Preparation of Candesartan Cilexetil Nanoparticles by Precipitation

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Abstract: The ADME of a drug involves its transport across cell membranes. This process is essential and influenced by the characteristics of the drug, especially its molecular size and shape, solubility at the site of its absorption, relative lipid solubility, etc. Over the last ten years, the number of poorly soluble drugs has steadily increased. One of the progressive ways for increasing bioavaibility is a technique of nanoparticles preparation, which allows many drugs to reach the site of action. Candesartan cilexetil (BCS class II) was chosen as a model compound. Twenty samples were prepared by precipitation with various surface-active excipients. All the prepared samples were analyzed by means of photon cross-correlation sensor Nanophox. Eighteen samples contained nanoparticles smaller than 200 nm. Relationships between solvents and used excipients and their amount are discussed.

Keywords: Candesartan cilexetil; Nanoparticles; Precipitation; Dynamic light scattering.

INTRODUCTION

Development in the field of pharmaceutical administration has resulted in the discovery of highly sophisticated drug delivery systems that allow for the maintenance of a constant drug level in an organism. Contrary to these revolution biopharmaceutical results, over the last ten years, the number of poorly soluble drugs has steadily increased. Estimates state that 40% of the drugs in the pipelines have solubility problems. Literature states that about 60% of all drugs coming directly from synthesis are nowadays poorly soluble [1,2].

Modification/optimization of unfavourable physico-chemical properties of these drugs is possible through increasing their water solubility or improving permeability. There are many ways to solubilize certain poorly soluble drugs.

Aqueous solubility can be increased by chemical exchange: (*i*) salts, co-crystals or solvates formation (affects also chemical stability, polymorphism, technological workability); (*ii*) substitution by hydrophilic groups (effect of drugs with small molecules can be decreased); (*iii*) prodrug preparation (hydrolyzable amides or semiesters with polybasic acids). In general, the following structural modifications are the best way to improve permeability: (*i*) replacement of ionisable groups by non-ionizable groups; (*ii*) increase of lipophilicity; (*iii*) isosteric replacement of polar groups; (*iv*) esterification of carboxylic acid; (*v*) reduction of hydrogen bonding and polarity; (*vi*) reduction of size; (*vii*) addition of a non-polar side chain; (*viii*) preparation of prodrugs. Pre-formulation/formulation can be another and mostly successful strategy for improving aqueous solubility and/or permeability and subsequently bioavailability. For example, selection of a suitable salt, particle size reduction (till nano size) connected with an increase of the surface area, change of polymorphic forms, selection of appropriate excipients to function as solubilizers/transporters (surfactants or pharmaceutical complexing agents, permeability enhancers) can be used for the oral dosage form [3].

As mentioned above, one of the progressive ways is a technique of nanoparticle drug delivery that allows many pharmacological agents to reach the site of action. Compounds are attached or incorporated into nanoparticles which serve as a universal drug delivery system. The advantages of nanotechnology are as follows: *i*) increased bioavailability (quick dissolution; improved penetration through membranes); *ii*) lesser doses; *iii*) lower toxicity; *iv*) targeted biodistribution; *v*) reduction of influence of food on variability; *vi*) quicker development of formulations [4]. Nanoparticles less than 200 nm are of practical importance [5].

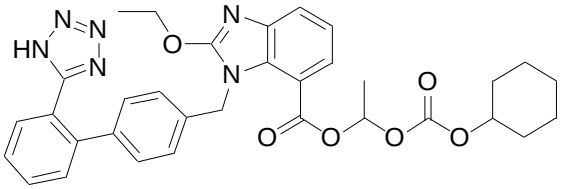
A wide range of techniques have been developed for the preparation of nanomaterials. Synthesis methods for nanoparticles are typically grouped into two categories: top-down and bottom-up. The first involves division of a massive solid into smaller portions. This approach may involve milling or attrition, chemical methods, and volatilization of a solid followed by condensation of the volatilized components, e.g. high-energy ball milling, high-pressure homogenization, emulsifying technology, microfluidization [6-16]. The second, bottom-up, method of nanoparticle fabrication involves condensation of atoms or molecular entities in a gas phase or in solution such as sol-gel synthesis [7,16] and precipitation processes, for example, spray freezing into liquid, evaporative precipitation into aqueous solution, precipitation with compressed antisolvent or rapid expansion of supercritical solution [7,9, 17-19]. The latter approach is far more popular in the synthesis of nanoparticles [20].

