



Preparation of Hydrochlorothiazide Nanoparticles

Eliska Vaculikova^{1,2}, Daniela Placha², Martin Pisarcik³ and Josef Jampilek^{1*}

¹ Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackeho 1/3, 612 42 Brno, Czech Republic; e-mail: josef.jampilek@gmail.com

² Nanotechnology Centre, VSB – Technical University of Ostrava, 17. listopadu 15/2172, 708 33 Ostrava – Poruba, Czech Republic

³ Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University, Odbojarov 10, 832 32, Bratislava 3, Slovakia

* Authors to whom correspondence should be addressed.

Abstract: Aqueous solubility and permeability through biomembranes are important parameters for drug bioavailability. Nanoparticles can be considered as a useful tool for improving properties of poorly soluble and/or permeable active ingredients. Hydrochlorothiazide (Class IV of BCS) was chosen as a model compound. Antisolvent precipitation – solvent evaporation and emulsion solvent evaporation methods were used as techniques for preparation of twelve samples containing hydrochlorothiazide nanoparticles. Water solutions of the surfactants sodium dodecyl sulfate and Tween 80 were used in mass concentrations of 1, 3 and 5%. Acetone and dichloromethane were used as solvents of model compound. The particle size of the prepared samples was measured by dynamic light scattering. The particle size ranged from 4.2 to 102.2 nm. Tween 80, that yielded nanoparticles <15 nm, was a preferable excipient to sodium dodecyl sulfate.

Keywords: Hydrochlorothiazide; Nanoparticles; Sodium dodecyl sulfate; Tween; Dynamic light scattering.

INTRODUCTION

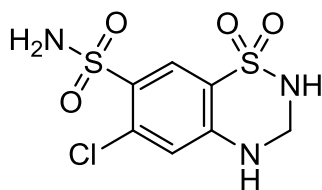
Hydrochlorothiazide (Figure 1), 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, is a thiazide diuretic that is frequently used in antihypertensive therapy in combination with antihypertensive agents. Hydrochlorothiazide affects the distal renal tubular mechanism of electrolyte reabsorption: it increases excretion of sodium and chloride ions [1]. Due to this increased excretion, it increases diuresis. Hydrochlorothiazide is not a first choice drug for hypertension treatment at patients with diabetes mellitus because of metabolic side effects. Hydrochlorothiazide can cause hyperglycaemia, it can worsen glucose tolerance and cause hypercholesterolemia [2]. There is a question if hydrochlorothiazide

nanoparticles would have less adverse effects in a range of metabolic side effects. Hydrochlorothiazide belongs to Class IV of the Biopharmaceutical Classification System (BCS). Drugs of the mentioned class are characterized by poor water solubility and low permeability; it means they are problematic from the point of view of oral bioavailability [3]. Some nanonized drug substances, such as megestrole acetate (Megace[®]), methylphenidate (Ritalin[®]), aprepitant (Emend[®]), sirolimus (Rapamune[®]) [4] and nanonized fenofibrate [5], have been used in human treatment. Doses of nanodrugs can be lower than doses of bulk drugs, which results in lower metabolic load and lower incidence of side effects [4].

The aim of this study was to investigate what surfactant is more convenient for preparation of stabilized hydrochlorothiazide nanoparticles via antisolvent precipitation with the following solubility and permeability experiments. It can be supposed that hydrochlorothiazide nanoparticles would have improved bioavailability. There are two main approaches of nanoparticles preparation: bottom-up and top-down. Top-down techniques are based on milling or high-pressure homogenization. The bottom-up approach is mainly based on precipitation [4,6–8]. This approach was used in this study.

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 hydrochlorothiazide; so two different possible mechanisms were used for the nanoparticle synthesis. When hydrochlorothiazide 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 [9,10] and the liquid antisolvent precipitation [11]. When hydrochlorothiazide is dissolved in dichloromethane and then mixed with water containing stabilizers, an emulsion (o/w type) is formed; hydrochlorothiazide is clustered by the excipient, which results in the encapsulation of hydrochlorothiazide into nano-vesicula. This combination of emulsification and solvent evaporation in nanoparticle synthesis is called emulsion solvent evaporation [12,13].

