Antimicrobial activity of novel chitosan/cloisite 10A nanocomposite: Preparation, optimization, characterization and drug delivery behavior

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Abstract: The objectives of the present research project were to formulate, evaluate and perform antimicrobial study and drug delivery behavior of nanocomposite material based on biopolymer chitosan and organically modified montmorillonite clay; i.e. cloisite 10A. In the present study, chitosan / cloisite 10A nanocomposite material was formulated by solution mixing and optimized. The nanocomposite material was characterized by FTIR, zeta sizer, XRD, and SEM. Polymer/clay nanocomposite material is evaluated for its antimicrobial activity against both gram- negative and gram- positive bacteria. It was also studied for potential drug carrier system using diclofenac sodium as a model drug. Drug incorporation efficiency and drug content were also determined. SEM provided the composite shape and its surface topography. XRD data revealed the nanocrystalline composition and crystallite size. The average diameters of particles in the nanocomposite were found to be around 80 nm from both XRD report, calculated by applying Scherrer equation and zeta sizer. The antimicrobial activity report revealed that nanocomposite exhibited stronger inhibition against the microorganisms as compared to that of pure chitosan. From the *in vitro* drug-release study, it is observed that biopolymer/clay nanocomposite exhibited extended release period of drug as compared to the pristine chitosan. This research work provides a platform for further research on the polymer/clay nanocomposites for biomedical and drug delivery applications.

Keywords: Chitosan, Clay, Nanocomposite, SEM, XRD, Diclofenac sodium

INTRODUCTION

Nanocomposites are defined as materials having a dispersed material that has one or more dimensions, such as length, width and thickness, in the nanometer size range. For their specific structure and properties, they have been used as biomaterials and as controlled drug delivery. They have emerged as perfect alternatives to overcome limitations observed in micro composite materials. Inorganic materials including mineral clays like bentonite, rectorite and montmorillonite (Wang *et al.,* 2010, Han *et al*., 2010, Wang *et al*., 2009, Wang *et al*., 2007, Tunc *et al*., 2011), hydroxyapatite, calcium deficient hydroxyapatite (Rajkumar *et al.,* 2011, Zhang *et al.,* 2010, Lazic *et al.*, 2001, Mizushima *et al.*, 2006, Xu *et al.*, 2008, Real *et al*., 2000) and silica (Khunawattanakul *et al*., 2010) have been used for the preparation of these special classes of novel nanomaterials. The biodegradable polymer nanocomposite materials possess better mechanical properties, swelling behavior, drug loading efficiency and controlled release behavior as compared to polymer matrices.

Chitosan is a biodegradable, biocompatible polymer derived primarily from chitin, which is present in shrimp, and crawfish (Raafat *et al*., 2008). It is derived from the alkaline deacetylation of chitin. It is considered as a partially N-deacetylated derivative of chitin (Kumar *et al*. 2000). Structurally, chitosan is a high-molecular-weight linear hetero-polysaccharide (Raafat *et al.*, 2009). It has been used in pharmaceutical, medical, cosmetic, and agricultural field due to its excellent properties like biocompatibility, biodegradability, and low toxicity (No *et al.*, 2007). Chitosan possesses antimicrobial, fungi static, CNS depressant, spermicidal, antitumor, anticholesteremic, immunoadjuvant and haemostatic properties. It can accelerate bone formation as well as exhibits regenerative effect on connective gum tissue (Dutta *et al.*, 2004).

The antimicrobial property of chitosan has received considerable attention in recent years due to severe adverse effects are associated with synthetic chemical agents. It shows greater potency, greater spectrum of activity, and lower toxicity than other natural antimicrobials. A number of studies have been conducted and reported regarding the assessment of antimicrobial potentials of chitosan (Jeon *et al*., 2001, Chhabra *et al*., 2006). Chitosan is a cationic antibacterial agent. It acts by interfering in bacterial cell permeability. Some scientists suggested that antimicrobial activity is due to the inhibition of the metabolic activity of the bacteria (Tokura *et al.*, 1997).

