Polyvinyl alcohol extrudates for improving the dissolution of main protease and reverse-transcriptase inhibitors.

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ABSTRACT

The aim of this work was to improve the solubility of various anti-HIV APIs extruding them with polyvinyl alcohol to allow a reduction in patient drug burden. There are no previously known cases of successful use of this polymer to improve the solubility of APIs using a convenient industrial technology. Anti-HIV API solid dispersion samples were obtained using hot melt extrusion with polyvinyl alcohol, and were studied with solubility testing, as well as various methods of confirming amorphousness (differential scanning calorimetry, X-ray powder diffraction). This study confirmed the presence of amorphousness and a significant increase in the solubility of the API in various dissolution media. According to the obtained data, the best samples were selected and utilized in tablet formulations, which successfully passed *in vitro* dissolution testing. The results of *in vitro* dissolution testing showed the improvement of dissolution kinetics for all APIs. These results showed the usefulness of hot melt extrusion with polyvinyl alcohol in a ratio of at least 1-3 or 1-5 for the studied APIs and improved the dissolution kinetics of the proposed tablet formulations compared to the controls.

KEY WORDS: Hot melt extrusion, differential scanning calorimetry, dissolution testing, X-ray powder diffraction, polyvinyl alcohol

INTRODUCTION

The number of known active pharmaceutical ingredients (APIs) classified as II and IV class of the BCS system continues to grow steadily from year to year (1). Thus, it is becoming increasingly difficult for pharmaceutical manufacturers to reach the quality of the finished drug product to satisfy the therapeutical requirements for the patient namely, good dissolution and, in part, associated bioavailability, using traditional technologies. In the past two decades, there has been an increase in the number of scientific publications proposing new methods to solve these problems. Among them, the utilization of inclusion complexes of cyclodextrins, self-emulsifying formulations, amorphous solid dispersions, coacervation, micronization, and nanocrystalline complexes have been used widely (1). Today there are amorphous solid dispersion systems used for finished drug products that were developed in the 1980s (2).

One of the most common methods for preparing solid dispersions is using hot melt extrusion. The essence

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of this method is mixing the molecular solution of the API with a polymer excipient (i.e., the carrier) at temperatures above the melting point of the API. The carrier-API mixture is fed through a hopper into a heated cylinder with a rotating screw, where the powder mixture is mixed intensively in a melted state and transferred to a die to get a granulate-like product (3). Despite a number of significant limitations in the applicability of this method, namely, the need to use APIs with relatively low melting temperatures and, at the same time, good stress resistance, the difficulty in choosing a combination of screw elements, the impossibility of conducting experiments with low material loads on laboratory equipment (2), it is the main production method for such FDA approved innovator drugs as NORVIR® (Ritonavir), KALETRA® (Lopinavir + Ritonavir), Onmel (Itraconazole), and so on (4).

Among the FDA-approved products developed using hot melt extrusion, the most commonly used carriers are hypromellose (HPMC), povidone, copovidone, HPMC acetate succinate and polyacrylate (5). The utilization of other polymers has been mainly on experimental level and does not go beyond the scope of a single scientific publication. One such poorly studied carrier is polyvinyl alcohol (PVAl), although there is a reasonable amount of references on the use of this polymer in hot melt extrusion with model substances such as celecoxib (1, 6), indomethacin (7) and diltiazem (8). In all these cases, the extrudates showed a significant improvement in the dissolution profile of the amorphous solid dispersions (ASD) as compared to pure API. Because the melting point of this polymer is relatively high (about 220-230°C), the utilization of PVAl is not suitable for APIs with a melting point well below this value. However, a new product, Parteck® MXP introduced by Sigma Millipore, has recently entered the pharmaceutical excipient market. This is a PVAl designed specifically for hot melt extrusion. The main difference between this brand and existing PVAls is a reduction of the melting point down to 170°C which is achieved through the unification of a certain particle sizes, according to the manufacturer.

The purpose of this study was to check the suitability of using PVAl class II and IV of the BCS system. Well-studied inhibitors of reverse transcriptase and protease such as, ritonavir, darunavir and efavirenz, used in HIV therapies, were selected as model APIs. This choice was driven by sufficient knowledge of these molecules using hot melt extrusion systems, confirming their ability to withstand the stress of the manufacturing process without significant degradation. Examples of successful extrudates of these molecules with copovidone, Eudragit® EPO, and poloxamers were found in recently published literature (9-11). Based on these data this study was carried out in a classical methodological framework for ASD investigation, with a first step a proof of the amorphousness of the obtained extrudates by differential scanning calorimetry (DSC), X-ray powder diffraction (XRD) and scanning electron microscopy (SEM). Then, a series of tests were carried out to dissolve extrudates in various buffer solutions. The ASD solubility test leaders were selected and included in tablet formulations for conducting the comparative dissolution kinetics test with original drugs. This work also continues previous study by the authors comparing two commercially available forms of darunavir, darunavir amorphous and darunavir ethanolate (12). The pseudopolymorphs of darunavir discovered during work carried out by the authors previously were not used here, since they required validation of the manufacturing technologies (13). A comparative study of ritonavir was carried out using two polymorphs in hot melt extrusion.

MATERIALS AND METHODS

Materials

Darunavir ethanolate (DE) was purchased from Mylan Laboratories Ltd. (batch number 22128670, Hyderabad, India). Darunavir amorphous (DA) was purchased from Hetero Labs Ltd. (batch number DV17060020, Hyderabad, India). Efavirenz (EFA) was purchased from Hetero Labs Ltd. (batch number EN17050069, Hyderabad, India). Ritonavir form I (RIT1) was purchased from Hetero Labs Ltd. (batch number R118070015, Hyderabad, India). Ritonavir form II (RIT2) was purchased from Mylan Laboratories Ltd. (batch number 20049428, Hyderabad, India).