Candesartan (2-ethoxy-1-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1*H*-1,3-benzodiazole-6-carboxylic acid) is an angiotensin II receptor antagonist used mainly for the treatment of hypertension. The prodrug candesartan cilexetil, see Fig. 1, is marketed by AstraZeneca and Takeda Pharmaceuticals, commonly under the trade names Blopress®, Atacand®, Amias®, and Ratacand®. Candesartan cilexetil is a BCS class II drug having low aqueous solubility and high permeability; hence its bioavailability is solubility rate limited [21,22]. For enhancement of solubility of candesartan cilexetil can be used various approaches, such as pectin complexes [23], self emulsifying drug delivery systems [24] or development of nanoparticle formulations [25].

This contribution deals with preparation of nanoparticles of candesartan cilexetil by precipitation. The procedure is in principle similar to the solvent evaporation process, e.g. evaporative precipitation into aqueous solution [26]. Methods based on the similar approach were described recently [27-29]. In the pilot screening various steroids as model compounds and a number of types of surface-active agents/pharmaceutical adjutants were

investigated [30]. These surfactants/excipients belong to GRAS (Generally Recognized as Safe) substances and were applied in various concentrations. This contribution is the result of our interest in primary screening of nanoparticle preparation. Relationships between a substance, a solvent and a used excipient are discussed.

Figure 1. Structure of candesartan cilexetil as prodrug.



RESULTS AND DISCUSSION

As discussed above, nanoparticle technology is emerging as a preferred approach to address challenges involved in the delivery of BCS class-II compounds (poorly soluble and highly permeable). Nanoparticles of candesartan cilexetil would result in enhanced bioavailability, reduced systemic variability and more convenient dosing regimen.

Based on pilot screening [30], a simple method of preparation of nanoparticles was developed. Candesartan cilexetil dissolved in polar (acetone) and nonpolar (dichloromethane) solvents (2% concentration) was added to aqueous solutions (5% or 10% concentration) of excipients such as Tween 80, sodium dodecyl sulfate, macrogol 6000, sodium carboxymethyl cellulose and sodium carboxymethyl dextran. Samples were obtained by mixing and simultaneous evaporation of organic solvent to final 10 mL sample volume and then characterized by dynamic light scattering [31]. The final relations candesartan cilexetil:excipient were 1:2.5 (2%:5%) and 1:5 (2%:10%). All the results are presented in Table 1 in detail and summarized in Table 2. Figure 2 illustrates the dependence of particle size expressed as the cumulative distribution x_{90} [nm] of candesartan cilexetil on the concentration [%] of excipients in both solvents. The particle size x_{90} was used for evaluation of the method success, since this value represents 90% of the cumulative particle size distribution in the measured sample.

The dispersity is a measure/degree of the homogeneity/heterogeneity of sizes of particles in a mixture/system. It is possible to see this feature on the width of the particle-size distribution, which is described as differences between cumulative distribution x_{10} and x_{90}, see Table 1. According to the results, the average relation of the cumulative distribution x_{10}/x_{90} ranged from 0.5 to 0.9. It is possible to suppose that nanoparticles are spheres, because the size in dynamic light scattering means the hydrodynamic diameter of the particle. All samples were dispersed by ultrasonics directly before the measurement to avoid possible re-agglomeration. Stabilization of the dispersed samples was achieved by surfactants and by the constant temperature. The measuring cell was equilibrated at 25 °C, so the Brown motion of nanoparticles is influenced just by their size.

