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Improved biopharmaceutical properties of oral formulations of 1,2,4-thiadiazole derivative with cyclodextrins: *in vitro* and *in vivo* evaluation

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ABSTRACT

The synthesized 1,2,4-thiadiazole derivative displaying biological activity has low aqueous solubility and dissolution rate. Novel oral formulations of thiadiazole with β- and hydroxypropyl-β-cyclodextrins were obtained by grinding and freeze-drying methods with the purpose to improve the aqueous solubility. Complex formation of 1,2,4-thiadiazole derivative with cyclodextrins was confirmed by means of solid-state ^{13}C MAS CP/TOSS NMR. Solubility, dissolution rate and permeability of the solid inclusion complexes were evaluated in different biorelevant media (SGF, FaSSGF, FaSSIF) simulating the conditions in the gastrointestinal tract. It was demonstrated that the content of biorelevant media affects the properties of the inclusion complexes. In particular, solubilizing effect of cyclodextrins became less pronounced when the micelles of taurocholic acid and lecithin are formed in the dissolution media. The inclusion of thiadiazole into cyclodextrin cavity is in competition with its partitioning into the micelles and this should be taken into account when the *in vivo* behavior is predicted. The results of *in vitro* and *in vivo* experiments were found to be in agreement and showed the highest solubility, dissolution rate and bioavailability of the freeze-dried complexes of thiadiazole with hydroxypropyl-β-cyclodextrin. These complexes can be proposed as more effective dosage forms for oral administration.

Keywords: biorelevant media, biovailability, thiadiazole, inclusion complexes, cyclodextrins

1. Introduction

It is known that effectiveness of most solid oral dosage forms is directly related to their absorption into the systemic circulation and delivery to the site of action. In so doing, the bioavailability of the active pharmaceutical ingredient (API) is affected by two processes: dissolution/release and permeability/absorption. The dissolution process involves extracting the API from the dosage form into the liquid contents of the digestive tract, and absorption is the process of transporting the substance from the gastrointestinal tract (GIT) into the systemic bloodstream. Very often newly synthesized APIs with confirmed biological activity do not reach the pharmaceutical market due to inadequate transport properties of dissolution and absorption.

Since dissolution and absorption of the drugs occur in different segments of the gastrointestinal tract, it becomes necessary to study these processes in biological liquids with different pH values. For this purpose, so-called biorelevant media developed by Prof. J. Dressman[1] are used. Biorelevant media are close as much as possible to the internal fluids of the human body (intestinal, gastric juice) in terms of chemical composition and physicochemical properties (pH, osmolality, buffer capacity, surface tension). Therefore, their use makes it possible to predict more accurately the bioavailability of oral drugs and to control the quality of medicines. Since the *in vivo* experiments are laborious and expensive, one of the main tasks in this direction is reliably modeling the drug behaviour *in vitro.*

The presence of the specific components of biorelevant media (bile salts and phospholipids) can significantly affect the solubility and dissolution rate of compounds. Fagerberg et al.[2] revealed that a majority of the compounds exhibited a higher dissolution rate and higher solubility in the simulated intestinal fluids (FaSSIF and FeSSIF) than in their corresponding blank buffers. Thus, the use of such dissolution media can significantly increase the degree of *in vitro–in vivo* correlation, as was noted by Galia et al.[3] in terms of a poorly soluble drug glibenclamide in biorelevant media. It should be noted that the physicochemical properties of substances affect the reasonability of using biorelevant media for predicting the *in vivo* behavior. As it has been shown,[1,2] the need for *in vitro* dissolution experiments in biorelevant media occurs in the case of both poorly soluble weakly basic substances and lipophilic ones. Moreover, for poorly soluble substances of weakly basic nature, the investigation in biorelevant media simulating both the stomach and intestines conditions should be performed. The bile acids in the intestinal juice emulsify a number of compounds increasing their permeability to the intestinal wall by forming a micellar solution of lipids in an aqueous medium. The authors[4,5] found a significant increase in the solubility of ketoprofen when 1.0% sodium lauryl sulfate was added to the dissolution medium and showed a combined effect of pH and surfactant on dissolution of drugs.

The 1,2,4-thiadiazole derivatives being the objects of our investigations[6,7] belong to an extremely important class of heterocycles having a wide application in pharmaceutics due to a diverse range of pharmacological action[8,9,10], including neuroprotective activity.[11]

Our previous study[6] showed that 1,2,4-thiadiazole derivatives are poorly soluble in aqueous media. The problem of low solubility of the lipophilic compounds in water can be successfully solved with the help of cyclodextrins (CDs). CDs are widely used as solubilizing and stabilizing agents in pharmacy,[12-14] cosmetics and food industry.[15]

The unique ability of cyclodextrin molecules to form inclusion complexes with lipophilic drug molecules both in solution[16] and in solid form[17] is widely described in the literature and serves as a convenient way to increase the solubility of lipophilic compounds in aqueous solutions. Along with this, complexation can result in changing the crystalline state of substances (for example, amorphization),[18] increasing the bioavailability and pharmacological activity,[19] prolonging the therapeutic effect[20] and alleviating undesirable side effects of drug substances by making the drug effective at lower doses. Moreover, as Leclercq[21] showed, CDs can be useful for a vast array of topics due to their ability to interact with endogenous substances that originate from an organism, tissue or cell. Due to a numerous advantages mentioned above, drug formulations based on CD and their derivatives as carriers being designed during several decades, succeeded in the development of new oral drug delivery systems.[22]

It is well known, that application of CD as solubility and dissolution enhancers in oral drug formulations can result in enhanced, unchanged, or even decreased oral bioavailability due to permeability variations.[23,24] This phenomenon tends to be complicated in biorelevant media containing bile salts and phospholipids. Namely, the drug molecule can be displaced from the CD cavity, leading to a decrease in solubility and an increase in intestinal absorption as a result of supersaturation stabilized by CD.[25] In this connection, Olesen et al.[26] emphasized that the presence of specific components may influence the concentration of CD necessary for the solubilization of the drug substance, which underlines the importance of conducting the experiments in biorelevant conditions. To obtain complete information about the behavior of a drug substance in the intestine for the purpose of objective prediction of the *in vivo* absorption, it is very useful to perform permeability assay in the presence of biorelevant media components. Miller et al.[27] investigated the effect of micellar solubilization by sodium taurocholate and sodium lauryl sulfate on the intestinal membrane permeability of the lipophilic drug progesterone and show that the diverse interplay between the apparent solubility and intestinal membrane permeability remains when the surfactants are used as drug solubility enhancers.

