2254	0.2266	0.2257	0.2291	0.2244	0.2243	0.2232	0.2237	0.2278
D	4	0.256	0.2561	0.2556	0.254	0.2489	0.259	0.263	0.2595	0.2608	0.2602	0.2603	0.2644
D	5	0.3322	0.3308	0.3318	0.3312	0.3328	0.3316	0.3427	0.332	0.3317	0.3305	0.3321	0.3468
D	6	0.199	0.1977	0.198	0.1968	0.2022	0.1994	0.198	0.1968	0.2016	0.2017	0.2025	0.2032
D	7	0.238	0.2412	0.2439	0.248	0.2556	0.2572	0.2618	0.2664	0.2741	0.2801	0.2865	0.2936
D	8	0.2793	0.2825	0.2849	0.2891	0.2913	0.2991	0.3027	0.3092	0.3168	0.3229	0.328	0.3351
D	9	0.2423	0.2448	0.2489	0.2559	0.2424	0.27	0.2759	0.2861	0.291	0.2995	0.3044	0.317
D	10	0.2546	0.2571	0.2602	0.2667	0.2588	0.2796	0.2885	0.2936	0.3	0.3066	0.3127	0.3229
D	11	0.1957	0.1976	0.1959	0.1945	0.1966	0.1968	0.1937	0.2009	0.1936	0.1982	0.1939	0.1949
D	12	0.2027	0.2016	0.2046	0.2058	0.2147	0.2102	0.2111	0.2131	0.2166	0.2181	0.2206	0.2238

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 31 - 5 h	Raw Data (595) 32 - 5 h 10 min	Raw Data (595) 33 - 5 h 19 min	Raw Data (595) 34 - 5 h 30 min	Raw Data (595) 35 - 5 h 40 min	Raw Data (595) 36 - 5 h 49 min	Raw Data (595) 37 - 6 h	Raw Data (595) 38 - 6 h 10 min	Raw Data (595) 39 - 6 h 19 min	Raw Data (595) 40 - 6 h 30 min	Raw Data (595) 41 - 6 h 40 min	Raw Data (595) 42 - 6 h 49 min
Time, hours		5.00	5.17	5.33	5.50	5.67	5.83	6.00	6.17	6.33	6.50	6.67	6.83
A	1	0.1728	0.1724	0.1719	0.172	0.1722	0.172	0.1707	0.1714	0.174	0.1712	0.1712	0.1714
A	2	0.2366	0.2339	0.2393	0.2359	0.2395	0.2324	0.2303	0.2328	0.2565	0.229	0.2344	0.2331
A	3	0.1972	0.1982	0.2014	0.1946	0.196	0.1965	0.1938	0.1947	0.1968	0.2034	0.1963	0.1952
A	4	0.2565	0.2552	0.2511	0.2496	0.25	0.2501	0.2522	0.2499	0.2608	0.2501	0.2547	0.2487
A	5	0.2491	0.2657	0.2483	0.2481	0.2475	0.2484	0.2471	0.248	0.2442	0.248	0.2468	0.2473
A	6	0.1988	0.1978	0.1965	0.1984	0.1995	0.1982	0.1972	0.1975	0.2015	0.1983	0.1995	0.1991
A	7	0.3336	0.3404	0.3461	0.35	0.3539	0.3564	0.3572	0.3583	0.3576	0.3586	0.3589	0.3569
A	8	0.3259	0.3329	0.3394	0.3471	0.3516	0.3592	0.3608	0.3676	0.368	0.3739	0.3768	0.3796
A	9	0.3231	0.3292	0.3323	0.3363	0.3391	0.3406	0.3394	0.3391	0.3376	0.3359	0.3344	0.3343
A	10	0.3803	0.3847	0.3911	0.3983	0.4056	0.4096	0.4164	0.4186	0.4293	0.4307	0.4368	0.437
A	11	0.1928	0.1932	0.1928	0.1879	0.1883	0.1887	0.1894	0.1873	0.1904	0.1894	0.1892	0.1885
A	12	0.2659	0.2712	0.2803	0.2826	0.2901	0.3011	0.3128	0.3542	0.3259	0.3182	0.3344	0.3392
B	1	0.1535	0.1536	0.1538	0.153	0.1534	0.1532	0.1518	0.1538	0.1484	0.1535	0.1534	0.1538
B	2	0.202	0.2018	0.2012	0.2013	0.2012	0.2015	0.2014	0.2015	0.1986	0.2009	0.2009	0.2017
B	3	0.2429	0.24	0.2458	0.2431	0.2394	0.2419	0.2385	0.2407	0.2382	0.2475	0.2474	0.2381
B	4	0.2316	0.2306	0.2304	0.2302	0.2335	0.2361	0.2396	0.2308	0.2354	0.2363	0.2346	0.2346
B	5	0.2804	0.2809	0.2835	0.2812	0.2802	0.2809	0.2797	0.2828	0.283	0.2804	0.2807	0.2812
B	6	0.1563	0.1565	0.1575	0.1566	0.1565	0.1568	0.155	0.1576	0.1549	0.1569	0.1563	0.1565
B	7	0.2994	0.305	0.3096	0.3141	0.3176	0.3224	0.3237	0.3307	0.3236	0.3385	0.34	0.343
B	8	0.3345	0.3479	0.3555	0.3628	0.3736	0.3798	0.3834	0.39	0.3845	0.3941	0.3958	0.3972
B	9	0.3173	0.3265	0.3318	0.3349	0.3392	0.3392	0.3444	0.344	0.3532	0.3448	0.3449	0.3431
B	10	0.3108	0.321	0.3274	0.3302	0.3388	0.3413	0.3465	0.3569	0.3552	0.37	0.3744	0.3748
B	11	0.1801	0.1813	0.1814	0.181	0.1815	0.1847	0.1796	0.1818	0.1816	0.1797	0.18	0.1845
B	12	0.2935	0.2977	0.3033	0.3068	0.3126	0.3236	0.3294	0.3462	0.3375	0.3412	0.3467	0.3482



## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 31 - 5 h	Raw Data (595) 32 - 5 h 10 min	Raw Data (595) 33 - 5 h 19 min	Raw Data (595) 34 - 5 h 30 min	Raw Data (595) 35 - 5 h 40 min	Raw Data (595) 36 - 5 h 49 min	Raw Data (595) 37 - 6 h	Raw Data (595) 38 - 6 h 10 min	Raw Data (595) 39 - 6 h 19 min	Raw Data (595) 40 - 6 h 30 min	Raw Data (595) 41 - 6 h 40 min	Raw Data (595) 42 - 6 h 49 min
Time, hours		5.00	5.17	5.33	5.50	5.67	5.83	6.00	6.17	6.33	6.50	6.67	6.83
C	1	0.1976	0.1983	0.1972	0.1966	0.1969	0.1963	0.196	0.1959	0.1955	0.1951	0.196	0.1958
C	2	0.2115	0.2108	0.2103	0.2104	0.2104	0.2101	0.2097	0.2105	0.2109	0.2103	0.2109	0.2117
C	3	0.2074	0.2004	0.2001	0.2001	0.2017	0.1998	0.2	0.2015	0.2008	0.2022	0.2002	0.2008
C	4	0.2232	0.2236	0.2229	0.2227	0.223	0.224	0.2229	0.2233	0.2234	0.2232	0.2229	0.2234
C	5	0.3043	0.3045	0.3058	0.3031	0.3047	0.3051	0.302	0.3029	0.3157	0.311	0.3109	0.3086
C	6	0.218	0.2175	0.2169	0.2165	0.2166	0.2172	0.216	0.2158	0.2199	0.2152	0.2154	0.2156
C	7	0.2924	0.2998	0.3033	0.3066	0.3109	0.3155	0.3175	0.3184	0.3206	0.3235	0.3254	0.326
C	8	0.3183	0.3287	0.3342	0.3399	0.3481	0.3561	0.36	0.365	0.3774	0.3735	0.3764	0.3822
C	9	0.3245	0.3335	0.3416	0.3467	0.3523	0.3588	0.3648	0.3685	0.3758	0.3784	0.3761	0.3805
C	10	0.3182	0.3275	0.3335	0.3346	0.3436	0.3467	0.3515	0.3532	0.3577	0.369	0.376	0.3844
C	11	0.2057	0.2056	0.2033	0.2065	0.2008	0.202	0.2025	0.2035	0.2012	0.2036	0.201	0.2018
C	12	0.2529	0.2597	0.2624	0.2648	0.2753	0.279	0.2873	0.2927	0.2852	0.2949	0.3029	0.3344
D	1	0.1755	0.1766	0.1757	0.1748	0.1753	0.1752	0.1738	0.1747	0.1752	0.1747	0.1737	0.1741
D	2	0.2304	0.2358	0.2303	0.2303	0.2293	0.2296	0.2294	0.2318	0.2477	0.2276	0.2267	0.23
D	3	0.2238	0.2266	0.2233	0.2286	0.2232	0.2263	0.2216	0.2249	0.2255	0.2246	0.2255	0.2248
D	4	0.2642	0.2643	0.2653	0.2657	0.2723	0.271	0.2718	0.2684	0.2752	0.2712	0.2766	0.2676
D	5	0.3315	0.3319	0.3307	0.3302	0.3301	0.3304	0.3321	0.3372	0.3327	0.3296	0.3303	0.3346
D	6	0.2022	0.2031	0.2028	0.2015	0.2027	0.2021	0.2012	0.2021	0.2081	0.2022	0.2007	0.2016
D	7	0.2999	0.3032	0.3087	0.313	0.3147	0.315	0.3182	0.3193	0.3258	0.325	0.3266	0.3257
D	8	0.3413	0.348	0.3552	0.3617	0.3672	0.3723	0.3751	0.3775	0.378	0.386	0.3887	0.3899
D	9	0.3211	0.3263	0.3318	0.3366	0.3371	0.3421	0.3384	0.3389	0.3245	0.3442	0.3541	0.3503
D	10	0.3266	0.3335	0.3384	0.3446	0.3517	0.3562	0.3586	0.3673	0.3567	0.3748	0.3812	0.3836
D	11	0.1938	0.1954	0.1946	0.1929	0.1935	0.1931	0.191	0.197	0.1928	0.195	0.1951	0.1948
D	12	0.2281	0.2315	0.2353	0.2385	0.2434	0.2493	0.2553	0.2574	0.2798	0.2883	0.294	0.3029