Twenty samples were prepared and analyzed by a Nanophox spectrometer. Sample **3** did not contain candesartan cilexetil nanoparticles (6.5 μm). Samples **1** and **12** contained nanoparticles greater than 200 nm (219 nm, 206 nm), and the rest of the samples (seventeen samples) contained nanoparticles less than 100 nm.

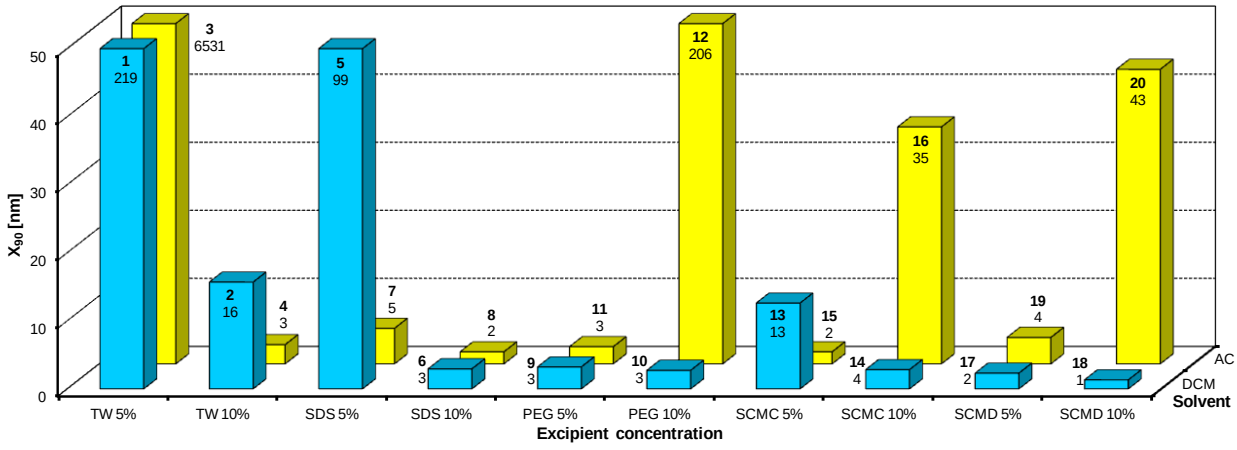
Table 1. Particle size (x_{10}, x_{90} [nm]) of candesartan cilexetil and concentration [%] of Tween 80 (TW), sodium dodecyl sulfate (SDS), macrogol 6000 (PEG), sodium carboxymethyl cellulose (SCMC), sodium carboxymethyl dextran (SCMD) in dichloromethane (DCM) or acetone (AC). All the presented results are reported as medium value of four independent measurements, repeatability was up to 7%. Samples that contained nanoparticles < 200 nm are bolded; nanoparticles < 10 nm are indicated by grey background. (S.No. = sample number)

Excipient/Solvent	Excipient concentration						
	S.No.	5%		S.No.	10%		
		x_{10}	x_{90}		x_{10}	x_{90}	
TW/DCM	**1**	160	219	**2**	14	**16**	Particle size [nm]
TW/AC	**3**	3183	6531	**4**	2	**3**	
SDS/DCM	**5**	90	**99**	**6**	2	**3**	
SDS/AC	**7**	4	**5**	**8**	1	**2**	
PEG/DCM	**9**	2	**3**	**10**	2	**3**	
PEG/AC	**11**	2	**3**	**12**	156	206	
SCMC/DCM	**13**	11	**13**	**14**	2	**3**	
SCMC/AC	**15**	1	**2**	**16**	32	**35**	
SCMD/DCM	**17**	2	**2**	**18**	1	**1**	
SCMD/AC	**19**	3	**4**	**20**	40	**43**	

Table 2. Summary of samples with formed nanoparticles. (dichloromethane = DCM, acetone = AC)

Excipient concentration [%]	Number of nanoparticle samples	Nanoparticle samples (DCM)	Nanoparticle samples (AC)
5	9	5	4
10	10	5	5

Figure 2. Dependence of particle size (x_{90} [nm]) of candesartan cilexetil on concentration [%] of Tween 80 (TW), sodium dodecyl sulfate (SDS), macrogol 6000 (PEG), sodium carboxymethyl cellulose (SCMC), sodium carboxymethyl dextran (SCMD) in dichloromethane (DCM) or acetone (AC). For clarity sake, the values on y-axis are only to 50 nm.



