Preparation of Glibenclamide Nanoparticles

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Abstract: One of the progressive ways for increasing oral bioavailability of drug substances with poor solubility is nanoparticles preparation. Glibenclamide (Biopharmaceutical Classification System class II) was chosen as a model compound with low solubility and high permeability. Nanoparticles were prepared either by the antisolvent precipitation – solvent evaporation method or by the emulsion solvent evaporation method. Sodium dodecyl sulphate and macrogol 6000 were used as stabilizing excipients in aqueous solutions with the mass concentrations of 1, 3 and 5%. Acetone and dichloromethane were used as glibenclamide solvents. Ten samples were characterized by dynamic light scattering. The particles size of seven samples ranged from 4.4 to 27.0 nm.

Keywords: Glibenclamide; Nanoparticles; Sodium dodecyl sulphate; Macrogol; Dynamic light scattering.

INTRODUCTION

Application of hardly soluble drugs in therapy remains challenging for science. Drugs with low solubility will have poor absorption and bioavailability [1]. Almost 40% of newly discovered drugs are poorly soluble in water, so their suitable dosage forms are being discovered for potential treatment [2]. Hydrophobic character is demanded for affinity toward the target receptors [3] and for intracellular targeting [4]. Poorly aqueous soluble drugs belong to Class II of the Biopharmaceutical Classification System (BCS). Drugs of the mentioned class are characterized by low aqueous solubility and high permeability [5]. In such case the dissolution rate of the drug becomes a limiting step for absorption [6].

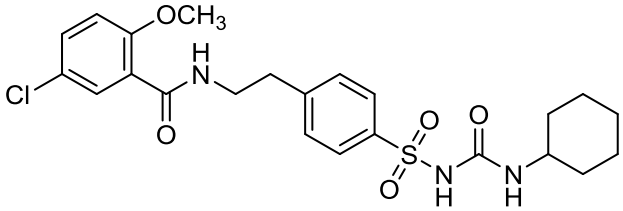
Glibenclamide, 5-chloro-*N*-(2-{4-[(cyclohexylcarbamoyl)sulfamoyl]phenyl}ethyl)-2-methoxybenzamide (see Fig. 1) was chosen as a representative of poorly aqueous soluble

compounds belonging to class II of BCS [7]. It is a sulfonylurea derivate that is used for treatment of diabetes mellitus of type II. It stimulates release of insulin from pancreatic β cells and enhances the sensitivity of peripheral tissue to insulin [8]. Glibenclamide is practically insoluble in water, which causes only 45% oral bioavailability of this drug. Some approaches of increasing its solubility can be found; preparation of glibenclamide nanoparticles via antisolvent precipitation using the high gravity technique [9] could be one of the examples. Other approaches of nanoparticle generation are based on preparation of nano-drug delivery systems. Glibenclamide was adsorbed to nanocarriers, such as polymeric solid self-emulsifying drug delivery system [10] or xanthan-grafted-C_{16} polymer [11]. Also solvent displacement method was used for preparation of glibenclamide nanoparticles [12].

The aim of this study was to prepare stabilized glibenclamide nanoparticles via antisolvent precipitation for the following solubility test. It can be supposed that glibenclamide nanoparticles would have improved solubility. Surfactant sodium dodecyl sulfate and polymer macrogol 6000 were used as excipients/nanoparticle stabilizers and acetone and dichloromethane were used as antisolvents.

Polar and nonpolar solvents were used in this investigation, therefore the exact principle of the applied solvent evaporation method is dependent on the water-based system, including or not an aqueous miscible organic solvent. Polar acetone and nonpolar dichloromethane were chosen as the most suitable solvents for easy dissolution of glibenclamide, so two different possible mechanisms were used for the nanoparticle synthesis. When glibenclamide is dissolved in acetone and then mixed with water containing a stabilizer, nanoparticles are formed spontaneously and immediately upon mixing. This method can be called antisolvent precipitation – solvent evaporation, and the procedure is in principle similar to the evaporative precipitation into aqueous solution [13,14] or the liquid antisolvent precipitation [15]. When glibenclamide is dissolved in dichloromethane and then mixed with water containing stabilizers, an emulsion (o/w type) is formed; glibenclamide is clustered by the excipient, which results in the encapsulation of glibenclamide into nano-vesicula. This combination of emulsification and solvent evaporation nanoparticle synthesis is called emulsion solvent evaporation [16,17].

Figure 1. Structure of glibenclamide.



