Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (6):37-44
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Functionalization of TiO_2 nanoparticles and curcumin loading for enhancement of biological activity

V. J. Sawant* and R. V. Kupwade

Department of Chemistry, Smt. Kasturbai Walchand College, Sangli, M.S., India

---

ABSTRACT

The hydrophobicity and limited bioavailability of both drug Curcumin and TiO_2 nanoparticles is destroyed herein this work by coating the anatase TiO_2 nanoparticles with biodegradable polymer Chitosan. TiO_2 nanocarriers for drug Curcumin had been synthesized by simple wet chemical co precipitation route and the nanoparticles functionalized in biological fluids by capping with Chitosan. The synthesized nanomaterials were characterized by UV-VIS, IR and XRD spectroscopic analysis. The morphology and particle sizes were determined on the basis of XRD. The primary biological activities of functionalized TiO_2 loaded with Curcumin were tested on Shiegella bacteria for antibacterial analysis. These functionalized TiO_2 nanoparticles exhibited higher antibacterial activities at MIC 1µg/ml. compared to free hydrophobic TiO_2 and drug Curcumin and shown particle size of 24 nm. and Spherical Morphology with anatase core. This work resulted in a simple drug delivery system of TiO_2 for future applications in delivery of hydrophobic drugs to biological systems for antibacterial and anticancer trials.

Keywords: TiO_2, Chitosan, Curcumin, Antibacterial activity.

INTRODUCTION

Researches have shown that titanium dioxide nanoparticles (TiO_2 NPs) are environment friendly and have good biocompatibility with weak or no toxicity in vitro and in vivo, which indicates its great probability of the applications in life science. However TiO_2 nanoparticles needs functionalization with surface coating by water dispersible agents like cyclodextrins, polymeric carbohydrate derivatives, and organic acids. Along with TiO_2 functionalization if drug loading performed on surface the biological activities may enhance and these nanosystems act as drug delivering platforms[2,5].

Turmeric is a spice derived from the rhizomes of *Curcuma longa*, which is a member of the ginger family (*Zingiberaceae*). Rhizomes are horizontal underground stems that send out shoots as well as roots. The bright yellow color of turmeric comes mainly from fat-soluble, polyphenolic pigments known as curcuminoids). Curcumin, the principal curcuminoid found in turmeric, is generally considered its most active constituent having therapeutic impact. Other curcuminoids found in turmeric include demethoxycurcumin and bisdemethoxycurcumin. In addition to its use as a spice and pigment, turmeric has been used in India for medicinal purposes for centuries[6,7]. More recently, evidence that curcumin may have anti-inflammatory and anticancer activities has renewed scientific interest in its potential to prevent and treat disease. Tumeric is the dried ground rhizome of *Curcuma longa*. It is used as a spice in Indian, Southeast Asian, and Middle Eastern cuisines. Curcuminoids comprise about 2-9% of turmeric. Curcumin is the most abundant curcuminoid in turmeric, providing about 75% of the total curcuminoids, while demethoxycurcumin provides 10-20% and bisdemethoxycurcumin generally provides <5%. Curry powder contains turmeric along with other spices, but the amount of curcumin in curry powders is variable and often relatively low. Curcumin extracts are also used as food coloring agents . The health benefits of curcumin are extremely well known, stretching back to ancient times. It has been widely used in medicine as an anti-inflammatory, to treat digestive and liver problems, skin diseases, biliary disorders, anorexia, cough, hepatic

disorders, bloody urine, hemorrhage, toothache, rheumatism, sinusitis, bruises and wounds. To take one example, curcumin has been found to inhibit the growth of the Helicobacter pylori bacteria, which has been linked to gastric ulcers and gastric cancer. Studies show that curcumin may help fight infections some cancers, reduce inflammation, and treat digestive problems. Shiegella is such a bacteria found in most of places in dirty and putrid water sources, and its infection is a cause of disorders of digestive systems.

