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Original Article

A PHYSIOCHEMICAL STUDY ON DRUG DELIVERY OF METFORMIN HCL-LOADED CS-PLGA NANOPARTICLES

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ABSTRACT

Objective: The current research was based on developing CS-PLGA nanoparticles (NPs) drug delivery system (DDS) for improving the bio-availability of metformin HCl, an anti-diabetic drug.

Methods: Nanoprecipitation method was utilized to prepare the metformin HCl-loaded CS-PLGA NPs DDS. The metformin HCl-loaded NPs were validated using an analytical method and characterization of NPs was also done. These polymers release the drug in a controlled manner.

Results: The correlation coefficient (R^2) value for the metformin HCl calibration curve was 0.9971 in phosphate buffer pH 6.8 at a concentration range of 0-12 µg/ml. Metformin HCl-loaded NPs release the drug at 144 h, approximately 90%. DSC tests were carried out for 50 mg and 75 mg of MET HCl incorporated NPs and FT-IR for 50 mg of MET HCl incorporated NPs, it was clear from the FT-IR and DSC spectra that there were no interactions between the metformin HCl and the polymer.

Conclusion: It was proven that metformin HCl-loaded NPs act as a prominent DDS by exhibiting extensive drug release and an increase in its bioavailability.

Keywords: Nanoparticles, Anti-diabetic agent, Type 2 diabetes, Drug delivery system, Bioavailability

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INTRODUCTION

The type 2 diabetes (T2D) is prevalent in diabetes patients worldwide and is also known as noninsulin-dependent [1]. It is a metabolic condition defined by abnormalities and anomalies that affect many major organs. It deals with insulin sensitivity in muscles, liver, and adipose and leads to a steady loss in β-cell performance as a consequence of the body's inefficient utilization of insulin. The T2D prevalence is still rising due to excessive weight gain, inactivity, and unhealthy eating habits [1].

Metformin is the widely prescribed pharmacological first-line medication for T2D, either solo or in conjunction with insulin or some glucose-lowering treatments. It is utilized to manage hyperglycemia in people with T2D and it enhances glycaemic regulation without causing hypoglycemia or weight accumulation [2]. Metformin HCl is indeed a biguanide compound that is commonly utilized to treat T2D and is recommended to about 120 million patients globally [2]. Certain investigations suggested that metformin may also be utilized to address polycystic ovary syndrome (PCOS) [3] and cancers, particularly breast and colon cancers [4]. Even though metformin was utilized to cure T2D as an antihyperglycemic medication since 1950s, its exact action mechanism is still unknown. But notable research has shown that metformin can effectively treat metabolic distinctions between healthy and unhealthy metabolic paths [5]. Metformin's ability to decrease blood sugar levels is greatly aided by the decrease in hepatic glucose production, plasma-fasting insulin levels and also insulin opposition [5].

Metformin has a comparatively minimal bioavailability after oral delivery and is mainly absorbed by the upper small intestine. Metformin holds 50% to 60 % absolute bioavailability. The loss of dosage proportionality with rising dose levels is caused by diminished absorption instead of a change in exclusion. Utilizing feasible drug delivery systems (DDS), like bio-adhesive and gastro-retentive DDS [6], helps to enhance its inadequate absorption [7]. DDS (microparticles, nanoparticles (NPs), etc.) are extremely helpful frameworks to get around the issues with traditional dosage types. In comparison to conventional dosage types, these frameworks offer several benefits. It leads to the efficient defence of medications against deterioration. It results in a decline of adverse drug events and repetitive dosage. It is also convenient for the patient. It also leads to an improvement in the drug's relative bioavailability [8, 9].

Many different DDS has been explored utilizing NPs. They benefit from a lot of things like:

- Strong stabilization in lyophilized or suitable preparation
- Practical aspects for the integration of both hydrophilic and hydrophobic active ingredients
- Large carrier potential of several therapeutic agents
- Practicability for a variety of administration pathways [9].

Metformin HCl-loaded NPs show minimal encapsulation efficiency and drug release characteristics from NP compositions were remarkably reproducible [10].

