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Core–shell nano-biomaterials for controlled oral delivery and pharmacodynamic activity of glibenclamide

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ABSTRACT

In this work, native xanthan polymer was chemically modified to xanthan-*grafted*-C_{16} amphiphilic copolymer. Microscopic examination revealed spherical core–shell micellar structures of the copolymer in water. The copolymer exhibited low critical association concentration (1.12 mg/l). The drug loading into copolymer was done by solvent evaporation method. Copolymer micellization enhanced water solubility of glibenclamide by 122 times. Moreover, aqueous dispersion of the copolymer showed negative zeta potential values (−25.9 to −26.6 mV). The micellar carrier extended the drug release profiles under simulated biological fluids up to 8 h. The dissolution efficiency was higher in phosphate buffer (pH 6.8) than in acidic (pH 1.2) solution. The drug release was dominated by super case II and anomalous transport mechanism depending upon the copolymer:drug ratio. DSC analysis suggested amorphous nature of the drug into the micelles. Furthermore, the micellar carriers were able to control hyperglycemia for a prolonged period and thus, had splendid outlook in diabetes management.

1. Introduction

The therapeutic application of hydrophobic drugs remains a challenge to the pharmaceutical scientists, since low water solubility is responsible for poor absorption and low oral bioavailability [1]. In reality, almost 40% drugs being discovered today are practically insoluble in water and thus the development of their suitable dosage forms is being halted [2]. The drug aggregation is associated with high local concentrations at the sites of deposition and often causes local toxicity and lower systemic bioavailability. Some situation demands their hydrophobic character to have considerable affinity toward the target receptors [3] and for intracellular targeting [4]. Therefore, a number of drug solubilization techniques have been described in the literature.

Chemically, glibenclamide is 5-chloro-*N*-(4-[*N*-(cyclohexylcarbamoyl)sulfamoyl] phenethyl)-2-methoxy benzamide. It belongs to second generation sulphonylurea class and frequently used in the treatment of type II diabetes mellitus. It causes hypoglycemia by stimulating release of insulin from pancreatic β-cells and by increasing the sensitivity of peripheral tissue to insulin [5]. It is practically insoluble in water and causes low oral bioavailability of the drug (∼45%) because in such cases, its dissolution rate becomes the rate limiting step for absorption. Therefore, improvement of its solubility and dissolution rate may lead to enhancement of bioavailability [6]. Many attempts have been made to increase the solubility of glibenclamide including β-cyclodextrin complexation [7,8], solid dispersion [9,10], surfactant micellization [11], and co-administration of water-soluble polymers such as HPMC [12], micronization [13], and others. Although a reasonable amount of glibenclamide can be solubilized by these techniques, the preparations are not investigated as drug delivery carriers.

Recently, the concept of polymer-based micelles have received much attention because of the high diversity of polymers, their biocompatibility, biodegradability, and the multiplicity of functional groups they display for the conjugation of pilot molecules [14]. Like surfactant molecules, amphiphilic polymers can also form core–shell structures [15]. However, they differ in their critical micellar concentrations. Polymer-based micelles offer low CAC values and thus remain stable upon dilution. Further, they are well protected from undesirable drug interactions with cells and proteins [16]. In the design of amphiphilic polymer, most of the workers have taken the help of poly (ethylene oxide) (PEO) chains as the hydrophilic outer shell. However, a wide range of hydrophobic blocks have been explored, resulting in different micellar systems with distinct physicochemical properties. These include propylene oxide [17], L-lysine [18], aspartic acid [19], caprolactone [20], and D,L-lactic acid [21].

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To date, most contributions in the area of polymeric micelles for oral formulations have been made with commercial Pluronic® triblock copolymers [22,23].

In spite of non-toxicity, biodegradability and biocompatibility of natural polysaccharides, their potential to serve as hydrophilic shell of the copolymer still remain to be investigated. Limited reports are available that focused on the design of natural polysaccharide-based copolymer micelles. These copolymers include: *N*-palmitoyl chitosan [24], stearyl chitosan and sulfated stearyl chitosan [25], dextran-*b*-poly (DL-lactide-co-glycolide) [26]; stearate-*g*-dextran [27]; octenyl succinate modified starch [28].

Xanthan is a hydrophilic polysaccharide, derived from the bacterial coat of *Xanthomonas campestris*. It finds its wide application in food, cosmetics and pharmaceuticals because of its encouraging reports on safety [29]. The primary structure of this natural polysaccharide contains a cellulosic backbone (β-D-glucose residues) and a trisaccharide side chain of β-D-mannose-β-D-glucuronic acid-α-D-mannose attached with alternate glucose residues of the main chain [30,31]. The terminal D-mannose residue may carry a pyruvate function. The non-terminal D-mannose unit in the side chain contains an acetyl function. The anionic character of this polymer is due to the presence of both glucuronic acid and pyruvic acid groups in the side chain.

Thus, the objective of this study was to design an amphiphilic xanthan copolymer with improved solubilization potential and pharmacodynamic activity of glibenclamide, a poorly soluble oral hypoglycemic.

