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Chitosan encapsulated curcumin loaded zinc ferrite core shell nanoassembly for biocompatible drug delivery on chicken embryonic stem cells

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ABSTRACT

Present work report the biocompatibility study of a nanoassembly of the drug Curcumin conjugated with Zinc Ferrite core and encapsulated by functionalizing agent Chitosan. Zinc Ferrite nanoparticles were synthesized separately by combustion route using urea and glycine mixture as fuel, and these nanoparticles were characterized on the basis of Transmission Electron Microscopy, FTIR spectra and X-Ray Diffraction. The average crystallite size of the ferrite nanoparticles were found to be 42 nm. The Chitosan capped and CTAB stabilized Curcumin loaded Zinc Ferrite Core shell CCZ nanoassembly were synthesized by In Situ Co precipitation. The mean particle size of these bioactive material was found to be 52nm. Slight cubical and spherical morphology of these nanostructure was estimated using Transmission Electron Microscopy , X ray Diffraction, and SEM. The formation of nanostructure was confirmed by UV-VIS, FTIR and Fluorescence spectra measurements. The drug delivery ability and biocompatibility on stem cells with plausible cancer therapy applications of these nanoassembly were elaborated on the basis in vitro test performed on Chicken Embryo Stem cells. The material had exhibited dose and time dependant biocompatibility with LD_{50} of 5 ng./ml. The internalization of Curcumin in the Stem cells elaborated on the basis of microscopic images. These functionalized nanoassembly had exhibited prominent applications in clinical trials for anticancer treatments.

Keywords: In Situ Co precipitation, Curcumin, Zinc Ferrite, Nanocomposites, Chitosan, biocompatibility.

INTRODUCTION

Ferrite nanoparticles with tailored surface chemistry have been widely used for various applications including magnetic resonance imaging, tissue repair, immunoassay, detoxification, hyperthermia and drug delivery. The applications of these capped nanoparticles in biomedical and bioengineering fields requires high magnetization and smaller size. Most importantly, the surfaces of these nanoparticles need to be tailored by coating with nontoxic and biocompatible polymers, not only to overcome the agglomeration resulting from a large surface-to-volume ratio, but also to meet the demands of some specific applications[5-8].

Among various coating materials, chitosan a natural polymer has been widely used because of its nontoxic, biodegradable, biocompatible properties . Curcumin is polyphenolic pigment found in turmeric [Curcuma longa], which is a traditional medicine found and used in south Asia and it has wide range of potent medicinal activities including antitumor, anticancer, antioxidant, anti-inflammatory, anti asthma and remedy for Alzheimer disease. Among the potent anticancer agents, curcumin has been found to be very efficacious against many different types of cancer cells. However, the major disadvantage associated with the use of curcumin is its low systemic bioavailability and aqueous solubility. So there is wide scope for the synthesis of encapsulated and drug loaded ferrite nanocomposites. Such nanostructures has got importance in effective and biocompatible drug delivery.

Although curcumin proves to be remarkably non-toxic and has promising anti-cancer activities, its application in anti-cancer therapies is limited due to its low aqueous solubility and poor bioavailability. More recently, the approach of biodegradable polymer materials has been developed for capping of ferrites. This offers promising therapeutic performance of anti-cancer drugs by increasing their bioavailability, solubility and retention time. These drug formulations are superior to traditional medicines with respect to control release, targeted delivery on the basis of ferrites as probes and therapeutic impact[9-11]. Polymeric nanoparticles act as nanocarriers with many advantages, such as low toxicity and high stability. Several drugs formulated in polymeric micelles are used in clinical trial development for the treatment of various cancers. Capped nanoparticles have attracted significant attention in the study of drug delivery systems as they offer a means for localized or targeted delivery systems of a drug to specific tissue, stem cells or tumor sites.

In the present work we have developed water dispersible Chitosan capped and functionalized Zinc Ferrite core shell nanocomposites loaded with drug Curcumin as novel targeted drug delivery system. After separate synthesis and physicochemical characterization of Zinc Ferrite nanoparticles and its Chitosan capped Nanocomposites loaded with Curcumin, the drug delivery ability and biocompatibity of these Nanostructures were estimated on the basis of in vitro test performed on Chicken Embryonic Stem Cells. The developed biocompatible drug delivery system shown the prominent future prospective for anticancer applications.

METERIALS AND METHODS

2.1. Materials and Instrument:

All the chemicals used were procured from S.D.Fine Chemicals ltd., Systronic double beam UV-VIS spectrophotometer, Perkin Elmer series IR spectrometer with KBr palette technique, Jasco type fluorescence spectrometer were used for structural characterization and Double Distilled Water was used for nanoparticles synthesis.

2.2. Preparation of bare $ZnFe_2O_4$ nanoparticles :

Zinc Ferrite nanoparticles were synthesized separately by combustion method. 0.1M Zn(II) and 0.2M Fe(III) nitrates were mixed together in a beaker and Urea + Glycine (0.1: 0.1 M) is added as a fuel, the mixture was homogenized to form emulsion. The emulsion first heated in air on hot plate to remove volatile gases by combustion and then ignited at 600^{o}C (Thermal Stability confirmed by TGA) in air in the alumina crucible with increase in heating rate in the muffle furnace for 3 hrs. So formed ferrite nanoparticles were used for physicochemical characterization.

2.3. Synthesis Chitosan coated Curcumin loaded Zinc Ferrite core shell nanoassembly (CCZ):

Chitosan coated curcumin loaded zinc ferrite core nanoparticles were synthesized using In situ co precipitation. Briefly, 15 ml. solution of 4.86 mg., 0.2 M $Fe(NO_3)_3$ and 2.96 mg. 0.1 M $Zn(NO_3)_2$ dissolved in double distilled water was heated to 80^{o}C for 10 min. and kept under constant stirring on magnetic stirrer for 3 hours. Then 15 ml. of 14M ammonium hydroxide were added drop wise followed by 100 mg. addition of Chitosan and 20 mg. CTAB surfactant to coat the chitosan on surface of ferrite nanoparticles and stabilize in colloidal state, the stirring continued for 30 min. The Chitosan coated magnetic nanoparticles were washed multiple times with Ethanol and acetone and then with Water to remove extra Chitosan and make them dispersible in water. These Chitosan capped ferrite nanoparticles dispersed in 25 ml. double distilled water and dispersion was formed. This Chitosan capped zinc ferrite nanoparticles dispersion further sonicated for 5 min. Then to load the Curcumin into these nanoparticles, 5 mg. of std. Curcumin dissolved in 1 ml . ethanol (about 5 ml., i.e. about 25mg./5ml.) was added drop wise to this suspension and kept stirring at 1000 rpm. on magnetic stirrer for 3 hrs. along with heating at 70-80^{0}C to allow the penetration of Curcumin into Chitosan coat. Finally the suspension cooled and centrifuged at 3000 rpm. and washed 3 times with 25 ml. portions of Double Distilled Water, and resuspended in sterile phosphate buffer solution with pH=7.4 until further use. whenever required, these CCZ designated nanocomposites were dried in desiccator.

