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Research paper

SRMS142-based solid lipid microparticles: Application in oral delivery of glibenclamide to diabetic rats

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ABSTRACT

P90Gylation refers to the modification of lipid molecules by one or more phospholipid chains. Phospholipon® 90G (P90G) contains about 94.0% of phosphatidylcholine stabilized with 0.1% ascorbyl palmitate and is a safe (GRAS) FDA-approved parenteral excipient with wide applications in drug delivery. P90Gylated-Softisan® 142 conjugate, otherwise referred to as (SRMS142), has numerous advantages: wetting, solubilization, drug stabilization, emulsification, and modified release. Here, we report an evaluation of solid lipid microparticles (SLMs) formulated from SRMS142 systems as an alternative carrier system for oral glibenclamide administration in diabetic rats. The result of our findings showed that SRMS142 generated an imperfect matrix with numerous spaces that accommodated glibenclamide in a concentration-dependent manner up to 60.58%. The blood glucose–lowering effect of the SLMs was higher than that of a commercial sample.

1. Introduction

Excipient modification has been justified and recently received increased attention as to create new entities that are recognized to perform better or different functions compared to the native materials [1–5], as to generally achieve wide applications: signaling [6,7], targeting [8,9], modification of circulation time [10], and better bioavailability among other things [11,12]. Synonymous with the term PEGylation as described in the 1970s by Davis and Abuchowsky [13,14] and which has recently expanded and developed tremendously [15–18], we have coined this term, P90Gylation to show a conjugate system of lipid matrix with good drug delivery potentials [19]. However, mixtures of Softisan® 142 and P90G as solidified reverse micellar solutions (SRMS142) have widely been employed in nanosuspension [1,2]. We report here solid lipid microparticles (SLMs) based on SRMS142 and evaluate the *in vivo* release of glibenclamide in alloxan-induced diabetic rats.

2. Materials and methods

2.1. Materials

Phospholipon® 90G (P90G) (Phospholipids GmbH, Germany) is a purified, deoiled, and granulated soy lecithin with phosphatidylcholine content of at least 90%. Glibenclamide was a kind gift from Juhel Pharmaceutical Nigeria Limited. Softisan® 142 (Pastillen, Germany), sorbic acid, sorbitol (BDH, England), and polysorbate 80 (Uniqema, Belgium) were used as procured from their manufacturers without further purification. Distilled water (Lion water, Nigeria) was used for SLM preparation.

2.2. Formulation of the lipid matrices

The lipid matrices consisting of 4:1 mixture of Softisan® 142 and P90G were prepared by fusion. Briefly, the lipids were weighed using an electronic balance (Mettler H8, Switzerland, melted together at 60 °C on a thermo-regulated water bath shaker (Heto, Denmark) and stirred until solidification to get SRMS142.

2.3. Differential scanning calorimetry (DSC)

Melting transitions and changes in heat capacity of the Softisan® 142 and SRMS142 were determined using a calorimeter (DSC) (NETZSCH DSC 204 F1, Germany). Approximately 3–5 mg of the lipid matrix was weighed (Mettler M3 Microbalance) into

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an aluminum pan, hermetically sealed, and the thermal behavior determined in the range of 35–190 °C under a 20 ml/min nitrogen flux at a heating rate of 10 °C/min. The thermal property of pure glibenclamide was also determined. This determination was extended to the SLMs formulated with or without drug. The baselines were determined using an empty pan, and all the thermograms were baseline corrected.

2.4. Formulation of SLMs

SLMs were formulated to contain 5% w/w of SRMS142, 1.5% w/w of polysorbate 80, 4% w/w of sorbitol, 0.1% w/w of sorbic acid and enough distilled water to make 100% w/w. The hot homogenization method was adopted.

In each case, the lipid matrix was melted at 60 °C, and the surfactant aqueous phase containing sorbitol and sorbic acid at the same temperature was added to the molten lipid matrix with gentle stirring with a magnetic stirrer (SR 1UM 52188, Remi Equip., India), and the mixture was further dispersed with a mixer (Silverson L4R, Adelphi Manufac., England) at 6200 rpm for 5 min to produce the hot primary emulsion, which was collected in hot containers and allowed to recrystallize at room temperature.