Chitosan is natural biodegradable carbohydrate polymer isolated from chitin source. It is used as capping and functionalizing agent. It can be used to deliver hydrophobic drugs and nano cores inside the cell. TiO_2 nanoparticles have good biocompatibility inside cell in bare form, but posses very low aqueous solubility and low bioavailability[10,11]. Yet these nanoparticles are used in biological applications. In continuation with these facts the work in this paper aimed to functionalize anatase TiO_2 nanoparticles in biological fluids by coating with Chitosan and after coating of these nanoparticles the loading with drug Curcumin to enhance its biological activities. We had synthesized water dispersible TiO_2 nanospheres by simple wet chemical route and functionalized them with Chitosan by in situ precipitation using CTAB surfactant. After their physicochemical characterization the antibacterial activities on Shiegella bacteria were tested and MIC of these drug delivery system was determined.

MATERIALS AND METHODS

2.1 Materials and Instruments : All the required TTIP (Ti^{4+} compound), std. curcumin, NH_4OH, organic solvents, Phosphate buffer solution (PBS) with pH 7.4,Chitosan and CTAB surfactant were procured from S.D.Fine Chemicals ltd. and Double distilled water was obtained with required minimum molar conductivity from Millipore distillation system then used in the synthesis of nanoparticles, drug nanocomposites. All other cell culture medium and reagents used were of analytical grade. The physicochemical characterization of synthesized nanomaterials were performed using Systronic double beam UV-VIS spectrophotometer, Perkin Elmer series type IR spectrometer and XRD spectrometer with Cu-Kα source on the basis of powder diffraction method.

2.2. Synthesis of TiO_2 nanoparticles by co precipitation route :

TiO_2 with rutile phase were synthesized using co precipitation method. In typical synthesis of TiO_2 bare nanoparticles, 5 ml. of TIIP (Titanium tertiary isopropoxide) placed in 100 ml. beaker and kept stirring on magnetic stirrer further 10 ml. ethanol and 2 ml. NH_4OH added successively till precipitation of hydroxide. Then total precipitate washed three times with 25 ml. portions of DD water and dumped in Petri plates to dry in oven at 85^OC. The TiO_2 nanoparticles further ignited at 450^OC in muffle furnace to get dry core anatase phase.

2.3. Synthesis of drug loaded Chitosan capped functionalized TiO_2 nanoparticles:

The curcumin loaded Chitosan coated functionalized TiO_2 nanocomposite material was prepared using simple co precipitation technique. Briefly, in typical synthesis of the nanomaterial 1 gm of dried TiO_2 nanoparticles powder was suspended in 10 ml. DDW and stirred at 450 rpm. then 0.6 gm. Chitosan was added to this mixture and immediately with 0.2 gm. of CTAB surfactant added and stirring speed increased to 850 rpm. the solution stirred for 3 hours and washed three times with DDW to remove unreacted Chitosan. This suspension again diluted by addition of 10 ml. DDW and then 2 ml. of Curcumin was added (1mg./ml. in ethanol) suddenly drop wise. The solution turned orange red and stirred for 6 hours then pH was adjusted to 7. Finally the nanomaterials washed with DDW and dried in oven at 90^oC.

The reactions taken place in the synthesis of these nanocomposites are explained as in scheme 1

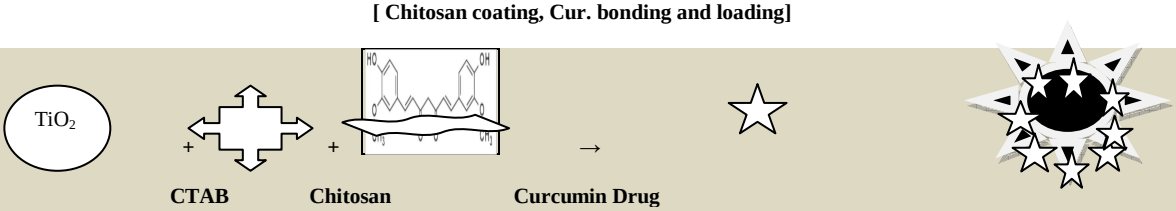
Scheme 1 : [A simple wet chemical Co-precipitation]

