Published: February 05th, 2019

RESEARCH ARTICLE

Citation: Amaral et al. (2019) The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat. SPG BioMed 1(2).

Corresponding Author:

Marco Chaud

Laboratory of Biomaterials and Nanotechnology. LaBNUS – University of Sorocaba- UNISO. Sorocaba-SP. Brazil.

Email:

marco.chaud@prof.uniso.br

Funding: None.

Copyright:

© 2019 Science Publishing Group

This open access article is distributed under the terms of the

[Creative Commons Attribution Non-Commercial License](#).

The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat

Amaral, V.A.¹, Rios, A.C.¹, Baldo, D.A.¹, Souza, J.F.¹, Crescencio, K.M.M.¹, Batain, F.¹, Gremiao, M.P.D.², Alves, T.F.R.¹, Chaud, M.V.^{1*}

Author affiliations:

¹ Laboratory of Biomaterials and Nanotechnology. LaBNUS – University of Sorocaba- UNISO. Sorocaba-SP, Brazil.

² Department of Drugs and Pharmaceuticals, Faculty of Pharmaceutical Sciences, São Paulo State University-UNESP, Araraquara, SP, Brazil.

Abstract

This study has been undertaken to investigate the influence of copolymers poly-L-co-D, L- lactic acid (PLDLA) 70:30 and Poly (ethylene glycol)-block-poly (propylene glycol)-block-poly (ethylene glycol) (P407) in the transmural transport of 3'-azido-3'-deoxythymidine (azidothymidine (AZT), zidovudine). Based on anterior studies we use the proximal duodenum to evaluate, *in vitro*, the apparent intestinal permeability. The binary solid dispersion (SD) was prepared with, AZT, PLDLA, and P407. The supercritical antisolvent (SAS) technique was used to obtain the SD, whose was studied by scanning electron microscopy (SEM). The differential scanning calorimetry (DSC) showed that pure AZT had an endothermic peak corresponding to AZT melting point of 125,10 °C, which indicated its crystalline nature. In the SD the same melting point peak was observed. DSC thermogram indicated that thermal properties of P407 were altered in the SD, but the SD did not alter the thermal properties of AZT and PLDLA. Powder X-ray diffraction (X-RD) was used to detect, qualitatively, a material with long-range order. Sharper diffraction peaks indicate more crystalline AZT and P407. The diffraction spectrum of pure AZT and P407 confirmed their crystalline nature. Sharper diffraction peaks indicate more crystalline AZT and P407. The diffraction spectrum of pure AZT and P407 confirmed their crystalline nature. The X-RD spectrum of SD showed modifications in the crystalline lattice, the physical mixture

(PM) X-RD spectrum showed the crystalline nature of AZT and P407. The Fourier Transform Infrared Spectroscopy (FTIR) analysis seems to indicate the absence of chemical interaction between copolymer and AZT, in binary solid dispersion and the presence of drug as a dispersion in the copolymer's matrix. The SD modifies the crystalline state of AZT and P407 modify the morphology of PLDLA (30:70), and inhibited the efflux pump.

The transmural intestinal wall permeability can be evaluated, *ex vivo*, in a site-specific manner. Increase in intestinal permeability was observed in tan association of AZT with PLDL and P407 in a binary solid dispersion. SD can be used to altering abnormally the properties of drugs whose bioavailability is limited by the effect of the efflux pump.

The supercritical fluid is an important technology to build particle design under strict quality assurance, environment-friendly, and scale-up with compliance to good manufacture products.

Keywords: Binary Solid Dispersion, Supercritical Fluid Technology, Azidothymidine Bioavailability, HIV/AIDS, Transmural Permeability.

Introduction

The first generation of antiretroviral agents, starting in 1985 with Retrovir® (zidovudine, azidothymidine, AZT or 3-azido-2,3-dideoxythymidine) manufacture name of Glaxo Smith Kline approval date is on March 1987. The AZT was the first nucleoside reverse transcriptase inhibitors to be administered to patients with HIV-1 infection and the first antiretroviral drug to be approved and is still in the part of the line regimen in Highly Active Antiretroviral Therapy ^{1,2}.

Since the initial placebo-controlled trial the AZT showed improved survival among patients with AIDS ³. In spite, AZT has a higher solubility, some factors that limit its clinical use are its toxicity, suboptimal bioavailability (50-75%), which is limited by intestinal permeation rate, bone marrow aplasia, inhibition of mitochondrial machinery, high hepatic first-pass metabolism. Despite the good water solubility (16.3 mg.mL⁻¹; log P = 0.3), the average bioavailability of the solid oral forms, tablets or capsule is 60% to 65%. Peak and trough levels of 5.5 ± 2.7 μmol and 0.3 ± 2.7 μmol respectively were noted with a dosage of 200 mg (~740 μmol) every four hours, and this finding corresponds to less than 1% of the administered dose. This found confirms that the bioavailability is limited by its permeation rate ⁴. This lack of permeation is partially attributed to drug efflux transporters. Thus, the inhibition of both ABCG2 and P-gp is critical for enhancing AZT bioavailability ^{4,5}. Even so, the management of adverse effects involves the development of anaemia later in the course of AZT therapy may be an early indication of underlying malignant disease or opportunistic. Studies of the granulocyte-macrophage- colony stimulating factor showed its potential role in the management of AZT-related neutropenia. The neutropenia, by the way, may be associated with an increased incidence of bacterial infection and mucosal thrush. A headache can be controlled with the use of such analgesics. Nausea and vomit may respond to dose reduction or the concomitant use of anti-nausea medications.

