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Research Article

# Influence of Poloxamer 188 on Design and Development of Second Generation PLGA Nanocrystals of Metformin Hydrochloride

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## Abstract

The poly(D,L-lactide-co-glycolide) (PLGA) based second-generation nanocrystals prepared by modified nanoprecipitation method, is the method of choice for encapsulation of both lipophilic and hydrophilic drugs. In this study, nanoprecipitation technique was adopted to develop second generation nanocrystals of PLGA loaded with metformin HCl (MHc). Poloxamer 188 with three different concentrations (0.5, 0.75, 1% w/v) in combination with PLGA at 1, 2, 3% concentrations (w/v) successfully produced MHc loaded PLGA second generation nanocrystals. The effects of poloxamer 188, amphiphilic triblock copolymer on carrier particle size, surface morphology, polydispersity index, zeta potential, drug entrapment efficiency and drug release of nanoformulation were investigated. The optimized formulation of second-generation nanocrystals with concentrations 0.75% w/v poloxamer 188 and 2% w/v PLGA, could produce particle size of 114.6 nm, entrapment efficiency of 63.48% and drug release 80.23% at 12 h. A blank formulation with the same composition as optimized formulation without addition of poloxamer188 compared with optimized formulation, exhibited nanoparticles of larger mean particle size of 212.9 nm with entrapment efficiency of 68.47% and 50.5% drug release at 12 h. Transmission electron microscopy (TEM) analysis of the nanoformulations revealed that poloxamer188 greatly contributed to smooth, spherical morphology of nanosize polymeric nanoparticles. Further Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) studies on nanoformulation emphasized the significance of poloxamer188 in formulation and development of optimized MHc loaded PLGA nanosuspensions of second generation nanocrystals. In conclusion, the study emphasizes that poloxamer 188 was a versatile excipient, which played a pivotal role in producing nanosize carrier with high drug release profile of MHc loaded PLGA nanosuspensions of second generation nanocrystals.

**Keywords:** Nanoprecipitation; Metformin HCl; Poloxmer 188; PLGA polymer; PLGA second-generation nanocrystals

## Introduction

Nanocrystals (NCs) of first generation are the carrier-free colloidal delivery systems of nanoscopic drug crystals with dimensions less than 1000 nm prepared by a nanosizing method. First generation of nanocrystal technology is produced by a single step either by bottom-up technologies or by top-down technologies [1]. The advancement of nanocrystals is the second-generation smarter nanocrystals which possess a specialized carrier developed with variety pre-combinative treatment strategies in producing particles with size less than 1000 nm. These second-generation nanocrystals provide faster dissolution, extra physical stability and meet needs of drug delivery challenges, which cannot be achieved via the single step techniques of first-generation nanocrystals. Second-generation smarter polymer coated or functionalized nanocrystals are utilized to enhance drug solubility and modify drug release profile, with improved bioavailability and absorption, elimination of food effects, enhanced safety, efficacy and tolerability profiles of orally administered drugs [2-4]. The drug with functionally modified polymer of second generation nanocrystals enable the greater potential applications in delayed and drug delivery system of therapeutics. Novel possibilities for second generation nanocrystal applications are expedited by the polymeric nano crystal composite system as a modified delayed drug delivery carrier for oral drug delivery [5].

Noninsulin-dependent, type 2 diabetes mellitus is a disease with high prevalence globally and has been a major concern to public health care provider for its mitigation and treatment. The contributors for this alarming increase of type 2 diabetes mellitus include aging, rapid population growth, overweight, genetic, lack of physical activity, improper diet management, stress, urbanization and sedentary lifestyle [6, 7]. Many new antidiabetic therapeutic agents have been introduced to the market for glycemic control, but the majority of them failed to achieve the goal due to their adverse effects and patient noncompliance [8-10]. There is a huge demand for design and development of formulation of antidiabetic therapeutic agent which is safe, established, with good glycemic control therapeutic profile and better patient compliance [11].

