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Development and Evaluation of Polymer-bound Glibenclamide Oral Thin Film

Raghavendra HL[1]* and Prem Kumar G[2]

[1]School of Medicine, Wollega University, Nekemte, Ethiopia
[2]Department of Toxicology, Bioneeds India Private Limited, Bangalore, Karnataka, India

Abstract

Glibenclamide (GLB) is a second-generation sulfonylurea oral hypoglycemic agent used in the treatment of non-insulin dependent diabetes mellitus. The use of this drug is limited due to its poor dissolution and pharmacokinetic profile. In order to nullify these drawbacks, GLB oral thin films were prepared and evaluated to study the influence of preparatory conditions on the physicochemical properties by using Scanning Electron Microscopy (SEM), Fourier Transform Infra-red Spectroscopy (FTIR) and Differential Scanning Calorimetric (DSC). The surface pH of the GLB thin film was found to be in the range of 6.50 ± 0.10 and it is non-irritant to mucosal lining of the oral cavity. The results of the SEM analysis showed uniform surface morphology with homogenous distribution of GLB pure drug in PVP matrix. The *in vitro* drug release profile at pH 5.0 and 7.5 revealed sustained release patterns for a period of 8 h. The GLB oral thin film showed an enhanced *in vivo* insulin release profiles as compared to pure GLB drug. Thus, the results of the present study revealed that, the prepared GLB oral thin film can be used as alternative strategy to deliver glibenclamide for diabetes mellitus as it showed a significant role in increasing *in vitro* dissolution and *in vivo* insulin release profiles.

Keywords: Sulfonylurea; Thin films; Drug release; Fourier transform infra-red spectroscopy (FTIR); Insulin release; Scanning electron microscopy (SEM); Differential scanning calorimetric (DSC)

Introduction

Glibenclamide (GLB) is a long-acting oral anti hyperglycemic agent used in the treatment of type II diabetes (noninsulin-dependent diabetes), closely related to sulfa drugs [1]. GLB acts through stimulating the insulin release from pancreatic β-cells by binding high-affinity receptors that are present in the K/ATP channels in β-cells of plasma membranes and it also increases the sensitivity of peripheral tissue to insulin [2]. Being a class II drug (it has high permeability and poor water solubility), the rate of drug dissolution is most certainly the principal limitation to its oral absorption [3,4]. The poor water solubility of GLB is liable for its poor dissolution rate, which ultimately leads to variable absorption of GLB [5]. The GLB showed to have a low oral bioavailability (40-45%), short biological half-life (3-5 h), and undergoes oxidative hepatic first-pass metabolism to yield metabolites having no hypoglycemic activity [6]. All these factors contributed to glucose variability and other gastric side effects viz., nausea, vomiting, anorexia and increased appetite [6,7].

In order to reduce the dosing frequency, improve patient compliance and drug dissolution or bioavailability of GLB, several strategies have been used by the researchers, such as micronization [8], co-precipitation [3], molecular dispersion [9], modification of crystal habits of drug substances [10,11], salt selection and amorphization of the compound [10,11], polymorphism [12], use of surfactants [13], glass formation [14], oil-in-water emulsion [15], freeze-drying [16] etc. The oral thin films prepared by solvent casting technique is emerged as one of the promising technique to circumvent high hepatic first-pass metabolism and harsh degradation of gastrointestinal environment [16,17] and at the same time helped to improve oral bioavailability and therapeutic effect to augment patient compliance.

Various synthetic and natural polymers are available for the preparation of thin films and are the backbone of thin film formulations. The polymers will be used either alone or in combination with other polymers to achieve the desired film properties of interest [18]. Over the years, the use of muco-adhesive polymers (methylcellulose, petrolatum, sodium alginate, cyanoacrylates, guar gum, care gum, pectin, gelatin, lectins, fimbrin, retene, tragacanth, polyacrylic acid, polyethylene glycol etc.) for the development of new pharmaceutical formulations have been employed to overcome conventional drugs drawbacks [19-22]. The employed polymers should be non-toxic, non-irritant, and non-immunogenic in nature to prepare oral thin films [18]. By keeping the above facts, the study was designed to prepare GLB oral thin films using PVP as a polymer matrix and to evaluate their physic-chemical properties by employing Scanning Electron Microscopy (SEM), Fourier Transform Infra-red Spectroscopy (FTIR) and Differential Scanning Calorimetric (DSC) processes. The thin films were also evaluated for *in vitro* drug release and *in vivo* insulin release process.

