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Pharmaceutical Nanotechnology

Glibenclamide Nanocrystals in a Biodegradable Chitosan Patch for Transdermal Delivery: Engineering, Formulation, and Evaluation

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ABSTRACT

Glibenclamide (GBD) nanocrystals (D_{50} = 429 nm) were engineered by applying combined precipitation and homogenization procedures. GBD crystallinity was maintained during the nanonization process as revealed by differential scanning calorimetry and X-ray analyses. Nanonized and micronized GBD were incorporated into chitosan solutions to fabricate transdermal delivery systems (TDDSs), nano- and micro-GBD, respectively. The fabricated TDDSs displayed satisfactory physicochemical characteristics without substantial aggregation of GBD nanocrystals during the casting and drying procedures. Within 24 hours, about 85 ± 3.1% of the GBD content was released from nano-GBD, compared to 61 ± 3.9% from micro-GBD. Cumulative permeation of GBD from nano-GBD after 24 hours was 498 ± 33.35 compared to 362 ± 25.25 μg/cm^2 from micro-GBD. The calculated flux across rat skin for nano-GBD was 23.14 compared to 13.64 μg/cm^2/h for micro-GBD, with an enhancement factor of 1.7. *In vivo* assessment clearly revealed the enhanced efficacy of nano-GBD to reduce blood glucose levels and counteract the induced hyperglycemia in tested animals compared to micro-GBD ($p < 0.5$). Simultaneously, the nano-GBD was able to maintain higher drug concentration for longer time (24 hours, $p < 0.5$) and minimize intense action and hypoglycemia associated with GBD oral therapy ($p < 0.5$).

Introduction

Particle size diminution to the nanostate is a well-established approach to enhance drug absorption, particularly when bioavailability is dissolution rate limited.[1] Basically, nanosized drug particles can be generated by top-down or bottom-up procedures. The top-down nanonization comprises breakup of drug macroparticles as in wet milling and high-pressure homogenization.[2] Alternatively, nanonization via bottom-up techniques involves building up of nanoparticles from molecules in drug solution through precipitation by admixing with miscible nonsolvents. A distinctive drawback of the bottom-up nanonization procedures is the application of organic solvents. Residues of organic solvents in the generated nanosuspensions represent a major difficulty especially in large-scale manufacturing. Despite the high energy consumption and costs, top-down processes are generally preferred in industry up till now.[3] Glibenclamide (GBD) is an oral hypoglycemic agent given to manage non−insulin-dependent (type II) diabetes mellitus.[4] As the Biopharmaceutics Classification System, GBD is a class II drug (i.e., poorly soluble with highly permeability). Accordingly, size diminution could be beneficial to improve bioavailability of the drug.[5]

Transdermal drug delivery is applied to topical administration of pharmaceutically active ingredients to the healthy skin mostly for systemic therapy. Transdermal drug delivery systems (TDDSs) have gained attention of pharmaceutical researchers for decades when transdermal patches were popular in systemic drug delivery.[6] TDDSs represent a noninvasive mean to avoid exposure to the first-pass metabolism and can sustain plasma levels within the therapeutic window over extended periods.[7] Transdermal patches are usually well accepted and easy to apply with the possibility of an ease and immediate cessation of drug administration by patch removal. TDDSs also offer an appreciated alternative when oral administration is not feasible or may result in erratic bioavailability.[8] Achieving these aims would lower variability in drug responses and considerably improve patient compliance.[9,10] However, transdermal rout remains a challenge for drug delivery through the impermeable epithelium of the skin. Chitosan, a polycationic polysaccharide, has been extensively used in transdermal

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formulations because of its favorable properties as biodegradability, bioadhesion, permeability-enhancing ability, and interesting physicochemical characters.[11,12]

Engineering of nanosized GBD through precipitation-homogenization processes was proposed in the present study. Aim was extended to preserve the size characteristics of the nanocrystals through fabrication of chitosan transdermal systems. *In vitro* and *in vivo* characteristics of the developed formulation were evaluated compared to the micronized drug formulation.

Methodology

Materials

GBD was kindly obtained from the European Egyptian Pharmaceuticals Company (Pharco Corporation; Alexandria, Egypt). Poloxamer 188 was obtained from Spectrum Chemicals (New Brunswick, NJ). Chitosan medium molecular weight was obtained from Sigma-Aldrich (Darmstadt, Germany). Lactic acid and dimethyl sulfoxide (DMSO) were obtained from Fisher Scientific (Tokyo, Japan). Other materials were of pharmaceutical grades and used as received.