The overall correlation of *in vivo* and *in vitro* results gains in understanding the limiting factors of drug absorption. Using such correlation in the case of different drug-CD solid forms

allows to propose the most effective oral formulations of poorly water soluble drug compound as a perspective candidate to the pharmaceutical market.

In this connection, the objectives of this study were: (1) to prepare the solid complexes of poorly soluble 1,2,4-thiadiazole derivative with β- and hydroxypropyl-β-cyclodextrins by grinding and freeze-drying techniques; (2) to investigate *in vitro* solubility, dissolution and permeability of the obtained solid dosage forms in compendial and biorelevant media; (3) to perform *in vivo* experiments in rats and evaluate the bioavailability of pure thiadiazole and its complexes with CD; (4) to analyze the influence of complex formation with CDs, method of the preparation of the complexes as well as the composition and properties of biorelevant media on the biopharmaceutical properties of 1,2,4-thiadiazole derivative; (5) to consider the *in vitro – in vivo* correlation and to propose the most perspective formulations for oral administration.

2. Materials and methods

2.1. Materials

1,2,4-Thiadiazole derivative (1-[5-(3-chloro-phenylamino)-1,2,4-thiadiazol-3-yl]-propan-2-ol, TDZ) was synthesized in the Institute of Physiologically Active Compounds of the Russian Academy of Sciences. The synthetic approach followed the method of Vivona et al.[28] and described by us earlier.[29] TDZ was obtained from 1-[5-(3-chloro-phenylamino)-1,2,4-thiadiazol-3-yl]-propan-2-one (2.0 g, 74.1%). Mp 390 K. Anal.: found, %: C 48.98; H 4.48; N 15.58. $C_{11}H_{12}ClN_3OS$ (C, H, N); calcd, %: C 48.88; H 4.62; N 15.46. ^{1}H NMR (200 MHz, $CDCl_3$) δ, ppm: 1.30 (d, *J*=6.17 Hz, 3 H, CH_3), 2.68 – 3.11 (m, 2 H, CH_2), 4.30 (m, 1 H, CH), 4.37 (br. s., 1 H, OH), 7.14 (d, *J*=7.50 Hz, 2 H, CH_2, HAr), 7.27 (s, 1 H, CH, HAr), 7.30 – 7.46 (m, 1 H, CH, HAr), 9.02 (br. s., 1 NH).

β-Cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) were obtained from Sigma-Aldrich. Average degree of molar substitution of HP-β-CD was 0.6 (average molecular weight is 1380 Da). The content of water in CDs was determined by thermogravimetry and was taken into account during calculation of the concentration.

Buffer components such as dibasic sodium phosphate (Aldrich), sodium hydroxide (Aldrich), sodium chloride (Aldrich), sodium dodecyl sulfate (Aldrich), lecithin (Fisher Chemical), sodium taurocholate (TCI), pepsin (Fisher Chemical) were of analytical grade and used as received. Table 1 shows the composition of biorelevant media SGF (Simulated gastric fluid), FaSSGF (Fasted state simulated gastric fluid), and FaSSIF (Fasted state simulation intestinal fluid), which were prepared according to the procedures proposed by Galia et al.[3] and Klein.[30]

Table 1
Composition of the media to simulate the conditions in the gastrointestinal tract

	SGF	FaSSGF	FaSSIF
Sodium dodecyl sulfate (mM)	8.7	–	–
Pepsin (mg/ml)	–	0.1	–
Sodium taurocholate (mM)	–	0.08	3
Lecithin (mM)	–	0.02	0.75
NaCl (mM)	34.2	34.2	105.85
Na_2HPO_4 (mM)	–	–	28.65
NaOH (mM)	–	–	8.7
pH	1.2	1.6	6.5

Freshly prepared distilled and degassed water was used for solutions preparation. The pH values of buffers were measured using Mettler Toledo Five Easy pH-meter, osmolality was controlled by means of Semi-Micro Osmometer K-7400 (Herbert Knauer GmbH, Berlin, Germany).

2.2. Preparation of TDZ/CD formulations

Physical mixture of TDZ and CD at molar ratio 1:1 was prepared. Complexes TDZ/CD were obtained by grinding (gr) and freeze-drying (fd) methods. Grinding and freeze-drying procedures of the physical mixtures has been described in our previous work.[31] The solid samples were subsequently sieving through an 80-mesh screen and stored in a desiccator until further evaluation. They have been characterized in detail by DSC, TG, hot-stage microscopy and PXRD.[6] In this work, complex formation of TDZ with CDs in the solid state was confirmed by ^{13}C MAS CP/TOSS NMR and biopharmaceutical properties of the complexes were examined *in vitro* and *in vivo*.

2.3. ^{13}C solid-state NMR

Solid-state ^{13}C MAS CP/TOSS NMR spectra were recorded at 30 °C on a Bruker Avance III 400WB spectrometer operating at 9.4 T (100.04 MHz for ^{13}C), using a $^{1}H/X$ dual probe with 4 mm zirconia rotors driven by dry air at 10 kHz. The CP contact time was 2 ms. The recycle delay was 5 s. ^{13}C chemical shifts were referenced with respect to external tetramethylsilane.

2.4. Solubility

Solubility of TDZ and TDZ/CD formulations in different buffers was measured by saturation shake-flask method at temperature of 25 ^{o}C. Excess amounts of the samples were shaken with appropriate solvents in glass ampoules until saturated solutions were obtained. Thermodynamic equilibrium between solids and solutions was determined from preliminary experiments on measuring the kinetic dependences of the solution concentrations. The time needed for reaching the plateau of drug concentration against time was considered as a suitable equilibration time. The time of 72 hours was estimated to be high enough for the equilibrium of all the systems studied to be reached. After equilibration solutions were centrifuged (Biofuge pico, Thermo Electron LED GmbH, Germany) at 6000 rpm for 20 min at 25 ^{o}C. Concentrations of TDZ in supernatants were determined spectrophotometrically (Shimadzu 1800, Japan). The experiments were performed in triplicate.