1) $Ti^{4+}_{(l)}$ **+** $NH_4OH_{(aq.)}$ **+ Ethanol → TiOH$_4$ [Precipitation by stirring at basic pH]**

2) TiOH$_4$ → TiO$_2$ (anatase) [Drying and Ignition at 450^{o}C for anatase phase]

3) TiO$_2$ + Chitosan + Curcumin $\xrightarrow{CTAB}$ **Chitosan Capped TiO$_2$ loaded with drug Curcumin**

[Chitosan coating, Cur. bonding and loading]



CTAB Chitosan Curcumin Drug

Chitosan coated curcumin loaded TiO$_2$ nanocomposite

Bare TiO$_2$ nanoparticles **Chitosan coated drug laoded TiO$_2$**

Image 1 : Synthesis of functionalized TiO$_2$ nanoparticles

2.4. Structural and morphological characterizations of Bare TiO_2 nanoparticles and functionalized nanoparticles:

The structures and morphology of bare TiO_2 nanoparticles and drug loaded chitosan coated TiO_2 nanocomposites were estimated and confirmed on the basis of UV-VIS spectroscopic and X-ray diffraction methods. Systronic type Double beam spectrophotometer was used to measure absorption spectra of these nanoparticles suspended in DDW with D D Water as blank from Millipore system. The maximum absorption wavelengths were compared to study bonding and spectra shifting interactions in materials. The formations of capping on surface of TiO_2 and bonding interactions of curcumin were confirmed on the basis of IR analysis using Perkin Elmer series type spectrometer with scanning from 400-1300 cm^{-1} range using KBr pallet technique. The mean particle sizes and morphology of these materials were confirmed using XRD spectra by powder diffraction method and particle sizes were estimated using famous Debye Sherrer's formula and the spectra matched with standard JCPDS card from literature to confirm phase of material.

2.5. Antibacterial analysis on Shiegella and determination of MIC:

The antibacterial activities of bare TiO_2 nanoparticles and its functionalized Chitosan coated drug loaded nanocomposites were determined on Shiegella by suspending the nanomaterials in Sterile phosphate buffer solution of pH of biological fluids. 1 to 5µg/ml. doses were given to grown cultures of bacteria using well method. Briefly, the Shiegella was grown on DMEA culture medium on culture plate in phosphate buffer by incubating it for 24 hours at 37^{o}C and 5%CO_2 environment in incubator. After dosing of nanomaterial solutions the plates were again incubated for 3 hours in same environment and zone of inhibition were analyzed under microscope. The images of culture plates were captured by camera have represented in figure. The minimum inhibition concentrations (MIC) of functionalized TiO_2 were determined from dosing of different concentrations.

RESULTS

3.1. Morphological and Physicochemical Characterization :

3.1.a. UV-Vis spectra :