Figure 1. Structure of hydrochlorothiazide.



RESULTS AND DISCUSSION

Based on the pilot screening [14,15], excipients such as sodium dodecyl sulfate (Series 1, 2) and Tween 80 (Series 3, 4) were chosen from the group of surfactants. Three water solutions of 1%, 3% and 5% mass concentration were prepared. Hydrochlorothiazide was solved in dichloromethane (Series 1, 3) as a non-polar solvent and acetone (Series 2, 4) as a polar solvent. Drug solution was added to the solution of excipient under continuous stirring. Organic solvent was evaporated in an ultrasonic bath that was used as a source of complementary energy for nanoparticles preparation. The combination of all excipients with hydrochlorothiazide provided twelve samples, see Table 1. All prepared samples were measured by dynamic light scattering [16], *i.e.*, the particle size and values of polydispersity index were determined (see Table 1). The suitability of the used surfactant and the organic solvent was analysed.

Table 1. Composition of samples (dichloromethane Series **1, 3**; acetone Series **2, 4**), concentration [%] of individual excipients in water samples relative to hydrochlorothiazide, particle size [nm] and polydispersity index (PDI) of hydrochlorothiazide samples expressed as mean \pm SD ($n = 5$ independent measurements). (SDS = sodium dodecyl sulfate, TW = Tween 80, DCM = dichloromethane, AC = acetone, n.d. = immeasurable due to crystallization)

Sample	Excipient/solvent/concentration [%]	Particle size [nm]	PDI
1a	SDS/DCM/1	24.5 \pm 3.6	0.654 \pm 0.073
1b	SDS/DCM/3	102.2 \pm 17.6	0.384 \pm 0.021
1c	SDS/DCM/5	21.0 \pm 1.2	0.356 \pm 0.011
2a	SDS/AC/1	9.6 \pm 0.4	0.302 \pm 0.014
2b	SDS/AC/3	4.2 \pm 0.6	0.300 \pm 0.027
2c	SDS/AC/5	7.6 \pm 1.2	0.271 \pm 0.012
3a	TW/DCM/1	10.5 \pm 0.5	0.177 \pm 0.020
3b	TW/DCM/3	13.6 \pm 0.7	0.189 \pm 0.008
3c	TW/DCM/5	n.d.	n.d.
4a	TW/AC/1	10.2 \pm 0.1	0.154 \pm 0.007
4b	TW/AC/3	9.3 \pm 0.3	0.128 \pm 0.057
4c	TW/AC/5	9.9 \pm 0.1	0.157 \pm 0.010

The investigated particles showed good particle size stability throughout the light scattering measurements, except for sample **3c** that could not be measured due to crystallization of hydrochlorothiazide. In the period of measurements, no significant deviations from the mean values of particle size, which could be a result of possible sample ageing, were observed. Also a regular visual check of the samples proved no changes in the sample structure, which was confirmed by the reproducible data obtained by the light scattering method. Nanoparticles of size ca. 100 nm were prepared only in the case of sodium dodecyl sulfate in 3% concentration (sample **1b**) in combination with dichloromethane. Sodium dodecyl sulfate in combination with dichloromethane provided particle size near 20 nm (samples **1a** and **1c**). Other prepared samples contained nanoparticles in the size range from 4.2 to 13.6 nm, mostly near 10 nm. These observations are shown in detail in Figures 2 and 3, where the dependences of particle size [nm] expressed as mean \pm SD of hydrochlorothiazide dissolved in dichloromethane or acetone on the concentration [%] of sodium dodecyl sulfate (Fig. 2) and Tween (Fig. 3) in water are illustrated.

