Original Article

Preparation And In Vitro Characterisation Of Metformin Loaded Polymeric Nanoparticles For Gastroretention

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Abstract

The objective of current study was to formulate and assess metformin hydrochloride (MET) nanoparticles (NPs) utilising various hydrophilic polymers. To formulate nanoparticles without the side effects on the body of surfactants and chlorinated solvents, the nano precipitation approach was employed. MET-NPs were synthesised by hydrophilic polymers, including gelatin, chitosan, and HPMC K100M. Particle size (PS), polydispersity index (PDI), zeta potential (ZP), loading capacity (LC), encapsulation efficiency (EE), and drug excipient compatibility were then assessed for the prepared formulations. Satisfactory results were obtained from the preparation of a nanoparticulate formulation using chitosan in a 1:1 ratio. The average PS was 321.14 ± 11.20 nm, the PDI was 0.432 ± 0.04, the ZP was 29.4±3.14 mV, the LC was 19.11 ± 0.43 %, and the EE was 78.9 ± 1.22 %. The drug and the polymers utilised in this investigation did not interact significantly, according to the results of the FTIR examination.

Keywords: Polymers, Drug release, Nano precipitation method, Nanoparticles, Drug formulation, *in vitro* study, gastroretentive

INTRODUCTION

Because oral drug administration offers significant therapeutic benefits such patient compliance, convenience of administration, and formulation flexibility, it is the most practical and widely used mode of drug delivery [1]. However, there are a number of physiological issues with this approach, including the inability to manage and locate the controlled drug delivery system inside the intended gastrointestinal tract region because of the erratic motility and gastric emptying.

Moreover, the relatively short human gastric emptying time—typically two to three hours—through the major absorption zone, which includes the stomach and upper portion of the intestine, can cause incomplete drug release from the drug delivery system, which lowers the effectiveness of the doses that are administered [2]. Due to these challenges, scientists have designed a drug delivery method that can remain in the stomach for an extended and consistent amount of time. Many efforts are being made to formulate a controlled drug delivery system that, by administering the drug in a regulated and repeatable way, can provide therapeutically effective plasma drug concentration for an extended length of time, decreasing the frequency of doses and minimising variations in plasma drug concentration at steady-state [3].

A variety of techniques, including intra-gastric floating systems, hydro dynamically balanced systems, expandable or extendable systems, microporous compartment systems, microballons, bio-adhesive systems, high-density systems, and super porous biodegradable hydro gel systems, have been documented in the literature to improve the gastric retention of drugs [4]. Such a dosage form would be kept in the stomach for a number of hours following oral administration, releasing the drug there in a controlled and prolonged manner.

This would allow the drug to be continuously supplied to the upper gastrointestinal tract's absorption sites. Extended stomach retention increases the solubility of drugs that are less soluble in high pH environments, decreases drug waste, and increases bioavailability [5]. MET was chosen as an appropriate drug for gastroretentive nanoparticles because of its short half-life, low bioavailability, frequent dosing schedule, and limited window of absorption in the upper region of the GIT and stomach.

One of the main challenges in developing new pharmacological formulations to increase the bioavailability of MET, an antidiabetic drug, is its limited aqueous solubility. MET poor water solubility has been addressed by several strategies, including chemical and physical alterations to the drug. The stomach will benefit from these mucoadhesive polymeric nanoparticles in a number of ways, including (i) a longer residence period for the dosage on mucosalt tissues. This will raise the drug's bioavailability and enhance its absorption. (ii) Increased drug concentration at the adhesion absorption site, which will act as a catalyst for the passive uptake by paracellular cells. (iii) Rapid absorption via the bioadhesive drug delivery method, avoiding dilution beforehand and potential degradation in the luminal fluids.

MATERIALS AND METHODS

Materials

Metformin hydrochloride (MET) was a gift sample obtained from SRL Labs, Maharashtra, India. Hydroxyl propyl methyl cellulose K100M and Gelatin were purchased from SD Fine Chem Limited, Mumbai, India. Chitosan was obtained from Loba Chemie Pvt Ltd., Mumbai, India. Dimethyl sulfoxide was purchased from Merck, Germany. Used chemicals and reagents were of analytical grade.

Preparation of MET-Nanoparticles (MET-NPs)

With a small adjustment, nanoparticles were made using the nano precipitation technique. In brief, each of the three polymers (HPMC, chitosan, and gelatin) was dissolved in 25 mL of acetone. A solution of 100 mg of MET was prepared using 2 mL of dimethyl sulfoxide. After the two solutions were combined, 50 mL of water were added, and the mixture was agitated for 30 min. The rotary flash evaporator was used to evaporate the acetone under low pressure, and 10 mL was the final volume of the suspension. After that, this suspension was centrifuged for 30 min at 4 ºC and 15000 rpm [6]. After discarding the supernatant, the precipitate underwent three rounds of distilled water washings. The resulting nanoparticles were then preserved in a desiccator after being dried for the entire night at 60 ºC in an oven.