The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat

Some antiretroviral drugs, including HIV protease inhibitors and reverse transcriptase inhibitors, such as abacavir and AZT are substrates as P-glycoprotein (Pgp, ABCB1) ⁶. Amphiphilic copolymers have shown that can effectively deplete tumorigenic intrinsically drug-resistant cancer stem cells. Interestingly, sensitization of multidrug resistance and inhibition of drug efflux transporters has similar activities in various experimental models⁷. Additionally, polymeric carriers can have a biological activity of their own. One such example is represented by a class of copolymers, called pluronic. Pluronics are the triblock polymers of poly (ethylene oxide) (PEO) and poly (propylene oxide) (PPO), arranged in PEO-PPO-PEO structure. The copolymer-cell interaction starts in the cell membrane, where drug efflux transporters are localized. Pluronics were shown to be potent inhibitors of ABC transporters, specifically Pgp, MRP, and BCRP⁸. However, increase in the permeation of AZT can lead to affect the margin of safety of the drug and make it difficult to manage the adverse effects. To meet this pharmaceutical challenge various solubilization technologies have been developed, including solid dispersions, nanocrystals, cyclodextrin complexes, and lipid formulations. With an accelerated increase in the number of FDA-approved products in recent years, solid dispersion is now firmly established as a platform technology for the formulation of poorly-soluble drugs ⁹. Solid dispersion technology has been applied to develop formulations with a high drug loading (e.g. Zidovudine Tablets USP 300 mg per tablet) or containing drugs with a high tendency to crystallize ¹⁰. However, the aim of this study was to improve the AZT permeation rate using Pluronic P-407 as an inhibitor of ABC transporters and PLDLA 30/70 to decrease the solubility of zidovudine and to prevent the re-crystallization with a crystal lattice different. To evaluate this proposal, we prepared a binary solid dispersion using supercritical fluid technology in mode anti-solvent system (SAS), employing dense carbon dioxide in its supercritical state as an anti-solvent,

Materials

Zidovudine (AZT) was gently donated by Cristália Products Chemical and Pharmaceutical LTDA, Brazil, Poly-L/D-lactide (PLDLA 70/30) was purchased of Sigma-Aldrich Chemical company, Pluronic P 407 was gently donated by Basf – Brazil.

Methods

Binary Solid Dispersion

The preparation of solid dispersion (SD) following model describe by Yoshida et al. (2015) with modifications. The solution of AZT-PLDLA and AZT-P407-PLDLA were prepared with solubilization of AZT and AZT-P407 (2:0.1) in ethanol. The PLDLA (3g) was dissolved in dichloromethane (DCM). The solutions of AZT or AZT-P407 were mixed with PLDLA/DCM and hermetically closed bottles. To prepare the solid dispersion using CO₂ supercritical in SAS mode (Waters Corporation, SAS-RESS Combined System, Pittsburgh, Pennsylvania), we used a shortcut method for predicting the solubility of solids in CO₂ ^{11,12}. Figure 1 shows the schematic representation of supercritical fluid machine. The correlative equation based on temperature, pressure, and density were used after the model had been “trained.” The experimental data points were fitted at 37 °C and 160 bars to that the SAS condition involves the phase transition from CO₂ supercritical (ScCO₂)-dissolved liquid solution to precipitates SD. As soon as the equilibrium conditions were reached the solutions of samples (AZT-PLDLA or AZT-P407-PLDLA) were released at a rate of 1.0 mL.min⁻¹ with the formation of the spray through a pneumatic nozzle. The flow of the solution passed through a capillary tube of 101.6 µm of diameter during 100 min. After this time the flow of CO₂ was kept at

The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat

60 min. The CO₂ flow rate (5.0 g.min⁻¹) was kept constant throughout the process. Finally, the vessel of precipitation was slowly depressurized. The solid dispersion (SD) samples were collected, fractionated, and stored in hermetically sealed vials and kept out of the light until the moment of use. To evaluate the effect of process was prepared samples of physical mixture (PM) of the formulations.

Figure 1: Schematic representation of supercritical fluid machine in mode SAS technique. Coll. Vess., means Collector Vessel

Chromatographic System and Instrumentation and Validation Method

A LC-10 AD system (Shimadzu – Kyoto- Japan) was used for the analysis of AZT, consisting of a degassing unit DGU-20A_{5R}, with SPD-M 10A Diode Array detector and SPD-10A-UV/VIS spectrophotometric detector, and a Rheodine VS 7125 injection valve with a 100 µL loop. The analytical column employed was Zorbax ODS (C18; 250 mm x 4.6 mm i.d. 5 µm particle size). The mobile phase was comprised of acetonitrile–water 15:85 (v/v). All separations were performed in isocratic elution mode at a flow-rate of 1.0 mL min⁻¹ and column condition was maintained at 25 °C.

