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Studies on biological macromolecules lipid-Gelucire based gastroretentive multiparticulate

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ABSTRACT

Studies on biological macromolecules lipid-Gelucire based sustained release gastroretentive multiparticulates of metformin hydrochloride (MH) were developed by dispersing MH in melted Gelucire 39/01 and 43/01 using the melt granulation technique while fast release solid dispersions gastroretentive multiparticulates of glibenclamide (GLB), poorly soluble drug were developed using Gelucire 50/13 and PEG 200, 400, 4000, 6000 as carrier at different ratios. Percent drug entrapment of MH was 99.6 ± 0.35% and *in vitro* floating ability was 11.3 ± 0.47 h. Model dependent analysis shows that zero order kinetics was followed while drug release mechanism was anomalous diffusion controlled. Combination of ethylcellulose, methylcellulose and microcrystalline cellulose with Gelucire were explored for release of drug, floatability and consistency for optimized formulation. While GLB multiparticulates showed entrapment efficiency of 99.8 ± 0.11%, *in vitro* buoyancy for 11 ± 0.2 h and improved solubility and dissolution rate. Zero order kinetics was promising for all formulations. Model independent analysis f_2 value for GIV was 40 while for M II was 54. Characterization was done by SEM, FTIR and PXRD. RP-HPLC method was adopted for simultaneous pharmacokinetic analysis of the drugs in rat plasma. In IVIVC studies confirm increased bioavailability of drugs in combination form and followed level A correlation using the diabetic type II Wistar rat.

1. Introduction

In order to increase the bioavailability of metformin hydrochloride (MH) and glibenclamide (GLB), the residence time of the orally administered dosage form in the upper gastrointestinal tract (GIT) needs to be prolonged. The main approaches to prolonging the gastric residence time of pharmaceutical dosage forms include bioadhesive drug delivery system, which adhere to mucosal surface; devices that rapidly increase in size once they are in stomach to retard the passage through the pylorus; and density control delivery system, which float on gastric fluid [1]. Biological macromolecules lipids have property of low density and insolubility in water. Unlike other classes of biological macromolecules, lipids do not form large polymers. Two or three fatty acids are usually polymerized with glycerol to create a triglyceride, but other lipids, such as steroids do not form polymers [2]. Interest in lipid based drug delivery has developed over the past decade fuelled by a better understanding of the multiple roles lipids may play in enhancing oral bioavailability. Lipids are fatty acids and their derivatives are generally insoluble in water and identified by their fatty acid composition, melting pint, hydrophilic–lipophilic balance (HLB), and solubility in non-polar organic solvents. Gelucire or polyoxylglycerides or macrogolglycerides is mixtures of mono-, di- and tri-glycerides and mono- and diesters of polyethylene glycol (PEG) [3]. These are available with range of properties depending on their HLB and melting point (°C) range (33–65 °C). Owing to their extreme hydrophilicity and low density, Gelucire 50/13 may be considered an appropriate carrier for designing fast release floating drug delivery system and also in combination with PEG polymers, as PEG have low melting point, low toxicity, wide drug compatibility and hydrophilicity [4]. Whereas Gelucire 39/01and 43/01 are considered as appropriate carriers for designing sustained release floating drug delivery system [5,6]. Gastric floating drug delivery system (GFDDS) having low density approach are able to prolong the retention time of a dosage form in the stomach, thereby improving the oral bioavailability of the drug [7]. Lipid based formulations

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positively influence drug absorption in a number of ways including increased solubilization capacity, preventing drug precipitation on intestinal dilution, enhancing membrane permeability and lymphatic transport [8].

Diabetes is one of the major causes of death and disability in the world. The global figure of people with diabetes is set to rise from the current estimate of 150 million to 220 million in 2010 and 300 million in 2025. Most cases will be of type II diabetes, due to sedentary lifestyle and obesity [9]. A plethora of antidiabetic drugs are used, of which glibenclamide and metformin hydrochloride is a very widely accepted combination of drugs [10]. The rationale of combinations of sulfonlyureas and biguanides is that, both are major oral antidiabetics and are used in the management of type 2 diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM). At present 4 combinations are marketed in India, which use a combination of metformin with glibenclamide. (i) Glucomide Lipha: GLB & MH (2.5 mg + 500 mg), (ii) Glibomet Guidotti: GLB & MH (2.5 mg + 400 mg), (iii) Suguan M Hoechst: GLB & MH (2.5 mg + 400 mg) and (iv) Bi-Euglucon M Boehringer: M-GLB & MH (2.5 mg + 400 mg) [11].

