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Self-Assembled Chitosan-*g*-Poly(itaconic acid) Nanoparticles: A Potential Drug Carrier for Docetaxel

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A series of chitosan-*g*-poly(itaconic acid) nanoparticles (PIACS) were prepared to evaluate their drug delivery application, which was prepared *via* free radical polymerization using ammonium persulphate as initiator in nitrogen atmosphere. The electrostatic attraction between carboxylic groups of poly(itaconic acid) and amine groups of chitosan lead to the size reduction. All the synthesized nanoparticles were characterized by FT-IR, particle size analyser, zeta potential analyzer, thermogravimetry, scanning electron microscopy and transmission electron microscopy. Drug was loaded to nanoparticle by co-precipitation method. Drug release studies were carried out in 6.8 and 7.4 pH. Drug entrapment efficiency of chitosan-*g*-poly(itaconic acid) nanoparticles was lying between 93-95 %. Predicted ADMET (PreADMET) was confirmed that plasma protein binding was less. These results highlight the potential of chitosan-*g*-poly(itaconic acid) nanoparticles as an efficient drug carrier.

Keywords: Chitosan, Itaconic acid, Nanoparticles, Docetaxel, Drug Carrier.

INTRODUCTION

Drugs have long been used to improve health and extend our lives. The practice of drug delivery has changed intensely in the last few decades. Researchers have contributed to the development of a number of new modes of drug delivery that have entered clinical practice. A new drug will enter into the market after a long screening process, which will take quite few years. Rather than going to such a tedious process, better we can give a chance to reduce its side effects. The development of efficient carrier-based delivery systems will be a suitable solution for this problem. Polymers are becoming increasingly important in the field of drug delivery. The pharmaceutical applications of polymers range from their use as binders in tablets to viscosity and flow controlling agents in liquids, suspensions and emulsions [1].

Chitosan is a natural linear aminopolysaccharide derived from chitin, the second most abundant polysaccharide on earth after cellulose. Chitosan is composed of randomly distributed (1→4) linked D-glucosamine and N-acetyl-D-glucosamine units. It is a biopolymer, as well as having non-toxic, biocompatible, biodegradable characteristics [2]. Chemically, modified chitosan derivatives have wide applications as drug-delivery carriers. A lot of research reports are available for chitosan as a drug carrier. Among them chitosan nanoparticle has received great attention. Presence of amine and hydroxyl groups will enhance the nanoparticle formulation through chemical and physical cross linking. It stimulates cross-linkage with various cross-linking agents, such as glutaraldehyde, sodium tripolyphosphate (TPP), geneipin, *etc*. to provide an efficient network [3].

Although preparation of poly(itaconic acid) grafts with chitosan had been studied earlier [4], there is no study available chitosan nanoparticle without addition of crosslinking agents. In this study, we have taken only itaconic acid to reduce the size of chitosan and not any cross linking agent and we have analyzed its pH sensitivity using docetaxel as a model drug.

Docetaxel, a second generation taxoid, is twice as potent as paclitaxel. It is the licensed drug for the treatment of breast cancer with a first-line chemotherapy regimen [5]. Docetaxel is highly lipophilic and practically insoluble in water, the main marketed product of docetaxel (Taxotere®) used clinically is formulated using Tween 80 [6]. High cost and lack of stability in the formulation are the major complications with the docetaxel.

EXPERIMENTAL

Chitosan with the viscosity of 200-600 cps, docetaxel was given as gift sample by I & DC formulation, itaconic acid, ammonium persulphate, glacial acetic acid, ethanol, Tween 80. All other chemicals and reagents used in the study were of analytical grade.

UV-visible spectroscopy, AU-2701UV-VIS Double beam spectrophotometer was used to measure the absorption beha-

viour of the products. The absorption was recorded in the range of 200 -800 nm. Fourier transform infrared (FTIR) spectra were obtained by a Jasco-FT/IR-4600 type 'A' spectrometer with the absorption range from 4000-400 cm^{-1}. The samples were pressed into potassium bromide and compressed into pellets for analysis. Average particle size, size distribution and zeta potential of DTX-CH-NP was analyzed by Malvern Instruments. Measurements were performed at 25 ºC after equilibrium. Thermogravimetric analysis was recorded in TA Instruments Exstar-SII-TG/DTA 6300. The nanoparticles (6-12 mg) was taken in a platinum pan for scanning under a dynamic nitrogen atmosphere at a heating rate of 10 ºC/min from 40 to 800 ºC.

SEM morphology were recorded using SIGMA HV-Carl Zeiss with Bruker Quantax 200-Z10 EDS Detector, to examine the morphology and surface properties of the nanoparticles with the energy dispersive analysis of X-rays (EDAX). The nanoparticles were deposited on a carbon tape and sputtered with the thin coat of gold under vacuum. 10kV was fixed as acceleration voltage. Transmission electron microscopy (TEM) images recorded with the FEI Technai instrument to view the internal features of the nanoparticles at different magnifications.

Preparation of chitosan-*g*-poly(itaconic acid) nanoparticles (PIACS): PIACS was prepared according to the previous report [4] with slight changes. Briefly 1g of chitosan was stirred with 1 % acetic acid for 1 h under mechanical stirring. Then certain amount of ammonium persulphate was added to the chitosan solution followed by the addition of 5 mmol of itaconic acid and the mixture was stirred well until it become clear. The mixture was stirred at 70 ºC for 6 h. Polymerization was carried out under nitrogen atmosphere. The reaction mixture will turn into a clear dark yellow colour which indicated the completion of the reaction. Then, the mixture was cooled and kept at room temperature for 12 h. Then the mixture was filtered to remove larger particles. Addition of acetone to the mixture will give the desired product of PIACS (Fig. 1).