To prepare the standard solution, the AZT was accurately weighed and dissolved in acetonitrile to prepare a primary stock solution at a concentration of 0.202 mg.mL⁻¹. This solution was diluted appropriately to obtain working standards in the concentration range of 0.8 – 12 µg.mL⁻¹. The methods validation was based on Cinto et al. with minor modification¹³.

The analytical method presented here was validated by confirming linearity over the tested concentration range and assessing specificity, precision, recovery and detection and quantitation limits. To examine the specificity of the proposed LC method, samples from inside the intestinal sac (without AZT) were analyzed to determine possible interferences and were used as blank and as blank plus analyte (with added to AZT). The linearity of the method was studied by injecting AZT

The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat

at five concentrations in the range 0.8 – 12 $\mu\text{g}.\text{mL}^{-1}$. Each concentration was prepared in triplicate. The mean peak areas versus concentration data was treated by least-squares linear regression analysis. The relative standard deviation for the slope and Y-intercept of the calibration curve was calculated. In linearity studies, the acceptance criterion is a correlation coefficient of at least 0.998. To examine the specificity of the proposed LC method, samples from inside the intestinal sac (without AZT) were analyzed to determine possible interferences and were used as blank and as blank plus analyte (with added to AZT). The linearity of the method was studied by injecting AZT at five concentrations in the range 0.8 – 12 $\mu\text{g}.\text{mL}^{-1}$. Each concentration was prepared in triplicate. The mean peak areas versus concentration data was treated by least-squares linear regression analysis. The relative standard deviation for the slope and Y-intercept of the calibration curve was calculated. In linearity studies, the acceptance criterion is a correlation coefficient of at least 0.998. The limit of detection (LOD) is expressed as a concentration at a specified signal-to-noise ratio, usually 2:1. The limit of quantitation (LOQ) is defined as the lowest concentration of the analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. The signal-to-noise ratio of 10:1 can be taken as LOQ of the method. The LOD and LOQ were determined using equations. (1) and (2), respectively:

$$\text{LOD} = \mu_{\text{br}} + K \sigma_{\text{br}} \quad (1)$$

$$\text{LOQ} = 10 \sigma/S \quad (2)$$

Where μ_{br} is the response of the white estimated as the mean of y_{br} , and σ_{br} is the standard deviation of measure of whit signal., estimated by S_{br} (slope of the standard curve), K is a constant witch was attributed the value 3.0. The correspondent LOD, express in unit of concentration (X_{LD}) was obtained by ($X_{\text{LD}} = K. S_{\text{br}} / b$), where b is the slope of the line. The correspondent LOQ, express in unit of concentration (X_{LQ}), where to K is attributed a value 10.0 was obtained by ($X_{\text{LQ}} = K. S_{\text{br}} / b$). To determine the intra-day and inter-day precision of the method, replicate sets (n= 3) of calibration samples were analyzed within the same day and between different days. Peak areas were determined and precision was expressed as relative standard deviation (RSD) of each curve. To confirm the accuracy of the proposed method, recovery studies were carried out by the standard addition technique in which quality control samples were prepared by spiking TC-199 with known amounts of the AZT to obtain three different concentration levels, within the calibration curve range. Each level of the drug was determined in triplicate by the LC assay. The amount of AZT recovered was calculated in relation to the added amount (recovery percent).

Drug content

Accurately weighed, each AZT loaded SD was entirely dissolved in 100 ml of acetonitrile, filtered and assayed for AZT content employing an HPLC method ¹⁴. The drug content was calculated as follows:

$$\text{DC} = \text{Ca AZT} / \text{Ct AZT} \times 100 \quad (3)$$

Where Ca and Ct are the actual and theoretical AZT concentration, respectively and filtered through a 0.45 μm membrane, were used throughout the study.

Solubility Equilibrium

The solubility equilibrium at AZT, AZT-PLDLA - SD and AZT-P407-PLDLA- SD, was performed using the analytical method of isothermal shaker and determination of AZT in aqueous solution by HPLC. After 48 h, the samples were filtered through hydrophobic polyvinylidene difluoride (PVDF) membranes (syringe filter - 0.45 μm), 0.1 mL was transferred to a volumetric flask, diluted to 10 mL with solution of acetonitrile: water (1: 1 v / v) and homogenized. The same process was applied to samples of 0.5 mL.

Scanning electron microscopy (SEM)

SEM photographs were obtained using a scanning electron microscope (LEO Electron Microscopy/ Oxford, Leo 440i, Cambridge, England) with a 15 kV accelerating electric tension. All samples were affixed to a brass specimen holder using double-sided adhesive tape, and the powders were made electrically conductive by coating with gold using a sputter-coater for 4 min at 15 mA.