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 43 - 7 h	Raw Data (595) 44 - 7 h 10 min	Raw Data (595) 45 - 7 h 19 min	Raw Data (595) 46 - 7 h 30 min	Raw Data (595) 47 - 7 h 40 min	Raw Data (595) 48 - 7 h 49 min	Raw Data (595) 49 - 8 h	Raw Data (595) 50 - 8 h 9 min	Raw Data (595) 51 - 8 h 20 min	Raw Data (595) 52 - 8 h 30 min	Raw Data (595) 53 - 8 h 39 min	Raw Data (595) 54 - 8 h 50 min
Time, hours		7.00	7.17	7.33	7.50	7.67	7.83	8.00	8.17	8.33	8.50	8.67	8.83
A	1	0.1704	0.1699	0.1707	0.1708	0.1701	0.1696	0.1697	0.1691	0.1698	0.1701	0.1696	0.1681
A	2	0.2304	0.2309	0.2297	0.2293	0.23	0.2257	0.2275	0.2268	0.225	0.2278	0.2277	0.2263
A	3	0.1953	0.1967	0.1949	0.1945	0.1969	0.1951	0.1957	0.1941	0.1958	0.1944	0.1939	0.1937
A	4	0.2591	0.2552	0.2516	0.2488	0.2495	0.2537	0.2486	0.2475	0.2469	0.2477	0.2471	0.2488
A	5	0.2484	0.2471	0.2534	0.251	0.2547	0.2483	0.2502	0.2496	0.2496	0.2512	0.2509	0.2533
A	6	0.1977	0.1972	0.1982	0.1998	0.1985	0.1969	0.1983	0.1975	0.1986	0.1984	0.199	0.1978
A	7	0.3565	0.3522	0.3514	0.3491	0.3465	0.3446	0.3442	0.3426	0.3392	0.3401	0.3373	0.3357
A	8	0.3836	0.3816	0.3858	0.3818	0.3797	0.3768	0.3765	0.3757	0.3733	0.3729	0.3717	0.368
A	9	0.3331	0.3309	0.33	0.3292	0.3274	0.3247	0.3241	0.321	0.3196	0.3197	0.3194	0.3175
A	10	0.4348	0.4366	0.4423	0.4429	0.4461	0.4486	0.4538	0.4556	0.4583	0.4639	0.4654	0.4715
A	11	0.1895	0.1872	0.1875	0.1897	0.1883	0.1879	0.1885	0.1897	0.1883	0.1893	0.1895	0.1886
A	12	0.3273	0.3504	0.3525	0.3525	0.3593	0.3598	0.3537	0.359	0.356	0.3605	0.3627	0.3729
B	1	0.1526	0.1515	0.1523	0.1525	0.1516	0.1509	0.1515	0.1522	0.151	0.1518	0.152	0.1498
B	2	0.2023	0.2008	0.2066	0.2023	0.2027	0.2045	0.2029	0.2033	0.2017	0.2014	0.2018	0.2005
B	3	0.239	0.2382	0.2397	0.2463	0.2413	0.2379	0.2378	0.2373	0.2375	0.2368	0.2388	0.2383
B	4	0.2319	0.2322	0.2327	0.2349	0.2326	0.2298	0.2293	0.2292	0.229	0.2299	0.2289	0.2292
B	5	0.2811	0.2822	0.2784	0.2814	0.2798	0.2789	0.2809	0.2817	0.2801	0.2786	0.2805	0.2793
B	6	0.1563	0.1551	0.1556	0.1559	0.1554	0.1551	0.1563	0.1563	0.1556	0.1552	0.1556	0.154
B	7	0.3443	0.3431	0.3423	0.3415	0.3404	0.3382	0.3386	0.3389	0.3389	0.3378	0.3386	0.3351
B	8	0.4015	0.402	0.4045	0.4054	0.4037	0.4042	0.4059	0.4041	0.4024	0.4008	0.3996	0.3969
B	9	0.3423	0.3393	0.3385	0.338	0.336	0.3342	0.3343	0.3326	0.3308	0.3288	0.3265	0.3252
B	10	0.3731	0.376	0.3763	0.3819	0.3826	0.3863	0.3918	0.3991	0.4007	0.4031	0.4104	0.4073
B	11	0.1866	0.1796	0.1808	0.1823	0.1815	0.1854	0.184	0.1834	0.1821	0.1804	0.1832	0.1833
B	12	0.3764	0.3779	0.3963	0.3951	0.3988	0.4038	0.4142	0.4054	0.4033	0.408	0.4078	0.4352

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 43 - 7 h	Raw Data (595) 44 - 7 h 10 min	Raw Data (595) 45 - 7 h 19 min	Raw Data (595) 46 - 7 h 30 min	Raw Data (595) 47 - 7 h 40 min	Raw Data (595) 48 - 7 h 49 min	Raw Data (595) 49 - 8 h	Raw Data (595) 50 - 8 h 9 min	Raw Data (595) 51 - 8 h 20 min	Raw Data (595) 52 - 8 h 30 min	Raw Data (595) 53 - 8 h 39 min	Raw Data (595) 54 - 8 h 50 min
Time, hours		7.00	7.17	7.33	7.50	7.67	7.83	8.00	8.17	8.33	8.50	8.67	8.83
C	1	0.1957	0.1943	0.1949	0.1949	0.1953	0.1942	0.1951	0.1955	0.1952	0.1949	0.1951	0.1947
C	2	0.2154	0.2129	0.2109	0.2158	0.2145	0.217	0.2155	0.2138	0.2095	0.209	0.21	0.2101
C	3	0.2058	0.2002	0.2045	0.2041	0.1994	0.1996	0.2	0.199	0.1992	0.2011	0.1991	0.2042
C	4	0.2243	0.229	0.23	0.2225	0.2238	0.2246	0.2247	0.2239	0.2242	0.223	0.224	0.2255
C	5	0.3028	0.3027	0.304	0.3026	0.3025	0.3026	0.3027	0.3017	0.3016	0.301	0.3023	0.3018
C	6	0.215	0.2139	0.2141	0.2145	0.2144	0.2133	0.2148	0.2134	0.2127	0.2129	0.2137	0.2123
C	7	0.3257	0.3242	0.3257	0.3259	0.3233	0.3224	0.3249	0.3231	0.3227	0.3233	0.3229	0.3218
C	8	0.3836	0.3842	0.3858	0.3863	0.3882	0.3842	0.3863	0.3845	0.3833	0.3838	0.3823	0.3785
C	9	0.3818	0.3801	0.3822	0.3788	0.3817	0.3798	0.3824	0.3814	0.3814	0.384	0.3856	0.3852
C	10	0.3834	0.3873	0.3935	0.393	0.3975	0.3937	0.3994	0.4041	0.4084	0.4134	0.4177	0.4169
C	11	0.2018	0.2031	0.2042	0.2	0.2011	0.2024	0.2032	0.2008	0.2002	0.2006	0.2025	0.1997
C	12	0.337	0.3442	0.3602	0.3724	0.3686	0.3644	0.3618	0.3722	0.3726	0.3668	0.3764	0.3705
D	1	0.1741	0.173	0.1748	0.1739	0.174	0.1733	0.1731	0.1732	0.1726	0.1732	0.1738	0.1722
D	2	0.2268	0.2234	0.2381	0.2309	0.2295	0.2309	0.2294	0.2331	0.2283	0.2272	0.2298	0.2286
D	3	0.2244	0.2253	0.2233	0.224	0.2253	0.2261	0.2226	0.2251	0.2279	0.2229	0.2232	0.2236
D	4	0.2675	0.2668	0.2712	0.2677	0.2677	0.2687	0.2655	0.2659	0.2687	0.2727	0.2671	0.2664
D	5	0.3284	0.3277	0.3463	0.3382	0.3307	0.3283	0.3296	0.3285	0.3283	0.3283	0.3294	0.3304
D	6	0.2011	0.1991	0.2019	0.2024	0.2023	0.1998	0.2018	0.2002	0.1999	0.2006	0.2017	0.2001
D	7	0.3255	0.3224	0.3236	0.3239	0.3234	0.3208	0.322	0.3212	0.3198	0.3193	0.3191	0.3177
D	8	0.393	0.3953	0.3964	0.3982	0.3979	0.3961	0.3947	0.3941	0.3917	0.3901	0.391	0.3876
D	9	0.352	0.3527	0.3495	0.3526	0.354	0.3545	0.3545	0.3506	0.3527	0.3476	0.3507	0.3504
D	10	0.386	0.3888	0.3897	0.3938	0.3962	0.4007	0.4074	0.4075	0.4141	0.4164	0.4229	0.4242
D	11	0.1946	0.1918	0.1911	0.1912	0.1913	0.1909	0.1915	0.1925	0.1924	0.1938	0.1897	0.1916
D	12	0.3048	0.2902	0.3085	0.3048	0.3157	0.3125	0.3195	0.3207	0.322	0.3218	0.325	0.3236

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 55 - 9 h	Raw Data (595) 56 - 9 h 9 min	Raw Data (595) 57 - 9 h 20 min	Raw Data (595) 58 - 9 h 30 min	Raw Data (595) 59 - 9 h 39 min	Raw Data (595) 60 - 9 h 50 min	Raw Data (595) 61 - 10 h	Raw Data (595) 62 - 10 h 9 min	Raw Data (595) 63 - 10 h 20 min	Raw Data (595) 64 - 10 h 30 min	Raw Data (595) 65 - 10 h 39 min	Raw Data (595) 66 - 10 h 50 min
Time, hours		9.00	9.17	9.33	9.50	9.67	9.83	10.00	10.17	10.33	10.50	10.67	10.83
A	1	0.1694	0.1693	0.1691	0.1694	0.1685	0.1725	0.1681	0.168	0.1685	0.1678	0.1678	0.1716
A	2	0.2301	0.2278	0.2308	0.2282	0.2259	0.2555	0.2275	0.2252	0.2265	0.2267	0.2286	0.253
A	3	0.194	0.196	0.1974	0.1961	0.1974	0.1949	0.1951	0.1955	0.1961	0.1937	0.1933	0.1927
A	4	0.2462	0.25	0.2463	0.2473	0.2471	0.249	0.2459	0.2455	0.2458	0.2459	0.2458	0.2477
A	5	0.2525	0.252	0.2489	0.2483	0.2484	0.2486	0.2478	0.2481	0.2485	0.2487	0.2482	0.2476
A	6	0.1983	0.1991	0.1994	0.2005	0.1992	0.204	0.2005	0.1994	0.2019	0.2004	0.1996	0.2053
A	7	0.3328	0.3317	0.3298	0.3293	0.3296	0.3311	0.3266	0.3262	0.3244	0.3236	0.3225	0.3225
A	8	0.3663	0.364	0.3609	0.3595	0.357	0.3553	0.3539	0.3508	0.3517	0.348	0.3464	0.3438
A	9	0.3155	0.313	0.3127	0.3114	0.3102	0.3104	0.3067	0.3058	0.3042	0.3045	0.3023	0.3024
A	10	0.4747	0.4755	0.4779	0.4843	0.4845	0.4906	0.4887	0.4895	0.4897	0.4899	0.4864	0.4884
A	11	0.1889	0.1898	0.1889	0.1896	0.1888	0.1906	0.1886	0.1883	0.1898	0.1886	0.189	0.1896
A	12	0.3574	0.3639	0.3715	0.3661	0.3735	0.3711	0.3615	0.3828	0.3659	0.3817	0.38	0.3588
B	1	0.1512	0.1523	0.1535	0.1536	0.1525	0.1479	0.1532	0.1532	0.1541	0.1528	0.1543	0.1481
B	2	0.2017	0.2022	0.201	0.2009	0.1995	0.1977	0.1995	0.2012	0.2002	0.2004	0.2016	0.1982
B	3	0.2372	0.2373	0.2366	0.2373	0.2361	0.2334	0.237	0.2369	0.2365	0.2364	0.2359	0.2323
B	4	0.2293	0.2296	0.2301	0.2293	0.2283	0.2289	0.2279	0.2287	0.2288	0.2273	0.2274	0.2272
B	5	0.2816	0.2816	0.2815	0.2806	0.2791	0.2732	0.2797	0.2778	0.2769	0.2767	0.2789	0.2723
B	6	0.1559	0.1563	0.1554	0.1547	0.1545	0.1541	0.1554	0.1542	0.1542	0.1544	0.1548	0.1531
B	7	0.3358	0.337	0.3374	0.3347	0.3335	0.3238	0.3327	0.3307	0.3312	0.3292	0.3293	0.3191
B	8	0.3951	0.3968	0.3938	0.3932	0.3908	0.3858	0.3896	0.3884	0.3879	0.388	0.387	0.3796
B	9	0.3236	0.3228	0.3195	0.3213	0.3195	0.3262	0.3163	0.3151	0.3148	0.3139	0.3141	0.3183
B	10	0.4146	0.4207	0.4239	0.425	0.4278	0.4251	0.4331	0.4342	0.4401	0.4498	0.4635	0.4686
B	11	0.1838	0.1837	0.1837	0.1799	0.1803	0.1828	0.1806	0.1799	0.1796	0.1799	0.1818	0.1829
B	12	0.3927	0.4009	0.4059	0.4038	0.4209	0.4109	0.4046	0.4174	0.405	0.4175	0.4121	0.4072

