

## Stabilizing and dispersing methods of TiO<sub>2</sub> nanoparticles in biological studies

Maliheh Hasan Nia<sup>1</sup>, Mostafa Rezaei-Tavirani<sup>2</sup>, Ali Reza Nikoofar<sup>3</sup>, Hamed Masoumi<sup>4</sup>, Reza Nasr<sup>1</sup>, Hadi Hasanazadeh<sup>5,\*</sup>, Majid Jadidi<sup>4</sup>, Mahdi Shadnush<sup>6</sup>

<sup>1</sup> Biotechnology Research Center, Semnan University of Medical Sciences, Semnan, Iran

<sup>2</sup> Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>3</sup> Department of Radiation Oncology, Iran University of Medical Sciences, Tehran, Iran

<sup>4</sup> Department of Medical Physics, Semnan University of Medical Sciences, Semnan, Iran

<sup>5</sup> Cancer Research Center & Department of Medical Physics, Semnan University of Medical Sciences, Semnan, Iran

<sup>6</sup> Department of Nutrition, Semnan University of Medical Sciences, Semnan, Iran

\*Corresponding Author: email address: [Hasanzadeh.h@semums.ac.ir](mailto:Hasanzadeh.h@semums.ac.ir) (H. Hasanazadeh)

### ABSTRACT

TiO<sub>2</sub> nanoparticles (NPs) might be considered as the most important photosensitizer due to high photocatalytic and sonocatalytic efficiency, low toxicity, excellent biocompatibility, low cost and high chemical stability. TiO<sub>2</sub>-NPs normally tend to aggregate in physiological medium and which results to decreased cell viability and inducing expression of stress-related genes. Thus dispersion and stability of TiO<sub>2</sub> NPs should be considered in biological application. This paper deals on various dispersing methods such as ultrasonication, electrostatic, steric electrosteric stabilization that suppress agglomeration and stabilizes the dispersed NPs in aqueous medium.

Sonication breaks up agglomerated NPs in a solvent. The results showed that probe sonication performs better than bath sonication in dispersing TiO<sub>2</sub> agglomerates, but sonication couldn't prevent long term aggregation of nanoparticles and in order to form stable dispersions, it is not enough to break nanoparticles apart. Agglomerated NPs can be separated by overcoming the weaker attractive forces by electrostatic, steric or electrosteric interactions. Electrostatic stabilization takes place when charges accumulate at the surface of particles. At values of potential more than 30 mV or less than -30 mV no agglomeration occurs. Ionic strength and pH influence on electrostatic stabilization; when pH is far from the isoelectric point, agglomeration is suppressed. In a sterically stabilized dispersion large molecules such as polymers, surfactants and biomolecules, adsorbed on the surface of particles suppress re-agglomeration. PEG is a hydrophilic polymer, non-toxic and non-immunogenic, and has favorable pharmacokinetics and tissue distribution. PEGylation of NPs not only prevents agglomeration, but also enhances their biocompatibility and increases the *in vivo* circulation time.

**Keywords:** TiO<sub>2</sub> nanoparticles; Dispersion; Stability.

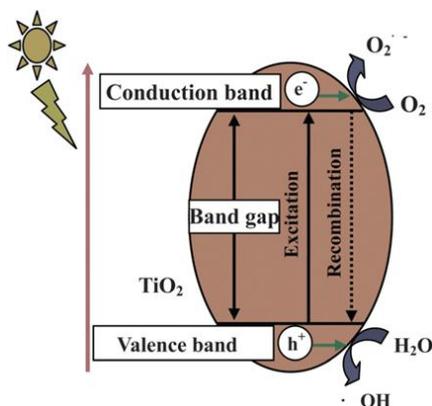
### INTRODUCTION

In recent years, Titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs) has been widely used in phototherapy of cancer cells, bacteria [1] and are potential photosensitizing agents for photodynamic therapy (PDT) due to their high stability and unique phototoxic effects upon irradiation [2]. After the first report of photo-catalytic splitting of water on a TiO<sub>2</sub> electrode under ultraviolet (UV) irradiation in 1972, TiO<sub>2</sub> due to its nontoxicity, strong optical absorption, high chemical stability

and low cost has been extensively used in photo-catalysis, photo-degradation, photovoltaic cells and electro-chromic devices [3]. Among all applications, using TiO<sub>2</sub>-NPs in medicine has also drawn significant attention, especially in treatment of cancer [4-6].

TiO<sub>2</sub>-NPs show photo-catalytic activity under UV irradiation with the band gap of 3.23 eV for anatase and 3.06 eV for rutile. Absorption of UV irradiation with energies higher than the band gap with wavelength of 385 and 400 nm for anatase

and rutile, results in the formation of electron ( $e^-$ ) and hole ( $h^+$ ) [7] (figure 1). In aqueous environments, these photo-induced electrons and holes could react with hydroxyl ions or water to form reactive oxygen species such as hydrogen peroxide, hydroxyl radicals and super-oxides which are capable of killing cancer cells and destroying the structure of bacteria [8].