The dispersity is a measure/degree of the homogeneity/heterogeneity of sizes of particles in a mixture/system. The uniformity of dispersed systems is expressed as polydispersity index (PDI), see Table 1. Low PDI values demonstrate narrow size distribution and uniformity of samples contrary to PDI \approx 1 that indicates that samples have a very broad size distribution, may contain large particles or aggregates and are not suitable for measurements [17,18]. In the prepared nanoparticles of hydrochlorothiazide PDI values ranged from 0.128 to 0.302, when the samples **1a–c** (with PDI values ranging 0.356–0.654) were eliminated.

It is evident that the combination of sodium dodecyl sulfate and dichloromethane was suitable at least within this study and the series of samples either for generation of samples with a larger size or for higher degree of heterogeneity. The results showed that acetone is a preferable solvent for generation of hydrochlorothiazide nanoparticles of a smaller size.

Nanoparticles prepared with acetone also featured little variation in the particle size. The combinations of Tween 80 in all the concentrations, except for 5% Tween 80 with dichloromethane that precipitated, provided a smaller particle size (<15 nm). On the other hand, nanoparticles stabilised with sodium dodecyl sulfate were more stable. No precipitation occurred after the preparation.

Figure 2. Dependence of particle size [nm] of hydrochlorothiazide dissolved in dichloromethane (DCM) or acetone (AC) on concentration [%] of sodium dodecyl sulfate (SDS) in water. Particle size is expressed as mean \pm SD ($n = 5$ independent measurements). Blue columns represent dichloromethane as hydrochlorothiazide solvent and orange columns represent acetone. Sample **1b** is not illustrated completely for the sake of better lucidity.

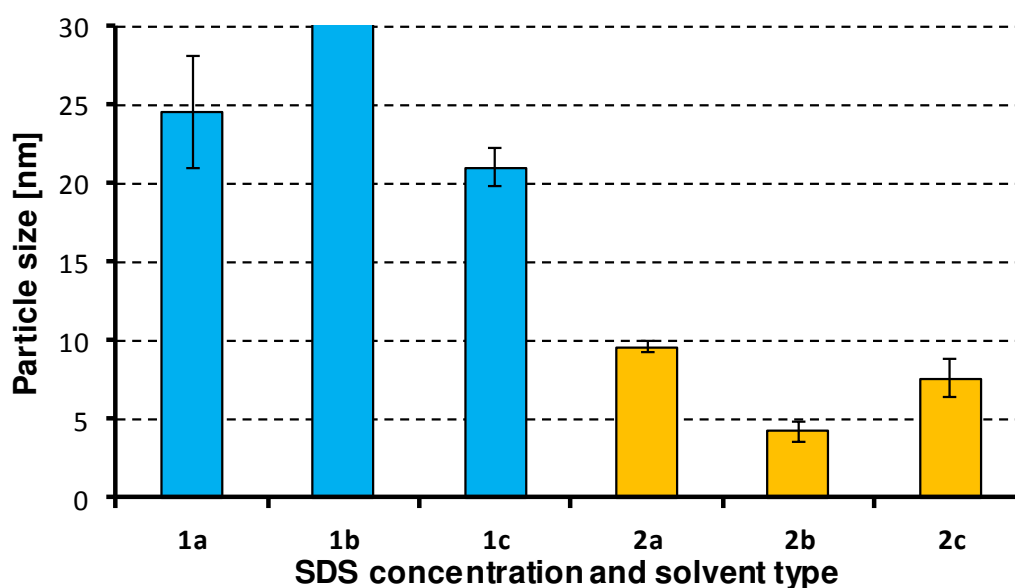
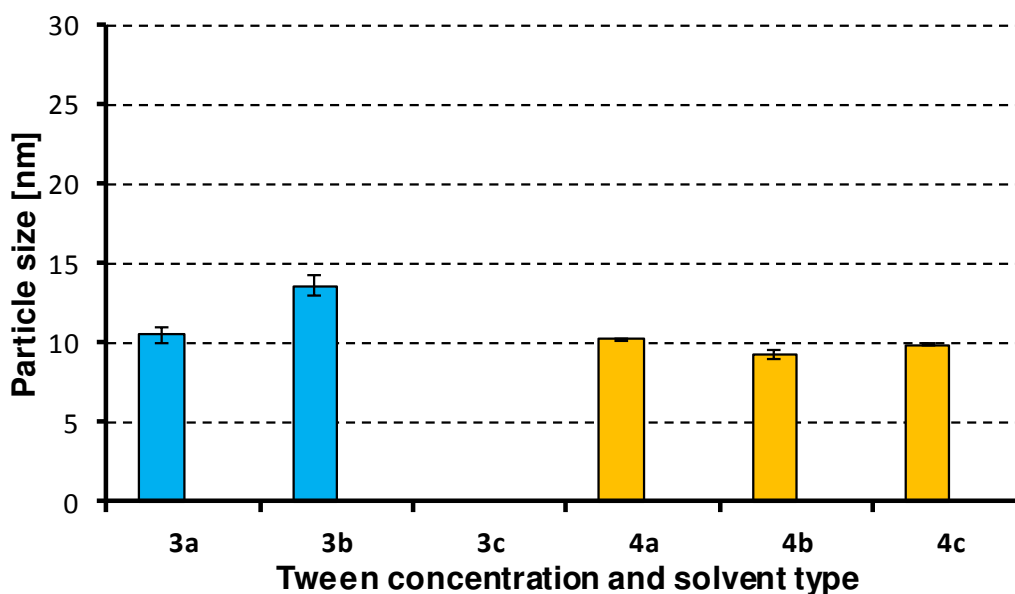


