Synthesis of N,O-Carboxymethyated chitosan and its application in the development of acyclovir loaded nanoparticles

Sherjeel Adnan, Nazar Muhammad Ranjha and Muhammad Hanif*
Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University, Multan

Abstract: Objective of the study was to perform a physico-chemical modification of low molecular weight chitosan (CTS) followed by its use in the formulation of nanoparticles carrier of Acyclovir (ACY). Modified polymer was used to develop ACY loaded nanoparticles in order to achieve optimal response and to minimize toxic effects of ACY. CTS were dissolved in varying concentrations of potassium hydroxide solution to synthesize N, O-carboxymethylated chitosan (N,O-CMC). Synthesized derivative was further processed with different concentrations of TPP (0.3%, 0.5% and 1%) and ACY to prepare nanoparticles. N,O-CMC and prepared formulations were characterized by Fourier transform infrared spectroscopy (FTIR). Furthermore, scanning electron microscopy (SEM) was done to observe the surface morphology, zeta size and zeta potential for particle size analysis, in vitro dissolution to find out the release pattern and kinetic modeling was done to observe the release mechanism and pattern of the drug. Result of FTIR was evidence of polymer modification when compared with chitosan which was the parent standard polymer as well as compatibility of the ingredients. Results of zeta size analysis have confirmed that the particles are of nanosized (109 - 125nm). Good controlled 98.77% over release of 24 h of formulation B observed in phosphate buffer of intestinal pH. Higuchi model with Fickian diffusion was dominating due to the formation of N, O-CMC complex which created smooth surface. All the results were significant and within the p value of 0.001. Conclusively, the modification of the CTS was in nanoparticle showed good sustained release.

Keywords: Chitosan, N,O-carboxymethyl chitosan, synthesis, nanoparticles.

INTRODUCTION

Polymer modification can be done by PEG (Polyethylene glycol)-protein complexes, conjugation of polymer with drug, polymer conjugated with protein, micelles of polymers, dendrimers and polyplexes. Due to unique property and high versatility, polymer conjugates have used to cure different diseases. Conjugation protects the body from undesirable and toxic effects of drug.

Chitosan (CTS) is a biodegradable polymer which is used for conjugation of drug. The $-NH^2$ and -OH groups in the CTS are suitable for modification (Nagpal *et al*., 2010). The initial amines in the chemical structure of CTS can be protonated under acidic conditions, that's why it is called as cationic polyelectrolyte (Mahoney *et al*., 2012) and also have ability of making inter and intramolecular H- bonding (Liu *et al*., 2015). CTS is deacetylated form of chitin and after cellulose it is the second most cationic polysaccharides in nature (Liu *et al*., 2015) which consists of β-1, 4-linked glucosamine with various N- acetyl glucosamine residues (Li *et al*., 2016). CTS also exists in various crystalline forms such as α, β and γ- form (Nagpal *et al*., 2010). The molecular weight of commercially available CTS has 3800-20,000 Dalton and 66% to 95% in deacetylated form (Sahoo *et al*., 2010). When amino group undergoes protonation in acidic (pH <6.5) condition, then it solubilize in water (Sahoo *et al*., 2010) . It is also solubilized in various acids such as dilute acetic acid, lactic acid and formic acid (Khalaf, 2016). It is obtained from crustacean shells and from the cell wall of certain fungi such as Zygomycetes species and yeast (Sahoo *et al*., 2010). Four steps are included in the production of CTS from crustacean shells such as Deproteinization, Demineralization, Decolouration and Deacetylation (Dutta *et al*., 2004).

Nanoparticles are defined as smaller particles having one dimension, smaller than 1 micron. Generally the size of nanoparticles ranges between 10 and 1000nm.(Mohanraj and Chen, 2006) Various terms are used to describe nanoparticulate drug delivery systems. Chitosan nanoparticles are the biodegradable, biocompatible, nontoxic carriers for different drugs (Kamat *et al*., 2016). Different mechanisms are involved in the release of drug from CTS nanoparticles such as inflation of the polymer, dispersion of the adsorbed drug, drug diffusion through the polymeric matrix and polymer abrasion (Mohammed *et al*., 2017).