The reactions taken place in the synthesis of nanoassembly are explained as
[In Situ Co precipitation]

1) $2\ Fe^{3+}_{(aq.)} + Zn^{2+}_{(aq.)} + 8\ OH^- \rightarrow ZnFe_2O_4 + 4H_2O$ [oxidation at basic pH and 80^{0}C]

2) $ZnFe_2O_4$ + CTAB + Chitosan → Chitosan Coated Ferrite [Phase transfer and adsorption]

2) Capped $ZnFe_2O_4$ + Curcumin → Chitosan coated -cur. Loaded -$ZnFe_2O_4$ [Cur. bonding and loading]

[Final CCZ Nanoassembly : $ZnFe_2O_4$ core coated by Chitosan and loaded with Curcumin]

2.4 Structural and Physicochemical Characterization :

2.4.1. UV-VIS spectra : UV-VIS spectra of curcumin and CCZ nanoformulation recorded separately with 0.1 mg./ml. dispersions in ethanol.

2.4.2. Fluorescence and FTIR spectra : Perkin Elmer Series FTIR spectrometer was used to study formation of nanoformulation compared with bare zinc ferrite nanoparticles using KBr palette method. The Jasco type Fluorescence spectrometer used to study the quenching of fluorescence due to bonding interaction of zinc ferrite with curcumin and capping material for 0.1 mg./ml. solutions in ethanol..

2.4.3. XRD analysis : The crystallite size of material, Packing and morphology tested using XRD spectrometer with Cu source on the basis of powder diffraction method.

2.4.4. SEM and TEM : The morphology of material confirmed on the basis of SEM analysis. The TEM image of CCZ nanoformulation sample was determined separately using JEUL type microscope from SAIF, IIT, Powai, Mumbai for the study of particle size.

2.5 In vitro Biocompatibility and drug delivery study of CCZ nanoassembly on Stem Cells :
In vitro biocompatibility of curcumin nanoformulation was tested on chicken embryonic stem cells. Briefly, the eggs were incubated to grow the stem cells at 37^0C in humid atmosphere in a sterile chamber. After 5 days variable concentration dosing of nanoformulation in sterile phosphate buffer medium[pH=7.4] was done on embryo and the stem cells which were incubated at same environment for 7 days. The embryo was slowly removed in sterile phosphate buffer solution and preserved in it to study cell internalization of nanoparticles and the morphology of embryo and stem cells were studied under microscope. The effect of nanoformulation on stem cells were checked on comparison with curcumin by separate dosing. The drug release and delivery effect for curcumin nanoformulation tested by using dialysis bag method and UV-VIS spectrometric assay.

2.6. In vitro drug release : (Dialysis bag method and UV-VIS spectrophotometric assay)
Dialysis bags of sizes of 12-14 kDa were cut off and filled with 2 mg. of CCZ nanoassembly and put into 40 ml. of sterile phosphate buffer with pH=7.4, this phase was stirred and incubated at 37^oC in 5% CO_2 atmosphere. After fixed time intervals in hours, the 2 ml. of this receptor phase was withdrawn and filled with fresh buffer. The curcumin release was assessed at these time intervals using UV-VIS spectrometer at 460 nm. The cumulative drug release profile for curcumin plotted against these time intervals in hours.

RESULTS AND DISCUSSION

3.1.Physicochemical and structural characterizations of Zinc Ferrite nanoparticles and CCZ Nanocomposites:
3.1.1. UV-Vis Spectra : The UV-Vis spectra of Std. curcumin and CCZ nanoacomposites were recorded for 0.1 mg./ml. suspension in ethanol. The spectra reveals that curcumin shows maximum absorption wavelength at 460 nm. and for CCZ nanostructure at 469 nm., due bonding of curcumin with Zinc Ferrite and Chitosan. While bare Cobalt Ferrite nanoparticles dispersed in ethanol and sonicated shown the λmax at 487 nm. The 9 nm. wavelength shift (Δλ) of curcumin to CCZ nanocomposite is due to formation of core shell nanocomposite. The decrease in the absorbance in the spectra supports the curcumin bonding and formation of Chitosan capped Curcumin and Zinc ferrite nanoconjugate (refer Fig.1).The magnetic nanostructure CCZ-MNC contain Van -Der Waals bonding interaction of curcumin with oxide and Chitosan coat as polymer capping material.