Metformin HCl (MHc) is an oral biguanidine class antihyperglycemic agent which is a first-line choice of drug for type 2 diabetes treatment [12, 13]. MHc lowers the plasma glucose in diabetic patient by different mechanisms; it reduces the absorption of glucose in GI, lowers both basal and postprandial plasma glucose which leads to decrease in hepatic glucose production, and it helps the body to utilize its own insulin more efficiently. It does not produce hypoglycemia effect in both normal subjects and diabetic patients [14, 15]. According to biopharmaceutics classification system (BCS), MHc belongs to BCS class III drug, having 50-60% bioavailability and short biological half-life (1.5-4.5 h) with per oral administration of 500 mg to 3000 mg daily, in divided doses [16-19]. MHc gets eliminated from the body through urine and faeces within 8-12 h after oral administration. Highly water soluble MHc gets absorbed mainly from the small intestine of gastrointestinal tract (GIT). It requires frequent administration of large doses to maintain the prolonged therapeutic efficacy [20]. A delayed release formulation of once daily dosing which maintains plasma MHc concentration of 8-12 h, would be an ideal situation for diabetes management. Development of MHc loaded polymeric nanoparticles for prolonged delivery system is more promising in type 2 diabetes therapy management and was selected for the current research investigation [21, 22].

Polymeric nanoparticle delivery system of biodegradable and biocompatible polymers developed based on nanotechnology have enormous potential in product development of Biopharmaceutics Classification System (BCS) class III drugs and beyond [23-26]. Polymeric nanocarrier drug delivery system is highly stable, has good drug entrapment efficiency and good feasibility in incorporation of hydrophilic, lipophilic therapeutic agents, vaccines and biological macromolecules with different routes of administration [27-29]. Novel polymeric nanocarrier drug delivery systems increase bioavailability, prolong drug release, minimize adverse effect, protect drug degradation, facilitate drug transport and improve site specificity for targeting, in comparison with other carrier based drug delivery systems. Polymeric nanocarrier drug delivery systems have been proved as a promising and worthwhile option in delivering of many therapeutic agents for chronic disorders and ailments, which are yet to be explored in the diabetic therapy management [30-32].

Many biodegradable, biocompatible and nontoxic polymers are approved by regulatory agencies for the development of polymeric nanoparticles as a drug delivery system. Among the various biodegradable, biocompatible and nontoxic polymers approved by

US FDA and European Medical Agency, PLGA (poly [D,L-lactide-co-glycolicacid]) is the most extensively and successfully studied polymer used in drug delivery and nanoformulation development [33-35]. PLGA gets metabolized by hydrolysis pathway and produces endogenous monomers, such as lactic acid and glycolic acid which are eliminated easily from the body. Biodegradation time of the commercially available PLGA polymer varies from days to months depending on its molecular weight and ratio of copolymers composition. Polymer PLGA is officially recognized by US, Japan, and Europe, as a stable and safe pharmaceutical excipient for device and formulation development for pharmaceutical use. Stability of polymeric nanocarrier drug delivery systems mainly depends on its method of preparation and formulation parameters [36-38].

Literature study of the present polymeric drug delivery systems revealed that the stabilizing agent played a vital role in characterization and stability of polymeric nanocarrier systems. These stabilizing agents were basically non-ionic surfactants which decreased the interfacial tension between hydrophilic and lipophilic phases of emulsion used in polymeric nanoformulation, influencing physical property and stability to the nanoformulation [39-42]. Poloxamer188 is a US FDA approved, triblock nonionic surfactant made of a central hydrophobic chain of poly (propylene oxide) flanked by two hydrophilic chains of poly (ethylene oxide); it was used in this study as the stabilizing agent for polymeric nanocarrier development [43]. The prime objective of the present investigation was to study the effect of poloxamer 188, a stabilizing agent, in the development of prolonged release Metformin HCl loaded PLGA second generation nanocrystals by nanoprecipitation method.

# Experimental

## Materials

Poly(D,L-lactide-co-glycolide) (PLGA; L/G:50:50, ester terminated, MW 7000-17,000) was purchased from Aldrich (Labchem Sdn Bhd, Selangor, Malaysia). Poloxamer 188 (Ph. Eur., NF grade), Metformin HCl (MHc) and acetone (EMSURE ACS, ISO, Reag. Ph. Eur) were obtained from Merck (Merck Sdn Bhd, Selangor, Malaysia). Milli Q water obtained from a Milli-Q® water purification system (Millipore Co., USA) was used for all aqueous solutions. All other chemicals of analytical grade were used in the study.