ACY is an antiviral drug (Ghosh *et al*., 2006) and Its chemical name is 2-amino-9-(2-hydroxyethoxymethyl)-1 H-purin-6-one. It is available in different dosage form such as tablet, suspension, intravenous injection, and ophthalmic ointment. (Susantakumar *et al*., 2011). It exists in hydrated form with 3:2 and 1:2 ratios of ACY to water molecules (Masuda *et al*., 2012). Passive diffusion mechanism has been involved in the absorption of ACY

**Corresponding author:* e-mail: muhammadhanif14@yahoo.com

due to its high hydrophilic nature (Chaudhari and Ubale, 2012). Plasma half-life of ACY in adult is about 3h-3.25h.

MATERIALS AND METHODS

Low molecular weight Chitosan, 100-150 KDa was obtained from MP Biomedicals, France. Acyclovir [2-amino-9-(2-hydroxyethoxymethyl)-1 H-purin-6-one] MW225.2 was purchased from Sigma-Aldrich Company. Potassium hydroxide, Monochloroacetic acid, Isopropyl alcohol (IPA), Acetic Acid, Ethanol, NaOH, Syringe filters (0.45-µm) were got from Merck Darmstadt, Germany. Potassium dihydrogen phosphate was obtained from Fluka, Germany. Pepsin purchased from Avon chemicals ltd, UK and pancreatin from Unichem, Korea. Similarly, TPP was bought from Dae- jung chemicals, Korea, while hydrochloric acid (HCl) from BDH, England. All other reagents were of analytical grade.

Synthesis of N, O-carboxymethyl chitosan (N, O-CMC)

Physiochemical modification of LMW chitosan was performed by NO-CMC is formed by dissolving 1g CTS into 10 ml potassium hydroxide solution of various strengths including 40, 50, 60, 65 and 70% (table 1) and stirred. Treated CTS was added to 10ml of IPA and stirred at room temperature. A mixture of monochloro-acetic acid and IPA was poured into reaction mixture solution and further stirred. The flask was kept in water bath under continue stirring and pH 9.5 was adjusted with 2% acetic acid. Obtained precipitates were filtered, washed with 70% ethanol (n=3) and isopropyl alcohol for the removal of hydroxide. Finally, the obtained mass of N, O-carboxymethalted chitosan was stored for further process and analysis.

Preparation of ACY-CS/NOCMC-NPs

Ionic gelation method was employed for the preparation of acyclovir loaded chitosan nanoparticles by the small modification of already reported method of Jain *et al.*, (Jain *et al*., 2016). Briefly, aqueous solution of 1mg ACY was made and mixed with CTS solution and stirred at 37^{o}C 600 rpm for 1h. Different concentration of TPP (table 2) and N, O-CMC (40 mg) were mixed together with previous mixture for overnight stirring at 600 rpm. Finally, the washing of resulting product ACY-CS/NOCMC-NPs were carried out with deionized water 3times at 12,000 rpm and freeze dried for 48 h. The loading efficiency (LE) and loading content (LC) of ACY was determined. For percentage calculation of LE and LC, the free ACY in supernatants (reaction water) was separated by filtration and centrifugation. The dilution (1:10 ratio) of both solutions made, whose concentration was examined spectrophotometrically at 254 nm (Feng *et al*., 2013). LE and LC were calculated as:

$$\text{LE}\,(\%) = \left(\frac{\text{Total addition of ACY free ACY}}{\text{Total addition of ACY}}\right) \times 100 \qquad (1)$$

$$\text{LE}\,(\%) = \left(\frac{\text{Total addition of ACY free ACY}}{\text{Total addition of ACY}}\right) \times 100 \qquad (2)$$

Fourier transform infra-red spectroscopy (FTIR) & Scanning electron microscope (SEM)

FTIR Spectroscopy was used for the compatibility study of drug, polymer and excipient. The spectra of polymer and dosage form was examined in the range of 3500 to 1000 cm^{-1} and mean of 10 spectra was reported (Selvaraj *et al*., 2010). SEM (Keyence, international Belgium) was used for determination of external structure, chemical composition and orientation of materials making up the sample. The morphology of CTS, NO-CMC, ACY and ACY-CS/NOCMC-NP determined after mounted in the aluminum stabs which was covered with the gold/palladium (200°A) and results of 20µm was reported.

