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Effect of UV Screening Nature of Cu-TiO_2 Thin Films on the Protection of Chlorophyll Content in Medicinal Plants

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Abstract: Titanium dioxide and copper doped Titanium dioxide thin films with different concentrations have been prepared by sol-gel dip coating technique and were annealed at 400°C for 3 hours in muffle furnace. The annealed films were characterized by X-Ray diffraction, UV-Visible spectroscopy, Atomic Force Microscopy, Scanning Electron Microscopy and Energy dispersive spectroscopy techniques. It was found that in Cu-TiO_2 thin films crystallite size shrinks and absorption raises with the increase in Cu concentration of the film. Chlorophyll pigments play an important role in photosynthesis. Chlorophyll benefits the human body in a number of distinct and unique ways. UV light impairs photosynthesis and increased exposure of UV light reduces size, productivity and quality of chlorophyll in many of the crop plants. Here, we have studied the efficiency of UV absorbing effect of Cu-TiO_2 thin films on the protection of chlorophyll content of medicinal leaves such as Acalyphaindica and Phyllanthusamarus.

Keywords: Cu-TiO_2 Thin films, UV radiation, chlorophyll, Acalyphaindica, Phyllanthusamarus**.**

1 Introduction

In recent years, titanium dioxide (TiO_2) has been extensively used because of its various qualities such as environment friendly nature, high photo catalytic activity, high refractive index, resistance to photo corrosion, low cost and chemical stability [1,2]. Doping the TiO_2 with transition metals (Cu) enhances its optical and chemical properties [3]. TiO_2 has strong ultraviolet (UV) absorbing capability [4]. Plants employ sunlight for the process of photosynthesis. As a result they are continuously exposed to ultraviolet (UV) radiation and the excess exposure of UV light shrinks the size, productivity and quality of chlorophyll in lots of the crop plants [5]. However many recent research shows that chlorophyll content of leaves has medicinal properties. Ayurveda medicine, unani medicine etc employs plant extracts for the cure of multitude of diseases [6, 7]. Also, a regular intake of chlorophyll keeps the circulatory and digestive systems of human body much healthier [8]. For this reason, shielding chlorophyll content of leaves from UV light is an essential task. Our interest is protection of chlorophyll pigment of plants from UV light using a suitable UV filter such as TiO_2 thin films since TiO_2 thin films efficiently alter destructive UV light energy into heat thus it can be utilized to protect chlorophyll content in leaves of medicinal plants [9]. In our present work, we have studied the properties of pure TiO_2 and Cu doped TiO_2thin films prepared with different Cu concentrations. The effect of Cu-TiO_2 thin films as an absorber of UV radiation to protect the chlorophyll content in Acalyphaindica and Phyllanthusamarus plants were also reported.

2 Experimental Procedure

2.1 Preparation of Cu doped TiO_2 thin film

TiO_2 and Cu doped TiO_2 thin films were prepared from a solution of analytical grade Titanium tetra isopropoxide (Ti $(OC_3H_7)_4$), Copper II nitrate trihydrate (Cu $(NO_3)_2.3H_2O$), ethanol and acetic acid using dip coating machine. The Cu doped TiO_2 sol was prepared by adding 6.3 ml of TTIP to 50 ml ethanol and 1.5 ml acetic acid was added to this mixture and stirred for 15 minutes. To this mixture, a suitable amount of Copper II nitrate trihydrate was added and the whole was stirring for one hour. The film was coated on glass substrates by dipping it into the solution and pulling it at a constant rate of 1.5mm/min using dip coating machine. The dip coated glass substrate was dried by heating at 100°C for 10 minutes and the process was repeated for different Copper concentrations (0, 0.02, 0.03, 0.04 and 0.05 mol%). Finally all the samples

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were annealed at 400°C for 3 hour in muffle furnace at air atmosphere. The annealed films were characterized by X-ray diffraction (XRD), UV-Visible Spectrophotometer (UV-Vis), Atomic Force Microscope (AFM), Scanning Electron Microscope (SEM) and EDX studies.

2.2 Material Characterization

The crystallite size of the Cu-TiO_2 thin films obtained from different concentrations were characterized by X–Ray diffraction method (XRD) using X'PERT PRO X–ray diffractometer which was operated at 40 KV and 30 mA with CuKα1 radiation of wavelength 1.5407Å. UV–visible spectra were recorded in the range of 300 – 800 nm using the Schimadzu 1800 UV–VIS – NIR spectrophotometer. The surface morphology observation and elemental analysis were done by Quanta SEG - 200 SEM and Bruker EDX respectively. Surface roughness of the films was recorded using NT-MDT, NTEGRA Prima-Modular AFM in semi contact mode using SiN cantilever.

