



Investigation of Statistical Experimental Design Results Showing UV-Vis Absorbance Values of Characterized Alpha and Gamma Fe₂O₃ Nanoparticles

Mehmet Ateş¹, Önder Aksu^{2*}, Durali Danabaş², Banu Kutlu², Işıl Canan Çicek-Çimen², İlkey Ünal³, Burcu Ertit Taştan⁴,

¹Department of Biotechnology, Graduate School of Natural and Applied Sciences, Munzur University, Tunceli, Turkey

²Fisheries Faculty, Munzur University, Tunceli, Turkey

³Department of Bioengineering, Munzur University, Tunceli, Turkey

⁴Vocational School of Health Services, Gazi University, Ankara, Turkey

*Corresponding Author

E-mail: onderaksu@munzur.edu.tr.

Abstract

Ultraviolet-visible (UV-Vis) spectroscopy is generally used in the characterization of metal and metal-based nanomaterials as they possess sensitive optic properties that vary with size, volume, aggregating state. UV-visible absorption spectra of α -Fe₂O₃ (20-40 nm), γ -Fe₂O₃ (20-40 nm), were determined between 300 and 800 nm using UV-visible spectrophotometer (Optima, SP-3000 Nano). It is concluded that among selected parameters for α -Fe₂O₃ (20-40 nm), particle dose and period have an impact decreasing absorbance value. When absolute values of coefficients are examined, effect order is period and dose. According to the results of statistical table provided for γ -Fe₂O₃ (20-40 nm) NP, correlation coefficient (R₂) value for appropriate Quadratic model is respectively determined as multiple R=89.78%, R₂=80.61% and adjusted R₂=66.76%. Determination of (R²) as 80.61% shows that compatibility between observed values and foreseen values is high and applied model takes place in confidence range. As a result of the evaluation, it is concluded that among selected parameters for γ -Fe₂O₃ (20-40 nm), particle concentration and period have an impact decreasing absorbance value. Considering all statistical data, it is concluded that maximum effect is observed in a period between 24-30 hours and 25 ppm absorbance concentration is more efficient. In all NPs, absorbance value decreases in periods below 24 hours and above 36 hours. Again likewise, the effect decreases in cases where absorbance dose is low or very high. Considering all data, it is concluded that the effect varies according to type and size of NP and this variance is meaningful.

Keywords: Iron Oxide, Nanoparticles, UV-Vis Absorbance Values, Statistical Experimental Design

INTRODUCTION

Nanoscience is becoming a prominent area of research in today's society. For years, nanoparticles (NPs), natural and manmade, have been entering the environment while their effects are unknown. The large increase in production, species, and utilization of manufactured NPs has raised concerns that the release of these materials into the environment may pose a serious threat, leading to calls for an environmental risk assessment of NPs [1-4]. As nanomaterials are being widely used in modern technology, there is an increasing need for information regarding their human health and environmental implications. To date, the human health impacts of NPs have received the greatest attention, which has been demonstrated through both in vivo and in vitro studies on mammalian test systems. The properties that make NPs so attractive for commercial applications (e.g., NPs' size and increased surface area) also can be potentially responsible for undesirable health effects [5-9].

The most commonly manufactured or synthesized forms of iron oxide NPs include magnetite (Fe₃O₄) and hematite (Fe₂O₃) that are also the major forms found in the environment originating from anthropogenic emissions and volcanic eruptions. Though aquatic ecosystems are the primary repositories for these NPs, their fate, transport/transformations in aquatic ecosystems and food web, and potential impact on aquatic microorganisms and fish are not fully understood. Bombin et al. [10] have reported that treatment with Fe₂O₃ NPs affected adversely reproductive capacity of *Arabidopsis thaliana*. Pollen viability increased up to 6% with up to 11% reduction in seedling yield at 3 mg/L levels, whereas exposure to 25 mg/L Fe₂O₃ NPs caused

significant reduction in root length besides reduced seedling. Fe₂O₃ NPs decreased the snout-vent length of *Xenopus laevis* tadpoles at 10 mg/L level, and caused significant reduction in total body length at 1000 mg/L levels [11]. Exposure to suspensions of Fe₂O₃ NPs caused significant mortality, hatching delay and malformation in zebrafish (*Danio rerio*) embryos at and above 10 mg/L levels [12]. More recently, Zhang et al. [13] reported that Fe₂O₃ and Fe₃O₄ NPs accumulated heavily in adult zebrafish (*D. rerio*) with total Fe content of as high as 1.32 and 1.25 mg/g, respectively during 28 days exposure. Guts were filled with aggregates of the NPs, but all cleared from the body within 24 days. With that, it was suggested that ingested NPs did not bioaccumulate in tissues rather they were stored or adsorbed through the gastrointestinal tract.

