Hepatotoxicity study of nanotitania on Mice and Zebra fish: A comparison

Vikash Kumar Sharma, Manoj Kumar Agrawal,
Department of Mechanical Engineering, GLA University, Mathura, U.P, India

* Corresponding author: vikhas.sharma@gla.ac.in

Abstract. The present paper for study aims the characterization and investigation of the oxidative stress induced toxicity of nanotitania in liver of mice and zebra fish models. Physical characterization for its size and the affirmation of phase by XRD, SEM, and TEM reveals, the particles as crystalline, predominant anatase phase with the mean size of about <50 nm. The toxic effect was assessed in vivo using the giant zebra fish 3-6 months old on two groups, viz, control and 24hr treated with 1ppm as experimental. Similarly 6-8 months old Mice with control and experimental groups (24 hour treatment of 50mg/kg bodyweight). Spectrophotometric analysis of the inductively coupled plasma optical emission confirmed the presence of nanoparticles in tissues. In both experimental models, a considerable raise in lipid peroxidation as well as liver marker enzymes ALT, AST and the decline within antioxidants such as SOD, Catalase, GPx due to TiO2 induced oxidant stress. Topographic evaluation of tissue through SEM reveals a remarkable uptake of nanotitania. Histopathological examination of TiO2 administrated groups infers cellular damage and DNA damage which was confirmed by COMET assay. It is substantial that nanoritania unlike to its bulk counterpart induces hepatotoxicity in both fish and Mice. The mice tissues are found to be more toxic than that of giant zebra fish in spite of better efficacy in its tissue.

Keywords: Hepatotoxicity, Antioxidant, Anatase, DNA damage, COMET assay.

1. Introduction
The photocatalytic and whitening property of titanium dioxide (TiO2) nano particles have enabled its industrial applications including manufacture of cosmetics, sunscreens, paints, papers and as a disinfectant in environment and wastewater [1-3]. Toxic effect of nano TiO2 is owing to its ability to absorb UVA light, which catalyzes the generation of reactive oxygen species (ROS). Several studies reported that even in the absence of UVA light, nano TiO2 is capable of producing toxic effect and it depends on the size and phase of the same [4-7]. Little size as well as high surface region, paired to additional physico-chemical characteristics like charged surfaces of nano TiO2 cause DNA damage indirectly, by promoting oxidative stress [25] and inflammatory responses (Singh et al. 2009). After entering the body via various exposure routes, Nano TiO2 is able to enter systemic circulation and will soon accumulate to secondary target organs such as liver, kidney, heart, spleen, and lung [8-10]. Non-soluble nano TiO2 is found to interact with epithelium cells, the interstitial tissue and vascular cells that cause these cells to respond pro-inflammatory (Borm et al. 2006). Cascade of inflammatory cytokines [24] can result in inflammatory cell chemotaxis, as well as apoptosis, causing severe tissue damage (Ma et al. 2009).

Nano TiO2 generates ROS by means of two different devices: oxidative burst (rapid reaction happening in minutes of exposure) as well as modification of the activity of the sequence of mitochondrial electron transport (constant reaction happening after almost one hour) [11-13]. In vivo,
nano TiO$_2$ induce Oxidative stress, is initiated by macrophages which is, in general, passive until stopped by potent injury, exogenous stimuli (e.g., xenobiotics, chemicals, particles) (Long et al. 2006). It was also showed that after activation of macrophages and epithelial cells by nano TiO$_2$ the production of both inflammatory cytokines, like tumor necrosis factor-α [14], changing increase factor β1 [26,27], granulocyte-macrophage colony-stimulating factor, platelet-derived increase factor, IL-6, IL-8, and (ROS) will occur (Becker et al. 1996; Fuji et al. 2001). Several in vivo studies reported that nano TiO$_2$ deposition will cause physical damage to DNA by direct interaction and indirectly by generating oxidative stress (Rahman et al. 2002). Liver, main detoxification tissue of the body generate free radicals with the involvement of kupffer cells (hepatic macrophage) on uptake of nano particles huhe above mechanism [15-18]. The present study was undertaken to characterize and compare the hepatic toxic effect of nano TiO$_2$ on aquatic and terrestrial environment by using zebra fish and mice models [19-21]. For this purpose, both the experimental groups were treated with nano TiO$_2$ for 24 hour. The content of TiO$_2$ was determined. Moreover biochemical assays, SEM, histopathology, DNA fragmentation and COMET assay were carried out to assess toxicity.

