Chitosan-poly (lactide-co-glycolide) (CS-PLGA) nanoparticles containing metformin HCl: Preparation and *in vitro* evaluation

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Abstract: In this study, the preparation and *in vitro* characterisation of metformin HCl-loaded CS-PLGA nanoparticles (NPs) were aimed. The prepared nanoparticles (blank nanoparticles (C-1), 50 mg of metformin HCl loaded nanoparticles (C-2) and 75 mg of metformin HCl loaded nanoparticles (C-3) ranged in size from 506.67±13.61 to 516.33±16.85 nm and had surface charges of 22.57±1.21 to 32.37±0.57 mV. Low encapsulation efficiency was observed for both nanoparticle formulations due to the leakage of metformin HCl to the external medium during preparation of nanoparticles. Nanoparticle formulations showed highly reproducible drug release profiles. ~20% of metformin HCl was released within 30 minutes and approximately 98% of the loaded metformin HCl was released at 144 hours in a phosphate buffer (PB; pH 6.8). No statistically significant difference was noted between the *in vitro* release profiles of the nanoparticles (C-2 and C-3) containing metformin HCl. Also, nanoparticles were characterised using FT-IR and DSC.

Keywords: PLGA, CS, Nanoparticles, FT-IR, metformin HCl.

INTRODUCTION

Metformin in the biguanide class has antihyperglycemic effect. Recent studies have shown that metformin has also antineoplastic and chemopretentive potentials (Emami Riedmaier, 2013; Spratt, 2012). Metformin, mainly absorbed from the small intestine, has a relatively low (50-60%) bioavailability. The absorption of metformin may be increased as the motility of gastrointestinal is slowed. The biological half-life ($t_{1/2}$) of metformin is range of 0.9-2.6 hours. Therefore, repeated applications of high doses of metformin are needed for an effective treatment. As a result, patient compliance reduces and/or the incidence of side effects (diarrhoea, nausea, anorexia, vomiting, lose weight and taste disturbance etc.) increase (Corti, 2008; Sweetman, 2007). Moreover, lactic acidosis, which is sometimes fatal, has occurred with biguanides (Sweetman, 2007). The development of different types of formulation for metformin is carried to improve its bioavailability, to reduce the dosing frequency of the drug and to decrease in gastrointestinal side effects (Marathe, 2000).

Particulate drug delivery systems have several advantages compared to traditional dosage forms (higher local drug concentrations, less variation in transit times along the gastrointestinal system (GIS), low variability among individuals, low risk of dose dumping) (Amorim and Ferreira, 2001). In particular, nanoparticles have been investigated for the application of many drugs (Cetin M, 2013; Gelperina S, 2005) As drug carriers, nanoparticles have the advantages of the application in different routes (oral, parenteral, inhalation etc.), possibility of the loading both hydrophilic and hydrophobic drugs, high encapsulation efficiency etc. (Gelperina 2005).

The aim of current study was to prepare and *in vitro* characterise metformin HCl-loaded CS-PLGA nanoparticles. The mucoadhesive and absorption enhancement properties of chitosan increase the bioavailability of metformin HCl by increasing the residence time of the nanoparticles in GIS. Therefore, the dosing frequency and oral application doses of metformin HCl to keep the concentration of metformin HCl in therapeutic range are reduced. Consequently, side effects are reduced and the patient compliance is increased. The size, zeta potential, surface morphology, encapsulation and drug loading efficiencies and drug release of prepared nanoparticles were examined. Besides, nanoparticles were characterised using FT-IR spectroscopy and DSC.

MATERIALS AND METHODS

Materials

Metformin HCl was a generous gift from Sandoz Ilac Sanayi ve Ticaret AS (Istanbul, Turkey). Chitosan (Protosan UP CL 113) was purchased from FMC Biopolymer-Novamatrix Co., Norway. Poly (D,L-lactide-co-glycolide) (PLGA) (Resomer® RG 502, MW 7,000-17,000 Da), acetone and polyvinyl alcohol (PVA, MW 30,000–70,000 Da) were obtained from Sigma-Aldrich Co. (USA). All other chemicals and reagents used as they were received were of analytical grade.

