TOXICITY STUDY OF $CaCe_2S_4$

by

WAFA TONNY

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Chemistry and Biochemistry

THE UNIVERSITY OF TEXAS AT ARLINGTON

August, 2017

Supervising Committee:

Robin Macaluso, Supervising Professor

Peter Kroll

Kayunta Johnson-Winters

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my research advisor, Dr. Robin Macaluso for her insight, leadership, and inspiration to be a better researcher and student. Thank you for your continued support throughout this project as it could not have been completed without it.

Thank you to Dr. Peter Kroll for challenging me and pushing me to think critically which has made me grow as a student. I thank you for motivating me to be a better solid state chemist and researcher. Thank you for taking the time to be on my thesis committee.

I have much appreciation to Dr. Kayunta Johnson-Winters for her support and kindness throughout my graduate years. She has taught me a great amount about biochemistry, which provides the fundamentals of this project. Thank you for being on my thesis committee.

I express gratitude to Dr. Kytai Truong Nguyen for guiding me through this project. Her knowledge and lab support was invaluable to my project.

Thank you Roshni Iyer for your time, effort and guidance during this project. Her expertise on this field were the biggest support to complete the project. I am grateful and thankful.

I thank my colleagues and lab mates for their great help and guidance during this project. Thank you my friends for their endless support and encouragement.

Last I would like to thank my parents Golam Mohammad and Sayeeda Akhter Banu and my family for their support. You have helped me get through this experience and complete this program. You believing in me has motivated my work here at the University of Texas at Arlington and I dedicate my thesis to you.

July 25, 2017

ABSTRACT

TOXICITY STUDY OF $CaCe_2S_4$

Wafa Tonny, Master of Science

The University of Texas at Arlington, 2017

Supervising Professor: Robin Macaluso

In recent years, rare earth sulfides have received attention for their interesting thermoelectric and optical properties.[1] They exhibit a fairly wide range of colors, which makes them promising candidates as pigments.[2] The demand for red colored pigments in industrial application is high but the current ones in use are not environmentally friendly. This has garnered attention towards the rare-earth sulfides. Among the rare-earth sulfide compounds, Ce_2S_3 is a high-performance pigment of red color. It is particularly interesting as doping with alkaline earth metal stabilizes the structure of Ce_2S_3 at low temperature and plays a role in tuning its color from red to orange. This thesis presents the cytotoxicity study of an alkaline earth doped rare earth sulfide of red color, $CaCe_2S_4$. Cytotoxicity of $CaCe_2S_4$ and CdS was evaluated on human dermal fibroblasts cells (HDFα) with Lactase dehydrogenase (LDH) assay. The study showed higher toxicity of $CaCe_2S_4$ compound at 1000 µg/mL-50 µg/mL. At concentrations of 1 ng/mL-200 ng/mL $CaCe_2S_4$ showed considerably low toxicity compared to commercially successful red pigment CdS, which makes it a reliable environment friendly pigment.

LIST OF ABBREVIATIONS

DMEM ……..…… Dulbecco's Modified Eagle's Medium

EDTA……..... Ethylene-di-amine-tetra-acetic acid) solution

FBS……..... Fetal Bovine Serum

HDFα……. Human Dermal Fibroblasts Adult

ICP-OES…… Inductively Coupled Plasma-Optical Emission Spectrometry

LDH……... Lactate Dehydrogenase

MTS..... 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)
-2-(4-sulfophenyl)-2H-tetrazolium

MTT……3-(4,5-Dimethylthiazol-2-yl)-2,5
-diphenyltetrazolium bromide

PBS……. Phosphate-buffered Saline

PDF……..….... Powder Diffraction File

SEC-ICP-MS……... Size Exclusion Chromatography-Inductively
Coupled Plasma-Mass Spectrometry

WST..... 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)
-2H-tetrazolium, monosodium salt

XRD…… X-ray Diffraction

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

CHAPTER I

INTRODUCTION

Inorganic pigments have been in use for some time in a multitude of industries such as ceramics, paints, and plastics. One major drawback to commercially or artistically important pigments is the presence of toxic metal ions, such as cadmium selenide in Cadmium Red, Cd(S, Se), and lead chromate in Chrome Orange, Pb_2CrO_5.[3]

Rare earth-based inorganic compounds, particularly rare-earth sesquisulfides, are a possible source of alternative pigments that have been explored, extensively because of their color and stability. Rare earth metals are capable of giving efficient absorption through charge transfer bands and some rare earth-doped inorganic compounds are actually used in the field of colored pigments such as $KCeS_2$[4] or $Zr_{1-x}Pr_xSiO_4$, both yellow pigments for the ceramic industry. A lot of attention is paid to Ce_2S_3 as a red pigment, but most of the rare earth sulfides can be candidates for obtaining a wide gamut of colors, e.g. La_2S_3 being yellow or Pr_2S_3 being green.[5]

It is well known that light rare earth sesquisulfides exist in several polymorphs which are commonly referred to as are α, β and γ. The α-phase has an orthorhombic structure and is a low temperature phase of RE_2S_3 (RE = rare earth element). The β phase has a tetragonal structure, with a limiting composition of $RE_{10}S_{14}O_{1-x}S_x$ ($0 \leq x \leq 1$). The γ phase has a cubic defect Th_3P_4 type structure and is a high temperature phase of RE_2S_3. The lesser known δ phase has a monoclinic structure; the ε-phase has a rhombohedral structure[6] and exists for heavier rare earth lanthanides.

The γ-phase of Ce_2S_3 is a non-toxic inorganic high performance pigment of dark red color and is used on an industrial scale in the coloration of plastics, paints, and coatings. γ-Ce_2S_3 is particularly interesting since alkali doping makes it possible to tune its color from maroon to

orange[7] and making it a very promising candidate to replace cadmium and organic red pigments because of its non-toxicity and bright color.[8] A slight distortion in the structure of γ-Ce_2S_3 from the confirmed γ-phase RE_2S_3 Th_3P_4 type structure makes it a potential compound for alkali- and alkaline earth doping.[9] Due to long exposure to moisture and high temperature treatment during the injection molding of pigments, rare-earth sulfides produce H_2S, which is a considerable challenge for the practical application of rare earth sulfides.[3] Doping of alkali or alkaline earth can stabilize the γ-phase Ce_2S_3 at low temperature[2] and reduce the release of H_2S.

Most inorganic pigments containing heavy metals or transition metals that can adversely affect the environment and human health if critical levels are exceeded. Cadmium-based pigments such as CdS or Cd(Se,S) in particular are of concern; although the pigments are not toxic due to their very low solubility in water, cadmium itself is toxic and can enter the environment in a bioavailable form through waste-disposal sites and incineration plants.[10] Rare earth sulfides may be a safer choice[4] but any relevant research or data claiming this possibility are near to non-existent. *In vitro* or cell-based assays are often used for screening collections of compounds to determine if they have effects on cell proliferation or show direct cytotoxic effects that eventually lead to cell death. Regardless of the type of cell-based assay being used, it is important to know how many viable cells are remaining at the end of the experiment. There are a variety of assay methods that can be used to estimate the number of viable healthy cells.[11] Cadmium toxicity is associated with several clinical complications depending on the dose, the route, and mainly duration of exposure[12] and recent publications explained well the extent of the toxicity.

Here we report, the very first time the cytotoxicity of $CaCe_2S_4$ and CdS. The cytotoxicity reports of CdS served as a guideline for the experimentation methods. Lactate dehydrogenase or LDH assay, a well-established cell-based assay was used for this study and at very low

concentrations a comparison with CdS are presented. The biocompatibility of pigments can be useful if the *in vitro* study can be done on healthy human skin cells. The toxicity of CdS nanocrystals are known in case of renal cells and the application of CdS as pigments are limited due to cadmium toxicity but lacking in the study of toxicity in case of healthy skin cells was also a motivation for this thesis.[13]

CHAPTER II

LITERATURE REVIEW

Rare earth elements can be used across different traditional industries such as metallurgy, machinery, chemicals, textile and agriculture. New rare earth products have emerged, and lead to the formation of a group of new industries.[14] One of the most promising examples of the development of rare earth compounds as specialties deals with the development of cerium sulfide as an alternative pigment to cadmium reds for the coloration of plastics and paints. As a matter of fact, the limitations on the use of heavy metal-containing pigments like cadmium sulfoselenides or lead molybdates induces some need for new yellow, orange, and red inorganic pigments, where very few alternatives exist. These compounds have excellent performance characteristics, but their toxicity is of environmental concern. Thus, driven by changes in legislation and government regulations, there is a strong incentive to develop new classes of inorganic pigments that are non-toxic and environmentally more compatible while preserving the optical, thermal, and chemical characteristics of present high-performance pigments.[7] The use of high-performance organics is one way to get the strong red color, but these pigments present some limitations, such as thermal and UV instability. When looking for color in rare earth compounds, RE_2S_3 (RE= Rare-Earth elements such as La, Ce, Pr, Nd, Sm,Eu, Gd, Dy etc.) sulfides appeared to be good candidates[5].

