BJPS

Brazilian Journal of
Pharmaceutical Sciences
vol. 52, n. 4, oct./dec., 2016
http://dx.doi.org/10.1590/S1984-82502016000400016

Article

A novel approach in mucoadhesive drug delivery system to improve zidovudine intestinal permeability

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Zidovudine (AZT) mucoadhesive solid dispersions (SD) were prepared using a sodium starch glycolate (SSG) and hypromellose phthalate (HPMCP) mixtures as carrier to enhance the intestinal permeability and bioavailability of zidovudine. SDs were prepared using the co-precipitation method followed by solvent evaporation and characterized according to their physicochemical properties such as particle size, crystallinity, thermal behavior, and liquid uptake ability. *In vitro* drug dissolution, mucoadhesiveness and AZT intestinal permeability were also determined. Thermal behavior and X-ray diffraction patterns demonstrated the amorphous state of AZT in SD systems. The HPMCP polymer restricted the liquid uptake ability in the acid medium; however, this property significantly increased with higher pH values. SDs allowed drug dissolution to occur in a controlled manner. HPMCP decreased the dissolution rates in the acid medium. The mucoadhesiveness of SDs was demonstrated and the permeability of AZT carried in solid dispersions was significantly improved. The effect of the SD carrier polymers on blocking efflux pump can be an important approach to improve the bioavailability of AZT.

Uniterms: Zidovudine. Solid dispersion. Mucoadhesion. P-glycoprotein. Intestinal permeability.

INTRODUCTION

Initially, advances in drug delivery systems were primarily based on the synthesis of functional polymers. Currently, the development strategies of these systems are strongly influenced by a detailed understanding of the underlying biological and molecular principles. With this knowledge, more sophisticated drug delivery systems have been designed that provide better pharmacokinetics, reduced toxicity, more focused drug targeting and controlled release of the drug (Grund, Bauer, Fischer, 2011).

The development of new strategies that overcome the low bioavailability of AZT mainly focus on the efflux mechanism mediated by P-glycoprotein (P-gp), which returns drugs absorbed by the intestinal membrane back to the lumen, increasing presystemic metabolism, and these strategies must overcome the significant challenges of reducing the adverse effects that hinder patient therapeutic compliance while improving drug bioavailability (Geocze *et al.*, 2010).

Zidovudine (Figure 1a) is the drug of choice for the treatment of Human Immunodeficiency Virus (HIV), which causes acquired immunodeficiency syndrome (AIDS). Zidovudine is a reverse transcriptase inhibitor that prevents the replication of the DNA strand from RNA through competitive inhibition of deoxynucleotide triphosphate, ultimately preventing the extension of the tape. Zidovudine needs to be converted into its triphosphate form for effective antiviral activity against the enzyme reverse transcriptase. For this to occur, zidovudine is first phosphorylated by thymidine kinase intracellularly and then transformed into diphosphate by thymidylate kinase. The triphosphate metabolite interrupts viral replication through competitive mechanisms.

Approximately 40% of the AZT dose is metabolized presystemically (Carvalho *et al.*, 2009; Teixeira *et al.*, 2011). Due to its low bioavailability, oral administration

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of AZT poses a major challenge to the development of new systems for controlled drug release. In addition, it is a substrate of various efflux mechanisms present in the central nervous system, immune system and intestinal epithelium, the latter being directly related to issues of drug bioavailability (Varatharajan, Thomas, 2009).

Mucoadhesive systems exploit the interaction between formulations containing polymers and the mucus layer that covers epithelial surfaces throughout the body. Due to their increased retention time and closer contact with the absorption site, mucoadhesive systems provide a promising approach for improving bioavailability by maximizing drug absorption (Carvalho *et al.*, 2013; Ivarsson, Wahlgren, 2012; Barbi *et al.*, 2015).

Solid dispersions (SD) are drug delivery systems in which the drugs are dispersed in an amorphous state in a hydrophilic polymer matrix. This approach has been traditionally used to improve the solubility of drugs with poor water solubility. In several drugs, it has been demonstrated that modifications of structure, solubility properties and drug release profiles can enhance bioavailability (Chen *et al.*, 2006; Giri *et al.*, 2012).

The SD preparation technique is a simple and low cost approach for preparing polymer matrix systems that contain drugs in an amorphous state. Studies investigating the influence of these structural changes on biological interactions, such as mucoadhesion and permeability of highly soluble drugs, have not been evaluated.

The polymer blend is a rational approach for obtaining novel materials with specific properties that allow suitable drug-release control that favors bioavailability (Carbinatto *et al.*, 2012; Soares *et al.*, 2013).