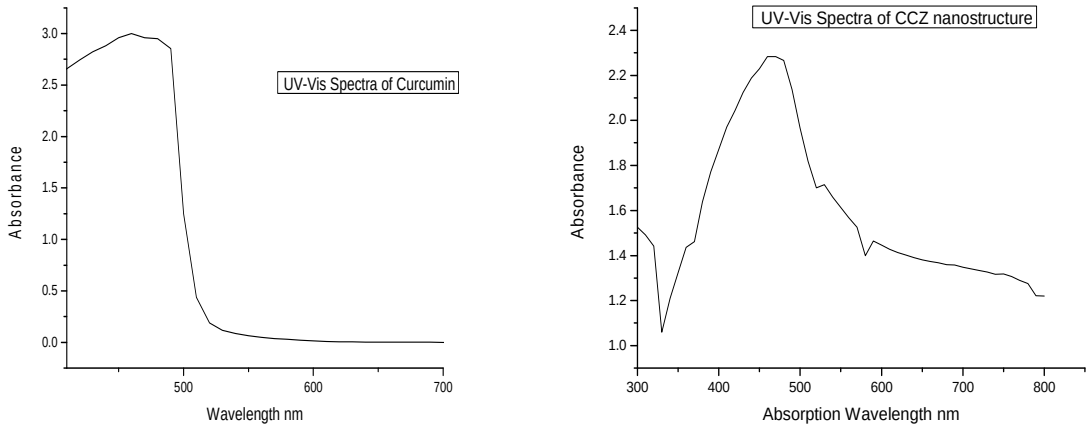


Figure 1 : UV-Vis spectra of curcumin and CCZ nanostructure

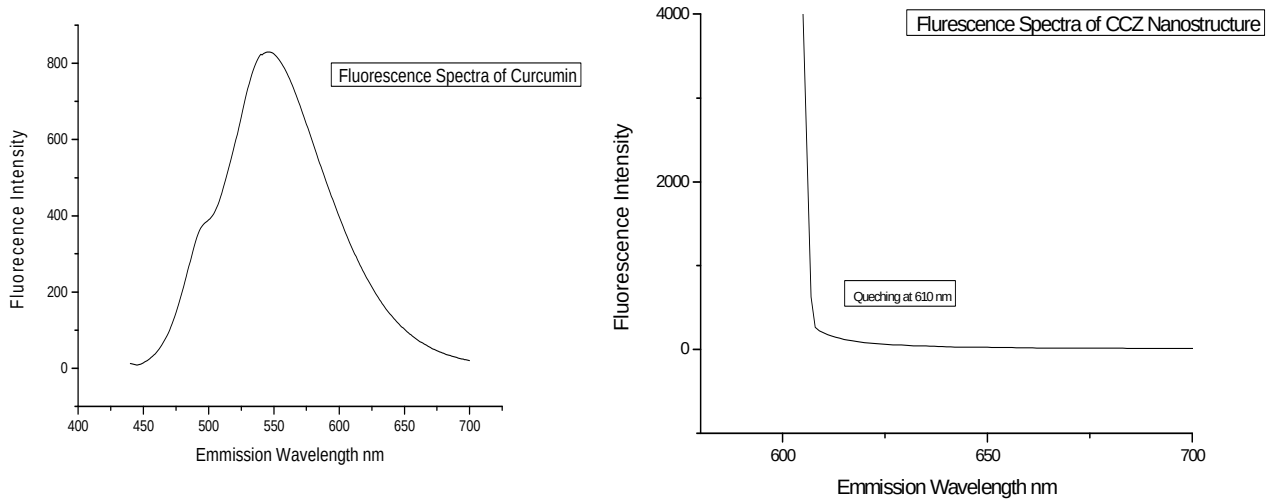


Figure 2 : Fluorescence spectra of curcumin and nanoassembly

3.1.2. Fluorescence Spectra of Curcumin and CCZ Nanocomposites:

Fluorescence spectra of curcumin recorded at 460 nm. and for CCZ nanocomposites it was recorded at 469 nm. for 0.1 mg./ml. solution in ethanol. The spectra revealed that curcumin shows maximum fluorescence at 560 nm. and CCZ nanostructure shown quenching of fluorescence, due to boding and capping of Curcumin. The fluorescence was quenched for nanoassembly at 610 nm. due bonding of curcumin with Zinc ferrite and Chitosan (refer Fig.2). It had proved the formation of Chitosan capped Curcumin loaded Zinc Ferrite core shell nanocomposites. The magnetic nanostructure contain electrostatic interaction of curcumin with ferrite and the polymer capping material.

3.1.3.FTIR Spectra of Zinc Ferrite and CCZ Nanostructure :

The FTIR spectra of Zinc Ferrite nanoparticles and Final CCZ nanocomposites were separately recorded on Perkin Elmer series spectrometer. Zinc Ferrite shown three strong peaks at 543 cm^{-1}, 1071 cm^{-1}, and 1115cm^{-1} in the fingerprint region because of Fe-O and Zn-O, Zn-Fe stretching vibrations respectively, and the two weak peaks at 2824 cm^{-1}, 3028 cm^{-1} were supposed due to Fe-O stretching in Fe_2O_3 entity present in zinc ferrite, while the broad peak at 3441 cm^{-1} was observed due to Zn-O-Fe stretching in zinc ferrite (refer Fig.3a). These values in the spectra supported tetrahedral group in the cubic spinal nature of zinc ferrite. Furthermore, In second FTIR spectra, –OH vibration Frequency of Curcumin was shifted at 3131 cm^{-1}, due to H-bonding of Curcumin in CCZ nanocomposite with Chitosan capping agent and Van- Der Waals interaction with Zinc Ferrite. The peak at 3441 cm^{-1} was due to –OH groups of Chitosan polymer which were H-bonded with Curcumin. The strong peak in second spectra at 3380 cm^{-1} was observed due to amine group interaction of Chitosan with Curcumin and Cobalt Ferrite. The strong peaks at 1265cm^{-1}, 1123cm^{-1}, 1069cm^{-1}, 992cm^{-1}, 956cm^{-1} were shown the N-H bending, O-H bending vibrations of Chitosan and Curcumin diketone bending vibrations which proved the weak bonding interactions of Curcumin with strong Chitosan cap and core shell of Cobalt Ferrite. The remaining peaks at 616 cm^{-1}, 518 cm^{-1}, and 854 cm^{-1} in second FTIR spectra (See Fig.3b) were due to aromatic-H and $–OCH_3$ groups of Curcumin bonded with Chitosan and Cobalt Ferrite core shell in final CCZ Nanostructure. So data suggested the Curcumin can be easily released in drug delivery, due to uniform distribution of Curcumin in the material.