2.5. Dissolution

The in vitro dissolution tests were performed using Labindia tablet dissolution tester (India). The rotation speed was set to 70 rpm and the water bath temperature was maintained at 37±0.5 ^{o}C. Tablets from TDZ and TDZ/CD formulations were prepared by direct compression method using hydraulic press. The content of TDZ was fixed in all tablets (~5±0.002 mg per tablet). The dissolution medium was 400 mL buffer.

At selected periods aliquots (5 mL) were withdrawn and replaced with the same volume of the medium. The aliquots were filtered, and absorbance at 248 nm was recorded using a UV–vis absorption spectrophotometer (Shimadzu UV-1800, Japan). The analyses were performed in triplicate. The dissolution profiles were plotted as the percentage of cumulated dissolution versus time.

2.6. Permeability

Permeability measurements were performed at pH 6.5 since the main absorption of drug takes place in the intestinal canal. All experiments were conducted using Franz-type diffusion cell (SES GmbH-Analytical Systems, Germany) through a regenerated cellulose membrane with a molecular weight cut off MWCO 12–14 kDa (Standard Grade RC Dialysis Membrane, Flat Width 45 mm). The membrane was pretreated with distilled water for 30 min and dried under air before use. The membrane was mounted between donor chamber and receptor chamber with an effective surface area of 0.64 cm^2. Uncomplexed TDZ and TDZ/CD complexes were dispersed in FaSSIF or blank buffer pH 6.5 to obtain sample suspensions which were placed in the donor compartment (bottom chamber). Acceptor compartment (upper chamber) was filled with the blank buffer pH 6.5. The suspension in the donor compartment was stirred vigorously. Thus, in

all cases a reverse dialysis set up was employed.[32] An aliquot of 0.5 µl of the receptor solution was withdrawn from the receptor chamber at predetermined time intervals (30 min) and replaced with fresh buffer (pH 6.5). Samples were analyzed spectrophotometrically (Shimadzu 1800, Japan). The permeation profiles were plotted as the amount of permeated compound over the surface area (dQ/A) versus the time (t). The flux (J) was calculated as slope of permeation profiles according to the equation:

$$J = \frac{dQ}{A \times dt} \quad (1)$$

The apparent permeability coefficient (P_{app}) was calculated by normalizing the flux J measured over the concentration of the drug in the donor compartment (C_0) as described by equation (2):

$$P_{app} = \frac{J}{C_0} \quad (2)$$

Each permeability experiment was repeated 3 times and the average value of P_{app} was determined. The experiments were performed under the sink conditions; that is, the drug concentration in the acceptor chamber did not exceed 10 % of the drug concentration in the donor chamber at any time.

2.7. In vivo pharmacokinetic study

All experiments were conducted in accordance with rules and regulations of the animal research ethics and Rules of laboratory practice in conducting preclinical research in the Russian Federation (GOST 51000.3-96 and 51000.4-96 3, GOST 50258-92). These experiments were also approved by the Ministry of Health of the Russian Federation (order №267 from 19.06.2003).

The pharmacokinetic study of TDZ and TDZ/HP-β-CD formulations was performed in white male rats (weight 150-200 g). Rats were maintained on a standard diet. They were fasted overnight with free access to water before the experiment. A single dose equivalent to 50 mg of TDZ per kg of rat body weight was administrated by oral gavage. Administration was made with starch 1.5%. Blood samples were retrieved from the tail of the rats at 15, 30, 60, 120, 180 and 300 minutes after oral administration. The blood was centrifuged at 3000 rpm for 15 min and the resultant plasma was stored at -20 ^{o}C until required for analysis.

Plasma samples (500 µL) were placed in Eppendorf tubes with 1000 µL water-acetonitrile (50:50 v/v) and thoroughly vortexed. Then the samples were centrifuged at 1200 rpm for 10 min and supernatant was filtered and analyzed by HPLC (Shimadzu LC-20, Japan). The mobile phase was water-acetonitrile (40:60 v/v). The resulting concentrations were plotted as

concentration-time profiles. The maximal concentration of TDZ in plasma (c_{max}), the time to reach the maximal concentration (t_{max}) and area under the concentration-time curve from zero to a definite time t (AUC_{0-t}) were obtained directly from the concentration-time profiles.

3. Results and discussion

The aim of this work was to increase the oral absorption of anti-Alzheimer drug candidate. For this purpose, inclusion complex formation with CDs was employed. The choice of CDs is determined by widespread application of these oligosaccharides as pharmaceutical excipients enhancing properties of poorly soluble drugs.[33] Solid inclusion complexes of TDZ with β-CD and HP-β-CD were prepared by grinding described in our recent work[6] and freeze-drying methods. Binding of TDZ with CDs in the solid state has been confirmed by DSC, powder X-ray diffraction and hot-stage microscopy methods.[6] The results of this study are given in Supporting Information (Figs S1-S3).

3.1. ^{13}C solid-state NMR study

Complex formation of TDZ with β-CD and HP-β-CD in the solid state was additionally confirmed by means of ^{13}C solid-state NMR. The ^{13}C MAS CP/TOSS NMR spectra of TDZ, CD (β-CD or HP-β-CD), their physical mixtures (TDZ+CD) and TDZ/CD complexes obtained by grinding (TDZ/CD_gr) and freeze-drying (TDZ/CD_fd) are shown in Fig. 1. The comparison of the spectra of physical mixture and complexes prepared with the same molar ratio confirms the complex formation in the solid state due to the following facts. On the contrary, upon grinding or freeze-drying signals of TDZ and CD carbons get significantly broader, which is especially noticeable for the signals of CD. Moreover the resonances of CD carbons at 101.5–103.5 ppm (C1 carbons[34]) are slightly shifted to the low field when compared to those of pure CD and (TDZ+CD) physical mixture. These changes in NMR spectra suggest that a weak binding of TDZ with CDs takes place in the solid state and causes a distribution of microenvironments for carbon atoms due to the formation of essentially amorphous or at least conformationally flexible inclusion complexes.[35]