Research Objectives of the study were

1. To design and analyze the pharmacological activity of metformin HCl-loaded CS-PLGA NPs DDS.

2. Developing CS-PLGA nanoparticles (NPs) drug delivery system (DDS) for improving the bio-availability of metformin HCl, an anti-diabetic drug.

The rationality of the research is to find efficient management for diabetics, especially T2D, which is a more prevalent disease in our fast-moving world. The novelty of this research is the study on bioavailability and drug release efficiency of DDS developed using metformin HCl incorporated CS-PLGA.

MATERIALS AND METHODS

Materials

MET HCl is gifted from Aurobindo pharma, Hyderabad India. Chitosan (CS), PLGA, acetone and polyvinyl alcohol (PVA) is obtained from Sigma Aldrich. All other ingredients used were of analytic grade and purchased from authentic suppliers.

Preparation of metformin HCl-loaded CS-PLGS NPs

In 4.0 ml of acetone, 100 mg of PLGA was dissolved. An ultrasonic probe was used to emulsify the organic phase for 7 min after it had been introduced to 6 ml of PVA (3%) comprising MET HCl (100 mg) and CS (30 mg). NPs were managed to recover by centrifugation at 15,000 rpm for 40 min after the organic part had evaporated under diminished pressure at 40 °C in a rotary evaporator. The NPs were subsequently given two washes in DH_2O. Following the last cleaning, the NPs were re-suspended in DH_2O and lyophilized for an extended period. At least triplicate batches of each NPs were generated [11]. The sample absorbance was seen at 231 nm in a UV spectrophotometer. The Metformin HCl standard calibration graph was developed by framing the concentration against the absorbance. From the plot, we get a regression equation.

Characterization on NPs

Surface morphology

A scanning electron microscope (SEM) was utilized to examine the NPs analytical image (Hitachi S-250, Japan). The NPs were put on aluminium stubs, vacuum-coated with gold and examined under an SEM [11].

Particle size and zeta (Z)-potential of NPs

Zetasizer 3000HS (Malvern apparatus, UK) was utilized to determine the grain size and Z-potential of the prepared NPs. At 25 °C, the measurements of NPs were done following a dilute suspension of NPs in DH_20 [11].

Drug content

10 mg lyophilized NPs were vortexed for 2 min in 5 ml acetone before being stirred at 750 rpm on a magnetic stirrer for 30 min. 10 ml phosphate buffer saline (PBS) (pH-6.8) was introduced to this mix and then combined at 750 rpm for an additional 30 min to obtain the metformin HCl. The residual aqueous distribution was centrifuged for 15 min at 10,000 rpm after the organic substance evaporation. Then each sample's drug content was assessed utilizing a verified UV technique at 232 nm. A calibration graph built within the level of 0-12 µg/ml was utilized to measure the quantity of metformin HCl connected with NPs [11]. Following were the formulas utilized to determine each formulation's drug loading (DL %) and encapsulation efficiency (EE %) [11].

$$DL\% = \frac{\text{drug weight in NPs}}{\text{weightof the NPs}} \times 100 \ldots\ldots (1)$$

$$EE\% = \frac{\text{drug weight in NPs}}{\text{theoretical drug weight}} \times 100 \ldots\ldots (2)$$

Drug release analyses

During the *in vitro* release research, an incubation mechanism was employed. 20 mg NPs was mixed in 4 ml phosphate buffer pH 6.8 and situated in vials. The vials were shaken at 37 °C in a water bath and agitated at 50 rpm. Then 1 ml of the sample was removed and 1 ml of brand-new buffer was introduced at fixed time intervals. At 10,000 rpm the samples were centrifuged for 10 min and UV methodology was utilised to determine the substances' drug content [11].

Differential scanning calorimetry (DSC)

To find out how metformin was distributed in the NPs matrix, DSC analyses were conducted. A DZ3335 differential scanning calorimeter (Jiangsu, China) was utilized to capture the thermal behaviours of the 10 mg of samples that have been mounted into the ceramic pans. At a scanning rate of 7 °C/min, the scanning range covered were between 25 °C-320 °C under a nitrogen environment [11].