By adding increasing concentrations of glibenclamide (100, 200, 300, 400 and 500 mg) to the SRMS142 and following the above-mentioned procedure, glibenclamide-loaded SLMs were obtained.

2.5. Morphology and particle size analysis

Particle size analysis was carried out on the SLMs within one week of production using a digital light microscope (Leica Diestar, Germany) and images captured with Moticam 1000 camera. The morphology and sizes of the particles were determined based on image analysis of the microparticles. The SLMs were also subjected to time-resolved particle size analyses for 12 months at 6-month intervals to check the effect of storage on the particle size.

2.6. Drug encapsulation efficiency

Approximately 6 ml of the glibenclamide-loaded SLMs was added into a microconcentrator (5000 MWCO Vivascience, Germany). This was centrifuged (TDL-4 B. Bran Scientific and Instru. Co., England) at 3000 rpm for 120 min. The supernatants were adequately analyzed by UV/Vis spectrophotometer (Unico 2102, England) at 300 nm. The amount of drug encapsulated in the microparticles was calculated referred to a standard Beer's plot to obtain the % encapsulation efficiency (EE) using the formula below:

$$\text{Encapsulation efficiency } (\%) = \frac{\text{Real drug loading}}{\text{Theoretical drug loading}} \times 100$$

2.7. In vivo antidiabetic study

2.7.1. Preparation of experimental rats

Clinically normal male Wistar rats weighing 200 ± 10 g were prepared for the experiment. *Ab initio*, the rats were supplied dry chick's mash finisher, for adult rats twice a day, and given free access to tap water. They were acclimatized to the new experimental environment for two weeks, housed separately in metabolic cages and their body weights, consumption of food and water, urine volume and the levels of serum glucose measured before the induction of diabetes. The rats were divided into nine groups of five rats each. The animal study complied with the ethics of animal use in the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

2.7.2. Induction of diabetes mellitus

The rats were fasted overnight prior to the induction of diabetes mellitus. Blood was collected for baseline glucose determination. The SLM formulations were administered to the rats and the blood glucose checked at predetermined time intervals of 0, 1, 3, 7, 9, 12, and 24 h.

Fresh solution of alloxan monohydrate (Sigma, USA) was prepared just prior to injection. A stock solution of alloxan monohydrate was made by dissolving alloxan in normal saline (0.9% w/v NaCl) at a concentration of 100 mg/kg. A volume equivalent to 1 ml of the stock solution was given intra-peritoneally after which the blood glucose levels were measured frequently for days using a glucometer (ACCU-CHECK, Roche, USA). Food consumption was measured in (g), water (ml), and urine volume (ml) on a daily basis. Diabetes was confirmed 3 days post-alloxan administration.

2.7.3. Oral administration of glibenclamide-loaded SLMs

Nine groups of five animals per group were involved in the investigation. The first rat group marked SLM-0 received blank SLMs (i.e. without glibenclamide, 2 ml p.o). The group marked DW received distilled water only (2 ml p.o), while that marked DW-G received pure glibenclamide dispersed in distilled water (5 mg i.p.), and the commercial sample (Daonil®) was given to another group. Then, the rest rat groups (SLM-1, SLM-2, SLM-3, SLM-4, and SLM-5) received increasing doses (1, 2, 3, 4, 5, mg/ml) of glibenclamide-loaded SLMs, respectively.

2.7.4. Pathological findings

After death or euthanasia, one rat in each group was selected for necropsy. Also, one normal rat was sacrificed to compare the pancreatic islets of Langerhans. The samples were fixed in 10% formalin solution, stained with hematoxylin & eosin, and examined by microscopy (Leica Galen III, Leica Inc., USA).

2.7.5. Storage stability studies of the formulations

The physical stability of the SLMs was evaluated for 12 months under different temperature conditions. Exact volumes of each SLMs were put in closed tubes and stored at 25 °C and 4–6 °C out from direct light. Aliquot samples were withdrawn every 6 months to determine particle size and morphology as described previously.

2.7.6. Determination of injectability

Injectability, defined as the smallest needle guage that a microparticulate sample can pass through [20], was carried out by pushing 4 ml of sample from a 5-ml plastic disposable syringe through hypodermic needles ranging from 18 to 27 G within 20 s. The formulation was first tested using the smallest needle (27 G). If the entire content of the sample passed through a 27 G needle, its injectability was recorded as 27, otherwise the study was repeated using 25 G needle, followed by the next smaller guage needle.

