Novel Preparation and Effective Delivery of Mucoadhesive Nanoparticles Containing Anti-diabetic Drug

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ABSTRACT

Background: Vildagliptin is an extensively studied DPP-4 inhibitors (anti-diabetic drug) which has contributed greatly to the understnading of molecular interatcion with the DPP-4 enzyme. However, Vildagliptin exerts glucose- lowering effect transiently due to short elimination half-life of 2-3 hrs. Consequently, strict adherence to the dosing schedule strongly emphasised to the diabetic patients. In order to sustain therapeutic effects of a drug at site of absorption, recent efforts have focused on development of mucoadhesive delivery systems using nanoparticles. Methods: Spray-drying method was used to prepare nanoparticles. The drug vildagliptin was incorporated into the nanoparticles and percentage yield, drug content, surface morphology studies, particle size, swelling rate, ex vivo, drug release, drug distribution, histopathology studies were carried out.

Results: The yield percentage and drug loading were determined as 95.8 ± 0.5% and 69 ± 0.3%, respectively. SEM microphotographs further revealed nanoparticles with smooth-surfaced spherical forms and particle size were typically about 450 nm with 375 - 525 range of scattering and swelling rate was found to be 171.5±12%. The ex vivo studies revealed superior mucoadhesive properties of Vildagliptin nanoparticles (8.02 ± 05 hr). The formulation prolonged the release of Vildagliptin to about 12 hrs (98.5 ± 2.5). Vildagliptin prolonged retention in GIT (~12 h) further suggests benefit associated with oral delivery of nanoparticles with higher efficiency than single dose administration. Conclusion: The mucoadhesive nanoparticles optimized in this study may potentially serve as the ideal technology for the effective Vildagliptin delivery specially for diabetes treatment.

Key words: Burst release, Diabetes mellitus, Nanoparticle, Muoadhesive, Vildagliptin, Spray drying.

INTRODUCTION

Diabetes is one of the most alarming public health threats of the current era with a rapidly rising prevalence of 425 million people worldwide projected to reach 629 million by 2045.1 The prevalence of diabetes in 2017 for the Middle East and North Africa (MENA) region reached 39 million people and forecasted to double by 2045 to 82 million.1 As part of MENA region, the fast-growing population of Saudi Arabia (over 32 million) is not immune to such serious diabetes epidemic with recent estimates indicating more than 25% of the population affected with diabetes.2 Furthermore, in recent years expenses associated with diabetes treatment in Saudi Arabia has surged greater than 500%,3 with the current economic burden estimated to be nearly 14%
of the total health care expenditure.4 According to the International Diabetes Federation data other countries in the Gulf Region with the highest diabetes prevalence include the United Arab Emirates (17.3%); Bahrain (16.5%); Qatar (16.5%); Kuwait (15.8%); Oman (12.6%) and Iraq (8.8%).1

Competent management of many patients affected by type 2 diabetes mellitus (T2DM) is restricted due to poor tolerance of some oral antidiabetic drugs during long-term pharmacotherapy. This is compounded by the presence of side effects as well as the inability of the existing drugs to restore secretory capacity of the pancreatic cells. As a result, a surge of interest in newer antidiabetic agents in treatment of T2DM capable of improving islet functions has emerged.57 The dipeptidyl peptidase-4 (DPP-4) inhibitors are one of the most recent class of anti-diabetic agents with different mechanism of action in management of T2DM that offer clinical benefits to complement activity of well-established drugs.8

The underlying therapeutic benefit of DPP-4 inhibitors (gliptins) depend on increasing endogenous availability of two incretin hormones mainly glucagon-like peptide-1 (GLP-1) and the gastric inhibitory polypeptide (GIP) by selective inhibitory effect on DPP-4 enzyme which rapidly inactives these hormones.910 The incretins in turn bind to their respective receptors promoting secretion of insulin from beta-cells.10

