Evaluation of Chitosan/Poly (lactic acid) Nanoparticles for the Delivery of Piceatannol, An Anti-cancer Drug by Ionic Gelation Method

Jeevitha.D and Kanchana.A*

***Abstract*---** Nanoparticles have resourceful potential for efficient development of different drug delivery formulations and routes because of the properties provided by their small size. Piceatannol (PIC), a phytocomponent of grapes (*Vitis vinifera*) possesses antioxidant, anti-inflammatory, anti-carcinogenic and anti-microbial properties. However, the clinical application of piceatannol in the treatment is considerably restricted due to its serious poor liberation characteristics. In order to augment the hydrophilicity and drug delivery capability, we developed a novel self-assembled nanoparticles by conjugating Chitosan (CS)–poly(lactic acid)(PLA) nanoparticles, as drug vehicle for the controlled release of piceatannol by ionic gelation method using sodium triphosphate (TPP) as a cross linker. The prepared chitosan/poly(lactic acid)-piceatannol nanoparticles(CS/PLA-PIC NPs) were evaluated for its functional groups (FTIR), surface morphology (FE-SEM), particle size and its potential. The preliminary study was determined for its drug loading capacity, encapsulation efficiency and *in vitro* drug release behaviour using UV spectrophotometer at 540nm.

Keywords— Chitosan, Ionic gelation, Nanoparticles, poly(lactic acid), Piceatannol.

I. INTRODUCTION

EPIDEMOLOGICAL studies strappingly support a role for phenolic compounds in the deterrence of cardiovascular diseases, cancers, osteoporosis, diabetes mellitus, arthritis and neurodegenerative diseases, which are related to oxidative stress and chronic inflammation[1]. PIC(3',4',3,5-Tetrahydroxy-*trans*-stilbene) is a stilbenoid phenolic compound, its occurrence has been described in berry fruits [2] peanut (*Arachis hypogaea*) [3], grape and wine [4,5], rhubarb [6], passion fruit seeds [7], *Euphorbia lagascae* seeds [8], and Japanese knotweed (*Polygonum cuspidatum*) [9]. Piceatannol has been exposed to have effective biological activities, including antioxidant [10, 11], anti-cancer [12,13], anti-inflammatory [14], and anti-obesity properties [15]. Interestingly, piceatannol has revealed more potent bioactivities than resveratrol in several studies [10-12, 14]. When compared to resveratrol, piceatannol possesses an supplementary hydroxyl group at position 30 in ring B forming a catechol structure. This is one of the most effective antioxidant configurations [5], which could partially elucidate the increased biological efficacy of piceatannol, as compared to resveratrol. Report says when PIC injected in rats, it's showed a hasty glucuronidation and a deprived bioavailability [16], and thus this study involves the encapsulation of PIC with CS/PLA nanoparticles for ever-increasing its bioavailability against the target cells.

Even though piceatannol proves to be remarkably non-toxic and has promising medicinal properties, its relevance in anti-cancer therapies is inadequate owing to its low aqueous solubility and poor bioavailability. To deal with this obstacle, a variety of methods together with the assimilation of phytocomponents into liposomes and into phospholipid vesicles are being studied [17-19]. Further recently, the approach of biodegradable polymer nanoparticles has been developed [20, 21]. This tenders potential therapeutic recital of anti-cancer drugs by increasing their solubility, retention time and bioavailability [22]. These drug formulations are higher to conventional medicines with deference to control release, targeted delivery and therapeutic impact. Polymeric nanoparticles have concerned noteworthy consideration in the study of drug delivery systems as they offer a means for localized or targeted delivery systems of a drug to specific tissue/organ sites of interest with an optimal release rate [23]. Polymeric nanoparticles act as nanocarriers with many advantages, such as low toxicity and high stability.