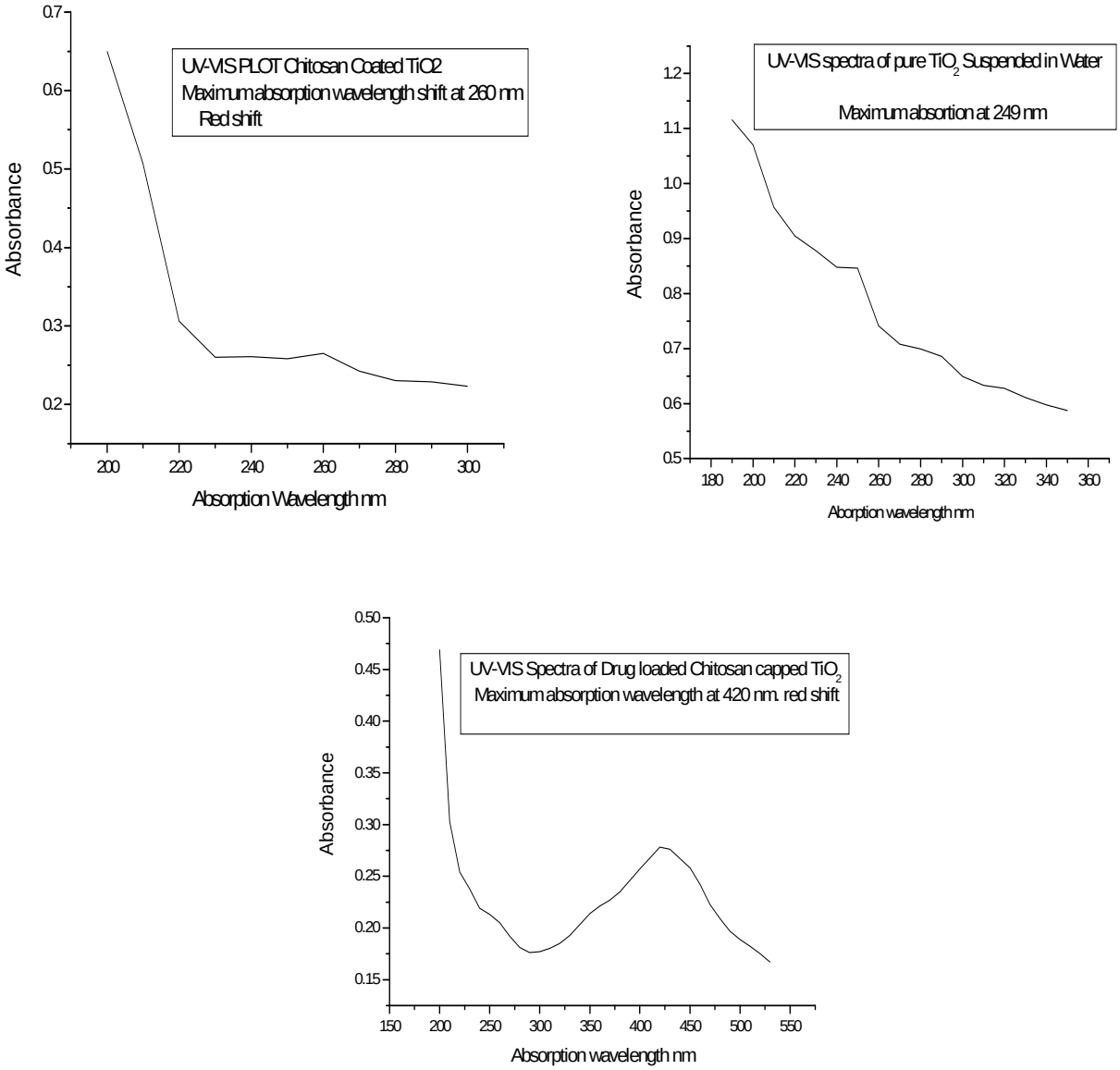


Figure 1: UV-VIS absorption spectra of bare TiO_2 , Chitosan coated and drug loaded TiO_2 nanomaterials

UV-Vis spectra of materials exhibit different absorption maxima at different wavelengths and shifting of signals of TiO_2 with functionalization and drug loading. As per Figure 1 the bare TiO_2 nanoparticles absorb and shows maxima at 249 nm. in UV region when suspended in water, while this absorption maxima shifted at 260 nm. after Chitosan being coating which proved strong bonding interaction and capping effect of Chitosan with TiO_2 and functionalization of material. When drug is loaded on Chitosan coated TiO_2 the quenching of drug absorption maxima take place along with shifting of signal of coated titanium dioxide nanoparticles. The signal is shifted from 260 nm. to 420 nm. with quenching of peak of drug Curcumin. Overall red shift may occur by bonding interactions.

