e-ISSN:2322-0139
p-ISSN:2322-0120

RESEARCH AND REVIEWS: JOURNAL OF PHARMACOLOGY AND TOXICOLOGICAL STUDIES

Influence of Darunavir: β-Cyclodextrin Complex on the Solubility of Darunavir.

Ana Carolina Kogawa*, Josilene Chaves Ruela Corrêa and Hérida Regina Nunes Salgado.

Department of Pharmaceutics, School of Pharmaceutical Sciences of Araraquara, Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP, Araraquara, São Paulo, Brazil.

Research Article

Received: 15/09/2014
Revised: 22/09/2014
Accepted: 29/09/2014

***For Correspondence**

Department of Pharmaceutics, School of Pharmaceutical Sciences of Araraquara, Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP, Araraquara, São Paulo, Brazil.
Phone: +55 16 3301 4681
Fax: +55 16 3301 6967

Keywords: Biopharmaceutics Classification System, child size, darunavir:β-cyclodextrin, solubility.

ABSTRACT

Darunavir, a protease inhibitor used in the treatment of HIV infection, was complexed with β-cyclodextrin because of its low solubility in water and poor bioavailability, with the objective of improving the solubility of darunavir aiming subsequently the administration of lower doses and increasing patient adherence to the treatment. Children under seven are usually unable to swallow the solid medications, especially tablets 300, 400 or 600 mg, such as darunavir. To make adult medicines suitable for children, tablets or capsules are often processed to adjust dosages and facilitate swallowing, but in most cases they do not support the medication. Therefore, complexation developed is extremely interesting. The last World Health Assembly launched the global campaign 'Make medicines child size'. The determination of the solubility of drugs is a fundamental part in Biopharmaceutics Classification System. In this research purified water with pH 8.0, acetate buffer 0.05 *M* pH 4.5, phosphate buffer 0.2 *M* pH 6.8 and phosphate butter 0.05 *M* with 0.5 % Tween pH 3.0 were evaluated in the solubility of darunavir:β-cyclodextrin complex. In all dissolution media tested complex showed solubility at least 5 times greater than the free drug.

INTRODUCTION

Darunavir (Figure 1), a protease inhibitor used in the treatment of HIV infection, has low solubility in water and poor bioavailability, therefore it requires administration in relatively high doses in order to exhibit therapeutic efficacy [1-2].

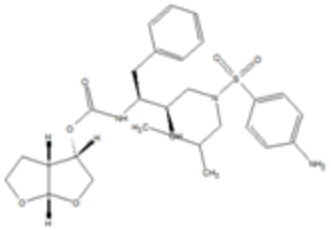

Figure 1: Chemical structure of darunavir (CAS 206361-99-1).

The important characteristics of a molecule that needs to be considered essential for anti-HIV effects are solubility, the extent to which a molecule of a solid is released from the surface by dissolution [3], and stability in biological fluids. When these properties are unfavorable for some specific drugs, the formulation and processing alternatives can be employed to achieve the maximum therapeutic gain [4].

e-ISSN:2322-0139
p-ISSN:2322-0120

The dissolution refers to the process by which drug molecules are released from the solid phase and enter the solution phase that is dependent on the time that represents the final step in the release of the active substance [5]. The drug release from the dosage form depends on the drug solubility characteristics as well as the formulation components and manufactory process.

The solubility of a drug depends on its molecular properties and its ability to form hydrogen bonds with water molecules. Factors such as pKa, pH, polymorphism, particle size, dosage form, excipients, manufacturing processes, intestinal transit, motility, volume and composition of intestinal fluids affect the solubility of the drugs.

According to the FDA [6], the solubility of a drug is determined by dissolving the higher dose of an immediate release dosage form in the volume of 250 ml, or less, of a buffer solution present in the range of pH 1.0 to 7.5 at a temperature of 37 °C.

The test of solubility of a drug must be performed by the methods of equilibrium, potentiometric or intrinsic dissolution. The equilibrium method is the most used, in which the saturation concentration of the drug, the speed and time of agitation are fundamental [6].

The solubility is a parameter used for the classification of drugs according to the Biopharmaceutics Classification System (BCS), as well as the intestinal permeability of the drug. Nowadays the BCS became an important tool for the development of formulations.

Darunavir: β-cyclodextrin complex (Figure 2) was carried out with the objective of improving the solubility of darunavir aiming subsequently the administration of lower doses and increasing patient adherence to the treatment [2]. Because using smaller doses the adverse drug reactions and drug interactions associated with antiretroviral therapy can be decreased. It is worth remembering that in chronic treatments even moderate toxicity can lead to serious complications [4].