Cloisite 10A is a natural montmorillonite modified with a quaternary ammonium salt. The organic modifier is 2MBHT: dimethyl, benzyl, hydrogenated tallow,

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quaternary ammonium. The modifier concentration is 125 meq/100g clay. Montmorillonite clays and their modified forms find wide range of applications, in various areas of science, like drug delivery systems because they are widely available in nature. It is normally chosen as the model clay in drug delivery systems because it exhibits both a high exchange capacity (W150mEq/100g) and a high surface area (700-800m2/g) (Han *et al.*, 2010, Tunc *et al.*, 2011).

Diclofenac sodium is a NSAID, widely used in long-term therapy for treatment of rheumatoid arthritis. It also reduces body pain, muscle pain and inflammation. It requires multiple dosing to maintain therapeutic drug blood level as the plasma half-life is about 1-2 hours. The most frequent adverse effects of this drug on long-term administration are gastro-intestinal disturbances, peptic ulceration etc. It is poorly water soluble in water.

The present study aims to develop, evaluate and perform antimicrobial study and drug delivery behavior of nanocomposite material based on chitosan and cloisite 10A and to find a new approach to control the release of drugs from the formulation and to overcome the limitations of conventional formulations which eliminates the need for multiple dosing thereby increasing patient compliance and decreasing the occurrence of adverse effects.

MATERIALS AND METHODS

Materials

Chitosan powder (deacetylation >85%, apparent density= 0.18-0.23gm/cc) was supplied as a gift sample by Everest Biotech (Bangalore, India). Diclofenac sodium was purchased from sigma Sigma-Aldrich, USA. Cloisite 10A was purchased from Southern Clay Products, USA. Other reagents and chemicals were obtained from Deepa industries ltd, Aurangabad, India. All chemicals were used as received, without further purification.

METHODS

Preparation of cloisite 10A-chitosan- diclofenac sodium nanocomposite materials by solution mixing method

Firstly, Cloisite 10A suspension was prepared by adding deionized water under vigorous stirring for 24 hours and kept for another 24 hours for swelling. Then chitosan solution is prepared by dissolving it in 1% (w/v) acetic acid to prepare 0.5% (w/v) solution. After that, chitosan solution was added slowly into the prepared cloisite 10A suspensions under magnetic stirring of 2000 rpm for 12 hour at 60^{o}C. Predetermined amount of diclofenac sodium was added slowly into the mixture. The resulting mixture was precipitated with 1 mol/l NaOH solution. The prepared nanocomposite materials were washed with deionized water until they became neutral. The formed nanocomposites were dried at 60^{o}C and made them to powders. The weight ratios of chitosan/cloisite 10A in different nanocomposites were 100:1, 50:1, 20:1, 10:1, and 5:1. The nanocomposite materials prepared were used for characterization. Chitosan/diclofenac sodium composites were also prepared by the same method without addition of cloisite 10A.

Entrapment efficiency determination

Drug entrapment (%) was carried out and calculated using the following equation.

Drug entrapment (%, w/w) = (Mass of the total drug −Mass of free drug) × 100/ Mass of total drug

Optimization of chitosan/clay ratio with regard to average particle size

For optimization of chitosan/clay ratio, the nanocomposite were prepared with 100:1, 50:1, 20:1, 10:1, and 5:1. The average particle size was determined using Particle size analyzer (LS230, Beckman Coulter, USA).

Optimization of stirring speed with regard to percent drug entrapment and average particle size

Nanocomposite formulation with optimized chitosan/cloisite 10A ratio was selected. After that nanocomposites were again prepared by same procedure however; with a varying stirring speed. Average particle size and drug entrapment were determined and recorded.

Antimicrobial activity study

Antimicrobial activities of the synthesized chitosan/cloisite 10A nanocomposite were performed by modified Kirby-Bauer disk diffusion method. These tests were done against *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus*. Tetracycline was used as a positive control.

In vitro drug release

Buffer solution of pH 7.4 was prepared by mixing 500ml of 0.1M KH_2PO_4 AND 391ml of 0.1M NaOH. The *in vitro* drug release tests were done using the USP six stage dissolution rate test apparatus by using dialysis bag technique. The amount of drug released was monitored by a UV Visible spectrophotometer (Shimadzu UV 1800 spectrophotometer) at 276nm.