2.3 Protection of chlorophyll content in plants from UV light using Cu-TiO_2 films

One gram of finely cut fresh leaves of Acalyphaindica was equally taken in 7 different 100ml conical flasks. One flask was completely exposed to UV light. Five flasks were covered with thin films prepared with 5 different concentrations (0, 0.02, 0.03, 0.04 and 0.05 mol %) and all were exposed to UV light of 260nm for 10 minutes in UV chamber. Final one flask was not exposed to UV light. Then the chlorophyll extraction process was done for all the samples. The absorbance of the chlorophyll extract was determined at 645, 663 and 653nm by using spectrophotometric techniques. Same procedure was repeated for the leaves of Phyllanthusamarus.

3 Results and Discussion

3.1 Structural analysis

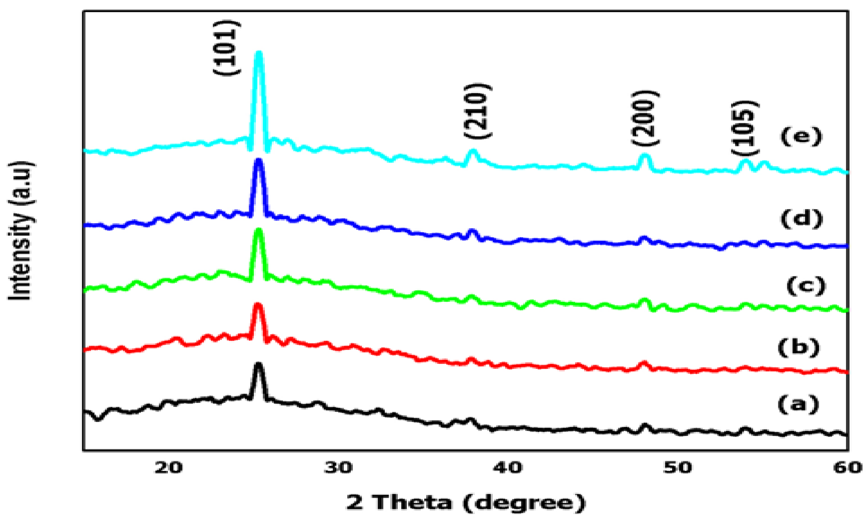

Figure.1. XRD pattern of thin films (a) TiO_2 (b) 0.02mol%Cu-TiO_2 (c) 0.03mol%Cu-TiO_2 (d) 0.04mol%Cu-TiO_2 (e) 0.05mol%Cu-TiO_2

XRD pattern of the films annealed at 400°C for different concentrations was displayed in Figure. 1. The dominant peaks were observed at (1 0 1) plane for all the films attributed to the tetragonal crystal structure with anatase phase which is in agreement with standard JCPDS data (File No.89-4203). The crystallite size of the films were determined for different proportions of Cu using the well-known Debye-Scherer's formula,

$$D = \frac{K\lambda}{\beta cos\theta} \ (nm)$$

Where K=0.94, λ= 1.5407°A, β= Full Width Half Maximum (FWHM) and θ= Diffraction angle. The average crystallite size of various mol ratios of Cu to TiO_2were 0, 0.02, 0.03, 0.04 and 0.05mol% are 64.5, 60.8, 58.3, 55.7 and 53.3 nm respectively. It was apparent that Cu added in TiO_2 has significant effect on crystallite size. It was found that TiO_2 doped with 0.05mol% of Cu show the smallest crystallite size. The crystallite size was decreases with increase in concentrations and the similar results of decrease of crystallite size by doping with several transition metals on TiO_2 are already reported [10]. The replacement of Ti with Cu causes the compressive stress which leads to the decrease in crystallite size [11]. No copper related peaks are visible, indicating the formation of a solid solution in the Cu-TiO_2 films. We believe that all the Cu is incorporated into TiO_2 as dispersed ions rather than cupric oxide crystallites [12].