Preparation of nanoparticles

In this study, PLGA (150mg) was dissolved in acetone. PLGA-CS nanoparticles loaded with metformin HCl were obtained by dropping 20mL aqueous solution (PVA; 3%

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w/v) containing metformin HCl (50 or 75 mg) and CS (30 mg) and stirred using an ultrasonic probe (with 60% power) (Sonoplus HD 2070; Bandelin Electronics, Berlin, Germany) for 7 minutes. The organic phase was then removed using rotary evaporator at 45°C. After pre-centrifugation at 5000 rpm for 5 minutes, NPs were centrifuged at 13500 rpm for 50 minutes. The NPs were re-suspended in distilled water and then lyophilised at −55 °C and 0.021 mbar for 24 hours. All batches of NPs were produced at least in triplicate (Parveen and Sahoo, 2011; Wang, 2011; Ravi Kumar, 2004).

Characterisation of nanoparticles

Surface morphologies of NPs

The nanoparticles were examined by SEM (Scanning Electron Microscope, NOVA Nano SEM 430, FEI, The Czech Republic). The SEM images of lyophilised nanoparticles mounted on metal stubs and spattered with gold were taken.

Size and zeta potential of prepared nanoparticles

The mean size and zeta potential of the nanoparticles were determined by the Zetasizer 3000HS (Malvern Instruments, UK). The measurements of the dilute suspensions of nanoparticles in pure water were performed in triplicate at 25°C.

Drug content of nanoparticles

Lyophilised nanoparticles (10mg) in 5mL of acetone were vortex-mixed for 2 minutes and then mixed on a magnetic-stirrer at 750 rpm for 30 minutes. To extract metformin HCl, PB (10mL; pH 6.8) was added into this mixture and mixed at 750 rpm for a further 30 minutes. After evaporation of organic solvent under vacuum, the remaining aqueous dispersion was centrifuged at 10,000 rpm for 15 minutes. The drug content of each sample was then measured using a validated UV method at 232 nm.

Drug Release study

An incubation method was used for *in vitro* release study. Nanoparticles (20 mg) in amber vials were suspended in PB (4 mL; pH 6.8) and the vials were placed horizontally shaking water bath at 37±0.5°C and agitated at 50 rpm. At predetermined time points, 1mL of sample was withdrawn from the medium and 1mL of fresh buffer was added into the medium. The samples were centrifuged at 10,000 rpm for 10 minutes, and their drug content was measured using the validated UV method at 232 nm.

UV method and validation

Absorbance measurement of metformin HCl in PB (pH 6.8) was carried out at 232 nm using Beckman Coulter-DU® 730 UV-Vis spectrophotometer (Beckman Coulter, USA). The calibration curve was obtained in the concentration range of 4-10 µg/mL (4, 5, 6, 7, 8 and 10 µg/mL, n=6 at each concentration). The line equation and corresponding correlation coefficient (r) were then calculated by least squares linear regression analysis. The method was validated for specificity, linearity, precision and accuracy according to the International Conference on Harmonization guidelines (ICH, 1997). Three different concentrations of standard metformin HCl (4.5, 6.5, 9 µg/mL, n=6 at each concentration) were analysed for evaluation of the intra- and inter-day precision and also intra- and inter-day accuracy of the method.

DSC and FT-IR analysis

DSC curves were recorded using a Netzsch STA 409 PC Luxx® model DSC. The instrument was calibrated using several standards (Bi, In, Ni, Zn, Al, Ag, Sn, Au). Alumina pan was used as reference. The DCS runs were conducted over a temperature range of 20-350°C under 60 mL/min of nitrogen flow.

A Perkin-Elmer Spectrum One model FT-IR was used to record the IR spectra of pure drug, pure polymers (chitosan and PLGA) and blank nanoparticles and metformin HCl-loaded nanoparticles formulations (50 mg and 75 mg) prepared in KBr disks in the region of 4000–400 cm^{-1}.