Drug-Excipient Compatibility Studies

The drug excipient compatibility investigations were conducted with a Perkin Elmer FT-IR spectrophotometer. The drug, polymer, and formulation FT-IR spectra (4000 to 400 cm^{-1}) were examined independently before being correlated to check for incompatibility [7].

Evaluation of MET-NPs

Loading capacity (LC %)

By using 0.1 M hydrochloric acid to remove the drug from the nanoparticles, the drug content of MET-NPs was ascertained. This approach involved stirring 50 mg of nanoparticles in 50 mL of 0.1 M hydrochloric acid until the particles were dissolved. The mixture was then filtered through a Millipore filter, and UV spectrophotometry was used to assess the drug content after the mixture had been diluted appropriately to a wavelength of 234 nm [8]. Utilising Equation 1, the LC of the MET-NPs was determined.

$$LC\ (\%) = (Qn / Wn) \times 100 \quad (1)$$

Where, Qn is the amount of drug contained in the nanoparticles and Wn is their weight.

Entrapment Efficiency (EE %)

The amount of drug in the clear supernatant following centrifugation was measured (w) using a UV spectrophotometer set to 234 nm to determine the level of drug entrapment. For this reason, a typical drug calibration curve was produced [8]. The total amount of drug added during the preparation (W) was then deducted from the amount of drug in the supernatant. In practical terms, (W-w) will provide the quantity of drug entrapped in the particles. Then, using Equation 2, the percentage of a drug that was entrapped was determined.

$$EE\ \% = (W-w/W) \times 100 \quad (2)$$

Particle Size (PS), Particle Size Distribution (SD), and Zeta Potential (ZP)

Using a zeta master (Malvern Instruments, UK) outfitted with the Malvern PCS software, photo correlation spectroscopy was used to ascertain the formulation's PS and SD. Distilled water was used to dilute each sample [9]. Using a Malvern zeta sizer (Malvern Instruments, UK), the electrophoretic mobility of the nanoparticles was measured in order to calculate the surface charge, or ZP. Distilled water was used to prepare the samples.

Polydispersity Index (PDI)

A measure used to characterise the SD of nanoparticles derived from photon correlation spectroscopic analysis is the PDI [9]. It is a dimensionless number that can be as high as 0.5–0.7 and starts at 0.01 for monodispersed particles, derived from the autocorrelation function. Samples possessing a very wide size distribution have PDI values greater than 0.7.

RESULTS AND DISCUSSION

In order to prevent surfactants and chlorinated solvents from having a harmful impact on the body, the nanoprecipitation approach was utilised instead. Every determination was made three times.

FTIR

The infrared data makes it evident that the drug's functions, including peak intensities, have not changed (Figure 1). This implies that the polymer has not reacted with the drug throughout the formulation process to produce reactive products. As a result, the mixture is purely physical and does not interact, which supports moving forward with formulation [10].

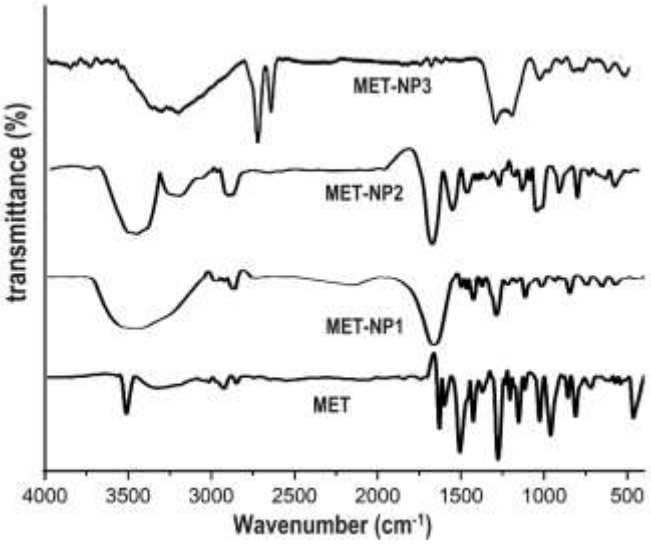

Figure 1. FT-IR spectrum of pure MET and various MET-NPs

LC (%) and EE (%)

While drug loading indicates the percentage weight of the active ingredient encapsulated to the weight of the nanoparticles, EE is the ratio of the percentage of drug content determined experimentally to the actual or theoretical mass of the drug used to prepare the nanoparticles. The combination of polymer and drug as well as the technique employed determine the LC. bigger amounts of hydrophobic pharmaceuticals are encapsulated by hydrophobic polymers, while bigger amounts of hydrophilic drugs are entrapped by hydrophilic polymers [11].
The amount of drug loading will depend on a number of formulation characteristics, including the kind of emulsifier, the weight ratio of polymer to drug, and the ratio of organic to aqueous phase. Drug loading and EE as a function of polymer are shown in Table 1 and illustrated in Figure 2.