3.1. b. IR Characterizations of bare TiO_2 and functionalized TiO_2 nanoparticles or drug loaded and chitosan coated TiO_2 :

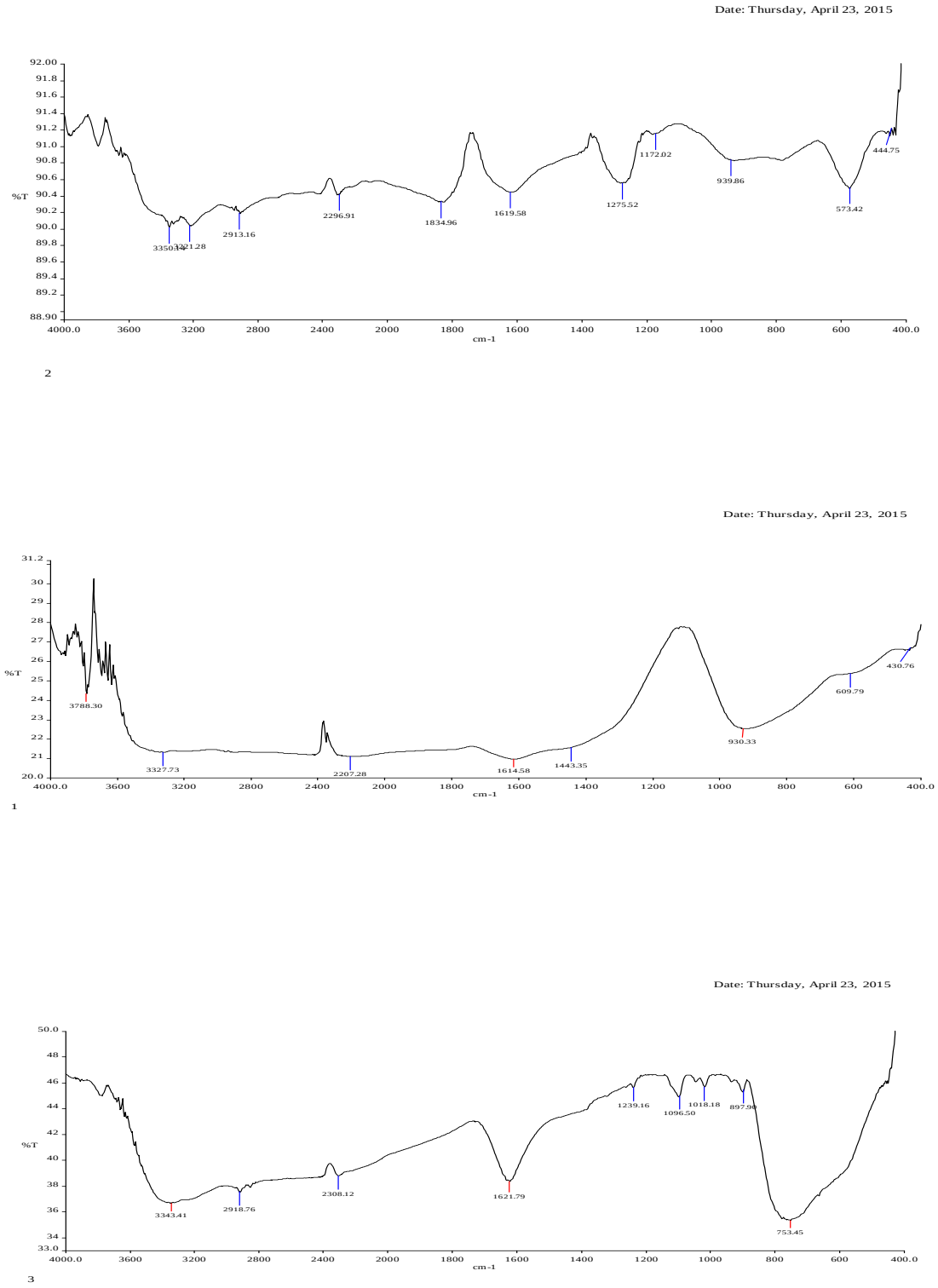


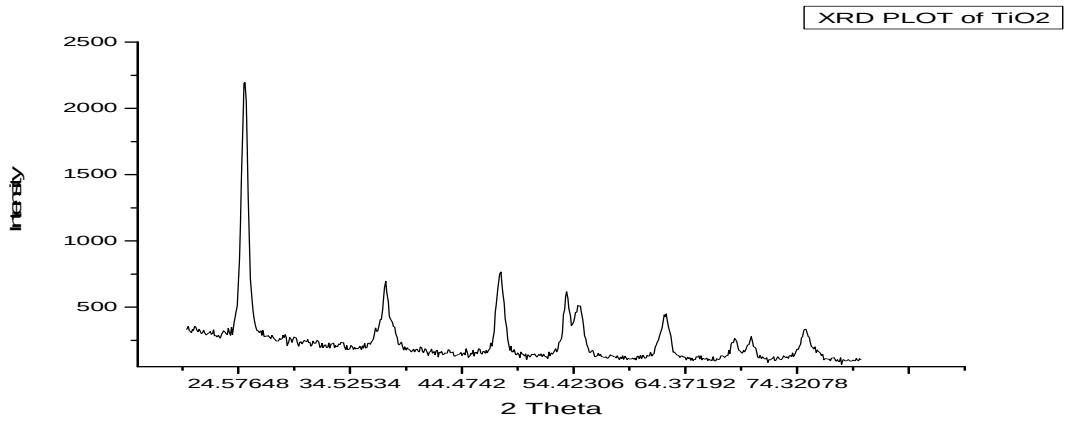
Figure 2: IR spectra of Chitosan coated (top), bare (middle) and curcumin loaded chitosan coated TiO_2 nanoparticles (bottom) from top respectively

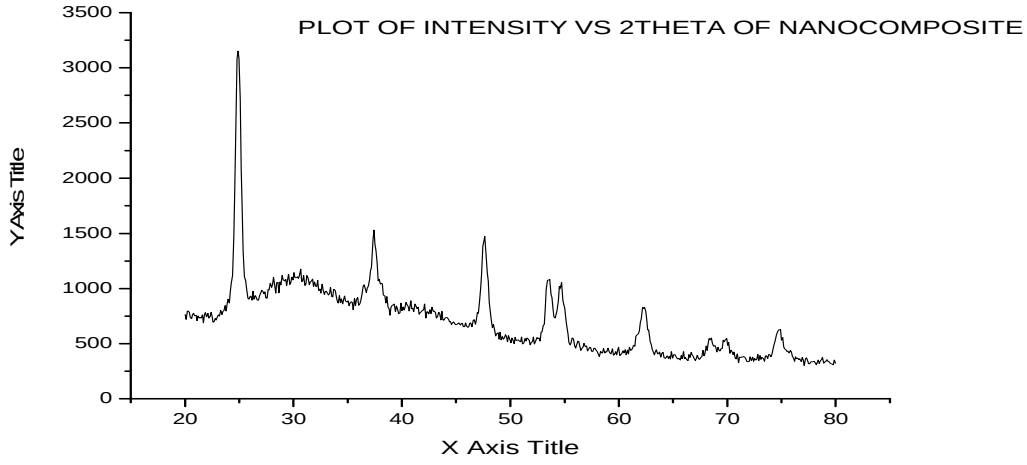
IR analysis of bare TiO2 nanoparticles and Chitosan coated material reveal that, The bare TiO_2 absorb between 500-650 cm^{-1} and 850-950 cm^{-1} region of IR spectra. The peak at 531 and 661 cm^{-1} suggest the Ti-O stretch band (Broad) in anatase phase. After coating of Chitosan of surface the peaks are weakened and one of the peak between 850-950 cm^{-1} decreased in its intensity which proved the formation of coating on TiO_2 for weakning of Ti-O strecth. The figure 2 indicate the IR spectra of bare Titanium dioxide nanoparticles and Chitosan coated titanium dioxide core in anatase phase. While curcumin loaded chitosan coated TiO_2 spectra revel that, -OH vibrations between 3300-3500 cm^{-1} are shifted and sharpened showing the bonding of –OH groups of chitosan with TiO_2 and of curcumin with chitosan and TiO_2. These IR peak predictions proved loading of curcumin over chitosan coated TiO_2 nanoparticles.