Loading of docetaxel into poly(itaconic acid): Docetaxel was trapped in the nanoparticle by co-precipitation method. 20 mg of nanoparticle was dissolved in minimum amount of dil. HCl. Docetaxel was prepared in ethanol medium of 1 mg/mL concentration. 1 mL of docetaxel was added to PIACS which will result the precipitation of PIACS-docetaxel nanoparticles. Keep this mixture for 24 h at 37 ºC and then centrifuge at 10000 rpm for 15 min. From the supernatant solution, the unbounded drug could be determined. After that the product was air dried. Entrapment efficiency and percentage of drug loading was calculated using following formula:

$$\text{Entrapment efficiency (\%, w/w)} = \frac{\text{Total amount of drug} - \text{Free drug}}{\text{Mass of drug in polymer matrix}}$$

$$\text{Loading efficiency (\%, w/w)} = \frac{\text{Total amount of drug}}{\text{Mass of polymer matrix}}$$

Calibration curve: Five different known concentrations of docetaxel solutions were prepared and considered as standard solutions. The absorbance was measured at 230 nm in AU-2701 UV-VIS Double beam spectrophotometer and calibration curve was constructed. From the above calibration curve, we could identify the unknown concentration of docetaxel. Two calibration curves were made for docetaxel to determine the amount of docetaxel released from the polymer nanoparticle in different medium (pH 6.8 and 7.4)

Drug release study: *in vitro* Release studies were carried out by placing the sample in 75 mL of release medium at 37 ºC for 7 days. The amount of docetaxel released was analyzed by spectrophotometrically using AU-2701UV-VIS Double beam spectrophotometer. The release studies were done in 6.8 and 7.4 buffer solutions. For each measurement 3 mL of release medium was taken out and refilled with fresh buffer solution.

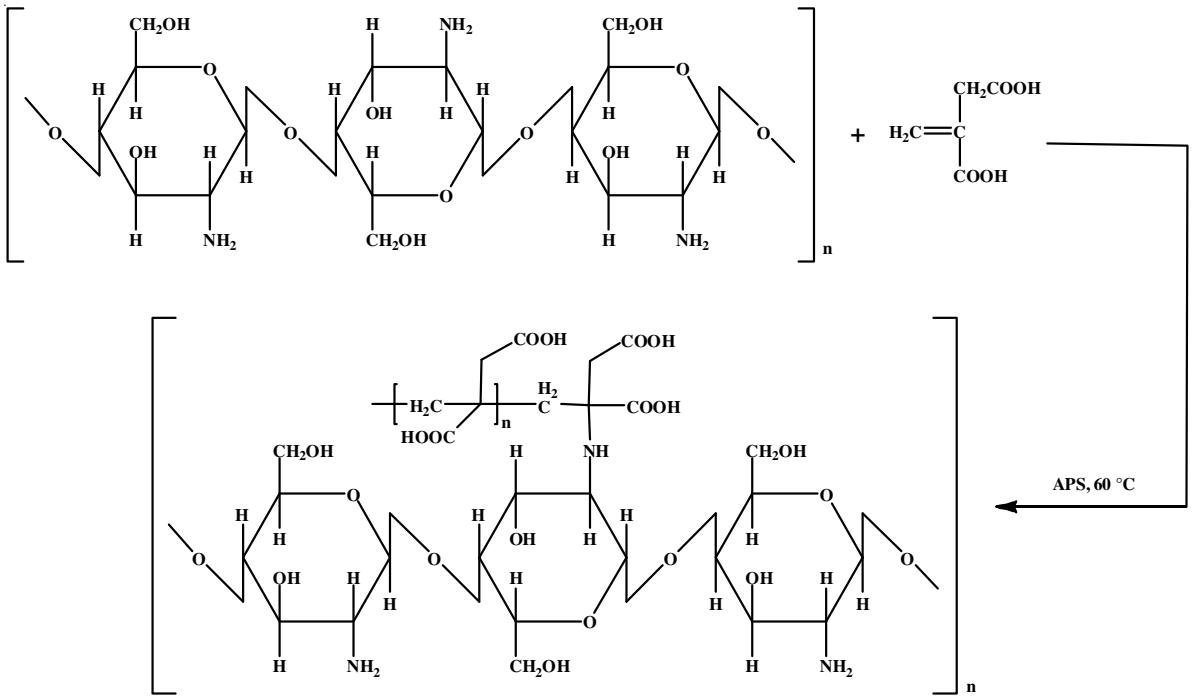

Fig 1. Synthetic scheme for chitosan-*g*-poly(itaconic acid) nanoparticles (PIACS)