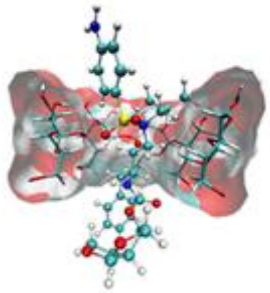

Figure 2: Darunavir: β-cyclodextrin complex, cluster-b.

Children under seven are usually unable to swallow the solid medications, especially high strength doses as 300, 400 or 600 mg, commonly doses for darunavir. To make adult medicines suitable for children, tablets or capsules are often processed to adjust dosages and facilitate swallowing. However, liquid formulations, essential for pediatric pharmacotherapy, are cause for growing concern in safety, efficacy and quality. Because organoleptic drawbacks that lead to avoidance and reduced adherence to ARV therapeutic regimes and a decrease in the bioavailability of the drug has been found in many cases [7].

As highlighted by Corrêa and coworkers, since the decrease of mortality from HIV/AIDS after introduction of antiviral treatment has a particular importance for the patients with high life expectancy as paediatric patients. However, treatment of HIV-positive children remains a challenge in HIV therapy, especially children younger than one year [1].

The last World Health Assembly approved the resolution “Best medicines for children” and has recently launched the global campaign ‘Make medicines child size’ [7]. Thus, the increased solubility of darunavir when complexed with β-cyclodextrin would contributes to a better treatment regimen for adults and children that are able to swallow small tablets or capsules.

The objective of this research was to determine the solubility of inclusion complex darunavir:β-cyclodextrin, according to the criteria established by BCS and to compare their results with those obtained with the free drug, previously studied by our research group [8].

e-ISSN:2322-0139
p-ISSN:2322-0120

EXPERIMENTAL

Materials

The materials used were darunavir:β-cyclodextrin complex [2], free darunavir lot SRP07000d (Sequoia Research Products, UK) and β-cyclodextrin (MW = 1135), kindly supplied by Roquette (France).

Method

For this solubility study the shake-flask method was applied. A shaker incubator MA 420 (Marconi™), a spectrophotometer UV 1800 (Shimadzu™) and quartz cuvettes with 1 cm optical path was used.

For the free drug solubility test, the drug was tested by equilibrium solubility measurement by saturation shake-flask method. The solubility was evaluated using physiologically relevant solution acetate buffer pH 4.5, phosphate buffer pH 3.0, phosphate buffer pH 3.0 with 0.5 % Tween and purified water. It was used 5 mL of medium were pre-heated to 37 °C ± 0.5 before adding a large amount of darunavir raw material. For darunavir:β-cyclodextrin complex an amount of powder equivalent to 5 mg of darunavir was weighed on an analytical balance model DV215CD (Ohaus™) from a pool of darunavir:β-cyclodextrin and transferred to each test tube containing 300 µL of the same test solutions.

The tubes were sealed with parafilm and rotated at 60 rpm in orbital shaking platform incubator. After 72 hours the samples were filtered through hydrophilic membrane of polytetrafluoroethylene (PTFE) with pore size of 0.45 m and diameter of 47.0 mm and some dilutions were carried out. For darunavir free drug, 0.2 mL of the filtered were pipetted into 5 ml volumetric flask, diluted with the same medium. For darunavir:β-cyclodextrin complex 20 µL of filtrate was diluted in varying amounts of dissolution medium to obtain absorbance of about 0.5 and the spectrophotometric reading performed at 267 nm in the zero order derivative UV spectrum. All solutions were measured at 276 nm in the first order derivative UV spectrum, always using dissolution media as blank. The used spectrophotometric method was previously validated [9]. The solubility in each medium was determined in triplicate.

To calculate the concentration was carried out a standard containing free darunavir at the concentration of 15 µg mL^{-1} for each tested solution. The results were obtained for darunavir free drug and darunavir:β-cyclodextrin complex were compared and the role performed by the complex over the darunavir solubility was evaluated.

RESULTS

The figure 3 shows the test tubes containing the darunavir:β-cyclodextrin and the excess at the bottom of the tube after 72 hours of test.