Materials and Methods

Materials

Glibenclamide (Assay purity ≥99%), Polyvinylpyrrolidone (Mw=13,00,000) and Dialysis tubing cellulose membrane were obtained from Sigma-Aldrich, Germany. Methanol, Chloroform, Monobasic sodium phosphate (NaH_2PO_4) and dibasic sodium phosphate (Na_2HPO_4) were procured from Emplura®, Merck Specialties Private Limited. Insulin was estimated using a Radioimmunoassay kit (RIAK-1) supplied by Bhabha Atomic Research Centre/Board of Radiation and Isotope Technology (BARC/BRIT; Mumbai, India).

***Corresponding author:** Raghavendra HL, School of Medicine, P.O. Box 395, Wollega University, Nekemte, Ethiopia, Tel: +251 922253091; E-mail: raghu.biogem@gmail.com (or) id: drpremkamal@gmail.com

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Preparation of GLB thin film

The GLB thin films were prepared using a solvent casting technique [23] by using PVP. PVP solution (10% w/v) was prepared by dissolving it in methanol by stirring it continuously for about 10 min to obtain a clear homogeneous solution and GLB solution (8% w/v) were prepared in a solvent mixture of chloroform: methanol (1:3) separately. The PVP solution and solvent mixture were mixed together in the ratio of 1:3 and stirred for about half an hour. Furthermore, the mixture was poured into a plastic mould, safely covered and kept overnight at room temperature for solvent evaporation. Finally, prepared GLB oral thin films were used to evaluate their physic-chemical properties, *in vitro* drug release and *in vivo* insulin release.

Characterization of GLB oral thin film

Scanning Electron Microscopy (SEM): The surface morphology of the GLB pure drug, GLB thin films and PVP thin films were characterized by using Field Emission Scanning Electron Microscope (FESEM, FEI Sirion) at operating voltage of 5-30 KV and all the images were recorded at working distance of 8-10 mm [23].

FTIR analysis: The procedure involving sample preparation and spectral recordings were carried out by using previously described method of Stuart [24]. IR spectra of GLB pure drug, PVP thin film and GLB thin film were recorded using FTIR Nicolet 6700 (Thermo Fisher Scientific, Madisson, WI, USA) operated by Omnic software 8.1. The spectra were obtained by Attenuated total reflectance (ATR) method using smart orbit diamond ATR. In Brief, each thin film was placed individually on the sample plate of the smart orbit and screwed lightly to record IR spectra in ATR mode.

Differential Scanning Calorimeter (DSC): The differential scanning calorimeter (Mettler-Toledo DSC 821e, Switzerland) analysis of thin films were carried out with initial and final temperatures at 0°C and 300°C respectively allowing rise in temperature to the tune of 10°C/min in argon atmosphere and DSC curves were evaluated with STARe software (Mettler-Toledo) [23].

Surface pH: The surface pH of GLB thin films were measured by using combined pH electrode and films were moistened with mille-Q-water and pH was measured at the inter-phase of water and film [25].

Measurement of oral thin film thickness: The thickness of oral thin films was measured in triplicate by using calibrated digital Verniar calipers (Mitutoyo 550-203-10, Mitutoyo, Japan) and mean value of thickness was recorded [26].

Folding endurance: The folding endurance capability of the thin films was determined by repeatedly folding the film (4 cm × 4 cm) at the same point until a breaking point was arrived. The total number of times, the film could be folded at the same point without breaking was considered as the folding endurance value. All the tests were performed for four times and mean of the values was reported [27].

Swelling percentage (% S): The swelling index of the films was conducted in simulated salivary fluid at pH 6.75. Briefly, the films (surface area 4 cm^2) were weighed and transferred onto a stainless-steel mesh (sieve size approximately 800 µm). This arrangement was later sunken to 50 ml of simulated salivary medium. At definite time interval (30 s), the stainless-steel mesh was removed; excess moisture was separated carefully with filter paper and reweighed. Increase in weight of the film was determined at every time interval until a constant weight was subsequently found. The swelling percentage was calculated by using the following formula [28,29].

$$\% S = \frac{(X_t - X_0)}{X_0} \times 100$$

Where, % S: Swelling percentage, X_t: The weight of swollen film after time t, and X_0: Weight of film at zero time.

***In vitro* disintegration time:** The *in vitro* disintegration time was measured by following the monograph of United States Pharmacopeia (USP) using disintegration test apparatus (LABINDIA, DS8000) [30].