Figure 3. Dependence of particle size [nm] of hydrochlorothiazide dissolved in dichloromethane (DCM) or acetone (AC) on concentration [%] of Tween 80 (TW) in water. Particle size is expressed as mean \pm SD ($n = 5$ independent measurements). Blue columns represent dichloromethane as hydrochlorothiazide solvent and orange columns represent acetone.



EXPERIMENTAL

General Procedure for Preparation of Nanoparticles

The model compound hydrochlorothiazide and the excipients sodium dodecyl sulfate and Tween 80 were purchased from Sigma-Aldrich (Germany). All compounds were of analytical grade. H₂O-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 water (10 mL), and three solutions with mass concentrations 1%, 3% and 5% were prepared. Hydrochlorothiazide (0.1 g) was dissolved in dichloromethane or acetone (10 mL), *i.e.* 1% solutions were prepared. The solution of hydrochlorothiazide in dichloromethane/acetone was slowly dropped (2 mL/min) to the aqueous solutions of excipients, and the solutions were stirred at 600 rpm for 15 minutes at 25 °C, after which the mixtures were transferred to the ultrasonic bath in the 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.

ACKNOWLEDGEMENTS

This study was supported by GACR P304/11/2246, by the IT4Innovations Centre of Excellence (CZ.1.05/1.1.00/02.0070), by SP2015/56 project and by Slovak Research and Development Agency Grant No. APVV-0516-12 (Small Molecules in Biomedical Research).

REFERENCES

1. Zestoretic SPC. Available online: <https://www.medicines.org.uk/emc/medicine/2309> (accessed on 31 August 2015).
2. Drugs.com-Know more. Be sure. Available online: <http://www.drugs.com/sfx/hydrochlorothiazide-side-effects.html> (accessed on 31 August 2015).
3. Reddy, B.B.K.; Karunakar, A. Biopharmaceutics classification system: A regulatory approach. *Dissol. Technol.* **2011**, *3*, 31–37.
4. Nekkanti, V.; Vabalaboina V.; Pillai R. Drug nanoparticles – An overview. In *The Delivery of Nanoparticles*; Hashim, A.A. (Ed.); InTech: Rieka, Croatia, 2012, pp. 111–132.
5. Maciejewski, S.; Hilleman, D. Effectiveness of a fenofibrate 145-mg nanoparticle tablet formulation compared with the standard 160-mg tablet in patients with coronary heart disease and dyslipidemia. *Pharmacotherapy* **2008**, *28*, 570–575.
6. Vaculikova, E.; Placha, D.; Cech-Barabaszova, K.; Jampilek, J. Cimetidine nanoparticles study. *Adv. Sci. Eng. Med.* **2014**, *6*, 477–481.