STATISTICAL ANALYSIS

All tabulated results were expressed as mean ± S.D. Differences between the characteristics of all nanoparticle formulations were evaluated by the Mann–Whitney U test ($p<0.05$ shows the statistical significance).

RESULTS

A simple UV method was developed and validated to determine the drug amount. The results of the method validation were given in table 1. Metformin HCl-loaded NPs were obtained to utilise the method used in previous studies by modifying and characterized in terms of size, zeta potential and in vitro release (Parveen and Sahoo, 2011; Wang, 2011; Ravi Kumar, 2004). The prepared nanoparticles had almost spherical morphology (fig. 1). The particle sizes of the nanoparticles ranged from 506.67±13.61 to 516.33±16.85 nm and had surface charges of 22.57±1.21 to 32.37±0.57 mV (table 2). The encapsulation efficiency values of C-2 and C-3 formulations were 4.311±1.101% and 4.480±0.559%, respectively (table 2). The in vitro release of nanoparticle formulations was investigated in PB (pH 6.8) at 37±0.5°C. ~20% of metformin HCl was released within 30 minutes and approximately 98% of the loaded metformin HCl was released at 144 hours. The release profiles of C-2 and C-3 formulations are shown in fig. 2. DSC thermograms were obtained for pure drug, pure polymers and drug-loaded nanoparticles and showed in fig. 3. Furthermore, the FT-IR spectra of pure drug, pure polymers, drug-polymers physical mixture and nanoparticle formulations were obtained and shown in figs. 4-9. The FT-IR spectra of drug-loaded NPs

formulations compared to the spectrum of the blank NP formulation showed the additional absorption bands at wavenumbers of 3288 cm^{-1}, 1655 cm^{-1}, 1563 cm^{-1} (C-2) and 3417 cm^{-1}, 1639 cm^{-1}, 1569 cm^{-1} (C-3) related to metformin HCl (figs. 7-9).

DISCUSSION

An analytical method was developed and validated to determine the drug amount. The proposed UV method was linear in the determined concentration range (4-10 µg/mL; y=0.076x+0.0008 r^2=0.9999 where y is absorbance and x is the concentration of metformin HCl in µg/mL). The precision and accuracy of the method for intra-and inter-day were determined using the samples of 4.5, 6.5, 9 µg/mL. The intra-day and inter-day accuracy values were -1.680– -0.710% and -1.738– -0.978%, respectively. The intra-day and inter-day precision values were 1.246– 1.585% and 1.254–1.490%, respectively (table 1). All these results indicate that precision and accuracy of the assay are satisfactory (Shabir, 2003).

PLGA has been extensively used in polymeric drug delivery systems for a variety of drugs. PLGA has favourable mechanical properties and predictable biodegradation behaviours and also shows high biocompatibility, the low risk of immunogenicity and toxicity. PLGA nanoparticles have also attracted considerable attention and interest (Song, 2008a; Muthu, 2009). Chitosan is a non-toxic, biocompatible and biodegradable natural polymer with positive charge (Channarong, 2011). The chitosan-coated nanoparticles were prepared to increase the absorption of drugs through the mucosa by prolonging the contact time at the absorption surface (Mazzarino, 2012).

Therefore, in this study, PLGA and chitosan were selected as the polymers for preparation of metformin loaded nanoparticles. In the current study, metformin HCl-loaded NPs were prepared to utilise the method used in previous studies by modifying. (Parveen and Sahoo, 2011; Wang, 2011; Ravi Kumar, 2004). SEM images clearly demonstrated that chitosan-coated PLGA nanoparticles were approximately spherical in shape (fig. 1).

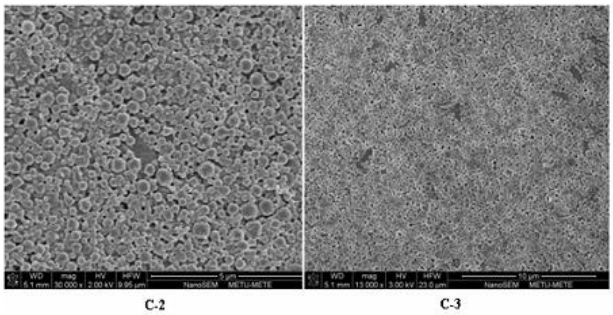

Fig. 1: Scanning electron microscopic photograph of the metformin HCl-loaded-CS-PLGA nanoparticles.