2. And Methods and Materials

2.1. Chemicals and Preparation

TiO$_2$ Nano particles employed in the reading had been bought and brought from Sigma-Aldrich [22]. For administration, TiO$_2$ nano particles were sonicated in saline to ensure uniform suspension. A mixture of 50mg of nano TiO$_2$ and 8ml of saline was sonicated using an ultrasonic grinding device (Branson Ultrasonics) at 22.5 kHz for 10 min under cooling condition for treating mice and 1mg/L were used for fish.

2.2. Physical characterization

**SEM:** The samples were prepared for analyzing in Joel 400N version. The nanopowders were placed on a glass plate after complete removal of moisture and placed on a conducting specialized grid for analysis.

**TEM:** By using the new TEM version of Philips, the nanopowders had been scattered within alcohol to ensure a consistent suspension few µl had been mounted upon a copper framework backed by carbon. The sample was obtained for analysis on drying.

**XRD:** The nanotitania powder samples dispersed on a glass plate and kept on XRD chamber for further analysis.

2.3. Animals and Treatment

Adult male mice of 28g were used. Animals were housed in a ventilated animal space by sex in stainless steel cages. The temperature of room had been kept at 20±20 C, relative humidity had been maintained at 60±10 percent and a light/ dark period of 12 h. The ad libitum was provided with purified water and sterilized food for mice. They had been acclimatized to this condition 5 days before dosing. All the experiments were carried out on clearance from Institutional ethical committee. The only dose of 50mg/kg body weight nano TiO$_2$ had been given intraperitonially.

Adult Giant Danio (Danio aequipinnatus) of 3-6 months old were obtained from Aliyar dam, Coimbatore and kept for 4 weeks in stock aquaria with running, aerated, dechlorinated tap water. Stock animals had been provided a commercial fish feed. The water temperature was maintained at 28±1°C, with a light/dark cycle 14/10 hr. Fish were then graded into an experimental glass aquaria (10fish/tank), and allowed to acclimatize for a week before addition of nanoparticulate metals. Dispersion in aquaria of 1mg/L was given to the experimental fish. Control group of mice were treated with saline and fishes were let in normal aquaria served as control. The animals and fishes were sacrificed after 24 hours of treatment. A part of liver was stripped and without delay set within the 10% formalin solution for histopathological sampling. The remaining
samples were taken for biochemical analysis and fresh samples were stored at -70°C prior to SEM studies.

2.4. Titanium content analysis
Tissues had been removed and melted. Around 0.1 g of liver-tissue was absorbed in 1ml of concentrated Nitric acid. Acid was evaporated off and the remaining residue was dissolved in 7.5 ml of distilled water. Digested sample was then analysed for the presence of nano titania by Inductively Coupled Plasma Optical Emission Spectroscopy by Optima5300DV ICP-OES analyser.

2.5. Antioxidant enzyme assay
100 mg of tissue was homogenized in 1 ml of 0.1 M Tris HCI (pH 7.4) with chilled mortar and pestle on ice and the homogenate had been centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant had been employed for antioxidant enzyme assays like SOD, catalase and GPx using a spectrophotometer (Genesys-2, Theremo spectronics). Serum was used to carry out liver marker enzyme assays such as ALT and AST.

2.6. Scanning electron microscopy
Fresh Liver and kidney tissues were freeze dried in suitable quantity of liquid nitrogen and the obtained moist free tissues were then subjected to Scanning Electron Microscope (JOEL 6360(LA)).

2.7. Histopathological examination
The tissues were coated in blocks of paraffins, then cut to a thickness of 5µm and placed on glass slides. The slides were analyzed after staining with Hematoxylin-eosin (HE), and the images were taken using an optical microscope.

2.8. DNA Fragmentation
Phenol / chloroform was derived from DNA and precipitate in ethanol. The removed DNA had been isolated by electrophoresis of the agarose gel (1.5 percent agarose gel holding 0.4 µg / mL bromide ethidium 100 V, 4h) and to make visible in the UV light.