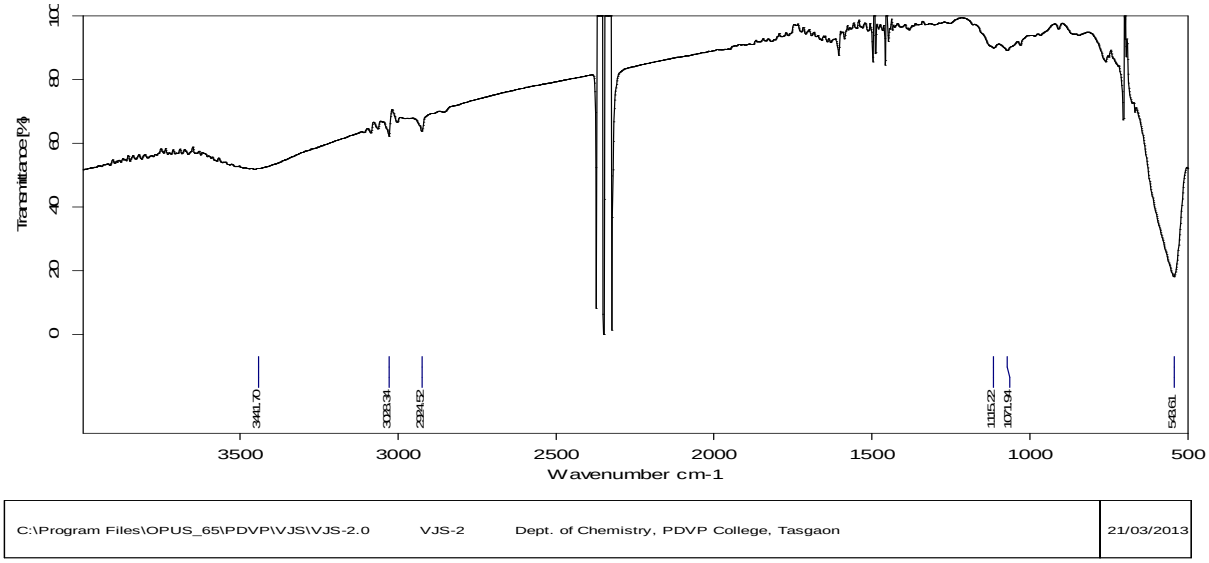


Figure 3 a : FTIR spectra of bare Zinc Ferrite nanoparticles

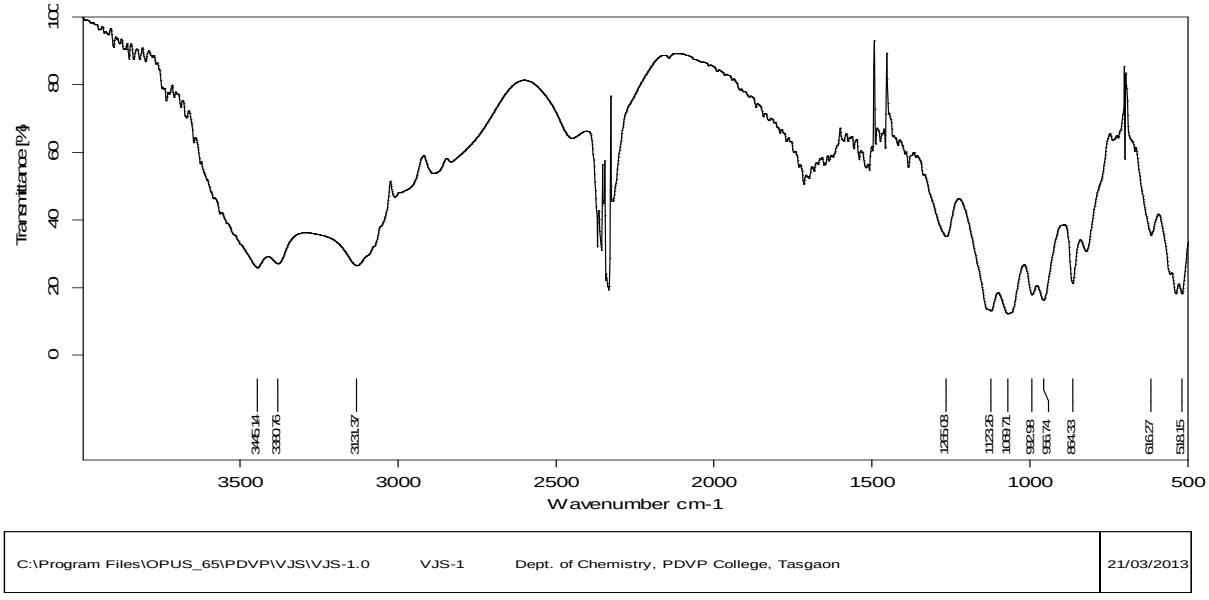


Figure 3 b : FTIR Spectra of Final CCZ Nanostructure

3.1.4. X ray diffraction :

X ray diffraction patterns of bare Zinc Ferrite nanoparticles and CCZ nanocomposites were determined separately by powder diffraction method using Cu kα source. The XRD spectra of Zinc Ferrite exhibited polycrystalline nature of bare oxide material with sharp peaks. The XRD pattern shown the sharp peaks with (311) plane as main peak. The spectra was matched with standard XRD pattern of Zinc Ferrite with JCPDS card no.-82-1049, having Cubic Spinal nature of material with crystalline lattice constant a = 8.440 A^0. All the peaks and lattice constant were in good agreement with standard XRD pattern. Hence synthesized bare Zinc Ferrite nanoparticles not shown any phase impurity, except some carbon impurity may be present due to combustion. Synthesized Zinc Ferrite nanoparticles shown Cubic Spinal structure with lattice constant a = 8.434 A^0, and the crystallite size was calculated on the basis of Debye Scherer's formula. It was found to be 42 nm.

The XRD pattern of CCZ nanocomposites shown slight decrease in the intensity of peaks, and some broadening of peaks in second spectra shown the amorphous coating of polymer and Curcumin loading on Ferrite core. The material had been shown Cubic spherical packing of Zinc Ferrite core with Curcumin in Chitosan. The sharp peaks in the XRD pattern were might be due to presence of Zinc Ferrite core in the nanocomposites. The 2 theta value of (311) plane of sharp peak was decreased in second XRD spectra of Nanocomposites showing bonding of Ferrite with Curcumin and Polymer. The final nanocomposite material shown the crystallite diameter of 52 nm. and lattice

constant of a = 8.556 A^0, which proved the presence of Zinc Ferrite core in the material without any phase impurity (refer Fig. 4). The XRD patterns proved the presence Zinc Ferrite core in the CCZ nano assembly.