Iron oxide nanoparticles having extensive applications are the current global demand. These particles have potential applications and are used as catalytic materials, adsorbents, pigments, flocculants, coatings, gas sensors and ion exchangers. They are also used in magnetic recording devices, in toners as well as in inks for xerography. Nanosized maghemite has excellent magnetic properties and is used for biomedical purposes. These particles have been widely researched for magnetic resonance imaging (MRI), which are becoming tools for brain tumor imaging and treatment [14-15]. Further, these particles are also used in transparent paints, in catalysis and in numerous synthesis processes [16]. In literature, iron oxide has been prepared by applying various techniques like microemulsion, precipitation, sol-gel, coprecipitation and hydrolysis methods [14-17].

Characteristic properties of materials vary depending on their structure and physicochemical processes used in their

formulation and manufacture. Materials in nano-scale could demonstrate entirely different properties than their bulk forms. Therefore, physical and chemical properties, such as particle size, shape, surface area, surface reactivity and solubility of NPs should be carefully identified when bio-assays to determine toxicity of NPs smaller than 100 nm are to be conducted.

Ultraviolet-visible (UV-Vis) spectroscopy is one of that identification/characterization analyses, usually used for measuring molecules or inorganic ions and complexes in the solutions. While the UV scan is corresponding normally to the wave length range of 200 - 400 nm, the visible light scan corresponds to the range 400-800 nm. Metal-based NPs have optical properties sensitive to size, shape, density, aggregation and refractive index close to the surface, which is why it is important to identify, characterize and examine

NPs by UV-Vis spectroscopy.

MATERIAL and METHOD

Nanoparticles

α -Fe₂O₃ (20-40 nm) and γ -Fe₂O₃ (20-40 nm) were obtained from commercial companies selling SkySpring products in our country. All chemicals of analytical reagent class are used without any purification or distillation.

Statistical Determination of UV-Vis Absorbance Values

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \dots \dots \dots (3)$$

$$Abs. = 0.13 - 0.0026 X_1 - 0.023 X_2 - 0.0067 X_{12} - 0.068 X_1^2 - 0.041 X_2^2 \dots (4)$$

As a result of the evaluation, it is concluded that among selected parameters for α -Fe₂O₃ (20-40 nm), particle dose and period have an impact decreasing absorbance value. When absolute values of coefficients are examined, effect order is period and dose.

UV – visible spectra of NPs were determined at 300-800 nm wavelengths in absorption mode using UV – Vis spectrophotometer (Optima, SP-3000 Nano). In order to determine the effect of NPs in the sizes of α -Fe₂O₃ (20-40 nm) and γ -Fe₂O₃ (20-40 nm), UV-Vis absorbance values were determined by Design Expert v.10., were evaluated statistically.

RESULTS and DISCUSSION

According to the results of statistical table provided for α -Fe₂O₃ (20-40 nm) NP, correlation coefficient (R²) value for appropriate Quadratic model is respectively determined as multiple R=81.65%, R²=66.67% and adjusted R²=42.87%. Determination of (R²) as 66.67% shows that compatibility between observed values and foreseen values is low and applied model does not take place in confidence range. The fact that multiple R value is 81.65% indicates that regression is important statistically and 19.35% of total variables cannot be explained with this model. In order to determine statistical value of this model, “Significance F” value in ANOVA test is examined. The fact that Significance F value is bigger than 0.05 (0.1060) and model F value is 2.80 indicates that the model in 95% confidence range is not valuable statistically (Figure 1).