Sodium starch glycolate (SSG) (Figure 1 b) is a polymer derived from starch largely used as a superdisintegrant in tablet formulations. It is practically insoluble in water and possesses high hydration capacity with the ability to absorb over 300 times its volume (Fransén, Björk, Edsman, 2007). The mucoadhesive properties of this polymer have been demonstrated in topical films and inhalant interactive mixtures that contain SSG (Repka, Mcginity, 2000; 2001). Furthermore, it has been reported that SSG can function as a polymeric excipient with properties that inhibit the intestinal efflux mechanisms of drugs (Takizawa *et al.*, 2013).

Hypromellose phtalate (HPMCP) (Figure 1 c) is an enteric polymer largely used to coat solid dosage forms. Due to its insolubility in acid medium, HPMCP confers gastro-resistance to drugs. In addition, its hydroxyl groups interact with the mucosa via secondary non-covalent bonds and form hydrogen bonds with the mucus, resulting in its mucoadhesive properties (Makhlof, Tozuka, Takeuchi, 2011).

P-glycoprotein (P-gp) is the major efflux transporter protein present in intestinal epithelial cells, particularly in the apical membrane. It is responsible for the efflux of a wide range of structurally unrelated drugs and xenobiotics and causes drugs to flow back into the gut lumen. Studies in animals and humans have indicated that P-gp plays a major role in limiting drug absorption and consequently oral bioavailability (Bansal *et al.*, 2009).

Thus, there is considerable interest in enhancing oral bioavailability by inhibiting P-gp-mediated drug efflux. Several studies have demonstrated that P-gp inhibitors (e.g., verapamil, VER) can improve the bioavailability of a large number of molecules (Werle, 2008; Ling *et al.*, 2010). Takizawa *et al.* (2013) demonstrated the effects of 20 common pharmaceutical excipients (diluents, disintegrants, binders, lubricants and sustained release substrate) on the mucosal membrane and how their effects differ in regions of the small intestine. The effects of these excipients on the membrane permeability of 5(6)-carboxyfluorescein (5-CF) were examined using the *in vitro* sac method in rat jejunum and ileum. The authors found that membrane permeability of sodium carboxymethyl starch, low-substituted hydroxypropyl cellulose and croscarmellose sodium was significantly increased in the jejunum.

However, such inhibitors have intrinsic pharmacological activity and, consequently, may cause toxic side effects. Moreover, evidence suggests that excipients of pharmaceutical formulations may block intestinal P-gp function enhancing the permeability of

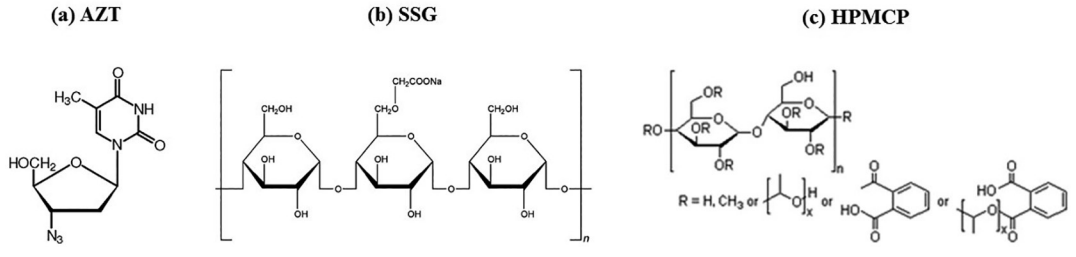

FIGURE 1 - Chemical structure of: (a) Zidovudine (AZT); (b) Sodium starch glycolate (SSG); (c) Hypromellose phthalate (HPMCP).

the intestinal substrate drugs (Shen *et al.*, 2006; Mudra, Borchardt, 2010).

The use of polymers for P-gp blockade has been extensively reported in the scientific literature. Studies have demonstrated that SSG and HPMCP enhance oral bioavailability through the inhibition of P-gp (Carreno-Gomez, Duncan, 2001; Takizawa *et al.*, 2013).

Despite the high solubility of AZT, the mucoadhesive SD approach could provide a simple and low cost method for preparing systems that could change the biological interaction of AZT and improve its bioavailability.

In this study, AZT SDs using SSG and HPMCP as polymer carriers were prepared and their mucoadhesiveness and intestinal permeability were evaluated. The influence of P-gp blockade on drug permeability was also investigated.

MATERIAL AND METHODS

Material

AZT was kindly provided by Fundação para o Remédio Popular - FURP, Guarulhos/SP, Brazil. Sodium starch glycolate was purchased from Henrifarma (São Paulo/SP, Brazil). Hypromellose phthalate and Verapamil were obtained from Sigma–Aldrich® (Hamburg, Germany). Other reagents were of analytical reagent grade.