**Figure 1.** Schematic illustration of main processes in a photo-catalytic reaction [9]

TiO<sub>2</sub>-NPs are typically insoluble in aqueous medium, hampering the utilization of TiO<sub>2</sub> in biological applications, so how to enhance the water-soluble activity of TiO<sub>2</sub> needs to be investigated [10]. This article summarizes important factors that control the state of TiO<sub>2</sub>-NPs dispersion in aqueous medium, such as solution ionic strength (IS), pH, surface charge and surface coating. We also review the recent biomedical applications of TiO<sub>2</sub> including photodynamic therapy, sonodynamic therapy and phototherapy of cancer and various method of water-dispersed TiO<sub>2</sub>-NPs for improving efficiency of cancer treatment [11-14].

#### **Stabilization of TiO<sub>2</sub>-NPs in aqueous medium**

Many nanoparticle samples used in toxicological studies should be effectively dispersed in water for in vitro and in vivo applications. In order to disperse nanoparticles, an external force is needed to overcome the van der Waals attractions. Electrostatic and steric stabilization suppresses agglomeration and stabilizes the dispersed NPs [15, 16]. It has shown that addition of as high as 90% of TiO<sub>2</sub>-NPs to water results to accumulation and particle size of

these agglomerated NPs would be estimated larger than 3  $\mu\text{m}$  which presumably might be due to hydrophobic nature of the TiO<sub>2</sub>-NPs surface and electrostatic attraction of the TiO<sub>2</sub>-NPs powders used [17]. Below, the influence of different modalities on resultant hydrodynamic size and agglomeration/aggregation state of NPs are discussed.

#### **Ultrasonic irradiation**

Sonication is commonly used to break up agglomerated NPs which usually performed in a solvent. The size of dispersed NPs depends on the suspension conditions such as solvent type, concentration and volume of suspension solution [18]. In sonication, oscillation of liquid cause nucleation and collapse of solvent bubbles; bubble formation and collapse at the surface of solids can be very effective in chopping solids. In this method, breaking the agglomerates is mainly controlled by power, time and dispersion volume [19]. Sonication is performed by two methods: bath sonication and probe sonication [11]. Oberdorster et al showed that probe sonication performs better than bath sonication in dispersing TiO<sub>2</sub> agglomerates when sodium pyrophosphate was used as stabilizing agent [11] but sonication couldn't prevent long term aggregation of nanoparticles and in order to form stable dispersions, it is not enough to break nanoparticles apart [20].

There exist several stabilization methods to disperse colloidal and synthesized metal oxide nano-powders such as electrostatic, steric and electrosteric interactions [16, 21-23].

The stability of nanoparticle dispersions and their tendency to agglomerate can be determined by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [24]; in this theory, attraction between particles is due to the van der Waals force and the electrical double layer surrounding each particle is called electrostatic repulsive force. Agglomerated NPs can be separated by overcoming the weaker attractive forces by electrostatic, steric or electrosteric interactions. Electrostatic stabilization takes place when charges accumulate at the surface of particles. In a sterically stabilized dispersion large molecules adsorbed on to the surface of particles suppress re-agglomeration. Electrosteric stabilization uses

a combination of electrostatic and steric mechanisms [25].

#### **Electrosteric stabilization**

Surface charge of the particles is a function of zeta potential. In dispersions where Zeta potential is close to zero (isoelectric point, the intermediate pH where a particle has zero surface charge), particles tend to agglomerate. At values of potential more than 30 mV or less than -30 mV no agglomeration occurs. The agglomeration depends on the effective pH, solvent, concentration of ions and the functional groups at the surface of nanoparticles [11]. The powders of different manufacturers due dissimilar surface chemistries have various isoelectric points [25].

#### **pH effects**

For dispersion of the nanoparticle in water, surface ionization controls their surface charge in the absence of soluble ions in solution [26]. The hydrodynamic size of nanoparticles can be adjusted by changing the solution pH [17]. Mineral oxides and sulfides such as  $\text{TiO}_2$ ,  $\text{SiO}_2$  and AsS have a positive surface charge at low pH and conversely at high pH, have a negative surface charge [26]. In the pH of lower than isoelectric point pH, particles indicate a positive surface charge, while the surface charge of particles is negative when pH is higher than isoelectric point pH [27].

The results of studies have shown a strong correlation between zeta potential and hydrodynamic size of nanoparticles [11, 17, 27]. When pH is far from the isoelectric point, the electrostatic repulsive force overcomes the van der Waals force, such that agglomeration is suppressed [27]. Adjustment of the pH to increase particle surface charge can increase the repulsive force between particles.

#### **Ionic strength effects**

According to the DLVO theory, increasing ionic strength is raising the aggregation of nanoparticles [24]. The studies indicate zeta potential decreases in ionic strength above 0.01M, while at low ionic strength there is no obvious change of zeta potential [11]. These results are consistent with the classical colloidal theory [28]. Increasing ionic strength leads to the compression of the electrical double layer [26]. Hence, the zeta potential decreases with increasing ionic strength

.The salts are containing the multiply charged ions such as polyphosphate, hexametaphosphate, pyrophosphate and polysilicate anions can be alternatively applied as dispersing salt. The multiple charged ions might be adsorbed by the particle in an aqueous environment and leads to an increase in particle surface charge and zeta potential [11].