3. Results and discussion

3.1. Characterization of lipid matrices and SLMs

The melting endotherm of Softisan® 142 was 46.8 °C with an enthalpy of −7.962 mW/mg (Fig. 1). This melting point value deviated from what is found on the product sheet or certificate of analysis (42–44 °C) probably due to variation in sensitivity of the DSC machine. However, SRMS142 gave a DSC trace of 43.3 °C and an enthalpy of −4.892 mW/mg (Fig. 2). This shows that SRMS142 is less crystalline than the bulk lipid due to its lower enthalpy value and suggests that mixture of lipids can produce matrices of low crystallinity. This means that SRMS142 generated an imperfect matrix (due to distortion of crystal arrangement of the bulk lipid after

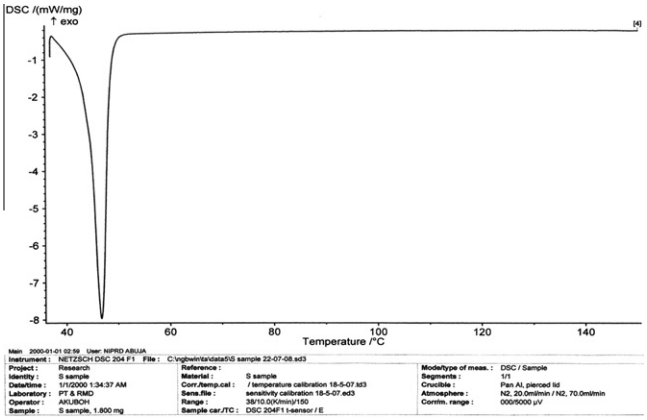

Fig. 1. DSC thermogram of pure Softisan® 142.

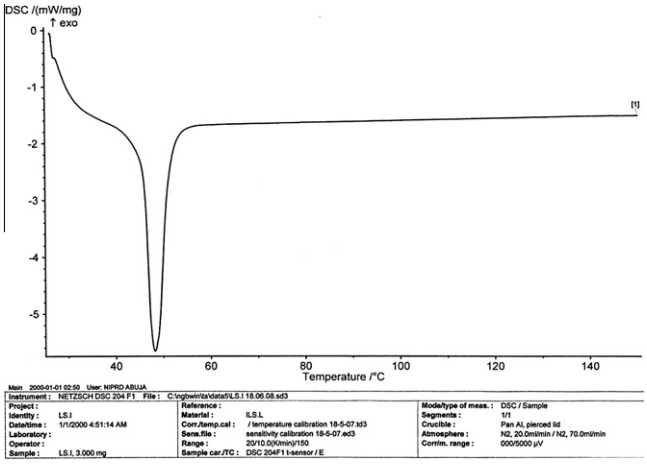

Fig. 2. DSC thermogram of SRMS142 matrix.

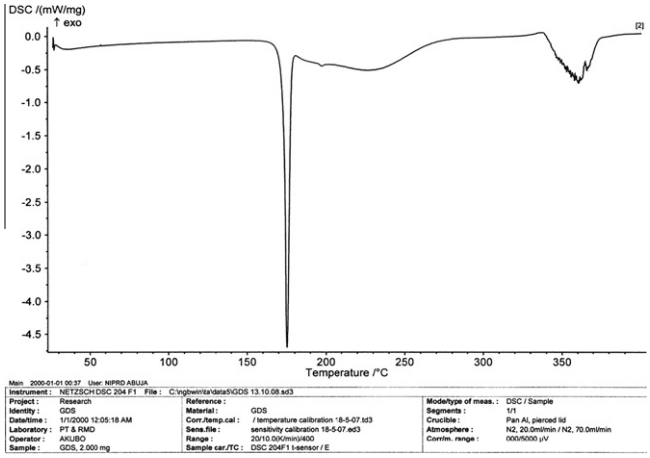

Fig. 3. DSC thermogram of pure glibenclamide.