Zeta potential and particle size

The surface charge of ACY-CS/NOCMC-NPs in mixture determined by zeta sizer Zetasizer Nano ZSP (Malvern Panalytical). Aqueous solution of 10mg/ml ACY-CS/NOCMC-NPs at 25^{o}C used and zeta potential was measured. Solubility and release of drug measured by Zeta size. Briefly, the photon correlation spectroscopy (PCS) with fixed angle at 250^{o}C demonstrated average diameter of nanoparticles. Sample was diluted with distilled water for methodical system and sample was run through zeta sizer to obtained results.

In vitro release studies and application of in vitro Kinetic Models

The *in vitro* releases of ACY from ACY-CS/NOCMC-NPs were studied by using simulated gastro intestinal media. The ACY-CS/NOCMC-NPs (0.044g) were kept into a cellulose membrane dialysis tube (12000-14000 kd) which acts as a donor compartment, tied and placed into 50ml of different simulated fluids act as receptor compartments at 37^{o}C. After appropriate interval samples (2ml each) were withdrawn and replaced with equal volume of fresh simulated fluids. Each sample was filtered by using syringe filters (0.22µm) and made dilution of each sample with distilled water. The amount of ACY released in medium was examined at 254 nm by UV spectrophotometer. Various kinetic models including zero order, first order, Higuchi, Hixson Crowell Model, Korsmeyer Peppas model and Weibull Model were used for understanding mechanism and kinetics of drug release. Higuchi Model is the better fit model which will represents kinetic release of drug where K_H is the Higuchi dissolution constant and 'Q' is the amount of drug release in time 't'.

STATISTICAL ANALYSIS

Microsoft Excel 2016 was used for the application of statistical approaches on results. Each experiment was

Table 1: Materials used in the synthesis of N, O-carboxymethylated chitosan

Formulation No	KOH pellets solution (%age)	CTS (g)	IPA (ml)	Mixture of IPA + monochloroacetic acid (ml+mg)
1	40	1	10	5+0.5
2	50	1	10	5+0.5
3	60	1	10	5+0.5
4	65	1	10	5+0.5
5	70	1	10	5+0.5

Table 2: Preparation of acyclovir loaded chitosan nanoparticles

Formulation code	TPP (mg)	ACY solution (mg/ml)	CTS solution (mg/ml)	N,O – CMC (mg)
A	5	1/10	3/10	40
B	3	1/10	3/10	40
C	10	1/10	3/10	40

Table 3: Kinetic modeling of the drug release data obtained from *in-vitro* drug release studies

Formulation Code	Zero order	First order	Higuchi model	Hixson crowell model	Korsmeyer peppas model	Weibull model	
	R^2	R^2	R^2	R^2	n	α	β
A	0.6345	0.6166	0.8136	0.6227	0.086	0.925	0.185
B	0.4348	0.4204	0.6462	0.4253	0.126	0.831	0.425
C	0.6262	0.5995	0.802	0.6084	0.107	1.082	0.210

Table 4: Zeta size and potential of ACY-CS/NOCMC-NPs

Formulation	Zeta size(nm)	Zeta potential(mV)
A	125.3±0.45	-25.4.±0.52
B	115.3±0.24	-23.8±0.5
C	109.23±0.2	-20.6±0.43

performed was performed threes and mean values with the standard deviation was reported in the text. One way ANOVA was applied with the p value of 0.001.

RESULTS

Synthesis of N,O-CMC from chitosan

N,O-CMC was synthesized from chitosan and its successful synthesis was confirmed by the infra-red spectroscopy. IR scan was performed in the range of 400 to 4000 and it was observed that there was a noticeable variation in the IR scan of pure chitosan and its modified form. The FTIR spectrum was used to assess the functional group present in the chitosan. The bands 3200 - 3358.60 cm-1 showed N-H and O-H stretching behavior as well as intermolecular hydrogen bonding. The C-H symmetric and asymmetric stretching was found at 2900 and 2877.45 cm-1respectively. A band 1589.63cm-1 corresponds to the N-H bending of primary amine. Similarly, the C-O stretching was observed at 1060.13 and 1025.27cm-1 (fig. 1).

