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Development, Characterization and *In Vivo* Evaluation of Metformin Lipid Nanoparticles Based on Stearic Acid

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ABSTRACT

Metformin HCl is an oral anti-diabetic drug used in the treatment of type-2 diabetes but has been linked with gastrointestinal disturbances and lactic acidosis. The aim of this study is to improve the efficacy and achieve dose reduction of metformin HCl by entrapping the drug in solid lipid nanoparticles (SLN). Solid lipid nanoparticles of metformin were prepared by hot homogenization method at different stearic acid and polysorbate 20 ratios. The formulated particles were characterized by particle size, time-dependent pH, differential scanning calorimetry, drug entrapment efficiency (EE) and loading capacity, and *in vivo* hypoglycaemic activity in mice. P-values less than 0.05 were considered statistically significant. Formulations prepared with lipid-surfactant ratios of 1:5 and 1:2 formed nanoparticles with sizes of 474 and 617 nm, respectively, with moderate entrapment efficiencies and loading capacities, while time-resolved pH showed dependence on surfactant proportion. The size and entrapment efficiency-optimized metformin HCl nanoparticles (1:2 lipid-surfactant ratio) showed faster, higher and sustained reduction of blood glucose in mice than pure metformin HCl powder. Metformin SLN improved the delivery of metformin and may reduce the occurrence of side effects associated with metformin. Solid lipid nanoparticles of metformin HCl may therefore be recommended for effective management of type-2 diabetes mellitus.

Keywords: Diabetes; entrapment; Hypoglycaemic activity.

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INTRODUCTION

Metformin is an oral anti-diabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type-2 diabetes, and in overweight and obese people with normal kidney function. The drug works by suppressing glucose production by the liver [1]. Metformin exerts its prevailing, glucose-lowering effect by inhibiting hepatic gluconeogenesis and opposing the action of glucagon. The inhibition of mitochondrial complex I results in defective cAMP and protein kinase-A signalling in response to glucagon[2]. Lactic acidosis, which involves a build-up of lactate in the blood, can be a serious concern in overdose and when it is prescribed to people with contraindications, but otherwise, no significant risk exists[3]. Metformin is contraindicated in people with any condition that could increase the risk of lactic acidosis ("metformin-associated lactic acidosis" or MALA), including kidney disorders where creatinine levels are over 150 μmol/l or 1.7 mg/dl [4], lung disease and liver disease. Lactate uptake by the liver is diminished with metformin administration because lactate is a substrate for hepatic gluconeogenesis, a process which metformin inhibits. In healthy individuals, this slight excess is simply cleared by other mechanisms (including uptake by the kidneys, when their function is unimpaired), and no significant elevation in blood levels of lactate occurs [5]. When impaired renal function is present, however, clearance of metformin and lactate is reduced, leading to increased levels of both, and possibly causing a build-up of lactic acid. Since metformin decreases liver uptake of lactate, any condition that may precipitate lactic acidosis is a contraindication to its use. Common causes of increased lactic acid production include alcoholism (due to depletion of NAD+ stores), heart failure, and respiratory disease (due to inadequate oxygenation of tissues). However, the most common cause of impaired lactic acid excretion is kidney disease[6]. The most common adverse effect of metformin is gastrointestinal irritation, including diarrhea, cramps, nausea, vomiting, and increased flatulence. Metformin is more commonly associated with gastrointestinal side effects than most other anti-diabetic drugs [7]. Gastrointestinal upset can cause severe discomfort and is usually observed when metformin is first administered, or when the dose is increased. Metformin has also been associated with a low risk of hypoglycemia. In this study, we investigated the effectiveness of solid lipid nanoparticles in the delivery of metformin to prolong its effect and possibly prevent the occurrence of gastric discomfort and lactic acidosis. Solid lipid nanoparticles are commonly defined as nano-scaled lipid matrices, solid at physiological temperatures and stabilized by surfactants [8, 9]. Solid lipid nanoparticles (SLN) are solid nano-scaled lipid matrices usually made from fatty acids, glycerides, fatty alcohols and solid waxes with high melting points. In recent years, biocompatible