Characterization of prepared nanocomposite

The nanocomposites were evaluated by powder x-ray diffraction (XRD), particle size analyzer (Beckman coulter), scanning electron microscopy (SEM) and fourier transmission infrared spectroscopy (FT-IR) techniques. The infrared absorption spectra of the polymer, clay, and the formulated nanocomposites were obtained using a FT-IR spectrophotometer (Perkin Elmer Spectrum Version 10.03.02). The intensity of distributions, average diameter and polydispersity index of particles in the nanocomposite

Table 1: Optimization of chitosan/cloisite 10A ratio with regard to average particle size

Formulation code	Chitosan/cloisite 10A	Average particle size (nm)	Polydispersive index	Diffusion constant (Cm^2/Sec)
CC I	100:1	394.6	0.275	1.247e-008
CC II	50:1	379.3	0.178	1.279e-008
CC III	20:1	378.4	0.191	1.300e-008
CC IV	10:1	81.2	0.180	6.058e-008
CC V	5:1	446	0.217	1.103e-008

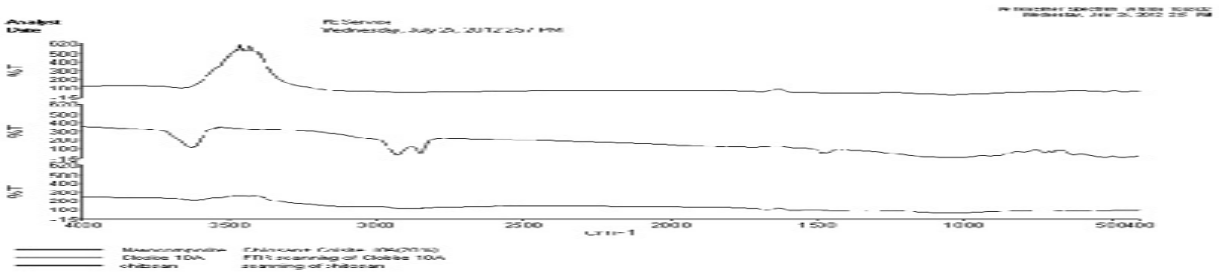

Fig. 1: FT-IR of chitosan, cloisite 10A and polymer/clay nanocomposite (weight ratio 10:1)

were determined by Particle size analyzer (LS230, Beckman Coulter, USA). The surface morphologies and surface topography were obtained using a scanning electron microscope (SEM). The powder X-ray diffraction (XRD) analysis was performed using a powder diffractometer with Cu target and Kα (λ=0.154056nm) at 40 kV with a slow scan of 0.3 degree/s in 2θ range 10-50 degree at room temperature. The crystallite size of the nanocomposite was determined from the XRD study by the Scherrer Equation.

$t = 0.9\,\lambda / B.\cos\theta$

Where, t = thickness of crystallite, λ = x-ray wavelength, B = (2θ High) – (2θ Low)

RESULTS

Optimization of chitosan/cloisite 10A ratio with regard to average particle size

The optimization of chitosan/cloisite 10A ratio is reported in table 1, considering average particle size.

Table 2: Optimization of stirring speed with regard to percent drug entrapment and average particle size

Formulation code	Stirring speed	% Drug entrapment	Average particle size (nm)
CC IV S1	500	66.6	686.0
CC IV S2	1000	74.0	605.8
CC IV S3	2000	75.3	81.2
CC IV S4	2500	72.5	347.3

Optimization of stirring speed with regard to percent drug entrapment and average particle size

The optimization of drug/chitosan-cloisite 10A ratio is reported in table 2, considering the stirring speed and average particle size.

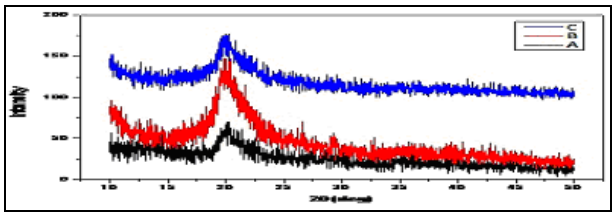

Fig. 2: X-ray diffraction patterns of chitosan/cloisite 10A/diclofenac sodium nanocomposite (A: chitosan, B: cloisite 10A, C: nanocomposite)