Differential scanning calorimetry (DSC)

The Differential Scanning Calorimetry was utilized to detect the presence of crystalline AZT and glass transition temperatures of prepared SD. Samples were tested by accurately weighing 10 ± 1 mg of processed SD, unprocessed AZT, and unprocessed copolymers (PLDLA and P407) into aluminum pans. After sealing the pans, five small holes were created in the lid of each pan to allow moisture to escape during the heating cycle. The thermic events were obtained using DSC (Thermal Analyzer TA 60WS, DSC-60, Shimadzu, Kyoto, Japan). The samples were heated from 25 to 200 $^{\circ}\text{C}$ at a ramp rate of 10 $^{\circ}\text{C}/\text{min}$ with a modulation amplitude and period of 0.75 $^{\circ}\text{C}$ and 45 s, respectively. The test was performed under a nitrogen atmosphere with a controlled flow at 50 $\text{mL}\cdot\text{min}^{-1}$.

X-Ray Diffraction Analyses (XRD)

The X-RD pattern was performed on a Philips Analytical X Ray, XPert-MPD (Almelo, Netherlands) using Ni-filtered $\text{CuK}\alpha$ radiation. The samples were analyzed over a 2θ scale range of 5 – 40, with a scan step size of 0.02 $^{\circ}\cdot\text{s}^{-1}$, 1.55 $\text{\AA}$, 40kV and 25 mA. The patterns were collected using a step width of 0.02 $^{\circ}\cdot\text{s}^{-1}$ over a range of 5 – 40 at room temperature.

Fourier transform infrared spectroscopy (FTIR)

A FTIR analysis (Shimadzu, IRAffinity-1, Kyoto, Japan) was used to collect FT-IR spectra via Labsolutions Software v.2.10. The samples were prepared in KBr discs by means of a hydrostatic press. The FTIR spectra were collected between 4000 – 400 cm^{-1} , with a resolution of 4 cm^{-1} and 32 scans.

Intestinal Permeation Assay

The intestinal permeation assay was performed as described by Chaud et al. 2010, with modifications. Male adults rats ($260 \pm 15\text{g}$) were kept in fasting state for 8 h, anesthetized with sodium pentobarbital and the thin intestine was immediately dissected, washed with TC 199 solution to 10 $^{\circ}\text{C}$ and oxygenated ($\text{O}_2:\text{CO}_2$ —95:5). The intestine was then, gently inverted with the aid of a flexible stem (2.5 mm of diameter) with its extremity protected by a fine fabric, one of the extremities was closed using suture wire and the intestinal segment filled with TC 199 without glucose. The other extremity was then closed so that the length of the intestinal segment was 6.4

The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat

± 0.21cm and 0.5 cm of external diameter. Incubation was carried through in TC 199 with glucose solution, containing 1 mg.mL⁻¹ of AZT in all samples (AZT, AZT-PLDLA SD and PM, AZT-P407-PLDLA SD and PM) by 90 min, under soft agitation. The TC medium was maintained at 37 °C and oxygenated (O₂:CO₂—95:5), the oxygen control was controlled with Oximeter DM4P (Digicrom Analitica- São Paulo, Brazil). The contents of intestinal sac were filtered through a membrane (cut off 0.45 mm) and the AZT absorption was determined based on a method developed and validated by HPLC systems. The equation obtained by analytical curve was used to determine the AZT concentration. Each permeation assay was performed six time (n= 6).

The protocol for the study of intestinal drug permeation using everted rat intestinal sac was duly approved by the Ethics Committee on Animal Use (CEUA) of the University of Sorocaba (UNISO), protocol N° 091/2016.

Statistical analysis

The main results were presented with their standard deviation. The statistical significance of the difference in the parameters was determined using ANOVA followed by the Tukey test. When p value of < 0.05 the difference of results was considered statistically significant.

Results

Binary Solid Dispersion

The binary solid dispersion of zidovudine was obtained with PLDLA and P407. The Figures 1 A - B shows the photographic images of SD obtained by FSC-SAS method. In panel 1A the particles of the SD appear adhered to the surface of the wall of the precipitation vessel. In Panel 1B shows the SD particles deposited on the bottom of the collector vessel. This result confirms the obtention of SD with AZT/PLDLA/P407 using CO₂ supercritical technology in the SAS technical.

Figure 1: Photographic images of SD after depressurization. Panel A shows the interior of collector vessel. Panel B shows the bottom of the collector vessel with SD particles.