According to mathematical modelling, it is found out that:

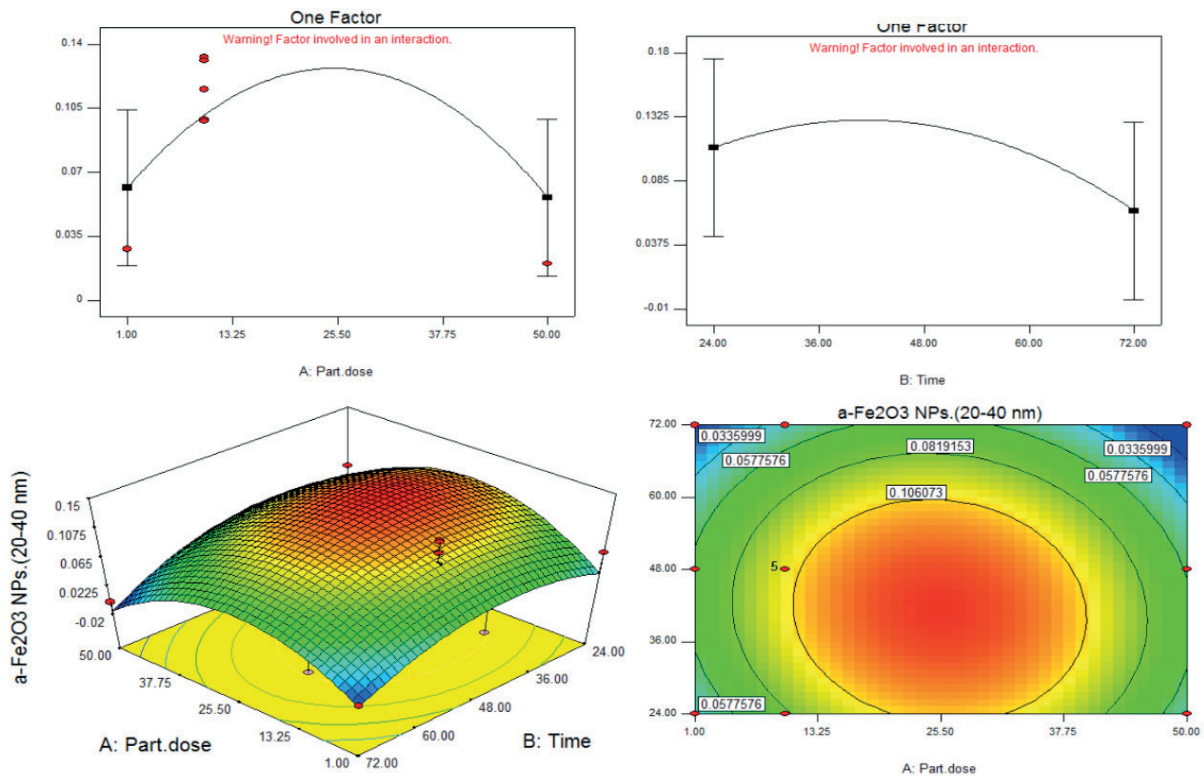


Figure 1.Graphics of statistical data for α -Fe₂O₃ NPs (20-40 nm)

According to the results of statistical table provided for $\gamma\text{-Fe}_2\text{O}_3$ (20-40 nm) NP, correlation coefficient (R^2) value for appropriate Quadratic model is respectively determined as multiple $R=89.78\%$, $R^2=80.61\%$ and adjusted $R^2=66.76\%$. Determination of (R^2) as 80.61% shows that compatibility between observed values and foreseen values is high and applied model takes place in confidence range. The fact that multiple R value is 89.78% indicates that regression is important statistically and 10.22% of total variables cannot

be explained with this model. In order to determine statistical value of this model, "Significance F" value in ANOVA test is examined. The fact that Significance F value is smaller than 0.05 (0.0195) and model F value is 5.82 indicates that the model in 95% confidence range is valuable statistically (Figure 2).

According to mathematical modelling, it is found out that:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \dots\dots\dots(3)$$

$$\text{Abs.} = 0.26 + 0.030 X_1 - 0.085 X_2 - 0.013 X_{12} - 0.25 X_1^2 + 0.037 X_2^2 \dots(4)$$

As a result of the evaluation, it is concluded that among selected parameters for $\gamma\text{-Fe}_2\text{O}_3$ (20-40 nm), particle concentration and period have an impact decreasing absorbance value. When absolute values of coefficients are examined, effect order is period and dose.