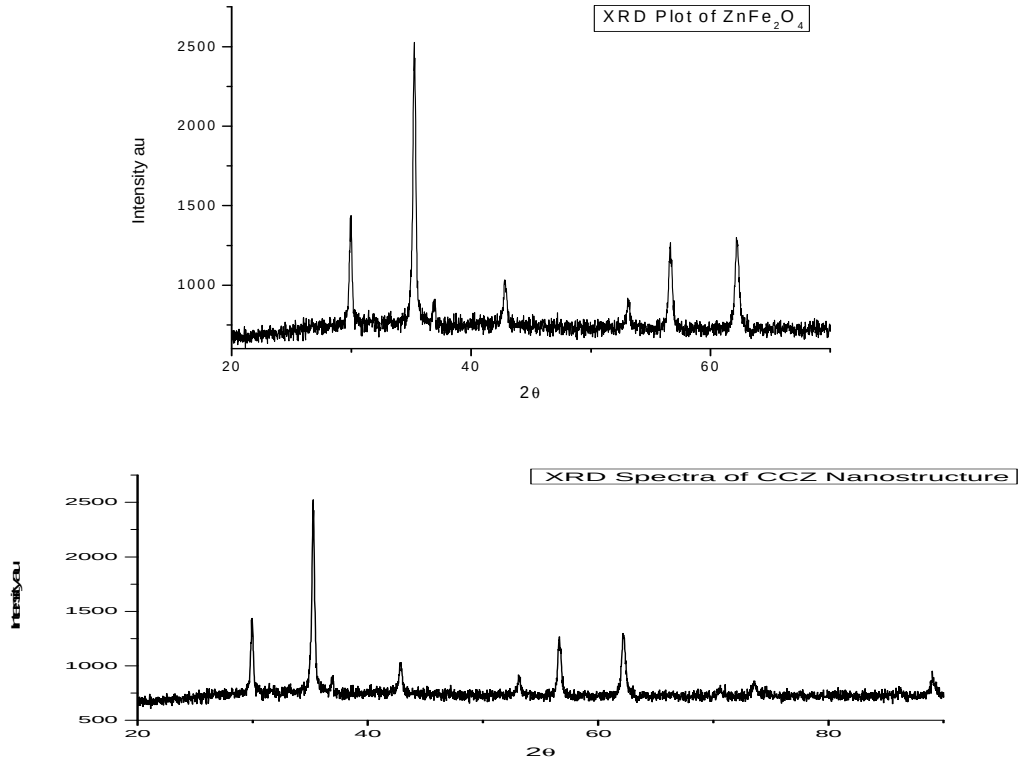


Figure 4 : XRD Patterns of Zinc Ferrite and Final CCZ Nano assembly

XRD Data of bare Zinc Ferrite :
Crystallite size calculated using Debye Scherer's formula, $K= 0.9\lambda/\beta.COS\theta=$ 42 nm.
Diffraction pattern of zinc ferrite,

Crystallite planes (Miller Indices) [h,k,l]	d Calculated A^0 $d = a/\sqrt{(h^2+k^2+l^2)}$ or $2dSin\theta= n\lambda$	d Standard A^0	Lattice Constant a A^0
220	2.9818	2.9840	a Standard = 8.440
311	2.5429	2.5447	
222	2.4346	2.4364	
400	2.1085	2.1100	
331	1.9349	1.9363	
333	1.6231	1.6242	a Calculated = 8.434
440	1.4909	1.4920	
622	------	1.2723	

3.1.5. SEM Images :
SEM images of CCZ nanostructure had shown the cubic, spherical and some elongated morphology of material suggesting the presence of zinc ferrite core. The nanocomposite shown chitosan coated curcumin loaded grains of zinc ferrite with non uniform size(See Fig.5a and 5b). The variable grain sized material had shown some amorphous aggregation of particles showing existence of chitosan encapsulation. While zinc ferrite shown single grain crystals with cubic packing and porosity (refer fig. 5a,b,c).