Method Validation Tests

Specificity retention time (t_R) of AZT was found to be 7.85 min with a flow rate of $1 \text{ mL} \cdot \text{min}^{-1}$ at detection wavelength 265 nm. The AZT peak did not overlap with peaks of any of the components of SD or TC-199 buffer, and TC-199 from inside the intestinal sac without AZT (blank), blank with AZT added and the sample from inside the sac incubated with AZT. The method is AZT specific and detects the peak of the drug in the SD and after intestinal permeation test. The analytical curve of AZT (Fig 2) was constructed in TC-199 buffer. This TC-199 medium was used as the solvent because this method is intended to be used to monitor concentrations of AZT rat intestinal sac absorption, *ex vivo* experiments. The coefficient of determination, denoted R^2 , was observed over the concentration range $0.8 - 12.0 \text{ } \mu\text{g} \cdot \text{mL}^{-1}$. Linear regression analysis data for the calibration plot (Fig 2) showed a good linear relationship between response and concentration; the determination coefficient was 0.9991 and the linear regression equation was $y = 23,127x + 145,430$. The limit of detection (LOD) and quantification (LOQ) of AZT were 2.99 and $14.47 \text{ } \mu\text{g} \cdot \text{mL}^{-1}$, respectively. This results indicated the excellent sensitivity of the analytical method. The intra- and inter-day precision of the method for AZT was evaluated by analyzing three replicates of 8, 10 and $12 \text{ } \mu\text{g} \cdot \text{mL}^{-1}$ quality control samples. Intra-day precision ranged from 3.1 to 6.0%, while the inter-day precision ranged from 2.8 to 4.7% as show Table 1. Given the complexity of the matrix, these results indicated the precision of method. The mean absolute recovery values of the present HPLC method throughout the linear range are presented in Table 2. The results show that the method is accurate and ensure reliable results since appropriate recovery values were 80–120%.

Figure 2: Analytical curve of AZT

Table 1: Intra-day and inter-day precision of quality control sample of AZT (n=3),

	<i>Theoretical ($\mu\text{g mL}^{-1}$).</i>	<i>Real ($\mu\text{g mL}^{-1}$)</i>	<i>Precision as RSD</i>
<i>Intra-day</i>	8.1	8.32	2.23
	10.09	10.18	2.09
	11.9	13.04	2.65
<i>Inter-day</i>	8.0	8.52	2.55
	10.1	10.15	3.1
	12.2	13.12	2.98

Table 2- Recovery of AZT from TC-199 buffer

<i>Concentration ($\mu\text{g mL}^{-1}$)</i>	<i>AZT recovery (%)</i>	<i>RSD</i>
8.1	99.86	2.07
10.09	102.55	2.42
11.9	102.89	2.66

Solubility equilibrium of AZT

The drug solubility is an equilibrium measure but also the dissolution rate at which the solid drug or the drug from the SD passes into solution is critically important when the dissolution time is limited, but this is no less important when the dissolution time is fast and of the drug has an important side effect. The apparent solubility of AZT (Figure 3) in the PM with PLDLA decreased when compared with. However, no significant difference statically ($p > 0.05$) was found. The apparent solubility of AZT in the SDs was more than AZT and PM ($p < 0.05$). Although, the apparent

The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat solubility of AZT-PLDLA-P407 SD is more than AZT-PLDLA SD no statistical difference was found ($p < 0.05$). Anyone statistical difference was found for SDs.

Figure 3: Apparent Solubility of AZT, PM and AZT-PLDLA-P407 SD (Different letters means that the results are statistically different).

Differential Scanning Calorimetry (DSC)

The thermograms of the PLDLA, P407, AZT, and SDs are illustrated in Figure 4. The DSC thermograms of each component, except PLDLA, exhibited a sharp endothermal peak to the melting point ($m.p$) of P407 (56.53 °C; onset: 49.28 °C; end set: 64.30 °C, and ΔH -116.59 J/g.), AZT (125.34 °C; onset: 122.62 °C, end set 128.07 °C, and ΔH -107.22 J/g. PLDLA thermogram exhibited an event correspondent at glass transition (T_g) onset 57.64 °C, end set 64.92 °C with the correspondent specific heat capacity of the 61.28 °C. The same result was found Motta et al. and Coimbra et al. 2008 ^{16,17}. The DSC thermogram of AZT-PLDLA SD exhibited a sharp endothermal peak to the melting point at 125.19 °C (ΔH -81.15 J/g correspondent at $m.p$ of AZT and a discrete T_g event of PLDLA, this result suggests that the fusion of AZT need of a minus fusion hate, when compared with AZT pure. In the AZT-PLDLA-P407 SD thermogram showed the same T_g event for PLDLA, and AZT melting point (125,10 °C, ΔH -91,16 J/g). However, the sharp event of the P407 not is present, suggesting a change to amorph state.

Figure 4: DSC thermographs of AZT, PLDLA, P407, AZT-PLDLA, and AZT- PLDLA- P407 SDs.