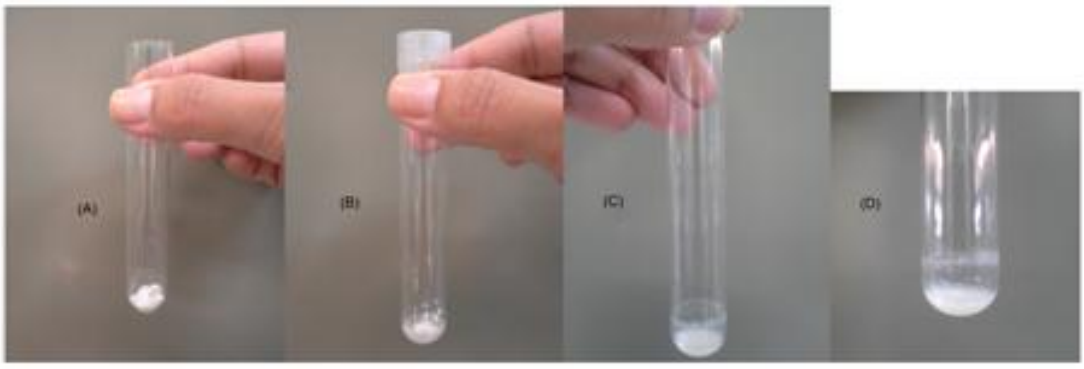

Figure 3: Tube containing (A) darunavir:β-cyclodextrin (B) in phosphate buffer solution 0.05 *M* with 0.5 % Tween pH 3.0, (C) after 72 hours on shaker at 60 rpm and 37 °C ± 0.5 °C. (D) Detail of the presence of precipitate, ensuring saturation of the solution.

The absorbance values of free darunavir at a concentration of 15 mg ml^{-1} in solvents tested are shown in Table 1.

e-ISSN:2322-0139
p-ISSN:2322-0120

Table 1: Absorbance values of free darunavir in different solvents at a concentration of 15 mg mL^{-1}

Concentration (µg mL^{-1})	Solvents	Average absorbance * 276 nm	Concentração (µg mL^{-1})
15	Purified water	0.024	17.98
	Acetate buffer, pH 4.5	0.021	16.83
	Phosphate buffer, pH 6.8	0.020	12.94
	Phosphate buffer, pH 3.0 (0.5 % Tween-20®)	0.023	16.83

*values corresponding to triplicate

Table 2 shows the comparisons of dilutions performed with the different solvents tested to obtain the same absorbance value.

Table 2: The needed dilution to obtain approximately the approximately the same absorbance value for each sample of darunavir:β-cyclodextrin complex at the end of the solubility test

Solvents	Diluition	Average absorbance * 276 nm	RSD (%)	Concentration (µg mL^{-1})
Purified water	6.0	0,017	1.16	3,834.72
Acetate buffer, pH 4.5	7.0	0,018	3.68	5,064.63
Phosphate buffer, pH 6.8	5.5	0,018	2.87	3,302.46
Phosphate buffer, pH 3.0 (0.5 % Tween-20®)	4.0	0,021	3.48	3,089.40

*values corresponding to triplicate

To prove that the β- cyclodextrin does not absorb at wavelengths used, the whole test was done using β- cyclodextrin alone. The values are shown in Table 3.

Table 3: Values of the absorbance of β-cyclodextrin in different solvents

Solvents	Average absorbance* 276 nm
Purified water	0,000
Acetate buffer, pH 4.5	0,000
Phosphate buffer, pH 6.8	0,000
Phosphate buffer, pH 3.0 (0.5 % Tween-20®)	0,000

*values corresponding to triplicate

The free darunavir had a relatively low solubility, mainly if compared to darunavir:β-cyclodextrin data. The dilution applied for the filtered samples of free drug was 0.2 mL of sample to 2 mL of tested solution. The results are shown in Table 4.

Table 4: Solubility of free darunavir accessed by equilibrium method

Solvents	Average absorbance* 276 nm	RSD (%)	Concentration (µg mL^{-1})
Purified water	0.009	3.45	166,83
Acetate buffer, pH 4.5	0.010	1.74	180,77
Phosphate buffer, pH 6.8	0.008	0.93	147,15
Phosphate buffer, pH 3.0 (0.5 % Tween-20®)	0.032	2.24	588.71

*values corresponding to triplicate

DISCUSSION

The solubility of a drug depends on its molecular properties and its ability to form hydrogen bonds with water molecules.

In the solubility test of darunavir:β-cyclodextrin complex were evaluated four solvents, purified water with pH 8.0, acetate buffer 0.05 *M* pH 4.5, phosphate buffer 0.2 *M* pH 6.8 and phosphate butter 0.05 *M* with 0.5 % Tween pH 3.0. The free darunavir presents pH 5.0 and five values of pKa 1.66, 1.76, 7.75, 11.43 and 14.31. The inclusion complex presents pH 4.5. Weakly acidic drugs, exposed to a

dissolution medium having a pKa greater than the drug pH tend to have increased solubility. Thus, the bioavailability is affected by changes in pH during the dissolution of the drug, and even by changes in the formulation of pharmaceutical forms [3].