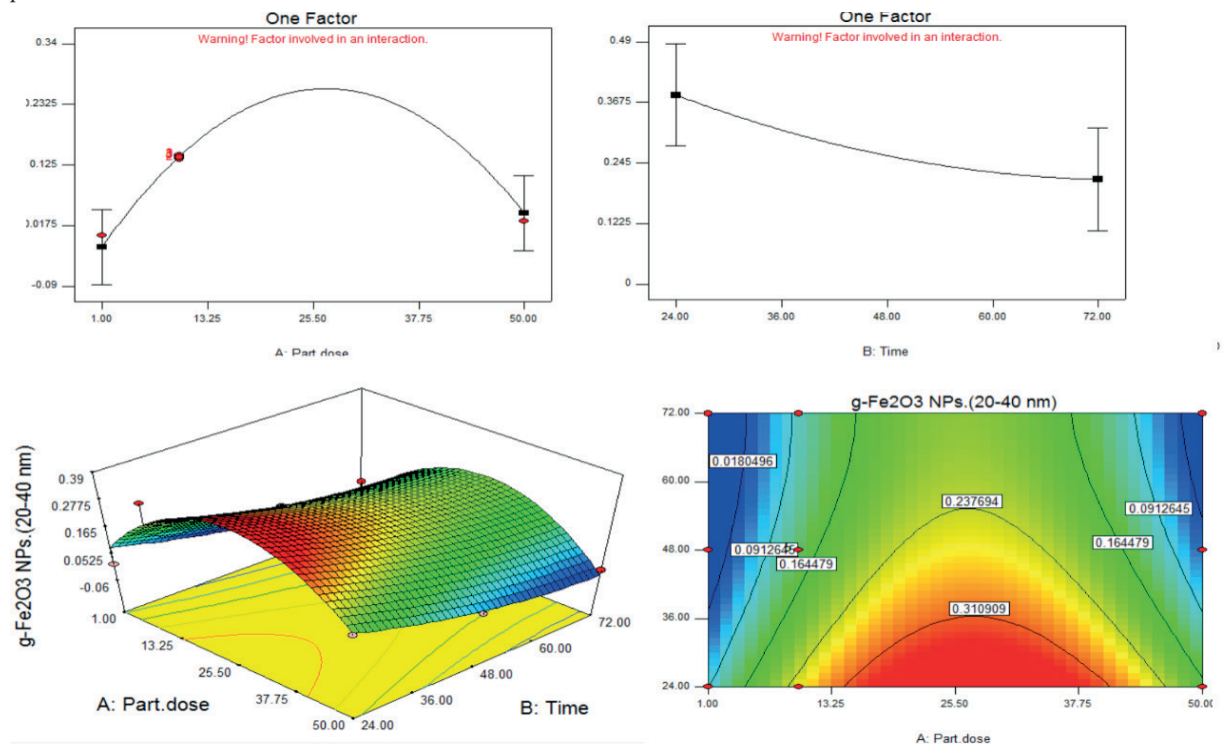


Figure 2. Graphics of statistical data for $\gamma\text{-Fe}_2\text{O}_3$ NPs (20-40 nm)

Considering all statistical data, it is concluded that maximum effect is observed in a period between 24-30 hours and 25 ppm absorbance concentration is more efficient. In all NPs, absorbance value decreases in periods below 24 hours and above 36 hours. Again likewise, the effect decreases in cases where absorbance dose is low or very high. Considering all data, it is concluded that the effect varies according to type and size of NP and this variance is meaningful.

ACKNOWLEDGEMENT

This study contains some of the results of a research, which was supported by TÜBİTAK-CAYDAG (project No: 114Y087).