lipid particles have been reported as potential drug carrier systems, and as viable alternative materials to polymer[10]. The solid lipid matrix protects loaded labile substances against degradation and can also offer controlled drug release and drug targeting. It can protect the GIT mucosa from the discomforting irritations of some drugs. The suitability of lipid particles as prolonged release formulation[11] for lipophilic drugs has been demonstrated[12]. Furthermore, the lipids in the products provoke delay in gastric emptying which results in increased residence time of the entrapped drug. This enables better dissolution of the drug at the absorptive site, thereby improving absorption[13]. Enclosure of metformin within a lipid matrix may reduce gastrointestinal irritations and the gradual but sustained release of the drug may reduce lactate accumulation. The aim of this research is therefore to improve the delivery and prolong the effect of metformin, possibly reduce side effects associated with the drug and improve patient compliance using solid lipid nanoparticles.

MATERIALS AND METHODS

Metformin hydrochloride (Michelle, Nigeria), Stearic acid, TWEEN® 20 - polysorbate 20 (Sigma-Aldrich, USA)

Preparation of metformin lipid nanoparticles

The hot homogenization method[14] was used for preparing the metformin SLN. The metformin HCl solid lipid particles were prepared according to the formula in Table 1, using varying quantities and ratios of stearic acid and polysorbate 20. Appropriate amount of stearic acid was heated with a thermo-regulated hotplate (Jenway, UK**)** to just above its melting point (69.5 °C) to melt it, after which the weighed metformin sample was dispersed into, and mixed with the lipid melt. The surfactant-water mix was maintained at the same temperature as the lipid melt. The surfactant-water system was added to the lipid melt, and the mixture was homogenized at 1500 rpm for 10 min using a homogenizer (Stuart, UK). During homogenization, the temperature of the system was kept above the melting point of the lipid by the thermo-regulated hot-plate. The emulsion was introduced in drops, using a 10 ml syringe, into 500 ml of ice-cold distilled water. The cold water was stirred slowly, on a magnetic stirrer (Jenway, UK**)**, to keep the particles dispersed. The final volume was determined. Thereafter, the dispersion was filtered using Whatman no.1 filter paper, and the residue particles were dried in a glass dessicator containing fused calcium chloride. Eight batches of solid lipid particles were prepared according to the quantities presented in Table 1.

Characterization of nanoparticles

Particle size analysis

The particle size and size distribution of the various batches were measured at 25 °C by dynamic

light scattering method using a Zetasizer (Malvern Instruments version 7.01, Worcestershire, UK)

Table 1: Quantities of ingredients used in preparing the different batches of nanoparticles

Batch	S1	S2	S3	S4	S5	S6	S7	S8
Metformin Hydrochloride (g)	2	2	2	2	2	2	2	2
Stearic acid (g)	1	1	1	1	2.5	2.5	2.5	2.5
Polysorbate 20 (g)	1	2.5	5	7.5	1.0	2.5	5.0	7.5
Dist. water to (g)	100	100	100	100	100	100	100	100

Time-dependent pH study

Using a calibrated pH meter (Jenway, UK**),** the pH of each formulation was measured by inserting the electrode part into the dispersions. This was done in triplicates in each case, and the mean values taken. The pH values of the eight (8) batches were measured after 7 days, 30 days and 60 days of preparation.

Thermal Analysis (Differential Scanning Calorimetry)

Differential scanning calorimetry (DSC) was performed for thermal analysis using the Calorimeter (Netzsch DSC 204 FI, Germany). A sample of the metformin HCl, stearic acid and lipid particles (1mg) was each individually weighed and sealed in aluminium pan with a similar empty pan serving as control. The machine was calibrated with indium and purged with nitrogen gas. Heating of the sample was carried out at the rate of 10 °C/min from 26 °C to 400 °C under nitrogen flow rate of 20 ml/min followed by cooling back to 30 °C at the same rate.

Entrapment Efficiency

Entrapment efficiencies of the samples were determined using the indirect method by measuring the concentration of the free unloaded drug in the supernatant after 10 ml of the filtrate was centrifuged at 4000 rpm for 50 minutes using an electronic centrifuge. After spinning, the filtrate was left to stand. After separation, a sample of the supernatant was withdrawn with a syringe, and the amount of free Metformin present was determined by measuring absorbance readings at 232 nm in a UV-Vis spectrophotometer (Shimadzu UV-1700, Japan).