2.9. COMET assay
Tissues were chopped in PBS and washed atleast thrice in order to get the cells in suspended form. Centrifuged at for 5 min and re-suspended in PBS. For the dose tested in this study, cell viability was over 85 percent, as evaluated utilizing staining with trypan blue dye-exclusion. A 75µl sample of a 50µl mix of cells at a density of 1x105 cells / ml and 500 µl of low melting point agarose was applied to the slides. The slides were immersed in a cold lysis buffer for 30 min, then in an alkaline solution (pH > 13), for 30 min, and then put in a fresh electrophoresis solution (300Mm NaOH, 1mM EDTA) filled with horizontal gel electrophoresis tank [23]. The electrophoresis was conducted at 23V and 300 mA at low temperature for 30 min. every step was carried out in the dim light [28,29]. The slides had been rinse with deionized water after electrophoresis and submerged for 5 min in 70 percent ethanol, then drained, and 50 µl of diluted ETBR was applied. At an enlargement of 200x employing a blue filter (450-490 nm), slides were scored with a fluorescence microscope (BX50, Olympus), and pictures taken on the high resolution.

3. Result
Nanotitania XRD patterns for size and phase suggested the presence of peaks (2θ = 25.4, 37.8 and 48.1) could be considered as an attributive predictor of crystalline anatase TiO2 anatase structure, and no other non-cristalline phases were found throughout. The particle size was around 31 nm, according to Scherrer formula, d = π / β cos.

SEM of the agglomeration state of nano titaniais as shown in Figure 2. The determination of grain size is further confirmed followed by TEM (3a and b) which shows that clear concentric circles that indicates the crystalline nature of anatase phase with the confirmed grain size of 51 nm. The size parity may reveal the agglomeration property which is the most characteristic feature of nanotitania.
The protein content, lipid peroxidation and antioxidative enzymes in liver tissues from mice were examined. Comparing with control, there is the considerable raise in protein, LPO, and decrease inside SOD, catalase, and GPx. A significantly upregulated processes of ALT and AST had also been observed with Mice liver (Table 2).

The ICP-OES signifies the efficacy of the nanoparticle in liver tissues at the extent of 24 hrs treatments in itself; the topographical evidence is further suggestive of uptake of nanoparticle through SEM/EDS status.

![Figure 1. XRD pattern of phase confirmation and grain size nanotitania.](image1)

![Figure 2. Scanning Electron micrographs of nanotitania illustrate individual and agglomerate grains.](image2)

![Figure 3. reveals the anatase crystalline nature of TiO₂](image3)
ICP-OES analysis values on tissue uptake efficacy.

$x \pm$ SEM of 6 Animals

**Table 1.** Protein content and redox status in mice liver (n=6)

| Groups | Protein (mg/g of tissue) | LPO (nM of MDA/mg of protein) | SOD (U/mg of protein) | Catalase (U/mg of protein) | GPx (U/mg of protein) |
|--------|--------------------------|-------------------------------|-----------------------|--------------------------|----------------------|
| Control | 36.6 ± 2.25             | 0.097 ± 0.006                | 2.53 ± 0.041          | 43.51 ± 3.67             | 1.56 ± 0.096         |
| Treated | 43.6 ± 1.36             | 0.15 ± 0.002                 | 2.48 ± 0.017          | 37.01 ± 2.68             | 1.48 ± 0.008         |

**Table 2.** Liver marker enzyme level in serum of mice (n=6)

| Groups | ALT (U/ml) | AST (U/ml) |
|--------|------------|------------|
| Control | 0.85 ± 0.017 | 0.96 ± 0.016 |
| Treated | 0.95 ± 0.54  | 1.17 ± 0.024 |

*p<0.05 significantly different from the control group.