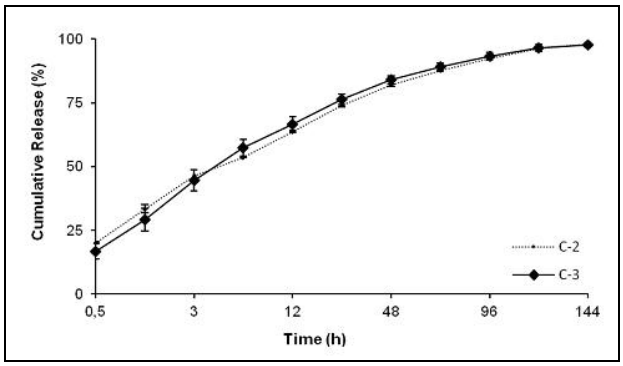

Fig. 2: Release profiles of nanoparticles (mean±SD; n=3).

The results of particle size measurements of metformin HCl-loaded nanoparticles prepared are given in table 2. Many factors (stabilising agent's type, stabilising agent's concentration, organic solvents' type, the organic phase/aqueous phase ratio, the concentration of polymer, the viscosity of media and drug polymer ratio, etc.) effect on the particle size of polymeric drug delivery systems (Khemani and Sharon, 2012; Jelvehgari, 2010). In our study, the mean particle sizes of nanoparticles containing metformin HCl were similar (p>0.05).

The zeta potential of freshly prepared nanoparticles was in the positive range, (22.57±1.21-32.37±0.57 mV) due to the chitosan, which bears positive charges, and there was no significant difference among the zeta potential values of all formulations (table 2). The similar result was previously reported (Parveen and Sahoo, 2011; Wang, 2011). Wang *et al*. (Wang, 2011) prepared the chitosan and aliginate-coated PLGA nanoparticles and reported that chitosan-coated PLGA nanoparticles and aliginate-coated PLGA nanoparticles have positive surface charge (18.8 mV, 0.2% w/v chitosan concentration), and negative surface charge (-23.4 mV, 0.2% w/v alginate concentration), respectively. Also, they reported that the positive surface charge of delivery systems increased with increasing the concentration of chitosan.

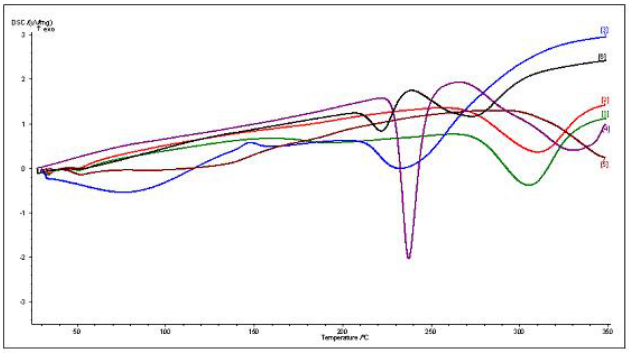

Fig. 3: DSC thermogram of metformin HCl, PLGA, CS, physical mixture of metformin HCl, PLGA and CS and metformin HCl loaded-CS-PLGA nanoparticles ([1]: C2 formulation, [2]: C3 formulation, [3]: CS, [4]: Metformin HCl, [5]: PLGA, [6]: PLGA-CS-Metformin HCl physical mixture).