Chemical compatibility of drug and polymer

The FTIR spectrum of the pure drug ACY was detected. The stretching region of functional group N-H ranges from 3600- 3200 cm^{-1}. Prominent peaks of hydroxyl group, carboxyl group aromatic amine were found at 1400cm^{-1}, 1320$cm^{-1}$and 1100cm^{-1} respectively. The stability of acyclovir during processing of acyclovir loaded microspheres was sustained by these peaks. The FTIR spectrum of CTS nanoparticles was assessed. The nanoparticles made up of CTS, N,O -CMC and different concentration of TPP. The N-H stretching was observed at peak 1050cm^{-1} for formulations A, B and C. A band 1590.10 cm^{-1} demonstrated to N-H bending of primary amines (fig. 1). The IR analysis revealed that the characteristics group exists in the product. It was also indicated that polymer showed interaction with N, O-CMC (Selvaraj *et al*., 2010).

In-vitro drug release studies

ACY release from ACY-CS/NOCMC-NPs was determined and the drug release characteristics were assessed *in vitro* at 37^{o}C using intestinal simulated fluid as dissolution mechanism. The factors which effect drug release during dissolution are the nature of the polymer matrix, porosity of release unit, solubility of drug and pH of the media. It was observed that drug release from formulation A, B and C displayed an initial burst of 60.92%, 54.20% and 57.63% respectively. Whereas 100%

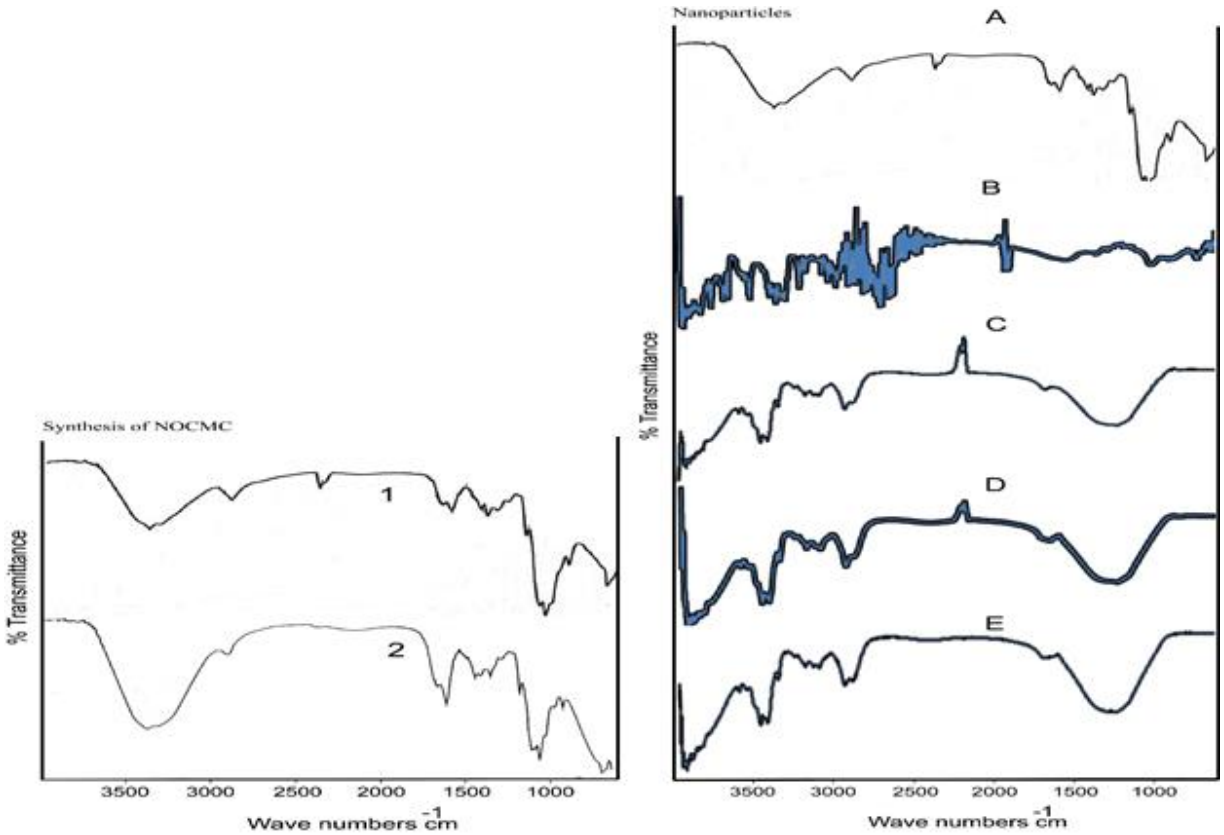

Fig. 1: FTIR reports of (1) Chitosan (2) N, O-CMC confirming the successful modification of chitosan, (A) chitosan, (B) ACY, (C) Formulation A, (D) Formulation B, (E) Formulation C).

drug release was observed from formulation B, 84.87% release from formulation A and a 80.925 release from formulation C after 8hrs of dissolution in simulated intestinal fluid (fig. 2).