AFM, SEM and EDX spectra of 0.04mol% Cu doped TiO_2 thin films are shown in Figure. 2. The surface morphology and roughness of the film was characterized using Atomic Force Microscopy. The film exhibits granular nanostructure. The surface roughness was found to be 5.58nm and RMS was about 7.33nm. SEM micrograph of Cu doped TiO_2 thin film shows loosely agglomerated grains with mosaic like structure. The dipping process was repeated seven times to make the coatings thicker. In this case small cracks, pin holes and island like structures form when the film thickness is increased [13]. The EDX spectrum demonstrates the presence of Cu, Ti and O in the ratio of Ti-32.57wt%, O-65.15wt% and Cu-2.28wt%.

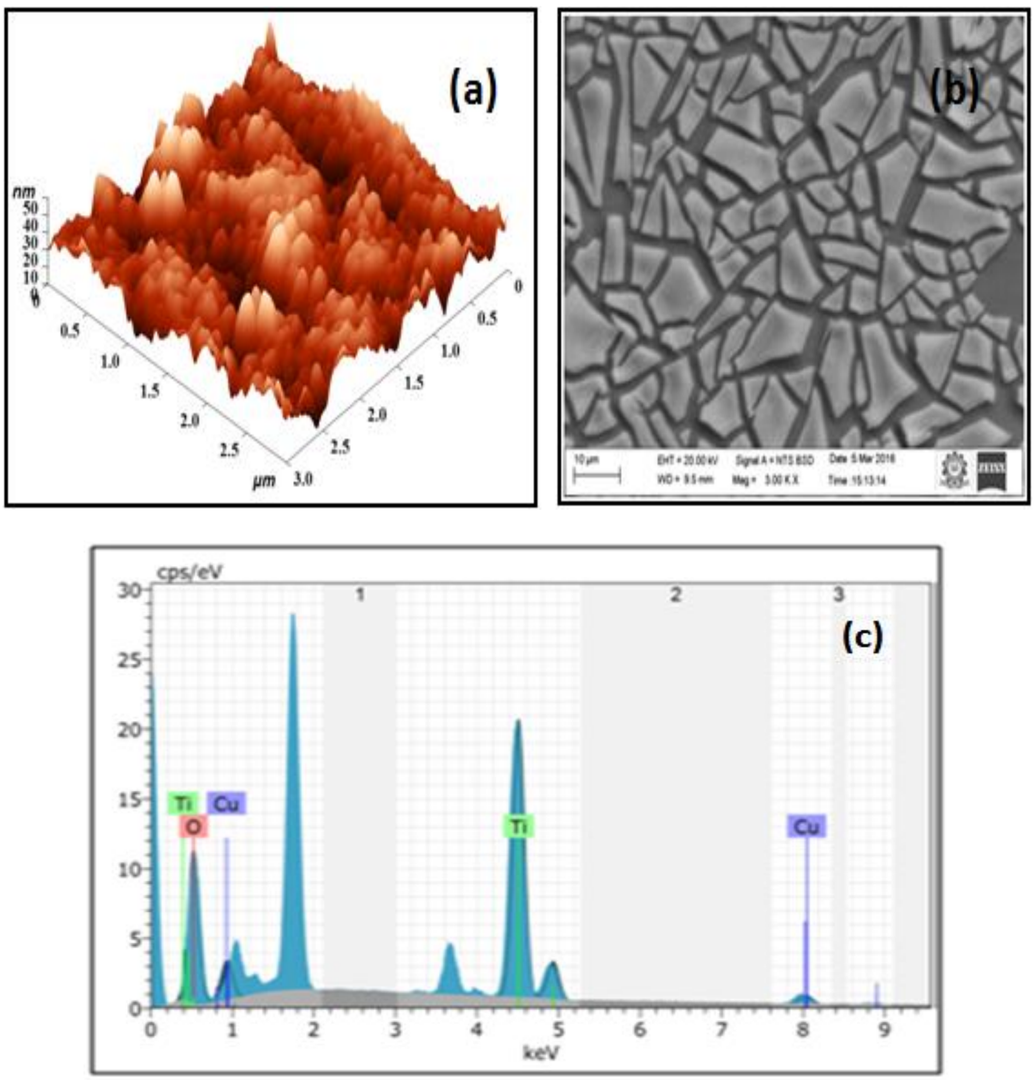

Figure.2. (a) AFM, (b) SEM and (c) EDX spectra of 0.04mol% Cu-TiO_2 thin film

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3.2 Optical analysis

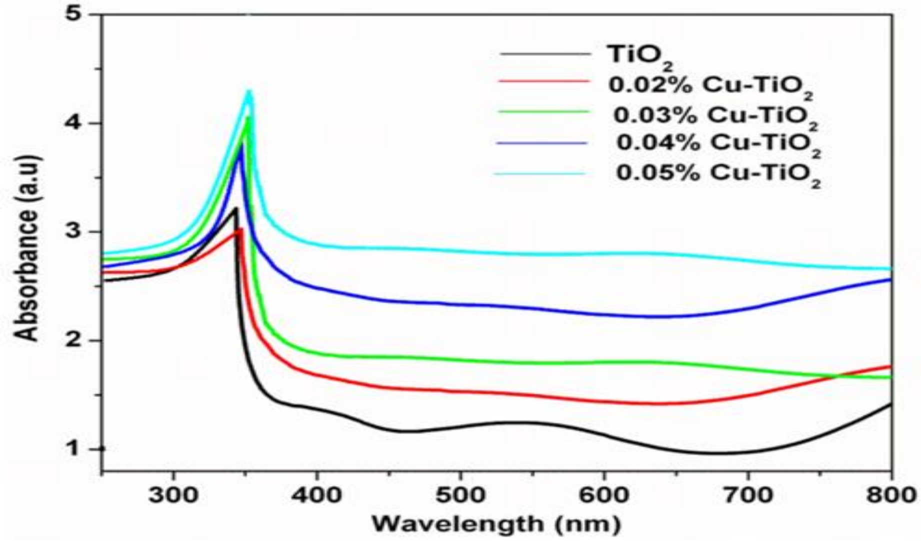

Figure.3. Absorption spectra of Cu-TiO_2 films with various Cu doping concentrations

The optical absorbance spectra of Cu doped TiO_2 thin films were measured in the region of 250-800nm by using UV-VIS-NIR spectrometer shown in Figure. 3. It has been noticed that absorption in the UV region was extended to the visible region for Cu doped TiO_2 and the shift was increased with increasing amount of dopant Cu. The strong absorption peak at 370nm is ascribed to pure TiO_2 film and new strong absorption band between 370-500nm might be assigned to the presence of Cu species in the TiO_2 film. In addition, the Cu-species also induced the formation of impurity inside TiO_2, resulting in a shift of adsorption edge to higher wavelength region (red shift), which is characteristic for the TiO_2 system after metal doping [14].

3.3 Extraction and estimation of chlorophyll content