## Preparation of MHc loaded PLGA nanoformulation

Nanoprecipitation method with minor modification of multiple emulsion technique was used in preparation of MHc loaded nanoformulations. In brief, the procedure for the preparation was as follows. The inner phase was prepared by dissolving MHc of 10 mg in 1 mL of distilled water emulsified with 8 mL of acetone containing polymer PLGA (160, 240 and 360 mg) under magnetic stirring at 1500 rpm. The inner phase emulsion was thereafter poured dropwise by means of syringe fitted with needle (27G×½") positioned directly in to the outer phase containing poloxamer 188 (80, 120 and 160 mg) in 15 mL of aqueous medium which was kept under magnetic stirring at 600 rpm. The nanosuspension was collected in a glass beaker and kept overnight on a magnetic stirrer (IKA-WERKE, RT10Power) at 300 rpm in a fume hood to evaporate acetone. The nanosuspension obtained was passed through 0.20 µm filter (Minisart, Sartorius Stedim) and the subsequent filtrate of nanoformulation was evaluated for various characterization studies [44, 45].

## Characterization of MHc loaded PLGA nanoformulation

### Particle size, polydispersity index and zeta potential analysis

MHc loaded nanoformulations were evaluated for particle size (Z-average), polydispersity index and zeta potential by a Zetasizer nano series (ZEN3600 Model), Malvern Instrument, Germany. Particle sizes (Z-average) and polydispersity index of drug loaded nanoformulations were characterized by photon correlation spectroscopy technique, whereas zeta potential was estimated on the basis of electrophoretic mobility principle under an electric field. Each sample was measured at 25 °C in triplicate and an average of each parameter was expressed [2].

### Entrapment efficiency study

Entrapment efficiency of nanoformulation was determined based on the ultra-centrifugation technique. The amount of MHc entrapped within nanosuspension was determined by ultra-centrifuge of the nanosuspension at 18,000 rpm for 15 min, and the supernatant containing free MHc was detected by spectrophotometry (Lamda XLS/Perkin Elmer) at the wavelength of 233 nm. The entrapment efficiency of each MHc loaded PLGA nanoformulation was

calculated as per the following equation [46, 47].

$$\text{Entrapment efficiency (\%)} = \left(\frac{\text{Mass of drug in nanoparticles}}{\text{Mass of drug used in formulation}}\right)\times 100$$

### In-vitro drug release study

In-vitro drug release was performed by using USP dissolution apparatus II, (paddle apparatus) for nanosuspensions and plain drug to study the effect of stabilizing agent on release profile of nanoformulation. Accurately weighed nanoformulations and plain drug were dispersed in 100 mL of pH 6.8 phosphate buffer saline at temperature 37 ± 0.5 °C with 50 rpm. As per predetermined time intervals, each time 5 mL of sample was collected and centrifuged at 10,000 rpm for 15 min. For each sampling point, 5 mL of drug sample was replaced by fresh phosphate buffer saline to reservoir dissolution media for maintaining the sink condition. The amount of drug released from nanosuspensions and plain drug in the buffer solution were measured by using a UV spectrophotometer (Lamda XLS/Perkin Elmer) at wavelength 233 nm. The in-vitro drug release studies of nanosuspensions and plain drug were carried out for 12 h. The conditions required to study the drug release pattern of nanoformulations were maintained constant during in-vitro drug release experiment [48].

### Fourier transform infrared spectroscopy analysis

Drug and excipients compatibility of MHc loaded PLGA nanoformulations, was studied using Fourier transform infrared spectroscopy (FTIR) analysis (FTIR spectrophotometer, Spectrum 100 Perkin Elmer) [49]. Analysis of FTIR spectra of MHc and optimized nanoformulation obtained from nanoprecipitation method were comparatively analyzed to know the drug-excipients compatibility. FTIR spectrum analysis of both the samples and background signals were measured. Each sample was 64 times scanned and % transmissions of spectra of each sample were recorded in the region of 4000 ~ 400/cm.

### Differential scanning calorimetry analysis

To study the compatibility between MHc and excipients such as PLGA and poloxamer 188 in MHc loaded PLGA nanosuspension, differential scanning calorimetry (DSC) (Mettler Toledo, DSC1, STAR System) studies were conducted. DSC thermal behaviors of the samples were studied in temperature ranged from 50 °C to 250 °C at a heating rate of 10 °C/min, under purging nitrogen gas as blanket gas. Samples of 2 ~ 8 mg were accurately weighed and placed into a standard aluminum pan against a reference empty pan to generate the thermograms [50].