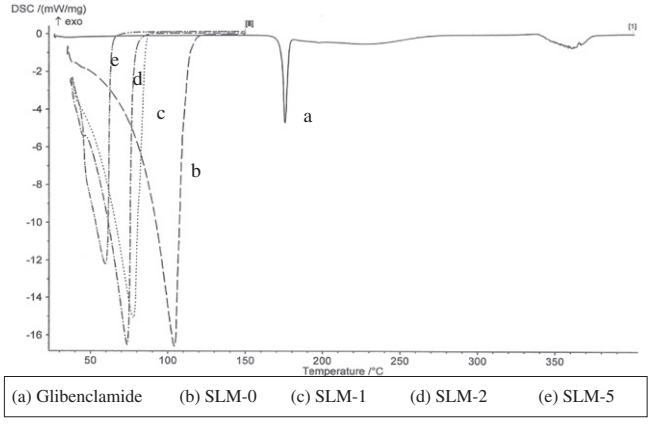

Fig. 4. DSC thermograms of SLM formulations based on SRMS142 containing increasing concentrations of glibenclamide.

melting and solidification), which may have created numerous spaces for drug localization [21–24]. It also suggests an alternative to lipid modification by chemical techniques because the latter often leads to products of decreased *in vivo* tolerability [22,23]. However, the DSC trace of glibenclamide shows a peak at 175.3 °C with an enthalpy of −4.696 mW/mg (Fig. 3). When the SRMS142 was employed to formulate SLMs, the DSC traces of the formulations showed various peaks according to drug concentration. It was observed that the highest drug loading of 0.5 g gave the least melting endotherm of 59.7 °C with an enthalpy of −12.19 mW/mg, whereas the blank SLMs showed the highest melting temperature (104.3 °C) together with highest enthalpy value of −16.58 mW/mg (Fig. 4). Lower enthalpy suggests less crystallinity and the possibility for retention of an entrapped drug over time, whereas high enthalpy means highly ordered crystalline arrangement (perfect crystals) which leads to drug expulsion upon crystallization of previously molten matrices [24].

3.2. Particle size analysis and morphology

The SLMs were well formed, smooth, and non-porous (Fig. 5). They were also stable and did not show sedimentation even after centrifugation (3000 rpm for 90 min). The particle sizes within one week of formulation were small and increased dose-dependently according to the concentration of entrapped glibenclamide as shown in Table 1. The particle sizes increased upon storage for 6 months, which may be due to crystallization of the formerly molten matrices [21,24]. The particle growth was only remarkable at 6 months beyond which it remained insignificant. The growth of the particles did not affect their shapes (Fig. 6). The best storage temperature for the SLMs was at 4–6 °C. At this temperature, the properties of the SLMs were best in terms of particle size, sedimentation, and drug content analysis.

3.3. Drug encapsulation efficiency

The drug-loading efficiency increased with increase in the concentration of the drug such that the maximum percentage drug loading was 60.58% (SLM-5 containing 0.5 g of glibenclamide), whereas the minimum percentage drug loading was 8.33% (SLM containing 0.1 g of glibenclamide) as shown in Table 1. It shows that the solubilizers (active and passive) in addition to the lipid matrix (SRMS142) promoted concentration-dependent drug solubilization. The lipid matrix accommodated more drug at higher drug loadings due to the low crystalline nature of the excipients. Despite achieving low EE (%), the formulation showing higher EE can be evaluated for further use. In other words, SRMS142 contains mixture of fatty acids of different chain length and degrees of saturation and thus formed less perfect crystals with many imperfections, which could have entrapped or localized the drug.

3.4. In vivo release studies of glibenclamide-loaded SLMs

3.4.1. Induction of diabetes mellitus in the experimental rats

Normal glucose level was measured as 160 ± 27.2 mg/dl. Diabetes was confirmed after 3 days post-alloxan injection. Daily con-