Recently, chitosan (CS) a natural biodegradable polymer has been used for drug delivery vehicles, wound healing accelerators and nerve regeneration agents [24] and could extort dose-dependent inhibitory effects on the proliferation of various tumor cell lines. However, the application of CS suffers severe limitations because of its poor solubility both in water and organic solvents due to its rigid crystalline structure. To conquer this deficit, assortment of embed copolymers of chitosan were synthesized and used for drug delivery systems [25, 26]. The most widely used classes of biocompatible and biodegradable polymers, approved by Food and Drug Administration (FDA), is that of aliphatic polyester, including poly (lactic acid) (PLA), poly (glycolic acid) (PLGA) and their

Jeevitha.D is with the School of Biochemistry, Sathyabama Dental College and Hospitals, Sathyabama University, Rajiv Gandhi Salai, Sholinganallur, Chennai-600119, Tamilnadu, India (Phone: +919940128141; e-mail: jivitha@hotmail.com).

Kanchana.A is with the School of Biochemistry, Sathyabama Dental College and Hospitals, Sathyabama University, Rajiv Gandhi Salai, Sholinganallur, Chennai-600119, Tamilnadu, India (e.mail: kanchibms@yahoo.co.in).

copolymers. Due to its good mechanical properties, like biodegradation, exceptional thermal/mechanical properties, biocompatibility, and higher transparency of these materials [27, 28] they have been widely used in surgical sutures, drug delivery systems and tissue engineering [29].

The present work aims to prepare novel self-assembled nanoparticles by conjugating chitosan and PLA by ionic gelation technique. The developed composite nanoparticles (CS/PLA-PIC NPs) have been characterized for their functional groups (FTIR), surface morphology (FE-SEM), particle size (zeta sizer). The preliminary study will be determined for its drug loading capacity, encapsulation efficiency and *in vitro* drug release behaviour using UV spectrophotometer at 540nm.

II. MATERIALS AND METHODS

Materials

Chitosan (Mw 80kDa), Poly lactic acid (Mw 60kDa), Piceatannol (Mw 244.24), and sodium tripolyphosphate were purchased from Sigma Corporation, USA. All other chemicals were of analytical grade and used without further purification.

Methods

A. Preparation of CS/PLA-PIC NPs

CS/PLA-PIC NPs were prepared by an ionic interaction method with slight modification in protocol [30]. Briefly, Chitosan and PLA was dissolved in distilled water, kept under sonicator till the solution was clear. The aqueous solution of CS and PLA was obtained at a concentration of 3mg/ml, about 12ml and 1.2mg/ml, about 30ml and TPP was added to the mixture. Piceatannol of about 2mg/ml was added into the CS/PLA suspensions with continuous stirring at room temperature for 45 mins. The nanoparticles suspensions was centrifuged at 10,000 rpm for 30 min, then washed twice with distilled water and dried. The dried nanoparticles were subjected to further analysis.

Characterization of nanoparticles

B. Particle Size and Zeta Potential

Dynamic light scattering measurements for evaluating the average size and size distribution of the CS/PLA NPs and CS/PLA-PIC NPs were performed using a Zetasizer (Malvern Instruments Ltd., Worcestershire, UK).The suspension of the nanoparticles was diluted with deionized distilled water, and the intensity of scattered light was detected at a scattering angle of 90^{o} to an incident beam at a temperature of 25^{o}C.

C. SEM and FT-IR Spectrum

CS/PLA NPs and CS/PLA-PIC NPs morphology was studied by field emission scanning electron microscopy (Hitachi). FTIR spectroscopy was obtained by using a FTIR spectrophotometer by KBr method (Bruker IFS 66V vacuum-type spectrometer).