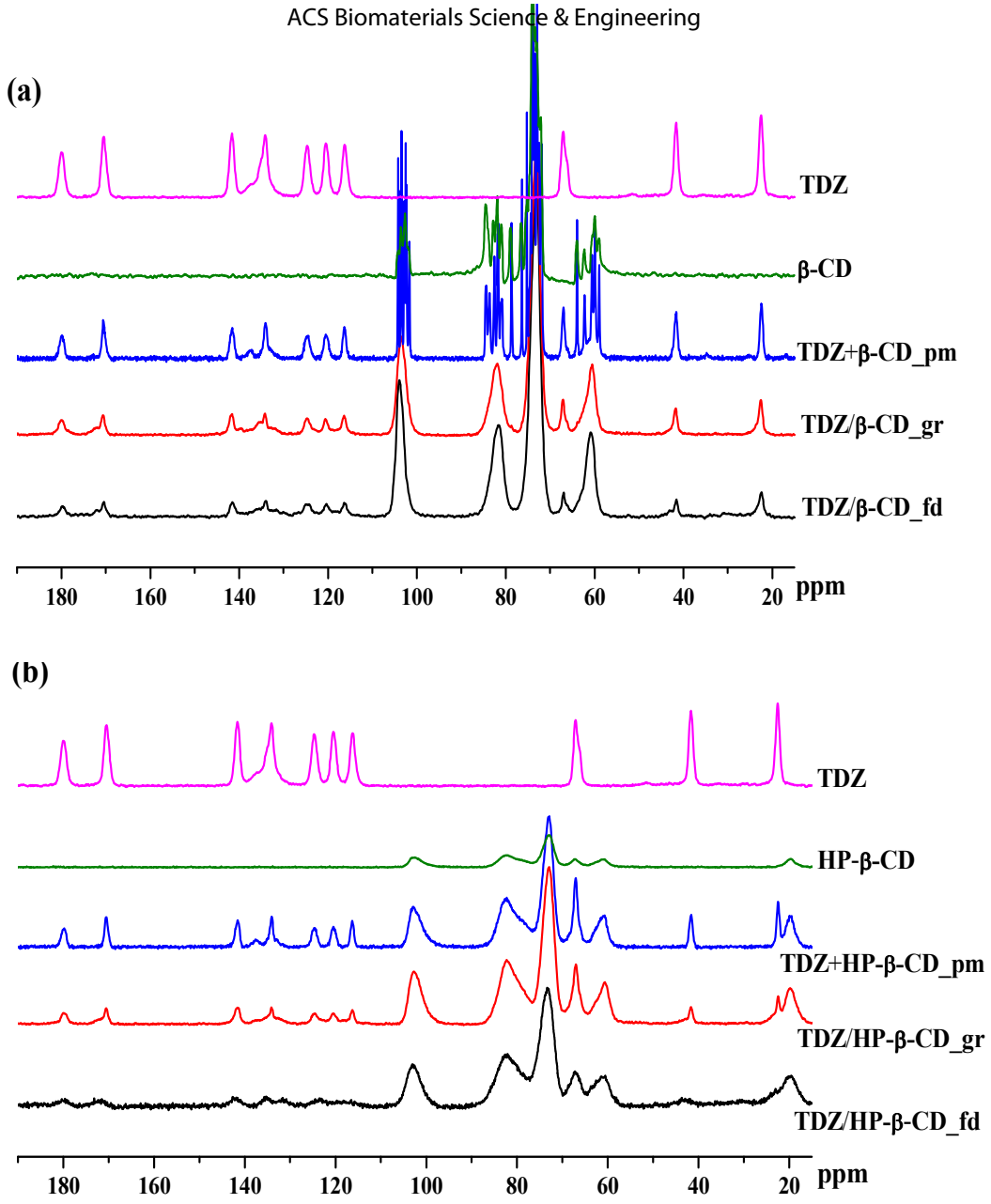

Fig. 1. ^{13}C CP/MAS NMR spectra of TDZ, CD, (TDZ+CD) physical mixture and TDZ/CD complexes (a – samples with β-CD; b – samples with HP-β-CD).

3.2. Solubility study

Solubility of TDZ, physical mixtures (TDZ+CD) and solid complexes TDZ/CD in different biorelevant media (SGF, FaSSGF and FaSSIF) and corresponding blank buffers such as hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 6.5) was measured at 25 ^{o}C. Despite the fact that blank buffers under study are widely used to mimic the biological liquids of the living organism, in this work they were considered as reference dissolution media having simplified composition. Really, the gastrointestinal liquids have more complicate composition and contain different organic ingredients. In this connection, biorelevant media developed by

Dressman[1] should be used to examine the solubility, dissolution and absorption of drugs in order to predict more precisely their *in vivo* behaviour. In this connection, solubility was determined in the biorelevant media such as SGF (pH 1.2), FaSSGF (pH 1.6) and FaSSIF (pH 6.5). The obtained results are shown in Fig. 2. It should be noted that solubility data reported in this study for blank buffer pH 1.2 are in a good agreement with our previous results.[6]

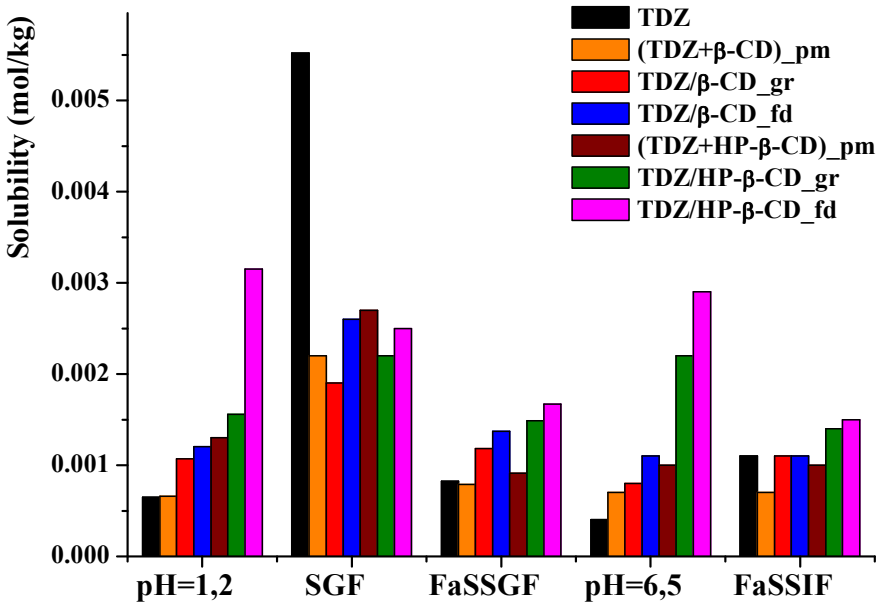

Fig. 2. Solubility of TDZ, (TDZ+CD) physical mixtures and TDZ/CD complexes at 25 ^{o}C.