Fourier transform infrared (FT-IR) spectroscopy

Fourier transform infrared (FTIR) analysis was done by FT-IR instrument (Spectrum one, PerkinElmer, USA) over the range of wave number 4000-400 cm^{-1}.

RESULTS AND DISCUSSION

Analytical technique and validation

To estimate the amount of the drug, an analytical technique (UV method) was created and validated.

MET HCl calibration curve

A calibration graph for MET HCl was done utilizing UV-spectrophotometry and detecting the absorbance at 231 nm. Fig. 1 shows the calibration curve for metformin HCl. The absorbance was measured at a concentration range of 0-12 µg/ml. The calculations of *in vitro* drug release and drug content were based on respective calibration curves. The curves follow Beer's Lambert's principle within concentration ranges of 0-12 µg/ml. The correlation coefficient (R^2) value for the metformin HCl calibration curve was 0.9971 in PBS pH 6.8.

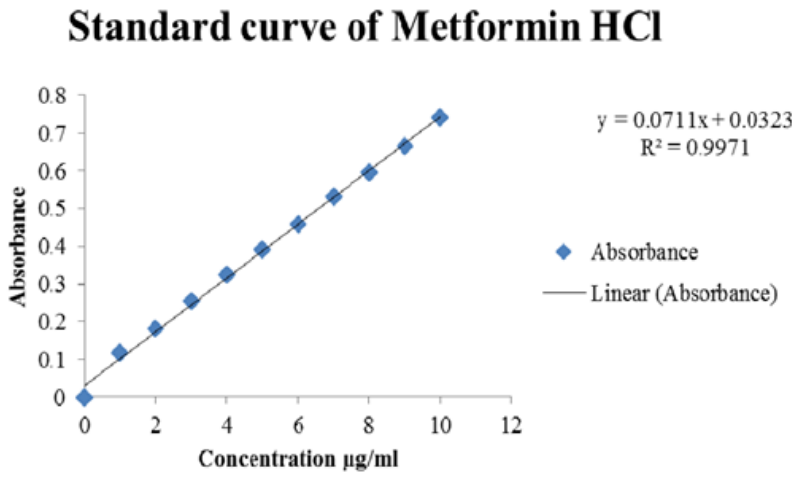

Fig. 1: Calibration curve for metformin HCl

Precession and accuracy

Utilizing the addition of 150, 75 and 15 µg/ml, the assay technique's accuracy and precision were assessed with six-replicated experiments for inter and intra-day variants. Comparing intra-day and inter-day mean±SD was observed higher in intra-day with mean±SD of (15.82±1.01, 75.86±3.11, and 151.40±6.38). Precision was 4.09 to 6.38%, and intraday accuracy falls from 5.46 to 0.93 %. The precision falls from 2.63 to 8.60 % and the inter-day accuracy was between-1.56 and 10 % (table 1). As per the outcomes, the accuracy and precision assay were satisfied.

Table 1: Accuracy and precision of metformin HCl

	Concentration addition (µg/ml)	Observed (mean±SD); (n=6)	[1]Accuracy (%)	[2]Precision (%)
[3]Intra-day	15	15.82±1.01	5.46	6.38
	75	75.86±3.11	1.14	4.09
	150	151.40±6.38	0.93	4.21
[4]Inter-day	15	16.50±1.42	10	8.60
	75	73.90±3.01	-1.46	4.07
	150	147.65±3.89	-1.56	2.63

[1]Accuracy was represented as percentage of relative error, [2]Precision was represented as percentage of standard deviation, [3]Intra-day data was calculated from six-replicated experiments (each concentration), [4]Inter-day data was calculated from six-replicated experiments (each concentration/day).

Characterisation of NPs

Surface morphology

Crystals with an orthorhombic morphology can be seen in the raw metformin HCl SEM image fig. 2(a). The raw PLGA in fig. 2(b) exhibits that the particles are spherical and sporadic ellipsoidal forms are most probably the result of the PLGA's temperature rise and selective melting during SEM. The SEM image of CS NPs exhibits a uniform size and spherical forms of NPs in fig. 2(c). CS-PLGA NPs were seen in SEM images to be roughly spherical in morphology [10].