### Surface morphology characterization

The effect of stabilizing agent on surface morphology of nanoformulation was carried out by using transmission electronic microscopy (TEM). A comparative morphological characterization of optimized nanoformulations and blank formulation (without poloxamer) prepared by nanoprecipitation method was performed by TEM (LEO-Libra 120). A drop of diluted PLGA nanosuspension (10 µL) was placed on carbon coated copper electron microscopy grids and stained with 2% w/v phosphotungstic acid solution (Sigma). After 30 sec, the sample grid was air dried and then viewed under TEM [51].

## Results and Discussion

In this study, nanoprecipitation technique was adopted to develop PLGA second generation nanocrystals loaded with hydrophilic, low molecular weight drug Metformin HCl (MHc). Poloxamer 188 with three different concentrations (0.5, 0.75, 1% w/v) in combination with PLGA at 1, 2, 3% concentrations (w/v) were successfully formulated as per the design of nanoformulation summarized in Table 1. The composition of nanoformulation as per the design, comprised of nine experiments (coded NP1 to NP9) and one blank formulation (coded NP10) composed of the same as optimized formulation without addition of poloxamer 188; in total 10 numbers were formulated as shown in Table 2. The resulting nanoparticles showed average particle size, entrapment efficiency and cumulative drug release at 12 h, in the range of 144.6 ~ 245.1 nm, 63.48 ~ 76.82%, and 52.47 ~ 80.23%, respectively. The effect of poloxamer 188 on critical evaluation of physicochemical properties

**Table 1** Design of nanoformulation

| Polymer PLGA (mg) | Poloxmer-188 (mg) | | |
|---|---|---|---|
| | Low (0.5%) | Medium (0.75%) | High (1%) |
| 160 (1%) | 80 (NP1) | 120 (NP2) | 160 (NP3) |
| 240 (2%) | 80 (NP4) | 120 (NP5) | 160 (NP6) |
| 320 (3%) | 80 (NP7) | 120 (NP8) | 160 (NP9) |

Note: PLGA = Poly(D, L-lactide-co-glycolide); NP = Nanoformulation.

**Table 2** The composition of PLGA nanoformulations

| | NP1 | NP2 | NP3 | NP4 | NP5 | NP6 | NP7 | NP8 | NP9 | NP10 (Blank) |
|---|---|---|---|---|---|---|---|---|---|---|
| Inner phase | | | | | | | | | | |
| Metformin HCl (mg) | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Water (mL) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| PLGA (mg) | 160 | 160 | 160 | 240 | 240 | 240 | 360 | 360 | 360 | 240 |
| Acetone (mL) | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Outer phase | | | | | | | | | | |
| Polaxomer-188 (mg) | 80 | 120 | 160 | 80 | 120 | 160 | 80 | 120 | 160 | -- |
| Water (mL) | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |

Note: PLGA = Poly(D, L-lactide-co-glycolide); NP = Nanoformulation.

of nanoparticles revealed that optimum concentration of poloxamer 188 had significant influence on carrier particle size, surface morphology, dispersity, zeta potential, drug entrapment efficiency and drug release of nanoformulations, which are presented in Table 3.

## Particle size, polydispersity index and zeta potential analysis

The MHc loaded nanoformulations prepared by nanoprecipitation method using PLGA and poloxamer 188 were able to produce particles in nano size range of 144.6 ~ 245.1 nm with narrow size distribution. The mean particle size of MHc loaded nanoformulations decreased with the increase in poloxamer concentration across all the concentrations of PLGA. Poloxamer had significant effect on mean particle size and distributions of MHc loaded nanoformulations prepared by nanoprecipitation method. The MHc loaded nanoformulation, NP5 composed of concentration 2% PLGA and concentration 0.75% poloxamer, produced smallest mean particle size of 114.6 nm. Poloxamer 188 showed good polydispersity index with narrow size distribution in the range of 0.196 to 0.495 in all the MHc loaded nanoformulations formulated by nanoprecipitation. Contributing factors for this narrow size distribution of nanosize colloidal fine dispersion of MHc loaded nanoformulation were the synergetic effect of poloxamer in stearic stabilization with PLGA and co-emulsification act of poloxamer in nanoprecipitation method.