Solubility studies: The drug concentration of 1 mg/ml GLB pure drug and GLB thin film was taken in a beaker containing Phosphate Buffer Solution (PBS) (pH 5.0 and 7.5) and were incubated at 37°C ± 0.1°C under dynamic conditions and amount of drug dissolved was noted visually [31].

Estimation of drug content

A sample of 4 cm^2 square of thin film was dissolved in 10 ml methanol and shaken it for 5 min to extract drug from the formulation. Finally, the solution was filtered through Whatman filter paper and the drug content was analysed spectrophotometrically at 228 nm using methanol as blank. The mean and standard deviation of drug content of three randomly selected films were calculated [32].

***In vitro* drug release:** The *in vitro* drug release studies of thin films were performed using USP Apparatus-I at 50 rpm and 900 ml of PBS (pH 5.0 and 7.5) at 37°C ± 0.5°C. The 10 mg (concentration 1 mg/ml) of GLB pure drug, GLB commercial drug and GLB thin film were separately placed in dialysis tube and immersed in PBS at pH 5.0 and 7.5. At pre-determined time intervals (0, 5, 15, 30 min, 1, 2, 3, 4, 6 and 8 h), an aliquot of 4 ml of the released media was withdrawn for determining the *in vitro* drug release. The concentration of the drug in release media was estimated by UV spectroscopy Evolution 300 (Thermo Fisher Scientific, Madisson, WI, USA) operated by Vision Pro® software at 228 nm and the regression equation of standard curve developed in the same media. The dissolution medium was replaced with fresh buffer (4 ml) to maintain constant total volume at every time interval [33,34].

***In vivo* insulin release assay:** Ten to twelve week old healthy Wister rats of either sex weighing around 220 g ± 20 g were procured from the Laboratory Animal Facility, Department of Pharmacology, College of Pharmacy, Bagalkot, Karnataka, India. All animals were maintained as per the protocol outlined in the publication of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) [35]. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) bearing approval No. HSKCOP/IAEC/2014/RP-14. The animals were acclimatized at temperature of 22°C ± 3°C and relative humidity of 50-60% under 12 h/12 h light/dark conditions for 5 days before the start of experiment. During experimentation all the animals were provided with a standard pellet diet (Provimi Animal Nutrition India Pvt. Ltd., Bangalore, Karnataka, India), and water ad libitum and same animals were fasted overnight (16-18 h) before the dose administration and water were made available ad libitum. The total animals (n=18) were arbitrarily distributed into three groups [Group I: Normal control, Group II: GLB pure drug, Group III: GLB thin film). The both formulations were administered orally using oral gavages at a calculated dose of GLB (100 mg/kg body weight). The blood sample (≈0.3 ml) were collected from the retro-orbital plexus under isoflurane anaesthesia at predetermined intervals of 0, 1, 2, 4, 8, 12 and 18 h into the micro centrifuge tubes

containing K2EDTA (2 mg/mL), as an anticoagulant. The blood samples were centrifuged at 5000 rpm for 10 min at 4°C to separate plasma from erythrocytes and stored at 80°C until assayed by RIA with rat insulin standards (RIAK-1, BARC/ BRIT; Mumbai, India).

Statistical analysis: The data of *in vitro* release and *in vivo* insulin release studies were subjected to Student's unpaired't' test. The P value <0.05 was considered as significant. Mean values and standard error of mean were calculated and all the values were expressed as Mean ± SEM (Graph Pad Prism 5 Software).

Results and Discussion

Oral thin films characterization

Visual examination of thin film: The visual observations of the thin films were showed that, there was no difference among the PVP and GLB thin film with respect to colour, flexibility, surface uniformity and transparency (Figure 1). The prepared thin films were elegant enough, transparent, flexible, smooth and homogeneous [36].

SEM analysis: The surface morphology of the GLB pure drug (Figures 2a and 2b) and GLB thin film (Figures 2c and 2d) with low and high magnification respectively were represented in Figure 2. The SEM image of GLB pure drug showed irregular particle size of diameters in the range of 15-45 µm (Image J software, provided by National Institutes of Health, USA) [37]. This irregular microcrystalline structure of the GLB pure drug might be one of the reasons to illustrate its poor pharmacokinetic profile. SEM image of GLB thin film showed uniform surface morphology with homogenous distribution of GLB pure drug in PVP matrix. The SEM results of the study, helped to understand PVP mechanism to formulate smaller drug particles with increased surface area and wet-ability; which in turn advantageous to enhance the dissolution rate of a poorly soluble drug [38].