Polyvinyl alcohol (PVAl) as brand Parteck® MXP was purchased from Merck Millipore (Burlington, MA, USA). Hydrochloric acid, sodium hydroxide, sodium acetate trihydrate, glacial acetic acid and monobasic potassium phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the organic solvents were of high-performance liquid chromatography (HPLC) grade. All standard buffer solutions were prepared accordingly to USP40-NF35 <2340>.

Low-substituted hydroxypropylcellulose (LH-11), crosspovidone (Kollidon CL), sodium stearylfumarate (Pruv), colloidal silicon dioxide (Aerosil® 200) and white coating mix Opadry® AMB II (88A180040) were purchased from Shin-Etsu Chemical Co., Ltd. (Japan), BASF SE (Germany), J. Rettenmaier & Söhne GmbH (Germany), Evonik Industries AG (Germany) and Colorcon, Inc. (USA), respectively.

Reference Prezista 800 mg tablets (Janssen Pharmaceuticals, batch number 561217, expiration date 12.2019), Norvir 100 mg tablets (AbbVie, batch 127678, expiration date 03.2022) and Stocrin 600 mg (Merck Sharp & Dohme, batch L008934, expiration date 03.2019) were bought in local Moscow drugstores.

Hot melt extrusion

All the equipment used to prepare the samples for analysis were calibrated and qualified.

Hot melt extrusion (HME) was performed using a Pharma 11® Twin-screw Extruder (Thermo Fisher Scientific, Germany). The apparatus was equipped with an 11 mm diameter screw. The extrusion was carried out at a constant powder feed rate of 500 g/h at 200°C maximum barrel temperature. Screw configuration consisted of ten standard conveying elements with a helix pitch of 1 L/D, one 1 L/D distributive feed screw, thirty 1 L/D conveying elements, one short pitch 0.5 L/D conveying element and two long pitch 2 L/D conveying elements. The temperature of the first section of the extruder was from 15 to 25°C with water cooling, the remaining sections at 205°C, screw rate 150 RPM and torque 7 Nm. Preliminary the dry powders for feed (API+PVAl) were blended in FTLMV-02 V-type mixer (Filtra Vibracion S.L, Spain). The process parameters were not measured during extrusion. Extrudates were ground using an IKA MF mill with a mesh size of 0.315 mm.

Solubility testing

The solubility of the pure APIs and the grinded API+PVAl samples was studied by measuring concentration levels in standard buffer solutions (Hydrochloric Acid Buffer pH 1.2, Acetate Buffer pH 4.5, Phosphate Buffer pH 6.8). Saturated DA and DE solutions were prepared by dissolving the DA+PVAl and DE+PVAl samples excess in USP dissolution apparatus 2 <711> for 30 minutes with 75 min^{-1} paddle rotating rate at 37°C in 900 mL of dissolution medium. Saturated EFA solutions were prepared by dissolving the EFA+PVAl samples excess in USP dissolution apparatus 2 <711> for 30 minutes with 50 min^{-1} paddle rotating rate at 37°C in 900 ml of dissolution medium. Saturated RIT1 and RIT2 solutions were prepared by dissolving RIT+PVAl samples excess in USP dissolution apparatus 2 <711> for 120 minutes with 75 min^{-1} paddle rotating rate at 37°C in 900 ml of dissolution medium. After the expiration of the test time, solution was extracted and filtered through PTFE filter with 0.45 μm pore diameter. After filtration, all samples were transparent and did not contain visible mechanical inclusions. Dissolution modes, stirrer rotation time, sampling points were reproduced according to FDA recommendations (14). The solubility data did not change after the first sampling point until the final one. The dissolution test was carried out in 18 test vessels of dissolution per sample, for each sample RSD did not exceed 5%.

The concentrations of APIs in saturated solutions were determined by HPLC methods presented in Table 1. All calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas.

Powder diffraction

The powder diffraction analysis was carried out in the Center of shared use at the Kurnakov Institute

Table 1 HPLC conditions for API determination

CONDITIONS	DARUNAVIR	EFAVIRENZ	RITONAVIR
HPLC system	LC-20 Prominence (Shimadzu)		
Column	X-Bridge C18, 150×4.6 mm, 3.5 µm	150×4.6 mm, 5 µm packing L10	150×4.6 mm, 5 µm packing L1
Column temperature	30°C	40°C	40°C
Flow rate	1.0 ml/min	1.5 ml/min	1.5 ml/min
UV detector wavelength	265 nm	250 nm	215 nm
Injection volume	20 µl	35 µl	25 µl
Mobile phase	Ammonium formate water solution, pH 3.0 : acetonitrile (55:45 v/v)	Solution A: Methanol, trifluoroacetic acid, and water (100:0.5: 900 v/v) Solution B: Methanol, trifluoroacetic acid, and water (900:0.5: 100 v/v)	Monobasic potassium phosphate water solution, pH 4.0 : acetonitrile (45:55 v/v)
Reference	(15)	(16)	(17)

of General and Inorganic Chemistry of the Russian Academy of Sciences.