Structure and morphology

In the spectra of chitosan, the broad band near 3500cm-1 corresponded to the amine and hydroxyl groups and the peak at 2950cm-1 is caused by -OH stretching. The peaks observed at 1080cm-1 and 1042cm-1 are the hydroxyl groups and the primary hydroxyl group. In the FT-IR spectra of cloisite 10A, sharp band at 3550cm-1 corresponds to N-H stretching where as peaks at 2900 cm-1 and 2950cm-1 corresponds to aliphatic C-H stretching. The peak observed at 1590cm-1 corresponds to N-H bending vibration and peak between 1000cm-1 to 1100cm-1 correspond to C-H stretching vibration. In the FT-IR spectra of nanocomposite, the broad band at 3510 cm-1 corresponds to the -OH and NH2 of chitosan, which has been shifted to 3440cm-1 in the nanocomposite, moreover the broadening of peak has also been reduced suggesting good interaction between cloisite 10A and chitosan. The typical FT-IR pattern of the nanocomposite is shown in fig. 1.

The typical XRD pattern of the nanocomposite is shown in fig. 2. It confirms the formation of nanocomposite material. The crystallite size of the nanocomposite was found to be 80 nm by applying Scherrer equation.

The average particle diameter of the nanocomposite was found to be 81.2nm, where as polydispersity index of particles was found to be 0.180. From the intensity of distribution table, it is found that the diameters of 10% particles were below 36.60nm, 50% particles were below 87.60nm, 90% particles were below 215.90nm. This analysis was carried out in 25^{o}C, with water as diluent, and scattering intensity 9950 cps.

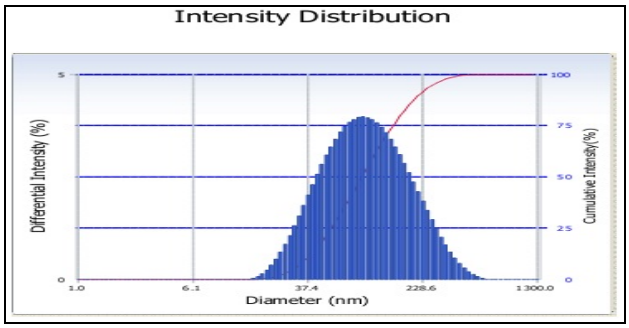

Fig. 3: Intensity of particle distributions in nanocomposite formulation

The surface morphologies of the prepared composites were examined using a scanning electron microscope (fig. 4a, 4b).

Chitosan/cloisite 10A nanocomposite and pristine chitosan exhibited bacterial growth inhibition 23 mm and 17 mm respectively against *B. subtilis*, where as in case of *E. coli* maximum growth inhibition zones were found to be 20 and 16mm respectively. Similar patterns were observed in the case of *P. aeruginosa* and *S. aureus*, where the maximum zone of inhibition was exhibited by chitosan/cloisite 10A nanocomposite followed by pristine chitosan. Generally all chitosan based materials, including pure chitosan and chitosan/cloisite 10A nanocomposite exhibit antimicrobial activity.

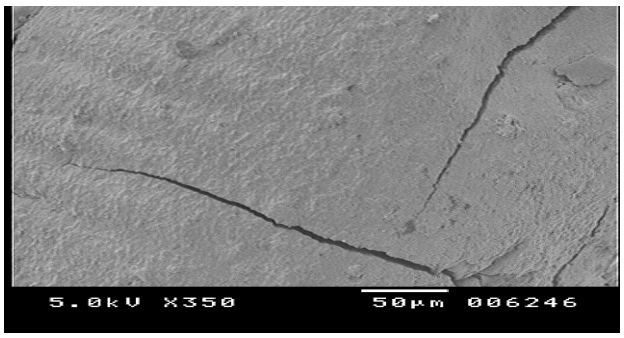

Fig. 4(a): SEM image of nanocomposite

The zone of inhibition produced by pure chitosan, chitosan/cloisite 10A nanocomposite against both gram positive and gram-negative bacterial strains is shown below in table 3.

In vitro drug release study

In vitro release profile of the chitosan/cloisite 10A/diclofenac sodium nanocomposite, chitosan-diclofenac sodium composite are shown in fig. 5. In case of chitosan-diclofenac sodium composite the release pattern was found to be biphasic with initial burst effect followed by a controlled release but in case of the chitosan/cloisite 10A/diclofenac sodium nanocomposite the release pattern was in a more controlled fashion. The drug release mechanism in chitosan/cloisite 10A/diclofenac sodium nanocomposite was non-fickian type (n=0.639) where as in the chitosan-diclofenac sodium composite was fickian type diffusion controlled (n=0.361).