FTIR analysis: The FTIR analysis was conducted to evaluate the physicochemical compatibility of GLB and PVP as a drug delivery system (Figure 3). The GLB pure drug (Figure 3a) showed characteristic amide peaks at 3362.54, 3317.59 and 1712.86 cm^{-1} (N-H stretching), urea carbonyl stretching (urea N-H stretching) vibrations at 1615.25 and 1276.75 cm^{-1}, SO_2 stretching vibrations at 1340.15 and 1156.07 cm^{-1}, C-H aliphatic stretching vibrations at 2933.48 cm^{-1}, O-H stretching vibrations at 2847.67 cm^{-1}, N-O stretching vibrations at 1518.35 and 1449.20 cm^{-1}, C-N stretching vibrations at 1243.58 cm^{-1} and C-O stretching vibrations at 1010.99 cm^{-1} [38-40]. The presence of various prominent functional groups in PVP thin film (Figure 3b) was observed and the broad absorption band at 3398.14 cm^{-1} was due to O-H stretching vibrations of hydrogen-bonded water. The absorption peak at 2950.87, 1372.25 cm^{-1} may be due to the C-H stretching vibration in PVP [41]. A peak at 1643.27 cm^{-1} was assigned to the stretching vibration of the C=O. Other imperative peaks at 1287.50 cm^{-1} and the doublet at 1438.03, 1422.04 cm^{-1} were assigned to the C-N stretching vibrations and the attachment of CH_2 groups in the pyrrole ring of PVP [42,43]. The spectra of optimized GLB thin film (Figure 3c) exhibited all the principle peaks present in the PVP thin film. This unchanged spectrum of PVP and GLB thin film showed the non-interactive nature of polymer and drug and, this non-interactive nature may be due to the encapsulation or masking nature of PVP with GLB pure drug.

Differential Scanning Calorimeter (DSC): The DSC analysis was performed to assess the thermal behaviour of the GLB pure drug, PVP thin film and GLB thin film as a drug delivery system. The DSC thermo grams of the GLB pure drug, PVP thin film and GLB thin film were shown in Figure 4. The DSC thermo grams of GLB pure drug indicated the melting onset, peak and end set at 173.84, 177.38 and 181.32°C respectively, which in turn showed the corresponding melting behaviour of the stable anhydrous form of crystalline GLB [44,45]. The DSC thermo grams of PVP thin film indicated melting onset, peak and end set at 146.22, 147.85 and 156.05°C respectively [46-48]. However, the DSC of GLB thin film indicated the GLB and PVP endothermic peaks at 172.80 and 122.61°C respectively, with slight decrease in the melting point (Figure 4). The observed slight decline in melting point and intensity of GLB endothermic peak of DSC thermo grams of thin film formulation might be partially attributed to the transformation of crystalline to amorphous drug or to the dissolution of drug in the carrier system at the temperatures below its melting point [49,50]. The broad endothermic peak ranging from 96.37-143.81°C might again be attributed due to the presence of residual moisture in PVP [51]. Finally, the observed peak of GLB in the DSC thermo grams of prepared thin film (GLB-PVP) was due to non-interactive nature of GLB pure drug and PVP polymer.

Evaluation of GLB containing-PVP oral thin films: The surface pH of GLB oral thin film was determined in order to examine the effect of pH on oral mucosa and surface pH of the GLB thin film was found to be in the range of 6.50 ± 0.10, which was almost neutral and non-irritant to the mucosal lining of the oral cavity [25]. As the thickness of film was directly concerned with drug content uniformity, then it was necessary to establish uniformity in the thickness of the film. The prepared films were evaluated for thickness using digital Vernier calipers and the mean value was found to be 0.136 mm ± 0.019 mm, which indicates the uniform surface nature of the prepared films [26]. The folding endurance determination will help to determine the mechanical strength like brittleness; flexibility etc. of the films and the increased folding endurance was directly proportional to its mechanical strength. The folding endurance of the drug loaded thin films was estimated and was found to be 460.75 ± 1.013, which indicates its acceptability for mechanical strength as necessitated in a drug delivery system. The penetrations of solvents into the polymer matrix led to increased weight of the films and facilitated diffusion of drug molecules from the thin film matrix to external environment [52]. The mean swelling percentage was determined and found to be 25.73%. The observed results entailed that, the absorption of water by the films was considerable for the drug release. Disintegration time will give an indication about the onset of action, which was desirable for oral thin films [53]. The calculated mean time for the film to disintegrate into complete fine particles was found to be 0.30 min ± 0.16 min and to completely disappear into the solution was found to be 1.00 min ± 0.21 min. These values indicated that, the films had optimum disintegration time.