Jiang et al indicated the adsorption of pyrophosphate ions at the  $\text{TiO}_2$  particle surfaces changes the zeta potential from positive (approximately 40 mV) to negative (approximately -53 mV). Though the ionic strength increases with increasing sodium pyrophosphate concentration, no change in the dispersion size distribution was observed up to the maximum  $\text{Na}_4\text{P}_2\text{O}_7$  concentration tested (0.01 M) [11].

#### **Steric stabilization**

Steric stabilization prevent the aggregation of nanoparticles in aqueous by coating them with polymers [29], surfactants [30] and biomolecules [31]. Unlike the electrostatic stabilization that is only efficient at ionic strength less than 0.1 M, steric stabilization is possible at all ionic strengths [11]. In recent years, scientists have used various materials for steric stabilization of nanoparticles in aqueous solutions which discussed below.

#### **Coating nanoparticles by polymers**

The polymer adsorbed on the  $\text{TiO}_2$  nanoparticle lead to steric repulsion between particles and prevents the agglomeration of nanoparticles [21]. Therefore nanoparticles are stable even at high ionic strength (0.1 M saline solution). Increased steric repulsion of the polymer reduces the size of the nanoparticles [21]. The researchers use several polymers for steric stabilization of  $\text{TiO}_2$  nanoparticles [29, 32, 33]. Diess et al used polyethylene glycol (PEG) and polyacrylamide (PAM) for steric stabilization  $\text{TiO}_2$  sols. Their results indicate the aggregation of  $\text{TiO}_2$  sol increases at high concentration and pH. The  $\text{TiO}_2$  sols Coated with PEG leads to stability of these sols at pH below 3 and concentration up to  $30 \text{ g l}^{-1}$  while the polyacrylamide (PAM) stabilized sols of concentration close to  $100 \text{ g l}^{-1}$  at pH up to 5, which lead to long-term stability of these sols [23].

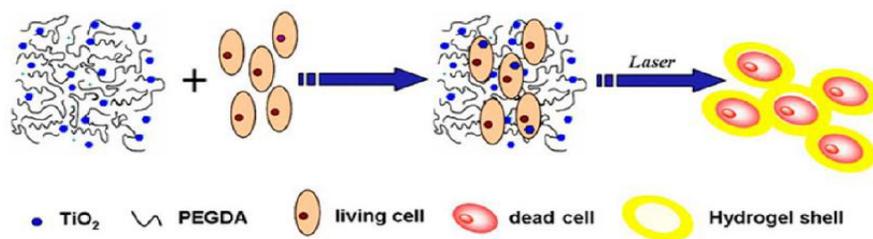
Jiang et al used quantum dot (QDs) nanocrystals with different surface coating (PEG, PEG-NH<sub>2</sub>, PEG-COOH) for stabilizing TiO<sub>2</sub> in physiological saline solution (0.15M NaCl). The QDs with different surface groups lead to the difference of the size distributions particles due to their different zeta potentials. Stabilizing TiO<sub>2</sub> particles with polymer-coated QD were due electrostatic repulsive forces and steric repulsive forces simultaneously. The TiO<sub>2</sub> particles coated with carboxylic-terminated polyethylene glycol surface-modified QDs had the highest surface charge (-40 mV) and the smallest hydrodynamic size, while QDs with PEG had the lowest surface charge (-0.3 mV) and the largest hydrodynamic size [11].

Terasaka et al constructed water-dispersed TiO<sub>2</sub> nanoparticles by the adsorption of chemical modified polyethyleneglycol (PEG) on the surface of TiO<sub>2</sub> nanoparticles (TiO<sub>2</sub>-PEG). They also investigated the photocatalytic antitumor effect of water-dispersed TiO<sub>2</sub> nanoparticles on C6 rat glioma cells to evaluate the treatment responses by the spheroid tumor models. Their results indicated that cytotoxic effects depend on both the concentration of TiO<sub>2</sub>-PEG and dose of UV irradiation. In TiO<sub>2</sub>-PEG-treated spheroids, the number of Annexin V-FITC-stained cells gradually increased during the first 6 h, and subsequently Propidium iodide stained cells appeared. The results of this study suggest that newly developed photo-excited TiO<sub>2</sub>-PEG have antitumor activity. Photodynamic therapy utilizing this material can be a clue to a novel therapeutic strategy for glioma [29].