**BIOCHEMICAL ASSAY ANALYSIS OF NANOTIO$_2$ TREATED ZEBRA FISH.**

**Table 3.** Protein content and redox status in fish liver (n=6)

| Groups | Protein (mg/g of tissue) | LPO (nM of MDA/mg of protein) | SOD (U/mg of protein) | Catalase (U/mg of protein) | GPx (U/mg of protein) |
|--------|--------------------------|-------------------------------|-----------------------|--------------------------|----------------------|
| Control | 3.133 ± 0.066654         | 7.27 ± 1.6513                | 4.343 ± 0.8563        | 46.05                     | 60.05                |
| Treated | 4.933 ± 0.04216          | 5.175 ± 0.85537              | 7.115 ± 0.00962       | 25.27                     | 19.106               |

**Table 4.** Liver marker enzyme level in serum of fish (n=6)

| Groups | ALT (U/ml) | AST (U/ml) | Ascorbic acid (mg/g) |
|--------|------------|------------|----------------------|
| Control | 0.0722 ± 0.00323 | 0.00890 | 79.14 ± 0.0083       |
| Treated | 0.0042      | 0.0062     | 7.08 ± 0.00391       |
Figure 4. SEM-EDS of liver Mice-Control

Figure 5. SEM-EDS Lever –Treated

Figure 6. SEM liver Zebra fish-Control
Figure 7. CV- Central vein, H- hepatocytes, S- Sinusoids, MI- Macrophage infiltration, FH- fused Hepatocytes

Figure 8. DNA fragmentation in mice (Picture1). Lane1: Marker, Lane 2: Control liver, lane 3: Test liver (24 hour)

Figure 9. DNA fragmentation in Zebra fish (picture2). Lane 1: Marker, Lane 2: Control liver, lane 3&4: Test liver (24 hour)
4. Discussion
The most conferring aspect at present behind the toxicity of nano TiO$_2$ is the induction of reactive oxygen species. In our study, role of persistence and the oxidative stress of nano TiO$_2$ on toxicity till DNA damage were studied in liver of mice and zebra fish. SEM/EDS analysis in the present study infer that nano TiO$_2$ got entered to liver. This internalization of nano TiO$_2$ brings about the oxidative stress induced tissue damage and DNA damage. Komatsu et al (2008) reported that the internalization of TiO$_2$ nanoparticles lead to cytotoxicity and gene expression changes in liver tissues. Titanium content analysis showed the accumulation of nano TiO$_2$ is high in liver of both the organisms in 24 hr treated groups. Liver is the major detoxification tissues and thus accumulating in them as a main route of metabolism and elimination of nano TiO$_2$ (Wang et al, 2007). The International Program on Chemical Protection (IPCS, 1982 ) showed that most of the titanium consumed was excreted with urine and was not absorbed by the organism. However, due to small size and difficult clearance of TiO$_2$, we got that nano TiO$_2$ was retained within both target tissues.

When compared to the control, a higher level of protein was identified in both samples of the liver. It is possibly because of the little stress connected with the entire system of the body exposure (Grassian et al , 2007) treated community for 24 hours as well as a persistent exposure to nano TiO$_2$ can enhance the expressions of mRNA and increase the protein levels of many inflammatory cytokines (Ma et al, 2009).

LPOs were identified to be substantially raised in 24 hours. MDA levels, the end product of LPO, had been utilized to represent an extent of oxidative injury in tissues, and exposure to intra-articular TiO$_2$ nanoparticles increased significantly in Mice synovium. It may be because of the fact that monocyte phagocytised nano-TiO$_2$, macrophages that result in phospholipid peroxidation (MDA elevation) (Wang et al, 2009).

In this study, GPX reported a small decline in its 24 hour community treated activity. This finding is controversial in Wang et al (2009) study where the remarkably increased GSH-Px, GSSG, suggested in the high TiO$_2$-exposed group synovium that the oxidative injury had been motivated due to the self-regulation of certain enzymes and antioxidants in organisms.

However, after treatment with [Gd@C$_8$2 (OH)$_{22}$] n nanoparticles, the GSH-Px, processes had been down regulated compared to the saline group. Similarly, the increase in hepatic lipid peroxide produced by TiO$_2$ nano-anatase indicated an oxidative attack, stimulated by reducing antioxidant enzymes such as superoxide dismutase, catalase , and glutathione peroxidase (Liu et al, 2009).

Indeed, our research is evidence on both catalase and SOD behaviors in 24 hours. This difference in our findings and that of Wang et al. (2009) is most likely due to the lack of self-regulation of antioxidant enzymes against the free radicals that are generated.