As one can see from Fig. 2, solubility of TDZ increases in the presence of β-CD and HP-β-CD in blank buffers pH 1.2 and 6.5. Enhancement of solubility of TDZ/CD complexes is more significant in comparison with the corresponding physical mixtures. This fact can serve as an additional confirmation of the binding of TDZ with CDs in the solid state. Freeze-drying is more appropriate method for preparation of water soluble complexes of TDZ. Solubility of freeze-dried TDZ/CD complexes is particularly noticeable. Solubilizing effect of HP-β-CD is more pronounced than those of parent β-CD. Compared with β-CD, HP-β-CD is better soluble and wettable in aqueous solutions and forms with TDZ more stable inclusion complexes in blank buffers pH 1.2 and 6.5.[6] This causes the observed difference in the solubilizing action of native and hydroxypropylated β-CDs.

As follows from Fig. 2, solubility of TDZ as well as the physical mixtures and complexes of TDZ with β-CD and HP-β-CD is considerably higher in SGF than in all other buffers under

study. This can be caused by the presence of sodium dodecyl sulfate micelles in the SGF buffer (Table 1). Micellar solubilization results in dramatic increase of the solubility of pure TDZ in SGF. It is interesting to note that solubility of the samples with CDs content in SGF is ~2 times lower in comparison with the pure TDZ. This can be explained by the competitive complex formation of CDs with TDZ and sodium dodecyl sulfate (SDS). Binding of SDS with β-CD and HP-β-CD has been well documented in the literature.[36,37,38] As follows from the published data, 1:1 complexes of SDS with β-CD and HP-β-CD predominate in aqueous solutions and magnitudes of their stability constants lay in the range of 10^3–10^4 M^{-1}. It is interesting to compare the binding of SDS with native and hydroxypropylated β-CDs. Bendazzoli et al.[38] investigated the complexation of SDS with native and modified CDs. Stability constants were found to be 3021±98 M^{-1} and 2871±107 M^{-1} of SDS complexes with β-CD and HP-β-CD, respectively. As one can see, binding affinity of SDS to both CDs is approximately the same. It is well known that critical micelle concentration (cmc) of the surfactants can be changed under addition of different additives. As rule, inclusion complex formation induces the increase of the cmc since the number of surfactant monomers decreases due to their competitive binding with CDs. Maeso et al.[39] determined the cmc of SDS (8 mM) in the aqueous solutions of HCl containing variable concentrations of α-CD, β-CD and γ-CDs. In the presence of 1 mM and 6 mM β-CD the cmc was equal to 5.07 mM and 8.81 mM, respectively. The cmc in 10 mM aqueous solutions of β-CD and HP-β-CD was 15.1 mM and 14.6 mM. As one can see, the difference is insignificant. Thus, we believe that SDS micelles exist in the SGF buffer and following equilibria take place in the systems under study:

$$SDS_{monomer} + CD = SDS_{monomer}/CD \quad (3)$$

$$TDZ + CD = TDZ/CD \quad (4)$$

$$TDZ + SDS_{micelle} = TDZ/SDS_{micelle} \quad (5)$$

Intermolecular interactions between positively charged TDZ and anionic SDS monomers are also possible in SFG. CDs and CD complexes are not incorporated into SDS micelles.[40] Thus, the observed decrease of the solubility of complexes and physical mixtures of TDZ with CDs is mainly caused by competitive binding of CDs with SDS monomers and TDZ. Complexes of β-CD and HP-β-CD with SDS are more stable than those with TDZ. In particular, stability constants of TDZ/β-CD and TDZ/HP-β-CD complexes have been found to be 278 M^{-1} and 378 M^{-1} in buffer pH 1.2[6] and they are 10 times lower compared with the SDS/CD complexes.[36,37] The preferred binding of β-CD and HP-β-CD with SDS molecules in aqueous solution is evident. This fact was confirmed by the results of UV-spectroscopic study on HP-β-CD complex

formation with TDZ in buffer SGF. The obtained dependence of an absorbance on HP-β-CD concentration is shown in Fig. 3. The linearity of this concentration dependence points out the weak complex formation between TDZ and HP-β-CD in the presence of SDS. Thus, TDZ/CD complexes dissociate in SDS solution and this does not result in the expected solubility increase (Fig. 2). In this case, the solubilizing effect of CDs becomes unimportant and mainly micellar solubilization occurs. However, the solubility of physical mixtures and complexes of TDZ with β-CD and HP-β-CD in buffer SGF is low compared with the pure TDZ. Weakening solubilizing effect of SDS micelles in the presence of CDs can be also caused by the inclusion complex formation of macrocycles with surfactant monomers. The SDS/CD binding results in decrease of the concentration of the micelles due to cmc increase.[38] Consequently, the SDS solubilizing capacity becomes weaker in CD solutions and this is accompanied by decrease of the solubility of the samples of TDZ with CDs (Fig. 2).