Fourier Transformation Infrared Spectroscopy (FTIR)

FTIR spectroscopic studies were conducted to detect possible AZT: carriers' interaction. The SD were compared with AZT, PLDLA, and P407. FTIR spectra of the binary SD (Figure 5) shown the IR of AZT, P407, PLDLA, AZT-PLDLA SD, AZT-PLDLA-P407 SD. The AZT spectrum showed the main peaks in 1087.85 cm^{-1} (C-OH), 1280.73 cm^{-1} attributed at C-O-C groups, a stretching band 1381.03 cm^{-1} (CH₃) and 1683.86 cm^{-1} correspondents at CH₂ of aromatic stretching. The C=O stretching was found in 1465.9 cm^{-1} , and the picks correspondent at the azide groups (C=N=N=N) was found in 2083.12 cm^{-1} . The chemicals groups O-H/N-H was found in 3462.22 cm^{-1} . P407 spectrum showed the specific stretching in 1107.14 cm^{-1} (C-O), and a sharp pick was observed in 2889.37 cm^{-1} attributed at aliphatic CH. The broad pick in 3485.37 cm^{-1} was attributed at OH. The bands corresponding to PLDLA occur at 1749 cm^{-1} and 1185 cm^{-1} (axial pick of C=O of ester grouping and COC, respectively). In the 1087.85 cm^{-1} and 1267.23 cm^{-1} the stretching is correspondent at =C-O.

The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat

Our results about AZT PLDLA and P407 FTIR are compatible with spectra shown by others authors¹⁷⁻²², confirmed the chemical stability of AZT in solid dispersion with PLDLA and P407 after SAS process.

Figure 5: FTIR spectra of PLDLA, P407, AZT, PLDLA-AZT SD and PLDLA-P407-AZT SD.

Powder X-ray Diffraction (XRD)

X-ray powder diffraction patterns were measured using a Shimadzu XRD 6000 diffractometer (Shimadzu Scientific Instruments, Columbia, MD) on PLDLA, P407, AZT, PLDLA-AZT SD, and PLDLA-P407-AZT SD samples. The results showed in Figure 6 confirmed the results obtained with DSC. The X-RD diffractogram of PLDLA confirmed its amorphous characteristics. The crystalline compounds and AZT were observed X-RD diffraction patterns. XRD of P407 the characteristics peaks in 19.07° and 23.15° with an intensity of the 1125 and 1671, respectively. This result also observed by Eloy et al.²². X-RD of AZT exhibited sharp and intense peaks confirming its crystalline property; in 8.89° (intensity 688); 14.73° (intensity 766); 15.57° (intensity 1508); 15.93° (intensity: 821); 17.17° (intensity: 672), 17.83° (intensity: 429), 21.43° (intensity: 1047), 22.27° (intensity: 1854), 27.85° (intensity: 847), 29.39° (intensity: 672) e 32.19° (intensity: 395). X-RD of AZT-PLDLA PM and AZT-PLDLA-P407 PM exhibited only the peaks of AZT. In XRD of AZT-PLDLA SD and AZT-PLDLA-P407-AZT was observed the characteristics peaks of AZT, however with appearance of halo in correspondent position at 15.93° , with an offset of angles (17.17° , 17.83° , 21.43° , 22.27° , 27.85° ,

The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat (29.39° e 32.19°), and with attenuated intensity. The changes in diffractogram pattern explain the discrete increase of solubility in the SD.

Figure 6: X-ray diffractograms of, PLDLA, P407, AZT, PLDLA-AZT PM and SD, and PLDLA-P407-AZTPM and SD.

Scanning Electron Microscopy (SEM)

The SEM photomicrographs of PLDLA, P407, AZT, AZT-PLDLA PM and SD, AZT-P407-PLDLA PM and SD are shown in Figure 7. The panel “A” shows shorter crystals with the specific morphology of AZT with cubic structure, but variable size and edges. In Panel 7-B the results showed the filamentous structure of PLDLA 70-30 with cylindrical morphology¹⁷. The P407 carrier has a spherical morphology and smooth surface as described by Gajera et al. 2016²³. The panels 7 C-F showed, respectively, AZT-PLDLA PM (C), AZT-PLDLA SD (D), AZT-P407-PLDLA PM (E), and AZT-P407-PLDLA SD. The PM no altered the morphology of formulation’s carrier. The significant morphological change can be seen in the solid dispersion, panel D and F. The FSC process in the SAS mode modify the crystalline structure of AZT, and the PLDLA morphologic aspect. The P407 cannot be seen in the SD, suggesting that carrier may have interacted physically with AZT, as demonstrate in X-RD and contributed to form an amorphous domain in the PLDLA-P407-AZT SD and increase de solubility of AZT, although the more solubility cannot be statistically confirmed.

Ex vivo Intestinal Permeability Assay

The studies of AZT transmural transport by everted intestinal sac method are shown in Figure 8. The permeation of AZT confirmed the results that shows the poor AZT intestinal permeability, which is a substrate for P-glycoprotein⁵. The results shows the poor intestinal permeability of AZT and AZT-PLDLA PM are not statistically different. The increase of intestinal permeability for AZT-PLDLA-P407 PM when compared with PM without P407 was attributed at partially inhibited efflux pump in the presence P407. However, no statistical difference was observed between AZT-PLDLA SD and AZT-PLDLA-P407 PM ($p < 0.05$). The presence of P407 in the SD increased the intestinal permeation in 76% and approximately 700% when compared, respectively, with AZT-PLDLA-P407 PM and AZT ($p > 0.05$).

Figure 7: SEM photomicrographs of PLDLA, P407, AZT, AZT-PLDLA PM and SD, AZT-P407-PLDLA PM and SD.