Solid dispersions preparation

SDs containing AZT were prepared using co-precipitation methods followed by solvent evaporation. Varying concentrations of SSG and HPMCP (1:10:10 and 1:15:15; AZT: SSG: HPMCP) were used as carrier polymers. AZT and SSG were dissolved in a sufficient amount of ethanol, under magnetic stirring (30 min). HPMCP previously dissolved in NaOH solution (0.05 M) was added to the ethanol solution containing AZT and SSG. The mixture was stirred for 15 min. Afterwards, the solvent was evaporated using a rotary evaporator (Tecnal®, Ourinhos/SP, Brazil) under reduced pressure at 45 ºC for 3 h. The samples were dried in an oven-drier at 60 ºC (Marconi MA 035®, Piracicaba/SP - Brazil) until the moisture content of the samples was less than 10%. The product was manually milled using a mortar. The particles were sieved (0.42 mm) and stored in a desiccator. Physical mixtures (PM) using the same drug:polymer ratios were prepared as the control.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded in the 4000–400 cm^{-1} range using an FT-IR spectrometer (Shimadzu® 8300, Kyoto - Japan). The samples were analyzed using KBr pellets. The powders were compressed at a pressure of 8 tons for 3 min using a hydraulic press (Shimadzu® SSP-10A, Kyoto - Japan).

Differential scanning calorimetry (DSC)

Thermal analyses were carried out using a DSC unit (DSC 1 Stare System, Mettler Toledo®, Zurich, Switzerland). Indium and zinc were used to calibrate the temperature scale and enthalpic response. Samples (5 mg) were placed in aluminum pans before heating under nitrogen flow (50 mL/min) at a scanning rate of 10 ºC/min from 25 to 200 ºC. An empty aluminum pan was used as the reference.

X-ray diffraction (DRX)

X-ray powder diffraction was performed at room temperature with an X-ray diffractometer (Siemens® D5000, Berlin, Germany) using monochromatic Cu-Kα radiation (λ = 1.5406 Å) operating at 40 kV and 30 mA. Samples were analyzed in the range of 2-60º (2θ) using a step size of 0.02º (2θ) and scan step time of 0.05/min.

Liquid uptake ability

The liquid uptake ability was assessed using an Enslin device (Cury *et al.*, 2009; Prezotti *et al.*, 2012). An accurately weighted mass of SD (0.05 g) was uniformly spread on a sintered glass filter. The volume of medium absorbed by the samples was measured using a pipette at predetermined times (5, 30, 60, 90 and 120 min). Two different media were tested: simulated gastric medium (0.1N HCl, pH 1.2) and simulated enteric medium (phosphate buffer, pH 7.4). Neither of the media contained enzymes. The tests were performed in triplicate and expressed as the percentage of liquid uptake relative to the initial mass of the samples, according to equation (Eq. 1):

$$\%S = \frac{V}{m} \quad (1)$$

where m is the initial mass of SD (g); V = volume (mL) of medium uptake, $\%S$ = percentage of media uptake of the samples.

In vitro adhesion test

Mucoadhesion properties were evaluated using a texture analyzer (TA.XT plus, Stable Micro Systems,

Godalming, UK) with 50 N load cell, according to the procedure described by Fransén, Björk and Edsman (2007). Mucin discs (11 mm diameter) were pre-hydrated in simulated enteric medium (phosphate buffer, pH 7.4) at 37 ºC for 60 seconds. Immediately after, the discs were attached to a metallic sample holder using double-sided adhesive tape.

The cylindrical ended probe (10 mm diameter) was covered with double-sided adhesive tape containing the sample. For this step, the adhesive tape was carefully pressed against a flat surface containing the powdered sample to create a monolayer of particles. The probe was moved downward with a speed of 2.0 mm/s, until contact was made with the mucin disc; contact was maintained for 600 seconds. Afterwards, the probe was raised at 2.0 mm/s. The maximum force required to detach the probe from the sample could be detected directly using Texture Exponent Lite software. The total amount of force involved in probe withdrawal (Wad - μJ) was determined by calculating the area under the force *versus* distance curve. The tests were repeated six times in *compression test mode* with 2 mN of triggered force.

In vitro drug release

In vitro drug release tests were performed using a Hanson Dissolution Test Station SR8-Plus (Chatsworth - USA) equipped with 150 mL vessels and mini paddles, based on the design of apparatus 2 (USP), to mimic system hydrodynamics. *In vitro* drug release was performed in simulated gastric medium (0.1N HCl, pH 1.2) for 2 h and in simulated enteric medium (phosphate buffer pH 7.4) for 4 h. The media were kept at 37 °C, while stirring at 50 rpm. AZT-free powder served as the control and was also tested.

All samples were put into hard gelatin capsules (size 0). The amount of AZT released was quantified at predetermined times using a UV spectrophotometer (Hewlett Packard 8453, California - USA) with an absorption of 267 nm. These tests were performed in triplicate under *sink conditions*.