Zeta potential of the polymeric nanosuspension is a critical factor which determines the physical stability and in-vivo performance of the carrier system

**Table 3** Physicochemical characterization of MHc loaded PLGA nanoformulations

| Formulation code | MPS (nm) ± SD | PDI ± SD | Zeta potential (mV) ± SD | Entrapment efficiency (%) | % Drug release at 12 h ± SD |
|---|---|---|---|---|---|
| NP1 | 157.1 ± 1.28 | 0.333 ± 0.032 | −10.1 ± 0.18 | 70.42 | 61.42 ± 1.13 |
| NP2 | 135.7 ± 0.63 | 0.261 ± 0.065 | −10.9 ± 0.04 | 68.33 | 62.84 ± 2.10 |
| NP3 | 135.9 ± 0.72 | 0.320 ± 0.015 | −11.2 ± 0.025 | 67.45 | 63.50 ± 1.75 |
| NP4 | 132.8 ± 0.52 | 0.209 ± 0.005 | −12.5 ± 0.014 | 66.80 | 74.60 ± 1.20 |
| NP5 | 114.6 ± 0.51 | 0.196 ± 0.012 | −14.4 ± 0.047 | 63.48 | 80.23 ± 1.25 |
| NP6 | 120.3 ± 0.42 | 0.495 ± 0.025 | −12.8 ± 0.085 | 65.28 | 76.92 ± 2.30 |
| NP7 | 245.1 ± 2.62 | 0.209 ± 0.054 | −8.44 ± 0.028 | 76.82 | 52.47 ± 1.03 |
| NP8 | 203.4 ± 1.85 | 0.435 ± 0.045 | −10.2 ± 0.085 | 76.11 | 56.80 ± 1.60 |
| NP9 | 182.7 ± 1.43 | 0.515 ± 0.045 | −12.6 ± 0.065 | 74.00 | 58.5 ± 2.50 |
| NP10 | 212.9 ± 1.92 | 0.833 ± 0.074 | −10.9 ± 0.275 | 68.47 | 50.5 ± 2.12 |

Note: PLGA = Poly(D,L-lactide-co-glycolide); MPS = Mean particle size; PDI = Polydispersity index; SD = Standard deviation (n =3).

[25]. All the formulations of MHc loaded PLGA nanosuspensions prepared by nanoprecipitation method exhibited negative zeta potential. In general, increase in concentration of poloxamer decreased the zeta potential in all the concentrations of PLGA nanoformulations. The surface charge of MHc loaded PLGA nanosuspension formulations ranged between −8.44 mV and −14.4 mV. The formulation NP5, composed of concentration 2% PLGA and concentration 0.75% poloxamer of MHc loaded nanoformulation produced −14.4 mV zeta potential, which was considered the optimized surface charge for better stability of nanosuspension. The stabilizing effect of poloxamer 188 on MHc loaded PLGA nanosuspension was greatly attributed to its hydrophilic poly (ethylene oxide) chain length.

## Entrapment efficiency study

Entrapment efficiency of MHc loaded PLGA nanosuspension prepared by nanoprecipitation method using poloxamer 188 and PLGA was evaluated. Entrapment efficiency of MHc loaded nanosuspension was found in the range of 63.48% to 76.82%. Increase in poloxamer concentration across all the concentrations of PLGA nanoformulation was found to decrease entrapment efficiency of metformin hydrochloride in PLGA nanosuspension. Poloxamer 188 and PLGA had synergetic effect in decreasing the mean particle size of nanoparticle and entrapment drug in MHc loaded PLGA nanosuspension.

## In-vitro drug release study

Drug release studies for all nine nanoformulations prepared by nanoprecipitation method as per formulation design were performed to visualize the effects of poloxamer 188 on drug release of PLGA nanosuspension. The in-vitro drug release study clearly depicted that increase in the % of PLGA decreased drug release, whereas increase in the concentration of poloxamer 188 had positive effect on drug release of MHc loaded nanoformulation. The cumulative % drug release of all nine nanoformulations at 12 h was found in the range of 52.47 to 80.23. Results of these studies signified that methods of preparation and concentration of poloxamer 188 significantly influenced the drug release of PLGA nanoformulations. The drug release study demonstrated poloxamer 188 as a versatile excipient which enhanced drug release by pore forming and acted as a drug release modulator for PLGA nanoformulation.