GBD Nanonization

GBD nanosized suspension was generated through combined bottom-up and top-down procedures. First, the organic phase was prepared by dissolving 250 mg of GBD in 1 mL of DMSO, whereas the aqueous phase was 49 mL of deionized water containing poloxamer 188 (0.5%, w/v) as a stabilizer. The GBD solution was then slowly infused at a rate of 0.5 mL/min using a syringe needle (0.5-mm diameter) into the aqueous phase under sonication at 25°C using energy output of 75 W (Ultrasons-HD; JP Selecta, Barcelona, Spain). The obtained GBD dispersion (5 mg/mL) was centrifuged at 4180 *g* for 30 minutes (Centrifuge Z 206 A; Hermle Labortechnik GmbH, Wehingen, Germany). Particles were carefully gathered, washed with deionized water, and recentrifuged similarly to remove residues of the organic solvent. The collected particles (about 80% of the initially added drug amount) were redispersed in an appropriate volume of aqueous solution of poloxamer 188 (1% w/v) to prepare a drug dispersion of 5 mg/mL. The formed dispersion was processed using an Ultra-Turrax T25 digital homogenizer (IKA-Werke, Staufen im Breisgau, Germany) at 10,000 rpm for 2 minutes. The resultant dispersion was then homogenized using Avestin C-5 homogenizer (Avestin Inc., Ottawa, Canada) with initial homogenization cycles performed at 100, 500, and 1000 bar. Finally, the suspension was homogenized for several cycles at 1500 bar until obtaining a drug nanosuspension of a constant particle size. For comparison, a slurry of the unprocessed GBD was homogenized similarly without the initial precipitation step.

Size Characterization

Particle size of the prepared GBD NS was determined by dynamic light scattering using Microtrac S3500 (Microtrac Inc., Montgomeryville, PA). Diluted samples (dilution factor = 2) of the prepared nanosuspension were measured 3 times for 120 seconds at 25°C.

Assessment of Drug Crystallinity

Differential Scanning Calorimetry

Thermal analysis by differential scanning calorimetry (DSC) was carried out using Netsch thermal analyzer (Netsch F3 Maia, Münster, Germany). GBD solid samples were collected from the dispersion media by centrifugation, washing with distilled water and recentrifugation. The residues were gathered and dried gently using a heated vacuum desiccator at 35°C and −25 inch Hg.[13] DSC thermograms of unprocessed, precipitated, and homogenized GBD were recorded. For each measurement, a sample of 5-mg weight was introduced in a sealed aluminum pan and scanned in the temperature range 30°C-300°C. A heating rate of 10°C/min was used, and the thermal analysis was performed under dynamic nitrogen atmosphere.

Powder X-Ray Diffractometry

X-ray powder diffraction patterns of unprocessed, precipitated, and homogenized GBD were generated using a Shimadzu XRD 6000 diffractometer (Shimadzu Corporation, Kyoto, Japan). Diffraction patterns were recorded over an angular range of 10°-60° 2θ using a graphite monochromator and a copper radiation source ($\lambda = 1.5418$ Å) with a scanning speed of 0.04°/min.

Formulation of GBD-Loaded TDDSs

Chitosan solutions, 2% w/w, were prepared in dilute lactic acid solution (2% w/w). Viscosity of chitosan solution was measured by the Cannon LV 2020 Rotary Viscometers (Cannon Instrument Company, State College, PA). Calculated volumes (25 mL) of the freshly formed GBD nanosuspension (5 mg/mL) and the unprocessed micronized GBD suspension (5 mg/mL) were added to 50 mL of chitosan solution and stirred by propeller stirrer (IKA, Staufen, Germany) for 10 minutes to disperse the drug. Mixtures were poured into Petri dishes and dried at 50°C (BINDER oven, Tuttlingen, Germany). Aluminum foil was used as a backing film. The developed TDDSs were coded as nano- and micro-GBD for formulations containing nanonized and micronized GBD, respectively.

Characterization of TDDSs

Thickness

Thickness of the prepared patches was determined by a digital Vernier caliper (Mitutoyo, Kawasaki, Japan) at 4 different locations. The average values and standard deviations were determined.[14]

Weight Variation

For weight variation test, 5 films (1 cm^2) were weighed separately by an analytical balance (ADAM Equipment, Milton Keynes, UK), and the average weight was calculated.

Drug Content

Known weights of the films (50 mg) were dissolved in a phosphate buffer (pH 7.4, containing 2%, v/v lactic acid). The solutions were then sonicated for 15 minutes and filtered and properly diluted (dilution factor = 1000). GBD was determined spectrophotometrically at λ_{max} of 237 nm against a blank prepared similarly without GBD. Formulations were tested in triplicate to calculate the mean and standard deviation.

Folding Endurance

The folding endurance is used to evaluate the mechanical strength and flexibility of the films that is needed for handling. Film strips (2 cm × 1 cm) were evenly cut and folded repetitively at the same place till they broke. The number of times the film could be folded without breaking was recorded.[15]

Moisture Content

Moisture content of the prepared films was determined gravimetrically at 105°C using a moisture analyzer (HB43-S Halogen Moisture Analyzer; Mettler Toledo, Greifensee, Switzerland).