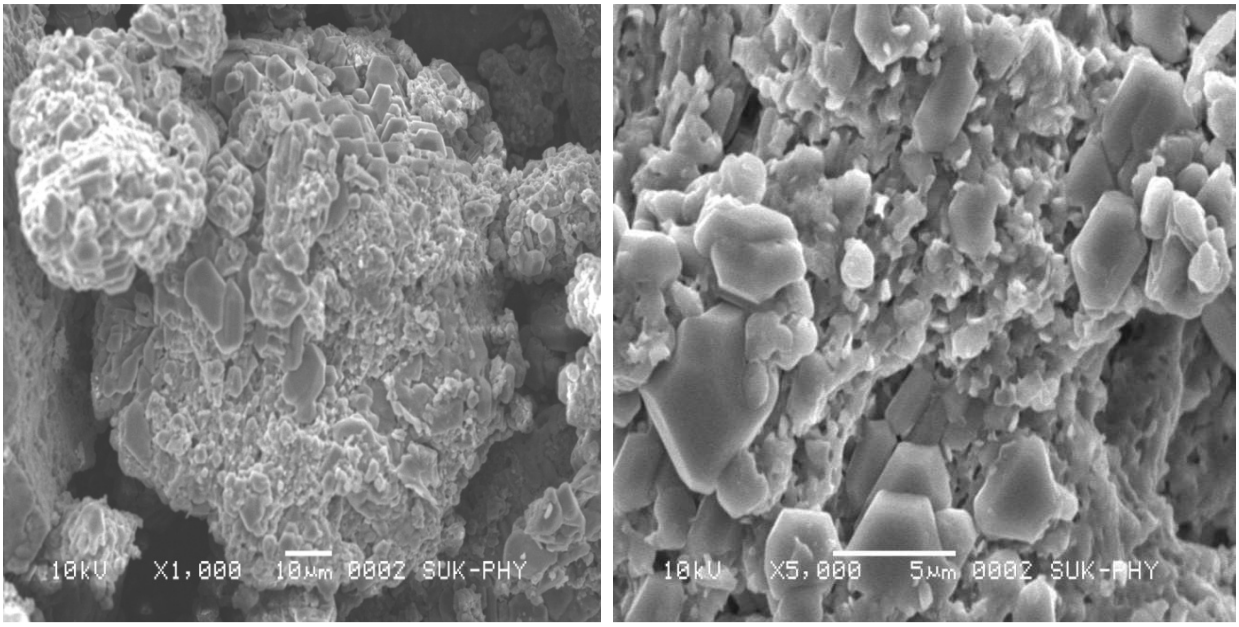


Figure 5 a) : SEM Images of CCZ Nanostructure

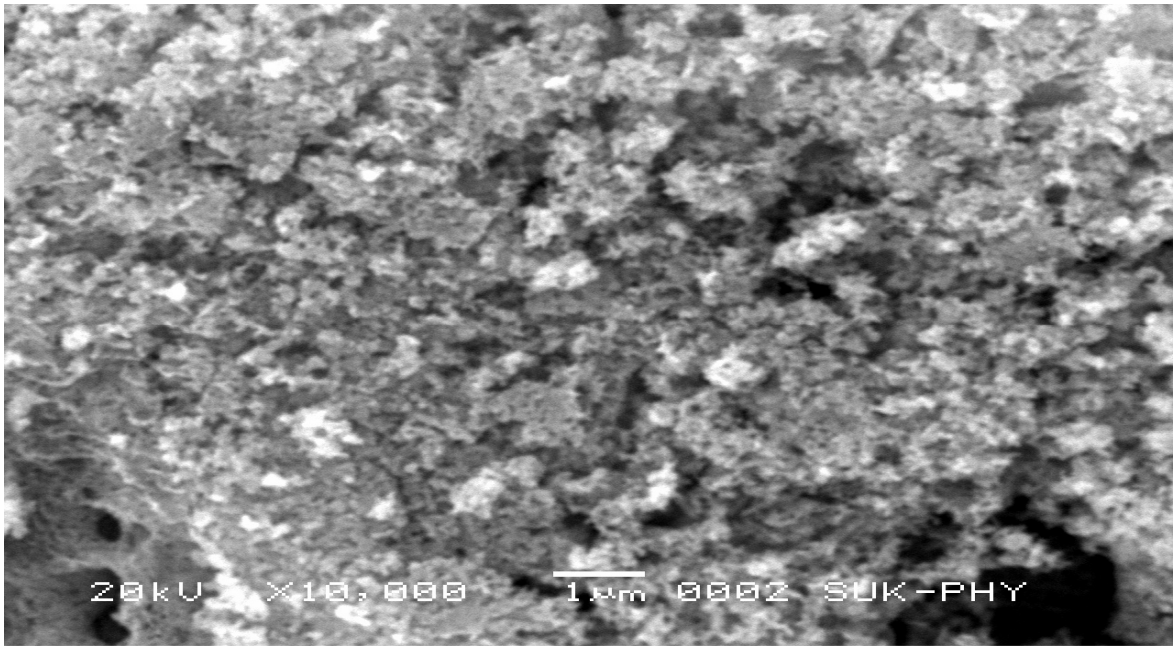


Figure 5 b) : SEM Image of CCZ Nanostructure

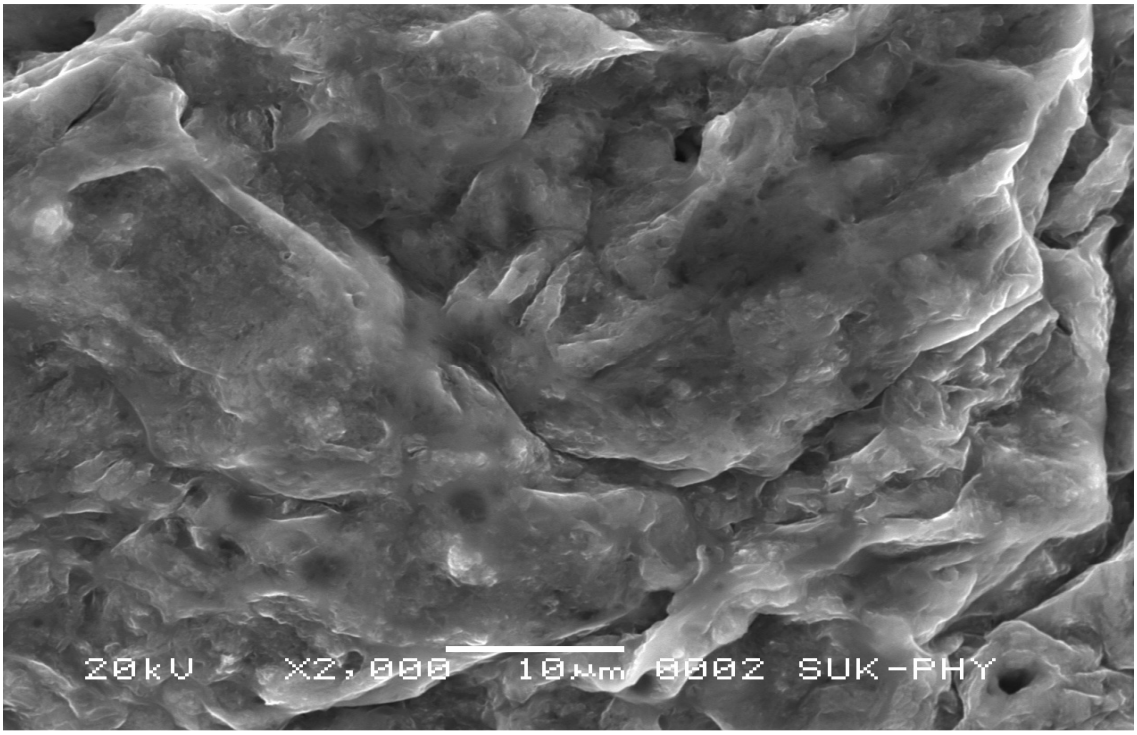
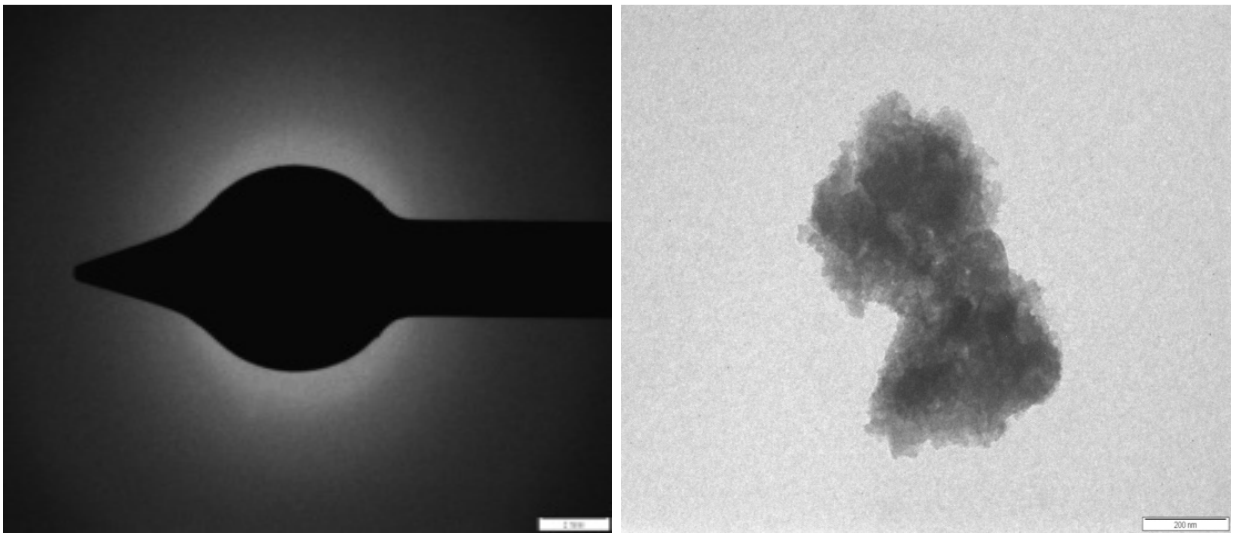