D. *Drug Loading and Encapsulation Efficiency*

Drug loading capacity and encapsulation was dogged by centrifugation method with slight modification in the protocol [31]. The nanoparticles mixture was centrifuged at 12,000 rpm for 1 hr to separate the free drug in the supernatant. The amount of PIC in the supernatant was determined by UV spectrophotometer (Shimadzu) at 540nm. The drug loading and drug entrapment efficiency were defined by the following equations respectively:

% Drug content =Total amount of drug loaded - free drug in supernatant ×100 / Weight of nanoparticles after freeze drying

% Encapsulation efficiency = Total amount of drug loaded - free drug in supernatant ×100/ Total amount of drug loaded

E. In vitro Drug Release

In vitro drug release was performed in phosphate buffered saline at pH5 and pH7.4 with slight modification in the protocol [32]. 5mg of drug loaded polymeric nanoparticles was suspended in 10 ml of PBS buffer and placed in a shaker for 72 hrs. At predetermined time intervals, medium was removed and replaced with the same amount of fresh saline which was monitored by UV spectrophotometer (Shimadzu) at 540 nm. The drug release can be determined by the following equation:

In vitro drug release (%) = D(t) / D(0) x 100

Where, D(0) is amount of drug loaded and D(t) is amount of drug released at a time, respectively.

III. RESULTS AND DISCUSSION

A. Synthesis of CS/PLA-PIC Nanoparticles

PIC loaded CS-PLA NPs were formed immediately due to ionic reaction between two oppositely charged polyelectrolyte polymers, PLA (negatively charged) and chitosan (positively charged). This method avoids the use of surfactants, which stay to be a toxin after preparation of nanoparticles. Ionic reaction results from the electrostatic interaction between the protonated amino groups of chitosan and carboxyl groups of PLA.

B. Surface Morphology

SEM images confirmed the formation of particles with a log-normal particle sized distribution. The SEM showed that the morphology of the polyelectrolyte complex (Fig.1a and 1b) appears homogenous, indicating a uniform distribution and a good compatibility between CS–PLA and CS–PLA-PIC. The surface morphology of CS/PLA-PIC NPs was spherical in shape and dispersed equally with aggregates (Fig.1b) when compared to CS/PLA NPs (Fig.1a).This observation is consistent with the general morphology of nanoparticles formed using combinations of emulsion solvent evaporation methodologies, by conjugating different bioactive compounds [33].