RESULTS AND DISCUSSION

Based on pilot screening [18,19] sodium dodecyl sulfate (SDS, sample series **1**, **2**) and macrogol 6000 (PEG, sample series **3**, **4**) were chosen as excipients. Three water solutions were prepared for each excipient with the mass concentration of 1% (samples **a**), 3% (samples **b**) and 5% (samples **c**). Glibenclamide as a poorly soluble drug was solved in dichloromethane (sample series **1**, **3**) as a non-polar solvent and acetone (sample series **2**, **4**) as a polar solvent. Drug solution was added to the solution of excipient under continuous stirring and stirred for 15 minutes. The organic solvent was evaporated in an ultrasonic bath that was used as complementary energy for nanoparticles preparation. Combination of all excipients with glibenclamide provided twelve samples, see Table 1. All prepared samples

were measured by dynamic light scattering [20]. The particle size distribution is presented in Table 1.

Table 1. Composition of samples, concentration [%] of individual excipients in water samples relative to glibenclamide, particle size [nm] of glibenclamide samples expressed as mean ± SD (n = 5 independent measurements). (SDS = sodium dodecyl sulfate, PEG = macrogol 6000, DCM = dichloromethane, AC = acetone, n.d. = immeasurable due to crystallization)

Sample	Excipient/solvent/ concentration[%]	Particle size [nm]
1a	SDS/DCM/1	95.4 ± 2.2
1b	SDS/DCM/3	81.3 ± 11.1
1c	SDS/DCM/5	4.4 ± 1.5
2a	SDS/AC/1	231.8 ± 18.1
2b	SDS/AC/3	7.0 ± 4.7
2c	SDS/AC/5	24.0 ± 8.4
3a	PEG/DCM/1	15.9 ± 6.9
3b	PEG/DCM/3	12.6 ± 0.2
3c	PEG/DCM/5	20.8 ± 0.2
4a	PEG/AC/1	27.0 ± 0.2
4b	PEG/AC/1	n.d.
4c	PEG/AC/1	n.d.

Due to crystallization of glibenclamide it was not possible to measure prepared samples **4b** and **4c**. Drug substance was not dissolved even after application of ultrasonic bath, thus only ten samples were evaluated. Nanoparticles of size under 200 nm were prepared in the case of nine samples. Sodium dodecyl sulfate in 1% concentration (sample **2a**) in combination with acetone provided the submicroparticles (232 nm) and in 1% and 3% concentrations (samples **1a**, **1b**) in combination with dichloromethane provided particle size near 100 nm. The smallest particles were found at 5% concentration of sodium dodecyl sulfate with dichloromethane (sample **1c**, 4.4 nm) and 3% concentration with acetone (sample **2b**, 7.0 nm). A significant effect of the applied excipient was observed for macrogol 6000 in combination with dichloromethane that gave nanoparticles in the size range from 12.6 to 20.8 nm.

These observations are shown in detail in Figures 2 and 3, where the dependences of particle size [nm] expressed as mean ± SD of glibenclamide dissolved in dichloromethane or acetone on the concentration [%] of sodium dodecyl sulfate (Fig. 2) and macrogol (Fig. 3) in water is illustrated.

The results showed that anti-solvent precipitation is an affordable method for preparation of nanoparticles. Chosen stabilizers are non-toxic and convenient for pharmaceutical and medical use. Glibenclamide nanoparticles prepared via anti-solvent precipitation can be used for pharmaceutical formulations and subsequent solubility tests.

Figure 2. Dependence of particle size [nm] of glibenclamide on concentration [%] of sodium dodecyl sulphate (SDS) in water. Particle size is expressed as mean ± SD (n = 5 independent measurements). Blue columns represent dichloromethane as glibenclamide solvent and red columns represent acetone. Sample **2a** is not illustrated completely for the sake of better lucidity.