## Comparison of optimized formulation with blank formulation (without poloxamer)

For the selection and optimization of PLGA and poloxamer 188 based nanoformulation, a set of predetermined desirability characteristics of nanoformulation were assigned, such as nanoformulation of smaller particle size and zeta potential (negative), with higher entrapment efficiency and drug release. Based on the desirability characterization of nanoformulation, NP5 formulation composed of 2% PLGA and 0.75% poloxamer was found to be the optimized nanoformulation, producing particle size of 114.6 nm, zeta potential −14.4 mV, entrapment efficiency of 63.48% and drug release 80.23 % at 12 h with 0.196 polydispersity index. To evaluate the effect of poloxamer 188 on the optimization of PLGA nanoformulation, a blank formulation composed of the same optimized formulation without addition of poloxamer188 was formulated (NP10) and comparatively analyzed with the optimized formulation (NP5), as presented in Table 4. Blank formulation NP10 produced nanoparticles of larger mean particle size of 212.9 nm, zeta potential −10.9 mV with entrapment efficiency of 68.47% and 50.5 % drug release at 12 h. Pure metformin (MHc) was released instantaneously and completed within 1 h of the drug release study. In-vitro drug release study of both the optimized and the blank (without poloxamer 188) nanoformulations indicated a biphasic

**Table 4** Comparison of the optimized formulation with the blank formulation (without poloxamer)

| Parameters | NP5 (Optimized) | NP10 (Blank, without poloxamer) |
|---|---|---|
| Mean particle size (nm) ± SD | 114.6 ± 0.51 | 212.9 ± 1.92 |
| PDI ± SD | 0.196 ± 0.012 | 0.833 ± 0.074 |
| Zeta potential (mV) | −14.4 ± 0.047 | −10.9 ± 0.275 |
| Entrapment efficiency (%) | 63.48 | 68.47 |
| Cumulative % drug release at 12 h ± SD | 80.23 ± 1.25 | 50.5 ± 2.12 |

Note: SD = Standard deviation (n =3); PDI = Polydispersity index; NP = Nanoformulation.

drug release of initial quick release following the first order drug release kinetics. The comparisons of drug release profile of the pure drug metformin (MHc), the optimized nanoformulation (NP5) and the blank formulation NP10 (without poloxamer 188) are depicted in Fig. 1.

Drug excipient compatibility study employing FTIR and DSC analysis were performed for MHc loaded nanoformulations prepared by nanoprecipitation method. The FTIR spectra of pure metformin (MHc) and optimized nanoformulation (NP5) of nanoprecipitation methods are shown in Fig. 2. The FTIR of spectrum for pure metformin showed typical characteristic peaks, such as N-H asymmetric stretching (3367.13/cm), N-H symmetric stretching (3296/cm and 3152/cm), C=N stretching (1661.38/cm) and N-H bending (1558.15/cm) 1419.21/cm colligated with C-H asymmetric bending (-CH3). The characteristic spectral peaks corresponding to metformin functional groups were present in both pure and optimized nanoformulations, indicating that metformin and excipients such as poloxamer 188, PLGA were compatible in design and development of MHc loaded PLGA second generation nanocrystals by nanoprecipitation method [52].

Comparative thermogram analysis by DSC of pure drug (MHc), physical mixer, optimized formulation (NP5) and blank formulation (without