Based on the results shown in Tables 1, 2 and Fig. 2, it can be stated that 10% concentration (*i.e.* candesartan cilexetil:excipient rate 1:5) of excipients seems to be more advantageous than 5%, nevertheless if samples **1** and **3** are eliminated also 5% concentration (*i.e.* candesartan cilexetil:excipient rate 1:2.5) of excipients is sufficient, which is advantageous from the point of view pharmaceutical formulations. If sample **3** is eliminated, both nonpolar dichloromethane and polar acetone seem to have convenient properties as media for nanoparticle generation. Generally (for both solvents and concentrations) the worst results provided Tween (samples **1** and **3**) as also discussed previously [30]. Macrogol (sample **12**) and sodium dodecyl sulfate (sample **5**) afforded satisfactory nanoparticle size. Sodium carboxymethyl cellulose and sodium carboxymethyl dextran showed the best, well-balanced and comparable results. When individual excipients were compared from the point of view of their used concentration, sodium dodecyl sulfate provided the best results at 10% concentration, while macrogol 6000 and sodium carboxymethyl dextran afforded the best results at 5% concentrations. Sodium carboxymethyl cellulose provided excellent results both at 5% and at 10% concentration.

It can be concluded that the investigated precipitation method can be used as an effective and an affordable technique for the preparation of nanoparticles. The selected conditions are convenient for formation of nanoparticles, and the used excipients (except Tween 80) are principally applicable as nanoparticle stabilizers. After optimization of the amount of the excipient and selection of a convenient non-toxic organic solvent this method can be scaled up. Nanoparticles of candesartan cilexetil prepared in this manner can be subsequently used for nanoparticle formulations with supposed enhanced bioavailability.

EXPERIMENTAL

General

Candesartan cilexetil was purchased from Zentiva Prague (Czech Republic). All the excipients were purchased from Sigma-Aldrich (Germany). Dichloromethane was purchased from Merck (Germany). Acetone was purchased from LachNer (Czech Republic). All compounds as well as solvents were of analytical grade. H_2O-HPLC – Mili-Q Grade was used as a solvent of excipients. Particle sizes of all the final samples were determined using dynamic light scattering in a Sympatec Photon Cross-correlation Sensor Nanophox (Sympatec GmbH, System-Partikel-Technik, Germany), He-Ne laser 632.8 μm, intensity max. 10 mW. The measuring cell was equilibrated at 25 °C.

Synthesis

General procedure for preparation of nanoparticles: Tween 80, sodium dodecyl sulfate (SDS), macrogol 6000 (PEG), sodium carboxymethyl cellulose (SCMC) and sodium carboxymethyl dextran (SCMD) were used as excipients. Each excipient (0.5 g or 1.0 g) was dissolved in water (10 mL), and two solutions with concentrations 5% and 10% were prepared. Candesartan cilexetil (0.2 g) was dissolved in dichloromethane and acetone (10 mL), *i.e.* 2% solutions were prepared. The solution of the candesartan cilexetil in dichloromethane (DCM) or acetone (AC) 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 10 min at 35 °C, after which the mixtures were transferred to an ultrasonic bath in the fume chamber, where they were mixed again for 40 min, and simultaneously organic solvent was evaporated. The final volume of the aqueous sample was 10 mL. The particle size of nanonized substances in samples was evaluated by means of Nanophox. All samples were dispersed by ultrasonics directly before the measurement. Measurements were repeated four times. All presented results are reported as medium value of these independent measurements. Repeatability was up to 7%. The results are summarized in Table 1 and Table 2 and illustrated in Figure 2.

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