The raised level of SOD was validated in their study since these antioxidant enzymes were initiated to antagonize the free radicals generated through phagocytosis. Wang et al (2008 ) reported that the raised CAT and declined SOD process was noticed when mice were exposed to TiO$_2$ particles, that showed that the oxidative stress in the murine brain was greatly regulated. Another report on the reduction of enzyme activity infer that nano-anatase TiO$_2$ induce inhibition of mRNA expression of SOD, CAT, APx, and GSHPx (Liu et al. 2009). The ratio of ALTIAST, a more sensitive pointer for hepatic injury, was raised after oral ingestion of TiO$_2$ particles (Wang et al, 2007). Intake of nano TiO$_2$ in liver tissues of both the organisms on histopathological profile findings shows considerable changes from that of control group. Macrophage infiltration can provide an early marker for tissue damage and disease.

A smear or ladder pattern of DNA fragments is the hallmark feature of apoptosis. Significant sheared pattern of DNA was observed with 24 hour group of liver. Vamanu et al . ( 2008) reported that the lower concentration range of nano TiO$_2$ suggested necrosis or secondary necrosis, while the cells displayed distinct signs of apoptosis at the higher end of the concentration range, such as increased DNA fragmentations.

Comet assay is a sensitive assay for detecting oxidative stress-induced genotoxicity. Inside our study, both a treated group showed comet like DNA damage in liver and kidney on treatment with nano TiO$_2$. Reeves et al, (2008) indicated that differences in the indicated genotoxic potential of TiO$_2$ nanoparticles can be explained by numerous variables, including the treatment regime for TiO$_2$, the
kind of cell used, the cell's metabolic / antioxidant efficiency, and ability to repair DNA. Our study reveals on prompt single cell DNA damage which is an excellent confirmation for induced toxicity till molecular level.

![Figure 10](image)

Figure 10. (a) Comet assay- mice control (b) treated (c) comet assay -zebra fish1 control (d) treated

5. Conclusion
From our studies it is evident that the nanotitania induces oxidative stress and apoptosis leading to liver damage which is the most significant tissue to reveal on toxicity. ALT and AST levels suggest the toxic potential along with histopathological and ICP-OES values as most indicative of the same. Further the nanomaterials should be prepared or synthesised in biocompatible green mode in order to save the eco system and thereby human society.

6. References
[1] Singh N, Manshian B, Jenkins GJS, Griffiths SM, Williams PM, Maffeis TGG, Wright CJ and Doak SH (2009). NanoGenotoxicology: The DNA damaging potential of engineered nanomaterials. Biomaterials 30 : 3891-3914.
[2] Ma L, Zhao J, Wang J, Liu J, Duan Y, Liu H, Li N, Yan J, Ruan J, Wang H and Hong F (2009). The acute liver injury in mice caused by nano anatase TiO$_2$. Nano scale Res. Lett. 4: 1275-1285.
[3] Reeves JF, Davies SJ, Dodd NJF and Jha AN (2008). Hydroxyl radicals (-OH) are associated with titanium dioxide (TiO$_2$) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells. Mutat. Res. 640 - 113-122.
[4] A Kumar, K Sharma, AR Dixit A review of the mechanical and thermal properties of graphene and its hybrid polymer nanocomposites for structural applications, Journal of materials science 54 (8), 5992-6026.
[5] K Sharma, M Shukla, Three-phase carbon fiber amine functionalized carbon nanotubes epoxy composite: processing, characterisation, and multiscale modeling, Journal of Nanomaterials 2014
[6] Borm PJA, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D and Oberdorster E (2006). Part. and Fibre Toxicol. 3:11.
[7] Ma L, Zhao J, Wang J, Liu J, Duan Y, Liu H, Li N, Yan J, Ruan J, Wang H and Hong F (2009). The acute liver injury in mice caused by nano anatase TiO$_2$. Nano scale Res. Lett. 4:1275-1285.
[8] K Sharma, KS Kaushalyayan, M Shukla, Pull-out simulations of interfacial properties of amine functionalized multi-walled carbon nanotube epoxy composites, Computational Materials Science 99, 232-241
[9] A Yadav, A Kumar, PK Singh, K Sharma, Glass transition temperature of functionalized graphene epoxy composites using molecular dynamics simulation, Integrated Ferroelectrics 186 (1), 106-114

[10] PK Singh, K Sharma, A Kumar, M Shukla, Effects of functionalization on the mechanical properties of multiwalled carbon nanotubes: A molecular dynamics approach, Journal of Composite Materials 51 (5), 671-680

[11] PK Singh, K Sharma, Mechanical and Viscoelastic Properties of In-situ Amine Functionalized Multiple Layer Graphene/epoxy Nanocomposites, Current Nanoscience 14 (3), 252-262

[12] Singh PK, & Sharma K, Molecular Dynamics Simulation of Glass Transition Behaviour of Polymer based Nanocomposites, Journal of Scientific & Industrial Research, 77 (10) (2018) 592-595.