Note: The arrow in the panel “E” indicates particles of P407 in the PM (Mag: 200x)

Figure 8: Cumulative amount (mg.ml^{-1}) of AZT permeated from AZT, PM and SD trough everted rat intestine at 37°C . (Different letters means that the results are statistically different).

The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat

The study has been undertaken to investigate the intestinal mucosal transport of AZT. The transmural transport of AZT was studied to evaluate the influence of efflux pump in the intestinal permeation. The AZT has good solubility in aqueous solution, but its bioavailability is poor because Pgp and ABCB1 influence its intestinal permeability. The PLDLA, a synthetic polymer, is biocompatible and biodegradable, and it was used to control the dissolution rate of AZT in SD and PM. However, the concentration of PLDLA was not sufficient to modify a significant form of the effect over dissolution rate and apparent permeability of AZT. Drug permeability has been widely accepted as a screening tool for determining intestinal absorption via the BCS groups during the drug development and regulatory approval processes. Amphiphilic polymers have shown similar results in various experimental models for apparent permeability. Pluronic P407 is a triblock copolymer of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), arranged in a triblock structure PEO- PPO-PEO, raised considerable interest in the drug delivery field. Others compound with inhibitory activity as cremophor EL, vitamin E TPGS 1000 and polysorbate 80 (at higher concentration) on both efflux transporters have been reported ^{24,25}. It has been observed that Pluronic PE 10300 and sucrose ester L-1695 inhibit ABCB1 but not ABCC2. However, cremophor RH 40 can inhibit ABCC2 but not ABCB1 (p-glycoprotein)²⁶. We demonstrated that P407 cause inhibition of the transporter activity, but the specific mechanism of inhibition cannot be explained in this study. Despite that, we show that P407 may increase the absorption of AZT due to the modulation of metabolism and transport by amphiphilic copolymers.

Currently, important research and development means are dedicated to the applications of supercritical fluids in the pharmaceutical industry, the numbers of commercial plants are now operating, but few researchers have acquired some know-how in process scale-up, especially in formulation and design²⁷. Hardly any articles were published on this domain, except for a dealing SAS process. We result corroborate to show that SAS process is not complex and can be conjugated with low cost, proven performances, design innovative, scale-up, and environment-friendly solutions.

Conclusion

There are many doubts about the effect of amphiphilic copolymers in the bioavailability of drugs. Our study contributes to show that the biological interaction of P407 in binary SD with AZT influencing in the apparent permeability of this drug, confirming the inhibitory effect over the efflux bomb. However, our study failed at increase also the solubility of AZT concomitantly. On the other side, the rise of permeability and solubility can be a way to reduction of dose, without prejudice to the therapeutic effects. The Supercritical fluid in mode SAS was used in the particle formation process was essential to obtain binary SD using hydrophilic and hydrophobic molecules.

References

1. Stürmer M, Staszewski S, Doerr HW. Quadruple nucleoside therapy with zidovudine, lamivudine, abacavir and tenofovir in the treatment of HIV. *Antivir Ther.* 2007;12(5):695-703.
2. Kumar P, Lakshmi YS, Bhaskar C, Golla K, Kondapi AK. Improved safety, bioavailability and pharmacokinetics of Zidovudine through lactoferrin nanoparticles during oral administration in rats. *PLoS One.* 2015;10(10):1-17. doi:10.1371/journal.pone.0140399.
3. Rachlis AR. Zidovudine (retrovir) update. *CMAJ.* 1990;143(11):1177-1185.