Intestinal permeability and P-glycoprotein influence

The intestinal permeability of AZT was evaluated using the everted gut sac model described by Barthe *et al.* (1998a) with modifications purposed by Quevedo *et al.* (2011). The animals were handled in accordance with the guide for the care and use of experimental animals (CEUA/UFSCAR Ethics in Research Committee, nº 003/2011).

Male adult Wistar rats (250-270 g) were kept under fasting conditions for 12 h before the assay and then anesthetized with sodium thiopental. Immediately after, a duodenum segment (approximately 6 cm length) was dissected, flushed with TC 199 buffer solution (culture medium) at 10 ºC and placed in oxygenated (O_2:CO_2 – 95:5) TC 199 buffer of the same temperature. This segment was gently inverted with the aid of a flexible cotton swab (mini brush, ~2.5 mm diameter). The intestinal segment was filled with fresh TC 199 medium and its ends were clamped to make a closed sac. The everted sac was incubated at 37 ºC in 20 ml of TC 199 with 10 mM oxygenated (O_2:CO_2 – 95:5) glucose containing 600 μg/mL of sample (AZT free powder, SD 1:15:15 or PM 1:15:15). The assay time was standardized in 90 minutes to maintain the viability of the intestinal tissue (Barthe *et al.*, 1998b; Barthe, Woodley, Houin, 1999). Afterwards, the everted sacs were removed from the incubation medium, externally washed with fresh TC 199 and cut to obtain the internal content. The content was filtered through a cellulose membrane filter (MilliPore 0.45 mm) and analyzed using the Varian ProStar® HPLC 330 UV-VIS PDA spectrophotometric detector and Rheodine VS 125 (SpectraLab, Toronto – Canada) (Carvalho *et al.*, 2009). The buffer solution (TC-199, pH 7.4) used in all preparations was freshly prepared and had a composition of 145 mM NaCl, 4.56 mM KCl, 1.25 mM CaCl2, 5 mM NaHPO4 and 10 mM glucose (Correa *et al.*, 2011).

To evaluate the influence of P-gp on AZT permeability, the same assay described above was performed with the addition of 50 μg/mL of VER added to the medium (Quevedo *et al.*, 2011). The tests were replicated six times.

Statistical analysis

One-way ANOVA followed by Tukey's multiple comparison procedure was used for statistical analysis of the data. A p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The AZT IR spectrum (Figure 2a) showed three characteristic peaks related to the stretching bands of the OH group, the C-O of OH groups and C=N=N=N (azide group) at 3550–3200 cm^{-1}, 1089 cm^{-1} and 2083 cm^{-1}, respectively (Araújo *et al.*, 2003). In the SSG IR spectrum (Figure 2 b) peaks assigned to C=C of the CH_2 group appeared approximately 1640 cm^{-1} while OH stretching bands were observed in the 3550–3200 cm^{-1} range. C-O of the ether group was observed in the 1275

at 1020 cm^{-1} range (Puttipipatkhachorn, Pongjanyakul, Priprem, 2005). The HPMCP IR spectrum (Figure 2 c) revealed absorption bands between 3500 and 3200 cm^{-1} reflecting O-H stretching while the peaks related to CH (sp^3) and C=O of the ester group were observed at 2800 cm^{-1} and 1700 cm^{-1}, respectively. The band at 1600 to 1550 cm^{-1} was assigned to an aromatic ring and 1000 to 1200 cm^{-1} bands were assigned to ether. A peak at 740 cm^{-1} was attributed to a monosubstituted aromatic ring (Miyazaki *et al.*, 2011). FTIR spectrum analyses were performed to identify the characteristic functional groups of the polymers and drug. FTIR spectrum analysis was also used to evaluate possible interactions between the drug and polymer in the SD samples. The characteristic peaks of the drug and isolated polymers were recorded. The SD and PM IR spectra were similar to those of AZT, SSG and HPMCP. The peaks indicated that there were no chemical interactions, only physical interactions between the components of the system (Araújo *et al.*, 2003).

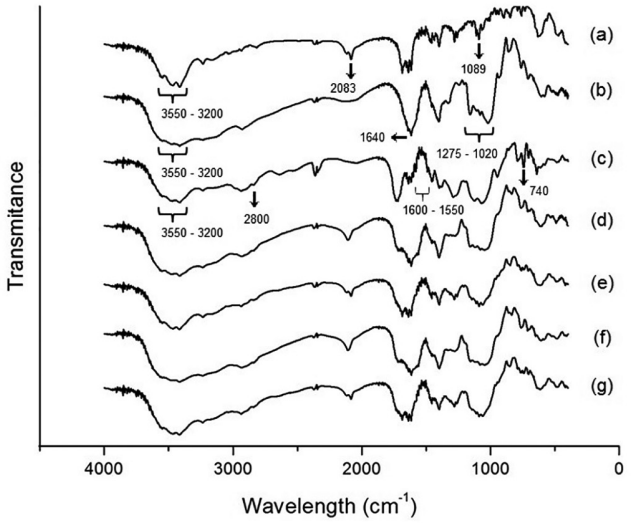

FIGURE 2 - FT-IR spectra of (a) AZT; (b) SSG; (c) HPMCP; (d) SD 1:10:10; (e) PM 1:10:10; (f) SD 1:15:15; (g) PM 1:15:15.

