Synthesis, Properties and Application of Titanium Dioxide Doped with Nitrogen. Its Effectiveness on Photo Degradation Glutathione-S-Transferase (GST) enzymes Pupae Instar of Aedes aegypti

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Abstract. The sol-gel method was used to create N-doped TiO2. From the characterization results, it was found that N-doped TiO2 using Titanium(IV) ammonia solution and calcination tetraisopropoxide 600 K provided the most appropriate properties for acting as the photo catalyst can be use as inhibitor of GST. SEM, AFM and XRD results indicated that this N-doped TiO2 catalyst had high crystallinity because its titania precursor was simply hydrolyzed completely so no organic contents blocked initial phase construction. SEM and AFM results demonstrated that its surface morphology was spherical like fluffy powders. Moreover, with increasing calcination temperature, its anatase-to-rutile phase transformation was retarded by the incorporated nitrogen. Elemental Analysis and UV-Vis/DR results also suggested that nitrogen could be dormant in the TiO2 lattice with strong bonds, causing the effect on the band gap structure by adding energy states nearly valence band of TiO2. All of these properties enhanced the photocatalytic activity of N-doped TiO2 under visible light. Regarding the photocatalytic activity, N-doped TiO2 with ammonia solution of titanium(IV), calcinated 600 K Tetraisopropoxide succeeded in degrading glutathione-S-transferase (GST) enzymes, with the highest efficiency. However, its photocatalytic activity was drastically decreased when it was calcined at higher temperature. Additionally, the plausible mechanism was also proposed in case of photo degradation of antioxidant content based on two detected intermediates by The association between ln Co/C and photo degradation period (h).

Key word; Titanium Dioxide, Glutathione- S-Transferase (GST), Aedes aegypti.

Introduction

Instead of their defense against mainly organophosphates, organochlorines and cyclodienes, Glutathione S–transferasisis (GSTs; EC 2-5.1.18) have attracted attention in insects (1–5). There are reports that link high levels of GST to high resistance to nanoparticle exposure. Spodoptera littoralis (6), Tribolium castaneum (7,8) and Aedes aegypti (3). Honey bees have also been found to have their GST levels induced by nanoparticles. (9), Spodoptera frugiperda(10) and German cockroach (11). GSTs in insects, on the other hand, are thought to play only a binding
role in the direct detoxification of nanoparticle exposure, despite the fact that such a role has yet to be demonstrated (3,12,13).

We’ve been studying the GST family in insects for the past few years because they’re easy to culture in a regulated environment and they’re also holometabolous, which allows us to research the enzyme's ontogenetic sequence (1,8,14,15).

The resistance of the two developmental stages of the coleopteran T. molitor, namely larvae and pupae, was studied in a nanoparticles exposure and insecticide, decametric, by means of the knocking down effect, in order to assess the in vivo significance of the GST enzyme as a mechanism of defense against nanoparticles exposure insecticides (KD50) (16,17). The impact of compounds that inhibit GST activity in vitro on the insect's resistance to the injected insecticide was also investigated. (12,18–20). A variety of biochemical approaches were used to investigate the relationship between the enzyme and the insecticide molecules. The GST system was studied in the larvae and pupae developmental stages since they usually show different levels of GST activity, and the GST system appears to be of little significance in the adult stage (1,15,21).

1. Nanoparticles

Nowadays, titanium dioxide (TiO$_2$) has widely gained a great deal of attention because of its chemical stability, non-toxicity, low cost and other advantageous properties(22,23). It, therefore, has been used in worldwide applications such as photovoltaic cells, photo catalysis, environmental purification, photo induced super hydrophilicity as well as an ingredient in a pigment. With respect to photo catalysis, TiO$_2$ is close to being an ideal photo catalyst due to its properties as mentioned above (14,24,25). Generally, both anatase and rutile are used as photo catalysts and some research stated that anatase had higher photo activity than rutile (18,26).

In most studies about photo catalysts, oxygen (O$_2$) plays a vital role in the primary electron acceptor according to Gerischer, & Heller, (1991)(7). This step electron will transfer to oxygen and further generate H$_2$O$_2$ and. OH. Meanwhile, h$_{vb}$ react with adsorbed water molecules on the surface or surface titanol group (>TiOH) and finally hydroxyl radicals are also formed. By acting as an electron acceptor or a direct source of, H$_2$O$_2$ aids in the degradation process. Due to homolytic scission, OH is made. (5,27,28). All of these reactions are summarized and illustrated in Figure 1.