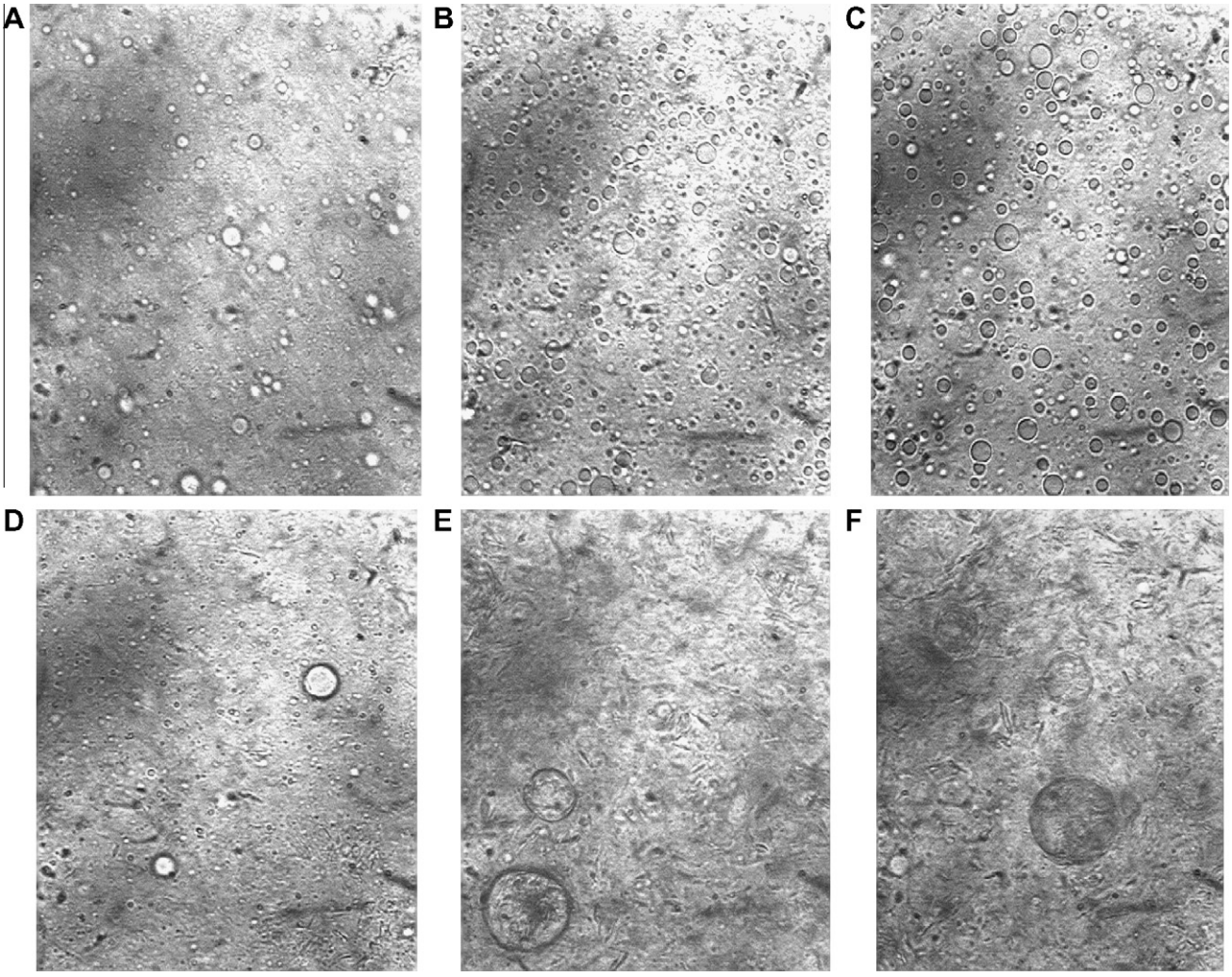

Fig. 5. Photomicrograph of the SLMs; (A) Blank SLM, (B) 0.1% w/w, (C) 0.2% w/w, (D) 0.3% w/w, (E) 0.4% w/w and (F) 0.5% w/w glibenclamide-loaded SLM within one week of preparation. Magnification 65×.

Table 1
Properties of the SLMs loaded with increasing concentrations of glibenclamide.