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 55 - 9 h	Raw Data (595) 56 - 9 h 9 min	Raw Data (595) 57 - 9 h 20 min	Raw Data (595) 58 - 9 h 30 min	Raw Data (595) 59 - 9 h 39 min	Raw Data (595) 60 - 9 h 50 min	Raw Data (595) 61 - 10 h	Raw Data (595) 62 - 10 h 9 min	Raw Data (595) 63 - 10 h 20 min	Raw Data (595) 64 - 10 h 30 min	Raw Data (595) 65 - 10 h 39 min	Raw Data (595) 66 - 10 h 50 min
Time, hours		9.00	9.17	9.33	9.50	9.67	9.83	10.00	10.17	10.33	10.50	10.67	10.83
C	1	0.1949	0.1946	0.1939	0.1958	0.1945	0.1933	0.1944	0.1944	0.1943	0.1948	0.1943	0.1925
C	2	0.2157	0.2134	0.2145	0.2145	0.2106	0.2156	0.214	0.2105	0.2108	0.2102	0.2117	0.2089
C	3	0.1996	0.1991	0.2006	0.2008	0.1991	0.2016	0.1991	0.1982	0.1997	0.1984	0.1979	0.1988
C	4	0.226	0.2237	0.2232	0.2245	0.223	0.2237	0.2223	0.2232	0.2224	0.222	0.2221	0.222
C	5	0.3012	0.3023	0.3008	0.3016	0.3014	0.3105	0.2997	0.3006	0.3015	0.3013	0.3007	0.3093
C	6	0.2125	0.2136	0.2122	0.2132	0.2134	0.2176	0.2121	0.2128	0.2123	0.2119	0.2121	0.2165
C	7	0.3217	0.3213	0.3209	0.3218	0.3189	0.3179	0.3169	0.3149	0.3166	0.3131	0.3121	0.3081
C	8	0.3802	0.3769	0.3778	0.3775	0.3754	0.3838	0.3742	0.3733	0.3744	0.3716	0.3699	0.377
C	9	0.3867	0.3871	0.3882	0.391	0.3919	0.3915	0.3946	0.3974	0.3974	0.3989	0.3998	0.3971
C	10	0.4247	0.4243	0.4308	0.4357	0.4358	0.4361	0.4471	0.4507	0.4562	0.4558	0.4566	0.4483
C	11	0.1995	0.2004	0.2011	0.1994	0.2	0.1985	0.1984	0.1981	0.1997	0.1987	0.1982	0.198
C	12	0.372	0.3603	0.3718	0.3685	0.3754	0.3644	0.3889	0.3762	0.3812	0.3829	0.3882	0.364
D	1	0.1733	0.1725	0.1725	0.1725	0.1718	0.1739	0.1723	0.1713	0.172	0.1723	0.1718	0.1715
D	2	0.229	0.2263	0.2276	0.23	0.2308	0.2509	0.2282	0.2251	0.2266	0.2279	0.2279	0.2451
D	3	0.2221	0.2256	0.2257	0.2237	0.2234	0.2276	0.2224	0.2239	0.2245	0.2224	0.2221	0.2248
D	4	0.2666	0.2669	0.2676	0.2699	0.2692	0.2624	0.2705	0.271	0.2711	0.2713	0.2695	0.2621
D	5	0.3288	0.3285	0.3287	0.3293	0.3278	0.3297	0.3274	0.3269	0.3287	0.3285	0.3276	0.3282
D	6	0.2006	0.2002	0.1995	0.2	0.1989	0.2106	0.2003	0.1989	0.1993	0.2005	0.2001	0.206
D	7	0.3173	0.3149	0.3136	0.3116	0.31	0.3154	0.3086	0.3065	0.3044	0.305	0.303	0.3061
D	8	0.3865	0.3837	0.3822	0.3804	0.3791	0.3711	0.3754	0.3736	0.3723	0.3714	0.3697	0.3602
D	9	0.3498	0.353	0.3503	0.3526	0.3563	0.3212	0.3516	0.3529	0.3575	0.3539	0.3544	0.3231
D	10	0.4279	0.4324	0.4349	0.4409	0.4437	0.4301	0.4484	0.4474	0.4488	0.4467	0.4465	0.4288
D	11	0.1906	0.191	0.1908	0.1904	0.1896	0.1898	0.192	0.1896	0.1899	0.1902	0.1895	0.1905
D	12	0.3242	0.3156	0.3245	0.3239	0.3233	0.3357	0.3251	0.3223	0.3255	0.3226	0.3285	0.3359

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 67 - 11 h	Raw Data (595) 68 - 11 h 9 min	Raw Data (595) 69 - 11 h 20 min	Raw Data (595) 70 - 11 h 30 min	Raw Data (595) 71 - 11 h 39 min	Raw Data (595) 72 - 11 h 50 min	Raw Data (595) 73 - 12 h	Raw Data (595) 74 - 12 h 9 min	Raw Data (595) 75 - 12 h 20 min	Raw Data (595) 76 - 12 h 30 min	Raw Data (595) 77 - 12 h 39 min	Raw Data (595) 78 - 12 h 50 min
Time, hours		11.00	11.17	11.33	11.50	11.67	11.83	12.00	12.17	12.33	12.50	12.67	12.83
A	1	0.1671	0.1682	0.1676	0.1672	0.1674	0.1671	0.1716	0.1673	0.1663	0.1673	0.1657	0.1668
A	2	0.2254	0.227	0.2273	0.2268	0.2278	0.2219	0.2489	0.2255	0.2239	0.228	0.2242	0.2255
A	3	0.1935	0.1936	0.1934	0.1923	0.1948	0.1952	0.1929	0.1955	0.1951	0.1947	0.1935	0.1946
A	4	0.2457	0.2455	0.2457	0.2448	0.2456	0.2461	0.2472	0.2446	0.2446	0.2431	0.2422	0.2435
A	5	0.2476	0.2483	0.2481	0.2476	0.2485	0.2486	0.2482	0.2486	0.2476	0.2485	0.2475	0.2478
A	6	0.2001	0.2001	0.2014	0.2005	0.2018	0.2002	0.2051	0.2002	0.2003	0.202	0.2004	0.2013
A	7	0.3209	0.3198	0.3197	0.3171	0.3181	0.3181	0.3186	0.3166	0.3156	0.3166	0.314	0.3141
A	8	0.3427	0.3419	0.342	0.338	0.3393	0.3357	0.3345	0.3345	0.3309	0.3332	0.3302	0.3288
A	9	0.3001	0.2992	0.2982	0.2959	0.2972	0.2966	0.2963	0.2949	0.2926	0.2934	0.2911	0.2902
A	10	0.4875	0.4866	0.4908	0.4964	0.5043	0.5119	0.5251	0.5348	0.537	0.5544	0.5593	0.5641
A	11	0.1888	0.1888	0.1899	0.1891	0.1899	0.1887	0.1905	0.1894	0.1887	0.1897	0.1892	0.189
A	12	0.3697	0.3588	0.3645	0.3594	0.3606	0.3772	0.3626	0.3762	0.3584	0.3566	0.3659	0.3609
B	1	0.1535	0.155	0.1547	0.1536	0.1544	0.1548	0.147	0.154	0.1553	0.1545	0.1553	0.1545
B	2	0.1997	0.2014	0.2025	0.2	0.201	0.1996	0.1988	0.2001	0.1991	0.1991	0.1991	0.1992
B	3	0.2358	0.2371	0.2363	0.2361	0.236	0.2351	0.23	0.2347	0.2348	0.2344	0.2347	0.234
B	4	0.2269	0.2276	0.2272	0.2262	0.2272	0.2258	0.2286	0.2267	0.2265	0.2256	0.2265	0.2252
B	5	0.277	0.2789	0.2762	0.2772	0.2768	0.2766	0.2704	0.2749	0.2785	0.2765	0.2775	0.276
B	6	0.154	0.1543	0.1542	0.1539	0.1537	0.1537	0.1529	0.1532	0.1536	0.154	0.1535	0.1531
B	7	0.3266	0.3288	0.326	0.3249	0.3257	0.3238	0.3074	0.3194	0.3213	0.3202	0.3201	0.317
B	8	0.384	0.3823	0.3819	0.3792	0.3777	0.3774	0.3681	0.3742	0.3708	0.3713	0.3699	0.3713
B	9	0.3108	0.3087	0.309	0.3065	0.3065	0.3058	0.3151	0.3057	0.3049	0.3095	0.3197	0.3377
B	10	0.4781	0.4805	0.4782	0.4748	0.4708	0.4673	0.4543	0.4573	0.4573	0.453	0.4498	0.4454
B	11	0.1818	0.1815	0.1848	0.186	0.1844	0.1842	0.1834	0.1802	0.1812	0.1816	0.1805	0.1799
B	12	0.4045	0.4022	0.4056	0.3975	0.3977	0.4201	0.3997	0.4115	0.4003	0.4018	0.4009	0.3977