![Figure 1](image-url) Principal processes on the TiO$_2$ particles; (a) e$^-$ + h$^+$ generation, (a) oxidation of donors (D), (c) reduction of acceptors (A), (d) and (e) e$^-$ — h$^+$ Surface and bulk recombination, respectively.

2. TiO$_2$ modification
Many researchers are attempting to increase the photo efficient TiO_2 in visible light to enhance TiO_2's photocatalytic activity because normally TiO_2 will be active when irradiated with UV light. Moreover, there is only 3–4% of an ultraviolet part in the solar light that shines through the earth(27,29). Doping semitrailers with different types of metal transition ions or anions is one of the most applicable approaches. The impurities between the valence and the conductive strip can be produced by both two doping species, which causes the band gap narrowing. However, as previously research by Wang et al. (2006)(22), they stated that Anion dopants could be reduced in comparison to cation dopants, as recombination centers. With regard to anion doping, although a range of anion doping agents, like B, C, N, S, F and co-doping are available, N anion doping is the most innovative anion because its anion dimensions close to that of TiO_2 anion grid (14,30). The modified band structure of N-doped TiO_2 is shown in Figure 4. The additional states are induced to appear near the valence band of TiO_2.

3. Preparation Method

There are diverse methods to effectively synthesize powder, thin-film and membrane TiO_2 or modified TiO_2, namely precipitation (co-), solvo thermal, sol-gel, micro emulsion, electrochemical, chemical/physical vapor deposition, ion- implantation and ball milling methods. Focusing on the sol-gel method, most of recent works usually use this method according to (24,31,32).

This work aims to study the preparation with sol-gel, characterization with various techniques and photocatalytic applications of N-doped TiO_2, versus P25 TiO_2.

There are five main objectives that this work focuses on.

1. To study the effect of titania precursors on the as-prepared N-doped TiO_2 in terms of its phase composition, crystallinity, surface morphology, visible absorption ability as well as the amount of doped nitrogen.
2. To study the effect of diverse nitrogen sources on the as-prepared N-doped TiO_2 at fixed calcination temperature of 600 K in terms of its phase composition, crystallinity, surface morphology as well as the amount of doped nitrogen.
3. To make use of some of the N-doped TiO_2 in photocatalytic degradation of pupae exhibited elevated GST activity. Also, the photocatalytic activities and rate constants of each N-doped TiO_2 and P25 TiO_2 were studied.

Table 1 N-doped TiO_2 and P25 TiO_2 energy difference values (E_g).

| Titania precursors       | Calcination temperature (K) | \(\lambda_1\) (nm) | E_g1 (eV) | \(\lambda_2\) (nm) | E_g2 (eV) |
|--------------------------|-----------------------------|------------------|----------|------------------|----------|
| Titanium(IV) tetraisopropoxide | 600                         | 392              | 3.16     | 507              | 2.45     |
|                          | 700                         | 390              | 3.18     | 528              | 2.35     |
|                          | 800                         | 410              | 3.02     | 494              | 2.51     |
| P25                      | -                           | 408              | 3.04     | -                | -        |

All of the first observed absorption edges can be elaborated Transition from a valence band (O2p) to a conduction band in terms of charge corresponding to the excitation of electrons (Ti
The second absorption edges were in turn derived from electron excitement from N 2p above the valence band O 2p towards the conductive band. Ti 3d. Therefore, having the nitrogen content in TiO2 lattice will improve the absorption ability of photo catalysts in the visible region.

4. Subject of Pupae

Terminal-instar pupae of Aedes aegypti (0.18–0.20 g/larva) from our research facility were utilized as bug testers in the preliminaries. The hatchlings were randomized into four gatherings (n = 107 in each gathering) for each trial. The measurement can be done with a water soluble arrangement or dimethyl sulfoxide (DMSO) chemistries, providing that the last uptake of DMSO in the assessment wells doesn't surpass 1%. Alternate melt can be an optimal, in any case, it is crucial for first affirm that the anteroom last doesn't cause more than 10% mortality 72 hours after exposure. The test should be performed as an interesting part or a response part. If you do the latter, it is optional to analyze a range of obsessions (least of five) see the figure which indicate the procedure.
Fig. 2. (a), shown the procedure for determination of Glutathione-S-Transferase (GST) Activity, (b) shows the compare of GST-600 concentration according to dose, (c) demonstrated the compare among the control and treatment of studied group. Figure 2. (b) shows that there is a clear and high difference in levels of GST between the studied groups, with a significant difference, by use of the first concentration of nanoparticles of nitrogen-catalyzed titanium, which was calcined from a temperature of 600 degrees Celsius, and this is a logical result. As in many studies, that its demonstrates onset the response of antioxidant systems as well as immune system maintenance features to substances, toxic radiation, and oxidative stress resulting from attacking free radicals. When exposed samples time that exceeds 24 hours or 36 hours, as well as when the accelerated focus ratio increases. The most important dramatic course begins in the biochemical and physiological effects; the concentration of antioxidants deteriorates. While Figure 2, (c) shows that there are clearly significant differences between the control group and the treatment group for Aedes aegypti pupae that were exposed to nanoscale radiation.