SRMS142	Drug composition (g)	Parameters					
		Particle size (μm) ± S.D		Drug encapsulation efficiency (%)	Injectability (Guage) at 25 °C		
		1 Week of preparation	At 6 months of storage		1 Week old	After 6 months	After 12 months
SLM-0	0.00	5.5 ± 1.60	95.4 ± 14.20	–	27	18	18
SLM-1	0.1	8.95 ± 1.51	50.9 ± 8.61	8.33	27	25	25
SLM-2	0.2	15.50 ± 2.18	205.6 ± 25.82	41.67	27	25	25
SLM 3	0.3	90.6 ± 15.23	278.3 ± 30.71	55.56	27	25	25
SLM-4	0.4	145.7 ± 18.50	369.6 ± 30.70	58.33	27	25	25
SLM-5	0.5	173.9 ± 19.30	450.8 ± 40.50	60.58	27	25	25

sumption of water and food in healthy adult rats were measured as 35 ± 5 ml and 11.3 g, respectively. Daily urine in healthy adult rats was measured as 11.1 ml, but in diabetic rats, the urine volume was measured as 130 ± 5 ml. The glucose level in diabetic rats was measured as 600 ± 25, and daily consumption of water and food in them was measured as 150 ± 5 ml and 50.6 ± 4 g, respectively (Fig. 7). There was also body weight change whereby diabetes was accompanied by loss of weight.

In addition, the changes in healthy and diabetic rats were apparently distinctive because in addition to thinness of diabetic rats, the tails of the healthy rats were pinkish with somewhat white velvet-like coat. Due to induction of diabetes, the tail became dark, stained and their coats rather pinkish than white behind the head and in the lower part of the body.

3.4.2. Fasting blood glucose reduction

The various batches of glibenclamide-loaded SLMs were shown to effectively lower the fasting blood sugar levels in the rats (Fig. 8). This shows that glibenclamide could effectively be delivered as SLMs using SRMS142. It further suggests that the release

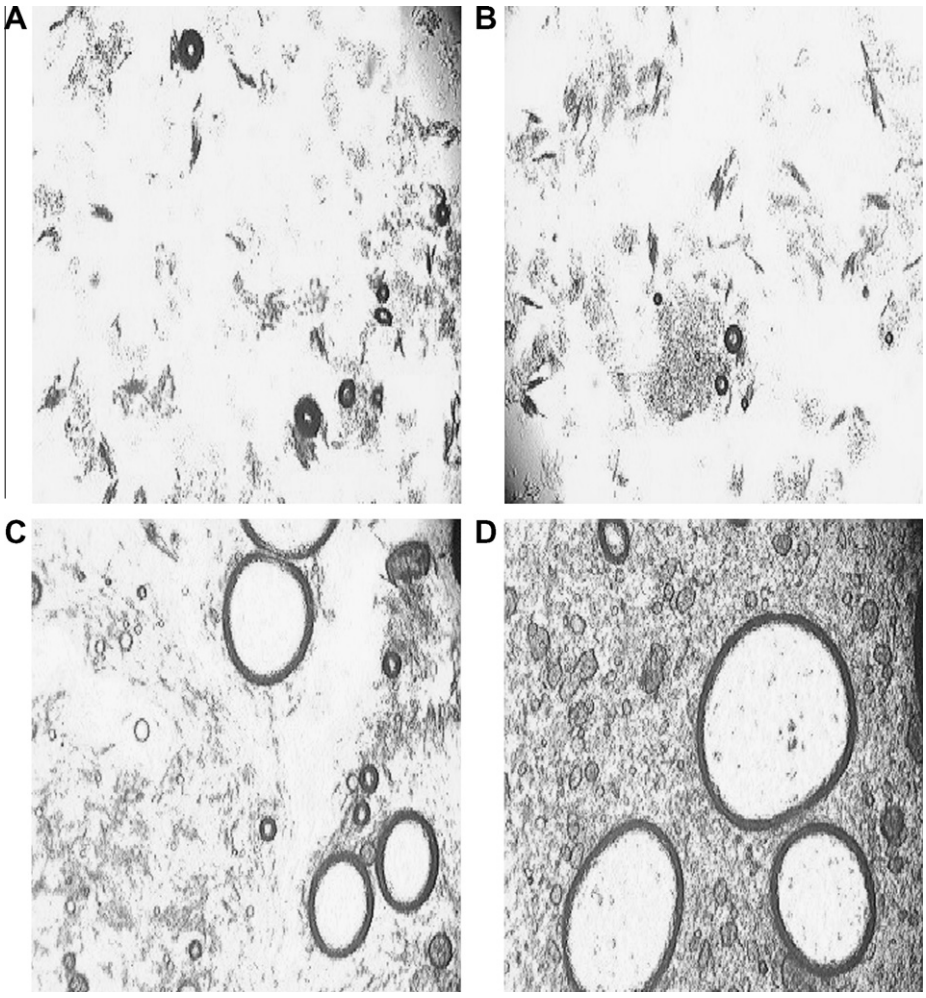

Fig. 6. Photomicrograph of the SLMs; (A) Blank SLM, (B) 0.1% w/w, (C) 0.2% w/w and (D) 0.5% w/w glibenclamide-loaded SLM after 6 months of preparation. Magnification 65×.