Vildagliptin is an extensively studied DPP-4 inhibitors (anti-diabetic drug) which has contributed greatly to the understanding of molecular interaction with the DPP-4 enzyme.1112 However, Vildagliptin exerts glucose-lowering effect transiently due to short half-life of 2-3 hrs.13 Consequently, strict adherence to the dosing schedule strongly emphasised to the diabetic patients.1415

In order to sustain therapeutic effects of a drug at sit of absorption, recent efforts have focused on development of mucoadhesive delivery systems using nanoparticles.1617 Such systems make use of a mixture of the mucoadhesive polymer to form mucoadhesive devices which adhere to mucous intestinal lining releasing drug load slowly.16 The mucoadhesive drug delivery systems are advantageous over the other existing delivery systems in that not only they target and localise dosage form specifically, but also result in higher drug flux at the absorption site in the Gastrointestinal tract (GIT), stabilise drug fluctuations in the plasma as well as reduce side effects.18

Therefore, this research was designed to evaluate the properties of Vildagliptin release in GIT using a rat model. In addition, a nanoparticle mucoadhesive formulation for Vildagliptin delivery was optimized. Recently albumin used extensively as bioadhesive with controlled release properties to significantly improve delivery of active constituents to various sites has been reported.19 On the basis of the published literature so far, this is the first report on delivery system using mucoadhesive drug preparation intended for Vildagliptin-albumin formulation (NVA) and administration.

**MATERIALS AND METHODS**

**Materials**

Vildagliptin, Albumin was purchased from Sigma-Aldrich, St. Louis, MO, USA. The remaining chemicals used were of analytical grade. Animals were 6-8 month old male Sprague Dewaley rats purchased from local market Al-Ahsa, Saudi Arabia.

**Characterization of formulations**

**Preparation of Nanoparticles**

The preparation of albumin-loaded Vildagliptin nanoparticles was performed according to the nano-spray drying method (BUCHI Labortechnik AG, Switzerland). Briefly, a spray nozzle of 4 µm was used at 100% relative fixed spray rate with a 120 L/min flow rate. The inlet and outlet temperatures were set at 100°C, Feed flow rate were between 20% respectively. The nano-spray drying technique is widely used in pharmaceutical and food science.20

A solution of Polymer (1000 mg of Polymer-A soaked in water for 120 mins and pre-warmed before spraying) containing equal amount of Vildagliptin was then filtered to prevent nozzle blockage and spray dried. Subsequently, using a powder scraper the chamber was scraped and dried particles collected to be stored at 25°C in a desiccator for later analysis.21

**Drug content estimation in the formulation**

A know amount of nanoparticles were crushed into fine powder in a mortar and pestle and 100 ml of Phosphate buffered saline (PBS) pH 7.4 and kept for 24 hrs. The solution was stirred for 15 mins and filtrate was analyzed by UV-1601 spectrophotometer (Shimadzu) at a wavelength of 244 nm.

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\% \text{ Drug content} = \frac{\text{Estimated Drug Content}}{\text{Total Drug Amount Taken}} \times 100
\]

**Percentage drug recovery**

The recovery of nanoparticles was measured as the relationship of weight of nanoparticles obtained associated with the total amounts of the drug and polymer at baseline in the preparation process.2223
Nanoparticle recovery = (Amount of nanoparticles collected)/(Total amount of the polymer and drug) × 100

**Characterization of Vildagliptin Nanoparticles**

The uncoated Vildagliptin nanoparticles were examined using scanning electron microscopy (SEM) at 20 kV (JSM-6390LA, Joel Ltd, Tokyo, Japan) viewed at 1,000x to 95,000x range of magnifications. Following platinum sputtering (JFC -1600, Joel Ltd, Tokyo, Japan) various batches were evaluated. To analyze the distribution of the nanoparticles both laser light diffraction and ultrasonic techniques were employed.