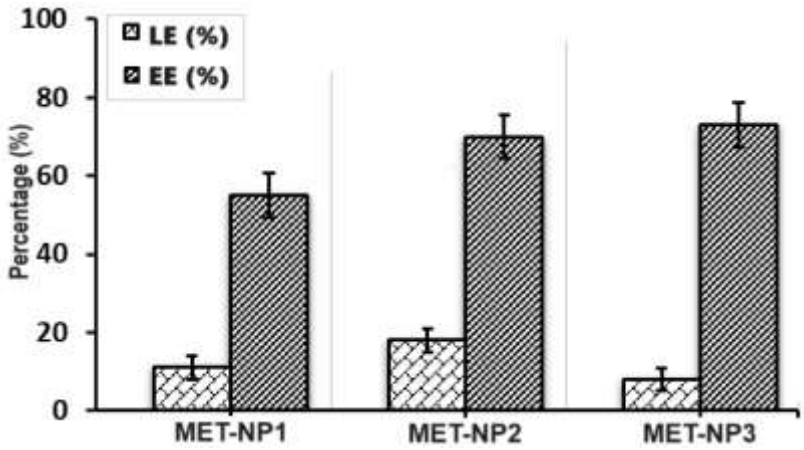

Figure 2. Effect of various polymers percentage on LE and EE

The ranges of the data were 9.22 ± 0.25%–19.11 ± 0.43% and 57.15 ± 2.24%–74.2%, in that order. Gelatin and HPMC nanoparticles had modest loading capacities (9.22 ± 0.25% and 12.11 ± 0.31%, respectively), but chitosan nanoparticles had high loading capacities (19.11 ± 0.43%). It was discovered that the formulations including gelatin and chitosan had high entrapment efficiencies (78.9 ± 1.22 % and 74.2%, respectively), while the formulation incorporating bovine serum albumin had poor entrapment efficiencies (57.15 ± 2.24%). Less EE may be caused by MET's hydrophilic nature; however, LC may be enhanced by raising the polymer ratio, ensuring that there is enough polymer available to entrap the drug present in the solution.

Table 1. LC (%) and EE (%) of prepared nano formulations

S.No.	Formulation code	LC (%)	EE (%)
1	MET-NP1	12.11 ± 0.31	57.15 ± 2.24
2	MET-NP2	19.11 ± 0.43	78.9 ± 1.22
3	MET-NP3	9.22 ± 0.25	74.3 ± 1.31

SD and PDI

PS and distribution are important determinants of nanoparticle performance, since batches with a broad spectrum of PS exhibit notable differences in drug loading, drug release, bioavailability, and efficacy. Transmission or scanning electron microscopy, as well as light scattering techniques, can be used to determine the size and distribution of particles. If emulsion with a narrow droplet size distribution cannot be formulated, then formulating nanoparticles with a narrow size distribution will be difficult [13]. An increase in PS will counteract the uptake of the drug and may have an impact on its bioavailability because nanoparticles are internalised into cells through the process of endocytosis. The kind of the target cell determines how much endocytosis occurs.

The outcomes of MET produced nanoparticulate formulations with various polymers are displayed in Figure 3 and Table 2. The PDI of the formulations was quite high, ranging from 0.432 ± 0.04 to 1.05 ±0.14. The data on SD makes it clear that, for HPMC nanoparticles, the mean particle diameter was 248.46 ± 8.99 nm, with the majority of the particles falling between 200 and 400 nm. Similarly, for chitosan nanoparticles, the mean particle diameter was 321.14 ± 11.20 nm, with the majority of the particles falling between 200 and 525 nm. The average diameter of the gelatin nanoparticles was 736.17 ± 21.90 nm, with the majority of the particles falling between 480 and 1200 nm in size.

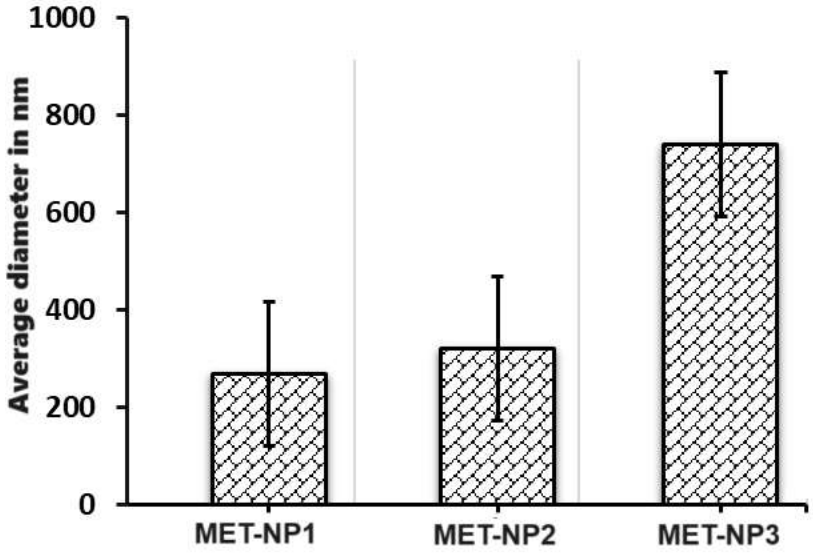

Figure 3. Effect of various polymers on average PS.