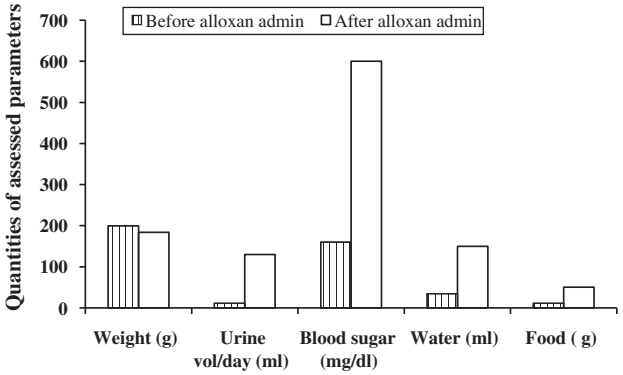

Fig. 7. Physiological parameters in normal and diabetic rats.

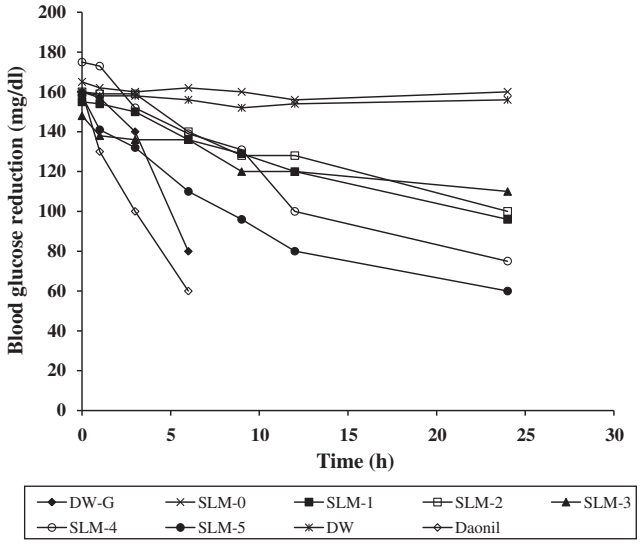

Fig. 8. Effect of glibenclamide-loaded SLMs on the fasting blood glucose of normoglycemic rats.

of glibenclamide from the SLMs stimulated the production of insulin from islet cells of Langerhans in a much more controlled manner than the conventional tablet form. The experimental rats had blood glucose levels within normal range before the alloxan injection and were further lowered in a gradual manner over a period of 24 h by the prolonged release of glibenclamide from the SLMS.

3.4.3. Oral administration of SLMs to diabetic rats

After alloxan monohydrate injection, the blood glucose levels of the rats increased and remained high 3 days post-alloxan administration. With the maintained hyperglycemia, the rats showed polyurea, polydepsia, and polyphagia in addition to weight loss.

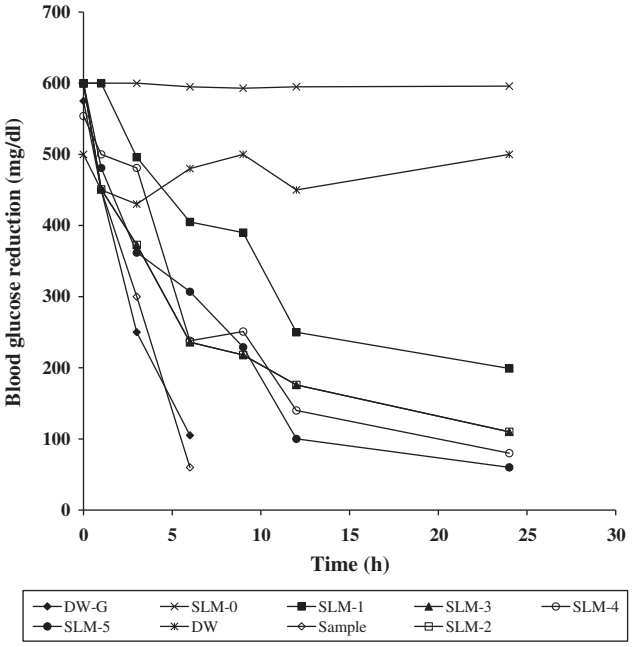

Fig. 9. Effect of glibenclamide-loaded SLMs on hyperglycemic rats.