**Particle size measurement**

Measurement of particle size were carried out using a Malvern Zetasizer (Malvern Zetasizer Nano. ZS, Westborough, MA). The reusable glass cuvette were filled 3/4th full with organic solvent and the sample (ultrasonicated), obtained from the spray dryer, were suspended and measured to determine the particle size distribution.

**Assessment of Nanoparticle Swelling**

In order to measure the degree of swelling of the nanoparticles, a 1:1 solution of ethanol: methanol was used. Baseline measurements and after incubation with ethanol/methanol solution for 0.15, 0.25, 0.5, 1.0, 3.0, 5.0, 8.0 and 12.0 hrs calculations were performed using microscopy techniques. The proportion of nanoparticle swelling at different time interval was determined by measuring the difference between diameter of nanoparticles at time ‘t’ (Nt) and the initial time (t=0 [M0]) as follows:

Swelling% = Nt - N0/N0 * 100

**Characterization of mucoadhesive nanoparticles**

To determine the mucoadhesion characteristics of nanoparticles an in vitro wash-off method described previously was employed. The basis of this experiment involves stimulation of a biological flow to wash test product fixed to a mucous membrane. Briefly, a piece of freshly cut rat stomach mucosa (7.5 cm) was prepared in isotonic saline solution and secured onto a glass slide with elastic bands. A measured quantity of the nanoparticles was spread over the tissue specimen which was fixed onto a polyethylene support and maintained at 85% relative humidity for 30 mins in a desiccator. A measured quantity of the nanoparticles was spread over the tissue specimen which was fixed onto a polyethylene support and maintained at 85% relative humidity for 30 mins in a desiccator. Phosphate buffer solution (PBS) at pH 7.4 was used to wash the mucosa meticulously at a rate of 10 ml/min using a peristaltic pump. Drug concentration was measured with a spectrophotometer and the quantity of nanoparticles corresponding to the differences in drug levels was calculated. An estimation of adhered nanoparticles was made from calculating the difference between the applied nanoparticles and the flowed nanoparticle amounts. Total proportion of mucoadhesion was then determined from the adhered nanoparticles compared to the actual total of applied nanoparticles.

**Drug release study of Vildagliptin**

The patterns of drug release for Vildagliptin was studied in vitro using conventional dialysis method and according to previously described method. Briefly, in a dialysis bag the Vildagliptin nanoparticles (50 mg equivalent to nanoparticles) were dialyzed against 250 mL of Phosphate buffer solution (PBS) which was diluted to a total volume of 1 L with water (pH 7.4). The temperature was kept at 37 ± 1°C with the dissolution conditions remaining the same for the remainder of the experiments.

**Drug distribution in GIT**

Prior to the experimentation, male Sprague Dawley (SD) rats (250-300 g) were abainted for 20 – 24 hr period with water provided ad libitum. For the in vivo experiments, a total of five groups each containing 15 rats were used. While the first group was administered aqueous Vildagliptin solution, the rest of the groups were administered Vildagliptin nanoparticles via oral routes. To administer nanoparticles, 50 mg of test sample was suspended in a ml of saline solution prior to oral administration. After 1, 3, 5, 8 and 12 hrs of oral administration, three rats at each time interval were sacrificed. The stomach tissue (section 1) together with the entire small intestine were isolated and further sectioned into six parts (sections 2–7; length of each section 12 cm). These sections were dissected to expose the inner mucosal surfaces which were scraped to collect all nanoparticles located in each part. The mixture was then pulverised with a homogenizer and Vildagliptin extracted which was then remained at room temperature for 24 hrs. Following centrifugation (805x g) for 20 min, supernatant was analysed spectrophotometrically. This study were conducted according to University Animal Ethical Committee protocol, College of Clinical Pharmacy, King Faisal University, Saudi Arabia approval number (VET/KFU/27/PR-4215).