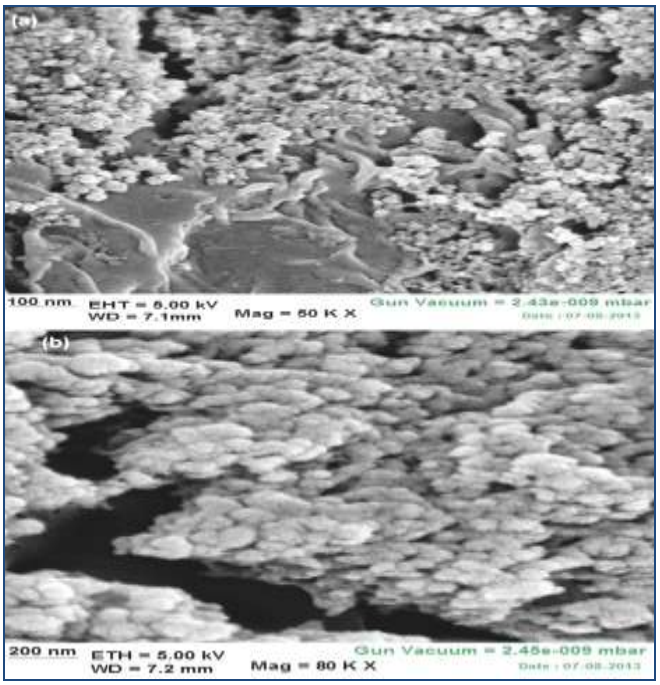

Fig.1 SEM images of (a) CS/PLA NPs and (b) CS/PLA-PIC NPs

C. Particle Size and Zeta Potential of CS/PLA and CS/PLA-PIC Nanoparticles

The *in vivo* performance of the nanoparticles can be studied by determining its size which is considered to be one of the most important properties. The smaller size improves the utilization of the drug, make sure the low uptake of RES and diminish drug side effects. The particle size of CS/PLA was evaluated by the source of dynamic light scattering which was found to be about 127nm (Fig.2a) that possessed a zeta potential of +1.97mV on the surface (Fig.2b & Table 1). However, the zeta potential value increased to +23.7mV in CS/PLA-PIC NPs with an increase in the size distribution of 253nm (Table 1).The value of zeta potential less than−30 mV or higher than +30 mV can be used to assure the stability of nanoparticles suspensions. The values obtained for the prepared nanoparticles were approximately to +30, so it was stable. The positive values obtained for zeta potential specifies that the nanoparticles surface was positively charged which may be attributed to the availability of chitosan free NH3+groups on the polymer surface [33].

TABLE I
PARTICLE SIZE, PDI AND ZETA POTENTIAL OF CS/PLA NPS AND CS/PLA-PIC NPS

Samples	Particle Size (nm)	PDI	Zeta potential (mV)
CS/PLA NPs	127	1	1.97
CS/PLA-PIC NPs	253	1	23.7

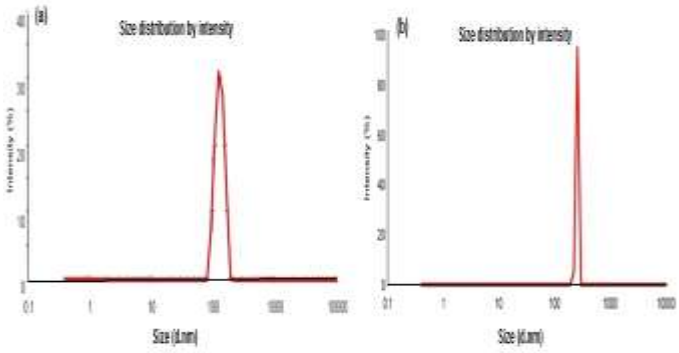

Fig. 2 DLS data of the prepared nanoparticles (a) CS/PLA NPs (b) CS/PLA-PIC NPs

D. FTIR Spectrum

In order to confirm the CS–PLA interaction to PIC, samples were analyzed by FT-IR spectroscopy shown in Fig. 4. CS is an amino glucose characterized by a small proportion of amide groups via an amide linkage with acetic acid. The FTIR of (Fig. 3a) CS/PLA NPs (Fig. 3b) PIC & (Fig. 3c) CS/PLA-PIC NPs showed peaks around 946 cm−1and1068 cm−1of assigned saccharine structure, and a strong amide. The CS–PLA NPs and CS–PLA/PIC NPs, FTIR spectra have several shifts as compared to those of free PIC. This indicates the formation of polymer-encapsulated PIC nanoparticles. For example, compared with that of pure PIC, the IR spectrum of CS–PLA–PIC showed a band shift from 3400 to 3417 cm^{-1}, which is probably due to the hydrogen bonding between–OH groups in PIC and CS. The FTIR spectrum of CS showed broad band at 3420 cm^{-1} due to the stretching vibration of hydroxyl group. A peak at 1634 cm^{-1} corresponds to the stretching vibrations of carbonyl group. The characteristic absorption band of CS-PLA that appeared at 1598 cm^{-1} was assigned to the N–H banding vibration of the primary amine. Comparing CS-PLA NPs and CS–PLA/PIA NPs, peak shifts were observed from 3420 to 3261 cm^{-1} and 1634 to 1625 cm^{-1}. The result confirmed the presence of PIC and CS/PLA in the CS–PLA/PIC mixture.

E. Drug Loading (DL) and Encapsulation Efficiency (EE)

Preliminary drug loading and encapsulation efficiencies was evaluated by UV spectroscopy method, which was found to be 20.4% of the drug loaded and 72.02% encapsulation efficiency (Table 2).The initial concentration of PIC plays an important role in the EE and LC of CS–PLA, nanoparticles. According to Desai & Park, 2005; Jelvehgari et al., 2010, when the concentration of drug is increased, the EE of CS–PLA, polymer combination is increased [34, 35]. Therefore, the increase in PIC concentration leads to increase of both encapsulation efficiency and loading efficiency of polymer nanoparticles. It was found that the encapsulation and loading efficiencies of CS–PLA combination nanoparticle could be attributed to the fact that CS–PLA has a more electrostatic attraction with PIC [33].