***In vitro* drug release:** The *in vitro* dissolution profiles of GLB and GLB thin film at pH 5.0 and 7.5 were illustrated in Figure 5. The dissolution rate of GLB thin film at 1 h (@ pH 5.0 and 7.5) were 66% and 69%; whereas, the GLB pure drug were 41% and 40% respectively. Additionally, it was noticed that a maximum percent release of GLB at pH 5.0 and 7.5 (@ 3 h) from the GLB thin film (76% and 90 %) and GLB pure drug (50% and 60%) respectively. The percent drug release at 8 h (@ pH 5.0 and 7.5) from GLB thin film and GLB pure drug remained same till 3 h. The obtained data of drug dissolution rate of GLB thin film was greatly influenced by the solvent cast technique. The better release profiles in the PVP oral film might be partially ascribed due to towering surface area, enhanced porosity and excellent wettability of the polymer matrix [54,55]. However, the release profiles assorted with pH (@ pH 5.0 and 7.5) of the release medium due to varied tortuosity and porosity of polymer matrix [56]. The drug release might have

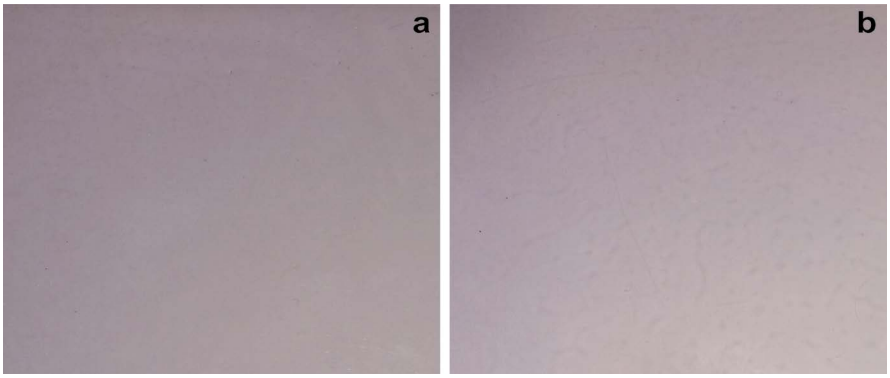

Figure 1: PVP thin film (a) and PVP-GLB thin film (b) prepared by solvent casting technique.