Rare earth sulfides are stable up to 350 °C in air and it shows a potential for pigments in paint and plastic industries. So far, cerium sulfide has gathered more attention but most of the rare-earth sulfides give a nice wide variation of colors presented in Table 1.

Table 1: Color of some rare earth sesquisulfides in their γ form[5]

La_2S_3	Yellow
Pr_2S_3	Green
Nd_2S_3	Light green
Gd_2S_3	Purple
Tb_2S_3	Light yellow
Dy_2S_3	Orange

Cerium sulfides and other similar sulfides were originally investigated in the 1940s in search for the development of new refractory materials as crucibles for the casting of plutonium and uranium. Although there are several sulfides in the phase diagram for the cerium–sulfur system, cerium(III) sulfide (Ce_2S_3) especially is considered to be useful as refractory materials, because they have high melting points and are thermodynamically stable at elevated temperatures.[8]

RE_2S_3 exists in three polymorphic forms that are α, β and γ phase. α which is stable at low temperature, is irreversibly transformed to β-phase of various compositions in the solid-solution range between R_2S_3 and R_3S_4 at high temperature. The β-phase is an oxysulfide having a limiting composition of $R_{10}S_{14}O$. Moreover, the β-phase is transformed to γ-phase, which has a Th_3P_4 structure, at higher temperature.[15] More detail information is given in Table 2:

Table 2: The polymorphs of the rare-earth sulfides[15] with crystal structures and transition temperatures

Phases	**Crystal Structures**	**Transition temperature**
α- RE_2S_3	Orthorhombic Gd_2S_3-type *Pnam*	Stable up to 900ºC
β- RE_2S_3 $RE_{10}S_{15-x}O_x$ ($0 \leq x \leq 1$)	Tetragonal $Pr_{10}S_{14}O$-type[47] *I41/acd*	~900ºC
γ- RE_2S_3 $RE_{3-x}R_xS_4$ ($0 \leq x \leq 1/3$) (R= RE vacancies)	Cubic Th_3P_4-type *I-43d*	~1300ºC

γ-phase which is semiconductor, is considered to be useful as a thermoelectric conversion materials, also because of the vivid color can be frequently used as pigment for plastics and paints.[15] The doped -and undoped γ-phase Ce_2S_3, and β- phase Ce_2S_3 have been used as a heavy metal free nontoxic red pigment in plastic industry. The color of undoped and alkali doped γ-Ce_2S_3 is due to electronic transitions from the Ce 4f level into the Ce 5d conduction band. The addition of alkali can stabilize the γ-phase Ce_2S_3 at low temperature and modify the color of the γ-phase

Ce_2S_3. Some heavy rare earth ions such as Dy^{3+}, Ho^{3+}, and Er^{3+} doped cerium sulfides have also been studied. It was found that the addition of these ions presents a stabilizing effect on the formation of the γ-phase Ce_2S_3.[2]

As an example, calcium doped Ce_2S_3 crystallized as $Ca_{0.89}Ce_{2.07}S_4$ with cubic symmetry in the *I-43d* space group and was isostructural with Th_3P_4 structure. The metal atom was surrounded by eight sulfur atoms forming a special type of dodecahedron named snub disphenoid. The snub disphenoid is a convex polyhedron with twelve equilateral triangles as its faces. It is not a regular dodecahedron because some vertices have four faces and others have five. Each dodecahedron shared with its neighbors either one triangular face or one edge. Between two neighboring dodecahedra sharing one edge[18], it was interesting to notice the much distorted empty tetrahedral cavities, S_4, the center of which lies at the Wyckoff position 12c.[19]

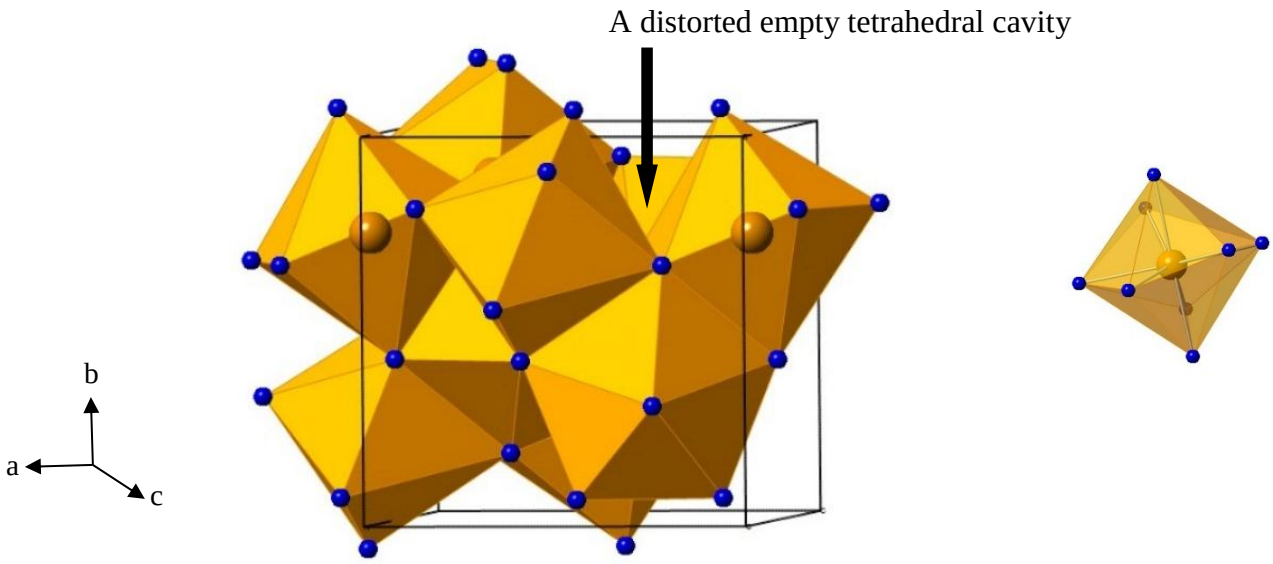


Figure 1: The unit cell of $CaCe_2S_4$ viewed down the z axis(left). Ca/Ce= yellow and S=blue sphere. The Ca/Ce atom is surrounded by eight S atoms forming a dodecahedron(right)

This was, with the center of the dodecahedra not fully occupied by a rare-earth atom, the second remarkable site of the structure. With the calcium-doped compound, it was confirmed that an insertion alkaline earth metal ions on empty dodecahedral sites occurs together with a substitution of cerium ions by alkali alkaline earth metal ions.[18]

Table 3: Important interatomic distances in the structure of $CaCe_2S_4$[18]

Interatomic distances in Ce_2S_3 and $CaCe_2S_4$		
Distances	Ce_2S_3	$CaCe_2S_4$
Ca/Ce-S	4 × 2.886(2) Å	4 × 2.9848(4) Å
	4 × 3.078(2) Å	4 × 2.9786(4) Å

Optical studies suggest that the dark red color of γ-Ce_2S_3 arises from dipole-allowed excitations of electrons from 4f-states into the empty 5d-states ($4f^15d^0 \rightarrow 4f^05d^1$) of the same cerium cation. The three modifications of Ce_2S_3 have an insulating nature, as is indicated from their color.[17] In contrast to compound semiconductors such as CdS and CdSe, where the color is controlled by interband transitions, the transitions from the anionic valence band into the cationic conduction band in Ce_2S_3 occur in the ultraviolet and the color of cerium-based pigments is explained in terms of atomic like *f→d* transitions. In other words, the color is largely an intrinsic property of cerium.[7]