Glucose levels above 180 mg/dl were considered as diabetic especially as the animals were fasted for 12 h with access to water only.

The rats that received SLM-0 continued to have elevated blood glucose levels throughout the 24-h sampling period. This is because there was no drug in the SLM (blank SLM). So the rats remained hyperglycemic all through the period and some even died as a result. The glibenclamide-loaded SLMs (SLM-1 to SLM-5) dose-dependently lowered the blood glucose levels of the rats. In other words, the release of glibenclamide showed somewhat multiphase patterns with initial burst effects at some points within the first 6 h but steadily maintained the release of the drug over an extended period within 24 h. Maximum blood glucose lowering (80 ± 22.6 mg/dl) was encountered in the SLM containing 500 mg of total formulation, and this was quite comparable to the blood glucose reduction (60 ± 18.32 mg/dl) encountered in the conventional tablet sample (Daonil®) which only released within 6 h while the SLM prolonged the drug release for 24 h (Fig. 9). However, the DW-G sample that was just a dispersion of drug powder in distilled water achieved 105 ± 15.6 mg/dl glucose lowering within 6 h.

3.4.4. Pathological finding

Necropsy was performed after euthanasia or death in one rat and in one normal rat. The histopathological examination showed that the pancreatic islets disappeared. This was probably due to destruction by the diabetogenic agent, alloxan monohydrate. The result is displayed in Fig. 10.

4. Conclusion

This study has shown that SLMs based on SRMS142 could conveniently deliver and sustain the release of glibenclamide in diabetic rats in a better manner than the conventional drug which releases glibenclamide within 6 h. This means that the conventional tablet form (Daonil®) will be taken several times a day, whereas an SLM suspension based on SRMS142 may only require once-daily dosing. It follows that this formulation could be an alternative to the tablet dosage form which can conveniently be taken once a day for the control of hyperglycemia, therefore providing a more patient-friendly compliance.

Conflict of interest

The authors have no conflict of interest regarding this experiment.

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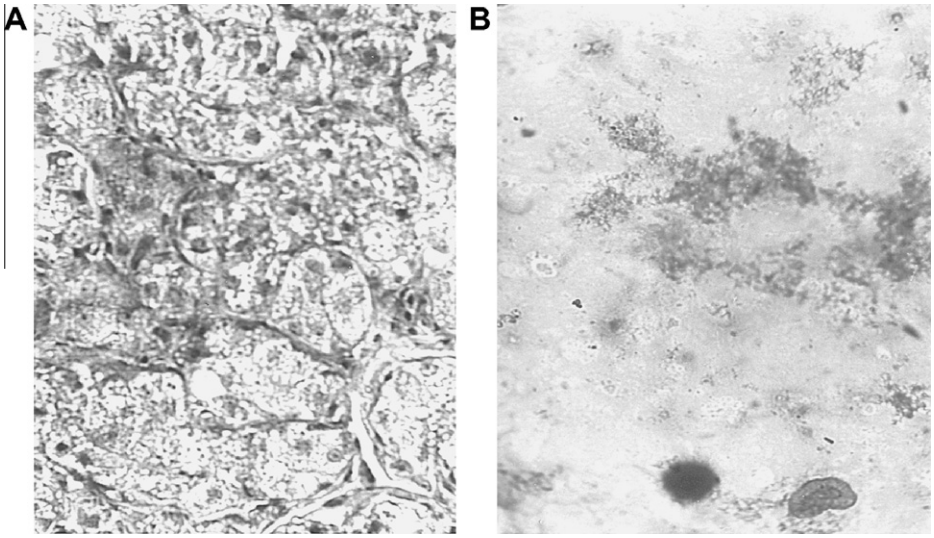

Fig. 10. Pancreatic biopsy: (A) normal rat and (B) diabetic rat. Magnification 65×.

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