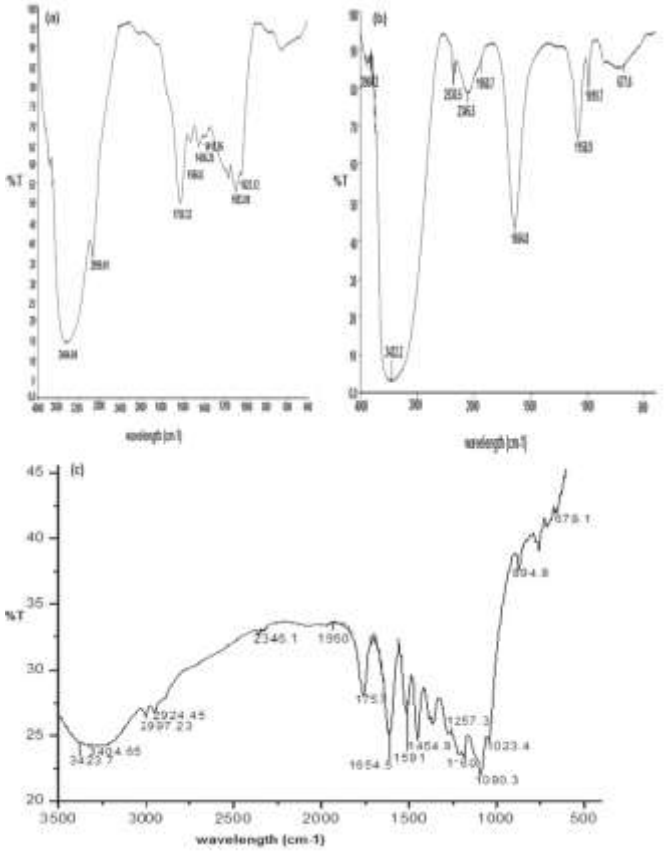

Fig. 3: FTIR spectra of (a) CS/PLA NPs (b) PIC & (c) CS/PLA-PIC NPs

TABLE II
DETERMINATION OF DRUG AND POLYMERS

Sample	Preliminary Evaluation	
	Drug Loading	Encapsulation Efficiency
CS/PLA-PIC NPs	20.4%	72.02%

F. In vitro Drug Release

Among the various techniques have been described for the study of drug release from nanocarrier systems the dialysis method has been frequently reported for this purpose as it facilitates the separation of the released drug from the bound drug. *In vitro* drug release studies were done via the dialysis method at pH 5 and pH 7.4 and the release pattern is shown in Fig. 4 for 24, 48 and 72h. After 24h, preliminary drug loaded sample released 35.5%, 72% after 48h, 93% after 72h of PIC from CS/PLA NPs. The release rate of the drug is usually affected by the diffusional confrontation of the membrane and by many factors such as polymer degradation, crystallinity, molecular weight the binding affinity between the polymer and the drug and so on [36]. Thus the drug release pattern showed a burst release in the first 24 h followed by a controlled release of PIC for 72 h and about 70% of drug was released during this time. PIC that is adsorbed on to the NPs surface and drug entrapped near the surface might be the reason for initial burst release, as the dissolution rate of the polymer near the surface is high, the amount of drug released will be also high.

The release was faster in acidic pH than in neutral, as in acidic environment the polymer medium swells due to protonation of amine group of chitosan, thereby facilitating the faster drug elution [37]. The lesser drug release rate in the acidic pH than the alkaline pH may be accredited to the repulsion between H+ ions and cations on the surface of CS, which slow down the hydrolysis [38, 39]. Drug loading is also a important factor for influencing the drug release. Higher drug loading caused the drug to be released more quickly. Henceforth the drug release rate was higher for alkaline pH than acidic pH.