Kinetic modeling on dissolution

It was noticed that the formulation A best perused higuchi model with regression coefficient R^2 0.8136 more than the regression coefficient of zero order 0.6345 and first order 0.6166 respectively (table 3). Therefore, it was observed that diffusion profile of the drug from the nanoparticles was better fitted for Fickian diffusion mechanism for the release of drug from nanoparticles. Weibull value for A followed steep curve because the value of β was less than model (table 3). The Korsmeyer values for the formulations were 0.107 demonstrated fickian diffusion mechanism. The Weibull method also applied for these formulation which followed curve shape was steep in case of C because the value of β was less than 1 (0.210). It was calculated from above observation that all formulations were followed higuchi model which elaborate ACY release from formulation was diffusion controlled release.

Surface morphological studies by using SEM

The morphological and surface characteristics of prepared particles from acyclovir loaded chitosan nanoparticles were evaluated by using SEM. The SEM provided images and information about nanoparticles surface through scanning (76). Different characteristics were analyzed in fig. 3. The SEM results displayed that nanoparticles had a solid compact structure with smooth surface. There is hardly evidence of traces of drug particles indicating that drug has been captured by the polymeric network suggesting suitable drug entrapment efficiency of the chitosan. The particle sizes of all formulations were established in 20µm.

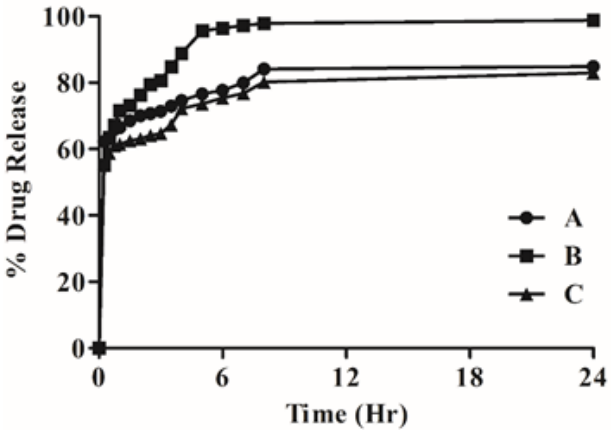

Fig. 2: Pictorial representation of drug release profiles of ACY released from different formulations