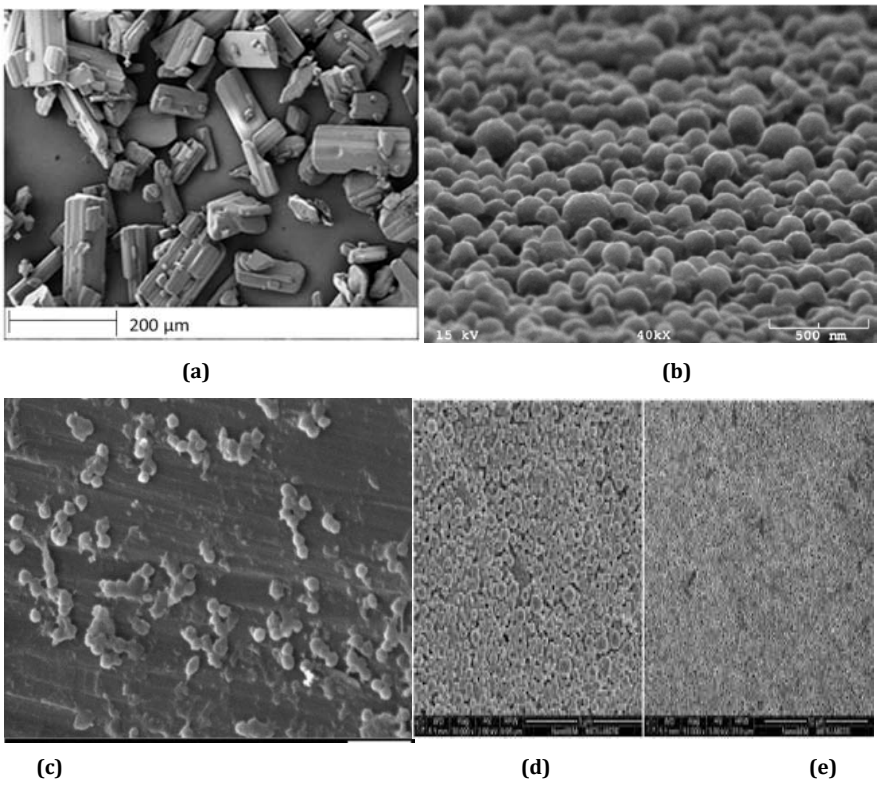

Fig. 2: (a) Raw metformin HCl, (b) Raw PLGA NPs, (c) Raw CS, (d) 50 mg metformin HCl loaded NPs, (e) 75 mg Metformin HCl loaded NPs

Particle size and Z-potential NPs

Table 2 displays the findings of particulate size analyses of prepared metformin HCl-loaded NPs. The particle size of polymeric DDSs is affected by a variety of factors, including the kind of stabilizing agent, the concentration of the stabilising agent, the organic solvent type, the drug-polymer proportion, etc. The mean grain sizes of NPs holding MET HCl in our research were comparable (p>0.05). Since CS carries positive charges, freshly made NPs had a positive Z potential (mean±SD) (22.57±1.21-32.37±0.57 mV). The Z potential readings of all preparations were similarly high after four-replicated experiments (table 2). Prior reports of the equivalent outcome were reported [12]. Alginate-coated PLGA NP and CS-incorporated PLGA NPs were created, and it was stated that both of these surface charges were positive (18.8 mV, 0.2% w/v CS content). Furthermore, they noted that as CS concentration was enhanced, the positive surface charge of DDS was also enhanced.