RESULTS AND DISCUSSION

FT-IR studies: The functional groups of chitosan, itaconic acid and chitosan-*g*-poly(itaconic acid) were characterized using FT-IR. Their spectra were compared as shown in Fig 2. The spectrum of chitosan presents the broad band between 3500-3100 cm^{-1} is mainly attributed to the stretching vibration of -OH groups, the extension of $-NH_2$ groups and the inter and -exter molecular hydrogen bonds. The peak at 2926 cm^{-1} is typical -CH stretching vibration. Peaks at 1645 are 1579 cm^{-1} are due to the stretching vibrations of amide I (-NH deformation of -NH-$COCH_3$) and amide II. In addition, the peaks at 1024 and 1060 cm^{-1} were associated with the primary hydroxyl groups (C6-OH) and secondary hydroxyl groups (C3-OH) [7]. Peak at 893 cm^{-1} is assigned to ring stretching, a characteristic band for 1→4 glycosidic linkage [8]. In itaconic acid spectrum, the broad band between 3200 and 2800 cm^{-1} is associated to -COOH group. The peaks at 1682 and 1625 cm^{-1} is attributed to -C=O and -C=C- stretching vibration, respectively [9]. The spectrum of chitosan-*g*-poly(itaconic acid) shows major peaks of chitosan. The disappearance of -C=C- group and the appearance of new peak at 1268 cm^{-1} due to the stretching vibration of -C-N- bond confirms the grafting of poly(itaconic acid) onto chitosan polymer chain. The NH symmetric vibration for chitosan-*g*-poly(itaconic acid) is 3187 cm^{-1} showing different value with the chitosan and it can prove some compound has been attached. The value of NH bending is different due to the attachment itaconic acid to the chitosan which is 1549 cm^{-1} [10].

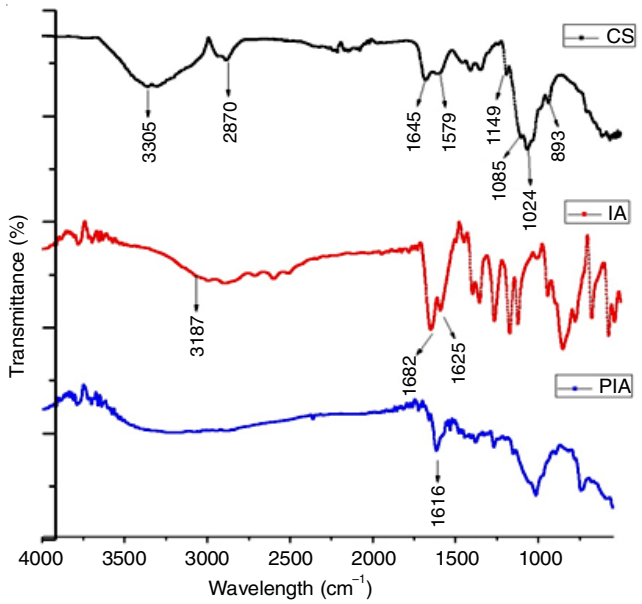

Fig. 2. FT-IR of chitosan-*g*-poly(itaconic acid) nanoparticles (PIACS) before loading

Fig. 3 shows the spectra of docetaxel and chitosan-*g*-poly-(itaconic acid)-docetaxel. Spectrum of docetaxel shows the absorption band at 3432 cm^{-1} for -N-H stretching of alkanes. Peaks at 1782 and 1700 cm^{-1} are assigned to -C-O- stretching vibrations and the peak at 1635 cm^{-1} is assigned to -N-H plane bending vibration. In chitosan-*g*-poly(itaconic acid)-docetaxel spectrum there is no significant difference between chitosan-*g*-poly(itaconic acid) and chitosan-*g*-poly(itaconic acid)-docetaxel, which indicated that there is no bonding between the polymer and drug moiety [2]. Disappearance of peak at 1700 cm^{-1} may be due to the interaction of -NH group of docetaxel with -COOH group of chitosan-*g*-poly(itaconic acid) polymer. Thus, FT-IR analysis confirms the successful grafting polymerization itaconic acid with chitosan and absence of drug excipient bond between the polymer and drug during the formulation.

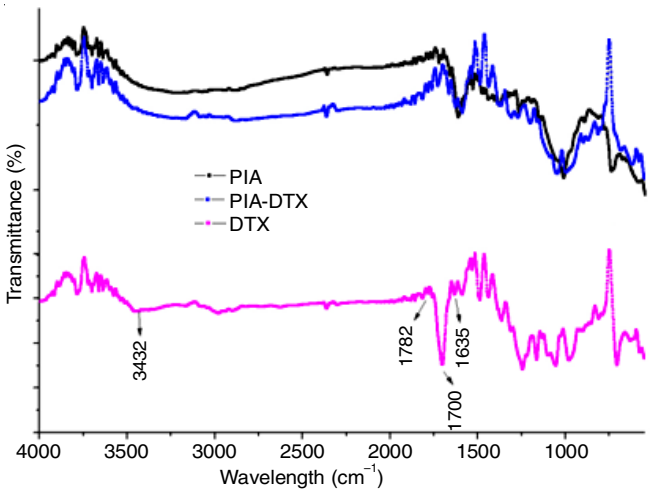

Fig. 3. FT-IR of PIACS-docetaxel after loading

Thermogravimetric analysis: Thermal stability patterns of chitosan, itaconic acid and poly(itaconic acid) are presented in Figs. 4 and 5. Thermogram of chitosan displayed three stage of degradation. First stage of weight loss around 2.9 % in the temperature range 68-118 ºC, which can be attributed to loss of moisture and other volatile impurities. Second stage of degradation is observed in the range of 255-348 ºC with the maximum decomposition rate around 302 ºC with the weight loss of 41.2 %. This resulted weight loss is due to the pyrolysis of polysaccharides with the random scission of glycosidic bonds [11], a complex process including dehydration of the saccharide rings, followed by a further decomposition forming acetic acid, butyric acids and depolymerization with the formation of water, CO_2, CH_4 [12]. From 350-560 ºC the final stage of