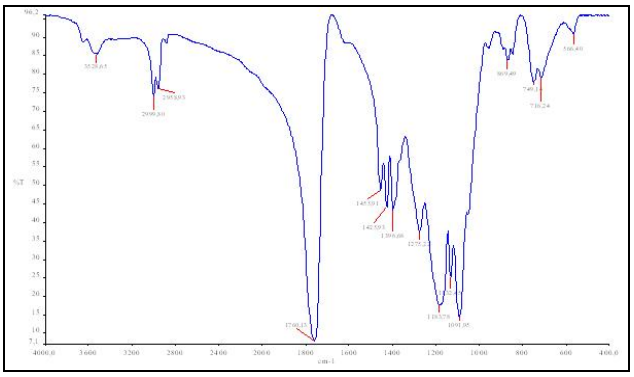

Fig. 4: FT-IR spectrum of PLGA

The encapsulation efficiency and drug loading of metformin HCl into nanoparticles are summarised in table 2. Slightly different drug loading values obtained for nanoparticles (50mg and 75mg) (table 2) ($p<0.05$). Various factors influence drug loading in particulate systems. The critical parameters for drug loading are the molecular weight and polymer type and the viscosity of phases used, drug-polymer ratio (Kilicarslan and Baykara, 2003; Fattal, 1999; Tuncay, 2000; Song, 2008b). Metformin HCl was poorly encapsulated in PLGA nanoparticles due to drug leakage to the external aqueous medium (Barichello, 1999).

The results of *in vitro* release of drug form nanoparticle formulation were given in fig. 2. The profile showed an initial burst release and later, a slow release (Makadia and Siegel, 2011). The fast release might be attributed to drugs adsorbed on the surface of NPs and ~20% of metformin HCL was released within 30 minutes. The about 98% of the loaded metformin HCl was released at 144 hours. The release results of C-2 and C-3 formulations were similar ($p>0.05$).

Fig. 3 displays DSC thermograms obtained for pure drug, pure polymers and drug-loaded nanoparticles. In DSC thermogram of only PLGA, there were two peaks at around 50°C and 285.4°C related to the glass transition and thermal decomposition of the polymer, respectively (Islam, 2011; Mi, 2003; Said, 2011). The DSC scan of pure chitosan presented an endothermic peak at around 100°C (Tpeak: 76.1°C) and an exothermic peak at around 300°C (fig. 3). The endothermic peak and exothermic peak are related to the evaporation of water, which forms the hydrogen bond with hydroxyl groups of chitosan and the degradation of chitosan, respectively (de Moura, 2008; Gazori, 2009). The pure metformin HCl gives rise to an endothermic characteristic peak at 237.5°C, which is near to its melting point (fig. 3) (Jain and Gupta, 2009; Corti, 2008).

The DSC scans of nanoparticle formulations (C-2 and C-3) showed the peaks at around 50°C and around 300°C associated with polymers. However, the endothermic peak characteristic of metformin HCl was not detected in the thermogram of drug-loaded nanoparticles, suggesting that the formation of a molecular dispersion of metformin HCl in the NPs (Pandit, 2013).