Table 2: A) Blank NPs, B) 50 mg MET HCl incorporated NPs, and C) 75 mg MET HCl incorporated NPs

Formulate	Particulate size (mean±SD); (n=4)	Z-potential (mean±SD) (mV); (n=4)	EE % (mean±SD); (n=4)	DL % (mean±SD); (n=4)
[1]A	514.63±5.73	25.6±0.6	-	-
[2]B	506.67±13.61	23.70±0.80	4.311±1.101	0.799±0.444
[3]C	516.33±16.85	22.57±1.21	4.480±0.559	1.318±0.165

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

Drug content

Table 2 summarises the effectiveness of DL and EE of metformin HCl NPs. In particle frameworks, drug content is impacted by several variables. The molecular mass, type of polymer, utilized phases' viscosity and drug-polymer proportion is crucial factors in drug loading. Because of drug leakage into the exterior aqueous phase, metformin HCl was only partially encapsulated in PLGA NPs. Minimal EE is the consequence of certain drugs inadvertently diffusing into the aqueous environment during the establishment of NPs, which take place at the interface among two phases. The tendency of DL differed from that of EE due to the alteration in CS's adjustment quantity. CS was modified, which enhanced the effectiveness of DL but boosted the overall mass of NPs. The DL value consequently dropped. Ultimately, the CS alteration had a favourable outcome.

Drug release

Fig. 3 displays metformin HCl NPs release. It displays the outcomes of drug form NPs formulation *in vitro* release. The profile revealed a burst release at first and a gradual release later. Drugs that have been adsorbed on the surface of NPs may be the cause of the quick release, and approximately 20% of the MET HCl was released within 30 min. At 144 h, approximately 90% or thereabouts of the incorporated MET HCl were discharged. The metformin discharge pathway from the NPs was recommended by the *in vitro* characteristics from the metformin HCl-packed NPs by the governed dispersion of the drug release, which follows the path from the interior of the particulates to its exterior.

DSC analysis

Fig. 4 implies the DSC thermograms of a) pure PLGA, b) CS, c) pure dug and d) drug-loaded NPs. The glass transition (Tg) of PLGA shows around 50 °C. Exothermic events start to happen between 280–380 °C, as shown by a conventional heating run of a crystalline sample. CS shows water entrapment at approximately 115 °C and a wide endothermic peak at 300 °C. Prior reports of the equivalent outcome were reported in [13], where the DSC and the CS show endothermic peak and exothermic peak justifying our result approximately at 310 °C and 117 °C. The water loss in PLGA CS sample 3 shifted to a higher temperature exhibiting multiple peaks from 120 °C to 150 °C. The raw MET HCL DSC graph displays a distinct endothermic peak at 224.63 °C, which is indicative of the medication's melting point. The crystallinity of the drug is also indicated by the sharp endothermic peak. The drug degrades most quickly above 300 °C. The DSC of the NPs preparations (C-2 and C3) revealed polymer-related peaks at about 50 °C and about 300 °C. C-2 and C-3 represent 50 mg and 75 mg of metformin HCl-loaded NPs respectively. Nevertheless, the thermogram of the drug-loaded NPs did not show the endothermic peak distinguishing feature of metformin HCl, indicating that the drug had not yet formed a molecular dispersion in the NPs.

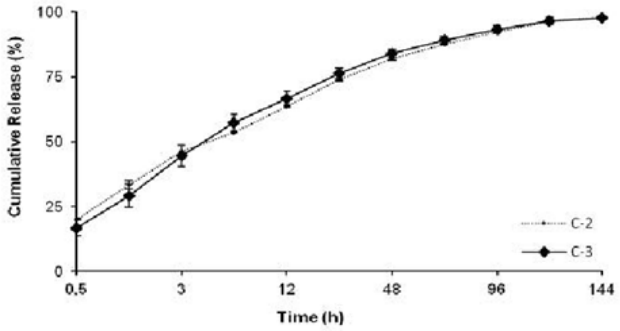

Fig. 3: Metformin HCl NPs drug release (mean±SD); (n=4)