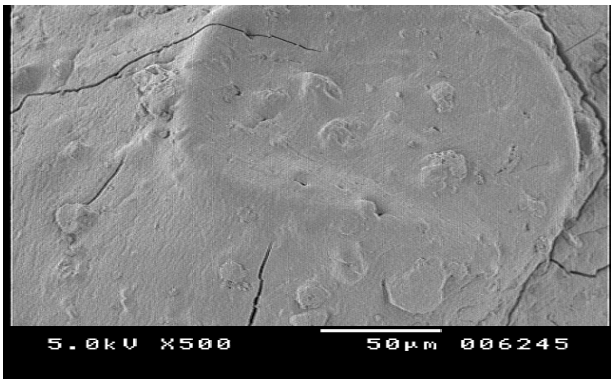

Fig. 4(b): SEM image of nanocomposite

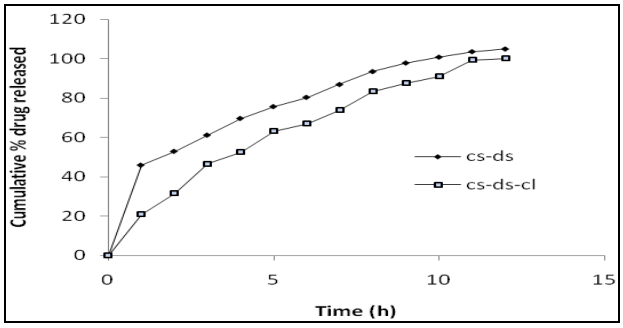

Fig. 5: Effect of cloisite 10A content on the release profile at pH 7.4.

DISCUSSION

In this study clay based nanocomposite as delivery carrier for diclofenac sodium was investigated. Nanocomposite was formulated by solution mixing technique in which drug was adsorbed onto clay. Higher drug loading capacity for clay was recognized. Chitosan was added to formulation to slow down the drug release. The successful intercalation of drug with polymer/clay was confirmed by XRD and FT–IR analysis. Formulation CC IV S3

provided optimized chitosan/clay ratio with regards to the average particle size. XRD peak confirmed that the size of particles in the formulation and their crystallinity. It was further confirmed by applying Scherrer's equation.

Table 3: Zone of growth inhibition (in mm) of pure chitosan and chitosan/cloisite 10A nanocomposite

Compound	Zone of inhibition in mm			
	Gram-positive		Gram-negative	
	Staphylococcus aureus	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*
Pure chitosan	15	17	17	16
Chitosan/cloisite 10A	23	23	20	20
Tetracycline	25	28	26	24

Furthermore, zeta sizer provided information regarding the particle size of this formulation. SEM reports revealed some cracks in the surface of nanomaterial, which may be due to rapid stirring and heating during the formulation process. From antimicrobial study, prepared nanocomposite showed significantly higher antibacterial activity against both gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria than pure chitosan. This novel nanocomposite triggered a slow release of the drug, as diclofenac sodium release was 92% from Chitosan/Clay (compared to 99% from chitosan) over a period of 12 h. We have shown that drug can be stored in the interlayer space of clay and sustainably released under simulated conditions, indicating that this type of material could play a significant role in developing new generations of drug delivery systems. Furthermore, the proposed delivery carrier has the advantage of easy processing as well as use of abundant, cheap and safe biopolymer and hydroxyapatite material.

CONCLUSION

A series of chitosan-cloisite 10A nanocomposites were prepared successfully by the solution mixing method which indicated a good intercalation of chitosan into layers of clay material. The nanocomposite offered a more prolonged drug release than pristine chitosan. Chitosan/clay nanocomposites also have promising antimicrobial potency against both gram-positive and gram-negative bacteria (*Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Escherichia coli, and Pseudomonas aeruginosa).* The outcome proposes possibilities of the newly prepared nanocomposite in developing drug delivery systems providing better-sustained release property with several drugs for providing better therapeutic action.