Chlorophyll was extracted using a method as described by Arnon (1949) [15]. 1gram of finely cut fresh leaves (after treatment to UV light) was ground to a fine pulp with the addition of 20ml of 80% acetone with a mortar and pestle. This paste was centrifuged for 5 minutes at 5000 rpm. The supernatant was transferred to a 50ml beaker. The residue was then ground with 20ml of 80% acetone, centrifuged for 5 minutes at 5000rpm and the supernatant was transferred to the same beaker. This process was repeated for 4 times till the residues became almost colorless. The inside of the mortar and pestle were also washed with 80% acetone and the clear washings were also collected in the beaker. The volume was made up to 100ml with 80% acetone. This was repeated for all the leaf samples. The absorbance of the extract solutions were read at 645, 663 and 652nm against the solvent (80% acetone) blank.
The amount of chlorophyll a, chlorophyll b and total chlorophyll in terms of mg/g was calculated using the following formula given by Goodwin (1965)

(a) $Chl.a = [12.7(A_{663}) - 2.69(A_{645})] \times [V/ (1000\times W)]$ (mg)/g
(b) $Chl.b = [22.9(A_{645}) - 4.68(A_{663})] \times [V/ (1000\times W)]$ (mg)/g
(c) $Total\ chl. = [20.2(A_{645}) + 8.02(A_{663})] \times [V/ (1000\times W)]$ (mg)/g

Where V = Volume of chlorophyll extract (100ml)
W = Fresh weight of leaf sample (1g)
A_{663} = Absorbance in 663 nm wavelength
A_{645} = Absorbance in 645 nm wavelength

Percentage of Chlorophyll content = (Total chlorophyll content after irradiation) / (Total chlorophyll for the control) × 100

And therefore, the amount of chlorophyll present in the respective samples of Acalyphaindica and Phyllanthusamarus leaves was calculated. The percentage of chlorophyll content was also calculated and listed in table 1. Here, each value is the mean of two replicate experiments (±SE).