REFERENCES

[1]. Moore, M.N., (2006). Do nanoparticles present toxicological risks for the health of the aquatic environment? *Environ Int*, 32:967–976.
 [2]. Nowack, B., and Bucheli, T.D., (2007). Occurrence, behavior and effects of nanoparticles in the environment. *Environ Pollut*, 150:5–22.
 [3]. Handy, R., von der Kammer, F., Lead, J., Hassellöv, M., Owen, R., Crane, M., (2008). The ecotoxicology and chemistry of manufactured nanoparticles. *Ecotoxicology*, 17:287–314.
 [4]. Klaine, S.J., Alvarez, P.J.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., (2008). Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. *Environ Toxicol Chem*, 27: 1825–1851.
 [5]. Oberdörster, G., Oberdörster, E., Oberdörster, J., (2005). Nanotoxicology: An emerging discipline evolving from studies of ultrafine particle. *Environ Health Perspect*, 113:823–839.
 [6]. Meng, H., Chen, Z., Xing, G., Yuan, H., Chen, V., Zhao, F., Zhang, C., Zhao, Y., (2007). Ultrahigh reactivity

provokes nanotoxicity: Explanation of oral toxicity of nano-copper particles. *Toxicol Lett*, 175:102–110.

[7]. Papageorgiou, I., Brown, C., Schins, R., Singh, S., Newson, R., Davis, S., Fisher, J., Ingham, E., Case, C.P., (2007). The effect of nano- and micron-sized particles of cobalt-chromium alloy on human fibroblasts in vitro. *Biomaterials*, 28:2946–2958.

[8]. Singh, S., Shi, T., Duffin, R., Albrecht, C., van Berlo, D., Hohr, D., Fubini, B., Martra, G., Fenoglio, I., Borm, P.J., Schins, R.P., (2007). Endocytosis, oxidative stress and IL-8 expression in human lung epithelial cells upon treatment with fine and ultrafine TiO₂: Role of the specific surface area and of surface methylation of the particles. *Toxicol Appl Pharm*, 222:141–151.

[9]. Poland, C.A., Duffin, R., Kinloch, I., Maynard, A., Wallace, W.A., Seaton, A., Stone, V., Brown, S., Macnee, W., Donaldson, K., (2008). Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat Nanotechnol*, 3:423–428.

[10]. Bombin, S., Le Febvre, M., Sherwood, J., Xu, Y., Bao, Y., Ramonell, K.M., (2015). Developmental and reproductive effects of iron oxide nanoparticles in *Arabidopsis thaliana*. *Int. J. Mol. Sci.*, 16, 24174–24193.

[11]. Nations, S., Wages, M., Canas, J.E., Maul, J., Theodorakis, C., Cobb, G.P., (2011). Acute effects of Fe₂O₃, TiO₂, ZnO and CuO nanomaterials on *Xenopus laevis*. *Chemosphere*, 83: 1053–1061.

[12]. Zhu, X., Tian, S., Cai, Z., (2012). Toxicity assessment of iron oxide nanoparticles in zebrafish (*Danio rerio*) early life stages. *PLoS One* 7, e46286.

[13]. Zhang, Y., Zhu, L., Zhou, Y., Chen, J., (2015). Accumulation and elimination of iron oxide nanomaterials in zebrafish (*Danio rerio*) upon chronic aqueous exposure. *J. Environ. Sci.*, 30: 223–230.

[14]. Lodhia, J., Mandarano, G., Ferris, N.J, Eu, P, Cowell, S.F., (2010). Development and use of iron oxide nanoparticles (Part 1): synthesis of iron oxide nanoparticles for MRI. *Biomed Imaging Interv J* 6:e12.

[15]. Hadjipanayis, C.G., Machaidze, R., Kaluzova, M., Wang, L., Schuette, A.J., Chen, H., Mao, X., Wu, H., (2010). EGFRvIII antibody-conjugated iron oxide nanoparticles for magnetic resonance imaging-guided convection-enhanced delivery and targeted therapy of glioblastoma. *Cancer Res*, 70:6303–6312.

[16]. Mohapatra, M., Anand, S., (2010). Synthesis and applications of nanostructured iron oxides/hydroxides—a review. *Inter J Eng Sci Technol*, 2:127–146.

[17]. Waseem, M., Munsif, S., Rashid, U. and ud-Din, I., (2014). Physical properties of α-Fe₂O₃ nanoparticles fabricated by modified hydrolysis technique. *Appl Nanosci*, 4:643–648