The DSC data of AZT, SSG, HPMCP, SDs and PMs are shown in Figure 3. The AZT thermogram revealed an endothermic peak at 126.7 °C, which was related to drug melting (Maru *et al.*, 2011), while the SSG and HPMCP exhibited a broad endothermic peak in the thermograms due to its amorphous nature (Puttipipatkhachorn *et al.*, 2005). The occurrence of these events is attributed to a significant amount of drug in its crystalline state (Cides *et al.*, 2006). The absence of any peculiar endothermic peaks in the SD thermograms suggests that AZT was predominantly in the amorphous form. In addition, the absence of these peaks has been attributed to the formation of solid drug solution within the polymer matrix (Soares *et al.*, 2013).

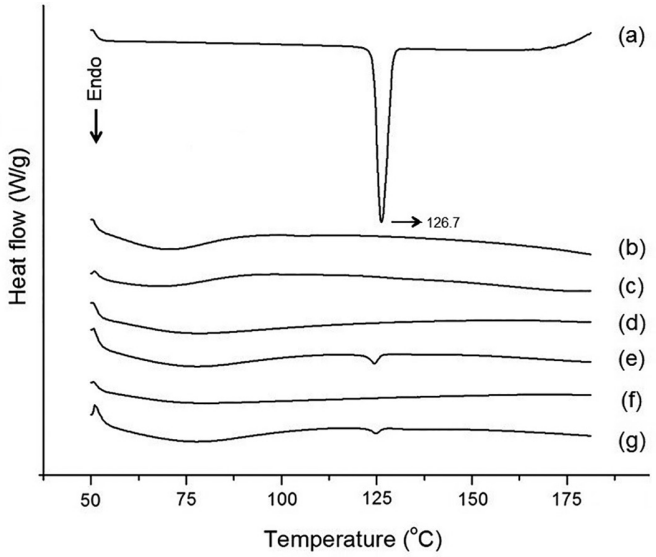

FIGURE 3 - DSC of (a) AZT; (b) SSG; (c) HPMCP; (d) SD 1:10:10; (e) PM 1:10:10; (f) SD 1:15:15; (g) PM 1:15:15.

The diffractograms of AZT, SSG, HPMCP, SDs and PMs are shown in Figure 4. AZT diffractograms present characteristic peaks approximately 21, 22, 24, 26, 30 and 32 (2θ). The diffraction patterns exhibited by the SDs and PMs were similar to the peaks isolated in the polymers (SSG and HPMCP). The SD and PM diffractograms were an addition of both isolated polymers (SSG and HPMCP). The absence of drug-associated peaks in SDs, a result of crystalline structure disruption, is consistent with

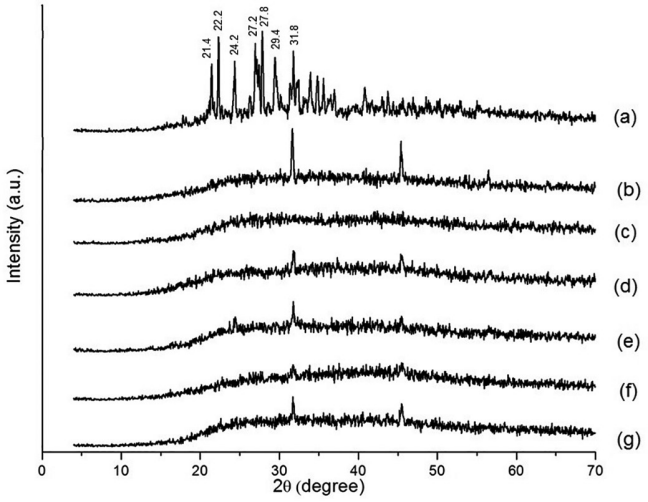

FIGURE 4 - X-ray diffraction patterns of (a) AZT; (b) SSG; (c) HPMCP; (d) SD 1:10:10; (e) PM 1:10:10; (f) SD 1:15:15; (g) PM 1:15:15.

the amorphous state of the drug in the solid dispersions revealed by the DSC analysis. In PMs, this absence can be the result of dilution due to the high proportion of polymers in the matrix.