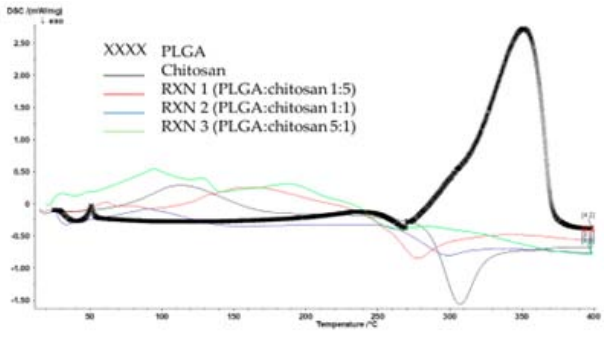

Fig. 4(a): DSC-pure PLGA, CS, and PLGA CS samples

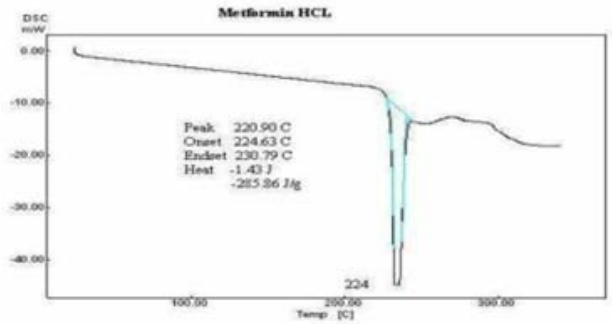

Fig. 4(b): DCS-pure metformin HCl

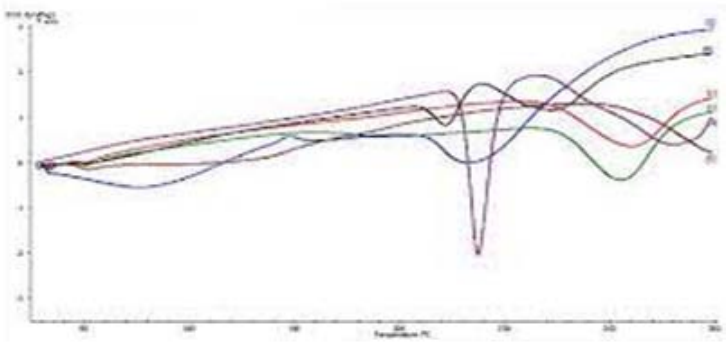

Fig. 4(c): DSC-metformin HCL loaded CS-PLGA

FT-IR studies

Fig. 5 implies the FTIR spectrum of (a) raw polymers, pure MET HCl, and drug-NPs incorporated compound. FT-IR spectrum of pure PLGA polymer is shown in fig. 5(a). At 1749 cm^{-1} (C=O, ester), the most noticeable peak for the presence of PLGA appears. The PLGA shows the broad characteristics peaks at 1164 C-O-, 1090 cm^{-1} cyclic ether, 1130 cm^{-1} CH_3; 1390 cm^{-1}, 745 cm^{-1} CH peak, and the 3020 cm^{-1}peaks, implied to CH_2 in glycolic acid part, and 2930 $cm^{-1}$for CH_3 in the lactic acid part. The spectrum of pure chitosan observed a broad peak at 1648 cm^{-1} (C=O amide group), 1588 cm^{-1} (NH+CN) of the N-acetyl, 1151 cm^{-1} (bridging-O-stretch), 1063 cm^{-1} (C-O-C stretching). The presence of NH_2 is also concealed by the band at 1585 cm^{-1}, which overlaps the amide II band, these results correspond to the prior studies in the reference database [14]. The PLGA-CS spectra show a shift in the amid bonds at 1588 and 1648 cm^{-1}, the novel peaks didn't correlate to the structure of any additional amid bonds. Rather, they resembled the establishment of a CS salt [15], as evidenced by bands farther downfield at 1627 and 1517 cm^{-1}.

The FT-IR spectrum of raw MET HCl shown in fig. 5(b) exhibits N-H stretching at 3371.41 cm^{-1}, C=N stretching at 1626.15 cm^{-1}, and 1580.41 cm^{-1}. Also exhibits 1567.84 cm^{-1} corresponding to N-H bending in the plane, 421.02, 543.21, and 584.15 cm^{-1} correspond to (C-N-C) bending and NH2 rocking at 636.27 cm^{-1}.

The FT-IR spectra of 50 mg metformin HCl loaded NPs shown in fig. 5(c) exhibits characteristic peaks 1563 cm^{-1}, 1655 cm^{-1}, 3288 cm^{-1}, relating to metformin HCl. So, fig. 5(c), confirms the presence of metformin HCl.