Figure 5 c) : SEM Image of Zinc Ferrite

3.1.6. TEM Images of CCZ nanoassembly :

TEM images of CCZ nanostructure shown that these nanocomposites of curcumin consist of zinc ferrite core with chitosan coating and curcumin loading and exhibited some cubical or elongated and spherical morphology(See Fig.6). The mean particle size these bioactive material found to be 50-60 nm , which matched with XRD crystallite size of 52 nm. The SAED pattern of material had shown that the CCZ nanocomposite possessed some amorphous nature due to biodegradable chitosan coat.



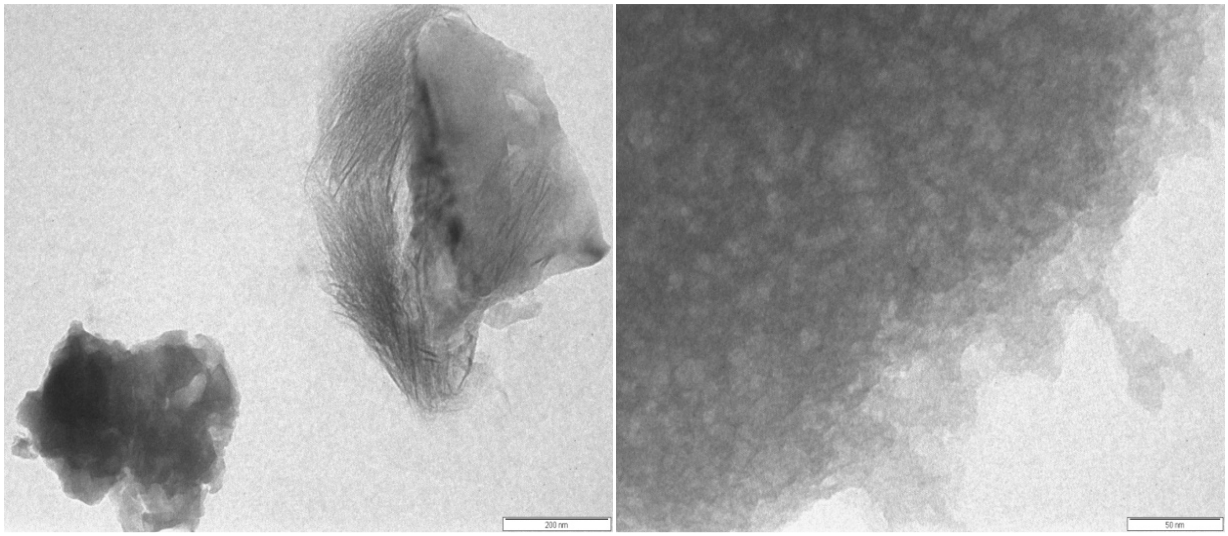


Figure 6 : TEM Images and SAED pattern of CCZ Nanostructure

3.2. Biocompatibility in vitro and drug delivery on chicken embryonic stem cells:

3.2.1. Dose and Time dependent biocompatibility :

The CCZ nanoformulation of curcumin shown dose dependant and time dependant biocompatibilities. The material shown cell internalization and growth inhibition of stem cells after incubation for 7 days. The CCZ nanocomposite exhibited slow and sustained drug release on the embryonic stem cells. The normal growth of embryo and stem cells was inhibited at very low doses of concentrations of 100 Ng./ml. to 5 Ng./ml. So this material is a best delivery system of anticancer drug curcumin. In combination with ferrite core the curcumin had proved plausible enhanced cell apoptosis effects. The microscopic images of embryo and stem cells demonstrates these effects with biocompatible drug delivery(refer the images 1 to 5).

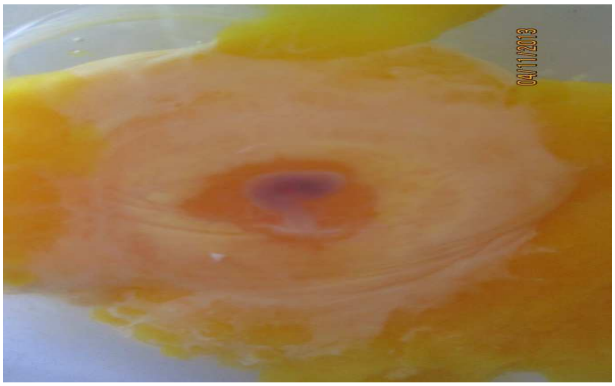


Image 1. :
Cell growth inhibition for CCZ [nanocomposites dose (100 Ng/ml.) in PBS with slow cell internalization]
(possible Cell apoptosis)

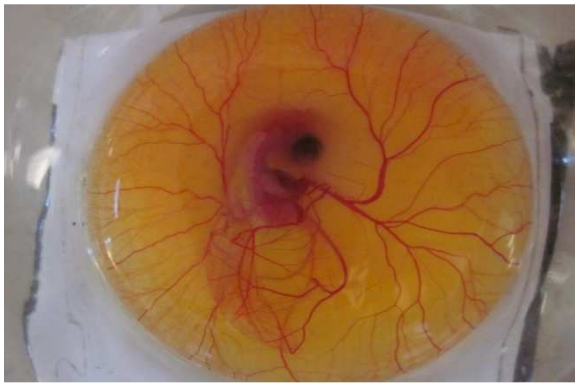


Image 2. :
Normal growth of Embryo

Image 3.: No growth for low soluble free Curcumin dose (100 Ng/ml.) in PBS With low cell internalization.