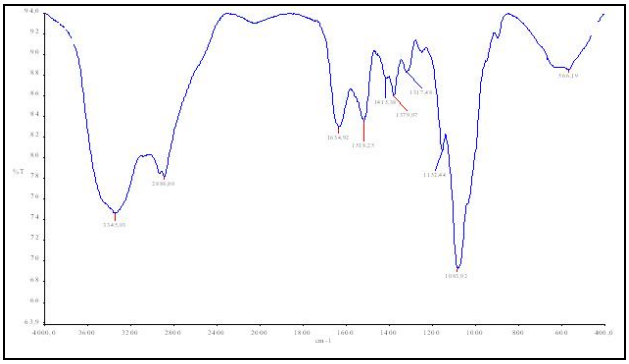

Fig. 5: FT-IR spectrum of CS

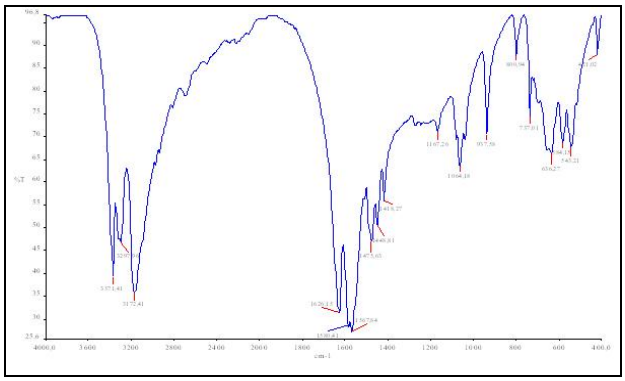

Fig. 6: FT-IR spectrum of Metformin HCl

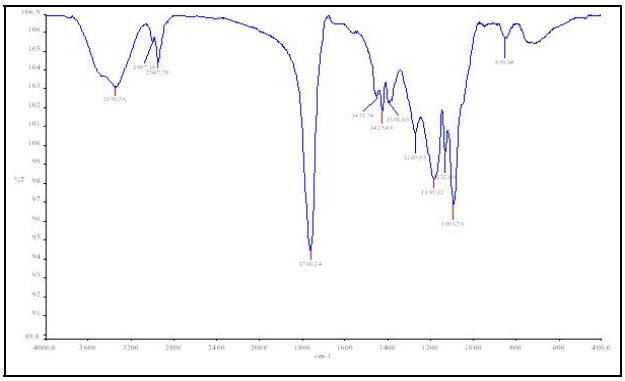

Fig. 7: FT-IR spectrum of C-1 formulation

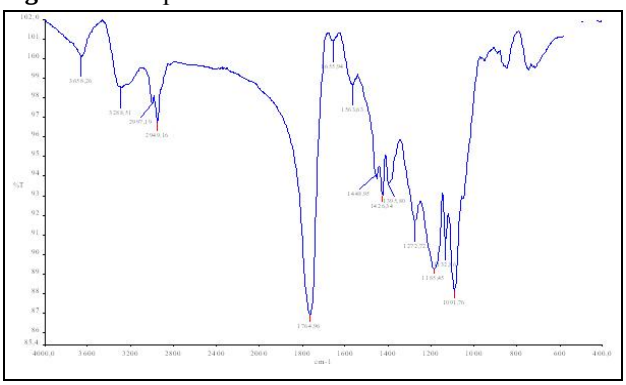

Fig. 8: FT-IR spectrum of C-2 formulation

Table 1: The accuracy and precision data of metformin HCl for intra-day and inter-day

	Concentration Added (µg/mL)	Concentration Measured (µg/mL) (mean ±SD)	[1]Accuracy	[2]Precision
[3]Intra-day	4.5	4.424 ± 0.070	-1.680	1.585
	6.5	6.431 ± 0.080	-1.062	1.246
	9	8.936 ± 0.126	-0.710	1.413
[4]Inter-day	4.5	4.436 ± 0.066	-1.422	1.490
	6.5	6.387 ± 0.080	-1.738	1.254
	9	8.912 ± 0.116	-0.978	1.299

[1](Relative error %):(found-added)/addedx100,[2] RSD (%):Relative standard deviation [3]Six replicates for each concentration,[4]Six replicates for each concentration per day.

Table 2: Mean particle size, zeta potential, drug loading and encapsulation efficiency of nanoparticles (mean±SD).

Formulation	Particle size (nm) (n=3)	Zeta potential (mV) (n=3)	Encapsulation efficiency % (n=4)	Drug loading % (n=4)
C-1	514.63 ± 5.73	32.37 ± 0.57	-	-
C-2	506.67 ± 13.61	23.70 ± 0.80	4.311 ± 1.101	0.799 ± 0.444
C-3	516.33 ± 16.85	22.57 ± 1.21	4.480 ± 0.559	1.318 ± 0.165

C-1: blank nanoparticles, C-2: 50 mg of metformin HCl loaded nanoparticles (polymer:drug ratio 3:1) and C-3: 75 mg of metformin HCl loaded nanoparticles (polymer:drug ratio 2:1).