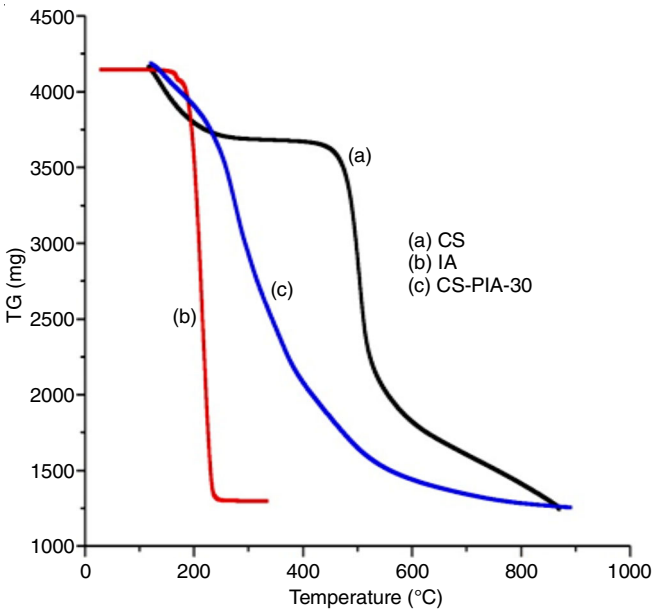

Fig. 4. TGA of chitosan-*g*-poly(itaconic acid) nanoparticles (PIACS)

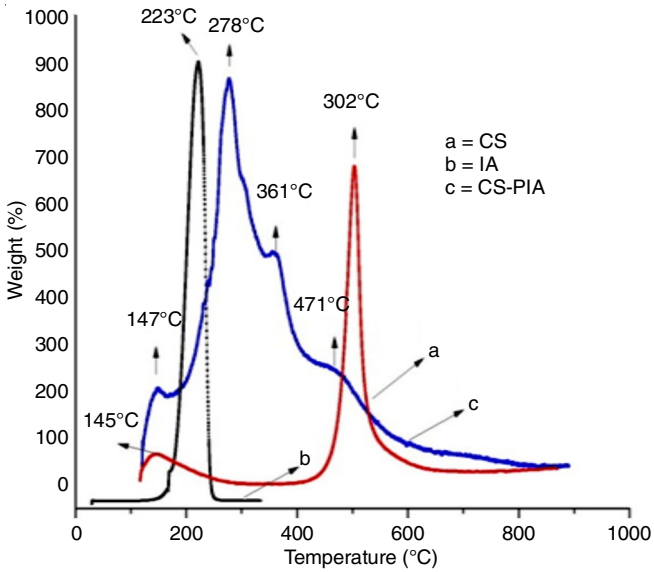

Fig. 5. DTG of chitosan-*g*-poly(itaconic acid) nanoparticles (PIACS)

decomposition occurred with 16.7 % of weight loss. This is because of the charring of degraded back bone of chitosan 30 % of the compound is still exist as a residue which needs oxygen to completely decompose.

TGA and DTG curve of itaconic acid shows only a one sharp degradation step at 223 ºC with 98 % of maximum weight loss. This is attributed towards the moisture, other type of gases like CO_2 and CH_4 and mainly as a result of decarboxylation with the maximum decomposition rate at 214 ºC. Remaining char of 2 % residue is believed to be a condensed crosslinked structure formed by decarboxylation or decarbonylation which needs oxygen to completely decompose [13].

Thermogram of chitosan-*g*-poly(itaconic acid) bring about two stage degradation in the temperature range of 30-120 ºC and 125-533 ºC with the weight loss of 6.3 and 61.4 %, respectively. Initial weight loss is ascribed to the desorption water that has absorbed onto the polymer. Second stage of degradation is a gradual weight loss with the maximum at 263 ºC. This process may be covering the breakage of chitosan back-bone, the release of itaconic acid moieties because of the depolymerization, the splitting of branches and side chain groups from the grafting site, decarboxylation of poly(itaconic acid) chain. Degradation beyond 535 ºC resulted marginal weight loss.

Chitosan-*g*-poly(itaconic acid) exhibited low thermal stability compared to chitosan. It is evident with the second T onset (onset temperature), where chitosan is higher than that of chitosan-*g*-poly(itaconic acid). As a result the thermal stability of chitosan-*g*-poly(itaconic acid) is decreasing with the grafting of poly-(itaconic acid) onto chitosan chain. This was reported by Naguib [14]. He has indicated that thermal stability of polymer reduced with the grafting of itaconic acid onto sisal fibre.

This revealed that grafting of poly(itaconic acid) decreased the thermal stability of chitosan with faster rate of degradation. Decrease of thermal stability due to grafting was reported by Naguib [14] where he grafted itaconic acid onto sisal fibre.

Particle size and zeta potential: Size of all synthesized nanoparticles is given in Table-1 and Figs. 6 and 7. In the present study, only one parameter was changed *i.e.*, concentration of itaconic acid and other parameters such as concentration of chitosan, docetaxel and ammonium persulphate are kept as constant. With increase the concentration of itaconic acid, the size of the nanoparticle is getting decreased and the polydispersity index is also decreasing which indicates that more -COOH groups of itaconic acid are available for the ionic interactions with -NH_2 groups of chitosan. Among six compositions, 10 mmol product shows better efficiency and also preferred size for the biomedical applications.