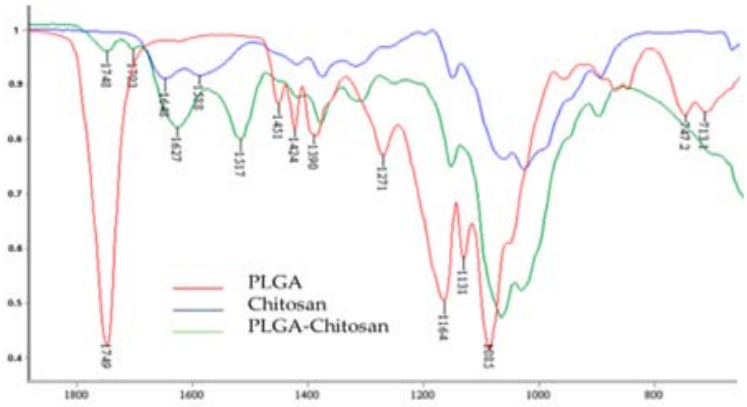

Fig. 5(a): FT-IR of pure PLGA, CS, and PLGA-CS

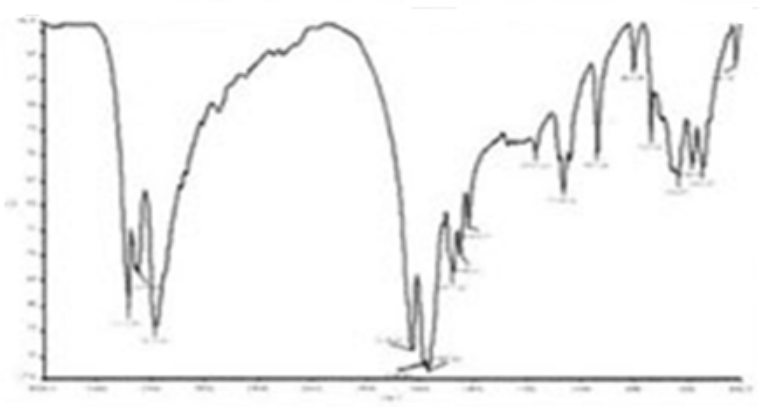

Fig. 5(b): FTIR of pure metformin HCl

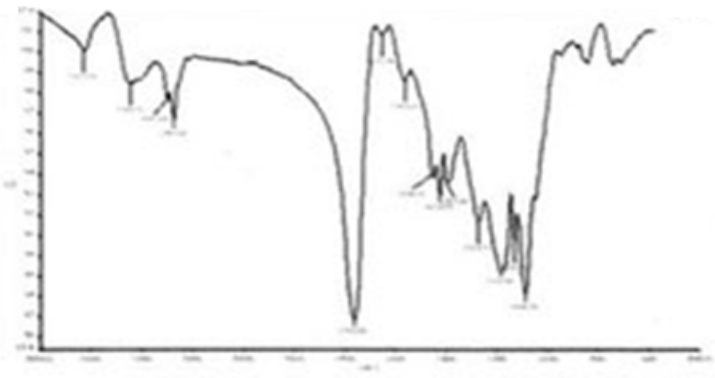

Fig. 5(c): FTIR of 50 mg metformin HCl loaded NPs

CONCLUSION

The antidiabetic drug metformin is the most frequently medicated one for T2D. CS and PLGA are utilized as polymeric DDS in this study due to their non-toxic, biocompatible and biodegradable nature. Hence in this research, the prepared metformin HCl-loaded NPs exhibited max particle size at 516 nm and surface NPs were positively charged due to CS. The effectiveness of processing metformin HCl NPs quick drug release formulation with improved solubility, bioavailability and dissolution rate was amply proven in the current investigation. New polymer-surfactant combinations were optimized and a successful stable system was created. It is anticipated that the nano-sized metformin HCl may have better bioavailability based on research on the drug and similar medications. This proves that DDSs are indeed very helpful mechanisms to get around the problems with traditional dosage aspects. Thus, developing DDSs (NPs) techniques for MET may be beneficial not just to increase its bio-availability but also to decrease the frequency of dosages and reduce toxic effects and gastrointestinal adverse reactions.