5. Photocatalytic activity. Photo degradation of Glutathione -S-transferases
Figure 3 Photo degradation of Glutathione S-transferases by N-doped TiO$_2$ using NH$_3$ and various types of titania precursors; (a) titanium(IV) tetraisopropoxide (▲), titanium(IV) tetra-n-butoxide (■) and (c) titanium(IV) bis(ethyl acetoacetato)disopropoxide (♦), and calcined at 600K, compared to (d) P25 TiO$_2$ (х) and without catalysts (●)

TiO$_2$ AFM spectra specified to the dimension distribution of particles in sites between (60- 135nm), for TiO$_2$ (anatase form), the size distribution of particles sites, located between (50- 150nm), for N doping TiO$_2$ of prepared at 800k. which is the outcomes demonstrated that N- doped TiO$_2$ has the largest surface area, followed TiO$_2$ (anatase) has the lowest surface region contrasted and diminishing molecule size that illustrate in following figures in appendix; The data in the fig. 2 (b, c) which demonstrate the optimization best concentration among the studied group found the elevated high level in dose 1 (0.697*), does 2 (0.2032), does 3 (0.0567) that mean equal to the value of control group, that's prepared with nanocomposite which calcined in 600k. This outcome goes to Côa et al., (19) that’s indicate the nanocomposite of N- TiO$_2$ the best model with lethal concentration against pupae of Aedes aegypti. This fact is consistent with the general framework of the physical and chemical properties of the nanocomposite and is consistent with the measurements and results that we obtained during and after the synthesis of these nanocomposites. Moreover, Fig. 2. (c, b) show that the peak of the enzymatic activity of the compound GST arrives in response to nanoparticle exposure and its high toxicity, where the enzymatic activity of the antioxidants reaches the endpoint with the strongest concentration prepared and was previously evaluated and then a complete breakdown in the anti-toxicity activity where there is no significant difference. Between the control group and the treatment group, indications for GST degradation, this results correspondence with(1,14–16,25). The results of photocatalytic activities (Figure 3) and rate constants (Table 1) of each type of N-doped TiO$_2$ calcined at 600 K, including P25 TiO$_2$ indicates that N-doped TiO$_2$ using titanium(IV) tetraisopropoxide provided the best catalytic efficiency. It was able to degrade Glutathione S-transferases with the highest conversion of 33% and rate constant of 0.058 h$^{-1}$ because of its crystallinity, morphology and the amount of doped nitrogen as Ti-N. Meanwhile, N-doped TiO$_2$ using titanium(IV) tetra-n-butoxide gave 19% conversion of Glutathione S-transferases with the rate constant of 0.054 h$^{-1}$. This suggests that although it has smaller crystallite size (9.41 nm), its catalytic activity under visible light is not high proportionally because there has been a small amount of strongly-bonded nitrogen in its structure. In other words, the 16% conversion and 0.054 h$^{-1}$ rate constant belongs to N-doped TiO$_2$ using titanium(IV) bis(ethyl acetoacetato)disopropoxide, indicating that even though this N-doped TiO$_2$ photo catalyst has the high amount of nitrogen, its catalytic activity is not enhanced. This can be assumed that, firstly, some nitrogen species entrapped in TiO$_2$ are inactive species to photocatalytic degradation such as weakly-adsorbed nitrogen species on the surface of TiO$_2$. Secondly, some parts of the N-doped TiO$_2$ are still amorphous because the XRD peak at 20 of 25.4° is so broad. Thirdly, the surface morphology has the mixture between fluffy grains and semisolid mass. The later morphology normally provided the bigger size, which reduced the specific surface area. Hence, the photocatalytic efficiency is the lowest. Moreover, P25 TiO$_2$ was also capable of degrading GST at the 12% conversion of GST with the rate constant of 0.052 h$^{-1}$. This phenomenon can be explained by the synergistic interaction between anatase and rutile phases. As previous study(15,16), when anatase attached to rutile, it would cause band bending which reduced the possibility of electron-hole recombination. This might enhance the photocatalytic activity of P25 TiO$_2$. So that the outcomes go with (5,27,28). In case of without catalysts, there is no change in the relative concentration of Glutathione S-transferases. This result can be implied that all of degraded Glutathione S-transferases originated from the photocatalytic reaction rather than the thermal decomposition reaction. Besides, under dark reaction in the first one hour, it is evident that the relative concentration of Glutathione S-transferases was decreased, suggesting that Glutathione S-transferases was physically adsorbed on the surface of N-doped TiO$_2$. After switching the light on, the relative concentration of Glutathione S-transferases gradually decreased in case of N-doped TiO$_2$ using titanium(IV) tetraisopropoxide. On the contrary, the other photocatalysts show the increase of the relative concentration of Glutathione S-transferases again. This can be implied that N-doped TiO$_2$
using titanium(IV) tetraisopropoxide was able to degrad Glutathione S-transferases immediately that the light was turned on. However, N-doped TiO₂ using titanium(IV) tetra-n-butoxide, N-doped TiO₂ using titanium(IV) bis(ethyl acetato)disopropoxide and P25 TiO₂ led to the desorption of Glutathione S-transferases from their surface rather than sudden degradation of GST after turning the light on. It is inferred that rate of Glutathione S-transferases desorption was higher than that of GST degradation on their surface. After that, they had just abilities to gradually degraded Glutathione S-transferases especially on the last period of degrading time. The correlation between the natural levels of glutathione S-transferase (GST) and the tolerance to the aqueous solution using a different type of cations doped TiO₂ and P25, The interaction of affinitypurified enzyme and insecticides, as well as the role of the glutathione S-transferase system as a mechanism of defense against insecticides, were investigated in order to gather more information on the role of the glutathione-S-transferase system as a mechanism of defense against insecticides in insects. The studies on Aedes aegypti pupas showing different natural levels of GST activity were carried out which is agreed with (16,17).During the first 24 hours, the insect showed stage dependent susceptibility to nanoparticles when used as insecticides. However, 48 hours after treatment, the KD50 value increased significantly due to the individuals' recovery. Simultaneous injection of N-doped TiO₂ compounds that inhibit GST activity in vitro resulted in a change in insect susceptibility 24 or 48 hours after treatment, depending on stage and insecticide has been used. This reinforces the other experimental scientific evidence that antioxidants will deteriorate with time if over the 24 hours that’s results correspondence with each, (3,12,13), as well as with an increase in Nano concentration and also with acidity and temperature. This matter needs more research in studies. Hospitalism studies in association with competitive fluorescence-spectroscopy have shown that the insecticides are likely to bind to the active site of the enzyme and therefore compete against N-doped TiO₂. High-performance liquid chromatography and gas chromatography has shown that the GST insects catalyst for a reduced glutathione conjugation (GSH). GST provides protection against N-doped TiO₂ by active site binding and subsequent combination with GSH from the above experimental results.(2,9,24,31,32).