[13] Long TC, Saleh N, Tilton RD, Lowry GV and Veronesi B (2006). Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ. Sci. Technol. 40 : 4346-4352.

[14] Long TC, Tajuba J, Sama P, Swartz C, Parker J, Hester S, Lowry GV and Veronesi B (2007). Nanosize Titanium Dioxide Stimulates Reactive Oxygen Species in Brain Microglia and Damages Neurons in Vito. Environ. Health Perspect. 115: 1631-1637.

[15] A Kumar, K Sharma, AR Dixit, Carbon nanotube-and graphene-reinforced multiphase polymeric composites: review on their properties and applications, Journal of Materials Science, 1-43

[16] Becker S, Soukup JM, Gilmour MI and Devlin RB (1996). Stimulation of human and rat alveolar macrophages by urban air particulates: effects on oxidant radical generation and cytokine production. Toxicol. Appl. Pharmacol. 141 : 637–648.

[17] Rahman Q, Lohani M, Dopp E, Pemsel H, Jonas L, Weiss DG and Schiiffmann D (2002). Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. Environ. Health Perspect. 110: 797-800.

[18] MK Shukla, K Sharma, Effect of carbon nanofillers on the mechanical and interfacial properties of epoxy based nanocomposites: A review, Polymer Science, Series A 61 (4), 439-460

[19] A Kumar, K Sharma, AR Dixit, A review on the mechanical and thermal properties of graphene and graphene-based polymer nanocomposites: understanding of modelling and MD simulation, Molecular Simulation 46 (2), 136-154

[20] K Mausam, K Sharma, G Bharadwaj, RP Singh, Multi-objective optimization design of die-sinking electric discharge machine (EDM) machining parameter for CNT-reinforced carbon fibre nanocomposite using grey relational analysis, Journal of the Brazilian Society of Mechanical Sciences and Engineering 41 ...

[21] MK Shukla, K Sharma, Improvement in mechanical and thermal properties of epoxy hybrid composites by functionalized graphene and carbon-nanotubes, Materials Research Express 6 (12), 125323

[22] Wang ML, Tuli R, Manner PA., Sharkey PF, Hall DJ and Tuan RS (2003). Direct and indirect induction of apoptosis in human mesenchymal stem cells in response to titanium particles. J. orth. Res. 21 : 697-707.

[23] Liu H, Ma L, Zhao J, Liu J, Yan J, Ruan J and Hong F (2008). Biochemical Toxicity of Nano-anatase TiO2 Particles in Mice. Biol. Trace. Elem. Res. 129: 170-180.

[24] K Kumar, K Sharma, S Verma, N Upadhyay, Experimental Investigation of Graphene-Paraffin Wax Nanocomposites for Thermal Energy Storage, Materials Today: Proceedings 18, 5158-5163

[25] Goyal, M., Shape, size and phonon scattering effect on the thermal conductivity of nanostructures, Pramana, 2018. 91(6): p. 87.

[26] Goyal, M. and B. Gupta, Study of shape, size and temperature-dependent elastic properties of nanomaterials. Modern Physics Letters B, 2019. 33(26): p. 1950310.
[27] Liu L, Takenaka T, Zinchenko AA, Chen N, Inagaki S, Asada H, et al (2007). Cationic silica nanoparticles are efficiently transferred into mammalian cells. Int. Symp. Micro-Nano Mechatronics Hum. Sci. 1-2:281-5

[28] Goyal, M. and B. Gupta, Analysis of shape, size and structure dependent thermodynamic properties of nanowires. High Temperatures--High Pressures, 2019. 48.

[29] Goyal, M. and M. Singh, Size and shape dependence of optical properties of nanostructures. Applied Physics A, 2020. 126(3): p. 1-8.