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DECLARATIONS

Author(s) declare that all works are original and this manuscript has not been published in any other journal.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all aspects of this work.

CONFLICT OF INTERESTS

Authors declare that they have no conflict of interest.

REFERENCES

1. Shivashankar M, Mani D. A brief overview of diabetes. Int J Pharm Pharm Sci. 2011;3(4):22-7.
2. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017 Sep;60(9):1577-85. doi: 10.1007/s00125-017-4342-z, PMID 28776086.
3. Viollet B, Guigas B, Sanz Garcia NS, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond). 2012;122(6):253-70. doi: 10.1042/CS20110386, PMID 22117616.
4. Spritzer PM, Motta AB, Sir-Petermann T, Diamanti Kandarakis E. Novel strategies in the management of polycystic ovary syndrome. Minerva Endocrinol. 2015;40(3):195-212. PMID 25781065.
5. Pernicova I, Korbonits M. Metformin-mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol. 2014;10(3):143-56. doi: 10.1038/nrendo. 2013.256, PMID 24393785.
6. N MP, S SC, SV, C PA, Ras N. Formulation and evaluation of simvastatin gastroretentive drug delivery system. Int J App Pharm 2017;9(3). doi: 10.22159/ijap.2017v9i3.18763.
7. Boldhane SP, Kuchekar BS. Gastroretentive drug delivery of metformin hydrochloride: formulation and *in vitro* evaluation using 3(2) full factorial design. Curr Drug Deliv. 2009;6(5):477-85. doi: 10.2174/156720109789941641, PMID 19863493.
8. Powar PV. Development status in the meadow of nanostructure magnetic drug delivery system and its promising applications. Int J Pharm Pharm Sci. 2017;9(12):10-7. doi: 10.22159/ijpps.2017v9i12.21544.
9. Gelperina S, Kisich K, Iseman MD, Heifets L. The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. Am J Respir Crit Care Med. 2005;172(12):1487-90. doi: 10.1164/rccm.200504-613PP, PMID 16151040.
10. Gundogdu N, Cetin M. Chitosan-poly (lactide-co-glycolide) (CS-PLGA) nanoparticles containing metformin HCl: preparation and *in vitro* evaluation. Pak J Pharm Sci. 2014;27(6):1923-9. PMID 25362616.
11. Pereira ASBF, de Souza Lima ML, da Silva-Junior AA, Dos Santos Silva E, de Araujo Junior RF, Martins AA. *In vitro-in vivo* availability of metformin hydrochloride-PLGA nanoparticles in diabetic rats in a periodontal disease experimental model. Pharm Biol. 2021;59(1):1576-84. doi: 10.1080/13880209.2021.2002369. PMID 34808068.
12. Wang Q, Jamal S, Detamore MS, Berkland C. PLGA-chitosan/PLGA-alginate nanoparticle blends as biodegradable colloidal gels for seeding human umbilical cord mesenchymal stem cells. J Biomed Mater Res A. 2011;96(3):520-7. doi: 10.1002/jbm.a.33000. PMID 21254383.
13. Mohammadzadeh S, Shahsavari S, Karimian F, Hashemi SJ, Akbari Javar H, Mollabagher H. Development and characterization of optimized sustained release voriconazole-loaded chitosan nanoparticles for ocular delivery. J Part Sci Technol. 2021;7(1):1-10.
14. Branca C, D'Angelo G, Crupi C, Khouzami K, Rifici S, Ruello G. Role of the OH and NH vibrational groups in polysaccharide-nanocomposite interactions: a FTIR-ATR study on chitosan and chitosan/clay films. Polymer. 2016;99:614-22. doi: 10.1016/j.polymer.2016.07.086.
15. Ma FK, Li J, Kong M, Liu Y, An Y, Chen XG. Preparation and hydrolytic erosion of differently structured PLGA nanoparticles with chitosan modification. Int J Biol Macromol. 2013;54:174-9. doi: 10.1016/j.ijbiomac.2012.12.019, PMID 23262384.