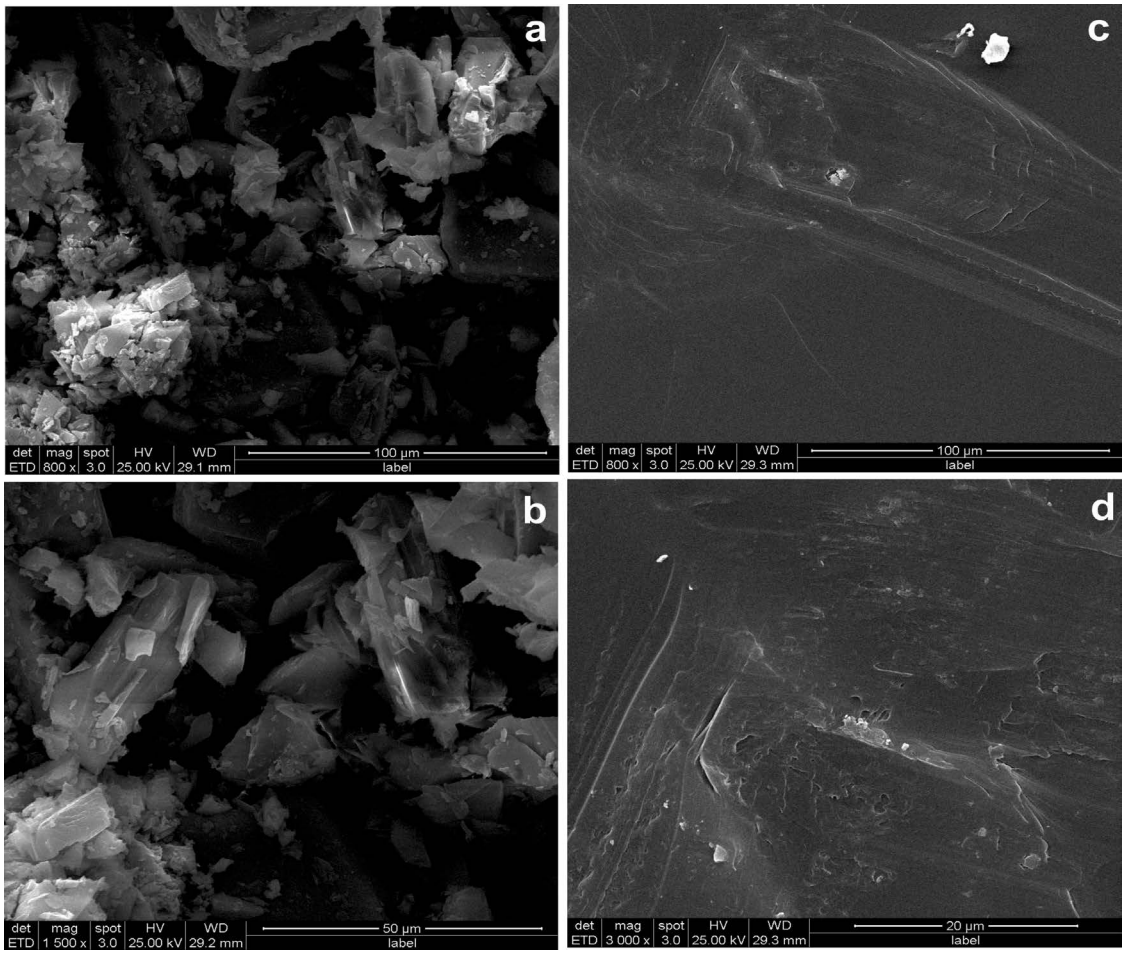

Figure 2: SEM images GLB (a & b) and PVP-GLB thin film (c & d) with low and high magnification.

occurred when the dissolution medium penetrated into the matrix to dissolve drug and created a channel through which diffusion occurred. The average diffusion path might have been increased with an increase in tortuosity. Furthermore, an augment in porosity helped in drug dissolution and drug release profile. On the whole, the PVP oral film exhibited better sustained drug release than GLB pure drug. This could be attributed to the less porosity and more tortuosity nature of the PVP polymer matrix as a drug delivery system [57,58].

***In vivo* insulin release assay:** Insulin resistance is a complex metabolic condition, which will affect the ability of peripheral tissues to respond to insulin. It is an important feature of metabolic syndrome and type 2 diabetes, and is a major risk factor for micro and macro vascular complications [59]. In the present study, the effect of GLB oral film on insulin release was compared with GLB pure drug (Figure 5). The estimated basal value of insulin in Group I (Normal control), Group II (GLB pure drug) and Group III (GLB thin film) was found