**Histopathological examination of mucosa**

For histopathological examination, after incubation of the collected tissues in PBS (pH 6.4), comparisons were made with the tissues incubated with formulation in the diffusion chamber. Once the collected tissue samples were fixed (10% buffered formalin at pH 7.2), using standard protocols the samples were then paraffin-
embedded in paraffin. The paraffin sections were then cut at 7.5 μm and stained with Hematoxylin and Eosin (HE) on glass slides before being evaluated with light microscopy. This was to identify potential tissue damage during the in vitro process which was performed by a pathologist blinded to the experiments.

RESULTS AND DISCUSSION

Drug loading and Percentage Yield

Spray-drying is a most common interesting techniques for many new drug development studies. The percentage yield and drug loading were determined as 95.8 ± 0.5% and 69 ± 0.3%, respectively. However, low powder yield are reported when prepared in conventional methods. This novel technology can be widely used in different pharmaceutical application due to volume can be as small as 200 mg.

The spray-drying method has attracted much attention due to the efficiency of particle generation process and capability for mass production. The nanoparticles of Vildagliptin prepared by this technology exhibited smooth to minor shriveled surfaces (Figure 1). This maybe possibly due to contractions during the drying process in the drying chamber.

Particle Size Analysis

The Vildagliptin nanoparticles were typically about 450 nm with 375 - 525 range of scattering (Figure 2). This suggests that the narrow particle size may allow constant release of the Vildagliptin from the nanoparticles at absorption sites.

Swelling Rate

The swelling rate of Vildagliptin nanoparticles formulation at various time intervals is presented in Figure 3. The outcome revealed that when immersion of the formulation in PBS at pH 7.4 was performed, rapid swelling of all nanoparticles occurred. Previously, swelling has been shown to influence adhesion and cohesion properties of mucoadhesive agents. As predicted, mucoadhesive nanoparticles tend to use capillary action to withdraw water from the underlying mucosa leading to rapid swelling resulting in a considerably stronger adhesion. The NVA formulation was found swell by 171.5±12%.

Mucoadhesion test

The wash-off studies revealed superior mucoadhesive properties of Vildagliptin nanoparticles (8.02 ± 0.5 hr). This adhesive bonding of Vildagliptin nanoparticles to mucin is largely from the existing electrostatic forces and creating hydrogen bonding with mucus molecules resulting in generation of strong adhesive forces. In turn, drug release into the GIT was made possible due to NVA mucoadhesive properties. In contrast, the control drug was instantly released from the rat gut mucosa supporting lack of mucoadhesive properties in the control drug.

In vitro Release studies

Nanoparticle formulation exhibits initial rapid release in vitro is depicted in Figure 4. During the first one hour of experimentation, from the total Vildagliptin
present as part of the NVA nanoparticles almost 32.6 ± 5 % of the drug was released. This indicated adsorption or weakly fused Vildagliptin on to the large surface of the polymeric nanoparticles. This could be explained as rapid initial burst release followed by prolong release of Vildagliptin to about 12 hrs (98.5 ± 2.5 %). In contrast, the release of pure drug was rapid (97.3 % released after half an hour). The obtained results from in vitro studies were fitted to different kinetic models (such as Korsmeyer-peppas model, Hixson-Crowell model, Zero order, Higuchi and first-order equations) in order to identify drug release mechanism as well as release rate by applying macros created for the Sigma Plot graphing tool (Systat Software, Trial Version 14.0). Accordingly, correlation coefficient value ($R^2$) is taken into account when selection of the most significant curve fit describing it's extent is required. The ideal fit with $R^2 = 0.9751$ was the Korsmeyer-peppas model which was determined by applying various data fits of NVA to identify suitable $R^2$ values (Figure 5). The drug release occurs by polymeric matrix diffusion according to the model of Korsmeyer-peppas. The drug release pattern follows the classical Fickian diffusion model whereby nanoparticles initial swelling together with the surface-adsorbed drug particles lead to a ‘burst’ release display. Subsequently, owing to diffusion activity, the drug release exhibits a much better control over longer period of time.