The API/carrier samples were radiographed using X-ray powder diffraction (XRD) using Bruker D8 ADVANCE diffractometer (Germany). The analysis was performed to examine the API/carrier samples for crystallinity and compare these data to previous research results. XRD patterns were obtained with CuKα radiation, accelerating tube voltage at 40 kV, current 40 mA, Ni-filter, LYNXEYE detector, reflection geometry, the angle range 2θ = 5-60°, step 0.01125°. The signal accumulation time was 0.22-0.4 sec/step; the rotation speed of the cuvette with the sample was 20 RPM. To obtain experimental diffraction patterns, the samples in this study were thoroughly ground in synthetic jasper immediately before recording. The powders obtained this way were placed in low-background cells with a substrate of oriented monocrystalline silicon.

Scanning electron microscopy

Powder samples were applied onto double-sided carbon tape (SPI Supplies, USA) attached to an aluminum table. Next, the samples were deposited with gold (layer thickness 5 nm) using an SPI-MODULE Sputter Coater (SPI Supplies, USA). The analysis of powder samples was carried out using a Quanta 200 3D double-beam scanning electron microscope (FEI Company, USA) in a high vacuum mode at an accelerating voltage of 10 kV.

Differential scanning calorimetry

Samples after SEM testing were by Q20PTA TA Instruments differential scanning calorimetry (DSC) instrument (USA). The samples were weighed accurately, 10.0±0.1 mg of binary physical mixtures of API with carriers were weighed and sealed into a platinum pan, then compressed in tightly closed state. The samples were heated from 50 to 200°C at 10°C/min ramp with second pot stated emptiness.

Final calculations and data systematization was performed using Microsoft Excel 2016 on Microsoft Windows 7 operating system. Significance levels of $P < 0.05$ denoted significance in all cases.

In vitro dissolution testing

In vitro dissolution testing in the form of comparative dissolution kinetics test was performed for comparison to Prezista® 800 mg tablets, Norvir® 100 mg tablets and Stocrin 600 mg according to FDA guidance for industry in the same methodology as the above mentioned solubility test (14). Reference tablets were used until their expiration date. After completion of the dissolution test, the suspensions from the dissolution vessel were filtered through 0.5 mm sieves to eliminate the influence of possible incomplete disintegration in the dissolution vessel.

For this experiment, best samples among certain APIs

were used, which were mixed with crospovidone (disintegrant), low-substituted hydroxypropylcellulose (additional disintegrant) and sodium strearyl fumarate (lubricant). The tablets were compressed using a Natoli Engineering Np-RD10 tablet press (St Charles, MO), punch size 10.25×20.5 mm modified capsule (EFA and darunavir) or 9.0×20.0 mm modified capsule (RIT), compression force – 20 kN (EFA and darunavir) or 10kN (RIT), die capability 14-16 mm. Both formulations passed successfully disintegration <701/2040>, tablet friability <1216> and tablet breaking force <1217> testing, using a Pharma Test DIST 3 (Hainburg, Germany), Pharma Test PTF 10E (Hainburg, Germany) and Copley Scientific TH3/500 (Nottingham, UK) apparatus, respectively. In all cases disintegration time was not more than 5 minutes and the friability was less than 0.1% without any significant deformations on tablet surface (breaking force was 180-210 N).

The tablets were film-coated using a SolidLab 1 coater (Bosch Hüttlin, Germany). Coating suspension was 15% (w/w) Opadry AMB II 88A180040 in water, prepared according to the instructions provided by Colorcon Inc. (Dartford, UK) manual guidelines. The coating process was performed with spray pressure and microclimate pressure had been set at 0.5 bar and 500 mbar respectively (nozzle was Schlick Nano ABC W6637), air flow rate was 30 m^3/h, process air temperature at 60°C, drum rotation rate at 25 RPM. The suspension was constantly stirred during the spray drying process on a magnetic stirrer Heidolph MR HEI TEC 145 (Schwabach, Germany). Peristaltic pump Watson-Marlow 323 U/D (Marlow, UK) feed rate was 3 g/min (1.6×1.6 mm hose).

Stability testing

For all film-coated tablet formulations, a stability study was carried out according to ICH Q1 Quality Guideline (accelerated conditions at 40°C ± 2°C at 75% RH ± 5% RH, 6 months) in a Memmert ICH 110 climatic chamber (Büchenbach, Germany). The tablets were filled into Duma Twist-Off 45035 containers with 3827 DES caps (both Gerresheimer, Germany). The stability testing included 1 month, 3 months and 6 months points where Related Substances, Assay, Dissolution, Disintegration and Description test results were specified according to corresponding USP monographs. The Assay and Related Substances tests was performed by HPLC methods with Mylan Laboratories Ltd. and Hetero Labs Ltd. API reference standards, respectively. The Dissolution test was performed in mentioned Solubility Test conditions in three standard buffer solutions (Hydrochloric Acid Buffer pH 1.2, Acetate Buffer pH 4.5, Phosphate Buffer pH 6.8).

In parallel to this experiment, the stability of the same ASD was studied. ASD samples were filled in Duma Twist-Off 45035 containers with 3827 DES caps and were tested as the same time points as the tablets in the stability testing (1 month, 3 months and 6 months, respectively). The ASD samples were evaluated for amorphousness using XRD.