TABLE-1
PARTICLE SIZE AND ZETA POTENTIAL OF THE PIACS

Conc. of IA (mmol)	DTX conc. (mg/mL)	Particle size (nm)	Polydispersity index (PDI)	Zeta potential (mV)
5	1	207	1.00	19.4
10	1	40	1.00	-3.98
15	1	323	0.68	12.4
20	1	509	0.11	12.6
25	1	628	0.45	23.1
30	1	441	0.72	26.8

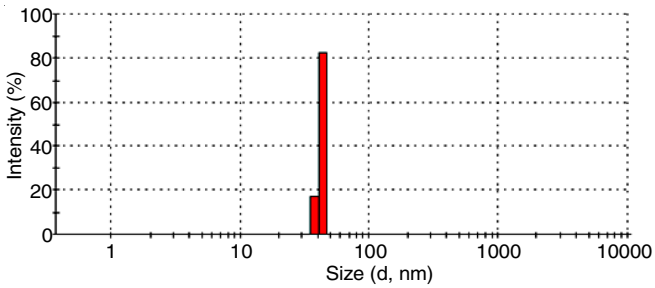

Fig. 6. Particle size distribution of chitosan-*g*-poly(itaconic acid) nanoparticles (PIACS)

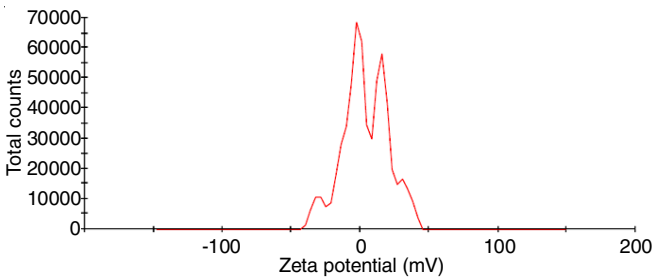

Fig. 7. Zeta potential of chitosan-*g*-poly(itaconic acid) nanoparticles (PIACS)

Encapsulation efficiency and drug loading: Drug entrapment efficiency of nanoparticles were analyzed using docetaxel as model drug in six different compositions of nanoparticle. Encapsulation and loading efficiency are given in Table-2. There is no significant difference in the encapsulation efficiency. All compositions show above 95 % of entrapment and it is getting increasing with increase in poly(itaconic acid) content. Drug loading efficiency was found to be lying in the range of 4-6 %, which is also, depends on poly(itaconic acid) concentration in the polymer composition. This may be due to the introduction of more -COOH group onto the chitosan, which will encourage formation of H-bonding within drug and polymer.

***in vitro* Drug release:** To evaluate *in vitro* drug release profile of docetaxel loaded CS-PIA nanoparticle was examined in 6.8 and 7.4 phosphate-buffered saline (PBS) solutions for 7

TABLE-2
ENCAPSULATION AND LOADING EFFICIENCY OF PIACS

Conc. of IA (mmol)	DTX conc. (mg/mL)	Encapsulation efficiency (%)	Loading efficiency
5	1	95	4.74
10	1	94	4.80
15	1	95	5.00
20	1	96	5.06
25	1	96	5.20
30	1	96	5.80

days. Among all composition chitosan-*g*-poly(itaconic acid)-5 and chitosan-*g*-poly(itaconic acid)-10 (Table-3) had shown cumulative release, which is ensuring the sustained drug release. A rapid release of docetaxel was observed in docetaxel injection, where 80 % of docetaxel were released in the first 12 h [5] and 100 % of release was found within 48 h [6] and with chitosan nanoparticle, it shows 77 % in 24 h [2]. However 76 % of docetaxel was released from chitosan-*g*-poly(itaconic acid)-10 in a 6.8 pH over a period of 7 days.

There was a low initial burst effect about 18-23 % drug was released for first 24 h which may be due to the entrapment of docetaxel located near the outer surface of nanoparticle than on the surface of the particle. This is also indicated that drug was stably incorporated in the core of polymeric nanoparticles. And the release of docetaxel was high in 6.8 (59 %) compared to 7.4 (76 %) medium. The enhanced drug release at acidic pH conditions might be attributed to pH-responsive nature of chitosan-*g*-poly(itaconic acid) nanoparticle [15] which results a complete dissociation of acidic groups of itaconic acid at this pH value, and also considered as loss of electrostatic interactions between the itaconic acid chain and docetaxel [16].

Percentage of cumulative release was decreased with increasing percentage of itaconic acid. This may be due to the enhanced drug-polymer interaction through H-bonding between -COOH and -OH groups of chitosan-*g*-poly(itaconic acid) and -OH groups of docetaxel [17]. This phenomenon is beneficial for drug delivery because the acidic extracellular and intracellular environments of tumours would be expected to accelerate drug release. Release profiles are given in Figs. 8-10.