Drug entrapment efficiency (E.E.) within the lipid was calculated using Equation 1:

$$\text{E.E (\%)} = \frac{Amount\ of\ drug\ entrapped\ in\ SLN}{Theoretical\ amount\ of\ drug\ added\ to\ formulation} \times 100 \quad \text{- - - - - - - - - - 1}$$

The amount of drug entrapped in SLNs was calculated by subtracting the amount of free drug in the filtrate from the theoretical amount of drug added to the formulation.

Drug Loading Capacity

The loading capacities of the eight formulations were determined from their entrapment efficiencies, by calculating the ratio of the amount of drug entrapped to total formulation mass using Equation 2:

$$\text{Loading Capacity (\%)} = \frac{\text{Weight of drug entrapped}}{\text{Weight of lipid added to system + Weight of drug entrapped}} \times 100 \text{ - - - -2}$$

***In vivo* hypoglycaemic activity**

The animals used in this study were cared for and all treatment protocols were performed in accordance with guidelines on animal ethics in Nigeria and Nigerian Universities, which complied with EU and American directives for all animal experiments. A total of sixteen albino mice (4 mice per group) were used for this study. The study involved induction of diabetes and test of hypoglycaemic activity. Diabetes was induced in the mice using alloxan. An alloxan stock solution of 15 mg/ml was prepared, and the dose volume corresponding to a loading dose of 190 mg/kg was administered to each mouse (starved overnight and weighed) through the intraperitoneal route. After administering the alloxan, the mice were left for 72 h. Thereafter, the fasting blood glucose (FBG) level of each mouse was measured using a glucometer (One Touch, UK). Diabetes was confirmed by a FBG level ≥ 9 mmol/L. For the hypoglyceamic activity testing, only SLN batch S7 was selected among the SLN formulations and used. The hypoglycaemic study was carried out over a period of 4 h. The treatments and the controls were administered orally using an intubation set constructed from a syringe and infusion set tube. The mice were divided into four groups; Groups A, B, C, and D. Group A mice were given pure Metformin at a dose equivalent to the normal adult dose of metformin in humans (500 mg/70 kg). This group served as the positive control. Group B mice were given the metformin nanoparticles at a dose equivalent to the normal adult dose of metformin in humans (500 mg/70 kg). Group C mice were given the metformin nanoparticles at a dose equivalent to 75% of the normal dose of metformin in humans (375 mg/70 kg) while Group D served as the negative control. The mice in Group D were given stearic acid at doses equivalent to the amount of stearic acid contained in the metformin nanoparticles administered to group B. At intervals of 30 min, 1 h, 2 h, 3 h, and 4 h, the glucose level in blood samples, collected from the tail of the mice, were measured using One Touch glucometer.

Statistical analysis

Statistical analysis was done using Microsoft excel 2007. Statistical significance was tested using SPSS version 16.0. P-values of less than 0.05 were considered significant.

RESULTS AND DISCUSSIONS

Particle size

The mean particle sizes of the metformin lipid particles are shown in Table 2. Formulations at lipid-surfactant ratios of 1:5 (S3) and 1:2 (S7) showed the least particle sizes of 0.474 and 0.617 µm, respectively, and were both in the nano-range. The intensity peaks showed that the sizes of

other batches were in micro-scale. This variation in size might be attributed to differences in the lipid-surfactant ratios and the respective excipient quantities used in the formulations. The stability of the nano-globules of the pre-emulsion may have resulted in maintenance of the nano-size during the cooling and solidification stage. Batch S3 had the least polydispersity index values (0.558) signifying that they had the highest population of particles of similar size than other batches. This may be attributed to formation of a relatively stable steric hindrance between nanoparticles of the former formulation. However, the formulations were generally polydispersed probably because stearic acid does not exhibit a very high melting point or the high dose of the entrapment-seeking metformin HCl affects the lipid solidification process.