The liquid uptake values (%) of SD, SSG and HPMCP in simulated gastric medium are shown in Table I. The SSG had the highest values (560.34%) and reached equilibrium in a short time (5 min). The %S values of SD 1:10:10 and SD 1:15:15 were 395.31% and 409.79%, respectively. The liquid uptake ability in simulated enteric medium showed that all samples (except HPMCP) exhibited significantly higher %S values ($p < 0.05$) than those in simulated gastric medium. In addition, SD 1:15:15 exhibited the highest %S value (2074.44) followed by SD 1:10:10, SSG and HPMCP, in that order. Polymeric swelling is an important property of the mucoadhesion process because it contributes to the mobility and expansion of polymer chains. Both of these factors allow for polymer interpenetration with mucin. Liquid absorption is the first step in the relaxation and expansion of polymer chains that characterize swelling behavior. The high swelling values of SSG in both media can be attributed to the peculiar behavior of swellable superdisintegrant polymers, such as SSG, because their action mechanism is based on the rapid expansion of the polymer matrix after contact with the aqueous environment. This behavior causes the development of tension in delimited regions that causes the structure to break into small particles (Bele, Derle, 2012).

The %S values of SD 1:10:10 and SD 1:15:15 demonstrated that the polymer concentration did not affect the liquid sorption ability of SDs ($p > 0.05$), while HPMCP can restrict it in acid medium due to its insolubility when the pH is low. Increased liquid uptake values in the simulated enteric medium indicated that increases in pH should favor carboxyl group repulsion in HPMCP, causing the chains to move farther apart loosening the polymer matrix. Both of these factors favor the entrance of water molecules (Oliveira *et al.*, 2010).

According to Table II, the highest W_{ad} value was exhibited by HPMCP while SSG and SD W_{ad} values were similar ($p > 0.05$). Despite the high W_{ad} values of HPMCP, its presence did not affect the adhesiveness of SD because the W_{ad} values of SD 1:10:10 and 1:15:15 were similar to SSG ($p > 0.05$). This indicated that this polymer determined this property of solid dispersions. The adhesiveness of polymeric systems is a very important property contributing to increased retention time and closer contact with mucous. These factors improve the permeability and bioavailability of drugs with typically poor bioavailability, such as AZT. The high adhesiveness of HPMCP (Table II) may be a result of interactions between the hydroxyl groups and mucin via non-covalent secondary connections that build the hydrogen bonds. In addition, HPMCP is a swellable bioadhesive polymer derived from cellulose. Swelling enhances the adhesiveness of HPMCP through the interpenetration of the swollen polymer flexible chains in mucin (Andrews, Laverty, Jones, 2009). However, the W_{ad} values of the SD samples examined in this study were similar to those of well-known adhesive polymers, such as carbopol 971P, polycarbophil AA1, sodium carboxymethylcellulose and carrageenan (Eouani *et al.*, 2001), verifying their significant mucoadhesive properties. Despite the higher liquid sorption ability of SD in enteric medium when compared to HPMCP, the mucoadhesiveness of SD was

TABLE I - Liquid uptake ability (%) of samples

Time (min)	Liquid Uptake Ability (%)							
	SSG		HPMCP		SD 1:10:10		SD 1:15:15	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
5	511.08 (± 76.90)	869.29 (± 10.83)	208.59 (± 21.25)	221.40 (± 18.45)	247.07 (± 38.57)	793.17 (± 211.35)	287.46 (± 21.18)	1015.23 (± 109.90)
30	529.56 (± 76.90)	875.55 (± 21.66)	214.72 (± 38.31)	258.30 (± 18.45)	284.13 (± 53.49)	1159.24 (± 211.35)	311.93 (± 18.34)	1269.04 (± 109.90)
60	541.87 (± 74.65)	894.31 (± 21.66)	220.86 (± 31.87)	270.60 (± 21.30)	339.72 (± 10.69)	1464.31 (± 317.03)	348.62 (± 18.34)	1649.75 (± 109.90)
90	554.19 (± 84.65)	919.32 (± 18.76)	220.86 (± 31.87)	295.20 (± 18.45)	376.78 (± 10.69)	1830.38 (± 317.03)	385.32 (± 18.34)	2030.46 (± 109.90)
120	560.34 (± 92.97)	938.09 (± 18.76)	220.86 (± 31.87)	313.65 (± 18.45)	395.31 (± 10.69)	2074.44 (± 211.35)	409.79 (± 10.59)	2284.26 (± 190.35)

(*a*) simulated acid medium 0.1N HCl (pH 1.2); (*b*) simulated phosphate buffer (pH 7.4).

lower than HPMCP. These results are consistent with findings regarding other crucial properties of adhesiveness in SD samples. In this sense, the SSG molecular weight could have contributed to steric hindrance that harmed the ability of swollen polymer flexible chains to interpenetrate into the mucin meshes of mucus, limiting the adhesiveness of SD samples (Lai, Wang, Hanes, 2009).