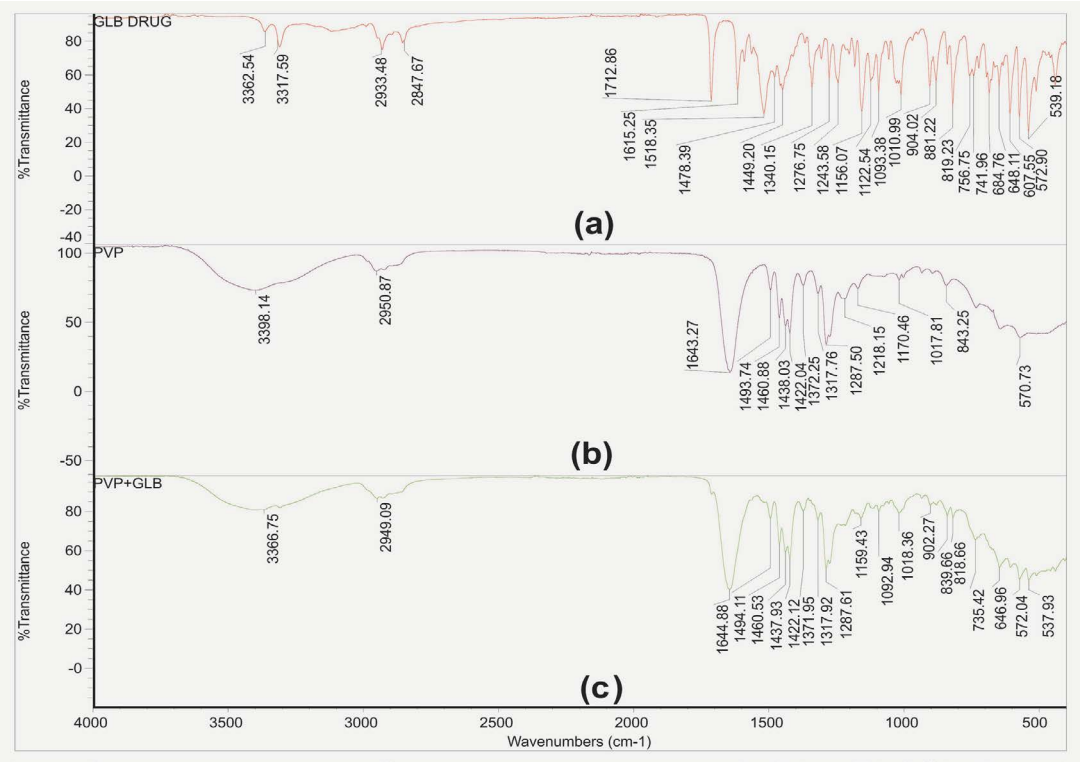

Figure 3: FTIR spectra of (a) GLB, (b) PVP thin film and (c) PVP-GLB thin film.

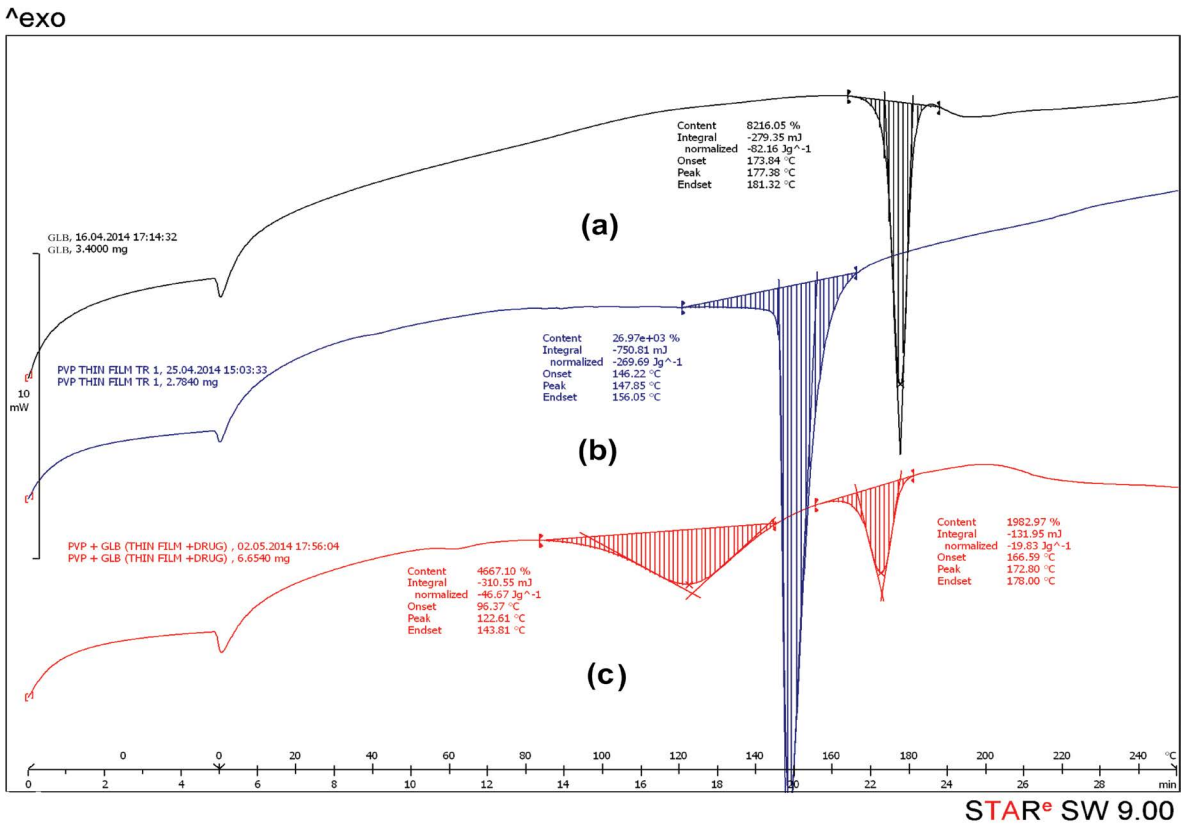

Figure 4: DSC plots of (a) GLB, (b) PVP thin film and (c) PVP-GLB thin film.