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CONFLICTS OF INTEREST

None declared

REFERENCES

Chhabra P, Huang YW, Frank JF, Chmielewski R and Gates K (2006). Fate of staphylococcus aureus, Salmonella enterica serovar typhimurium and vibrio vulnificus in raw oysters treated with chitosan. *J. Food Prot.*, **69**: 1600-1604.

Dutta PK, Dutta J and Tripathi VS (2004). Chitin and chitosan: Chemistry, properties and applications. *J. Sci. Ind. Res.,* **63**: 20-31.

Han YS, Lee SH, Choi KH and Park I (2010). Preparation and characterization of chitosan-clay nanocomposites with antimicrobial activity. *J. Phys. Chem. Solids,* **71**: 464-467.

Jeon YJ, Park PJ and Kim SK (2001). Antimicrobial effect of chito-oligosaccharides produced by bioreactor. *Carbo. Poly.*, **44**: 71-76.

Khunawattanakul W, Puttipipatkhachorn S, Rades T and Pongjanyakul T (2010). Chitosan-magnesium aluminum silicate nanocomposite films: Physicochemical characterization and drug permeability. *Int. J. Phar.,* **393**: 219-229.

Kumar MNV (2000). A review of chitin and chitosan applications. *React. Funct. Poly.,* **46**:1-27.

Lazic S, Zec S, Miljevic N and Milonjic S (2001). The effect of temperature on the properties of hydroxyapatite precipitated from calcium hydroxide and phosphoric acid. *Thermo. Acta,* **371**(1): 13-22.

Mizushima Y, Ikoma T, Tanaka J, Hoshi K, Ishihara T, Ogawa Y and Ueno A (2006). Injectable porous hydroxyapatite microparticles as a new carrier for protein and lipophilic drugs. *J. Cont. Rel.,* **110**: 260-265.

No HK, Meyers SP and Prinyawiwatkul W (2007). Applications of chitosan for improvement of quality and shelf-life of foods: A Review. *J. Food Sci.,* **72(5)**: 87-100.

Raafat D and Sahl HG (2009). Chitosan and its antimicrobial potential-a critical literature survey. *Micro. Biotechnol.,* **2**(2): 186-201.

Raafat D, Bargen KV, Haas A and Sahl HG (2008). Insights into the mode of action of chitosan as an antibacterial compound. *Appl. Environ. Microbiol.*, **74**: 3764-3773.

Rajkumar M, Meenakshisundaram N and Rajendran V (2011). Development of nanocomposites based on hydroxyapatite/ sodium alginate: Synthesis and characterisation. *Mater. Charact.,* **62**: 469-479.

Real RP, Padilla S and Vallet-Regi M (2000). Gentamicin release from hydroxyapatite/ poly (ethyl methacrylate)/poly (methyl methacrylate) composites. *J. Biomed. Mater. Res.,* **52**: 1-7.

Tokura S, Ueno K, Miyazaki S and Nishi N (1997). Molecular weight dependent antimicrobial activity by Chitosan. *Macromol. Symp.,* **120**: 1-9.

Tunc S and Duman O (2011). Preparation of active anti-microbial methyl cellulose/carvacrol/montmorillonite nanocomposite films and investigation of carvacrol release. *LWT Food Sci. Tech.,* **44**: 465-472.

Wang X, Liu B, Ren J, Liu C, Wang X, Wu J and Sun R (2010). Preparation and characterization of new quaternized carboxymethyl chitosan/rectorite nanocomposite. *Comp. Sci. Tech.,* **70**: 1161-1167.

Wang X, Du Y, Luo J, Yang J, Wang W and Kennedy JF (2009). A novel biopolymer/rectorite nanocomposite with antimicrobial activity. *Carbo. Poly.,* **77**: 449-456.

Wang X, Du Y, Luo J, Lin B and Kennedy JF (2007). Chitosan/organic rectorite nanocomposite films: Structure, characteristic and drug delivery behaviour. *Carbo. Poly.,* **69**: 41-49.

Xu QG and Czernuszka JT (2008). Controlled release of amoxicillin from hydroxyapatite-coated poly (lactic-co-glycolic acid) micro spheres. *J. Control. Rel.,* **127**(2): 146-153.

Zhang J, Wang Q and Wang A (2010). In situ generation of sodium alginate/hydroxyapatite nanocomposite beads as drug-controlled release matrices. *Acta. Biomater.,* **6**: 445-454.