Titanium dioxide (TiO<sub>2</sub>) is a type of nanoparticle that is widely used in biomedical applications and thus, knowledge about its effects on human health

and the environment is necessary. Hence, Taniguchi et al focused on understanding the mechanism of TiO<sub>2</sub> NP-induced Nano-toxicity through the evaluation of biomarkers. They indicated that modifying TiO<sub>2</sub> NPs with PEG reduces their cytotoxicity and stress-related genes [34]. TiO<sub>2</sub> NPs tend to aggregate in aqueous media, and these aggregates decrease cell viability and induce expression of stress-related genes, such as those encoding interleukin-6 (IL-6) and heat shock protein 70B' (HSP70B'), indicating that TiO<sub>2</sub> NPs induce inflammatory and heat shock responses. Polyethylene glycol (PEG) is a hydrophilic polymer with repeating ethylene ether units. PEG is inexpensive, versatile, and FDA-approved for many applications [35]. In addition, PEG is non-toxic and non-immunogenic, and has favorable pharmacokinetics and tissue distribution [36]. Modifying the surface of NPs with PEG not only prevents agglomeration [37], but also renders NPs resistance to protein adsorption and enhances their biocompatibility [38]. Coating nanomaterials with PEG also increases the in vivo circulation time, thereby likely reducing clearance via the reticuloendothelial system (RES) [39].

Li et al prepared a novel inorganic/organic hybrid hydrogel system containing poly (ethylene glycol) double acrylates (PEGDA)-modified titanium dioxide (TiO<sub>2</sub>) and applied it for photodynamic therapy (PDT) (Fig. 2). Drug-loaded hydrogel shell formed around tumor cells were preventing TiO<sub>2</sub> from migrating to normal tissue. The hybrid hydrogel system created a high concentration of singlet oxygen (<sup>1</sup>O<sub>2</sub>) under NIR irradiation, Also, the hydrogel shell were photochemically recyclable and could be reused in regular treatment [33].



**Figure 2.** TiO<sub>2</sub>/PEGDA Hydrogel Formed on Tumor Cells by Photo-polymerization [33]



Shimizu et al immobilized pre-S1/S2 protein from hepatitis B virus to the surface of TiO<sub>2</sub> nanoparticles using an amino-coupling method. The protein-modified TiO<sub>2</sub> nanoparticles could be identified by liver cells that were confirmed by surface Plasmon resonance analysis and immunostaining analyses [12]. Sonication also utilized instead of UV to produce OH radicals by activating protein-modified TiO<sub>2</sub> nanoparticles. This method was applied for sonodynamic therapy of HepG2 cancer cells by sonication intensity of 0.4 W/cm<sup>2</sup> [12].

In another study, this group investigated the uptake behavior of TiO<sub>2</sub> NPs modified with pre-S1/S2 by HepG2 cells for 24 h [43]. Their results indicated that uptake of the TiO<sub>2</sub> NPs by HepG2 cells was performed for 6 h. Then, the application of sonodynamic therapy on HepG2 cell growth was checked for 96 h after the 1 MHz sonication (0.1 W/cm<sup>2</sup>, 30 s) by pre-S1/S2 -modified TiO<sub>2</sub> nanoparticles. Apoptosis was observed at 6 h after this treatment. Although no apparent cell-injury was observed until 24 h after the treatment, the viable cell concentration had reduced to 46% of the control at 96 h. Finally pre-S1/S2 -modified TiO<sub>2</sub> nanoparticles (0.1 mg) was directly injected into tumors of a mouse Xenograft model and sonication was performed at 1 MHz (1.0 W/cm<sup>2</sup> for 60 s). As a result of the treatment repeated five times within 13 days, tumor growth could be hampered up to 28 days compared to the control group [43].

Elvira et al successfully coupled monoclonal antibody (mAb) Nilo1 to TiO<sub>2</sub> nanoparticles which can recognize the surface antigen in neural stem cells (44). Nilo1-TiO<sub>2</sub> complexes can be used to specifically deplete in vitro cancer stem cells upon UV-irradiation. mAb-TiO<sub>2</sub> complexes were activated under UV-irradiation led to selective removal of the antibody-targeted cells. Furthermore, TiO<sub>2</sub> nanoparticles can be directed to a particular target by coupling them to an appropriate monoclonal antibody recognizing cell surface molecules [44].

Shimizu et al prepared TiO<sub>2</sub> NPs modified with avidin protein and investigated its application in sonodynamic therapy of the breast cancer cells (MCF-7). They first, modified the surfaces of the TiO<sub>2</sub> NPs with polyacrylic acids (PAA) to prevent

the aggregation of TiO<sub>2</sub> NPs under physiological conditions [32]; then Avidin protein (68 kDa) was immobilized on the surfaces of the PAA-modified TiO<sub>2</sub> NPs via chemical coupling at the carboxyl residue of PAA [12, 32]. The uptake of avidin immobilized TiO<sub>2</sub> NPs by healthy and cancerous cells was examined. The results of this study indicated that 30% of the normal breast cells (human mammary epithelial cells) exhibited the uptake of avidin-modified TiO<sub>2</sub> NPs, although over 80% of the breast cancer cells (MCF-7) exhibited the uptake of avidin-TiO<sub>2</sub>NPs. After that, avidin-modified TiO<sub>2</sub> NPs were activated by external sonication (TiO<sub>2</sub>/US treatment) to generate hydroxyl radicals. Next the TiO<sub>2</sub>/US treatment on MCF-7 cell growth for up to 96 h after 1-MHz ultrasound (0.1W/cm<sup>2</sup>, 30 s) was investigated; the results of which indicated no apparent cell injury until 24 h after the treatment, but the viable cell concentration declined to 68% compared with the control at 96 h [45].