The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat

4. Quevedo MA, Nieto LE, Briñón MC. P-glycoprotein limits the absorption of the anti-HIV drug zidovudine through rat intestinal segments. *Eur J Pharm Sci.* 2011;43(3):151-159. doi:10.1016/j.ejps.2011.04.007.
5. Namanja HA, Emmert D, Davis DA, et al. Toward eradicating HIV reservoirs in the brain: Inhibiting P-glycoprotein at the blood-brain barrier with prodrug abacavir dimers. *J Am Chem Soc.* 2012;134(6):2976-2980. doi:10.1021/ja206867t.
6. Namanja-Magliano HA, Bohn K, Agrawal N, Willoughby ME, Hrycyna CA, Chmielewski J. Dual inhibitors of the human blood-brain barrier drug efflux transporters P-glycoprotein and ABCG2 based on the antiviral azidothymidine. *Bioorganic Med Chem.* 2017;25(19):5128-5132. doi:10.1016/j.bmc.2017.07.001.
7. Alakhova DY, Kabanov A V. Pluronics and MDR reversal: An update. *Mol Pharm.* 2014;11(8):2566-2578. doi:10.1021/mp500298q.
8. Chiappetta DA, Sosnik A. Poly(ethylene oxide)-poly(propylene oxide) block copolymer micelles as drug delivery agents: Improved hydrosolubility, stability and bioavailability of drugs. *Eur J Pharm Biopharm.* 2007;66(3):303-317. doi:10.1016/j.ejpb.2007.03.022.
9. Huang Y, Dai W-G. Fundamental aspects of solid dispersion technology for poorly soluble drugs. *Acta Pharm Sin B.* 2014;4(1):18-25. doi:10.1016/j.apsb.2013.11.001.
10. Chadha R, Arora P, Saini A, Bhandari S. Crystal Forms of Anti-HIV Drugs: Role of Recrystallization. *Recrystallization.* 2012. doi:10.5772/33777.
11. Vetere A. A short-cut method to predict the solubilities of solids in supercritical carbon dioxide. *Fluid Phase Equilib.* 1998;148(1-2):83-93. doi:10.1016/S0378-3812(98)00180-0.
12. Jouyban A, Rehman M, Shekunov BY, Chan HK, Clark BJ, York P. Solubility prediction in supercritical CO₂ using minimum number of experiments. *J Pharm Sci.* 2002;91(5):1287-1295. doi:10.1002/jps.10127.
13. Cinto PO, Souza ALR, Lima AC, Chaud M V., Gremião MPD. LC Evaluation of Intestinal Transport of Praziquantel. *Chromatographia.* 2009;69(S2):213-217. doi:10.1365/s10337-009-1019-x.
14. Carvalho FC, Barbi MDS, Gremião MPD. LC Evaluation of In Vitro Release of AZT from Microemulsions. *Chromatographia.* 2009;69(S2):207-211. doi:10.1365/s10337-009-1015-1.
15. Solid dispersions with hydrogenated castor oil increase solubility, dissolution rate and intestinal absorption of praziquantel. doi:http://dx.doi.org/10.1590/s1984-82502010000300010.
16. Coimbra MER, Elias CN, Coelho PG. In vitro degradation of poly-L-D-lactic acid (PLDLA) pellets and powder used as synthetic alloplasts for bone grafting. *J Mater Sci Mater Med.* 2008;19(10):3227-3234. doi:10.1007/s10856-008-3425-2.
17. Motta AC, Duek EAR. Síntese e caracterização do copolímero poli (L-co-D,L Ácido Láctico).

The effect of efflux bomb and the transmural potential difference in the permeation of azidothymidine across the small intestine of the rat

Polimeros. 2007;17(2):123-129. doi:10.1590/S0104-14282007000200011.

18. Mara S, Cattani DM, Ribeiro GDA, et al. Surface Characterization and Osteoblast-like Cells Culture on Collagen Modified PLDLA Scaffolds. *Mater Res*. 2014;17(6):1523-1534.
19. Komatsu D, Mistura D V., Motta A, Domingues JA, Hausen MA, Duek E. Development of a membrane of poly (L-co-D,L lactic acid-co-trimethylene carbonate) with aloe vera: An alternative biomaterial designed to improve skin healing. *J Biomater Appl*. 2017;32(3):311-320. doi:10.1177/0885328217719854.
20. Bansal R, Guleria A, Acharya PC. Analytical Method Development and Validation for Quantitative Estimation of Torsemide in Bulk and Pharmaceutical Dosage Form. *Drug Res*. 2013;63(1):165-170.
21. Peepliwal A, Vyawahare SD, Bonde CG. A quantitative analysis of Zidovudine containing formulation by FT-IR and UV spectroscopy. *Anal Methods*. 2010;2(11):1756-1763. doi:10.1039/c0ay00341g.
22. Eloy JO, Marchetti JM. Solid dispersions containing ursolic acid in Poloxamer 407 and PEG 6000: A comparative study of fusion and solvent methods. *Powder Technol*. 2014;253:98-106. doi:10.1016/j.powtec.2013.11.017.
23. Gajera BY, Dugar RP, Dave RH. Formulation Development and Optimization of Ibuprofen Poloxamer Melt Granules Using Hydrophilic Excipients. *Br J Pharm Res*. 2016;13(6):1-20. doi:10.9734/BJPR/2016/29048.
24. Park GB, Mitra AK. Mechanism and Site Dependency of Intestinal Mucosal Transport and Metabolism of Thymidine Analogues. *Pharm Res An Off J Am Assoc Pharm Sci*. 1992;9(3):326-331. doi:10.1023/A:1015882617066.
25. Arca HÇ, Mosquera-Giraldo LI, Dahal D, Taylor LS, Edgar KJ. Multidrug, Anti-HIV Amorphous Solid Dispersions: Nature and Mechanisms of Impacts of Drugs on Each Other's Solution Concentrations. *Mol Pharm*. 2017;14(11):3617-3627. doi:10.1021/acs.molpharmaceut.7b00203.
26. Gaikwad VL, Bhatia MS. Polymers influencing transportability profile of drug. *Saudi Pharm J*. 2013;21(4):327-335. doi:10.1016/j.jsps.2012.10.003.
27. Clavier J-Y, Perrut M. Scale-up issues for supercritical fluid processing in compliance with GMP. In: York P, Kompella UB, Shekunov BY, eds. *Supercritical Fluids Technology for Drug Product Development*. 1st ed. London: Informa Healthcare; 2008:666.