Inorganic pigments have been utilized by mankind since ancient times, and are still widely used to color materials exposed to elevated temperatures during processing or application. Indeed, in the case of glasses, glazes and ceramics, there is no alternative to inorganic pigments for coloring. However, most inorganic pigments contain heavy metals that can adversely affect the human health if critical levels are reached. Although cadmium-based pigments are not harmful

due to their very low solubility in water or dilute mineral acids, cadmium metal itself is toxic and can enter the ecosystem through waste-disposal sites. Based on the precautionary principle the use of cadmium pigments has been restricted and heavily regulated. Recent studies have concluded that the risk to humans or the environment might be not as impactful as originally feared, still a strong demand for inherently safer substitutes remains.[10]

Cadmium exposure has been established to induce carcinogenic effect in laboratory animals, but data on human studies are lacking, so it is difficult to conclude the toxicity effect on human cells. Data on Cd for development of quantum-dots showed great promise for the diagnosis of cancer and targeted drug delivery. However, after intravenous administration of quantum-dots, Cd content has been observed in the liver and the kidneys even after 30 days, suggesting that these particles can pass through the kidneys, so studies have been made on renal cell line with MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and WST [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt]assays.[12] No information on cytotoxicity of rare earth sulfide compounds is available, which makes it a promising field to study.

CHAPTER III

EXPERIMENTAL METHODS

Synthesis of $CaCe_2S_4$

CaS (≥ 99.9% trace metal basis, Sigma-Aldrich) and Ce_2S_3 (99.9% metals basis, Alfa Aesar) were combined in 1:1 molar ratio and were ground thoroughly with a mortar and pestle inside a nitrogen-filled glove box. Then the powder mixture was placed into a Ta foil (99.9%, Sigma-Aldrich) pouch and sealed under vacuum in a quartz tube. The ampoule was then heated to 1100ºC for 100 h at 7ºC/h and cooled at 20ºC/h to the room temperature. The resulting red-orange colored powder was not air sensitive.

Characterization Techniques

Sample purities were monitored by X-ray diffraction (XRD) using a PANalytical Empyrean series 2 Diffractometer (45 kV, 40 mA, Cu-Kα radiation, λ= 1.54056 Å) with reflection-transmission-spinner stage (the incident beam module was Bragg-Brentano). To confirm phase purity of the synthesized powder the diffraction pattern was recorded in a 2θ range of 20–90◦ (step size of 0.008°). The diffraction pattern is shown on next page in Figure 2.

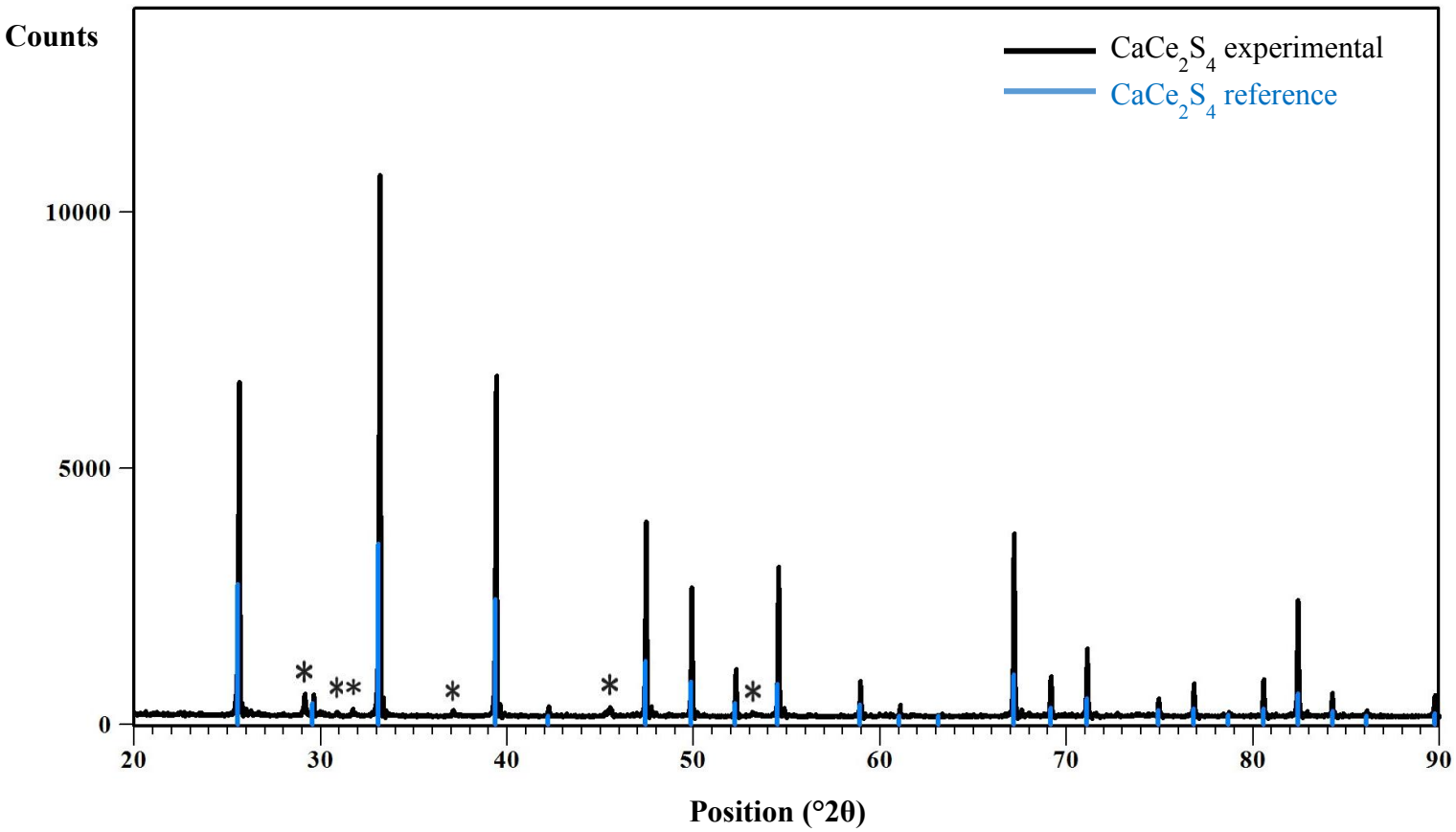


Figure 2: The powder diffraction pattern of $CaCe_2S_4$ matched with a reference pattern. The experimental pattern shown in black, the reference pattern shown in blue colored sticks and the additional peaks in the experimental diffraction pattern marked by asterisks (*).

The reference pattern has been taken from Powder Diffraction File (PDF 4+ 2016) database, PDF card no. 01-077-7217. Experimental powder pattern is mostly in good agreement to the reference pattern. There are some additional peaks pattern marked by asterisks (*) indicating presence of small amount of impurities but the main phase of the synthesized powder is $CaCe_2S_4$. The additional peaks can be attributed to the formation of oxysulfides e.g. Ce_2O_2S[8] or $Ce_{10}S_{15-x}O_x$ with $(0\leq x\leq 1)$[17].

Cytotoxicity Study

The focus of the study was to evaluate the cytotoxicity of $CaCe_2S_4$ because this compound is being considered as a potential pigment to be used in paint and ceramic industry. The toxicity studies were also performed using CdS, a well-known cadmium-based pigment which is now under strict regulation due to cadmium toxicity.[10]

The toxicity tests were done on Human Dermal Fibroblasts (HDFα) primary cells provided by American Type Culture Collection, Virginia.

Procedures for cell culture:

Thawing and culturing of HDFα cells:

HDFα cells were grown in T75 (growth area 75 cm^2, working volume 20 mL) flask under normal cell culture conditions. Cells were thawed and grown in a humidified cell culture incubator at 37°C with 5% CO_2 using Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (containing 10,000 units/mL penicillin and 10,000 µg/mL streptomycin), followed by subculture upon 85% confluency. In cell culture confluency is commonly used to describe the density of adherent cells and it is used as a measure of their proliferation. It is usually combined with an estimated (or counted) percentage, so 10% confluency means that 10% of the surface the dish or flask used is covered with cells, 100% means that it is entirely covered.