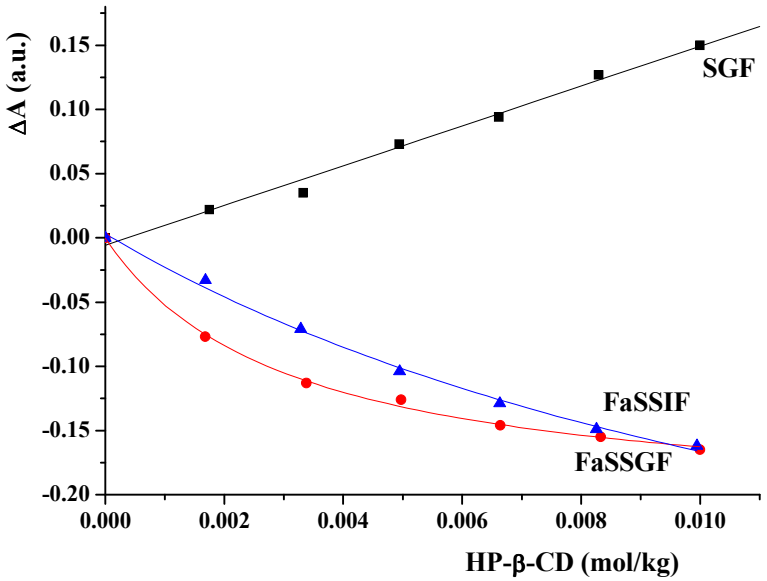

Fig. 3. Dependences of the TDZ absorbance change on HP-β-CD concentration in different buffers.

Buffer FaSSGF simulating the gastric juice is composed of sodium taurochorate, lecithin and pepsin (Table 1). As it is well known, taurocholate and lecithin are able to form micelles in aqueous solutions.[41] However, their concentration in FaSSGF is below cmc.[42] Thereby, micellar solubilization does not occur in FaSSGF and observed enhancement of the solubility of samples containing CDs is attributed to complex formation of TDZ with CDs. Comparison of the solubility data obtained for blank buffer pH 1.2 and FaSSGF gives the insignificant difference (Fig. 2). It can be assumed that components of FaSSGF have no pronounced effect on the

solubility due to the absence of their specific interactions with TDZ. However, it should be mentioned that 1:1 inclusion complexes between β-CD and sodium taurocholate are formed in aqueous buffered solution and it has been documented in the literature.[43,44,45,46] Stability constants of taurocholate/β-CD complexes obtained by calorimetry were found to be 2630 M^{-1} [44] and 2290 M^{-1} [45] in buffer solution pH 7.2. In FaSSGF buffer the concentration of taurocholate is very low (Table 1). Therefore, concentration of CDs is sufficient for binding both taurocholate and TDZ. Occurrence of complex formation between TDZ and CDs was evidenced by UV-spectroscopic study. Fig. 3 demonstrates the binding isotherm of TDZ with HP-β-CD in buffer FaSSGF. Calculated stability constant of TDZ/HP-β-CD complexes (K=325 M^{-1}) is close to those obtained in blank buffer pH 1.2. Thus, the behavior of TDZ/CD complexes in FaSSGF and blank buffer pH 1.2 is approximately the same.

Let us consider the FaSSIF buffer composed of the lecithin and taurochlate taken at higher concentration in comparison with the buffer FaSSGF (Table 1). The available in the literature values of the cmc of taurocholate lay in the rather wide range of 3–11 mM.[47,48,49] For instance, cmc at 3–5 mM was observed by means of the spectral shift of rhodamine 6G probe.[50] The cmc of sodium taurocholate in pure water and aqueous solution of NaCl (0.15 M) was found to be 10 mM and 8.5 mM, respectively.[48] Funasaki et al.[49] determined the cmc 8 mM using ^1H NMR technique. FaSSIF contains an array of mixed micelles such as spherical, rod-like, disk, and bilayer micelles and liposomal structures.[51] Another point of view is an absence of critical micellization phenomenon since sodium taurocholate is able to continuous self-association over a wide concentration range.[52] It has been documented that simple taurocholate micelleas are the only aggregates.[53] The bile salt association is gradual process which occurs through the formation of primary and secondary micelles.[54] Moreover, in the presence of lecithin, the formation of the taurocholate/lecithin micelles is possible, the cmc of which has been reported as 3.1 mM.[42] The most striking feature of lecithin-cholate micellar systems is the variation in size of the particles depending on the microenvironment.[41] As a rule, these mixed micelles have larger size and higher solubilizing capacity.[54] Additionally, phospholipid molecules intrinsically are arranged into bilayered aggregates[55] and lecithin forms liposomes when hydrated.[51] Taking into account the 3 mM concentration of taurocholate in FaSSIF (Table 1) and possibility of rearrangement of the taurocholate and lecithin aggregates in thiadiazole solution, and one can assume that FaSSIF is a complicate medium with a variety of microstructures. Therefore, the observed increase of solubility of pure TDZ in FaSSIF can be caused by the interactions of thiadiazole with both monomers and aggregates from lecithin and taurocholate. As can be seen from Fig. 2, the solubilizing effect of CDs is not visible in FaSSIF. It was discussed above that stable inclusion complexes are formed between CDs and taurocholate.[43,44,45,46] This process is in

competition with TDZ/CD complexation as it was confirmed using UV-spectroscopy. As follows from the experimental results presented in Fig. 3, stability constant of TDZ/HP-β-CD inclusion complexes (K=56 M^{-1}) is lower in FaSSIF than in blank buffer pH 6.5. Thus, the competing interactions of CDs with TDZ and taurocholate occur in buffer FaSSIF and prevent the inclusion of the thiadiazole into macrocyclic cavity. Similar results were obtained by Matsiu et al.[56] during the evaluation of the solubility and dissolution of oral dosage forms of itraconazole in biorelevant media. Itraconazole oral solution with HP-β-CD content displayed faster precipitation in FaSSIF. This observation was explained by the molecular interaction between taurocholic acid and HP-β-CD. It was also reported[56] that complex of HP-β-CD with taurocholic acid is formed in place of complex with itraconazole in FaSSIF buffer.

3.3. Dissolution study

Dissolution rate is an important parameter which determines the bioavailability of drugs. It is known that poor soluble drugs can reveal rapid dissolution On the contrary, the dissolution of highly soluble compound can be very slow. Dissolution of pure TDZ and TDZ/CD complexes prepared by grinding and freeze-drying methods was studied in biorelevant media and corresponding blank buffers. Dissolution profiles are shown in Fig. 4. According to USP, the drug is considered rapidly dissolving when 80 % or more of the substance dissolves within 30 minutes.[57] The values of drug amount dissolved in 30 minutes (D_{30min}) are summarized in Table 2. As one can see, the dissolution of pure TDZ is rather slow process in all buffers under consideration. It occurs slightly faster in SGF due to a powerful solubilizing effect of SDS micelles (Fig. 2). Complexes of TDZ with β-CD and HP-β-CD display the improved dissolution behaviour. In particular, the freeze-dried complexes of TDZ with HP-β-CD completely dissolved within 10 min in all media under study. According to the results of powder X-ray diffraction (Fig. S3), pure TDZ is a crystalline substance and, therefore, its solubility and dissolution rate are not high. On the contrary, complexes of TDZ with CDs are amorphous (Fig. S3) and display better dissolution rate since the necessary energy for molecule separation is less than that of the crystalline form. The improved dissolution behaviour of the complexes with HP-β-CD is caused by the higher wettability and aqueous solubility of hydroxypropylated β-CD as compared with the native one.