Release kinetics: The drugs release from nanoparticles depends on the physical and chemical parameters such as drug diffusion rate, partition coefficient between the drug and hydrophobic segment and polymer degradation [18]. To understand the drug release profile and mechanism from the nanoparticle,

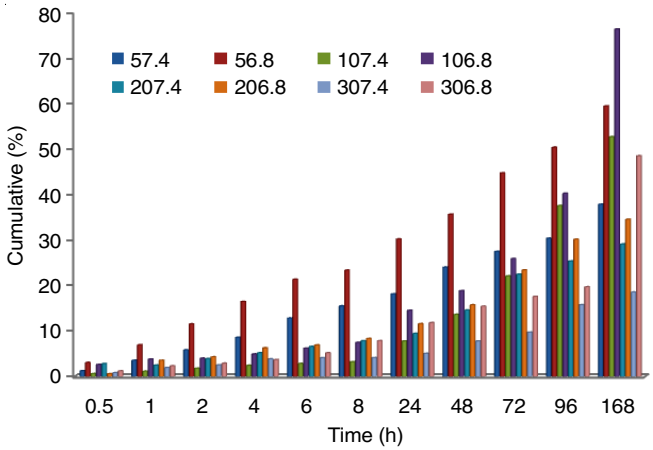

Fig. 8. Cumulative percentage of chitosan-*g*-poly(itaconic acid) nanoparticles (PIACS) in 6.8 and 7.4

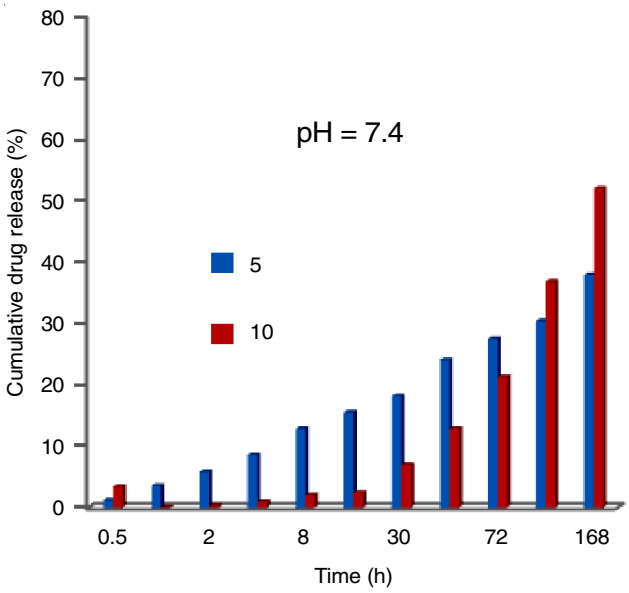

Fig. 9. Release in 7.4 buffer

the release data were fitted into various kinetic models. Table-4 shows the kinetic values for all mathematical models. The release pattern was well fitted with the Korsmeyer-Peppas model with 'n' value lying in the range of $0.45 \leq n \leq 0.89$ except chitosan-*g*-poly(itaconic acid)-10 in 7.4 medium. The Korsmeyer-Peppas model equation is follows as:

$$M_t/M_a = kt^n$$

TABLE-3
RELEASE PERCENTAGE OF PIACS

Conc. of itaconic acid	5		10		20		30	
	7.4	6.8	7.4	6.8	7.4	6.8	7.4	6.8
0.5	1.2000	3.0206	0.5863	2.5900	2.7569	0.5487	0.7862	1.1711
1	3.5005	6.9075	1.1114	3.7602	2.4379	3.4989	1.9032	2.2781
2	5.7922	11.485	1.7535	3.9578	3.8819	4.2602	2.4933	2.8466
4	8.5497	16.401	2.3861	4.8903	5.1476	6.2315	3.8008	3.6466
6	12.771	21.286	2.8020	6.1623	6.5321	6.8718	4.0658	5.1459
8	15.434	23.307	3.1935	7.4191	7.7978	8.2885	4.0787	7.8075
24	18.092	30.151	7.7280	14.473	9.4092	11.540	5.0107	11.767
48	23.954	35.591	13.576	18.791	14.537	15.689	7.7361	15.389
72	27.427	44.670	22.020	25.845	22.415	23.348	9.6712	17.523
96	30.296	50.292	37.485	40.187	25.290	30.114	15.708	19.636
168	37.777	59.375	52.617	76.243	29.030	34.490	18.473	48.420

TABLE-4
DRUG RELEASE KINETICS VALUES

Conc. of IA	Drug release kinetics					
	First order	Zero order	Higuchi mode	Hixon-crowel	Korsmeyer-peppas	'n' value
			7.4			
5	0.8376	0.8201	0.9499	0.8319	0.9389	0.5170
10	0.8293	0.8337	0.7748	0.8308	0.9244	1.2810
20	0.7938	0.7829	0.9310	0.7902	0.9474	0.4341
30	0.5909	0.5862	0.7880	0.5893	0.9156	0.4510
			6.8			
5	0.7926	0.7604	0.9108	0.7822	0.9438	0.45032
10	0.8208	0.8200	0.8497	0.8207	0.8956	0.56520
20	0.7793	0.7670	0.8968	0.7753	0.8864	0.55689
30	0.9313	0.9284	0.9578	0.9304	0.9672	0.55438