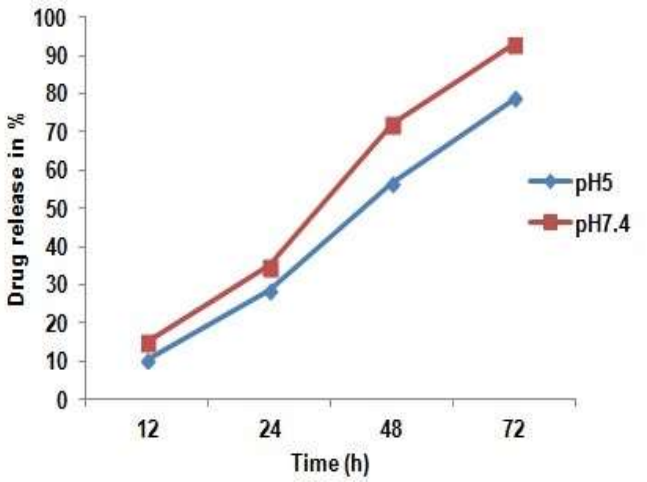

Fig. 4 *In vitro* Drug Release of PIC from CS-PLA NPs

IV. CONCLUSION

In the present studies copolymer CS-PLA NPs encapsulated PIC were prepared successfully by ionic gelation method. It was found that these particles have a good solubility in PBS. As spherical particles with an average size from 250 to 300 nm, they are also believed to be suitable for drug delivery applications. With all these excellent features our future study will be focused on the efficacy of the developed towards cancer therapy on different cancer cell lines.

ACKNOWLEDGMENT

We are thankful to our Chancellor Dr.Jeppiaar, and Directors Dr.Marie Johnson and Dr.Mariazeena Johnson, Sathyabama University, Chennai for providing the infrastructure for carrying out this research work.

REFERENCES

[1] Cicerale, S., Lucas, L. J., Keast, R. S. J. Current Opinion in Biotechnology, 2012; 23: 129–135.
[2] Rimando, A. M., Kalt, W., Magee, J., Dewey, J., Ballington, J. R. Journal of Agricultural and Food Chemistry, 2004;52: 4713–4719.
[3] Ku, K. L., Chang, P. S., Cheng, Y. C., Lien, C. Y. Journal of Agricultural and Food Chemistry, 2005; 53: 3877–3881.
[4] Guerrero, R. F., Puertas, B., Fernández, M. I., Palma, M., Cantos-Villar, E. Innovative Food Science and Emerging Technologies, 2010; 11: 231–238.