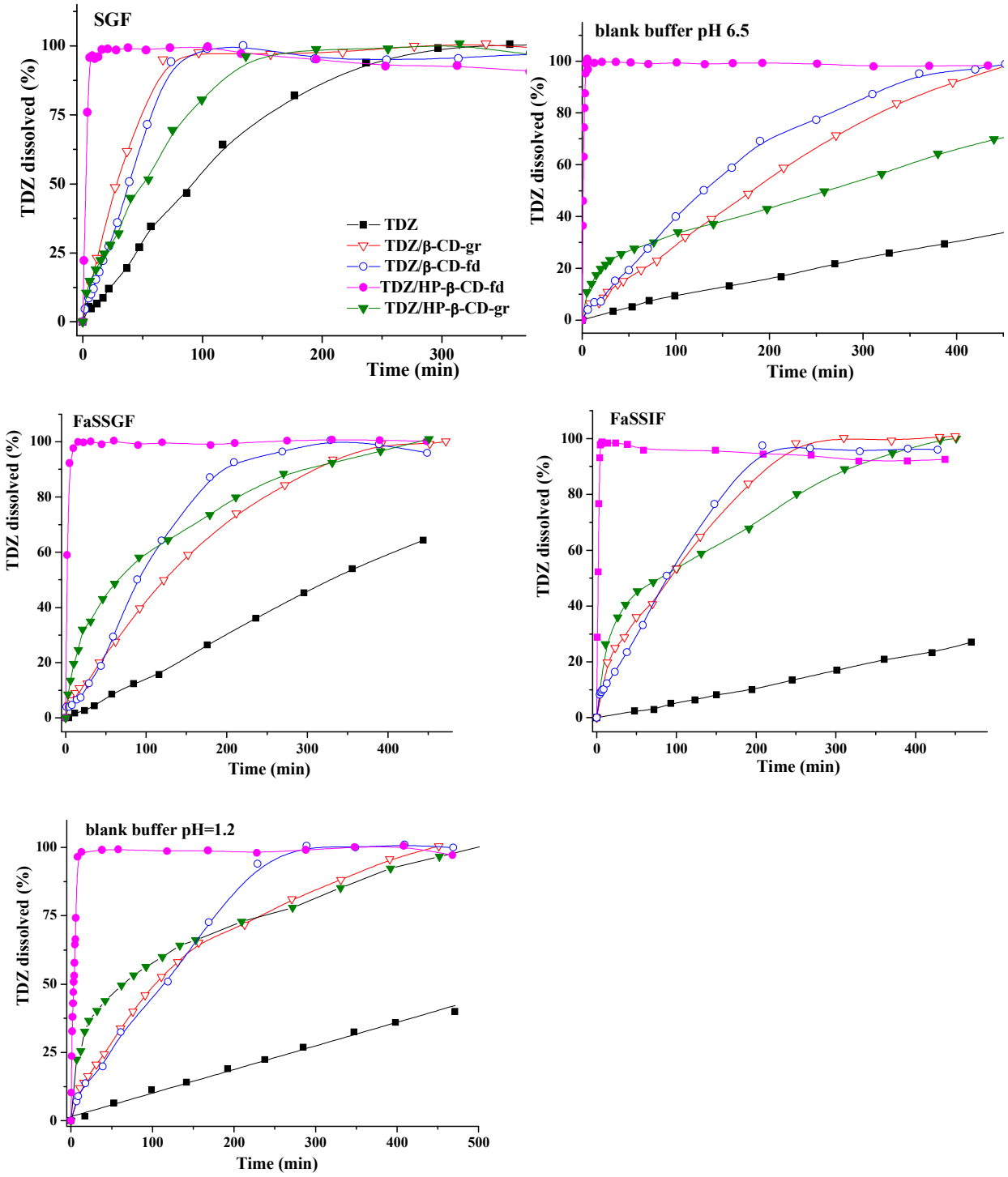

Fig. 4. Dissolution profiles of 1,2,4-thiadiazole derivative and its complexes with cyclodextrins in different buffers at 37 °C.

Table 2
Amount (w, %) of the dissolved 1,2,4-thiadiazole derivative within 30 minutes (D_{30min}) in different buffers at 37 ^{o}C

Sample	blank buffer pH 1.2	SGF	FaSSGF	blank buffer pH 6.5	FaSSIF
pure TDZ	3.5	15.8	3.9	2.9	1.4
TDZ/β-CD_gr	20.1	52.6	14.7	11.6	27.6
TDZ/β-CD_fd	17.5	37.6	13.5	12.9	20.3
TDZ/HP-β-CD_gr	38.7	31.7	34.7	23.5	37.4
TDZ/HP-β-CD_fd	98.8	99.3	99.9	99.3	98.3

It is interesting to compare the influence of buffer composition on the kinetics of dissolution. There is no significant influence of buffer pH on the dissolution process (Fig. 4) due to the close ionization state of TDZ at different pH as it has been demonstrated by us before.[6] However, the dissolution is affected by the buffer composition. Dissolution of TDZ and TDZ/CD complexes is accelerated in SGF and FaSSIF (Fig. 4). Faster dissolution in SFG can be explained by micellar solubilization of TDZ. Similar results were demonstrated by de Smidt et al.[58] when they studied the dissolution of gliseofulvin in SDS solutions.

The dissolution rate can be expressed via the Noyes-Whithey equation:

$$\frac{dm}{dt} = A\frac{D}{d}(c_S - c_b) \quad (6)$$

where m is the mass of the substance dissolved; t is the time; A is the surface area of the solute particle; D is the diffusion coefficient which is dependent on several parameters such as temperature, viscosity of the medium, radius of the solute molecule; d is the thickness of the boundary layer of the solvent at the surface of the dissolving substance; C_S is the concentration of solute in the solvent near the solute surface; C_b is the concentration of solute in bulk solvent.