#### **Electrosteric stabilization**

Electrosteric stabilization prevents nanoparticles from agglomeration by the combination of electrostatic and steric mechanisms [25]. Proteins, serum, and chemical surfactants are often used as Electrosteric stabilization [46].

Bihari et al used various stabilizing agents such as human, bovine, and mouse serum albumin, Tween 80, and mouse serum to prevent nanoparticle agglomeration (TiO<sub>2</sub> in the form of anatase and rutile), ZnO, Ag, SiO<sub>x</sub>, SWNT, MWNT in distilled water, PBS, or RPMI 1640 cell culture medium. The optimal sequence was first sonication of nanoparticles in water, adding dispersion stabilizers and finally addition of buffered salt solution. This study indicated 1.5 mg/ml of human, bovine or mouse serum albumin, or mouse serum to TiO<sub>2</sub> (rutile) prevented agglomeration in PBS or RPMI medium. The required concentration of albumin to stabilize the nanoparticle dispersion depended on the concentration of the nanoparticles in the dispersion. TiO<sub>2</sub> (rutile) particle dispersions at a concentration lower than 0.2 mg/ml could be stabilized by the addition of 1.5 mg/ml albumin. TiO<sub>2</sub> (rutile) particle dispersions prepared by this method were stable up to at least 1 week. This

method was suitable for stabilized nanoparticle dispersion (average diameter < 290 nm) TiO<sub>2</sub> (rutile), ZnO, Ag, SiO<sub>x</sub>, SWNT, MWNT, and diesel SRM2975 particulate matter [30].

Ji et al applied bovine serum albumin (BSA) as a model protein and fetal bovine serum (FBS) as a protein rich serum to improve the TiO<sub>2</sub> (p25) nanoparticle in six different mammalian, bacteria, and yeast cell culture media [31].

Protein molecules could be absorbed onto a nanoparticle's surface through electrostatic interaction, hydrophobic interaction, or specific chemical interaction [13, 47]. pH and ionic strength of the media determine the type of interaction between protein and nanoparticles. At pH close to the isoelectric point (IEP) of BSA (~4.7) is hydrophobic and the adsorption can occur through hydrophobic interactions. Above this pH, BSA becomes negatively charged and below the IEP, BSA is positively charged; in these condition electrostatic forces become dominant over hydrophobic. Thus steric or electrosteric interactions of proteins may also play an important role during the nanoparticle dispersion. The type of TiO<sub>2</sub> nanoparticle dispersion from medium to medium depend on the water chemistry and therefore different adsorption mechanisms of BSA. In general, the maximum adsorption of BSA occurred around the IEP of BSA, in the pH range of 4-5 [14]. Their results also indicated that phosphate ions play an important role in the adsorption/desorption process of BSA and consequently the stability of the TiO<sub>2</sub> nanoparticle suspension. FBS acted as best dispersing agent in these studies for stabilizing TiO<sub>2</sub> nanoparticles in all six cell cultures due the synergistic effect of various proteins in FBS. The results of this study indicated 2 mgmL<sup>-1</sup> of BSA and 1% V/V of FBS (with an average hydrodynamic diameter of 200-300 nm) was already sufficient for stabilizing 50 µgmL<sup>-1</sup> of TiO<sub>2</sub> in all six cell culture media [31].

An increasing selective antitumor activity and decreasing non-selective cell death of TiO<sub>2</sub> nanoparticles can specifically identify and bind with the receptors of cancer cells [40].

## RESULTS AND DISCUSSION

Titanium dioxide nanoparticles have been widely used in biomedical due to low toxicity, excellent biocompatibility, high photocatalytic and sonocatalytic efficiency. TiO<sub>2</sub>-NPs are typically accumulated in biological medium; hence nanoparticle samples used in toxicological studies should be effectively dispersed in water for in vitro and in vivo applications. Ultrasonic irradiation, electrostatic, steric and electrosteric stabilization suppresses agglomeration and stabilizes the dispersed NPs in aqueous medium. Sonication breaks up agglomerated NPs in a solvent. The results showed that probe sonication performs better than bath sonication in dispersing TiO<sub>2</sub> agglomerates [11], but sonication couldn't prevent long term aggregation of nanoparticles and in order to form stable dispersions, it is not enough to break nanoparticles apart [20].

The stability of nanoparticle dispersions and their tendency to agglomerate can be determined by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [24]; in this theory, attraction between particles is due to the van der Waals force and the electrical double layer surrounding each particle. Agglomerated NPs can be separated by overcoming the weaker attractive forces by electrostatic, steric or electrosteric interactions.