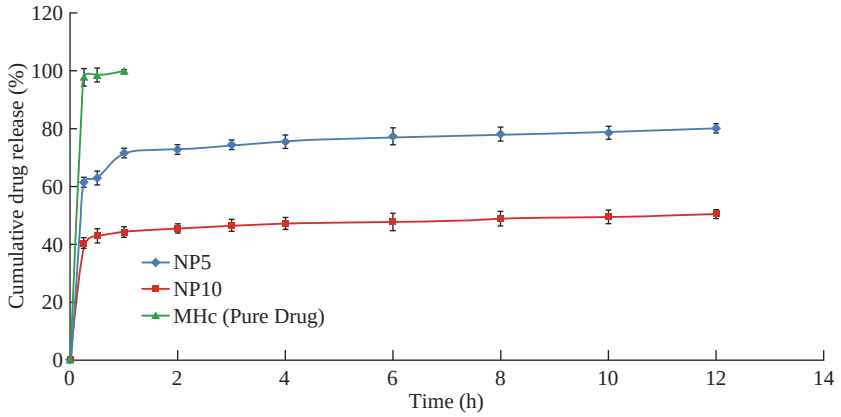


**Fig. 1** Cumulative drug release profiles of pure drug MHc, optimized formulation NP5, and blank formulation NP10.

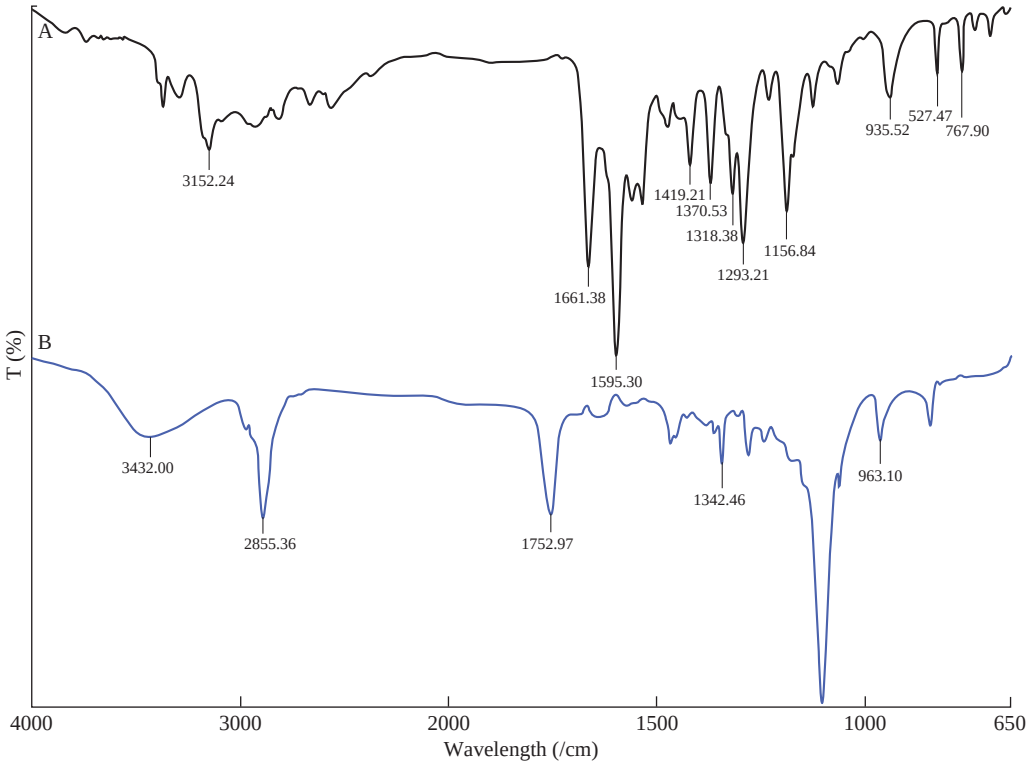


**Fig. 2** FTIR spectra of **(a)** metformin HCl and **(b)** optimized nanoformulation, NP5.

poloxamer) (NP10) were investigated for drug excipient compatibility, as shown in Fig. 3. Pure drug metformin displayed a sharp endothermic peak at 232 °C, which corresponded to the characteristic thermal behavior of metformin. DSC thermogram scan of both the optimized nanoformulation NP5 and the blank formulation NP10 produced a single endothermic peak without detection of metformin characteristic peak (Fig. 4). The DSC thermogram assay study signified that the drug and excipients were compatible for nanoformulation development; the single endothermic characteristic peak of nanoformulations showed that drug metformin was in a molecular dispersion, or in an amorphous form, or in a solid solution state.

Morphological characterizations by TEM analysis of optimized formulation (NP5) and blank formulation (NP10) were conducted to investigate the effect of poloxamer 188 on the morphology of MHc loaded nanoformulations, as illustrated in Fig. 4. Images of nanoformulations evident from transmission electronic microphotographs revealed that the nanoparticle of optimized formulation (NP5) was spherical with smooth morphology with smaller particle size compared to the formulation without poloxamer (NP10). The comparative particle size analysis of nanoformulations proved the size of particle reported by TEM analysis was comparable with the particle size measured by dynamic light scattering technique of zetasizernano. FTIR, DSC and TEM studies on nanoformulation emphasized the significance of poloxamer188 in the formulation and the development of optimized MHc loaded PLGA second generation nanocrystals.