Table 2: Average size of metformin-loaded SLN systems formulated by varying the relative amounts of stearic acid and polysorbate 20

Batch	S1	S2	S3	S4	S5	S6	S7	S8
Average size (µm)	2.512	3.471	0.474	2.421	3.675	8.640	0.617	4.827
Polydispersity index	0.950	1.0	0.558	0.953	1.0	0.728	0.724	0.749

Time-dependent pH study

Time-resolved pH determinations showed changes in acidity or alkalinity of the formulated dispersions over time. Fig. 1 shows how changes in pH occurred in the particulate dispersions over a period of two months. The pH of the dispersions reduced with increasing proportions of polysorbate 20. Polysorbate 20 is a polyoxyethylene sorbitan fatty acid ester containing 20 units of oxyethylene. These constituents of polysorbate 20 may have caused this partial pH reduction. The time-resolved pH measurements showed varying responses. Some batches (S4, S5 and S7) remained pH-stable after 7 days, 30 days and 60 days while others showed reduced or increased pH values. Results revealed that slight time-dependent reductions in pH values were observed at lower polysorbate concentrations while increases in pH values were observed at higher proportions of polysorbate. This pattern may suggest that at lower polysorbate concentrations, the polysorbate partially loses its physical character in water over time, thereby releasing its own fatty acid components, SLN stearic acid base, and permitting partitioning of the metformin HCl into aqueous phase. However, at higher proportions of polysorbate 20, the surfactant may approach its critical micelle concentration, with its surface excess solubilizing free acidic molecules over time.

Drug entrapment efficiency (EE%) and loading capacity (LC)

The entrapment efficiencies of the entire batches were low (Table 3) because metformin hydrochloride is hydrophilic, and it is not highly soluble in hydrophobic matrices. During the production process, some of the drug molecules may have dissolved in the aqueous compartment

as free drug while others remained within the lipid phase. Also the hot homogenization process may have facilitated partitioning into the aqueous water phase. The amount of drug partitioning to the water phase increased with the solubility of the drug in water at high production temperature. Although, some of the drug molecules may try to re-partition into the molten lipid phase during the cooling process, they usually settle at the surface of the solidifying lipid nanoparticles. Polysorbate 20 as surfactant, helped to reduce lipid repulsion in relation to the highly hydrophilic core as observed in batches S7 and S4 which showed the highest drug entrapments (EE% of 52% and 51% respectively). The relatively low entrapment efficiency of the formulations was because of the low proportion of stearic acid (lipid) which was necessary to avoid diabetic patient discomfort after administration of formulation. The loading efficiency of the metformin in the SLM formulations was highest in batch S4 (stearic acid and polysorbate 20 at the ratio of 1:7.5). This showed that more of the metformin HCl was entrapped by relatively smaller amount of lipid.

Table 3: Drug entrapment efficiency and loading capacity of different SLM dispersions

Batch	S1	S2	S3	S4	S5	S6	S7	S8
Stearic acid-polysorbate 20 ratio	1:1	1:2.5	1:5	1:7.5	2.5:1	2.5:2.5	1:2	1:3
Encapsulation efficiency (%)	43	27	41	51	24	48	52	20
Loading capacity	46	35	45	51	16	28	29	14

Thermal stability (Differential Scanning Calorimetry)

The thermal properties of the metformin sample, stearic acid and SLN S7 (optimized by particle size and drug encapsulation efficiency) are deduced from their DSC thermograms (Figures. 2 and 3). The thermogram of metformin showed a sharp peak at 233.4 °C with an enthalpy of −42.63 mW/ mg, while stearic acid showed a sharp endothermic transition at 61.8 °C with enthalpy of −60.17 mW/ mg. The sharpness, small area under peak and symmetry of the peaks showed that the materials have high percent purity. Other very small and broad peaks observed may be artifacts and residues in the sample holder of the equipment. The transition peaks of metformin HCl (233.4 °C) was definitely absent in the thermogram of the SLN and shows that metformin was now in a predominantly amorphous state. This physical transformation to a less crystalline form with low enthalpy facilitates drug entrapment [15, 16]. However, it seems the peak corresponding to stearic acid shifted to 70.7 °C and another smaller peak emerged at 92.3 °C with a low enthalpy which may be either due to re-crystallization of the metformin HCl from solid lipid solution or a trace of polysorbate surface residue on the SLN