Electrostatic stabilization takes place when charges accumulate at the surface of particles. Surface charge of the particles is a function of zeta potential. Particles tend to agglomerate when Zeta potential is close to zero. At values of potential more than 30 mV or less than -30 mV no agglomeration occurs. pH and Ionic strength influence on electrostatic stabilization. When pH is far from the isoelectric point, the electrostatic repulsive force overcomes the van der Waals force, such that agglomeration is suppressed [27]. Increasing ionic strength leads to the compression of the electrical double layer [26] thus, the zeta potential decreases with increasing ionic strength. The salts are containing the multiply charged ions such as polyphosphate, hexametaphosphate, pyrophosphate and polysilicate anions can be alternatively applied as dispersing salt. The studies indicate electrostatic stabilization can be used at ionic strength less than 0.1 M, While ionic strength of the physiological saline solution (0.15 M NaCl), for in vitro and in vivo studies is often

higher than 0.1 M [48]. In these conditions, dispersed nanoparticles are highly agglomerated and unstable [48]. Also, in many biological studies, pH adjustment range is limited for healthy function of test cells and organisms [11]. In a sterically stabilized dispersion large molecules such as polymers [29], surfactants [30] and biomolecules [31], adsorbed on to the surface of particles suppress re-agglomeration. PEG is a hydrophilic polymer, non-toxic and non-immunogenic, and has favorable pharmacokinetics and tissue distribution [36]. PEGylation of NPs not only prevents agglomeration [37], but also renders NPs resistance to protein adsorption and enhances their biocompatibility [38]. Coating nanomaterials with PEG also increases the *in vivo* circulation time, thereby likely reducing clearance via the reticuloendothelial system (RES) [39].

Chemical surfactants such as Tween 80 and sodium dodecyl sulfate are toxic; while the nontoxic biologically relevant species such proteins or antibodies would be the most suitable candidate [31].

In recent years, scientists prepared the modified TiO<sub>2</sub> nanoparticles with monoclonal antibody proteins such CEA [40], pre-S1/S2 [12], IL13a2R [41] and EGFR [42]. These proteins are existed at the surface of certain cancer cells leading to directing nanoparticles towards the specific cell population [40].

## REFERENCES

1. Tsuang YH, Sun JS, Huang YC, Lu CH, Chang WHS, Wang CC. Studies of photokilling of bacteria using titanium dioxide nanoparticles. *Artif Organs*. 2008; 32(2): 167-74.
2. Wamer WG, Yin J-J, Wei RR. Oxidative damage to nucleic acids photosensitized by titanium dioxide. *Free Radic Biol Med*. 1997; 23(6): 851-8.
3. Pang CL, Lindsay R, Thornton G. Chemical reactions on rutile TiO<sub>2</sub> (110). *Chem Soc Rev*. 2008; 37(10): 2328-53.
4. Rezaei-Tavirani M, Dolat E, Hasanzadeh H, Seyyedi S-S, Semnani V, Sobhi S. TiO<sub>2</sub> Nanoparticle as a Sensitizer Drug in Radiotherapy: *in Vitro* Study. *Iran J Cancer Prev*. 2013; 6: 37-44.

## CONCLUSION

In this review, the influence of important parameters on state and stability of TiO<sub>2</sub> nanoparticle dispersions have been discussed including solution ionic strength, pH, surface charge, and surface modification. Ionic strength leads to changing the electrical double layer thickness, while pH can change the dispersion state. Increasing ionic strength or pH around of the nanoparticle isoelectric point will enhance agglomeration and result in larger hydrodynamic sizes. Adsorbed multiply charged ions, polymer coatings, proteins and antibody on nanoparticle surfaces can suppress agglomeration and stabilize nanoparticle dispersions. The modified TiO<sub>2</sub> nanoparticles with monoclonal antibody and proteins in addition to stabilizing of TiO<sub>2</sub> nanoparticles in physiological solutions lead to directing the nanoparticles towards the specific cancer cells due to binding with the cancer cells receptors. These studies have important implications in performance of toxicological studies, such as preparing nanoparticle dispersions for *in vitro* or *in vivo* studies and interpretation of biological responses.

## ACKNOWLEDGMENTS

This work was a part of Mr. Masoumi thesis for the degree of M.Sc. in Medical Physics and also a common project supported by Semnan University of Medical Sciences and Shahid Beheshti University of Medical Sciences.

5. Dolat E, Hasanzadeh H, Tavirany MR, keshel SH, Arfaee AJ, Seyyedi S, et al. Evaluation of Synergistic Effect of TiO<sub>2</sub> Nanoparticles and Gamma Rays on Human Breast Cancer Cell Line. *Scientific Journal of Ilam University of Medical Sciences*. 2013; 20(4): 223-30.
6. Dolat E, Hasanzadeh H, Rezaei Tavirany M, Semnani V, Jabbari Arfaee A, Seyyedi S, et al. Evaluation of radio-sensitizing effect of TiO<sub>2</sub> nanoparticles on human gastric cancer cell line. *Scientific Journal of Ilam University of Medical Sciences*. 2013; 20(4): 121-8.
7. Ashikaga T, Wada M, Kobayashi H, Mori M, Katsumura Y, Fukui H, et al. Effect of the photocatalytic activity of TiO<sub>2</sub> on plasmid DNA. *Mutat Res*. 2000; 466(1): 1-7.