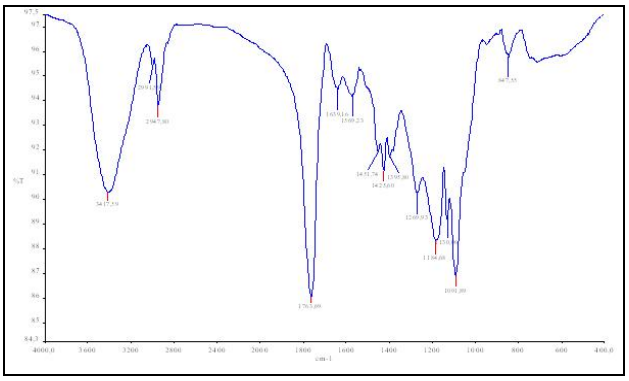

Fig. 9: FT-IR spectrum of C-3 formulation.

The FT-IR spectra of pure drug, pure polymers, drug-polymers physical mixture, blank and drug-loaded nanoparticle formulations were shown in figs. 4-9. The PLGA spectrum presents characteristic peaks at 3528.65 cm^{-1} due to -OH stretching, at 2999.80 cm^{-1} and 2958.93 cm^{-1} assigned to -CH, -CH2, -CH3 stretching, at 1760.13 cm^{-1} and range of 1091.95 cm^{-1}-1275.22 cm^{-1} associated with –C=O and CO stretching, respectively, at 1425.93 cm^{-1} associated with C-H bending in the -OCH_2 (fig. 4) (Mainardes, 2006; Kang 2008, Kiremitci-Gumusderelioglu and Deniz, 1999). The spectrum of pure chitosan displayed peaks at 3345.03 cm^{-1} (NH_2 and O-H stretching), 2888.80 cm^{-1} (C-H stretching), 1634.92 cm^{-1} and 1379.97 cm^{-1} (C=O and C-O stretching of amide group), 1152.44 cm^{-1} (bridge-O stretching), 1083.92 cm^{-1} (C-O-C stretching) (fig. 5) (Mouryaa, 2010; Salehizadeh, 2012).

The FT-IR spectrum of metformin HCl shows peaks at 3371.41 cm^{-1} corresponded to N-H asymetric stretching, at 3297.96 cm^{-1} and 3172.41 cm^{-1} related to N-H symetric stretching, 1626.15 cm^{-1} and 1580.41 cm^{-1} associated with C=N stretching. Furthermore, the absorption band at wavenumber 1567.84 cm^{-1} corresponded to N-H bending in plane, 1475.63 cm^{-1}, 1448.81 cm^{-1} and 1418.27 cm^{-1} associated with C-H asymetric bending (-CH_3), 1167.26 cm^{-1} and 1064.18 cm^{-1} associated with C-N stretching, 937.58 cm^{-1} and 737.01 cm^{-1} related to N-H wagging, 584.15, 543.21 and 421.02 cm^{-1} assigned to C-N-C bending and 636.27 cm^{-1} associated with NH_2 rocking (fig. 6) (Gunasekaran, 2006; Banerjee, 2012).

The C-1 formulation spectrum (fig. 7) showed characteristic peaks at 3528.65 cm^{-1} and range of 794-566 cm^{-1} due to PLGA and 3350.56cm^{-1} associated to chitosan. The FT-IR spectra of C-2 and C-3 (drug-loaded formulations) compared to C-1 formulation spectrum present the additional absorption bands at wavenumbers of 3288 cm^{-1}, 1655 cm^{-1}, 1563 cm^{-1} (C-2) and 3417 cm^{-1}, 1639 cm^{-1}, 1569cm^{-1} (C-3) related to metformin HCl. The bands confirm the presence of metformin HCl in C-2 and C-3 formulations (figs. 8 and 9).

CONCLUSION

In this study, we prepared and characterised metformin HCl-loaded CS-PLGA nanoparticles. The mean particle size ranged from 506.67 to 516.33 nm and the nanoparticle surface was positively charged (22.57 to 32.37 mV) due to chitosan. The encapsulation efficiencies for both formulation (C-2 and C-3) were low because of the leakage of metformin HCl to the external medium,

resulting in decreased drug content in the nanoparticles. Positively-charged chitosan can be used to increase the bioavailability of metformin HCl. This study will be useful for the future studies aiming at the development of metformin HCl-loaded nanoparticles with the high encapsulation efficiency and investigating in vivo efficiency of the NPs.

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