3.1.c. X ray diffraction (XRD) analysis :

X ray diffraction patterns of bare TiO_2 nanoparticles, Chitosan coated nanoparticles were determined separately by powder diffraction method using Cu kα source.

Crystallite sizes were calculated using Debye Scherer's formula, $K= 0.9\lambda/\beta.COS\theta$.





Intensity

2 Theta

Figure 3: XRD plots of pure bare TiO_2 and Chitosan coated TiO_2 nanoparticles.

'Table 1 : Diffraction pattern of crystalline TiO_2 and slight amorphous Chitosan coated TiO_2:

Crystallite planes (Miller Indices) [h,k,l] Anatase	d Calculated A^0 for TiO_2 $d = a/\sqrt{(h^2+k^2+l^2)}$ or $2dSin\theta = n\lambda$	d Calculated A^0 for Chitosan coated TiO_2 $d = a/\sqrt{(h^2+k^2+l^2)}$ or $2dSin\theta = n\lambda$	d Standard A^0 JCPDS card no.-84-1286	Lattice Constant $a\ A^0$
				a Standard = 6.440 a Calculated = 6.436
101	2.9828	2.9810	2.9840	
103	2.5440	2.5427	2.5447	
004	2.4358	2.4347	2.4364	
112	2.1089	2.1072	2.1100	
200	1.9349	1.9338	1.9363	
105	1.6234	1.6225	1.6242	
211	1.4909	1.4888	1.4920	

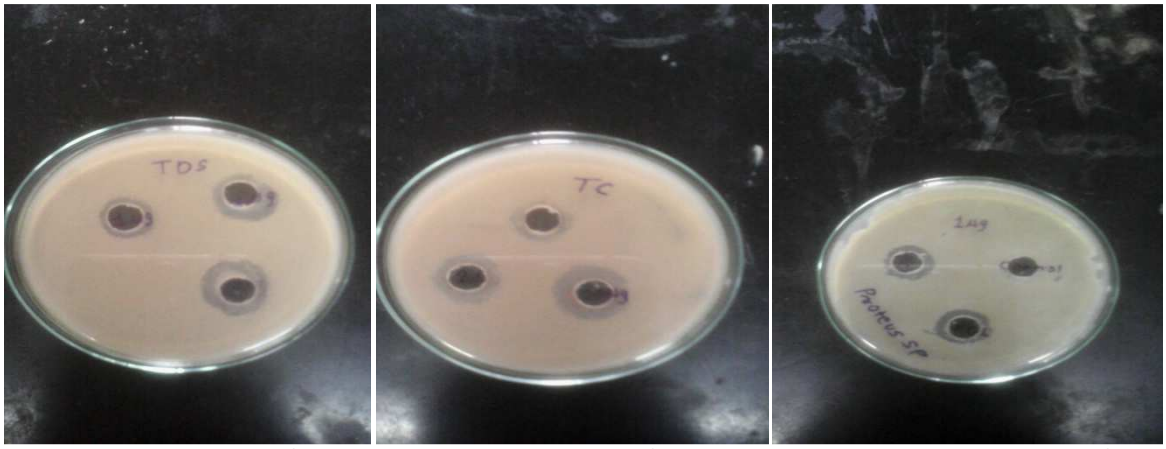
3.2. Antibacterial activities of functionalized drug loaded TiO_2 nanoparticles:

Activity of nanoparticles in phosphate buffer pH=7.4 on Shiegella .

A] Pure TiO_2 5 g/ml. Small inhibition

B] Chitosan coated TiO_2 5 g/ml. medium zone of inhibition on shiegella

C] Large antibacterial activity of Chitosan coated drug loaded Drug loaded TiO_2 at 5 g/ml.