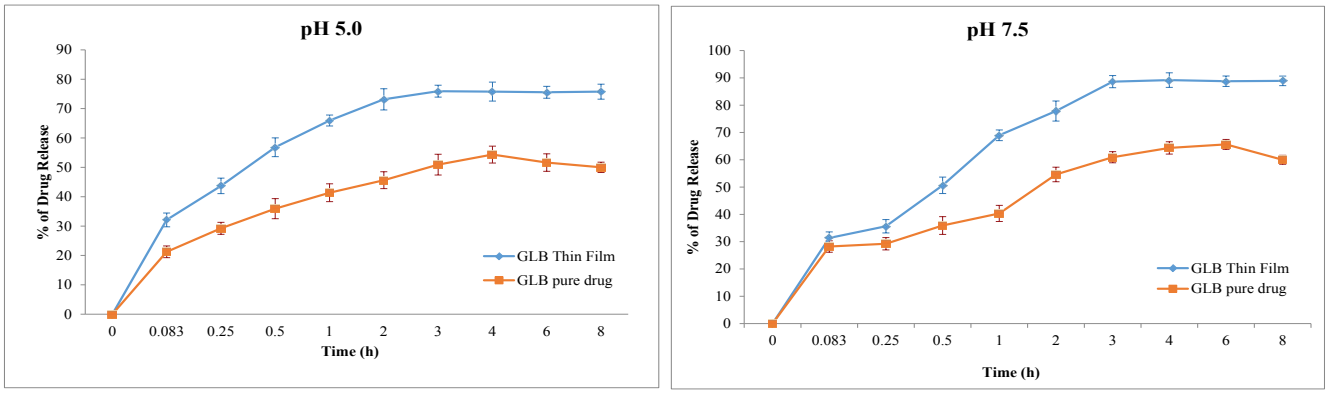

Figure 5: *In vitro* dissolution profiles of GLB and PVP-GLB thin film at pH 5 and 7.5.

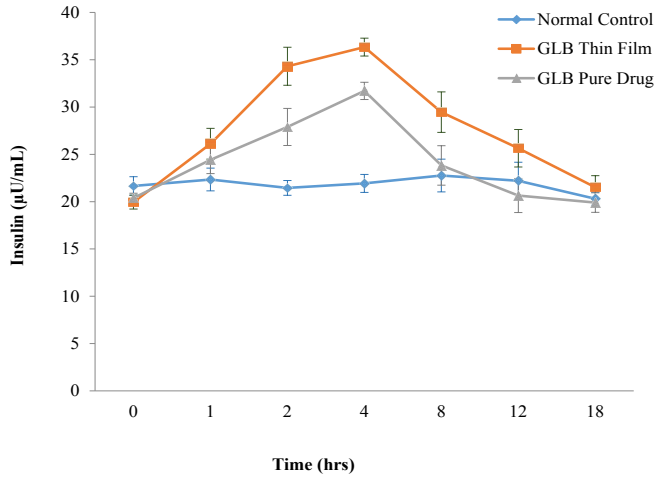

Figure 6: *In vivo* insulin release profile of normal control, GLB pure drug and PVP-GLB thin film.

to be 11.66 µU/ml ± 0.023 µU/ml, 9.38 µU/ml ± 0.23 µU/ml and 9.35 µU/ml ± 0.163 µU/ml respectively. The estimated insulin release was observed maximum at 4 hrs for GLB pure drug (21.71 µU/ml ± 0.175 µU/ml) and GLB thin film (26.34 µU/ml ± 0.216 µU/ml). The estimated insulin release of GLB pure drug retuned to basal value (8.90 µU/ml ± 0.372 µU/ml); whereas of GLB thin film (12.52 µU/ml ± 0.216 µU/ml) was persistent at 18 h after drug administration. The results of the present study showed the enhanced insulin release properties of GLB thin film, when compared to GLB pure drug at the selected dose and route of administration (Figure 6).

Conclusions

In this research work, GLB thin film was prepared by using PVP as a polymer matrix. The preparation of GLB thin film was performed by using simple solvent casting technique, which could have been easily scaled up. The SEM analysis confirmed the uniform distribution of the drug in the film. The FTIR spectra and DSC thermo grams results indicated the absence of interaction between the GLB and PVP as a drug delivery system. GLB thin film enhanced the drug release process to a considerable extent, as confirmed by *in vitro* drug release study. *In vivo* insulin release profile of GLB oral thin film had shown superior release process against GLB pure drug. Therefore, this solvent casting technique could have been among useful alternative strategies to deliver glibenclamide for diabetes mellitus and as well as for other drugs to relieve chronic illness viz., hypertension, hypothyroidism, multiple sclerosis, Parkinson's, rheumatoid arthritis etc. Finally, solvent casting technique served as a useful simple technique to afford patients compliance by easing off drawbacks of glibenclamide.

Conflict of Interest

The author reported no declarations of conflict of interest.

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