Passaging of cells:

Passaging, also referred to as subculturing, is the removal of the medium and transfer of cells from a previous culture into fresh growth medium, a procedure that enables the further

propagation of the cell line or cell strain. A routine procedure for passaging would be, cells are washed with 1X Phosphate-buffered Saline, PBS (pH 7.4), followed by detachment using trypsin/ EDTA (Ethylene-di-amine-tetra-acetic acid) solution. The trypsin/EDTA solution was neutralized with DMEM (Dulbecco's Modified Eagle's Medium) supplemented with FBS (Fetal Bovine Serum), cell suspension collected and centrifuged at 1000 rpm for 5min at 250g to pellet the cells from cell membrane. Cell density was determined using a hemocytometer.

In vitro cytotoxicity assay:

To study the dose dependence on cytotoxicity of $CaCe_2S_4$, cells were first seeded onto 96-well plate (10,000 cells/well) overnight in DMEM. The following day, media in the wells were replaced with various concentrations of $CaCe_2S_4$ ranging from 1ng/mL to 1 μg/mL. Also at low concentrations of 200 ng/mL, 50 ng/mL and 1 ng/mL $CaCe_2S_4$ was compared with CdS, Cells exposed to triton-1X (cell lysing agent) were used as positive control. Cells were then incubated in these solutions for 24 hours. Cytotoxicity was analyzed by Lactate dehydrogenase (LDH, Takara Pharmaceuticals Ltd.) cytotoxicity assay following the manufacturers' protocol.

LDH assay:

The LDH Cytotoxicity Detection Kit uses lactate dehydrogenase, LDH a stable cytoplasmic enzyme that is present in large amounts in most cells. LDH is released into the cell culture supernatant during cytoplasmic membrane damage and can be easily measured using standard reagents. The Detection Kit provides a colorimetric measure of cell cytotoxicity based on the measurement of LDH activity in the cell culture supernatant. The cell culture supernatant is collected and incubated with the kit's reaction mixture.

Components of LDH assay were Catalyst (diaphorase/NAD^+, lyophilized) and Dye solution containing iodotetrazolium chloride (INT) and sodium lactate. For long-term storage, assay kit is usually stored at -20°C, protected from light. Lyophilized Catalyst can be stored for one year at -20°C, or for several weeks at 4°C, dissolved Catalyst can be stored for several weeks at 4°C and thawed Dye solution can be stored for several weeks at 4°C.

LDH Assay protocol:

Cells (5-100 x 10^3/well) were cultured in a 96-well microtiter plate in a final volume of 200 µL/well in the absence or presence of various factors to be tested and were incubated for 20-48 hours. After incubation cells are removed from the culture medium by centrifugation at 250g prior to assay. Prepared culture supernatant can be stored at 4ºC for a few days without loss of LDH activity. The reaction mixture must be prepared immediately before use because the reaction mixture can't be stored. Prior to use 250 µL of Catalyst were mixed with 11.25 mL of Dye solution and that served as the reaction mixture.

Fifty µL of cell-free culture supernatant were transferred to clear, flat bottom microtiter plate and 50 µL of reaction mixture were added and incubated for 10-30 minutes at room temperature protected from light. Then absorbance was measured at about 490 nm. The reference wave length was >600 nm.

Principle of LDH Assay:

When the cell membranes are compromised or damaged LDH is released into the surrounding extracellular space. Since this only happens when cell membrane integrity is compromised, the presence of this enzyme in the culture medium can be used as a cell death marker. The relative amounts of live and dead cells within the medium can then be quantitated by

measuring the amount of released LDH using the colorimetric assay. When using an LDH colorimetric assay, the amount of LDH released in the surrounding environment is measured with an enzymatic reaction which converts iodonitrotetrazolium or INT (a tetrazolium salt) into a red color formazan. When LDH is present in the cell culture, it reduces NAD^+ to NADH and H^+ through the oxidation of lactate to pyruvate. Afterward, the catalyst (diaphorase) then transfers H/H^+ from NADH + H^+ to the tetrazolium salt INT to form the red colored formazan salt. The intensity of color produced is measured at 490nm by standard spectroscopy and is proportional to the number of damaged cells in the culture. Due to the inherent linearity of the assay, it can be easily used to accurately determine the percentage of damaged or injured cells in a sample.

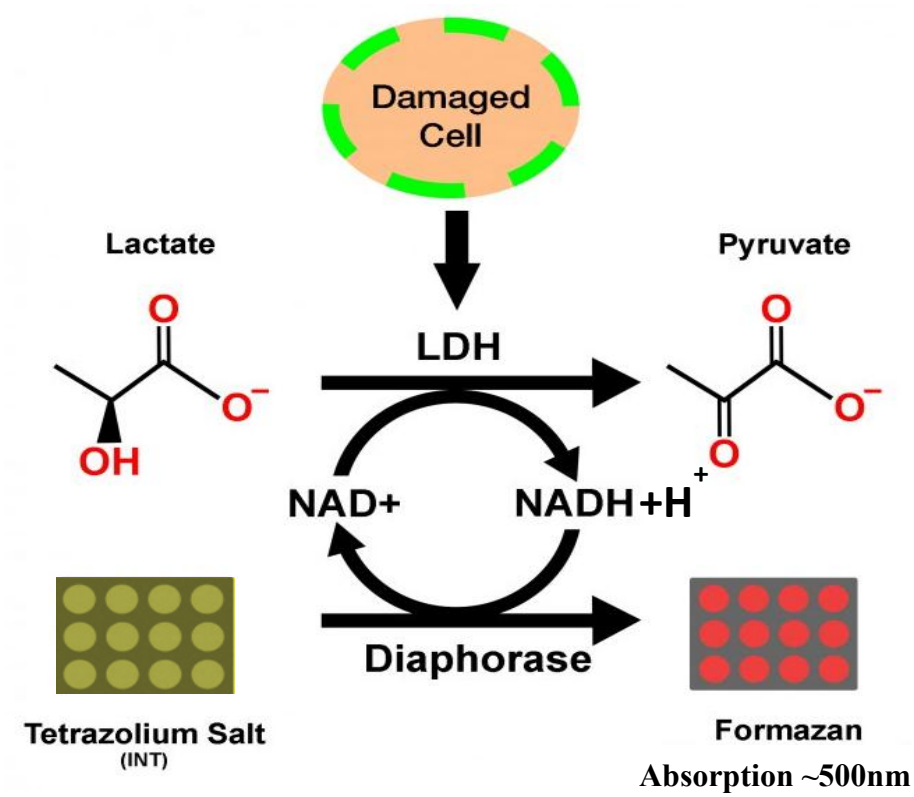


Figure 3: A pictorial representation of principle of LDH assay

Calculation of cytotoxicity:

$$\text{Cytotoxicity (\%)} = \frac{(\text{average exp. value})_{abs} - (\text{background/low control value})_{abs}}{(\text{positive/high control value})_{abs} - (\text{background/low control value})_{abs}} \times 100$$

Average experimental value: the average absorbance value of cells in a column of 5 wells for each concentration

Background control: absorbance value of LDH activity in the assay medium

Low control: absorbance value of spontaneous LDH release from untreated normal cells

Positive/High control: absorbance value of LDH released by Triton 1X treated cells

To calculate the toxicity either low control or background control is considered. For this experiment background control was measured.

CHAPTER IV

RESULTS AND DISCUSSION

Powder X-Ray Diffraction

The refined powder X-ray diffraction data showed about 98% phase purity. The unidentified peaks can be of some known byproduct such as cerium oxysulfides e.g. Ce_2O_2S[8] or $Ce_{10}S_{15-x}O_x$ with $(0 \leq x \leq 1)$[17] , a common compound formed in the sulfurization reaction of C_2S_3[8].

Toxicity Study

The column of well treated with Triton-1X (cell lysing agent) served as the positive control for LDH assay as LDH assay indicates the percentage cell death. Triton-1X is one of the most widely used nonionic surfactants for lysing cells to extract protein and other cellular organelles or to permeabilize the living cell membrane for transfection. However, if large amounts are added or the cells are subject to prolonged exposure to Triton 1X, the cells die.