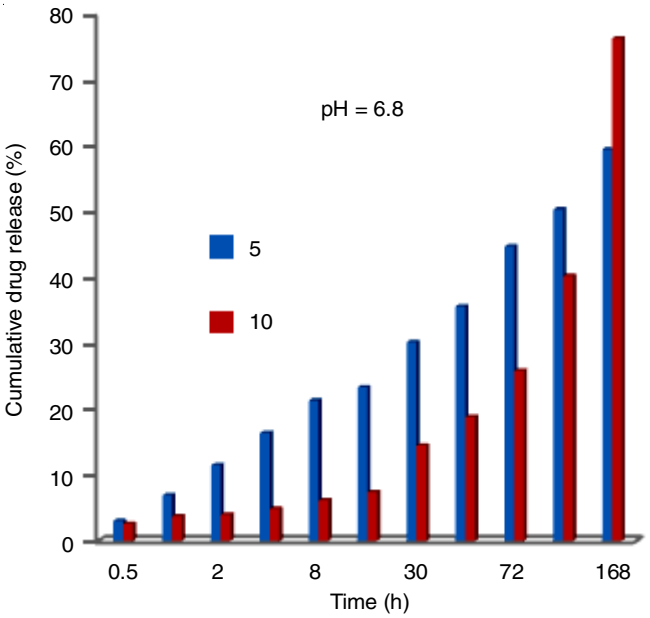

Fig. 10. Release in 6.8 buffer

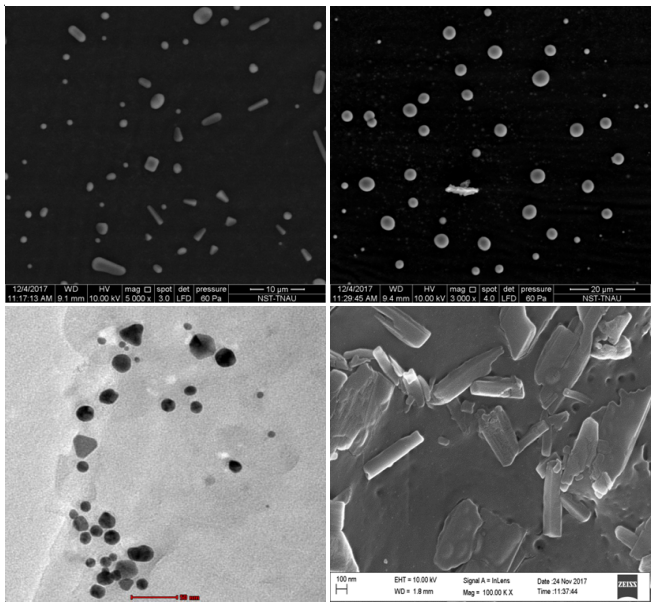
Fig. 11. SEM and TEM images of chitosan-*g*-poly(itaconic acid) nanoparticles (PIACS) loaded with docetaxel

where M_t/M_a is fraction of drug released, t = time, k = constant includes structural and geometrical characteristics of the nanoparticles; n = release component which is indicative of drug release mechanism, it is a diffusion exponent. Peppas equation could adequately described the release of solutes from slabs, spheres, cylinders and disks, regardless of the release mechanism. The slope of linear plot log M_t/M_1 *versus* log t, for drug release < 50 % gives the power law exponent (n) value.

The equation is physically realistic for n = 0.45 (pure diffusion-Fickian controlled drug release) and n = 1 (swelling-controlled drug release or Case II transport: non-Fickian). For other values of 'n' anomalous transport kinetics, *i.e.* a combined mechanism of pure diffusion and Case II transport will be operating. Range of n values 0.44 to 1.28 was observed which indicated that a combined mechanism of pure diffusion and non-Fickian (Case II transport) is the driving force for the drug release from chitosan-*g*-poly(itaconic acid) nanoparticle.

SEM Morphology: SEM images of the nanoparticles are shown in Fig. 11. These images confirm the size distribution and the shape of the nanoparticles. Nanoparticles are not only in the spherical shape, it is also in the triangle shape, square and rod likes structures. A porous structure of the nanoparticle is confirmed by the image which will enrich the drug loading of the nanoparticle. EDAX results are shown in Fig. 12. Table-5 gives the composition of chitosan, chitosan-*g*-poly(itaconic acid) and drug loaded nanoparticle.

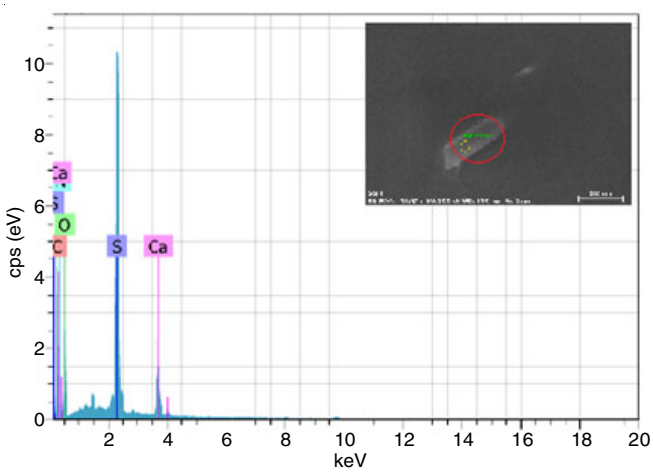

Fig. 12. EDAX results

There is a decrease in the percentage of carbon and nitrogen, however the percentage of oxygen is getting increased id due

TABLE-5
EDAX RESULTS

Element	Before Loading		After Loading
	PIACS	CS	Sphere
C	57.20	62.07	69.6
O	27.05	21.29	14.9
S	5.99	5.78	–
N	8.27	10.29	4.44

to the incorporation of itaconic acid into the chitosan backbone, which confirms the grafting of poly(itaconic acid) into chitosan backbone. After drug loading again carbon content got increased from 57-69 %, this proved the evidence of appropriate drug loading. TEM image confirmed that the size of nanoparticles is below 50 nm and the shapes are sphere, triangle and irregular shape.