**In vivo studies**

Figure 5 depicts the percentage of drug concentration, NVA and Vildagliptin administered to Sprague Dewaley rats under experimental conditions as above. The nanoparticles mucoadhesive properties were examined at 30 min, 1, 3, 5, 7, 10 and 12 hrs The residual percentage of NVA in the GIT were considerably higher ($P<0.05$) compared to the Vildagliptin suspension. The findings indicated that presence of Albumin in the nanoparticles occurs as a result of their inherent chemical nature. Albumin has no immunogenicity, has half-life of 19 days and has much stronger adhesion under protonated conditions in acidic environments. At time intervals of 30 min NVA was found to be – 22%, 1 h – 32%, 2 h – 52%, 3 h – 59%, 4 h – 67%, 5 h – 78%, 6 h – 78%, 8 h – 78%, 10 h – 96% and at 98 h – 97%. Compared to the Vildagliptin nanoparticles the drug released at 30 min was 91.5% with the total drug released 98.4% at 3 hr. At the same time NVA nanoparticles showed 22% drug release, indicating the great amount of Vildagliptin adsorption or fused near the nanoparticles. This is known as a ‘initial rapid release effect’ in clinical practice nanoparticles shows faster and effective effect on the patients treatment.

**CONCLUSION**

We have developed and characterised an optimized mucoadhesive formulation by evaluation of Vildagliptin release kinetics (in vitro) as well as its distribution in GIT (in vivo) and mucoadhesion properties (ex vivo). The SEM images indicate that nanoparticles were transformed to an almost spherical 375 - 525 nm particle size. In addition, the pattern of Vildagliptin release as evident from the in vitro studies follows Korsmeyer-peppas model. At the same time, the nanoparticles exhibit significantly stronger and greater mucoadhesive bonds lasting approximately 12 hrs. Vildagliptin prolonged retention in GIT (~12 h) further suggests benefit associated with oral delivery of nanoparticles with constant drug delivery. We therefore conclude the mucoadhesive nanoparticles in this study may potentially serve as the ideal technology for the effective Vildagliptin delivery specially for diabetes treatment.
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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

T2DM: Type 2 Diabetes Mellitus; DPP-4: Dipeptidyl Peptidase-4 (DPP-4); GLP-1: Glucagon-Like Peptide-1; GIT: Gastrointestinal Tract; SD: Sprague Dawley; NVA: Vildagliptin Albumin Nanoparticles.

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Spray-drying is a most common interesting techniques for many new drug development studies. Vildagliptin prolonged retention in GIT (~12 h) further suggests benefit associated with oral delivery of nanoparticles with constant drug delivery.

Dr. Sree Harsha received his (ranked top 5) Master of Pharmacy Degree and subsequently earned a doctorate in Pharmaceutics from Rajiv Gandhi University of Health Sciences, Bangalore, India in 2006. He came to King Faisal University in 2007 as an assistant professor in the Department of Pharmaceutical Sciences, bringing with him several years’ worth of teaching experience in fundamentals of pharmaceutics and drug delivery systems. He was actively participated in Accreditation Council of Pharmacy Education (ACPE) and Canadian Council for the Accreditation of Pharmacy Programs (CCAPP). His primary area of focus is pharmaceutical technology and novel/targeted drug delivery systems. For this research, he received grants (20 number) from Deanship of Scientific research, King Faisal University. The author contributed so far to 50 peer-reviewed full papers on a variety of topics in lung targeting, topical drug delivery, and mucosahesive drug delivery systems, He has contributed in writing a book chapter titled “Targeted Drug Delivery System” and “Microspheres” in Textbook of Industrial Pharmacy, Publisher-Orient Longman Private Ltd. In addition, he is an Ad-hoc reviewer for scientific journals. He has attended many seminars and Workshop both national and international on Pharmaceutical Technology and Public health issues.