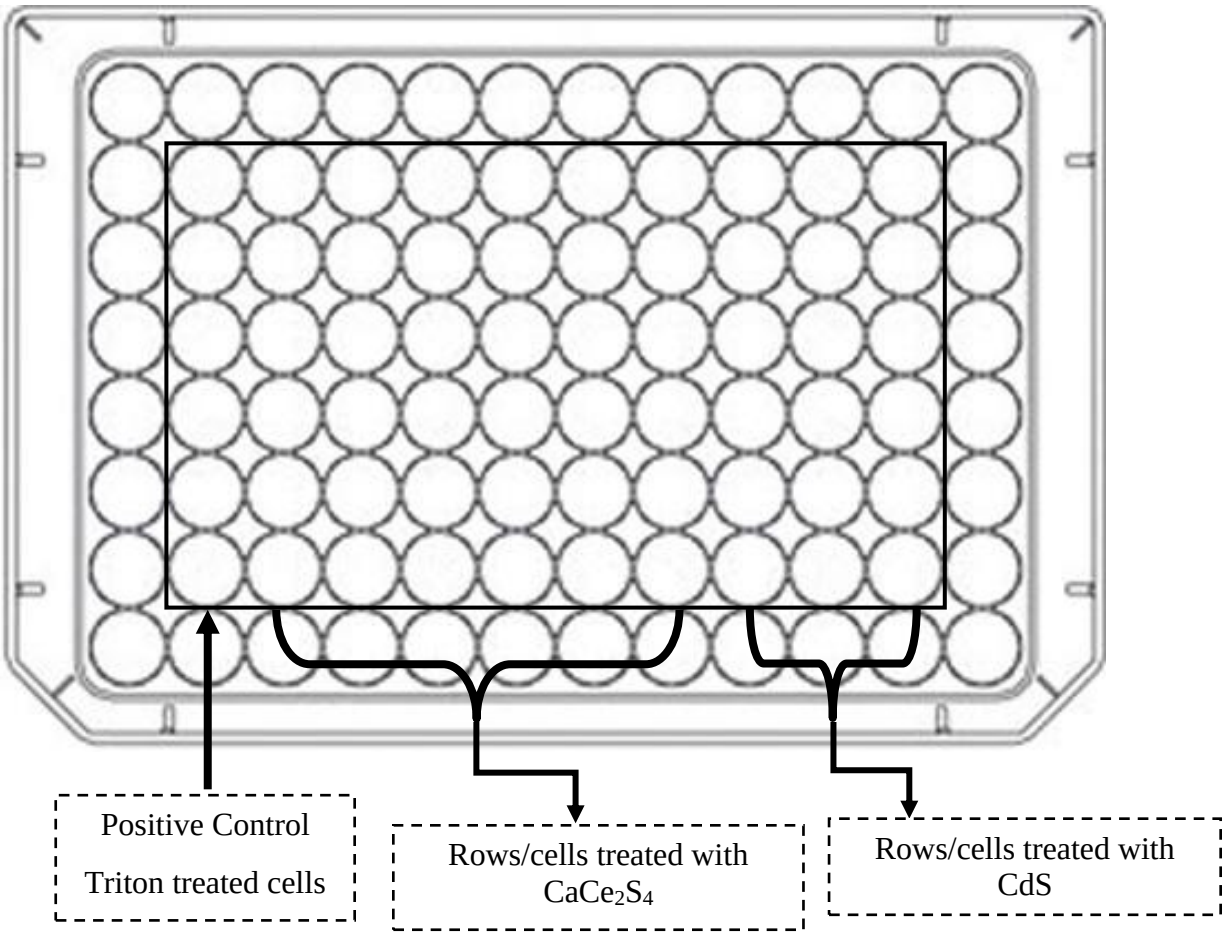
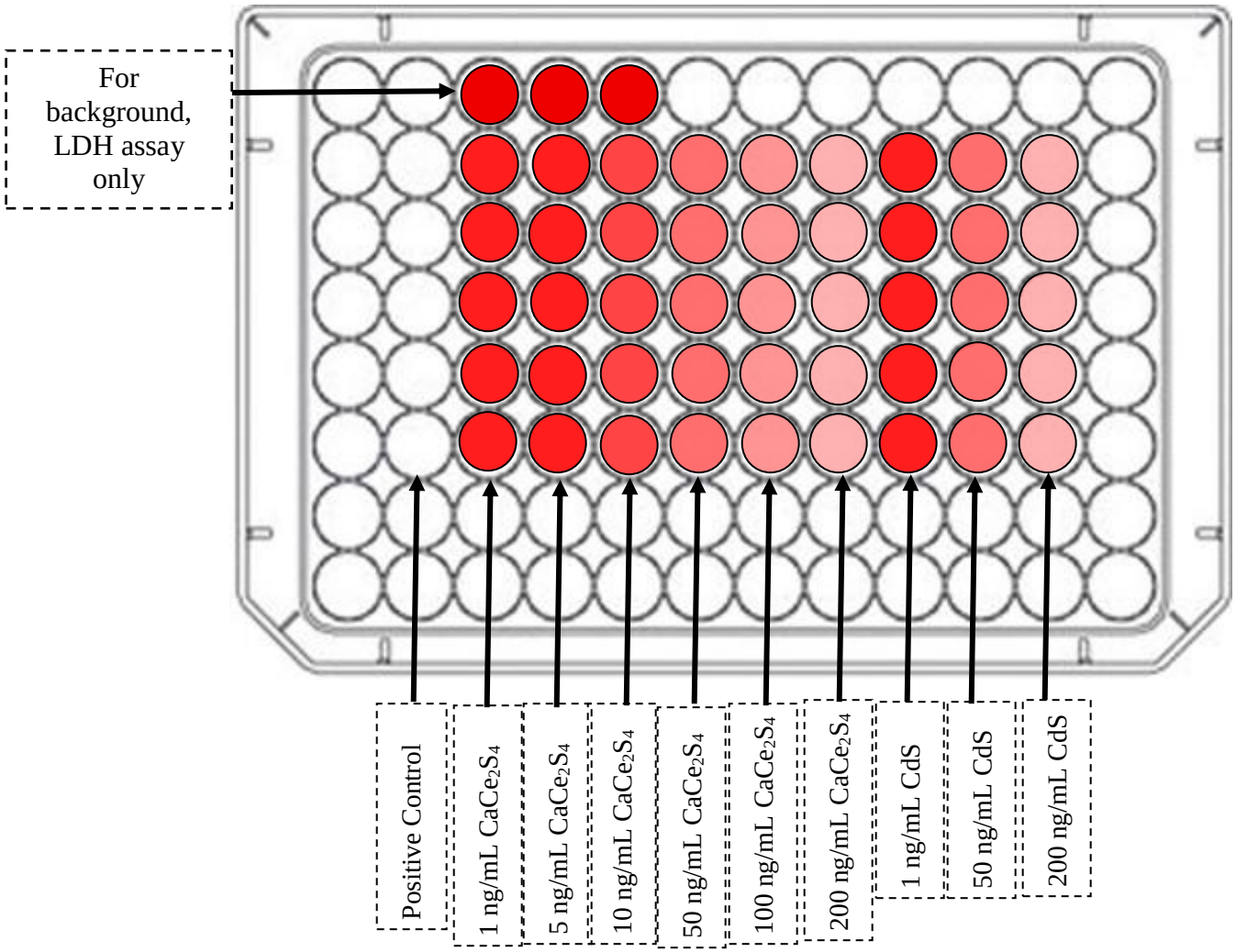


Figure 4: Ninety-six-well microtiter/well plate with seeded cells and media. Usually to avoid contamination and due to faster evaporation of assay, the rows of wells across the border of the well plate were not considered. Cells of first column served as the positive control and the rest of the columns were treated with serially diluted solution of $CaCe_2S_4$ and CdS.

LDH assay presented the % cell death in presence of samples or upon other treatment. The positive control showed 100% death as expected, as the cells in this row were exposed to triton 1X for 24 hours. So, there was no cell proliferation and the rest of the data sets can be compared to this percentage, that's why it was the positive control.



***** Colors used only for presentation; they do not represent actual color change.

Figure 5: Ninety-six-well microtiter plate after LDH assay treatment. First column with positive control white color i.e. no color because of nearly 100% cell death. For $CaCe_2S_4$ treated column of wells, upon serial dilution the color faded with higher concentration, similar trend was observed for CdS. In three wells, only LDH assay reaction mixture were added for background correction.