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 67 - 11 h	Raw Data (595) 68 - 11 h 9 min	Raw Data (595) 69 - 11 h 20 min	Raw Data (595) 70 - 11 h 30 min	Raw Data (595) 71 - 11 h 39 min	Raw Data (595) 72 - 11 h 50 min	Raw Data (595) 73 - 12 h	Raw Data (595) 74 - 12 h 9 min	Raw Data (595) 75 - 12 h 20 min	Raw Data (595) 76 - 12 h 30 min	Raw Data (595) 77 - 12 h 39 min	Raw Data (595) 78 - 12 h 50 min
Time, hours		11.00	11.17	11.33	11.50	11.67	11.83	12.00	12.17	12.33	12.50	12.67	12.83
C 1		0.1939	0.1933	0.1944	0.1921	0.1935	0.1932	0.1915	0.1939	0.1929	0.1933	0.1923	0.1923
C 2		0.2121	0.212	0.2119	0.2098	0.2118	0.2106	0.2086	0.2114	0.2112	0.211	0.2099	0.2094
C 3		0.1989	0.198	0.1984	0.1966	0.1981	0.1973	0.1981	0.1995	0.1974	0.1976	0.1971	0.1961
C 4		0.2217	0.2213	0.2216	0.2207	0.2213	0.2212	0.2207	0.2216	0.22	0.2209	0.2205	0.221
C 5		0.301	0.3015	0.3019	0.2995	0.3007	0.3007	0.3085	0.2998	0.2992	0.2993	0.2987	0.299
C 6		0.2124	0.2119	0.2124	0.2117	0.2129	0.2148	0.2196	0.2159	0.2162	0.2186	0.2188	0.2189
C 7		0.3099	0.3082	0.3082	0.3065	0.3058	0.3042	0.301	0.3029	0.3024	0.3022	0.3015	0.3014
C 8		0.3681	0.3657	0.3654	0.3636	0.3636	0.3617	0.3671	0.3612	0.3597	0.3589	0.3598	0.3621
C 9		0.4019	0.4021	0.4008	0.3988	0.3955	0.3918	0.3834	0.3864	0.3821	0.3831	0.3926	0.4029
C 10		0.4561	0.4526	0.4523	0.4488	0.4497	0.4435	0.4336	0.4402	0.436	0.4332	0.4318	0.4306
C 11		0.1985	0.1991	0.1979	0.1976	0.1984	0.1983	0.1959	0.1984	0.1975	0.1976	0.1971	0.1967
C 12		0.3975	0.3836	0.3874	0.3938	0.3861	0.3837	0.3669	0.388	0.3806	0.3812	0.3859	0.3864
D 1		0.1713	0.1713	0.171	0.1706	0.1717	0.1712	0.171	0.1707	0.1706	0.1712	0.1698	0.1706
D 2		0.2282	0.2259	0.2283	0.2249	0.2289	0.2264	0.2434	0.2271	0.2254	0.225	0.2241	0.2247
D 3		0.2231	0.223	0.2237	0.2212	0.2232	0.222	0.2236	0.2223	0.2223	0.2221	0.221	0.221
D 4		0.2691	0.2699	0.2694	0.2691	0.2707	0.2692	0.2622	0.2696	0.2691	0.2682	0.2676	0.2689
D 5		0.3274	0.3276	0.3281	0.3271	0.3288	0.3282	0.3281	0.3281	0.3278	0.3278	0.3266	0.3289
D 6		0.1992	0.1991	0.1988	0.1985	0.1998	0.1996	0.2058	0.1995	0.1994	0.1978	0.1981	0.1991
D 7		0.3015	0.3001	0.2987	0.2978	0.297	0.2967	0.2997	0.2968	0.2949	0.2946	0.2943	0.2956
D 8		0.3663	0.3635	0.3634	0.3621	0.3607	0.3599	0.3511	0.3597	0.3592	0.359	0.3576	0.3603
D 9		0.3548	0.3484	0.34	0.3256	0.317	0.3183	0.2897	0.3319	0.3393	0.3447	0.3484	0.359
D 10		0.4428	0.4402	0.4417	0.4363	0.4352	0.433	0.4151	0.4303	0.4262	0.4236	0.4204	0.4188
D 11		0.1907	0.1898	0.191	0.1938	0.1957	0.197	0.1948	0.1956	0.1951	0.1951	0.1939	0.195
D 12		0.3305	0.3307	0.3342	0.3292	0.3298	0.3305	0.3355	0.3322	0.3356	0.3333	0.335	0.3347

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 79 - 13 h	Raw Data (595) 80 - 13 h 9 min	Raw Data (595) 81 - 13 h 20 min	Raw Data (595) 82 - 13 h 30 min	Raw Data (595) 83 - 13 h 39 min	Raw Data (595) 84 - 13 h 50 min	Raw Data (595) 85 - 14 h	Raw Data (595) 86 - 14 h 9 min	Raw Data (595) 87 - 14 h 20 min	Raw Data (595) 88 - 14 h 30 min	Raw Data (595) 89 - 14 h 39 min	Raw Data (595) 90 - 14 h 50 min
Time, hours		13.00	13.17	13.33	13.50	13.67	13.83	14.00	14.17	14.33	14.50	14.67	14.83
A	1	0.1656	0.1665	0.166	0.1658	0.1664	0.1668	0.1666	0.1659	0.1654	0.1654	0.1653	0.1654
A	2	0.2258	0.2236	0.2249	0.2243	0.2241	0.2236	0.2242	0.2218	0.2219	0.2237	0.2219	0.2251
A	3	0.1944	0.1946	0.1942	0.1943	0.1945	0.1951	0.1943	0.1949	0.1941	0.1949	0.1941	0.1947
A	4	0.2434	0.2434	0.2428	0.2433	0.2434	0.2432	0.2428	0.243	0.2426	0.2422	0.2422	0.2423
A	5	0.2479	0.2477	0.2484	0.2477	0.2486	0.2483	0.2477	0.2479	0.2476	0.2479	0.2479	0.2482
A	6	0.2003	0.2012	0.2003	0.2016	0.2004	0.2011	0.2023	0.1995	0.1987	0.2004	0.1988	0.2013
A	7	0.3136	0.3133	0.3127	0.3127	0.3121	0.3124	0.3113	0.3112	0.3099	0.3109	0.3102	0.3101
A	8	0.3299	0.3274	0.3273	0.3276	0.3254	0.3249	0.3253	0.3226	0.3212	0.3224	0.3203	0.3211
A	9	0.2903	0.2888	0.2882	0.2885	0.2877	0.2874	0.2867	0.2863	0.2853	0.2848	0.2836	0.2841
A	10	0.5677	0.5666	0.5662	0.5673	0.5683	0.5688	0.5685	0.5679	0.5682	0.5701	0.5691	0.5716
A	11	0.1902	0.1902	0.1897	0.1899	0.1899	0.191	0.1906	0.1893	0.1896	0.1904	0.19	0.1911
A	12	0.3606	0.3573	0.3572	0.3584	0.3597	0.3587	0.3541	0.3615	0.3627	0.3557	0.3597	0.3491
B	1	0.1551	0.1545	0.1536	0.1549	0.1535	0.1543	0.1549	0.1542	0.1541	0.1548	0.1545	0.1546
B	2	0.1991	0.1992	0.1989	0.1988	0.1991	0.2	0.1988	0.1993	0.1987	0.1994	0.1985	0.1992
B	3	0.2345	0.2349	0.2329	0.2343	0.2332	0.2342	0.2332	0.2325	0.2326	0.2337	0.233	0.2328
B	4	0.2253	0.2255	0.2249	0.2256	0.2249	0.2259	0.2247	0.2251	0.225	0.2256	0.2249	0.2251
B	5	0.2754	0.2756	0.2748	0.2749	0.2743	0.2757	0.2766	0.2753	0.274	0.274	0.2746	0.2743
B	6	0.1536	0.1533	0.1523	0.1532	0.1529	0.1534	0.1534	0.1536	0.1526	0.1535	0.1527	0.1527
B	7	0.3177	0.3172	0.3145	0.317	0.3141	0.3142	0.3152	0.3137	0.3131	0.3148	0.3139	0.3151
B	8	0.3698	0.3702	0.3679	0.3676	0.367	0.3668	0.3652	0.3654	0.3637	0.3642	0.3619	0.3618
B	9	0.3442	0.3499	0.352	0.3576	0.3591	0.3625	0.3623	0.3654	0.3664	0.368	0.3675	0.3696
B	10	0.4415	0.44	0.4364	0.4336	0.4325	0.431	0.4307	0.4274	0.425	0.424	0.4228	0.4234
B	11	0.1794	0.1808	0.18	0.179	0.1805	0.1812	0.1795	0.1804	0.1789	0.179	0.1785	0.1793
B	12	0.3978	0.3948	0.3922	0.3965	0.3981	0.396	0.3913	0.4021	0.4021	0.3993	0.408	0.3992



## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 79 - 13 h	Raw Data (595) 80 - 13 h 9 min	Raw Data (595) 81 - 13 h 20 min	Raw Data (595) 82 - 13 h 30 min	Raw Data (595) 83 - 13 h 39 min	Raw Data (595) 84 - 13 h 50 min	Raw Data (595) 85 - 14 h	Raw Data (595) 86 - 14 h 9 min	Raw Data (595) 87 - 14 h 20 min	Raw Data (595) 88 - 14 h 30 min	Raw Data (595) 89 - 14 h 39 min	Raw Data (595) 90 - 14 h 50 min
Time, hours		13.00	13.17	13.33	13.50	13.67	13.83	14.00	14.17	14.33	14.50	14.67	14.83
C	1	0.1927	0.1926	0.1922	0.1924	0.1923	0.1929	0.1926	0.1923	0.1923	0.1926	0.1915	0.1921
C	2	0.2094	0.2091	0.2087	0.2094	0.2087	0.2096	0.2087	0.2092	0.2086	0.2092	0.2088	0.2093
C	3	0.1977	0.1973	0.1974	0.1971	0.1965	0.1976	0.1971	0.1965	0.1966	0.1972	0.1951	0.1965
C	4	0.2209	0.2202	0.2204	0.2199	0.2208	0.2207	0.221	0.2211	0.2205	0.2205	0.2202	0.2203
C	5	0.2991	0.299	0.2983	0.2989	0.2984	0.2989	0.2982	0.2988	0.2978	0.2989	0.2973	0.2982
C	6	0.2197	0.22	0.2203	0.2208	0.2205	0.2221	0.2209	0.2223	0.2216	0.2221	0.2221	0.2225
C	7	0.3022	0.3028	0.3025	0.3043	0.305	0.3057	0.3069	0.3049	0.3081	0.3096	0.3095	0.3118
C	8	0.3634	0.3652	0.3655	0.3664	0.3692	0.3706	0.3715	0.3714	0.3734	0.3736	0.3732	0.3745
C	9	0.407	0.4087	0.4079	0.4093	0.4122	0.4128	0.4128	0.4124	0.4135	0.4128	0.4135	0.4131
C	10	0.4299	0.4275	0.4251	0.425	0.4247	0.4232	0.4231	0.4188	0.4207	0.4196	0.4179	0.4175
C	11	0.1975	0.1976	0.1978	0.1972	0.1981	0.1986	0.1978	0.1976	0.1974	0.1976	0.1974	0.1978
C	12	0.3906	0.3806	0.3726	0.3739	0.3893	0.3817	0.391	0.3735	0.3907	0.3847	0.3846	0.3886
D	1	0.1704	0.1706	0.1697	0.1699	0.1701	0.1704	0.1696	0.1698	0.1699	0.1692	0.1689	0.1697
D	2	0.2249	0.2237	0.2249	0.2237	0.2242	0.223	0.2256	0.2235	0.2247	0.2229	0.223	0.2266
D	3	0.2207	0.2208	0.2211	0.2211	0.2213	0.2217	0.2214	0.2222	0.2226	0.2217	0.2209	0.221
D	4	0.2696	0.2689	0.2681	0.269	0.2693	0.2697	0.269	0.269	0.27	0.2689	0.2684	0.2696
D	5	0.3284	0.3284	0.3276	0.3283	0.329	0.3299	0.331	0.3315	0.332	0.3317	0.3309	0.3318
D	6	0.1991	0.1995	0.1987	0.1999	0.1999	0.1995	0.2001	0.1992	0.1993	0.1979	0.1989	0.2001
D	7	0.2957	0.2947	0.2941	0.2943	0.2952	0.2953	0.2961	0.296	0.296	0.2962	0.2963	0.2971
D	8	0.3606	0.3619	0.3624	0.3643	0.3648	0.3676	0.3687	0.3712	0.372	0.3753	0.3765	0.3751
D	9	0.361	0.3662	0.3692	0.3748	0.3773	0.3793	0.3808	0.3829	0.3854	0.3915	0.3857	0.3883
D	10	0.4177	0.4154	0.4135	0.4126	0.4131	0.4125	0.41	0.409	0.4083	0.4083	0.4065	0.4058
D	11	0.1958	0.1963	0.1953	0.1936	0.1956	0.1952	0.1951	0.1934	0.1933	0.1944	0.1937	0.1954
D	12	0.3353	0.3347	0.3324	0.332	0.3373	0.3365	0.3367	0.3263	0.3403	0.3376	0.3396	0.3399

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 91 - 15 h	Raw Data (595) 92 - 15 h 9 min	Raw Data (595) 93 - 15 h 20 min	Raw Data (595) 94 - 15 h 30 min	Raw Data (595) 95 - 15 h 39 min	Raw Data (595) 96 - 15 h 50 min	Raw Data (595) 97 - 16 h	Raw Data (595) 98 - 16 h 10 min	Raw Data (595) 99 - 16 h 19 min	Raw Data (595) 100 - 16 h 30 min	Raw Data (595) 101 - 16 h 40 min	Raw Data (595) 102 - 16 h 49 min
Time, hours		15.00	15.17	15.33	15.50	15.67	15.83	16.00	16.17	16.33	16.50	16.67	16.83
A	1	0.1657	0.1657	0.165	0.1649	0.165	0.1644	0.1636	0.1638	0.1649	0.1637	0.1641	0.1636
A	2	0.2228	0.2234	0.2208	0.2211	0.2224	0.2213	0.2202	0.2202	0.2209	0.2198	0.2203	0.2199
A	3	0.1943	0.1943	0.1942	0.1935	0.1942	0.1933	0.1931	0.1929	0.1935	0.194	0.1937	0.1934
A	4	0.2422	0.242	0.2421	0.2408	0.242	0.241	0.2408	0.2401	0.2413	0.2415	0.2407	0.2404
A	5	0.2482	0.2477	0.2472	0.2468	0.2473	0.2476	0.2467	0.2469	0.2481	0.2478	0.2477	0.2477
A	6	0.2005	0.2002	0.1983	0.2006	0.2003	0.1993	0.1983	0.1991	0.1986	0.1988	0.2	0.1979
A	7	0.3091	0.3086	0.3083	0.3073	0.3077	0.307	0.3067	0.3074	0.3063	0.3069	0.307	0.3057
A	8	0.3199	0.3181	0.3157	0.3159	0.3155	0.3137	0.3122	0.3124	0.3125	0.3114	0.3109	0.309
A	9	0.2836	0.2824	0.2817	0.2819	0.2812	0.2809	0.279	0.2787	0.2794	0.2787	0.278	0.2766
A	10	0.5701	0.5724	0.5692	0.5747	0.5717	0.5727	0.5719	0.5728	0.5726	0.5738	0.5733	0.5745
A	11	0.1895	0.1898	0.1893	0.191	0.191	0.1901	0.1894	0.1903	0.1899	0.1896	0.1905	0.1901
A	12	0.3526	0.347	0.3629	0.3424	0.3444	0.3448	0.3431	0.3392	0.3401	0.3452	0.3414	0.3422
B	1	0.1553	0.1548	0.1536	0.1534	0.1532	0.1537	0.1538	0.154	0.1542	0.1533	0.1545	0.1541
B	2	0.1987	0.1987	0.201	0.2007	0.2009	0.2003	0.2001	0.1996	0.201	0.2013	0.2015	0.2007
B	3	0.233	0.2329	0.2319	0.2307	0.2312	0.2319	0.231	0.2315	0.2313	0.2304	0.2319	0.2317
B	4	0.2253	0.2255	0.2254	0.2245	0.224	0.2244	0.224	0.2234	0.2241	0.224	0.2255	0.2241
B	5	0.276	0.2754	0.2732	0.2726	0.2728	0.2731	0.2728	0.274	0.2743	0.2717	0.2721	0.2732
B	6	0.1535	0.1539	0.1521	0.1513	0.1521	0.1524	0.1522	0.152	0.1525	0.1517	0.1522	0.1526
B	7	0.3154	0.3151	0.3142	0.3123	0.3129	0.3137	0.3139	0.3132	0.3139	0.3118	0.3137	0.3133
B	8	0.3616	0.3607	0.359	0.3579	0.3585	0.3571	0.3572	0.3565	0.3564	0.3563	0.3562	0.3561
B	9	0.3705	0.3714	0.3716	0.3717	0.374	0.3724	0.3726	0.3724	0.3738	0.3753	0.3752	0.3761
B	10	0.4233	0.4214	0.4201	0.4181	0.4177	0.4178	0.4171	0.4149	0.4157	0.4135	0.4136	0.4147
B	11	0.1797	0.1816	0.1814	0.1779	0.179	0.178	0.1777	0.178	0.1781	0.1768	0.1774	0.1779
B	12	0.4023	0.3961	0.413	0.4	0.3991	0.401	0.3971	0.3944	0.3962	0.3997	0.3976	0.3972

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 91 - 15 h	Raw Data (595) 92 - 15 h 9 min	Raw Data (595) 93 - 15 h 20 min	Raw Data (595) 94 - 15 h 30 min	Raw Data (595) 95 - 15 h 39 min	Raw Data (595) 96 - 15 h 50 min	Raw Data (595) 97 - 16 h	Raw Data (595) 98 - 16 h 10 min	Raw Data (595) 99 - 16 h 19 min	Raw Data (595) 100 - 16 h 30 min	Raw Data (595) 101 - 16 h 40 min	Raw Data (595) 102 - 16 h 49 min
Time, hours		15.00	15.17	15.33	15.50	15.67	15.83	16.00	16.17	16.33	16.50	16.67	16.83
C 1		0.1923	0.1922	0.1918	0.1907	0.1917	0.1911	0.1904	0.1911	0.1911	0.191	0.1917	0.1912
C 2		0.2089	0.2091	0.2093	0.2079	0.2084	0.2091	0.2087	0.2084	0.2087	0.2092	0.2092	0.2086
C 3		0.1967	0.1964	0.1961	0.1958	0.1966	0.1952	0.1946	0.1955	0.1954	0.1952	0.1961	0.1955
C 4		0.2203	0.2204	0.2201	0.2193	0.2192	0.2192	0.2187	0.2194	0.2192	0.2191	0.2196	0.219
C 5		0.2975	0.2978	0.2971	0.2965	0.297	0.2964	0.2965	0.2966	0.2963	0.2967	0.2968	0.2966
C 6		0.2235	0.2235	0.2227	0.222	0.2234	0.223	0.2228	0.2233	0.2236	0.2235	0.2238	0.2237
C 7		0.3131	0.3132	0.3146	0.3172	0.3189	0.3202	0.3199	0.3208	0.3251	0.3244	0.3271	0.3297
C 8		0.3758	0.376	0.3756	0.3781	0.3785	0.3778	0.3772	0.3782	0.3812	0.3809	0.3816	0.3815
C 9		0.4143	0.4135	0.4145	0.4134	0.4147	0.415	0.4139	0.4139	0.416	0.4167	0.4157	0.4176
C 10		0.4185	0.4168	0.4143	0.415	0.4149	0.4123	0.4109	0.4107	0.4127	0.4114	0.4109	0.4104
C 11		0.1972	0.1976	0.1981	0.1971	0.1976	0.1969	0.1966	0.1968	0.1981	0.1978	0.1978	0.1971
C 12		0.3932	0.3949	0.3899	0.4003	0.3972	0.3956	0.3942	0.396	0.4014	0.4013	0.4057	0.4102
D 1		0.1693	0.1696	0.1692	0.1689	0.169	0.1687	0.1672	0.1684	0.168	0.1688	0.168	0.1681
D 2		0.2253	0.2255	0.2253	0.2257	0.2257	0.2243	0.2234	0.2236	0.2224	0.2244	0.2245	0.2262
D 3		0.2209	0.222	0.2217	0.221	0.2202	0.2202	0.2192	0.2192	0.2202	0.2199	0.2201	0.2199
D 4		0.2699	0.2705	0.2698	0.269	0.268	0.2687	0.2686	0.2684	0.2685	0.269	0.2691	0.2698
D 5		0.3324	0.3319	0.3324	0.3315	0.3318	0.3305	0.33	0.3303	0.3315	0.3309	0.3307	0.3308
D 6		0.2004	0.2005	0.1977	0.1993	0.1994	0.1981	0.1978	0.1986	0.1973	0.1991	0.1991	0.1989
D 7		0.2981	0.2987	0.2992	0.2996	0.3005	0.3012	0.3007	0.3018	0.3037	0.3048	0.3056	0.3064
D 8		0.3782	0.3782	0.3804	0.3806	0.3819	0.3824	0.3827	0.3824	0.3858	0.3858	0.3873	0.3876
D 9		0.3933	0.3911	0.4004	0.3958	0.3962	0.4002	0.3973	0.3969	0.4047	0.3999	0.3997	0.4029
D 10		0.4057	0.4058	0.406	0.404	0.4043	0.4029	0.4008	0.4002	0.4041	0.4009	0.4011	0.4009
D 11		0.1936	0.1942	0.1929	0.1935	0.1944	0.1933	0.1918	0.1929	0.1941	0.1943	0.1931	0.1931
D 12		0.3447	0.3454	0.3457	0.3554	0.3531	0.3534	0.3559	0.3536	0.3639	0.358	0.3679	0.3777