RESULTS

The study began with the preparation of extrudates, which used as samples for further investigation of their solubility in standard buffer solutions, as well as using DSC, XRD, and SEM to determine their final amorphousness. Opaque extrudates were excluded from the number of fill-course studied samples, since it was assumed that the inclusion presence in the extrudate indicates the crystallization of the API in the polymer environment, which automatically meant that there would not be shown significant improvement in the solubility of the API in buffer solutions. Opaque extrudates were those extrudates that showed clearly visible traces of white API crystals on their surfaces that had become visible during the discharge of the extrudate from the extruder. The examples of opaque and transparent obtained extrudates are presented in Figure 1. The selection of extrusion parameters for these ratios of opaque extrudates did not lead to the production of transparent samples, thus it was concluded that the concrete API-PVAl ratio itself was unsuccessful. For all cases, the ratios 1:1, 1:3, and 1:5 of the API-PVAl were studied. A higher PVAl ratio was considered unreasonable, since it would necessitate further increasing the tablet weight, which required at

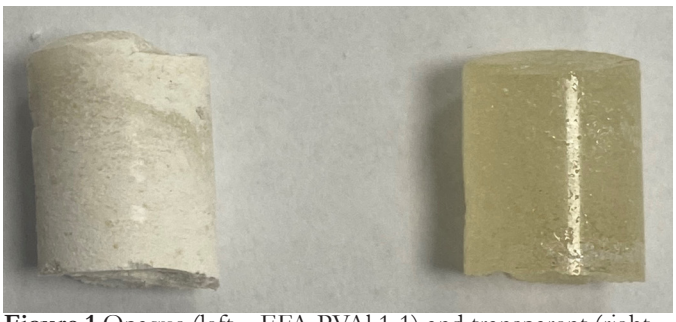

Figure 1 Opaque (left – EFA-PVAl 1-1) and transparent (right - EFA-PVAl 1-5) extrudates obtained in the experiments.

least an additional disintegrant, which would cause difficulties for the patient to swallow.

Immediately after receiving the extrudates, it was noted that all samples with a 1:1 ratio of API-PVA, without exception, had a dull white color and were classified as opaque extrudates. For a 1:3 API-PVAl ratio, such an observation was made only for RIT samples, both for RIT1 and RIT2. As will be shown below, all the results of RIT1 and RIT2 with insignificant deviations were almost identical, despite the different initial polymorphic nature. For the rest of the APIs, the extrudates at a 1:3 ratio were transparent and, at the 1:5 ratio, no opaque samples were found.

Based on the transparent extrudates, an experiment was carried out to study the solubility in standard buffer solutions, the results are presented in Table 2. For comparison, Table 2 also presents the results of the pure APIs solubility in the same media. It was recorded that in all extrudate cases there was a solubility improvement in all media compared to pure APIs, and samples with a 1:5 ratio showed higher API solubility than samples 1:3. Regardless of the type of medium, at least a twofold increase in solubility compared to the API was achieved. It was noted that each of the APIs had the best solubility in the pH 1.2 dissolution medium, and in the same medium, the best results among the extrudates were obtained. However, the largest relative increase was observed in a pH 4.5 environment, where the growth for samples with a 1:5 ratio was almost tenfold in all cases.

To further explain the behaviors of the extrudate results from the solubility tests, an XRD study was carried out. The results are presented in Figure 2. All the APIs, with the exception of DA, confirmed their crystalline nature, while DA and PVAl, on the contrary, appeared to be amorphous halos in the diffractograms. The extrudates of the 1:5 ratio also had extremely amorphous halos, similar to the results of PVAl, which indicated their complete amorphousness, confirming the results of the dissolution test. The extrudates of the 1:3 ratio, however, had less pronounced PVAl halos; however, the inclusion of individual intensity peaks was not found among them, which should indicate the presence of a crystalline phase.

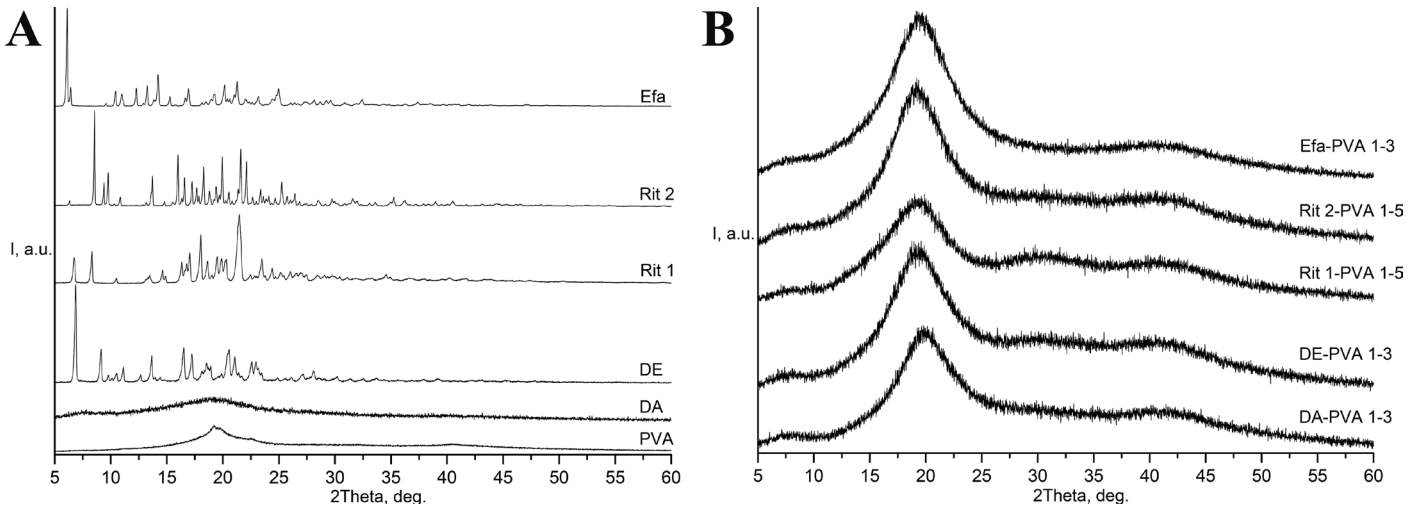

Figure 2 XRD diffractograms of initial components (left) and API+PVAl samples at a raito of 1:3 and 1:5 right).