TABLE II - Mucoadhesive performance of polymers and SDs

SAMPLE	W_{ad} (μJ)
SSG	1896 (±0.69)
HPMCP	3137 (±0.51)
SD 1:10:10	1507 (±0.25)
SD 1:15:15	1702 (±0.29)

In acid medium, the AZT-free drug release profile (Figure 5) showed that approximately 50% of the drug was released in 15 minutes. On the other hand, SD 1:10:10 and SD 1:15:15 released only 11% (± 0.0178) and 19% (± 0.0100) of the drug in 15 minutes, and 19% (± 0.0012) and 37% (± 0.0184) in 30 minutes, respectively. In enteric medium, the drug release profiles demonstrated that SD 1:10:10 was also able to release 81% (± 0.0716) of the drug at 60 minutes and SD 1:15:15 was able to release 80% (± 0.1097) in 90 minutes. The AZT-free drug release profile reached 80% in 45 minutes in acid medium (Figure 5) because it is highly soluble in this medium. SD 1:10:10 and SD 1:15:15 exhibited low drug release rates in acid media releasing 19% (± 0.0012) and 37% (± 0.0184) of drug, respectively in 30 min. This behavior can be attributed to the gastro-resistance properties of HPMCP, which exhibited lower swelling in this medium (Table 1), avoiding erosion and contributing to decreasing drug release rates (Oliveira *et al.*, 2010). Indeed, it can be observed despite the presence of HPMCP, an enteric polymer, the drug release rates in the first 15 minutes were very similar for both acid and enteric media. However, after 30 minutes, the drug release rates were higher in enteric than in acid medium, suggesting HPMCP dissolution. HPMCP dissolution increases the drug dissolution rate.

HPMCP is used in SDs to control drug release rates due to its insolubility in gastric fluid and its capacity to dissolve in the upper intestine at pH values higher than 5.5 (Giri *et al.*, 2012). The association of HPMCP with SSG, a polymer that quickly swells in aqueous medium, allows a swollen matrix to form that entraps the drug decreasing the drug release rate. The pH-dependence of HPMCP leads to polymeric hydration in the enteric medium, enabling the formation of water-filled pathways by which AZT can be released (Kim *et al.*, 2007).

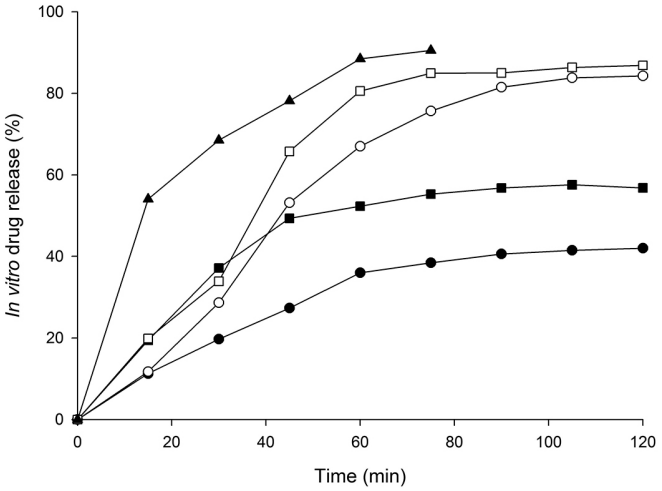

FIGURE 5 - AZT release profiles from SD: (▲) AZT; (●) SD 1:10:10, (■) SD 1:15:15 in simulated gastric medium; (○) SD 1:10:10, (□) SD 1:15:15 in simulated enteric medium.

The intestinal permeability of free AZT, SD and PM (shown in Figure 6), was significantly higher in SD (65.2%) than in free AZT (33.9%) ($p<0.05$). The drug permeability was reduced in PM (45.5%); however, the permeability of PM did not differ significantly from other samples ($p>0.05$). The presence of VER, a P-gp blocker, improved the permeability of the free drug (43.5%) ($p<0.05\%$); however, it did not influence SD drug permeability (69.7%, $p>0.05\%$). In Figure 6, it is clear that the permeability of AZT carried in SD was significantly greater (69.6%) when compared to free AZT (33.9%) and PM (45.4%). This sharp increase in permeability of AZT carried in SD may be a result of the formation of a solid amorphous drug solution in the polymer matrix that modified the biological interaction improving its permeability.

The permeation values of PM (45.4%) were similar to those observed in free AZT in the presence of VER (43.5%). The presence of VER did not affect the permeation of SD ($p>0.05$). VER is a known P-gp inhibitor that blocks the efflux pump and improves AZT permeation (Quevedo *et al.*, 2011). These findings alerted us to the similarities between the P-pg blocking effects of VER and carrier polymers.