PreADMET predictions: PreADMET is a web-based application for predicting ADME data and building drug-like library using *in silico* method. It will calculate the adsorption distribution metabolism and excretion properties [19]. This will also give values for pharmacological parameters like plasma protein binding (PPB), human intestinal absorption (HIA), penetration of blood-brain barrier (C_{brain}/C_{blood}) and cellular permeability (P_{caco-2}). Toxicological properties such as mutagenic and carcinogenetic nature can also be able to predict through this online software. This nanoparticle showed 24 % of plasma protein binding which is lying in the category of weakly bound chemicals. Drugs which are binding to the plasma proteins are not available for therapeutic activity, simply they are inactive. Hence, drug entrapped with in the nanoparticle is getting protected from drug protein binding and drug is completely available for therapeutic activity.

Conclusion

In this study, a series of chitosan-*g*-poly(itaconic acid) nanoparticles (PIACS) with the different concentration of itaconic acid from 5-10 mmol by graft copolymerization using ammonium persulphate as initiator is successfully prepared. FT-IR confirms the graft polymerization of PIACS. Particle size analysis and zeta potential report, we have confirmed that 10 mmol itaconic acid content shows better size and stability of the nanoparticle. Thermogravimetric analysis also confirms the formation of PIACS. Entrapment and loading efficiency was found to be in the expected range. Morphology studies were coincided with the particle size analysis and it revealed the shape of the nanoparticles. From these findings, it is concluded that this nanoparticle could be a good drug carrier for anticancer drug.

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REFERENCES

1. N. Debotton and A. Dahan, *Med. Res. Rev.*, **37**, 52 (2017); https://doi.org/10.1002/med.21403.
2. A. Jain, K. Thakur, G. Sharma, P. Kush and U.K. Jain, *Carbohydr. Polym.*, **137**, 65 (2016); https://doi.org/10.1016/j.carbpol.2015.10.012.
3. M. Prabaharan, *Int. J. Biol. Macromol.*, **72**, 1313 (2015); https://doi.org/10.1016/j.ijbiomac.2014.10.052.
4. H. Ge, T. Hua and J. Wang, *Int. J. Biol. Macromol.*, **95**, 954 (2017); https://doi.org/10.1016/j.ijbiomac.2016.10.084.
5. H. Wang, Y. Xu and X. Zhou, *Int. J. Mol. Sci.*, **15**, 3519 (2014); https://doi.org/10.3390/ijms15033519.
6. Z. Liu, D. Liu, L. Wang, J. Zhang and N. Zhang, *Int. J. Mol. Sci.*, **12**, 1684 (2011); https://doi.org/10.3390/ijms12031684.
7. Y. Yin, Q. Dang, C. Liu, J. Yan, D. Cha, Z. Yu, Y. Cao, Y. Wang and B. Fan, *Int. J. Biol. Macromol.*, **102**, 10 (2017); https://doi.org/10.1016/j.ijbiomac.2017.04.005.
8. M. Bajpai, S.K. Bajpai and P. Jyotishi, *Int. J. Biol. Macromol.*, **84**, 1 (2016); https://doi.org/10.1016/j.ijbiomac.2015.11.088.
9. D.M. Raþă, J.-F. Chailan, C.A. Peptu, M. Costuleanu and M. Popa, *J. Nanopart. Res.*, **17**, 316 (2015); https://doi.org/10.1007/s11051-015-3115-1.
10. S.F.H. Syed Haron, M.M. Sanagi and A.S. Abdul Keyon, *eProceed. Chem.*, **1**, 18 (2016).
11. N.B. Milosavljevic, L.M. Kljajevic, I.G. Popovic, J.M. Filipovic and M.T. Kalagasidis-Krusic, *Polym. Int.*, **59**, 686 (2010); https://doi.org/10.1002/pi.2756.
12. N. Isiklan, F. Kursun and M. Inal, *Carbohydr. Polym.*, **79**, 665 (2010); https://doi.org/10.1016/j.carbpol.2009.09.021.
13. S. Shang, S.J. Huang and R.A. Weiss, *Polymer*, **50**, 3119 (2009); https://doi.org/10.1016/j.polymer.2009.05.012.
14. H.F. Naguib, *J. Polym. Res.*, **9**, 207 (2002). https://doi.org/10.1023/A:1021399826969.
15. S.L. Tomic, M.M. Micic, S.N. Dobic, J.M. Filipovic and E.H. Suljovrujic, *Radiat. Phys. Chem.*, **79**, 643 (2010); https://doi.org/10.1016/j.radphyschem.2009.11.015.
16. X. Hu, Y. Wang, L. Zhang, M. Xu, W. Dong and J. Zhang, *Carbohydr. Polym.*, **155**, 242 (2017); https://doi.org/10.1016/j.carbpol.2016.08.077.
17. M. Sen and O. Guven, *Radiat. Phys. Chem.*, **55**, 113 (1999); https://doi.org/10.1016/S0969-806X(98)00315-6.
18. K. Subramanian and V. Vijayakumar, *Saudi Pharm. J.*, **20**, 263 (2012); https://doi.org/10.1016/j.jsps.2011.09.004.
19. S. Balachandar, M. Sethuram, P. Muthuraja, T. Shanmugavadivu and M. Dhandapani, *J. Photochem. Photobiol. B*, **163**, 352 (2016); https://doi.org/10.1016/j.jphotobiol.2016.08.045.