At low concentrations of 200 ng/mL to 1 ng/mL range, $CaCe_2S_4$ was compared with CdS. The concentrations that these two were compared were 1ng/ml, 50ng/ml and 200 ng/ml. The concentrations with the % cell death results are given on next page in Table 4.

Table 4: LDH Assay Results with $CaCe_2S_4$ and CdS at low concentrations

Treated cells in LDH assay		% Cell Death	Standard Deviation
Positive Control, Triton treated		100	
Concentration, ng/mL			
$CaCe_2S_4$	1	11.5145	4.255759
	5	11.0524	4.325109
	10	12.6191	2.628072
	50	13.0166	1.699
	100	13.7731	4.28066
	200	14.4047	5.10988
CdS	1	14.8903	5.876748
	50	17.9205	6.444254
	200	21.1395	5.174048

On this trial the concentration of $CaCe_2S_4$ was as low as 1ng/ mL and the cell viability was much higher compared to CdS of same concentration. For 5 ng/mL, 10 ng/mL and 50 ng/mL concentrations of $CaCe_2S_4$ compound, despite not following a trend the cell damage/death was as

low as 11-14%. Compared to CdS treated cells for the same concentrations, at 50 ng/mL and 200 ng/mL the percentage was much lower for $CaCe_2S_4$.

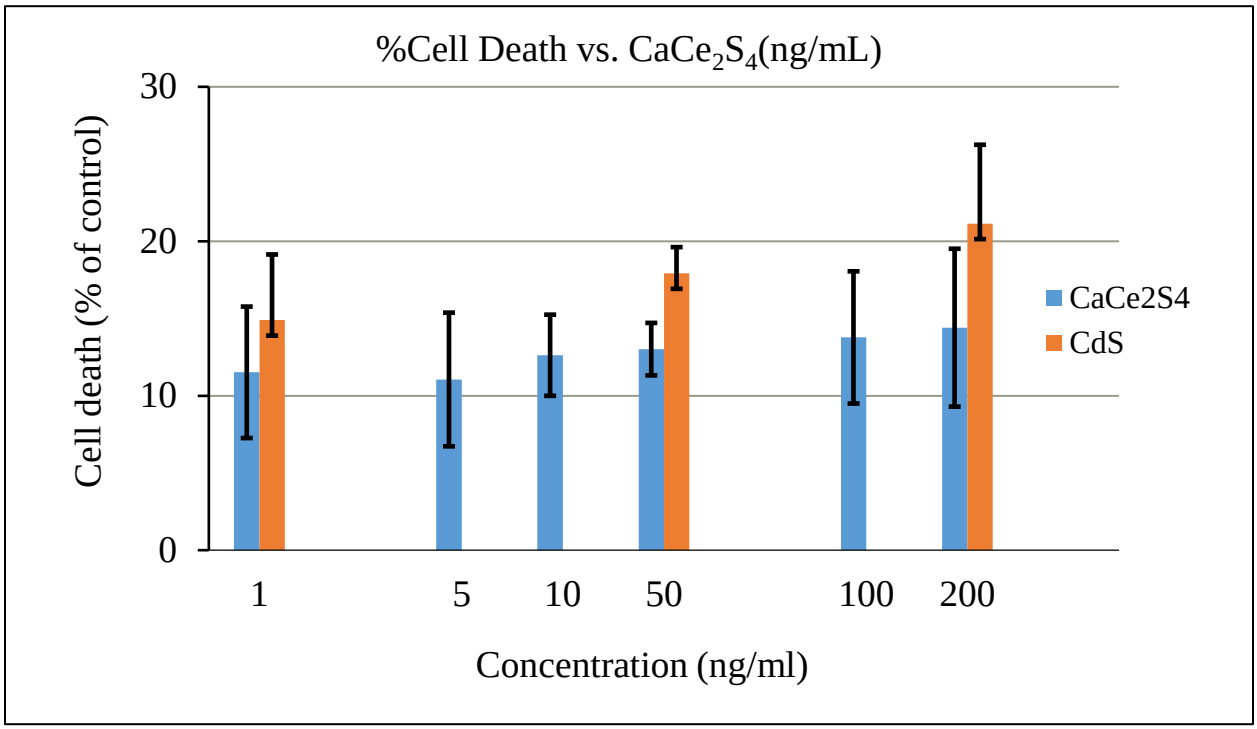


Figure 6: LDH assay data chart presented with error bars. The % cell death of cells treated with serially diluted $CaCe_2S_4$ (ng/mL) showed in blue column and CdS (ng/mL) showed in orange column respectively.

The % cell death of cells treated with CdS followed a trend of lower-concentration, higher-viability. CdS in micro and nano gram level gave high cell mortality in renal cell lines in MTS assay but for other cell lines it had not been reported yet.[12] For $CaCe_2S_4$ treated cells there was not a specific trend but it showed % cell mortality in the range of 11.5-14.5 % for all concentrations. The very low % of cell viability could be attributed to cadmium toxicity.[20]

On the next trial, the concentration range for $CaCe_2S_4$ was much higher i.e.1000 µg/mL to 50 µg/mL. At high concentration, such as 1000µl/mL the LDH assay showed about 75% cell deaths and at 50µl/ml the cell death went down to 34%.

Table 5: LDH results with $CaCe_2S_4$ at high concentrations

$CaCe_2S_4$, µg/mL	% Cell Death	Standard Deviation
50	34.4063	6.541513179
100	35.15957	8.331856138
200	50.9666	10.78148494
500	60.4975	12.08026242
1000	74.6109	13.58902142

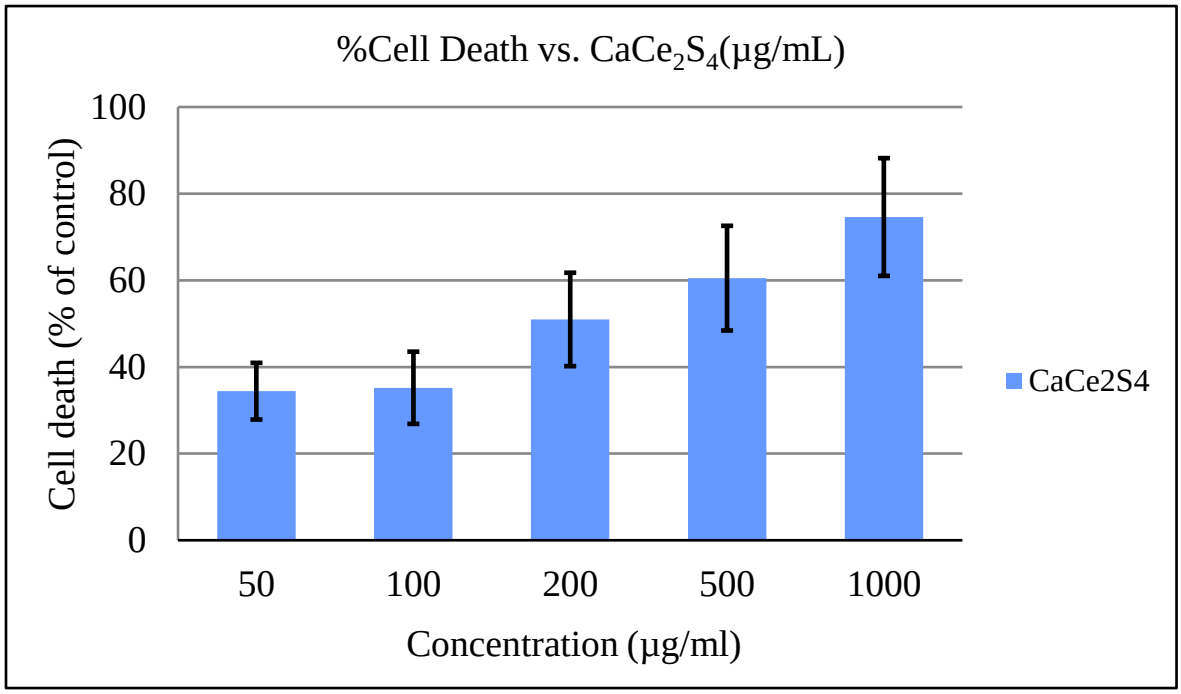


Figure 7: LDH assay data chart presented with $CaCe_2S_4$ at high concentrations

During the cell treatment, the distribution of the sample on media was noticeably low and even with frequent resuspension, the sample settled down immediately. The solubility of sulfide compounds was always very low which makes them almost insoluble. Also, the rare earth in the sample makes it heavy, it settled down quickly. So, the uneven distribution of the sample across the cell wall might contribute to this high percentage of cell death. With serial dilution as the concentration decreased, the percentage cell death declined too.