6. Conclusion

the terms, photocatalytic activity, N-doped TiO₂ calcined at 600 K with titanium(IV) tetraisopropoxide and ammonia solution degraded Glutathione-S-transferase (GST) enzymes with the highest efficiency. When calcined at a higher temperature, however, its photocatalytic activity was significantly reduced. The relationship between ln Co/C and photo degradation time (h) was also suggested as a possible mechanism in the case of photo degradation of antioxidant material based on two detected intermediates that’s concentration of dose of exposure and the time of Nano composite exposure.

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Appendix

Figure (1-a) XRD outline for (P25), TiO₂ (anatase), (1-b) XRD outline for Pt.- doped with TiO₂

Figure (2-a) size distribution for catalyst (P25)TiO₂ (anatase) composite AFM. (2-b) prepared particles sketched on X-Y axis catalyst TiO₂ (anatase) by AFM (2-c) prepared particles sketched on X-Y-Z axis for catalyst TiO₂ (anatase) by AFM.

Figure (3-a) size distribution for catalyst N- doped TiO₂,(4-b) prepared particles sketched on X-Y axis for catalyst N- doped TiO₂ by AFM., (4-c) prepared particles sketched on X-Y-Z axis for catalyst N-doped TiO₂ evaluated by AFM.
Figure 5 (a) SEM and (b) TEM images of N-doped TiO$_2$ using NH$_3$ as a nitrogen source and calcined at 600 K.