***In vivo* hypoglycaemic activity**

The metformin SLN administered at the same dose with pure metformin HCl showed a faster and extended blood glucose reduction compared to the pure metformin (Figure 4). This is evident as

the SLN immediately and continuously reduced the blood glucose with time. The pure drug only reached peak oral absorption and glucose reduction after 3 hours. The SLN may have improved the rate and extent of absorption of metformin through the GIT of the mice. Studies have shown that lipid particles can improve effectiveness of drugs [16]. The metformin particles may have been small enough (617 nm) to pass through the GIT mucosa or interact with mucosal transporters. Rapid uptake or internalization of SLN by cell lines have been reported [17]. Therefore, the bioavailability of the drug may have increased. The SLN formulation in group C (given at a reduced dose) also showed a faster absorption and glucose lowering than the pure drug, but a peak hypoglycaemic activity lower than the pure drug. Since nanosystems increase efficiency of drug delivery, the doses may need to be recalibrated [18]. Therefore, the use of 75% drug dosage presented as SLN showed that SLN can be used to reduce the dose of metformin without compromising effectiveness while facilitating reduction in undesirable effects. The negative control produced higher percent glucose levels in the mice blood, which increased gradually over time. This showed that unloaded stearic acid alone cannot reduce blood glucose levels. Metformin inhibits gluconeogenic enzymes and stimulates glycolysis by altering the activity of multiple enzymes in these pathways [19]. Solid lipid nanoparticles of metformin HCl may have interacted more closely with the enzymes, thereby providing a targeted and more effective delivery of the drug. The uptake of gluconeogenic substrates, such as lactate is usually reduced in the presence of metformin, possibly owing to depolarization of the hepatocyte membrane through metformin-stimulated Cl^- efflux [20]. However since SLN provides a time-dependent controlled release of the drug, the inhibition of hepatic lactate uptake may be more quantitatively precise and specific, therefore allowing excess lactate to be gradually cleared by the kidney. This implies that metformin SLNs may cause reduced occurrence of lactic acidosis. Incorporation of drugs into nanocarriers offers a new prototype in drug delivery that could be used for effective drug targeting [21]. Therefore solid lipid nanoparticles can be employed to improve metformin delivery and possibly reduce the side effects of the drug.