D] antibacterial activity of 3 g/ml. Chitosan coated drug loaded TiO2 nanoparticles

E] antibacterial activity of 5 g/ml. Chitosan coated drug loaded TiO_2 Zone of inhibition on shiegella

F] Large antibacterial activity of Chitosan coated and the Drug loaded TiO_2 at 1 g/ml. [MIC]

Image 2: Antibacterial activities and MIC determination for nanoparticles on Shiegella

As a primary biocompatibility study of bare TiO_2 nanoparticles and its functionalized nanocomposite we had tested the antimicrobial activity of both materials on Shiegella using well culture plate method and determined MIC values. As shown in image 2, very active zones of inhibition were observed for material dosing in phosphate buffer medium. The functionalized and drug loaded TiO2 nanoparticles had shown higher antimicrobial activities than bare TiO_2 and capped TiO_2. Free TiO_2 is insoluble hence shown lesser activity, while functionalized TiO_2 is hydrophilic and shown high activity. These nanomaterials exhibited a platform for drug delivery of hydrophobic molecules like

Curcumin. Here dual antimicrobial effect of Curcumin drug and TiO_2 was observed on Shiegella. The minimum inhibition concentration optimum value (MIC) was obtained as 1µg./ml.

DISCUSSION

In this paper a very simplest in situ co-precipitation method has been reported for the synthesis and functionalization of TiO_2 in biological fluids. Herein this work enhanced antibacterial activity of drug delivery system of TiO_2 coated with Chitosan have been reported for Curcumin. This is a trial to functionalize TiO_2 nanoparticles in biological fluids for delivery of hydrophobic drugs. This nanocomposite system proved future trial as an anticancer drug delivery system. Here this system with functionalized TiO_2 proved enhanced antibacterial activity on Sheigella.

CONCLUSION

In the present work we had functionalized anatase TiO_2 nanoparticles in biological fluids by capping with biodegradable polymer Chitosan. Here we reported a simple wet chemical co precipitation route for functionalization and drug loading of TiO_2. After functionalization these nanoparticles exhibited higher antimicrobial activity against Shiegella. The functionalized TiO_2 nanoparticles shown a platform for delivery of hydrophobic drugs as well as anticancer drug delivery system.

Acknowledgments
The author greatly acknowledge the moral support and provision of facilities of Biotechnology and Microbiology departments of Smt.K.W.College, Sangli, M.S., India. and help of the UG student K. Mohanani for this work

REFERENCES

[1] Lausmaa, J.; Löfgren, P.; Kasemo, B. *J. Biomed. Mater. Res*. **1999**, 44, 227−242.
[2] Tsud, N.; Acres, R. G.; Iakhnenko, M.; Mazur, D.; Prince, K. C.; Matolín, V. *J. Phys. Chem*. B **2013**, 117, 9182−9193.
[3] Delay, M.; Frimmel, F. H. *Anal. Bioanal. Chem*. **2012**, 402, 583−592.
[4] Domingos, R. F.; Tufenkji, N.; Wilkinson, K. J. *Environ. Sci. Technol*. **2009**, 43, 1282−1286.
[5] Stebounova, L.; Guio, E.; Grassian, V. *J. Nanopart. Res.* **2010**, 1−12.
[6] Xu, M.; Li, J.; Iwai, H.; Mei, Q.; Fujita, D.; Su, H.; Chen, H.; Hanagata, N. *Sci. Rep*. **2012**, 2, 406.
[7] Vertegel, A. A.; Siegel, R. W.; Dordick, J. S. *Langmuir* **2004**, 20, 6800−6807.
[8] Kumar, S.; Aswal, V. K.; Callow, P. *Langmuir* **2014**, 30, 1588−1598.
[9] Schmidt, M. M.; Koehler, Y.; Derr, L.; Treccani, L.; Rezwan, K.; Dringen, R. *J. Phys. Chem*. C **2012**, 116, 23136−23142.
[10] Xia, X.-R.; Monteiro-Riviere, N. A.; Riviere, J. E. *Nat. Nanotechnol*. **2010**, 5, 671−675.
[11] Xia, X. R.; Monteiro-Riviere, N. A.; Mathur, S.; Song, X.; Xiao, L.; Oldenberg, S. J.; Fadeel, B.; Riviere, J. E. *ACS Nano* **2011**, 5, 9074−9081.