8. Serpone N, Salinaro A, Horikoshi S, Hidaka H. Beneficial effects of photo-inactive titanium dioxide specimens on plasmid DNA, human cells and yeast cells exposed to UVA/UVB simulated sunlight. *Journal of Photochemistry and Photobiology A: Chemistry*. 2006; 179(1): 200-12.
9. Yin ZF, Wu L, Yang HG, Su YH. Recent progress in biomedical applications of titanium dioxide. *Phys Chem Chem Phys*. 2013; 15(14): 4844-58.
10. Seo Jw, Chung H, Kim My, Lee J, Choi Ih, Cheon J. Development of Water Soluble Single Crystalline TiO<sub>2</sub> Nanoparticles for Photocatalytic Cancer Cell Treatment. *Small*. 2007; 3(5): 850-3.
11. Jiang J, Oberdörster G, Biswas P. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. *J Nanopart Res*. 2009; 11(1): 77-89.
12. Ogino C, Shibata N, Sasai R, Takaki K, Miyachi Y, Kuroda S-i, et al. Construction of protein-modified TiO<sub>2</sub> nanoparticles for use with ultrasound irradiation in a novel cell injuring method. *Bioorg Med Chem Lett*. 2010; 20(17): 5320-5.
13. Cedervall T, Lynch I, Foy M, Berggård T, Donnelly SC, Cagney G, et al. Detailed identification of plasma proteins adsorbed on copolymer nanoparticles. *Angew Chem Int Ed Engl*. 2007; 46(30): 5754-6.
14. Kopac T, Bozgeyik K, Yener J. Effect of pH and temperature on the adsorption of bovine serum albumin onto titanium dioxide. *Colloids Surf A Physicochem Eng Asp*. 2008; 322(1): 19-28.
15. Heijman S, Stein H. Electrostatic and sterical stabilization of TiO<sub>2</sub> dispersions. *Langmuir*. 1995; 11(2): 422-7.
16. Widegren J, Bergström L. Electrostatic stabilization of ultrafine titania in ethanol. *J Am Ceram Soc*. 2002; 85(3): 523-8.
17. Tso C-p, Zhung C-m, Shih Y-h, Tseng Y-M, Wu S-c, Doong R-a. Stability of metal oxide nanoparticles in aqueous solutions. *Water Sci Technol*. 2010; 61(1): 127-33.
18. Kusters KA, Pratsinis SE, Thoma SG, Smith DM. Energy—size reduction laws for ultrasonic fragmentation. *Powder Technol*. 1994; 80(3): 253-63.
19. Thoma SG, Ciftcioglu M, Smith DM. Determination of agglomerate strength distributions Part 3. Application to titania processing. *Powder Technol*. 1991; 68(1): 71-8.
20. Baveye P, Laba M. Aggregation and toxicology of titanium dioxide nanoparticles. *Environ Health Perspect*. 2008; 116(4): A152.
21. Mates TE, Ring T. Steric stability of alkoxy-precipitated TiO<sub>2</sub> in alcohol solutions. *Colloids and Surfaces*. 1987; 24(4): 299-313.
22. Wassell DTH, Hall RC, Embery G. Adsorption of bovine serum albumin onto hydroxyapatite. *Biomaterials*. 1995; 16(9): 697-702.
23. Deiss J, Anizan P, El Hadigui S, Wecker C. Steric stability of TiO<sub>2</sub> nanoparticles in aqueous dispersions. *Colloids Surf A Physicochem Eng Asp*. 1996; 106(1): 59-62.
24. Derjaguin B. Theory of the stability of strongly charged lyophobic sols and the adhesion of strongly charged particles in solutions of electrolytes. *Acta Physicochim USSR*. 1941; 14: 633-62.
25. Mandzy N, Grulke E, Druffel T. Breakage of TiO<sub>2</sub> agglomerates in electrostatically stabilized aqueous dispersions. *Powder Technol*. 2005; 160(2): 121-6.
26. Morrison ID, Ross S. *Colloidal dispersions: suspensions, emulsions, and foams*: Wiley-Interscience; 2002.
27. French RA, Jacobson AR, Kim B, Isley SL, Penn RL, Baveye PC. Influence of ionic strength, pH, and cation valence on aggregation kinetics of titanium dioxide nanoparticles. *Environ Sci Technol*. 2009; 43(5): 1354-9.
28. Hunter RJ. *Zeta potential in colloid science: principles and applications*. 1981.
29. Yamaguchi S, Kobayashi H, Narita T, Kanehira K, Sonezaki S, Kubota Y, et al. Novel Photodynamic Therapy Using Water dispersed TiO<sub>2</sub>-Polyethylene Glycol Compound: Evaluation of Antitumor Effect on Glioma Cells and Spheroids In Vitro. *Photochem Photobiol*. 2010; 86(4): 964-71.
30. Bihari P, Vippola M, Schultes S, Praetner M, Khandoga AG, Reichel CA, et al. Optimized dispersion of nanoparticles for biological in vitro