CHAPTER V

CONCLUSIONS

The demand for commercially available and environment friendly red pigment in paint, ceramic and plastic industry is rising every day and as an alternative to other toxic heavy metal based pigments, alkaline earth doped rare earth sulfides has a potential for this particular application for their interesting properties.

Here the possibility of using the red colored member of alkaline earth doped rare earth sulfides family, $CaCe_2S_4$ as a pigment has been evaluated by studying its toxicity in a common cell-based assay. The synthesis route that has been followed is a standard solid-state synthesis reaction and the information on crystal structure has also been reported. The emphasis of the study was on the cytotoxicity test done on Human Dermal Fibroblasts cell line and evaluated by Lactate dehydrogenase assay. At high concentrations of 1000 µg/mL, the low solubility of $CaCe_2S_4$ led to low distribution of sample in the cell culture media. As the compound settled, it showed higher cell mortality in higher concentration. At low concentration of 200-1 ng/mL range, it was easier to distribute the sample in the media and it showed very low cell death of about 11.5-14.5%. At this low concentration range CdS showed higher cell death which proves, $CaCe_2S_4$ is less toxic.

Further Work

A more detailed analysis on the cytotoxicity study based on every varying concentration of $CaCe_2S_4$ and CdS would give more reliability to the study. Although the starting materials CaS and Ce_2S_3 are air sensitive, a cytotoxicity evaluation on these compounds would enrich the study. Also, cytotoxicity on the impurities such as the oxysulfides would give more clear indication of the source of the toxicity. The particle size of $CaCe_2S_4$ plays an important role in its use as pigment, also it would help to understand the dispersity of the compound in media. Dynamic light scattering(DLS) technique or inductively coupled plasma mass spectrometry(ICP-MS) could be used to measure the particle size. In addition to lactate dehydrogenase, LDH assay, other cell-based assay study such as MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] or WST [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] could give more insight to how $CaCe_2S_4$ reacts and affects the cells. Cellular effects can investigated by means of size exclusion chromatography coupled with inductively coupled plasma mass spectrometry (SEC-ICP-MS) and intracellular sample content can be measured by inductively coupled optical emission spectrometry (ICP-OES)[12]. Besides Human Dermal Fibroblasts cell line, any known renal cell line would give a better comparative data set as studies on cytotoxicity study of CdS on renal cell line have been done[12]. As this study showed that $CaCe_2S_4$ has a low toxicity on healthy skin cells, the other members of alkaline earth doped rare earth sulfides family of vibrant color would be the next targets for cytotoxicity study. The toxicity study of this compound will lead the way to the application of the alkaline earth doped rare earth sulfides as commercial pigments.

APPENDIX

A-1. Crystallographic data for γ-Ce_2S_3

Formula	$Ce_{2.666}S_4$
Molecular weight(g)	501.90
Space group	*I-43d*
a (Å)	8.651(2)
V ($Å^3$)	647.4(4)
Z	4

Atomic positions and displacement parameters of γ-Ce_2S_3

Atom	x	y	z	B_{iso} ($Å^2$)	Site Occupancy
Ce	3/8	0	1/4	1.08(3)	0.888
S	0.0731(2)	0.0731	0.0731	0.058(5)	1

A-2. Structure refinement results for the X-ray diffraction diagram of the $Ca_{0.89}Ce_{2.07}S_4$[18]

Atoms	x	y	z	B_{iso} ($Å^2$)	Site Occupancy
Ce	3/8	0	1/4	0.43	0.69(6)
Ca	3/8	0	1/4	0.43	0.29(6)
S	0.072(7)	0.072	0.072	0.34	1

REFERENCES

(1) Schevciw, O.; White, W.B. The Optical Absorption Edge of Rare Earth Sesquisulfides And Alkaline Earth- Rare Earth Sulfides. *Mater. Res. Bull.* **1983**, *18*, 1059-1068.

(2) Haibin, Y.; Jianhui, Z.; Ruijin, Y. U.; Qiang, S. U. Preparation of ternary rare earth sulfide $La_xCe_{2-x}S_3$ as red pigment. *J. Rare Earths* **2013**, *31* (3), 327.

(3) Mao, W.; Zhang, W.; Chi, Z.; Lu, R.; Cao, A.; Wan, L. Core–shell structured Ce2S3@ZnO and its potential as a pigment. *J. Mater. Chem. A* **2015**, *3*, 2176.

(4) Aubert, M.; Macaudiere, P. U.S. Patent No. 6,221,473 B1. Washington, DC: U.S. Patent and Trademark Office. **2001**

(5) Maestro, P.; Huguenin, D. Industrial applications of rare earths : which way for the end of the century ? *J. Alloys Compd.* **1995**, *225*, 520.

(6) Xian, Z.; Wenhua, G. U. I.; Qian, W. U.; Qingfeng, Z. Structural , phonon , mechanical , optical , and thermodynamic properties of stable β-La_2S_3 from first-principles calculations. *J. Alloys Compd.* **2017**, *35* (3), 271.

(7) Perrin, M. Color of pure and alkali-doped cerium sulfide : A local-density-functional study. *Phys. Rev. B: Condens. Matter* **1996**, *54* (4), 2428.

(8) Brewer, L. Synthesis and Sintering of Cerium(III) Sulfide Powders. *J. Am. Ceram. Soc.* **1998**, *51* (191212), 145.

(9) Zhukov, V.; Mauricot, R.; Gressier, P.; Evain, M. Band Electronic Structure Study of Some Doped and Undoped γ-Ln_2S_3 (*Ln*=La, Ce, Pr, and Nd) Rare Earth Sulfides through LMTO-TB Calculations. *J. Solid State Chem.* **1997**, *204* (128), 197.

(10) Jansen, M.; Letschert, H. P. Inorganic yellow-red pigments without toxic metals. *Nature* **2000**, *404* (6781), 980.

(11) Riss, T. L.; Moravec, R. A.; Niles, A. L.; Duellman, S.; Benink, H. A.; Worzella, T. J.; Minor, L. Cell Viability Assays. *Assay Guidance Manual* **2004**, *740*, 33.

(12) L'Azou, B.; Passagne, I.; Mounicou, S.; Tréguer-Delapierre, M.; Puljalté, I.; Szpunar, J.; Lobinski, R.; Ohayon-Courtès, C. Comparative cytotoxicity of cadmium forms ($CdCl_2$, CdO, CdS micro- and nanoparticles) in renal cells. *Toxicol. Res.* **2014**, *3* (1), 32.

(13) Lin. C.;Wu, K.; Yew, F.; Lee, T. Differential Cytotoxicity Cadmium To Rat Embryonic Fibroblasts and HumanSkinFibroblasts. *Toxicol. Appl. Pharmacol.* **1995**,*133*, 20-26

(14) A, Q. G.; Xikun, L.; Tai, Q.; Haitao, Z. Application of Rare Earths in Advanced Ceramic Materials. *J. Rare Earths* **2007**, *25*, 281.

(15) Ohta, M.; Yuan, H.; Hirai, S.; Uemura, Y.; Shimakage, K. Preparation of R_2S_3 (R : La , Pr , Nd , Sm) powders by sulfurization of oxide powders using CS_2 gas. *J. Alloys Compd.* **2004**, *374*, 112.

(16) Wimmer, E.; Pourovskii, L.; Biermann, S.; Georges, A. Structure and optical properties of α- and γ-cerium sesquisulfide. *J. Alloys Compd.* **2008**, *459*, 438.

(17) Macaudie, P. Low-temperature process of the cubic lanthanide sesquisulfides : remarkable stabilization of the γ-Ce_2S_3 phase. *J. Mater. Chem.* **1997**, *7* (8), 1541.

(18) Mauricot, R.; Gressier, P.; Evain, M.; Brec, R. Comparative study of some rare earth sulfides: doped γ-[A]M_2S_3 (M = La, Ce and Nd, A = Na, K and Ca) and undoped γ-M_2S_3 (M = La, Ce and Nd). *J. Alloys Compd.* **1995**, *223* (1), 130.