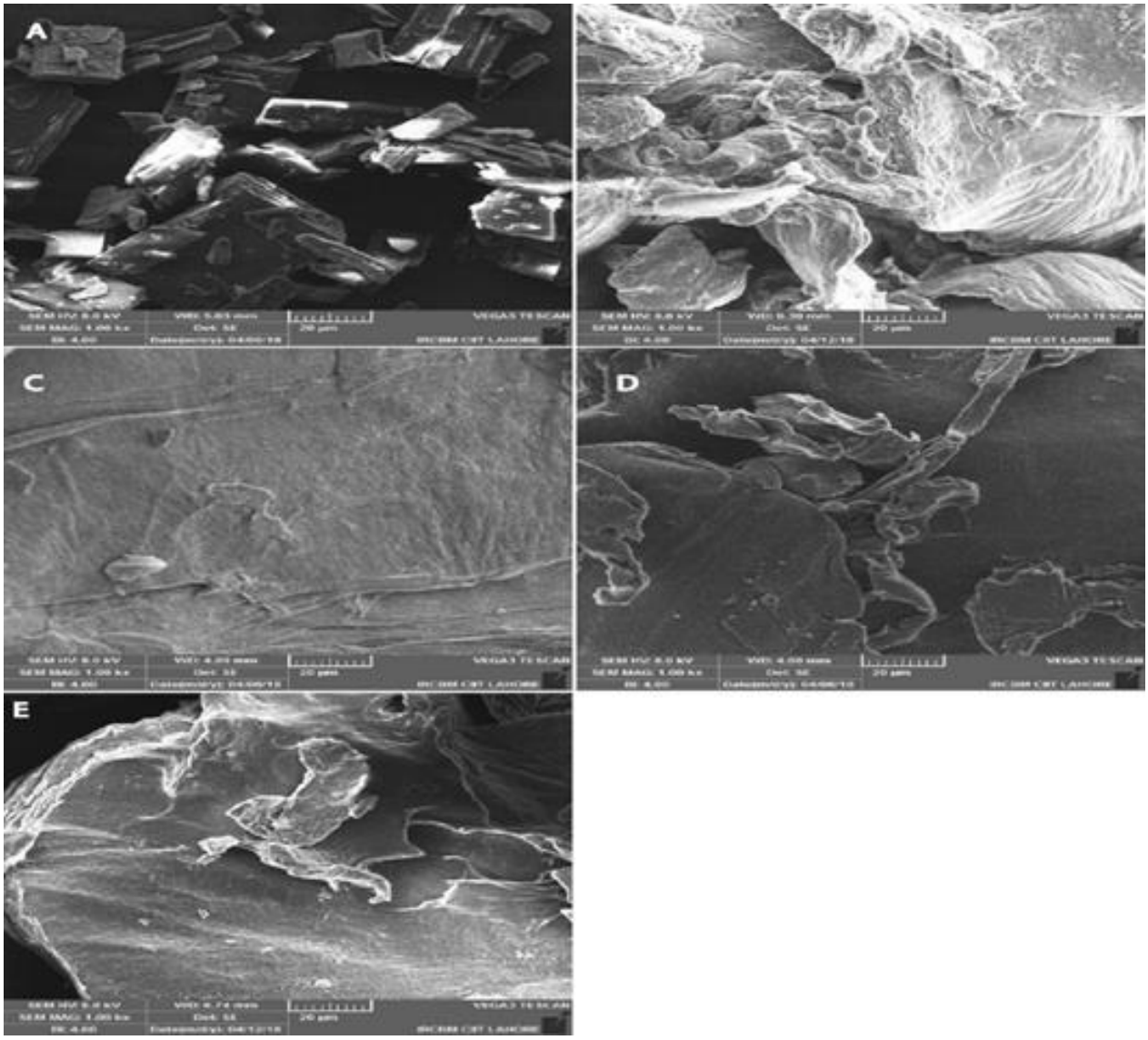

Fig. 3: SEM photographs of (A) ACY, (B) N,O-CMC, (C) Formulation A, (D) Formulation B and (E) Formulation C

Zeta potential and Zeta size of ACY-CS/NOCMC-NPs

The element charge and rate of particle movement in electronic area showed by zeta potential. The surface charge of nanoparticles in mixture was determined by zeta potential procedure. If Zeta Potential rates of nanoparticles more than +25 mV or less than -25 mV, they usually showed high degrees of stability. The negatively charge zeta potential principle were determined to observe strength of ACY. The results of formulations A, B and C were -25.4.±0.52,-23.8±0.5 and -20.6±0.43 respectively as shown in table 4. The photon correlation spectroscopy (PCS) with fixed angle at 250^{o}C demonstrated average diameter of nanoparticles. Sample was diluted with distilled water for methodical system and sample was run through zeta sizer to obtained results. The particle size of acyclovir-loaded chitosan nanoparticles (A– C) was determined to be 125.3±0.45, 115.3±0.24 and 109.23±0.21 respectively as described in table 4.8. The extreme size was observed in formulation A as compared to other formulations.

DISCUSSION

Results show a successful structural change of N, O-CMC confirmed by FTIR. The band at 3361.89 cm-1 showed the stretching vibrational absorption overlapping peaks of amino and hydroxyl bonds formed by hydroxyl groups (O-H, N-H stretch). Methylene stretching was observed at 2889.45 cm-1(CH2 stretch in CH2OH group). Stretching vibration of amides was shown at 1582.59cm-1. The band 1413.75 cm-1 indicated the carboxylation of N, O-CMC. This stretching was the main difference of N, O-CMC as compared to CTS.