All of the formulations did, however, include a small minority of nanoparticles in a significantly smaller range. For HPMC, roughly 11% of the particles fell between 15 and 30 nm, for chitosan, roughly 7.1% of the particles fell between 48 and 90 nm, and for gelatin, 14.1% of the particles fell between 70 and 160 nm. The higher overall polydispersity indices of the formulations are caused by these minority populations [14].

Table 2. Average PS, SD, PDI and ZP of prepared nano formulations

Formulation code	Polymers	Mean PS (nm)	SD	PDI ± SD	ZP (mV) ± SD
MET-NP1	Ethyl cellulose	248.46 ± 8.99	11 ± 0.54 % (15-30 nm) 89± 0.89 % (200-400 nm)	1.05 ±0.14	20.4 ± 1.88
MET-NP2	Chitosan	321.14 ± 11.20	8 % (48-90 nm) 92 % (200-525 nm)	0.68 ±0.15	29.4±3.14
MET-NP3	Gelatin	736.17 ± 21.90	15.5% (70-160 nm) 84.5% (480-1200 nm)	0.77±0.14	13.7±2.4

In order to boost the yield of the particles in the smaller range and produce much smaller nanoparticles with a higher degree of monodispersity, we are currently investigating the process variables affecting the relative amounts of various populations. Such nanoparticles can be readily isolated from the broader population using straightforward techniques like filtration.

The aforementioned data makes it evident that the mean nanoparticulate diameter and granulometric distribution of the nanoparticles made with chitosan and HPMC were reduced. However, the nanoparticle population of big particles was produced when gelatin was used as a polymer in the preparation of the nanoparticles. The lack of an emulsifier may be the cause of the higher PS and PDI because it reduces the surface tension between the aqueous phase and the organic phase, acetone, and causes smaller solvent droplets to form, which in turn results in smaller PS [15]. According to earlier studies, it also stabilises freshly formed surfaces and stops the particles from aggregating. Thus, the study's results could be enhanced by increasing the drug:polymer ratio, utilising alternative formulation strategies like desolvation (for gelatin and albumin) or counter-ion-induced aggregation (for chitosan and sodium alginate), using a cross-linking agent and then neutralising the remaining cross-linking agent with cysteine, and vigorously stirring.

ZP

Predictions on the storage stability of colloidal dispersions can be made through the measurement of the ZP. Due to electrostatic repulsion, charged particles (those with a high ZP) are generally less prone to aggregate. ZP levels (positive or negative) greater than 30 mV typically result in more stable nanocapsule suspensions because the particles' repulsion keeps them from aggregating. ZP, or electrostatic repulsion, was thought to have decreased and was the reason behind the aggregation process. The distribution of the nanospheres throughout the body and the degree of cell absorption will be influenced by their surface charge. Positively charged nanoparticles have a higher electrostatic affinity due to the negatively charged cell membrane [15]. Consequently, to increase effectiveness, the surface of cationic or neutral nanoparticles can be changed to impart a positive charge. The colloidal suspension may not be stable and may cause aggregation, according to the ZP values, which ranged from

13.7±2.4 to +20.4 ± 1.88 mV. Zeta power can be changed by adjusting the principal constituents, which include the polymer, surfactants, and surface composition of the nanoparticles; also, the presence or absence of adsorbed compounds, the dispersing phase's composition, primarily the ionic strength and pH, can all be changed.

CONCLUSION

Formulation NP 2, one of the various nanoparticulate formulations made using the nanoprecipitation method, demonstrated satisfactory results with a mean particle size of 321.14 ± 11.20 nm (the majority of the particles fell within the 200–525 nm range), a polydispersity index of 0.432 ± 0.04, a zeta potential of 29.4±3.14, a loading capacity of 19.11 ± 0.43 %, and an EE of 78.9 ± 1.22%. The drug and the polymers utilised in this investigation did not interact significantly, according to the results of the FTIR analysis.

Declarations

Acknowledgments

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Ethical Approval

Not Applicable in this section.

Competing Interest

Author declares that there is no potential conflict of interest in this paper

Author Contribution

All the authors are equally contributed and approved the manuscript.

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