(19) Mosset, A.; Macaudiere, P.; Trombe, J. C.; Romero, S. Effect of some dopant elements on the low temperature formation of γ-Ce_2S_3. *J. Alloys Compd.* **2000**, *302*, 118.

(20) Chen, N.; He, Y.; Su, Y.; Li, X.; Huang, Q.; Wang, H.; Zhang, X.; Tai, R.; Fan, C. The cytotoxicity of cadmium-based quantum dots. *Biomaterials* **2012**, *33* (5), 1238.

(21) Sis, C.; S, C. S.; Materials, C. S. S.; Gauthier, G.; Jobic, S.; Evain, M.; Koo, H.; Whangbo, M.; Fouassier, C.; Brec, R. Syntheses , Structures , and Optical Properties of Yellow Ce_2SiS_5,$Ce_6Si_4S_{17}$, and $Ce_4Si_3S_{12}$ Materials. *Chem. Mater.* **2003**, No. 15, 828.

(22) Sreeram, K. J.; Aby, C. P.; Nair, B. U. Synthesis and Characterization of Doped Rare Earth Oxides for Environmentally Benign Nontoxic Reddish-yellow Pigments. *Chem. Lett.* **2008**, *37* (9), 902.

(23) Alanko, G. A.; Butt, D. P. Mechanochemical synthesis of cerium monosulfide. *J. Am. Ceram. Soc.* **2014**, *97* (8), 2357.

(24) Zhao, Y.; Rabouw, F. T.; Puffelen, T. Van; Walree, C. A. Van; Gamelin, D. R.; De Mello Donegá, C.; Meijerink, A. Lanthanide-doped CaS and SrS luminescent nanocrystals: A single-source precursor approach for doping. *J. Am. Chem. Soc.* **2014**, *136* (47), 16533.

(25) Pujalté, I.; Passagne, I.; Brouillaud, B.; Tréguer, M.; Durand, E.; Ohayon-Courtès, C.; L'Azou, B. Cytotoxicity and oxidative stress induced by different metallic nanoparticles on human kidney cells. *Beilstein J Nanotechnol* **2014**, *5* (1), 1590.

(26) Tsay, B. J.; Wang, L. H.; Hon, M. H. Formation and densification of $CaLa_2S_4$ powders by sulfidization of modified metal alkoxides in different atmospheres. *Mater. Sci. Eng., B* **2000**, *72* (1), 31.

(27) Urones-garrote, E.; Fern, F.; Otero-d, L. C. New inorganic pigments in the Ca – Nd – S system : Stabilization of γ phase. *J. Alloys Compd.* **2006**, *418*, 86.

(28) Trombe, J. C.; Verelst, M.(2001). Stabilization of the γ-Ce_2S_3 phase. *J. Alloys Compd.* **2001**, *324*, 66.

(29) Adachi, G.; Imanaka, N.; Tamura, S. Research trends in rare earths : A preliminary analysis. *J. Rare Earths* **2010**, *28* (6), 843–846.

(30) Henderson, J. R.; Muramoto, M.; Gruber, E. L. B. Electronic Structure of Rare-Earth Sesquisulfide Crystals. *J. Chem. Phys.* **1967**,*47*(9), 3347.

(31) Li, K. G.; Chen, J. T.; Bai, S. S.; Wen, X.; Song, S. Y.; Yu, Q.; Li, J.; Wang, Y. Q. Intracellular oxidative stress and cadmium ions release induce cytotoxicity of unmodified cadmium sulfide quantum dots. *Toxicol. In Vitro* **2009**, *23* (6), 1007–1013.

(32) Jood, P.; Ohta, M. Hierarchical Architecturing for Layered Thermoelectric Sulfides and Chalcogenides. *Materials* **2015**,*8*, 1124–1149.

(33) Kumta, P. N.; Risbud, S. H. Low temperature synthesis of cubic lanthanum sulfide (La_2S_3) powders. *Mater. Sci. Eng., B* **1989**, *2* (4), 281–286.

(34) Gibbard, K. B.; Allahar, K. N.; Kolman, D.; Butt, D. P. Kinetics of thermal synthesis of cerium sulfides. *J. Nucl. Mater.* **2008**, *378* (3), 291–298.

(35) Suresh, K.; Murthy, K. V. R.; Atchyutha Rao, C.; Poornachandra Rao, N. V. Rare Earth Doped Alkali Earth Sulfide Phosphors for White-Light LEDs. *ISRN Condens. Matter Phys.* **2011**, *2011*, 1–3.

(36) Lehnert, G.; Drexler, H.; Hartwig, A. Cadmium and its inorganic compounds. *MAK Commission* **2006**, Vol. 22.

(37) Beman, A.; Mahmodabady, Z.; Ph, D.; Saberi, M.; Eimani, H.; Pyrzad, J.; Sc, M.; Sharifabady, R. R. Cytotoxic and Oxidative Stress Caused by Cadmium and Lead on Human Skin Fibroblast Cells. *Yakhteh* **2006**, *8* (3), 172–177.

(38) Satoh, M.; Koyama, H.; Kaji, T.; Kito, H.; Tohyama, C. Perspectives on cadmium toxicity research. *Tohoku J. Exp. Med.* **2002**,*196*, 23–32.

(39) Crosera, M.; Bovenzi, M.; Maina, G.; Adami, G.; Zanette, C.; Florio, C.; Filon Larese, F. Nanoparticle dermal absorption and toxicity: A review of the literature. *Int. Arch. Occup.*

Environ. Health **2009**, *82* (9), 1043–1055.

(40) Jin, C. Y.; Zhu, B. S.; Wang, X. F.; Lu, Q. H. Cytotoxicity of titanium dioxide nanoparticles in mouse fibroblast cells. *Chem. Res. Toxicol.* **2008**, *21* (9), 1871–1877.

(41) Srivastava, V.; Gusain, D.; Sharma, Y. C. Critical Review on the Toxicity of Some Widely Used Engineered Nanoparticles. *Ind. Eng. Chem. Res*. **2015**, *54* (24), 6209–6233.

(42) Zhang, L. W.; Yu, W. W.; Colvin, V. L.; Monteiro-Riviere, N. A. Biological interactions of quantum dot nanoparticles in skin and in human epidermal keratinocytes. *Toxicol. Appl. Pharmacol.* **2008**, *228* (2), 200–211.

(43) Bae, D. S.; Gennings, C.; Carter, W. H.; Yang, R. S. H.; Campain, J. A. Toxicological interactions among arsenic, cadmium, chromium, and lead in human keratinocytes. *Toxicol. Sci*. **2001**, *63* (1), 132–142.

(44) Monteiro-Riviere, N. A.; Inman, A. O.; Zhang, L. W. Limitations and relative utility of screening assays to assess engineered nanoparticle toxicity in a human cell line. *Toxicol. Appl. Pharmacol* **2009**, *234* (2), 222–235.

(45) Dechsakulthorn, F.; Hayes, A.; Bakand, S.; Joeng, L.; Winder, C. In vitro cytotoxicity assessment of selected nanoparticles using human skin fibroblasts. *Proceeding of 6th World Congress on Alternatives & Animal Use in the Life Sciences* **2007**,*14*, special issue, 397–400.

(46) Lewinski, N.; Colvin, V.; Drezek, R. Cytotoxicity of nanopartides. *Small* **2008**, *4* (1), 26–49.

(47) Urones-garrote, E.; Ávila-brande, D.; Varadé-lópez, R.; Fernández-martínez, F.; Otero-díaz, L. C. Stabilization of the g form of the rare earth sesquisul fi des at lower temperature by doping with Calcium. *Solid State Sci.* **2012**, *14* (7), 897–902.

Wafa Tonny joined the graduate program of Department of Chemistry and Biochemistry at the University of Texas at Arlington in Fall, 2015. She earned her Master's and Bachelor's from Department of Applied Chemistry and Chemical Engineering, University of Dhaka, Bangladesh. Prior to coming to USA, she was a senior lecturer of Chemistry in Department of Natural Sciences in American International University-Bangladesh. Her research work includes synthesis of semiconductor type oxynitrides, thermoelectric materials, biodegradable polymer synthesis for packaging application and energy efficiency study of urea manufacturing plant. Her motivation to research is to apply her academic knowledge and experience into practical applications that offer a career opportunity and contribute to the betterment of the future.