2. Materials and methods

2.1. Materials

Glibenclamide was a gift from Mylan Labs, R&D, Hyderabad, India. Xanthan gum, cetyl alcohol, and alloxan were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Dioxane and *N*, *N* dimethyl formamide (DMF) were purchased from Merck Specialties Pvt. Ltd., Mumbai, India. Thionyl chloride ($SOCl_2$) and sodium hydride (NaH) were from Spectrochem Pvt. Ltd., Mumbai, India. Benzoyl acetone was purchased from SRL Lab, Pvt. Ltd., Mumbai, India. All other reagents were of analytical grade.

2.2. Synthesis of xanthan copolymer

For the preparation of cetyl chloride, cetyl alcohol (3.33%, w/v) in chloroform and $SOCl_2$ (2:1) mixture was reflux condensed for 2 h at room temperature. Xanthan gum (3%, w/v) was gradually dispersed in DMF and stirred for 1.5 h and cooled (15 °C). To this, sodium hydride (1.67%, w/v) was added. After 20 min, cetyl chloride dispersion in DMF (6.67%, w/v) was transferred to the xanthan gum dispersion. Then, the resulting mixture was put into 50 ml of water and the pH was adjusted to 7.0 using glacial acetic acid. The copolymer was isolated by filtration, washed with ethanol (2× 50 ml) and dried at room temperature.

2.3. Characterization of the copolymer

Silver nitrate in ethanol test was carried out to confirm chlorination of cetyl alcohol. To a chlorinated sample of 10 mg, two to three drops of ethanol was added. Then, 1 ml of 0.1 M $AgNO_3$ solution in ethanol was added to the test sample, mixed well for few minutes to ensure precipitation. In case, no precipitation occurred after 5 min, the sample was heated at boiling point of water for 3–4 min in order to observe white color of silver chloride (AgCl).

Fourier transform infrared (FTIR) spectra of xanthan gum, cetyl alcohol and copolymer were recorded for the changes in functional groups (Perkin-Elmer, Spectrum RX1, UK) using potassium bromide pellets.

2.4. Preparation of drug-loaded micelle

Initially 10 mg of the copolymer was added to 10 ml of distilled water and allowed to dissolve by stirring in a magnetic stirrer for 1 h, with occasional moderate heating. In another test tube, the required amount of drug was dissolved in 3 ml of chloroform. The organic drug solution was added to the aqueous copolymer solution and stirred for 4 h till the complete evaporation of solvent. After that, the dispersion was filtered by a Whatman filter paper (No. 41). Different copolymer:drug weight ratio (1:0, 1:0.5, 1:1, and 1:1.5) was used for the micellar formulations and was denoted by S1, S2, S3 and S4, respectively.

2.5. Estimation of drug solubility

Accurately measured, 4 ml of the micellar dispersion was evaporated to dryness. To it, 10 ml of methanol was added and the absorbance was determined after appropriate dilution. The drug content was measured using the slope of standard curve. To judge the reliability of this estimation method, a recovery analysis was done by taking a known sample of copolymer and drug (1:1). The recovery averaged 98.26 ± 2.01%.

2.6. Determination of critical association concentration (CAC)

CAC of the copolymer was determined by a method reported earlier [32]. A concentrated solution of benzoyl acetone (BZA) in dioxane (5 mg/ml) was prepared. From this solution, 0.4 ml was pipette out into 25 ml volumetric flask and diluted to the mark with water (80 μg/ml) and was kept as stock solution. The various quantity of aqueous copolymer solution (100 μg/ml): 0.01, 0.1, 0.2, 0.3, and 0.4 ml was diluted to 10 ml.

In a series of test tubes, 3.6 ml of the diluted copolymer solution and 0.4 ml of the BZA stock solution was added. The samples were scanned in the UV range of 200–400 nm against respective blanks (UV1, Thermo Scientific, UK) and the absorbance values were noted at 245 nm and 319 nm. Instead of using 0.4 ml BZA stock solution, the same volume of water was used in the preparation of blanks.

2.7. Microscopic evaluation of micelles

The drug-loaded and the drug-free micelles were dispersed in distilled water and the dispersion was examined under Magnus digital microscope (Magnus MLX, Olympus, India). An optical combination of 10× eye piece and 4× objective was used. The photographs were captured by Moticam 1000 camera.

2.8. Measurement of size and zeta potential

Measurements were carried out with Malvern Zetasizer Nano ZS 90 apparatus (Malvern Instruments, Worcestershire, UK) equipped with a DTS 1060 cell. A 1:11 (v/v) dilution of the dispersion of nanoparticles in 1 mM NaCl was done. Other measurement conditions: temperature 25 °C; dielectric constant of 78.5; refractive index of 1.33; viscosity of 0.8872 cP; and cell voltage of 150 V. The zeta potential was calculated from the electrophoretic mobility by the Smoluchowsky equation.

2.9. In vitro drug release study

Paddle-type dissolution rate test apparatus (VDA-6D, Veego Instruments Corporation, Mumbai, India) was used to test the