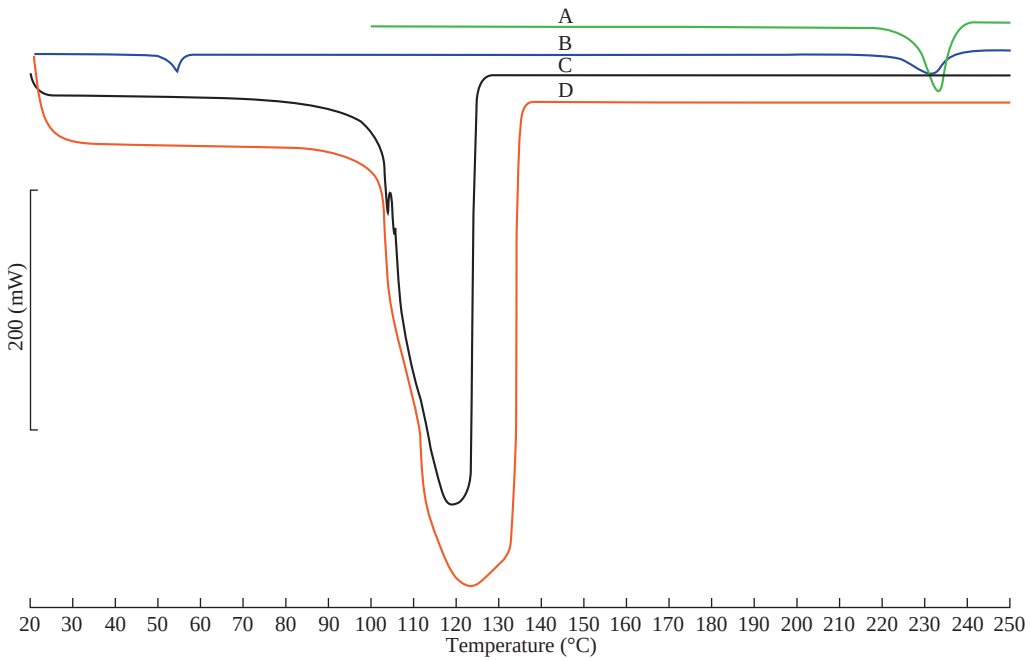


**Fig. 3** DSC thermograms of **(a)** metformin HCl, **(b)** physical mixture, **(c)** optimized nanoformulation, NP5 and **(d)** blank formulation, NP10.

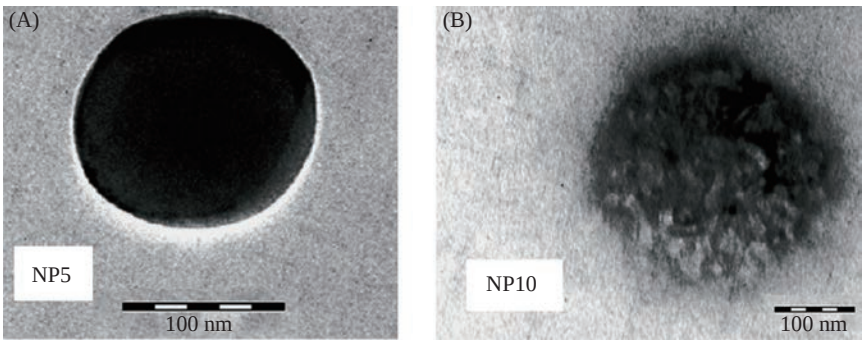


**Fig. 4** Transmission electronic microphotographs of **(a)** optimized nanoformulations NP5 and **(b)** blank formulation NP10 (without poloxamer).

## Conclusions

In conclusion, present findings of the research study emphasized the potential applications of poloxamer 188 and nanoprecipitation method in successful formulation development and optimization of nanosuspension formulation of metformin hydrochloride loaded PLGA second generation nanocrystals. The formulation containing concentration of 0.75% w/v poloxamer 188 and 2 % w/v PLGA was considered to be the optimized formulation which could produce particle size of 114.6 nm, entrapment efficiency of 63.48% and drug release of 80.23% at 12 h. FTIR, DSC and TEM studies on nanoformulation further confirmed the role of poloxamer 188 in the optimization of metformin hydrochloride loaded PLGA nanoformulation. The stabilizing agent, poloxamer 188 proved to be a versatile excipient which regulated the nanonizing of polymeric nanocrystals with smooth, spherical morphology with ideal dispersibility. It also modulated the drug release and entrapment efficiency of drug loaded PLGA nanoformulations prepared by nanoprecipitation method, which can be explored for its potential in design and development of second generation nanocrystals drug delivery.

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## Conflict of interests

The authors declare that this paper content has no conflicts of interests.

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