- and in vivo studies. *Part Fibre Toxicol.* 2008; 5(1):14.
31. Ji Z, Jin X, George S, Xia T, Meng H, Wang X, et al. Dispersion and stability optimization of TiO<sub>2</sub> nanoparticles in cell culture media. *Environ Sci Technol.* 2010; 44(19): 7309-14.
32. Kanehira K, Banzai T, Ogino C, Shimizu N, Kubota Y, Sonezaki S. Properties of TiO<sub>2</sub>-polyacrylic acid dispersions with potential for molecular recognition. *Colloids and Surfaces B: Biointerfaces.* 2008; 64(1): 10-5.
33. Zhang H, Shi R, Xie A, Li J, Chen L, Chen P, et al. Novel TiO<sub>2</sub>/PEGDA Hybrid Hydrogel Prepared in Situ on Tumor Cells for Effective Photodynamic Therapy. *ACS Appl Mater Interfaces.* 2013; 5(23): 12317-22.
34. Mano SS, Kanehira K, Sonezaki S, Taniguchi A. Effect of polyethylene glycol modification of TiO<sub>2</sub> nanoparticles on cytotoxicity and gene expressions in human cell lines. *Int J Mol Sci.* 2012; 13(3): 3703-17.
35. Jokerst JV, Lobovkina T, Zare RN, Gambhir SS. Nanoparticle PEGylation for imaging and therapy. *Nanomedicine.* 2011; 6(4): 715-28.
36. Wang W, Xiong W, Wan J, Sun X, Xu H, Yang X. The decrease of PAMAM dendrimer-induced cytotoxicity by PEGylation via attenuation of oxidative stress. *Nanotechnology.* 2009; 20(10): 105103.
37. Matsumura S, Sato S, Yudasaka M, Tomida A, Tsuruo T, Iijima S, et al. Prevention of carbon nanohorn agglomeration using a conjugate composed of comb-shaped polyethylene glycol and a peptide aptamer. *Mol Pharm.* 2009; 6(2): 441-7.
38. Eck W, Craig G, Sigdel A, Ritter G, Old LJ, Tang L, et al. PEGylated gold nanoparticles conjugated to monoclonal F19 antibodies as targeted labeling agents for human pancreatic carcinoma tissue. *Acs Nano.* 2008; 2(11): 2263-72.
39. Prencipe G, Tabakman SM, Welsher K, Liu Z, Goodwin AP, Zhang L, et al. PEG branched polymer for functionalization of nanomaterials with ultralong blood circulation. *J Am Chem Soc.* 2009; 131(13): 4783-7.
40. Xu J, Sun Y, Huang J, Chen C, Liu G, Jiang Y, et al. Photokilling cancer cells using highly cell-specific antibody-TiO<sub>2</sub> bioconjugates and electroporation. *Bioelectrochemistry.* 2007; 71(2): 217-22.
41. Rozhkova EA, Ulasov I, Lai B, Dimitrijevic NM, Lesniak MS, Rajh T. A high-performance nanobio photocatalyst for targeted brain cancer therapy. *Nano Lett.* 2009; 9(9): 3337-42.
42. Matsui K, Segawa M, Tanaka T, Kondo A, Ogino C. Antibody-immobilized TiO<sub>2</sub> nanoparticles for cancer therapy. *J Biosci Bioeng.* 2009; 108(1): S36-S7.
43. Ninomiya K, Ogino C, Oshima S, Sonoke S, Kuroda S-i, Shimizu N. Targeted sonodynamic therapy using protein-modified TiO<sub>2</sub> nanoparticles. *Ultrason Sonochem.* 2012; 19(3): 607-14.
44. Elvira G, Moreno B, del Valle I, Garcia-Sanz JA, Canillas M, Chinarro E, et al. Targeting neural stem cells with titanium dioxide nanoparticles coupled to specific monoclonal antibodies. *J Biomater Appl.* 2011; 26(8):1069-89.
45. Ninomiya K, Fukuda A, Ogino C, Shimizu N. Targeted sonocatalytic cancer cell injury using avidin-conjugated titanium dioxide nanoparticles. *Ultrason Sonochem.* 2014; 21(5):1624-8.
46. Nativo P, Prior IA, Brust M. Uptake and intracellular fate of surface-modified gold nanoparticles. *ACS nano.* 2008; 2(8):1639-44.
47. Patil S, Sandberg A, Heckert E, Self W, Seal S. Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential. *Biomaterials.* 2007; 28(31): 4600-7.
48. Murdock RC, Braydich-Stolle L, Schrand AM, Schlager JJ, Hussain SM. Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. *Toxicol Sci.* 2008; 101(2): 239-53.