Glibenclamide (GLB) is a low dose, poorly soluble drug with possible content uniformity problems and dissolution rate-limited bioavailability [12]. The therapeutic dose of GLB is 5–15 mg daily, with low bioavailability (30%) and half-life around 10 h. GLB shows poor solubility in GIT fluids, but by preparation of solid dispersions using Gelucire, it was reported enhancement in dissolution rate of drug [13]. Combination use of surfactants and PEG were previously examined for increased solubilization behavior of poorly soluble drug [14]. Lipid based delivery system are finding increased application in oral delivery of poorly water-soluble, lipophilic drugs via several mechanism [15]. The ability of biological macromolecules lipid-Gelucire based formulations to facilitate gastrointestinal absorption of lipophilic or poorly soluble drug candidates and *in vitro–in vivo* correlation has been thoroughly documented in the published literature [16]. Therefore, it becomes necessary to examine the solid-state properties of the solid dispersion system of GLB using various grades of PEGs and Gelucire 50/13 prepared at different ratios. Moreover, solubility and dissolution rate study were performed to qualify the solid dispersions multiparticulates comparing with the drug alone or as physical mixtures. Thus, in current study it is desirable to improve the earlier studies by formulating fast release gastroretentive multiparticulate system. On the other hand, there has also been contradictory report on the utilization of Metformin hydrochloride (MH) in single unit control release dosage form [17]. MH is a highly water-soluble anti-hyperglycaemic agent used in the treatment of type II diabetes mellitus. MH dose ranges from 0.5 to 2.5 g per day divided in twice or thrice taken with meals [18]. The low bioavailability (50–60%) and short plasma half-life (1.7–4.5 h) of MH make the development of sustained-release gastroretentive dosage forms desirable using Gelucire 39/01 and 43/01[19,20]. As gastroretentive swelling tablets of MH showed mean bioavailability of 115% relative to the immediate-release tablets [21]. Therefore, in the current study it forms the evidence to improve the bioavailability of drugs and compare to market formulations by formulating MH and GLB in gastroretentive multiparticulates system using Gelucire and also optimize the pharmacokinetics and pharmacodynamic of the drugs and using them in combination form. Glibenclamide and metformin hydrochloride quantification in plasma samples is required for pharmacokinetic studies [22], therapeutic drug monitoring [23], tests of new anti-diabetic drugs [24] and bioequivalence assays. Several HPLC methods have been used for this purpose. Techniques used are chromatography-tandem mass spectrometry (LC–MS–MS) [24], reversed-phase HPLC [25], ion-pair HPLC [22,23] and cation-exchange HPLC [26]. Sample pre-treatment methods include protein precipitation [22,24].

The present study also suggests that the pharmacokinetic study should be explored in view to check the increased bioavailability of GLB and MH drugs released from gastroretentive multiparticulates after being orally administered in Wistar rats. In this study, a simple, rapid (short elution time) and adequate sensitive isocratic reversed-phase HPLC method with UV detection has been adopted for determination of glibenclamide [27] and metformin [28] in rat plasma. Further, GLB and MH released from the biological macromolecules lipid-Gelucire based gastroretentive multiparticulates can be explored *in vivo* individually as well as in combination for improved bioavailability by their pharmacokinetic evaluation using our developed method with modification in Wistar rats. Finally, *in vitro–in vivo* correlation (IVIVC) study will be performed [16].

2. Experimental

2.1. Materials

Metformin hydrochloride: gift sample (Sohan Healthcare Pvt. Ltd., Pune, India), glibenclamide: gift sample (Prudence Pharma Chem, Ankleshwar-393002, India), Gelucire 39/01, 43/01 and 50/13: gift sample (Gattefosse-St. Priest, Cedex, France), PEGs (CDH, India), Glyciphage (Franco-Indian Pharmaceutical Pvt. Ltd., India), Betanase (Zydus Cadila Healthcare Ltd., India), Streptotozotocin (Sigma, USA). Hydrochloric acid: analytical grade, methanol: HPLC grade, acetonitrile: HPLC grade and phosphate buffer: analytical grade (CDH, India), ultra-pure water: HPLC Grade (Qualigens, India).

Dissolution tester (TDT06N, Electrolab, India), UV Spectrophotometer (UV-1800, Shimadzu, Japan), Scanning electron microscope (JSM-6610 LV/A/LA, Jeol, Bruker 127 eV), Fourier-transform infrared spectrometer (FTIR-8400, Shimadzu, Japan), HPLC equipment (D-20A UV visible detector and LC-20 AT solvent delivery system, Shimadzu, Japan).

2.2. Formulation methodology

2.2.1. Preparation of GLB loaded multiparticulates solid dispersion (SDs) and physical mixtures (PMs)

The GLB was dispersing in melted Gelucire 50/13 at various ratio 1:1, 1:2, 1:4, 1:6, 1:8 and 1:10 by using hot melt technique [29]. After cooling on aluminium foils and keeping in refrigerator, solid lump was passed through fine mesh (150 μm) to obtain multiparticulates and were desiccated for 48 h. The saturation solubility and melting point was determined further of above prepared multiparticulates of GLB [30]. Each experiment was performed in triplicate (Table 1). On basis of saturation solubility and melting point, the 1:10 ratio was optimized to prepare physical mixtures and solid dispersions multiparticulates of GLB and hydrophilic additives PEG 200, 400, 4000, 6000 and Gelucire 50/13 [31] as following batches (Table 2). Physical mixtures of drug and Gelucire were prepared by triturating them on mortar and pestle for 15 min, followed by sieving (150 μm).

2.2.2. Preparation of MH loaded multiparticulates

MH loaded multiparticulates were prepared by dispersing MH in melted Gelucire 39/01 and 43/01. After cooling on aluminium foil in refrigerator, solid lump was passed through sieves to obtain multiparticulates and kept in desiccator. All the samples were passed through fine mesh (150 μm) and stored in desiccated environment until further study. By using hot melt technique [29], a number of formulations were prepared using different drug: lipid ratio [32]. Lipid was melted at 50 °C and the drug or drug additive mixture was added to the melt. The system was mixed well and cooled to room temp. The mass so prepared was passed through sieve of appropriate size (20–40) to obtain uniform size multiparticulates system.