Table 2 Extrudates and pure API solubility in standard buffer solutions.

RATIO	pH 1.2	pH 4.5	pH 6.8	pH 1.2	pH 4.5	pH 6.8
	RIT1 mg/ ml	RIT2 mg/ ml				
Pure API	0,377	0,017	$0{,}244 \cdot 10^{-3}$	0,098	$0{,}053 \cdot 10^{-3}$	$0{,}047 \cdot 10^{-3}$
1-1	Opaque samples	Opaque samples				
1-3						
1-5	2,930	0,510	0,270	2,925	0,514	0,273
-	DE (for base darunavir) mg/ ml	DA mg/ ml				
Pure API	0.821	0.101	0.112	1.212	0.391	0.183
1-1	Opaque samples	Opaque samples				
1-3	1,672	0,831	0,437	1,669	0,840	0,433
1-5	1,761	1,331	0,631	1,760	1,357	0,628
-	EFA mg/ ml					
Pure API	0,012	0,011	0,011			
1-1	Opaque samples					
1-3	0,870	0,895	0,021			
1-5	1,239	1,203	0,035			

The DSC results looked similar and are presented in Figure 3. DSC allows to measure glass transition temperatures (T_g) as well as regular crystalline melting point (T_m). For API molecules, the corresponding melting points were obtained, while the extrudates showed smooth curves without extrema and maxima.

The analysis of SEM photos did not deviate from

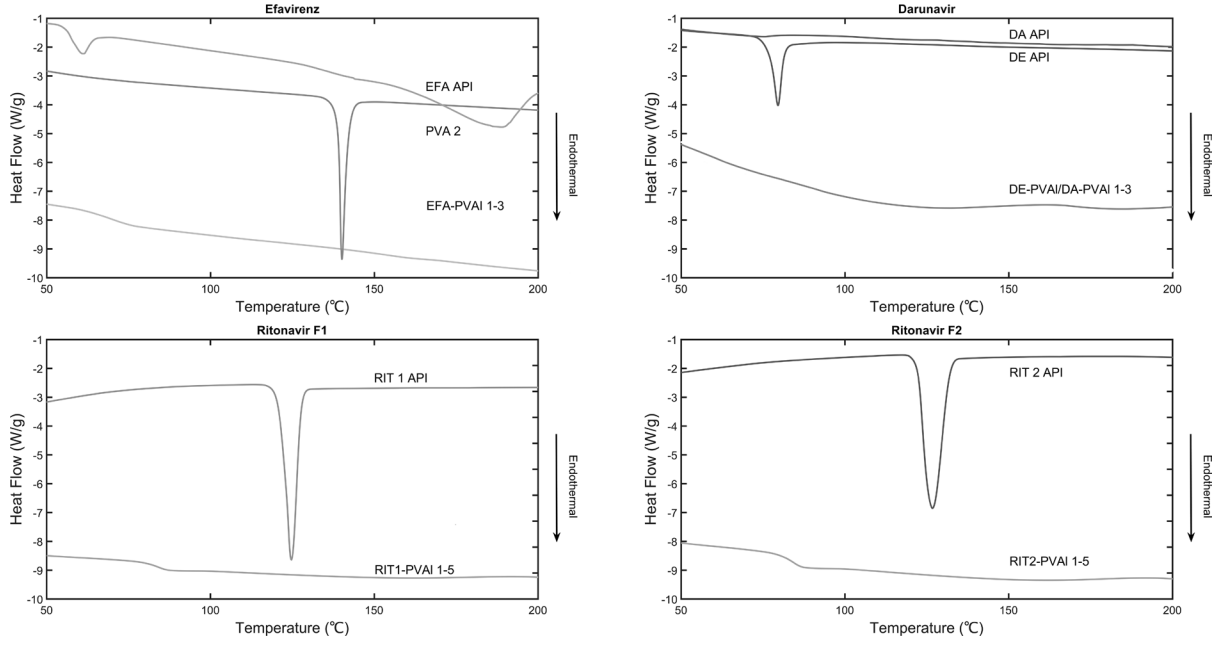

Figure 3 DSC thermograms of initial components and the resulting API+PVAl samples.