Table 1: Chlorophyll content in the leaves of Acalyphaindica and Phyllanthusamarus

	Acalyphaindica		*Phyllanthusamarus*	
Treatments	**Total chlorophyll (mg/g)**	**Chlorophyll content (%)**	**Total chlorophyll (mg/g)**	**Chlorophyll content (%)**
UV unexposed	3.723 ± 0.09	100	2.9572 ± 0.13	100
UV exposed	1.159 ± 0.34	31	1.0643 ± 0.25	36
TiO_2	1.6620± 0.18	45	1.4420± 0.37	49
0.02mol%Cu-TiO_2	2.2375± 0.26	60	2.0137± 0.15	68
0.03mol%Cu-TiO_2	2.4583± 0.12	66	2.2681± 0.09	77
0.04mol%Cu-TiO_2	2.9006± 0.08	78	2.5271± 0.21	85
0.05mol%Cu-TiO_2	3.1885± 0.23	86	2.6932± 0.38	91

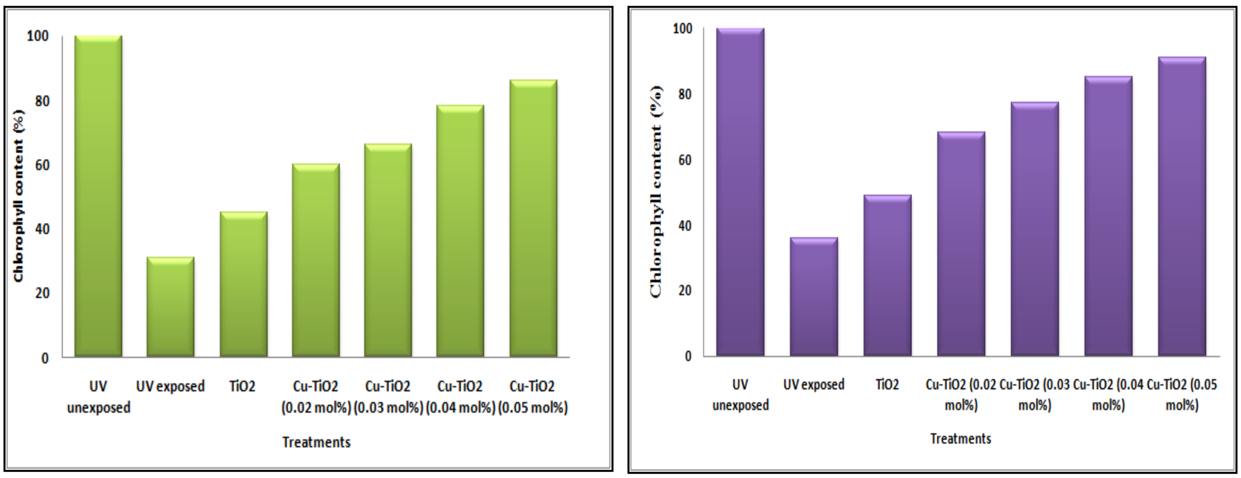

Figure.4. Chlorophyll content in the leaves of *Acalyphaindica* and *Phyllanthusamarus* for different concentrations of thin films.

In the fresh leaves completely exposed to UV light, the chlorophyll content is minimum, whereas in the leaves unexposed to UV light the chlorophyll content is maximum [5]. But when the leaves with different concentrations of Cu doped TiO_2 thin films were exposed to UV light the chlorophyll content gradually increases with increase in concentrations of the films as shown in Table 1.

After estimating the values of total chlorophyll content among different concentrations, it was observed that the higher concentrations of Cu-TiO_2 showed the maximum chlorophyll content in both medicinal plants and thus the film act as a protective layer to UV light. The present study showed that the Cu doped TiO_2 thin films protect from UV rays i.e. it acts as an UV absorber and preserves the chlorophyll content in the leaves of Acalyphaindica and Phyllanthusamarus.

4 Conclusion

In this work, TiO_2 and Cu-TiO_2 thin films of various concentrations were deposited on glass substrates by sol-gel dip coating technique. It was found that crystallite size decreases and absorbance increases with increase in concentration of the films. SEM image shows that the films are crack with mosaic like morphology. The presence of Cu, Ti and O elements has been confirmed by EDX analysis. AFM images confirm the roughness of the surface. The present study shows that the properties of Cu-TiO_2 thin films are considerably influenced by varying copper concentrations. The percentage of chlorophyll content was increases with increase in concentrations for both plants. The highest concentration film is having high absorbance of UV radiation and high percentage of chlorophyll content of plants. Thus Cu-TiO_2 thin films act as protective layer by absorbing UV light and protect the chlorophyll content in the leaves of medicinal plants.

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