The similarity difference between drug permeability from SD and PM (Figure 6) indicated that the drug amorphization (Figure 3) inherent to SD preparation did not affect this property. Based on this finding, carrier polymers are imperative for increasing permeability for

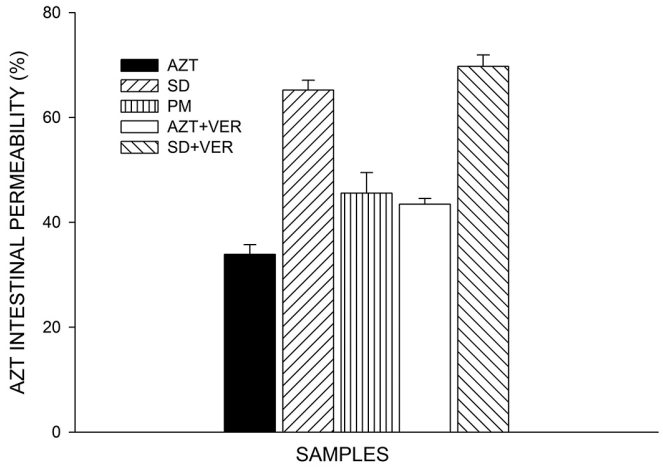

FIGURE 6 - Intestinal permeability of AZT by everted intestinal sac.

the following reasons. First, the high liquid absorption ability of SD should allow a high degree of swelling in polymers, particularly of SSG, contributing to the mucoadhesion of the system. Closer contact between the drug and epithelium and a high local concentration gradient enhances AZT permeability. In addition, the high hydration capacity of polymers can lead to the accumulation of liquid around the intestinal membrane. This accumulation results in high hydrostatic pressure in the paracellular junctions, promoting their opening. Thus, the passage of drug through the intestinal membrane is favored and drug permeation is increased.

The influence of P-gp on AZT permeation was examined by examining the permeability of free AZT in the presence of VER, a known P-gp inhibitor. Addition of VER improved AZT permeation; however, the same trend was not observed in the SD samples, which were unaffected by VER ($p>0.05$). These findings pointed to the possibility that carrier polymers and VER exert similar influences by inhibiting the efflux pump via P-gp inhibition thereby improving AZT permeation.

In a recent study, Takizawa *et al.* (2013) investigated the influence of pharmaceutical excipients, such as microcrystalline cellulose (MCC), starch sodium glycolate (SSG), hydroxypropylmethyl cellulose (HPMC), ethyl cellulose (EC), and others on the membrane permeation of 5(6)-carboxyfluorescein (5-CF) in rat jejunum and ileum using the in vitro sac method. SSG significantly increased the membrane permeability of 5-CF in the jejunum, demonstrating that this polymer influences the intestinal efflux pump mechanism and enhances drug permeation. However, the membrane permeation-enhancing mechanism has not yet been elucidated. Complementary studies will be necessary to determine the effects of pharmaceutical excipients on drug transport via paracellular and transcellular mechanisms and membrane transporters.

This interesting efflux pump inhibitor effect has also been reported in studies of several polymers, including Pluronic P85, Myrj 52 and chitosan-4-thiobutylamidine, polyethylene glycols, amphiphilic block copolymers, dendrimers, thiolated polymers, and dextran sulphate-PLGA hybrid (Werle, 2008; Ling *et al.*, 2010).

Jodoin, Demeule, Béliveau (2002) and Honda *et al.* (2004) also demonstrated that polyphenols of green tea and compounds of grapefruit juice represent natural polymeric efflux pump inhibitors. Carreno-Gomez and Duncan (2001) have patented the use of polysaccharides, dendrimers and surfactants as efflux pump inhibitors for the oral delivery of several drugs with low absorption. It has been revealed with experimental data that anionic gums (polysaccharides), dextran and sodium alginates possess the ability to inhibit efflux pumps.

According to the permeability data, the effects of SD carrier polymers on efflux pumps can be an additional important approach to be further exploited to improve the bioavailability of AZT.

CONCLUSION

DSC and XRD data demonstrated the amorphous state of drugs in SD and the lack of chemical interaction between drugs and carriers. The liquid uptake ability was sensitive to pH and was limited in acid medium because of HPMCP. The high adhesiveness of SDs was demonstrated, and steric hindrance seems to be a key factor in this process. The dissolution rates in acid medium were decreased probably due to the properties of HPMCP. Increases in polymer concentration contributed to the control of drug release rates. The intestinal permeability of AZT carried in SD was enhanced. The results indicated that the roles of polymer carriers and P-gp blockers are similar, improving this property. This study provides valuable insights for future experiments and provides a promising tool to increase the bioavailability of drugs.

ACKNOWLEDGEMENTS

The authors are grateful to Fundação para o Remédio Popular (FURP), Brazil, for the donation of AZT and gratefully acknowledge the financial support provided by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes). We would also like to thank Fabíola Garavello Prezotti.

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Received for publication on 30th September 2015
Accepted for publication on 25th August 2016