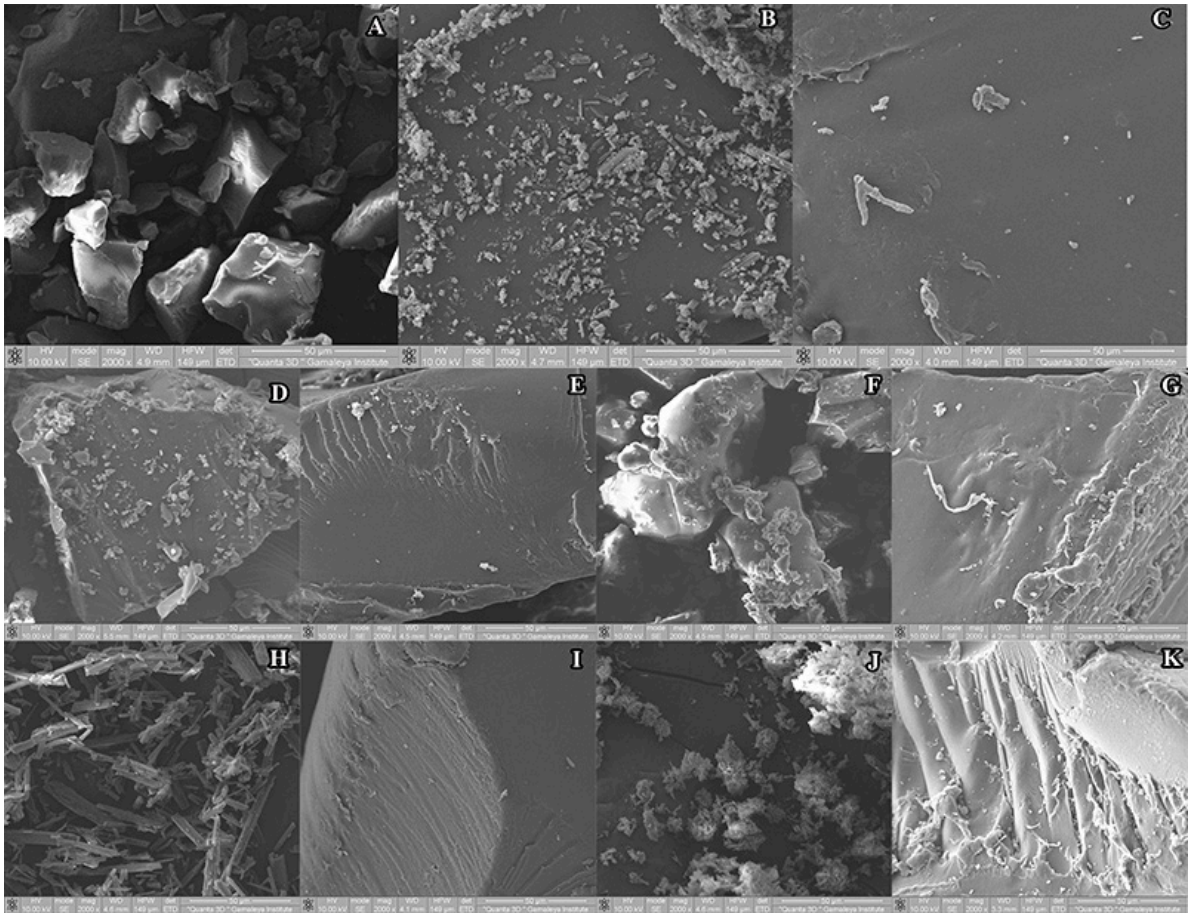

Figure 4 SEM photos of initial components and obtained API+PVAl dispersion samples (50 μm scale). (A) PVAl, (B) EFA, (C) EFA+PVAl 1:3, (D) DE, (E) DE+PVAl 1:3, (F) DA, (G) DA+PVAl 1:3, (H) RIT2, (I) RIT2+PVAl 1:5, (J) RIT1 and (K) RIT1+PVAl 1:5)

preliminary research. SEM analysis of photographs did not deviate from preliminary research. The SEM results are presented in Figure 4. The obtained transparent extrudate was analyzed using the lowest polymer content, on the assumption that if the amorphous structure was confirmed, then at a higher polymer concentration there is a limited chance that crystallinity would occur. EFA and RIT2 appeared to be represented by prismatic crystals of various sizes, while RIT1 turned out to be a cluster of irregularly shaped particles. A detailed analysis of the morphology of DA and DE particles was given by us earlier in the corresponding article (12). Particles of PVAl in the photographs were shown as fragments of irregular shape and different sizes. The extrudates as one turned out to be almost identical in the photographs, i.e. all of them were represented by large fused blocks, similar to the largest PVAl particles, without any presence of external fragments and especially without crystalline inclusions.

The next step was formulating tablets based on extrudates. For all of them, a system of two disintegrants was used, that is crospovidone and a low-substituted hydroxypropyl cellulose, which was supposed to simultaneously maintain the tablet strength and easy disintegration when it enters the dissolution medium. It was assumed that the obtained tablets for the convenience of the patient should be dispersible, which led us to the use of a large number of disintegrants to achieve the required time for obtaining the suspension of the drug. Earlier, we had tested a similar system on the example of darunavir on mesoporous carriers (12), however, in comparison with that case, the need to introduce an additional carrier was lost, because PVAl, in contrast to mesoporous

carriers, possessed good compressibility in itself, but required an increase in the amount of disintegrant due to good adhesion ability. Moreover, it was precisely a disintegrant without a pronounced ability to play the role of a dry binder in pressing was required. Crospovidone was chosen for this role, and extrudate particles were pre-sieved on a sieve with a 0.315 mm mesh size to facilitate the destruction of the extrudate particles. The final formulations are presented in Table 3. A sample of only RIT2 was chosen, and not of both polymorphic forms, since it was believed that, due to the same dissolution results, the polymorphic form does not have any effect on the extrudates properties and one test will be more than enough for comparison with the original preparation. The choice of DE was motivated by the same considerations that were previously outlined by us in the corresponding article (12).

All dissolution kinetics curves are shown in Figure 5. It should be noted that the RIT2 samples were compared with 100 mg Norvir, while 800 mg Prezista with 4 tablets of 200 mg DE, and 600 mg of Stocrin with 3 tablets of EFA. This methodology for the last two cases is due to the production of extremely large tablets weighing more than 1000 mg, which, while maintaining the dosage, would have to be increased by another 3 or 4 times making them difficult to manufacture, as well as, difficult for patients to swallow.