[5] Piotrowska, H., Kucinska, M., Murias, M. Mutation Research, 2012; 750: 60–82.
[6] Ye, M., Han, J., Chen, H., Zheng, J., Guo, D. Journal of The American Society for Mass Spectrometry, 2007;18: 82–91.
[7] Matsui, Y., Sugiyama, K., Kamei, M., Takahashi, T., Suzuki, T., et al. Journal of Agricultural and Food Chemistry, 2010; 58: 11112–11118.
[8] Duarte, N., Kayser, O., Abreu, P., Ferreira, M.-J. U. Phytotherapy Research, 2008; 22: 455–457.
[9] Lin, L.-L., Lien, C.-Y., Cheng, Y.-C., Ku, K.-L.. Journal of Chromatography B, 2007;853: 175–182.
[10] Murias, M., Jäger, W., Handler, N., Erker, T., Horvath, Z., et al. (2005). Biochemical Pharmacology, 69, 903–912.
[11] Ovesná, Z., Kozics, K., Bader, Y., Saiko, P., Handler, N., et al. Oncology Reports, 2006;16: 617–624.
[12] Kita, Y., Miura, Y., Yagasaki, K. Journal of Biomedicine and Biotechnology, 2012: 1–7.
[13] Wieder, T., Prokop, A., Bagci, B., Essmann, F., Bernicke, D.,et al. Leukemia, 2001;15:1742–1753.
[14] Son, P.-S., Park, S.-A., Na, H.-K., Jue, D.-M., Kim, S., et al. Carcinogenesis, 2010; 31: 1442–1449.
[15] Kwon, J. Y., Seo, S. G., Heo, Y.-S., Yue, S., Cheng, J.-X., et al. The Journal of Biological Chemistry, 2012;287:11566–11578.
[16] Roupe, K.A., Yáñez, J. A., Teng, X.W, Davies, N. M. Journal of Pharmacy and Pharmacology, 2006; 58: 1443–1450.
[17] Takeuchi H., Yamamoto H., Kawashima Y. Advances in Drug Delivery Reviews, 2001;47: 39-54.
[18] Sou, K., Inenaga, S., Takeoka, S., Tsuchida, E. International Journal of Pharmacy, 2008; 352:287.
[19] Li, L., Braiteh, F. S., Kurzrock, R. Cancer, 2005;104: 1322.
[20] Anand, P., Nair, H. B., Sung, B., Kunnumakkara, A. B., Yadav, V. R., et al. Biochemistry and Pharmacology, 2010;79: 330.
[21] Yallapu, M. M., Gupta, B. K., Jaggi, M., Chauhan, S. C. Journal of Colloid Interface Science, 2010;351: 19.
[22] Shenoy, D, B., Amiji, M.M. International Journal of Pharmacy, 2005; 293: 261.
[23] Mu, L., Seow, P. H. Colloids and Surface B, 2006;47: 90.
[24] Geng, L., Yonglong, Z., Qing, M., Mozhen, W., Yuee, F. Carbohydrate Polymer, 2008; 72: 60–66.
[25] Jayakumar, R., Reis, R. L., Mano, J. F. Journal of Bioactive and Compatible Polymers, 2006;21: 327–340.
[26] Kweon, D. K., Kang, D. W. Journal of Applied Polymer Science, 1999;74: 458–464.
[27]Tzong-Ming, W., Cheng-Yang, W. Polymer Degradation and Stability, 2006; 91: 2198.
[28] Yan, S., Changsheng, L., Yuan, Y., Xinyi, T., Fan, Y., et al. Biomaterials, 2009;30: 2340–2348.
[29] Rahul, M. R., Amol, V. J., Douglas, E. H. Progress in Polymer Science, 2010; 35: 338.
[30] Hu, B., Pan, C., Sun, Y., Hou, Z., Ye, H., et al. Journal of Agriculture and Food Chemistry, 2008; 56: 7451–7458.
[31] Tamizhrasi, S., Shukla, A., Shivkumar, T., Rathi, V., J. Rathi, J.C. International Journal of PharmTech Research, 2009;1: 411-415.
[32] Ashish, D., Binulal, N.S., Anitha, A., Nair, S.V., Furuike, T., et al. Carbohydrate Polymers, 2010;80: 833–838.
[33]Rajan, M., Raj, V. International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 4: 255–259.
[34] Desai, K. G. H., Park, H. Journal of Drug Development Research, 2005; 64: 114–128.
[35] Jelvehgari, M., Nokhodchi, A., Rezapour, M., Valizadeh, H. Indian Journal of Pharmaceutical Sciences, 2010; 72: 72–78.
[36] Vassilios, K., Evangelos, K., Dimitrios, G., Sofia, P., Dimitrios, B. International Journal of Nanomedicine, 2011; 6: 3021–3032.
[37] Jayakumar, R., Reis, R. L., Mano, J. F. Drug Delivery, 2007;14: 9–17.
[38] Proikalis, C. S., Tarantalli, P. A., Andreopoulos, A. G.. European Polymer Journal, 2006;42: 3269–3276.
[39] Zhao, Y., Fu, J., Dennis, K. P. N., Wu, C. Macromolecular Bioscience, 2004;4: 901–906.