Regarding chemical compatibility of ACY with N.O-CMC a characteristics peak identified in formulation B

which clearly indicated a shift to 1582cm^{-1} and 1023 cm^{-1} for N-H bending of primary amines and C-O stretching on CTS respectively. The characteristic peak of N, O-CMC was also shifted to 3375cm^{-1}in nanoparticles. A new sharp peak at 1322 on ACY was also raised. It was found that all characteristic peaks of ACY, polymer and combination of pure drug with CTS confirming compatibility of the pure drug and chitosan (Selvaraj *et al*., 2010). The results confirmed that there was a linkage between hydroxyl group of ACY and amino group of CTS (Selvaraj *et al*., 2010). The absorption peak of 1589 cm^{-1} (amino group) and at 1025 cm^{-1} (C-O group) on CTS was absent in the spectrum of acyclovir-loaded chitosan nanoparticles in formulation C as shown in fig. 1. From all above discussion, the results confirmed that there was a linkage between hydroxyl group of ACY and amino group of CTS.

The mechanism of initial burst of drug is due to drug content which is closer to the surface of chitosan nanoparticles will be dissolved and drug released rapidly. Thereafter, the drug present in the nanoparticle's core showed prolonged release of drug (fig. 2). The drug release depends upon drug loading efficiency. If the amount of drug loading is low, then the rate of drug release becomes also slow because more void spaces available for transportation of less number of drug molecules (Naik and Raval, 2016). The electrostatic force of interaction between ACY and chitosan show association between drug and polymer which resulted in drug retardation from nanoparticles (Rajendran *et al*., 2014). The drug release from nanoparticles also depends upon matrix erosion (Bhosale *et al*., 2013). Here formulation B has shown sustained effect in simulated intestinal fluid in 24 h. At this pH nanoparticle became uncertain and decompose resulted in a fast release of ACY. That's why, ACY-CS/NOCMC-NPs have the capability to release ACY to small intestine and also able to release the loaded ACY into the intestinal mucosa after infiltration (Feng *et al*., 2013).

Results evaluated that the release of drug from nanoparticles is composed due to drug diffusion through pores. The kinetic models used were zero order, first order, hixon Crowell, and Higuchi and Korsmeyer Peppas equation. For planery geometry, if value of n=0.5 it illustrates Fickian diffusion system. For value 0.5<n<1.0 then it represents non Fickian and n=1 showed class II transport (Selvaraj *et al*). Greater association was experiential in the Higuchi equation. It was calculated from above observation that formulation followed higuchi model which elaborate ACY release from formulation was diffusion-controlled release. Therefore, it observed that diffusion profile of the drug from the nanoparticles was better fitted for Fickian diffusion The Weibull method also applied for formulation, which followed steep curve because the value of β was less than 1.

Similarly, for formulation B best-fit model was higuchi model representing diffusion-controlled system with regression coefficient 0.6462. The Korsmeyer values for the formulation was 0.126 elaborated the release of drug from the nanoparticles was through Fickian diffusion. Weibull model for the formulations was 0.425 and has followed the steep curve shape. Similar type of behavior was observed as C has also followed higuchi model (R^2=0.802) Furthermore surface morphology exhibited a solid structure with smooth surface. Size of nanoparticles formulated were in nano size, indicated the formation of required nanoparticles that carries negative charges.

CONCLUSION

CTS nanoparticles with excellent carrying capacity of ACY was successfully prepared. Effect of increased concentration of TPP in combination with ACY, CTS and N, O-CMC showed direct effect of crosslinking capacity. The absorption peaks of nanoparticles confirming the interaction and compatibility of the pure drug and CTS. The SEM results demonstrated suitable drug entrapment efficiency of the CTS. Zeta potential and zeta size definite the electronic charge and confirmed nanosized. Negative charge of modified CTS made them a potential candidate having improved mucoadhesive nature from positively charge mucous membrane which increased the residence time of ACY. The *in vitro* release in simulated fluids represent entrapment efficiency for ACY and measured the effect of particle size on drug release from polymer matrix. The *in vitro* releases Kinetics methods of ACY from formulations have given sustain drug release proceeding higuchi model with fickian diffusion mechanism. Prepared nanoparticles may be used for the improved permeation of ACY in experimental animals.

REFERENCES

Bhosale UV, Devi K and Choudhary S (2013). Development and *In vitro-In vivo* Evaluation of Oral Drug Delivery System of Acyclovir Loaded PLGA nanoparticles. *Int. J. Drug Deliv*, **5**(3): 331-343.

Chaudhari V and Ubale M (2012). A validated stability-indicating HPLC assay method for acyclovir in bulk drug. *Int.. J. Anal Pharm BiomedSci*, **1**(2): 5-12.