Image 4:
Slow growth inhibition for Only free Curcumin dose (5ng./ml.) in PBS With high cell internalization.

Image 5:
Fast growth inhibition for CCZ Nanocomposites dose (5ng./ml.) in PBS [LD_{50}] with high cell internalization.

[a preliminary observation for cell internalization, biocompatibility and plausible anticancer potential]

3.2.2. Drug release profile : The in vitro drug release profile of curcumin from CCZ nanoassembly revealed that these water soluble nanoassembly release curcumin in large percentage after 72 hours., equal to about 64 %. After three days the curcumin release increased in PBS or aqueous medium. After 7 days the highest drug release was observed that of 74.8 % . Hence these nanoassembly have ability to release drug curcumin on stem cells or cancer cells effectively. The slow and sustained release of curcumin in release profile exhibited the potent ability of these nanoassembly in therapy (refer drug release profile graph of Fig.7). As curcumin have different therapeutic applications, these nanoassembly are novel water soluble formulation model for targeted delivery of curcumin on various types of cancer cells on the basis of zinc ferrite as probe. The sustained release of curcumin was due to coating of biocompatible and biodegradable polymer Chitosan.

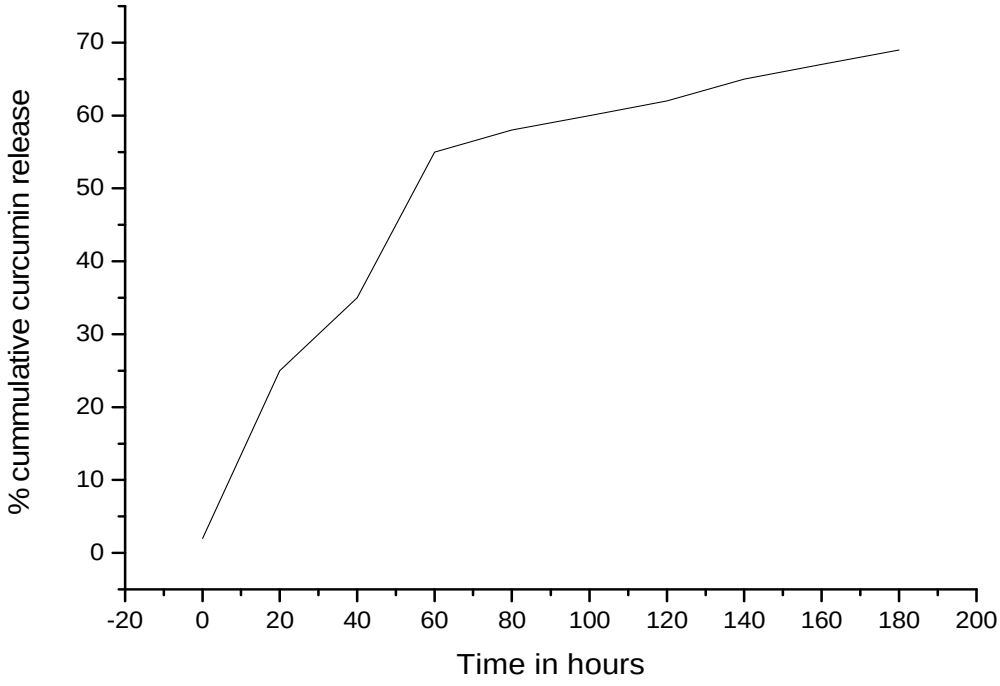


Figure 7 : In vitro drug release profile of curcumin from CCZ nanoformulation.

CONCLUSION

In the present work we had synthesized a drug delivery system of anticancer drug curcumin. It was containing chitosan as biocompatible, non-toxic and hydrophilic encapsulation natural polymer agent and zinc ferrite as magnetic core. These hydrophilic nanocomposite drug targeting delivery system was synthesized through simple in situ wet chemical co precipitation route. As synthesized nanoassembly and bare zinc ferrite nanoparticles synthesized by combustion route were characterized on the basis of UV-VIS, FITR, Fluorescence spectra measurements and formation of material was confirmed . The nanocomposite exhibited cubic and spherical morphology and bare zinc ferrite nanoparticles exhibited spinal structure confirmed on the basis of XRD pattern. Further the morphology of material conformed by SEM analysis. The crystallite size of bare zinc ferrite was found 42 nm. TEM analysis for spherical CCZ nanocomposite of curcumin were shown mean particle size of 52 nm and cubic structure which was estimated on the basis of XRD pattern. The CCZ nanocomposite had shown concentration and time dependent drug release ability. The biocompatibility of these material tested on chicken embryonic stem cells. The material shown Lethal Dose, LD_{50} value equal to 5 ng/ml. The material had shown prospective for biocompatible drug delivery to anticancer treatments. Furthermore there is need of cytotoxicity study for these biocompatible drug delivery system.

Acknowledgments
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REFERENCES

[1] Namdeo M, Saxena S, Tankhiwale R, Bajpai M, Mohan YM, Bajpai SK., *J Nanosci Nanotechnol.* **2008**,*8(7),3247–3271.*
[2] Banerjee SS, Chen DH., *Nanotechnology*, **2008**,*19(26),265602.*
[3] Jain TK, Foy SP, Erokwu B, Dimitrijevic S, Flask CA, Labhasetwar V., *Biomaterials*, **2009**,30(35),6748–6756.
[4] Yallapu MM, Foy SP, Jain TK, Labhasetwar V., *Pharm Res.*, **2010**,*27(11),2283–2295.*
[5] Ly JD, Grubb DR, Lawen A., *Apoptosi,*. **2003**,*8(2),*115–128.
[6] Manju S, Sreenivasan K*J Colloid Interface Sci.*,**2011**,359(1),318–325.

[7] Marcolino VA, Zanin GM, Durrant LR, Benassi Mde T, Matioli G., *J Agric Food Chem.*, **2011**,59(7),3348–3357.
[8] Hegge AB, Schuller RB, Kristensen S, Tonnesen HH., *Pharmazie.*, *200,63(8),*585–592.
[9] Shahani K, Panyam J., *J Pharm Sci,.* **2011**,*100(7),*2599–2609.
[10] Utreja P, Jain S, Tiwary AK., *Curr Drug Deliv.*, **2010**,*7(2),152–161.*
[11] Sawant V. J., Bamane S. R., *Int.J.Pharm.Sci.Rev.Res.*, **2013**,*20(1), 159-165* .