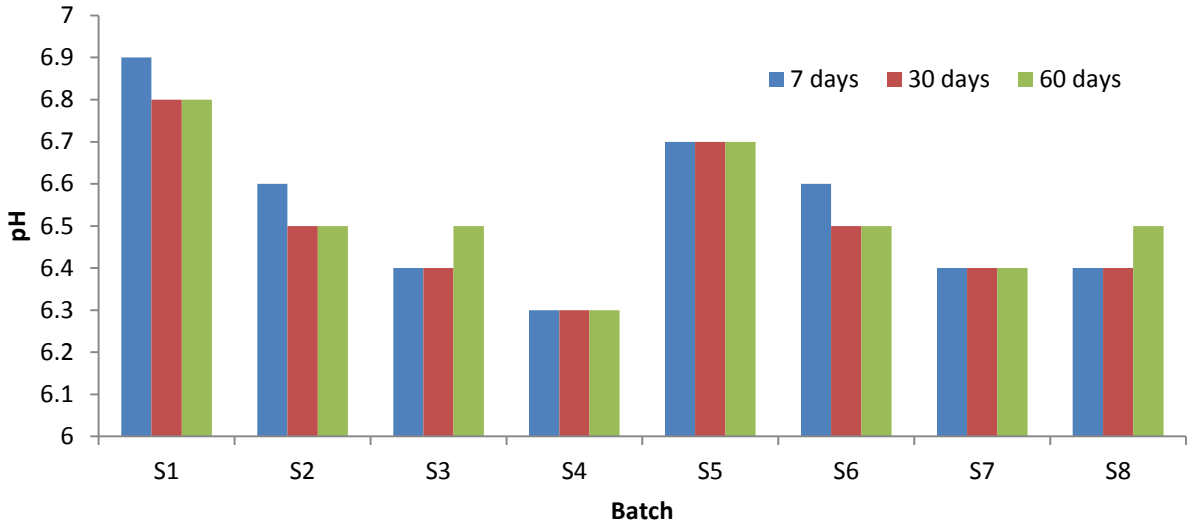
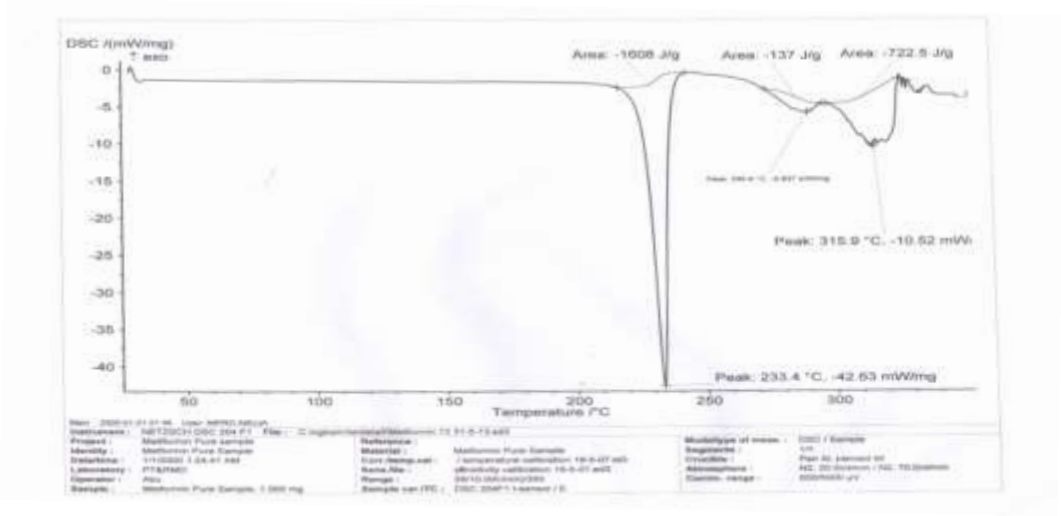
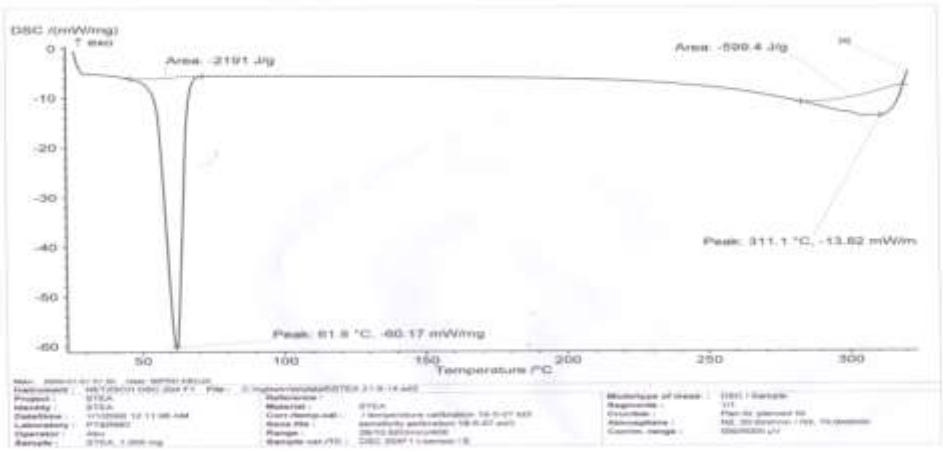


Figure 1: Time dependent change in pH of the SLN formulations



a



b

Figure 2: DSC thermograms of metformin (a) and stearic acid (b)