Experimental formulations in the test have shown dominance over the original ones in all investigated dissolution media. The results obtained by dissolving the experimental tablets exceeded the original Prezista, Norvir and Stocrin formulations, and the maximum concentration in the solution was reached after 10 minutes of the experiment. Both the original and the experimental tablets completely disintegrated in the dough, as no particles with a diameter of more than 0.5 mm were found. The increase in EFA dissolution was greatest compared to Stocrin in media with pH 1.2 and pH 4.5, where the API concentration increased by more than an order of magnitude. In other cases, the increase was not as drastic, but only for RIT2 in pH 1.2, Norvir showed close values, while in other cases, the increase in concentration was at least twofold.

All the tablet formulations were tested for stability under accelerated conditions. No fundamental changes were noted during the storage of each formulation. After 6 months, the developed RIT and EFA tablets still met the USP specifications by Related substances level (16, 17), and in the case of DAR, single impurities were no more than 0.1% and the sum of impurities was no more than 0.3%. The dissolution profile of the tablets was the same at all points of the stability study. The phase stability was also proven because the formations of crystalline phase was not detected after the same storage conditions for uncompressed ASDs.

DISCUSSION

The transparent and opaque extrudates was the first of the theoretical problems considered in this study. It is the understanding that such extrudates were out of

Table 3 Experimental tablet formulations used in the *in vitro* dissolution testing

COMPONENT	RIT2	EFA	DE
API	100.0 mg	200.0 mg	216.82 mg
PVAl	500.0 mg	1000.0 mg	1000.0 mg
Low-substituted hydroxypropylcellulose	50.0 mg	100.0 mg	100.0 mg
Crospovidone	300.0 mg	500.0 mg	500.0 mg
Sodium stearilfumarate	10.0 mg	20.0 mg	20.0 mg
Film coating: Opadry II 88A180040 (polyvinyl alcohol – 37.00%, Talc – 31.00%, Titanium dioxide – 25.00%, GMCC type I – 4.00%, Sodium laurylsulphate – 3.00%)	20.0 mg	40.0 mg	40.0 mg

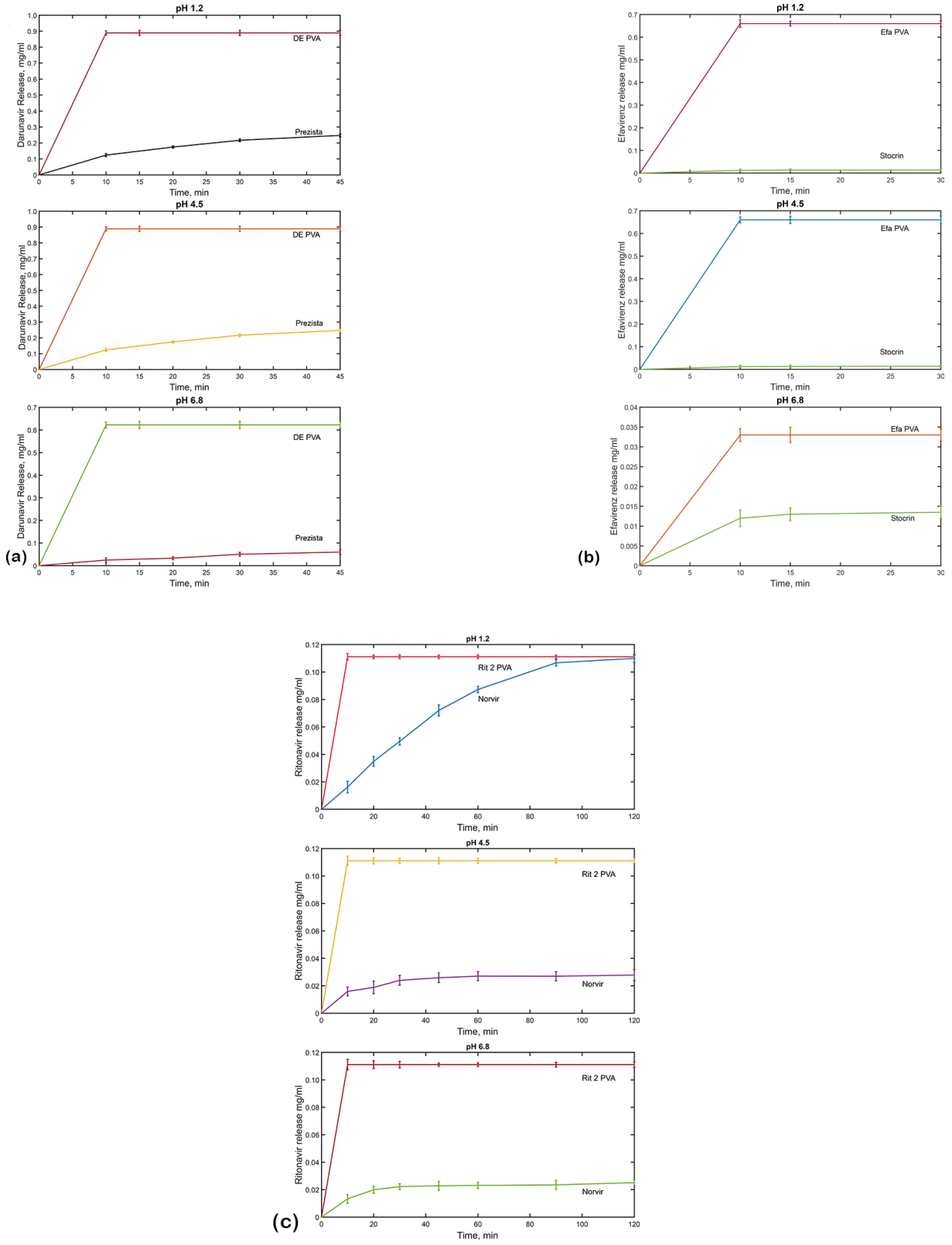

Figure 5 Release profiles for tablet formulations (a) Darunavir, (b) Efavirenz and (c) Ritonavir alongside the original tablets each at pH 1.2 (top), pH 4.5 (middle) and pH 6.8 (bottom) respectively. Each data point represents the mean of 10 repeats.