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 103 - 17 h	Raw Data (595) 104 - 17 h 10 min	Raw Data (595) 105 - 17 h 19 min	Raw Data (595) 106 - 17 h 30 min	Raw Data (595) 107 - 17 h 40 min	Raw Data (595) 108 - 17 h 49 min	Raw Data (595) 109 - 18 h	Raw Data (595) 110 - 18 h 10 min	Raw Data (595) 111 - 18 h 19 min	Raw Data (595) 112 - 18 h 30 min	Raw Data (595) 113 - 18 h 40 min	Raw Data (595) 114 - 18 h 49 min
Time, hours		17.00	17.17	17.33	17.50	17.67	17.83	18.00	18.17	18.33	18.50	18.67	18.83
A	1	0.1644	0.1637	0.1639	0.1637	0.1632	0.1638	0.1642	0.1629	0.1629	0.1636	0.1627	0.1633
A	2	0.2211	0.2216	0.2197	0.2192	0.2198	0.2198	0.2202	0.218	0.2168	0.2175	0.2172	0.2188
A	3	0.1936	0.1936	0.1936	0.1932	0.1932	0.1938	0.1929	0.193	0.1925	0.193	0.1926	0.1934
A	4	0.2401	0.2406	0.2409	0.2402	0.2398	0.2405	0.24	0.2401	0.2402	0.2401	0.2398	0.2394
A	5	0.2475	0.2471	0.2483	0.2476	0.2478	0.248	0.2472	0.2474	0.2471	0.2472	0.2474	0.2477
A	6	0.1998	0.1999	0.1987	0.1996	0.1993	0.1995	0.1993	0.1975	0.1983	0.1979	0.1975	0.1993
A	7	0.3059	0.3059	0.3042	0.3044	0.304	0.3039	0.3023	0.3019	0.3012	0.3012	0.3008	0.3006
A	8	0.31	0.3075	0.3068	0.3061	0.3059	0.3062	0.3045	0.3035	0.3016	0.3009	0.2996	0.3003
A	9	0.2777	0.2765	0.2765	0.2756	0.2752	0.2751	0.2737	0.2724	0.2715	0.2724	0.2707	0.2711
A	10	0.5766	0.5756	0.5747	0.5749	0.5773	0.5782	0.5796	0.5759	0.5748	0.5726	0.5769	0.5761
A	11	0.1908	0.1907	0.1902	0.1907	0.1917	0.1918	0.191	0.1903	0.1902	0.1896	0.1906	0.1905
A	12	0.3395	0.3396	0.3403	0.3386	0.343	0.3442	0.3443	0.344	0.35	0.3457	0.35	0.3506
B	1	0.1546	0.1537	0.1534	0.154	0.1541	0.1529	0.1537	0.1531	0.1533	0.1536	0.1534	0.1537
B	2	0.2002	0.2005	0.2007	0.2001	0.2003	0.2006	0.2003	0.1999	0.2002	0.2001	0.199	0.2004
B	3	0.2316	0.2317	0.2302	0.2317	0.2302	0.2296	0.2312	0.23	0.2306	0.2298	0.2301	0.2293
B	4	0.2246	0.2244	0.2241	0.2246	0.2241	0.224	0.2239	0.2232	0.2235	0.2235	0.2237	0.2235
B	5	0.2738	0.2722	0.2713	0.2717	0.2718	0.2711	0.2721	0.2703	0.2713	0.2714	0.271	0.2701
B	6	0.1524	0.1517	0.1519	0.1519	0.1517	0.1516	0.1524	0.1512	0.1518	0.1518	0.1512	0.1513
B	7	0.3144	0.3137	0.3101	0.3136	0.3118	0.3107	0.3129	0.3113	0.3102	0.3107	0.3107	0.3094
B	8	0.3554	0.3563	0.3542	0.3552	0.3535	0.3549	0.3542	0.3536	0.3531	0.3547	0.3561	0.3551
B	9	0.3755	0.3756	0.3775	0.3759	0.3761	0.3768	0.3756	0.3765	0.3767	0.3781	0.3775	0.3789
B	10	0.4142	0.4116	0.4095	0.4097	0.4098	0.4101	0.411	0.4085	0.4087	0.4101	0.4083	0.4086
B	11	0.1778	0.1777	0.1785	0.178	0.1787	0.1796	0.1794	0.1788	0.1785	0.1795	0.1786	0.1796
B	12	0.3941	0.3959	0.3973	0.3934	0.3973	0.3957	0.397	0.3973	0.3994	0.4002	0.4025	0.403

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 103 - 17 h	Raw Data (595) 104 - 17 h 10 min	Raw Data (595) 105 - 17 h 19 min	Raw Data (595) 106 - 17 h 30 min	Raw Data (595) 107 - 17 h 40 min	Raw Data (595) 108 - 17 h 49 min	Raw Data (595) 109 - 18 h	Raw Data (595) 110 - 18 h 10 min	Raw Data (595) 111 - 18 h 19 min	Raw Data (595) 112 - 18 h 30 min	Raw Data (595) 113 - 18 h 40 min	Raw Data (595) 114 - 18 h 49 min
Time, hours		17.00	17.17	17.33	17.50	17.67	17.83	18.00	18.17	18.33	18.50	18.67	18.83
C 1		0.1912	0.1909	0.1909	0.1908	0.1908	0.1907	0.1905	0.19	0.1902	0.1903	0.1898	0.1904
C 2		0.209	0.208	0.2088	0.2084	0.2087	0.2084	0.2085	0.2077	0.2067	0.208	0.2079	0.2075
C 3		0.1954	0.1957	0.1949	0.1954	0.195	0.1947	0.1953	0.1945	0.1953	0.1954	0.1944	0.1948
C 4		0.2189	0.2184	0.219	0.2194	0.2193	0.2194	0.2186	0.2185	0.2175	0.2191	0.2179	0.2189
C 5		0.2967	0.2955	0.2964	0.2962	0.2954	0.2956	0.2955	0.2947	0.2945	0.2955	0.2953	0.2951
C 6		0.2237	0.2238	0.224	0.2238	0.2245	0.2235	0.2238	0.223	0.2233	0.2243	0.2239	0.2243
C 7		0.3296	0.3319	0.3316	0.3332	0.333	0.3359	0.3374	0.338	0.3392	0.3423	0.3421	0.3448
C 8		0.3815	0.3836	0.3835	0.384	0.384	0.3851	0.3859	0.3869	0.3876	0.3889	0.3885	0.3913
C 9		0.416	0.4168	0.4177	0.4173	0.4177	0.4185	0.4199	0.4188	0.4184	0.4198	0.4185	0.4195
C 10		0.4085	0.4111	0.4096	0.4091	0.4077	0.4093	0.4078	0.4078	0.4065	0.4074	0.4053	0.4067
C 11		0.198	0.1984	0.1977	0.1972	0.1977	0.1976	0.1972	0.1973	0.1962	0.198	0.1966	0.1972
C 12		0.4029	0.4077	0.4108	0.4125	0.4122	0.4133	0.4155	0.4146	0.4073	0.4178	0.412	0.4168
D 1		0.1689	0.1678	0.1683	0.1678	0.1679	0.1677	0.1678	0.1668	0.167	0.1676	0.1673	0.1664
D 2		0.2254	0.2225	0.2242	0.2244	0.2247	0.2251	0.2223	0.2217	0.2226	0.2237	0.2225	0.2216
D 3		0.221	0.2211	0.22	0.2198	0.2203	0.2198	0.2204	0.2195	0.2201	0.2212	0.2227	0.2227
D 4		0.2697	0.2692	0.269	0.2699	0.2693	0.2691	0.2682	0.2687	0.2685	0.2697	0.2685	0.2679
D 5		0.3318	0.3312	0.3308	0.3312	0.331	0.3301	0.33	0.3288	0.3291	0.33	0.33	0.329
D 6		0.1982	0.1977	0.1986	0.199	0.1988	0.1986	0.1974	0.1979	0.1981	0.1982	0.1973	0.1966
D 7		0.3071	0.3078	0.3083	0.309	0.3096	0.3116	0.3131	0.3128	0.3151	0.3178	0.3192	0.3208
D 8		0.3884	0.3899	0.3901	0.3902	0.3908	0.3922	0.3949	0.3938	0.3953	0.3977	0.3985	0.4001
D 9		0.4037	0.4075	0.4062	0.405	0.4048	0.4066	0.41	0.4068	0.4089	0.4113	0.4121	0.4144
D 10		0.4005	0.4029	0.4014	0.3993	0.4004	0.4013	0.4009	0.4001	0.3982	0.3994	0.3999	0.4012
D 11		0.1931	0.1929	0.1937	0.194	0.1937	0.1943	0.1928	0.1913	0.1916	0.1927	0.1919	0.1922
D 12		0.3684	0.3761	0.3824	0.3832	0.3881	0.3919	0.3995	0.3971	0.399	0.4085	0.3959	0.4023