Dutta PK, Dutta J and Tripathi V (2004). Chitin and chitosan: Chemistry, properties and applications. *J. Sci Ind. Res.*, **63**(1): 20-31

Feng C, Wang Z, Jiang C, Kong M, Zhou X, Li Y, Cheng, X and Chen X (2013). Chitosan/o-carboxymethyl chitosan nanoparticles for efficient and safe oral anticancer drug delivery: *In vitro* and *In vivo* evaluation. *Int. J. Pharm*, **457**(1): 158-167.

Ghosh PK, Majithiya RJ, Umrethia ML and Murthy RS (2006). Design and development of microemulsion drug delivery system of acyclovir for improvement of oral bioavailability. *AAPS Pharmscitech*, **7**(3): E172-E177.

Jain A, Thakur K, Sharma G, Kush P and Jain UK (2016). Fabrication, characterization and cytotoxicity studies of ionically cross-linked docetaxel loaded chitosan nanoparticles. *Carbohydr. Polym.,* **137**: 65-74.

Kamat V, Bodas D and Paknikar K (2016). Chitosan nanoparticles synthesis caught in action using microdroplet reactions. *Scientific Reports,* **6**: 22260.

Khalaf MN (2016). Green Polymers and Environmental Pollution Control. CRC Press, pp.1-46.

Li Y, Qin Y, Liu S, Xing R, Yu H, Li K and Li P (2016). Preparation, Characterization, and Insecticidal Activity of Avermectin-Grafted-Carboxymethyl Chitosan. *Bio. Med. Res. Int.,* org/10.1155/2016/9805675

Liu J, Wu HT, Lu JF, Wen XY, Kan J and Jin CH (2015). Preparation and characterization of novel phenolic acid (hydroxybenzoic and hydroxycinnamic acid derivatives) grafted chitosan microspheres with enhanced adsorption properties for Fe (II). *Chem Eng. J.,* **262**: 803-812.

Mahoney C, Mccullough M, Sankar J and Bhattarai N (2012). Nanofibrous structure of chitosan for biomedical applications. *J. Nanomedic Biotherapeu Discover,* **2**(1): 1-9

Masuda T, Yoshihashi Y, Yonemochi E, Fujii K, Uekusa H and Terada K (2012). Cocrystallization and amorphization induced by drug excipient interaction improves the physical properties of acyclovir. *Int. J. Pharm.,* **422**: 160-169.

Mohammed MA, Syeda J, Wasan KM and Wasan EK (2017). An overview of chitosan nanoparticles and its application in non-parenteral drug delivery. *Pharmaceutics,* **9**: 53.

Mohanraj V and Chen Y (2006). Nanoparticles: A review. *Trop. J. Pharm Res,* **5**: 561-573.

Nagpal K, Singh SK and Mishra DN (2010). Chitosan nanoparticles: A promising system in novel drug delivery. *Chem. Pharm. Bull,* **58**: 1423-1430.

Naik DR and Raval JP (2016). Amorphous polymeric binary blend pH-responsive nanoparticles for dissolution enhancement of antiviral drug. *J. Saudi Chem Soc,* **20**(1): S168-S177.

Rajendran N, Natrajan R, Kumar S and Selvaraj S (2014). Acyclovir-loaded chitosan nanoparticles for ocular delivery. *Asian J. Pharm,* **4**(4): 220-226

Sahoo S, Sasmal A, Nanda R, Phani A and Nayak P (2010). Synthesis of chitosan polycaprolactone blend for control delivery of ofloxacin drug. *Carbohydr Polym,* **79**(1): 106-113.

Selvaraj S, Niraimathi V and Nappinnai M Formulation and evaluation of acyclovir loaded chitosan nanoparticles. *Int. J. Pharm. Pharm. Sci*., **5**(4): 619-629.

Selvaraj S, Saravanakumar N, Karthikeyan J, Evangeline DD, Rajendran NL and Lathamary D (2010). Acyclovir loaded chitosan nanoparticles for ocular delivery. *Der. Pharmacia Lettre,* **2**: 420-431.

Susantakumar P, Gaur A and Sharma P (2011). Comparative pharmacokinetics, safety and tolerability evaluation of acyclovir IR 800 mg tablet in healthy Indian adult volunteers under fasting and non-fasting conditions. *J. Bioequiv. Availab.,* **3**: 128-138.