The results showed that the β-cyclodextrin complex contributes to a huge increase in drug solubility. The complex has 28 times higher solubility than free darunavir in acetate buffer solution 0.05 *M* at pH 4.5; 23 times when using the water purified, 22 times when using phosphate buffer solution 0.2 *M* at pH 6.8 and 5 times when using phosphate buffer solution 0.05 *M* with 0.5 % Tween at pH 3.0.

This amazing improvement in solubility of darunavir can be explained by changes in the crystalline form of the drug. Darunavir:β-cyclodextrin has amorphous form. Amorphous form drugs has rapidly aqueous dissolution and are generally better absorbed, since the molecules are randomly arranged, requiring less energy to separate each other, resulting in a faster dissolution [10]. The darunavir solvated form, the commercial form of the drug [11], has a crystalline form with ordered form of molecules which requires more energy to break them apart. May be due to all this, the inclusion complex showed higher solubility in all solvents tested when compared to the free darunavir.

The determination of the solubility of drugs is a fundamental part in BCS and currently also assumes great political importance, since this test, together with the permeability, constitutes an essential criterion for bio-exemption in obtaining registration of generic and similar medicines. The bio-exemption enables the reduction of cost and time of drug development, which benefits the patient by having access to cheaper drugs that leads to compliance with pharmacotherapy, and the public health, by having greater diversity of drugs for the treatment of a large number of diseases.

CONCLUSION

Solubility test inclusion complex darunavir:β-cyclodextrin was performed by the equilibrium method and showed higher solubility than the free drug in all dissolution media tested, which makes this recent advancement of pharmaceutical technology an important milestone for the use of antiretroviral and for the public health.

We must remember that treatment failure not only affects the quality of life of patients, but also contributes significantly to the economic burden of the health system [4]. Therefore, complexation developed is extremely interesting, both from technological point of view and financial [2].

ACKNOWLEDGMENTS

The authors acknowledge CNPq (Brasília, Brazil), FAPESP (São Paulo, Brasil), CAPES (São Paulo, Brasil) and PADC/FCF/UNESP (Araraquara, Brazil).

REFERENCES

1. Corrêa JCR, D'arcy DM, Serra CHR, Salgado HRN. Darunavir: a critical review of its properties, use and drug interactions. Pharmacol. 2012;90:102-9.
2. Kogawa AC, Zoppi A, Quevedo MA, Longhi MR, Salgado HRN. Complexation between darunavir ethanolate and β-cyclodextrin. Experimental and theoretical studies. WJPPS. 2014;3(6):298-309.
3. Martinez MN, Amidon GL. A mechanism approach to understanding the factors affecting drug absorption: A review of fundamentals. J Clin Pharmacol. 2002;42:620-43.
4. Sharma P, Garg S. Pure drug and polymer based nanotechnologies for the improved solubility, stability, bioavailability and targeting of anti-HIV drugs. Adv Drug Delivery Rev. 2010;62:491-502.
5. Martin A, Sinko PJ. Martin: Físico-farmácia e Ciências Farmacêuticas. 5th Ed. Porto Alegre:Artmed; 2008.
6. FDA. Food and Drug Administration. Guidance for Industry. Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System; 2000. Available from http://www.fda.gov
7. Sosnik A, Chiappetta DA, Carcaboso AM. Drug delivery systems in HIV pharmacotherapy: What has been done and the challenges standing ahead. J Controlled Release. 2009;138:2-15.
8. Corrêa JCR, Serra CHR, Singh RSP, Derendorf H, Salgado HRN. Development of *in vitro* and *in silico* tools for the prediction of *in vivo* pharmacokinetics of darunavir tablets. II Congress of Brazilian Association of Pharmaceutical Sciences. 2014a. Abstract S11-15. Available from: http://www.congressoabcf.com.br/

9. Corrêa JCR, Serra CHR, Salgado HRN. Development and validation of first derivate spectrophotometric method for quantification of darunavir in tablets. Br J Pharm Res. 2014b;4(6):722-30.
10. Stulzer HK, Tagliari MP, Silva MAS, Laranjeira MCM. Desenvolvimento, avaliação e caracterização físico-química de micropartículas constituídas de aciclovir/quitosana desenvolvidas pela técnica de spray-drying. Lat Am J Pharm. 2007;26(6):866-71.
11. Gyseghem EV, Stokbroekx S, Armas HN, Dickens J, Vanstockem M, Baert L, et al. Solid state characterization of the anti-HIV drug TMC114: Interconversion of amorphous TMC114, TMC114 ethanolate and hydrate. Eur J Pharm Sci. 2009;38:489-97.