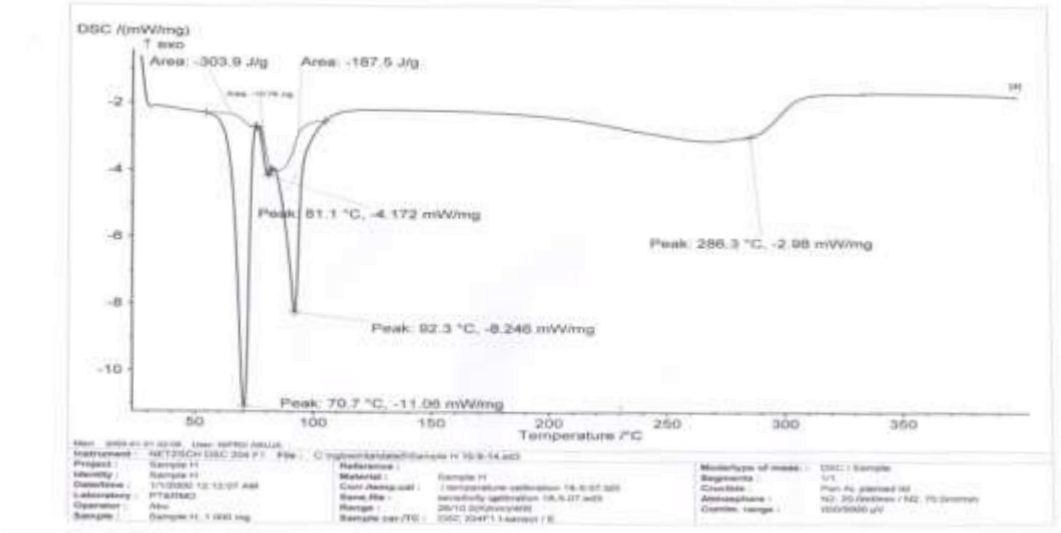


Figure 3: DSC thermogram of metformin SLN S7

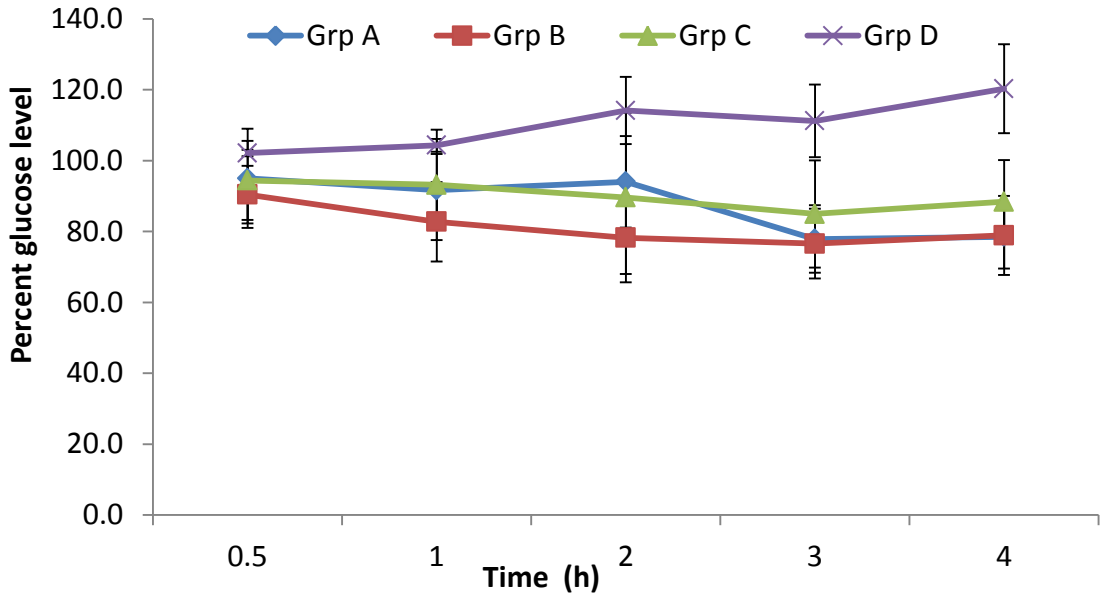


Figure 4: Graph of percentage glucose level against time

CONCLUSION

Metformin HCl solid lipid nanoparticles based on stearic acid showed a faster, higher and more sustained reduction of blood glucose level than pure metformin HCl. This suggests that presentation of metformin HCl as solid lipid nanoparticles improved the delivery of metformin and may reduce the occurrence of side effects associated with metformin.

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