7. Vaculikova, E.; Placha, D.; Pisarcik, M.; Peikertova, P.; Dedkova, K.; Devinsky, F.; Jampilek, J. Preparation of risedronate nanoparticles by solvent evaporation technique. *Molecules* **2014**, *19*, 17848–17861.
8. Vaculikova, E.; Placha, D.; Pisarcik, M.; Jampilek, J. Preparation of glibenclamide nanoparticles. Proceedings: *The 18th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-18)*, November 1-30, 2014, b025, <http://www.sciforum.net/conference/ecsoc-18/paper/2573>. Editors: Julio A. Seijas, M. Pilar Vazquez Tato, Shu-Kun Lin, CD-ROM edition. Published in 2015 by MDPI, Basel, Switzerland.
9. Sanggu, K.; Waikiong, N.; Yuancai, D.; Surajit D.; Tan, R.B.H. Preparation and physicochemical characterization of trans-resveratrol nanoparticle by temperature-controlled antisolvent precipitation. *J. Food Eng.* **2012**, *108*, 37–44.
10. Chin, S.F.; Pang, S.C.; Tay, S.H. Size controlled synthesis of starch nanoparticles by a simple nanoprecipitation method. *Carbohydr. Polym.* **2011**, *86*, 1817–1819.
11. Thorat, A.A.; Dalvi, S.V. Liquid antisolvent precipitation and stabilization of nanoparticles of poorly water soluble drugs in aqueous suspensions: Recent developments and future perspective. *Chem. Eng. J.* **2012**, *181/182*, 1–34.
12. Zielinska-Jurek, A.; Reszczynska, J.; Grabowska, E.; Zaleska, A. Nanoparticles preparation using microemulsion systems. In: *Microemulsions – An Introduction to Properties and Applications*; Najjar, R. (Ed.); InTech: Rijeka, Croatia, 2012, pp. 229–250.
13. Lee, M.; Cho, Y.W.; Park, J.H.; Chung, H.; Jeong, S.Y.; Choi, K.; Moon, D.H.; Kim, S.Y.; Kim, I.S.; Kwon, I.C. Size control of self-assembled nanoparticles by an emulsion/solvent evaporation method. *Colloid Polym. Sci.* **2006**, *284*, 506–512.
14. Vaculikova, E.; Grunwaldova, V.; Kral, V.; Dohnal, J.; Jampilek, J. Primary investigation of the preparation of nanoparticles by precipitation. *Molecules* **2012**, *17*, 11067–11078.
15. Vaculikova, E.; Grunwaldova, V.; Kral, V.; Dohnal, J.; Jampilek, J. Preparation of candesartan and atorvastatin nanoparticles by solvent evaporation. *Molecules* **2012**, *17*, 13221–13234.
16. Merkus, H.G. *Particle Size Measurements: Fundamentals, Practice, Quality*. Springer Science+Business Media B.V.: Dordrecht, The Netherlands, 2009.
17. Win KY, Feng SS. Effects of particle size and surface coating on cellular uptake of polymeric nanoparticles for oral delivery of anticancer drugs. *Biomaterials* **2005**, *26*, 2713–2722.
18. Malvern Instruments Ltd: Dynamic Light Scattering Common Terms Defined. Available online: http://www.biophysics.bioc.cam.ac.uk/wp-content/uploads/2011/02/DLS_Terms_defined_Malvern.pdf (accessed on 6 September 2015).