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 115 - 19 h	Raw Data (595) 116 - 19 h 10 min	Raw Data (595) 117 - 19 h 19 min	Raw Data (595) 118 - 19 h 30 min	Raw Data (595) 119 - 19 h 40 min	Raw Data (595) 120 - 19 h 49 min	Raw Data (595) 121 - 20 h	Raw Data (595) 122 - 20 h 10 min	Raw Data (595) 123 - 20 h 19 min	Raw Data (595) 124 - 20 h 30 min	Raw Data (595) 125 - 20 h 40 min	Raw Data (595) 126 - 20 h 49 min
Time, hours		19.00	19.17	19.33	19.50	19.67	19.83	20.00	20.17	20.33	20.50	20.67	20.83
A	1	0.1624	0.1631	0.1626	0.1628	0.1637	0.1631	0.1622	0.1625	0.1626	0.1617	0.1619	0.1676
A	2	0.2169	0.217	0.2191	0.216	0.2171	0.2181	0.2179	0.2176	0.2176	0.2166	0.2173	0.2438
A	3	0.1928	0.1929	0.1927	0.1928	0.1929	0.1921	0.1926	0.1923	0.1927	0.1919	0.1921	0.1906
A	4	0.2395	0.2397	0.2394	0.2393	0.239	0.2394	0.2396	0.2385	0.2384	0.2383	0.2391	0.2409
A	5	0.2475	0.2477	0.2472	0.2473	0.2478	0.2472	0.2474	0.2471	0.2468	0.2457	0.247	0.2484
A	6	0.1979	0.1971	0.1982	0.1968	0.1979	0.1979	0.1972	0.1966	0.1977	0.1972	0.1953	0.2016
A	7	0.2999	0.2995	0.2997	0.299	0.2991	0.2987	0.2983	0.297	0.2961	0.2964	0.2957	0.2972
A	8	0.2988	0.2989	0.2992	0.2964	0.2968	0.2973	0.2959	0.2961	0.2952	0.294	0.293	0.2954
A	9	0.2688	0.2689	0.2696	0.2681	0.2687	0.2685	0.2687	0.2687	0.2682	0.269	0.2678	0.2708
A	10	0.574	0.5728	0.576	0.5706	0.5736	0.5723	0.5734	0.572	0.569	0.5707	0.5688	0.5731
A	11	0.1901	0.1902	0.1916	0.1902	0.1909	0.1911	0.1906	0.1907	0.191	0.1907	0.1902	0.1935
A	12	0.3528	0.3518	0.3546	0.3584	0.3557	0.3568	0.3645	0.3662	0.3731	0.3813	0.3799	0.3965
B	1	0.153	0.1531	0.1532	0.1532	0.1537	0.1524	0.1528	0.1535	0.154	0.1537	0.1532	0.1434
B	2	0.2001	0.2	0.1998	0.1993	0.1989	0.1995	0.2005	0.1991	0.1984	0.199	0.1991	0.1984
B	3	0.23	0.2285	0.2301	0.2288	0.2295	0.2293	0.2288	0.2297	0.2289	0.2294	0.229	0.2231
B	4	0.2236	0.2232	0.2229	0.2233	0.2222	0.2223	0.2232	0.2227	0.2219	0.222	0.2219	0.2246
B	5	0.2707	0.2696	0.2708	0.271	0.2711	0.2689	0.2688	0.2705	0.2714	0.2715	0.2704	0.2635
B	6	0.1506	0.1516	0.152	0.1516	0.1516	0.1507	0.1511	0.151	0.1516	0.1513	0.1514	0.1505
B	7	0.3105	0.3078	0.3105	0.3101	0.3097	0.3102	0.3084	0.3125	0.311	0.3119	0.3109	0.2943
B	8	0.3568	0.3566	0.3612	0.3698	0.3774	0.3849	0.3884	0.3954	0.3961	0.3983	0.4005	0.3886
B	9	0.3782	0.38	0.3788	0.3783	0.378	0.3783	0.3813	0.3806	0.3826	0.3836	0.3868	0.395
B	10	0.4083	0.4075	0.4083	0.4117	0.4139	0.4113	0.4112	0.4153	0.418	0.4178	0.4186	0.4103
B	11	0.18	0.1806	0.1811	0.1822	0.1824	0.1817	0.1824	0.1826	0.1825	0.1826	0.182	0.183
B	12	0.4049	0.4048	0.4069	0.4145	0.407	0.4085	0.4156	0.4167	0.4211	0.4292	0.4309	0.4447

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 115 - 19 h	Raw Data (595) 116 - 19 h 10 min	Raw Data (595) 117 - 19 h 19 min	Raw Data (595) 118 - 19 h 30 min	Raw Data (595) 119 - 19 h 40 min	Raw Data (595) 120 - 19 h 49 min	Raw Data (595) 121 - 20 h	Raw Data (595) 122 - 20 h 10 min	Raw Data (595) 123 - 20 h 19 min	Raw Data (595) 124 - 20 h 30 min	Raw Data (595) 125 - 20 h 40 min	Raw Data (595) 126 - 20 h 49 min
Time, hours		19.00	19.17	19.33	19.50	19.67	19.83	20.00	20.17	20.33	20.50	20.67	20.83
C 1		0.1898	0.1901	0.1906	0.1905	0.1902	0.1898	0.1899	0.19	0.1888	0.1889	0.1901	0.1881
C 2		0.2074	0.2076	0.2077	0.2073	0.207	0.2066	0.207	0.2071	0.2065	0.2066	0.2071	0.2042
C 3		0.1938	0.194	0.1952	0.1947	0.1945	0.1945	0.1944	0.1943	0.1946	0.1931	0.1945	0.194
C 4		0.2179	0.2185	0.2183	0.219	0.2182	0.2182	0.2182	0.2183	0.2178	0.2173	0.218	0.2194
C 5		0.2951	0.2947	0.2948	0.2961	0.2938	0.2942	0.2941	0.2951	0.2948	0.2943	0.2939	0.3019
C 6		0.2233	0.2235	0.2234	0.2243	0.2228	0.223	0.2232	0.2235	0.2238	0.2235	0.2237	0.2294
C 7		0.3454	0.3456	0.3486	0.3503	0.3518	0.3539	0.355	0.3548	0.357	0.3552	0.3581	0.3526
C 8		0.3912	0.3936	0.3936	0.3957	0.3991	0.3988	0.4	0.3985	0.3989	0.3981	0.4007	0.4088
C 9		0.4194	0.4202	0.4189	0.4216	0.421	0.4184	0.4197	0.418	0.4191	0.4168	0.4202	0.416
C 10		0.4054	0.4057	0.4057	0.4058	0.4074	0.4046	0.4057	0.4051	0.4059	0.4032	0.4047	0.3976
C 11		0.1962	0.1968	0.1963	0.1968	0.1967	0.1961	0.1961	0.1966	0.1965	0.1959	0.1957	0.1948
C 12		0.4184	0.4192	0.42	0.4153	0.4258	0.4168	0.4221	0.4213	0.4324	0.4198	0.4301	0.4185
D 1		0.1667	0.1667	0.167	0.1664	0.1662	0.1659	0.1661	0.1659	0.1659	0.1662	0.1655	0.165
D 2		0.222	0.2228	0.2233	0.2226	0.2209	0.2226	0.2233	0.2208	0.2226	0.2197	0.2206	0.2386
D 3		0.2224	0.2237	0.2252	0.2248	0.2248	0.2249	0.2248	0.2246	0.2236	0.2241	0.2244	0.2263
D 4		0.2682	0.2693	0.2691	0.2678	0.2688	0.2685	0.2688	0.2684	0.2684	0.2677	0.2684	0.2624
D 5		0.3292	0.3292	0.329	0.3292	0.3287	0.3283	0.3291	0.3286	0.329	0.3272	0.3282	0.3279
D 6		0.1973	0.1977	0.1977	0.1968	0.1965	0.1964	0.1969	0.1968	0.1976	0.1968	0.1966	0.2023
D 7		0.3216	0.3231	0.3254	0.3273	0.3285	0.3296	0.3329	0.333	0.3356	0.3356	0.338	0.3397
D 8		0.4011	0.4027	0.4022	0.4058	0.4082	0.4096	0.4112	0.412	0.415	0.414	0.4156	0.407
D 9		0.4121	0.4168	0.4139	0.4185	0.4207	0.418	0.4199	0.4173	0.4196	0.4172	0.4204	0.3956
D 10		0.3993	0.3999	0.3993	0.4006	0.4005	0.3988	0.4	0.4001	0.3993	0.3992	0.4027	0.3919
D 11		0.1913	0.1923	0.192	0.1923	0.1916	0.1915	0.192	0.1915	0.1919	0.1913	0.1925	0.1927
D 12		0.4052	0.4096	0.4079	0.4001	0.4137	0.4012	0.4101	0.4061	0.4166	0.4115	0.4133	0.4213

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 127 - 21 h	Raw Data (595) 128 - 21 h 10 min	Raw Data (595) 129 - 21 h 19 min	Raw Data (595) 130 - 21 h 30 min	Raw Data (595) 131 - 21 h 40 min	Raw Data (595) 132 - 21 h 49 min	Raw Data (595) 133 - 22 h	Raw Data (595) 134 - 22 h 10 min	Raw Data (595) 135 - 22 h 19 min	Raw Data (595) 136 - 22 h 30 min	Raw Data (595) 137 - 22 h 40 min	Raw Data (595) 138 - 22 h 49 min
Time, hours		21.00	21.17	21.33	21.50	21.67	21.83	22.00	22.17	22.33	22.50	22.67	22.83
A	1	0.1623	0.1611	0.167	0.1615	0.161	0.1612	0.1615	0.1609	0.1608	0.1614	0.1606	0.1625
A	2	0.2178	0.2164	0.2428	0.2156	0.2157	0.2175	0.2184	0.215	0.219	0.2169	0.2188	0.2173
A	3	0.1918	0.1917	0.1903	0.1925	0.1922	0.1925	0.1916	0.1919	0.1916	0.192	0.1909	0.1921
A	4	0.2386	0.2382	0.2411	0.2389	0.2386	0.2383	0.2382	0.2384	0.2384	0.2383	0.2367	0.238
A	5	0.2469	0.2469	0.2476	0.2479	0.2473	0.2474	0.2469	0.2469	0.2467	0.2468	0.246	0.2473
A	6	0.1975	0.1964	0.2013	0.1951	0.1949	0.1962	0.1963	0.1947	0.1963	0.1949	0.1962	0.1959
A	7	0.2953	0.294	0.2966	0.2946	0.293	0.2932	0.2923	0.2924	0.2929	0.2921	0.2918	0.292
A	8	0.2934	0.2935	0.2936	0.291	0.2902	0.2915	0.2916	0.2885	0.2908	0.2872	0.2895	0.2891
A	9	0.2688	0.2678	0.2703	0.2681	0.2675	0.2672	0.2666	0.2663	0.266	0.2653	0.266	0.265
A	10	0.571	0.5704	0.5697	0.5683	0.5647	0.5675	0.5663	0.5587	0.5648	0.5628	0.5651	0.5623
A	11	0.1912	0.191	0.1928	0.191	0.1905	0.1915	0.191	0.1892	0.1915	0.1897	0.1917	0.1915
A	12	0.3817	0.3824	0.3969	0.3953	0.3931	0.4015	0.4046	0.4163	0.4111	0.4095	0.4136	0.4141
B	1	0.1538	0.153	0.1437	0.1528	0.153	0.1524	0.153	0.1529	0.1521	0.1526	0.1524	0.1526
B	2	0.1988	0.1989	0.1976	0.1995	0.1983	0.1988	0.1982	0.1983	0.199	0.1987	0.1986	0.1986
B	3	0.23	0.2282	0.2234	0.2282	0.229	0.2271	0.2282	0.228	0.2273	0.2286	0.2281	0.2272
B	4	0.2231	0.2213	0.2244	0.2218	0.2218	0.2218	0.2215	0.2207	0.2214	0.2218	0.2209	0.2218
B	5	0.2711	0.2692	0.2642	0.2682	0.2701	0.2678	0.2693	0.2689	0.2673	0.2702	0.2689	0.2669
B	6	0.151	0.1512	0.1494	0.1507	0.1515	0.1506	0.1511	0.1508	0.15	0.1514	0.1512	0.1504
B	7	0.3115	0.3103	0.2955	0.3119	0.3121	0.311	0.3113	0.3092	0.3096	0.3112	0.3108	0.3084
B	8	0.4064	0.4054	0.3978	0.4083	0.4107	0.411	0.4109	0.4119	0.4142	0.4158	0.4157	0.4164
B	9	0.3881	0.3882	0.3978	0.394	0.3944	0.3943	0.3948	0.3972	0.3972	0.3998	0.4005	0.3997
B	10	0.4204	0.4212	0.4148	0.4243	0.4287	0.4268	0.4287	0.43	0.4262	0.4292	0.4309	0.4275
B	11	0.1813	0.1808	0.1824	0.182	0.1822	0.1814	0.1816	0.1819	0.1806	0.1815	0.182	0.1809
B	12	0.4351	0.4335	0.4449	0.4398	0.4408	0.4513	0.4558	0.4637	0.4654	0.462	0.4663	0.4663



## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 127 - 21 h	Raw Data (595) 128 - 21 h 10 min	Raw Data (595) 129 - 21 h 19 min	Raw Data (595) 130 - 21 h 30 min	Raw Data (595) 131 - 21 h 40 min	Raw Data (595) 132 - 21 h 49 min	Raw Data (595) 133 - 22 h	Raw Data (595) 134 - 22 h 10 min	Raw Data (595) 135 - 22 h 19 min	Raw Data (595) 136 - 22 h 30 min	Raw Data (595) 137 - 22 h 40 min	Raw Data (595) 138 - 22 h 49 min
Time, hours		21.00	21.17	21.33	21.50	21.67	21.83	22.00	22.17	22.33	22.50	22.67	22.83
C	1	0.1889	0.1891	0.1888	0.1892	0.189	0.1889	0.1887	0.1889	0.1889	0.1889	0.1885	0.189
C	2	0.2066	0.2059	0.2045	0.2064	0.2068	0.2063	0.2058	0.2068	0.2057	0.2057	0.2061	0.2058
C	3	0.1933	0.1935	0.1945	0.1935	0.1929	0.1932	0.1932	0.1935	0.1933	0.1928	0.1929	0.1933
C	4	0.2168	0.2172	0.2197	0.2176	0.2179	0.2177	0.2172	0.2174	0.217	0.2174	0.217	0.2173
C	5	0.2937	0.2931	0.3006	0.2938	0.2938	0.2931	0.2927	0.2934	0.2927	0.2926	0.2926	0.2924
C	6	0.2237	0.2238	0.2291	0.2238	0.2239	0.2236	0.2241	0.2245	0.2243	0.225	0.225	0.2255
C	7	0.3574	0.3585	0.3541	0.36	0.3597	0.3606	0.3609	0.3638	0.3628	0.3638	0.363	0.3638
C	8	0.3982	0.4	0.4104	0.4004	0.4009	0.4005	0.4011	0.4024	0.4002	0.4018	0.402	0.4015
C	9	0.4179	0.4195	0.4179	0.4206	0.421	0.4207	0.4199	0.4225	0.4209	0.4221	0.4214	0.4213
C	10	0.4036	0.4032	0.396	0.4034	0.4018	0.4023	0.4006	0.4023	0.4017	0.4016	0.4007	0.4014
C	11	0.1949	0.1957	0.1951	0.1954	0.195	0.1953	0.1945	0.195	0.1953	0.1955	0.1949	0.1951
C	12	0.4214	0.4286	0.4158	0.4296	0.4311	0.4346	0.4269	0.4389	0.4406	0.4366	0.4419	0.4461
D	1	0.1655	0.1655	0.1653	0.1657	0.1654	0.1655	0.1649	0.1649	0.1654	0.1651	0.1648	0.1653
D	2	0.2209	0.2219	0.2379	0.2201	0.2208	0.2201	0.2199	0.2198	0.221	0.2203	0.221	0.2217
D	3	0.2242	0.2233	0.2265	0.2241	0.224	0.2232	0.2237	0.2238	0.2238	0.2241	0.224	0.2237
D	4	0.2667	0.2683	0.2619	0.2676	0.2681	0.2685	0.2675	0.2679	0.2684	0.2683	0.2684	0.2688
D	5	0.3281	0.3284	0.3278	0.3284	0.3288	0.3277	0.3275	0.3273	0.3284	0.3274	0.3282	0.328
D	6	0.1965	0.1966	0.2027	0.1957	0.1973	0.1962	0.1955	0.196	0.1962	0.1971	0.1967	0.1967
D	7	0.3398	0.3407	0.3428	0.3446	0.347	0.3469	0.3487	0.3503	0.3517	0.3516	0.3525	0.3532
D	8	0.4167	0.4163	0.4097	0.4222	0.4234	0.4239	0.4237	0.4261	0.4257	0.4251	0.4266	0.427
D	9	0.4187	0.4169	0.3967	0.4237	0.4257	0.4222	0.4286	0.43	0.4265	0.4256	0.4285	0.4242
D	10	0.4025	0.4031	0.3935	0.4048	0.4033	0.4025	0.4028	0.4049	0.4036	0.406	0.4062	0.4064
D	11	0.1914	0.1913	0.1926	0.1915	0.1914	0.1909	0.1907	0.1899	0.1907	0.1907	0.1901	0.1907
D	12	0.4102	0.4072	0.417	0.4038	0.4072	0.4147	0.411	0.4172	0.4247	0.425	0.4315	0.4348

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 139 - 23 h	Raw Data (595) 140 - 23 h 10 min	Raw Data (595) 141 - 23 h 19 min	Raw Data (595) 142 - 23 h 30 min	Raw Data (595) 143 - 23 h 40 min	Raw Data (595) 144 - 23 h 49 min	Raw Data (595) 145 - 24 h
Time, hours		23.00	23.17	23.33	23.50	23.67	23.83	24.00
A	1	0.1606	0.1611	0.1602	0.1611	0.1609	0.1599	0.1603
A	2	0.2165	0.2159	0.2159	0.2165	0.2164	0.2147	0.2154
A	3	0.1913	0.1919	0.1912	0.1915	0.1918	0.1912	0.1918
A	4	0.2378	0.2381	0.2378	0.2369	0.2378	0.2373	0.2371
A	5	0.2465	0.2474	0.2465	0.2464	0.2469	0.2466	0.2466
A	6	0.1955	0.1958	0.1952	0.1956	0.1961	0.1938	0.1955
A	7	0.2908	0.291	0.2902	0.2905	0.2904	0.289	0.2889
A	8	0.2861	0.2865	0.2846	0.2859	0.2859	0.2837	0.2848
A	9	0.2651	0.2651	0.2645	0.2653	0.2652	0.2641	0.2639
A	10	0.5559	0.5575	0.5542	0.5563	0.553	0.551	0.5522
A	11	0.1903	0.1913	0.1906	0.1915	0.191	0.19	0.1899
A	12	0.4153	0.4209	0.4261	0.424	0.4256	0.4246	0.4304
B	1	0.152	0.1527	0.1527	0.1529	0.1533	0.1521	0.1514
B	2	0.1982	0.199	0.1976	0.1983	0.1981	0.1977	0.1978
B	3	0.2267	0.227	0.2278	0.2281	0.2282	0.227	0.2262
B	4	0.2213	0.2208	0.2214	0.2218	0.2214	0.2205	0.2208
B	5	0.2674	0.2682	0.2693	0.2702	0.2679	0.2664	0.2671
B	6	0.1496	0.1506	0.1505	0.1512	0.1505	0.1494	0.1498
B	7	0.3093	0.3117	0.3139	0.3137	0.3128	0.3114	0.3112
B	8	0.4163	0.4184	0.4197	0.4207	0.4218	0.4216	0.4234
B	9	0.4013	0.4014	0.4025	0.4029	0.4037	0.4027	0.4049
B	10	0.4298	0.4312	0.4319	0.4342	0.4341	0.429	0.4322
B	11	0.1812	0.1813	0.1814	0.1816	0.1815	0.1798	0.1811
B	12	0.4675	0.4779	0.4817	0.478	0.4821	0.4791	0.4837

## Appendix 7.2

Test Name: Pseudomonas

Absorbance

Well Row	Well Col	Raw Data (595) 139 - 23 h	Raw Data (595) 140 - 23 h 10 min	Raw Data (595) 141 - 23 h 19 min	Raw Data (595) 142 - 23 h 30 min	Raw Data (595) 143 - 23 h 40 min	Raw Data (595) 144 - 23 h 49 min	Raw Data (595) 145 - 24 h
Time, hours		23.00	23.17	23.33	23.50	23.67	23.83	24.00
C	1	0.1886	0.1886	0.1884	0.1876	0.1884	0.1881	0.188
C	2	0.2062	0.2064	0.206	0.2058	0.2056	0.2047	0.2047
C	3	0.1934	0.1928	0.1926	0.1933	0.1931	0.1923	0.1928
C	4	0.2169	0.2169	0.2169	0.2164	0.2167	0.2159	0.2161
C	5	0.2925	0.2929	0.2923	0.292	0.2927	0.2916	0.2911
C	6	0.226	0.2264	0.2273	0.2264	0.2265	0.226	0.2265
C	7	0.3636	0.3643	0.3648	0.3656	0.3659	0.3654	0.3663
C	8	0.4025	0.4018	0.4023	0.4023	0.4023	0.3998	0.4011
C	9	0.4218	0.4223	0.4217	0.4219	0.4228	0.4202	0.4225
C	10	0.4008	0.4001	0.4004	0.4005	0.4014	0.3993	0.3993
C	11	0.1947	0.1944	0.1945	0.1948	0.1947	0.1936	0.1939
C	12	0.4401	0.4437	0.4367	0.4452	0.444	0.4442	0.4515
D	1	0.1648	0.1653	0.1642	0.1648	0.1646	0.1639	0.1641
D	2	0.2187	0.2208	0.2188	0.2194	0.2216	0.2199	0.2187
D	3	0.2227	0.2242	0.2229	0.2237	0.2225	0.223	0.2226
D	4	0.268	0.2681	0.2673	0.2685	0.2688	0.2674	0.2674
D	5	0.3279	0.3289	0.3273	0.3273	0.3272	0.3275	0.3269
D	6	0.1972	0.1974	0.1966	0.196	0.197	0.1964	0.1971
D	7	0.3544	0.3559	0.3556	0.3564	0.3569	0.3576	0.3574
D	8	0.4297	0.4299	0.4296	0.4296	0.4295	0.4309	0.4312
D	9	0.4254	0.4284	0.4273	0.4307	0.4281	0.4296	0.431
D	10	0.4073	0.4082	0.408	0.4092	0.4099	0.4116	0.41
D	11	0.1899	0.1901	0.1901	0.1898	0.1911	0.1904	0.1902
D	12	0.435	0.441	0.4299	0.4433	0.4422	0.4398	0.4458