interest for further study, due to the lack of homogeneity of the dispersion with separated API and PVAl phases. From this observation, it can be concluded that the 1:1 ratio for specific molecules is insufficient to prevent crystallization because of the steric effects of PVAl. On the contrary, the higher the PVAl content in the sample, the more the final mixture acquires properties similar to those of the polymer. A literature search showed no comparative information on the ratios of PVAl extrudates or some other polymers, however, the very fact of obtaining extrudates of various types is not unique. A. Forster already noted that only in the case of turbid and opaque PVP extrudates were traces of crystallinity found (18). J. Albers *et. al.,* found that the properties of turbid extrudates during DSC analysis are a kind of middle state between the completely amorphous state of the extrudate and a banal physical mixture of the starting components (19). Based on these data, the focus of this study shifted towards extremely transparent extrudates, and the assumption was indirectly confirmed. Transparent homogeneous results were obtained for different polymorphic (ritonavir) or solvate (darunavir) forms in identical API-PVA ratios. This circumstance allowed for the assumption that the key factor for stabilizing the extrudate as a dispersed system is precisely the amount of PVAl per one API molecule and the properties of the main API molecule itself, and not the solvates or the crystal lattice of the raw material.

The transparent extrudate in the course of this study did not provide any conflicting information that would agree to shake these theses. Therefore, based on XRD results, it can be concluded that 1:3 samples are also amorphous, and the best results for 1:5 samples in solubility are a consequence of the influence of polymer concentration, which directly affects the solubilization process during dissolution. In the case of API-polymer systems with a predominant polymer content, it was considered doubtful whether SEM is necessary for initially transparent extrudates, since it is difficult to expect the detection of real crystalline inclusions even at high scaling with complete fusion of the components. For example, J. Albers *et. al.,* presented photographs of both transparent and opaque extrudates (19) and the differences between these images were manifested only in the presence of slightly noticeable tiny white dots on the surface of the opaque extrudates. However, in practice, extrudates of different polymers can have various surface defects that are completely unrelated to the presence of crystallinity. E. Ashour *et. al.,* produced both transparent and opaque extrudates, but the SEM photographs contained similar dots and defects in both cases (20). Therefore, it is considered that SEM should be used primarily as a secondary indirect method, which is lower in importance relative to XRD and DSC.

The analysis of the DSC and XRD results or the DA and DE extrudates, concurs with a previous article on micronization of these molecules with mesoporous carriers during thermal exposure above 80°C that DE transforms into DA (12).

The thermograms and diffraction patterns of them are fundamentally identical. However, if earlier it was possible to write off this to the influence of the porous carrier, now, when the result is repeated on a different system, the rest of the interpretation of this phenomenon should be discarded. In general, the DSC results are fully consistent with the description of X-ray diffractograms, i.e., systems with a high content of PVAl almost completely repeat the curvature of the pure PVAl thermogram. At the points corresponding to the pure APIs melting points, no signs of the crystalline phase presence were found. The absence of extrema does not indicate the total absence of glass transition temperature because they can be located outside of limits of measurement range, which was insignificant for our experiment due to relatively high temperatures.

The results of the *in vitro* dissolution test demonstrated a strong superiority in the rate at which the API reaches its maximum concentration in the dissolution media. Of course, this behavior is directly related to the faster disintegration of the experimental tablets, in contrast to the fact that the original formulations were dissolved layer by layer for a longer time. However, decomposition in itself does not guarantee the transition of the substance itself into solution, which is associated with the need to destroy the crystal lattice.

It should be noted that the reference drugs Stocrin and Prezista are based on crystalline APIs, while Norvir is an extrusion product with copovidone (21). This fact, in the case of ritonavir, makes it possible to achieve similar results of the test formulation with the original one in pH 1.2 dissolution medium. Thus, in the above examples, the effect of ASD on dissolution was observed, which also indicates the potential use of systems similar to the formulation presented here to create dispersible tablets for substances with low solubility. The very use of the resulting composition requires mandatory *in vivo* studies in order to prove the effectiveness and the absence of new previously unknown side effects.

CONCLUSIONS

As a result of this study, extrudates were obtained, among which amorphism was positively observed only at an API-PVA ratio of 1:5 in all studied systems. The study of the obtained extrudates by the SEM method showed fundamentally identical structures of fused polymers. The best absolute increase in solubility of all APIs was obtained using a pH 1.2 standard buffer solution, while the best relative increase was found in pH 4.5 dissolution medium.

The resulting tablet formulations were used for dispersible tablet preparation. A comparative test with the original drugs revealed a multiple superiority in the concentration of API in all cases studied, as well as a surprisingly fast peak in concentration as early as 10 minutes. This makes it possible to consider essentially new approaches to the dosage regimen of drugs with low solubility and a sufficiently fast absorption time as it would reduce the drug load to the patient.

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