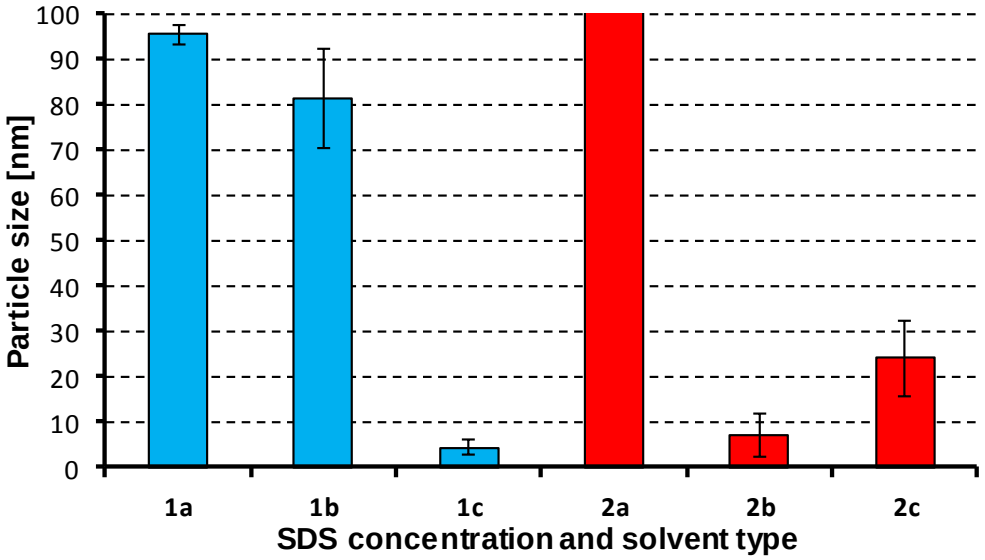
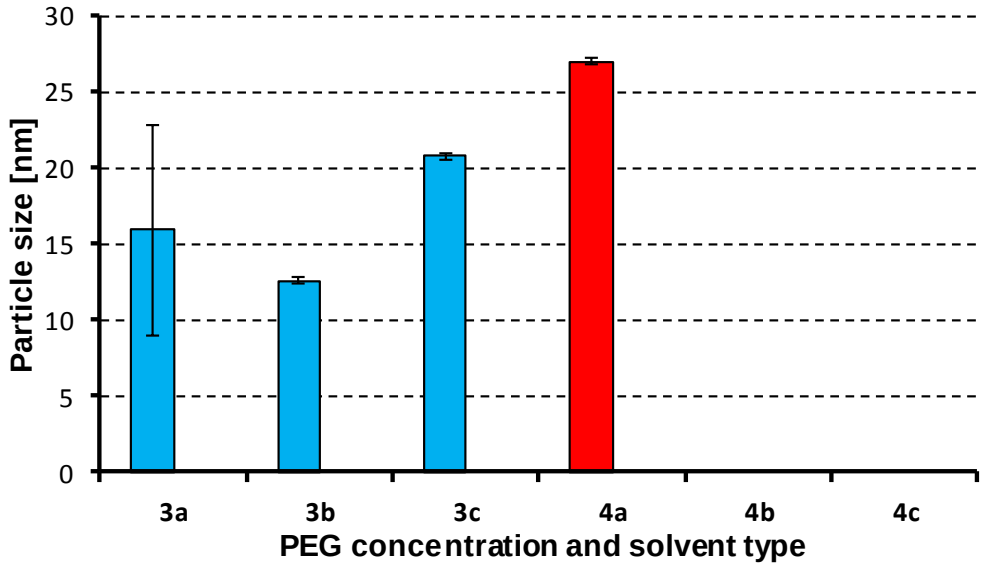


Figure 3. Dependence of particle size [nm] of glibenclamide on concentration [%] of macrogol 6000 (PEG) in water. Particle size is expressed as mean ± SD (n = 5 independent measurements). Blue columns represent dichloromethane as glibenclamide solvent and red columns represent acetone.



EXPERIMENTAL

Standardized General Procedure for Preparation of Nanoparticles

Glibenclamide and both excipients, sodium dodecyl sulfate and macrogol 6000, were purchased from Sigma-Aldrich (Germany). All compounds were of analytical grade. H_2O-HPLC – Mili-Q Grade was used as a solvent of excipients. Each excipient (0.1 g, 0.3 g or 0.5 g) was dissolved in the corresponding amounts of water, and three solutions with mass concentrations 1%, 3% and 5% were prepared. Glibenclamide (0.1 g) was dissolved in

dichloromethane or acetone, and solutions with mass concentrations 1% were prepared. The solution of glibenclamide in dichloromethane/acetone was slowly dropped (2 mL/min) to the aqueous solutions of excipients that were stirred (600 rpm). Then the system was stirred (600 rpm) for 15 min at 25 °C, after which the mixtures were transferred to the ultrasonic bath in a fume chamber, where they were mixed again for 20 minutes for homogenization of the samples. Finally the solvent was evaporated.

Dynamic Light Scattering Measurements

The particle size was determined using a Brookhaven dynamic light scattering system BI 9000 (Brookhaven Instruments Corporation, Holtsville, NY, USA) with a goniometer SM-200 and an argon gas laser (Lexel 95, wavelength 514.5 nm). Scattered intensity was registered at the scattering angle 90° and the temperature of 25 °C. All the samples were dispersed by sonication and additionally filtered directly before the measurement through syringe filters with 0.45 μm pore size to remove mechanical impurities. Five independent recordings of the autocorrelation function were done for each investigated excipient concentration. The particle size was calculated from the translational diffusion coefficient using the Stokes-Einstein formula. The translational diffusion coefficient was obtained based on the cumulant expansion of the autocorrelation function up to the second cumulant. The presented particle sizes are reported as the mean values taken of the set of five independent measurements. The results are summarized in Table 1 and illustrated in Figures 2 and 3.

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