Taking into account Eq. (6), one can assume that fast dissolution in SGF is determined by the dramatic increase of TDZ solubility in the presence of SDS (Fig. 2). The TDZ solubility increases also in FaSSIF (Fig. 2). Therefore, the accelerated dissolution of the samples in this buffer can be also explained by the buffer solubilizing capacity. In addition to this, bile acids can increase the dissolution rate via the enhanced wetting of the compound.[54]

It is important to evaluate the difference between the dissolution profiles. In order to simplify a comparison of TDZ dissolution in different media it was useful to perform a pair-wise

procedure based on the estimation of the difference and similarity factors (f_1 and f_2, respectively) proposed by Moore and Flanner:[59]

$$f_1 = \frac{\sum_{j=1}^{n}\left|R_j - T_j\right|}{\sum_{j=1}^{n} R_j} \times 100 \quad (7)$$

$$f_2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{j=1}^{n}\left|R_j - T_j\right|^2\right]^{-0.5} \times 100\right\} \quad (8)$$

where n is the sampling number, R and T are the percents of the reference and test products dissolved at time point j. It is believed[60] that values of $f_1 < 15$ and $f_2 > 50$ show the similarity of the dissolution profiles. The obtained in our work and summarized in Table S1 values of f_1 and f_2 were used to compare the dissolution behavior of TDZ and TDZ/CD complexes in SGF, FaSSGF, FaSSIF and correspondent blank buffers. The data in Table S1 clearly demonstrate the similarity of the dissolution behavior of all investigated TDZ/CD complexes in blank buffer and FaSSGF. At the same time, the difference between blank buffer and SGF as well as between blank buffer and FaSSIF was observed (except freeze-dried TDZ/HP-β-CD complex). This is caused by the TDZ micellar solubilization in these buffers. The absence of the micelles in FaSSGF determines the similarity of dissolution behavior of the samples in this biorelevant medium and corresponding blank buffer. High degree of similarity of the dissolution profiles of the freeze-dried TDZ/HP-β-CD complexes can be explained by their extremely rapid dissolution in all media under study.

3.4. Permeability study

It was shown above that complex formation with β-CD and HP-β-CD results in the enhancement of aqueous solubility and dissolution rate of TDZ and this is favorable for increase of TDZ bioavailability. Meanwhile, CD containing formulations with improved solubility for oral administration can show complicated and unpredictable gastrointestinal behavior because an increase in solubility is often accompanied by a decrease in the free, bioavailable fraction of drug.[61,62] Holm et al.[63] assessed these opposing effects on danazol and cinnarizine drug formulations with CD and indicated that CD concentration can have a major effect on the pharmacokinetic profile of one compound and a minor effect on the pharmacokinetic profile of another. Taking into account very low intestinal absorption of CDs (and CD complexes) the drug has to be released from the hydrophobic cavity. Therefore, high binding constants as well as large CD concentrations may impede intestinal absorption.[64] From the above argumentations, it

was necessary to take into account the influence of the inclusion complex formation on the membrane permeability of TDZ both in blank buffer and biorelevant medium.

To this end, firstly, in order to elucidate the CD concentration influence, the apparent permeability coefficients P_{app} of TDZ were determined in the presence of variable amounts of CDs. Dependences of P_{app} on CD concentration are given in Fig. 5 along with the solubility diagrams obtained in our previous study[65] in order to evaluate the solubility–permeability interplay in the systems under study. As one can see, complex formation with CDs weakens the ability of TDZ to pass through an artificial membrane. Permeability coefficients decrease with the increase of CDs concentration (Fig. 5). The obtained results clearly confirm that β-CD and HP-β-CD are not the penetration enhancers and can be only employed as solubilizers. In so doing, the use of high concentrations of CDs in order to achieve the large soubilizing effect is not recommended because of the decrease in permeability and bioavailability of TDZ. As Fig. 5 illustrates, expected solubility–permeability interplay is characteristic for the complexes of TDZ with both CDs used.

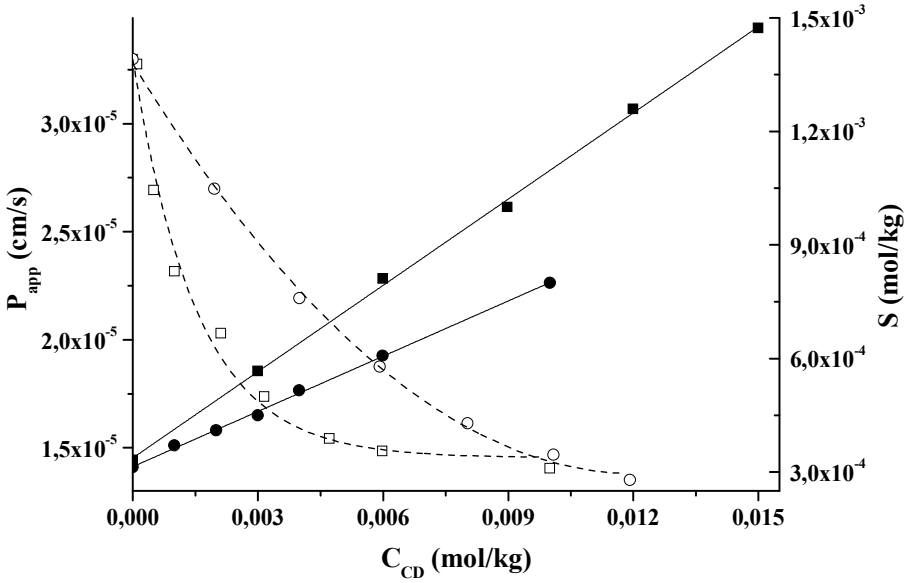

Fig. 5. The effect of β-CD (○, ●) and HP-β-CD (□, ■) concentration on TDZ permeability coefficients (open symbols) and solubility (filled symbols).

In view of the importance of the solubility–permeability interplay in case of solubility enabling formulations it is interesting to consider the permeability of suspensions prepared from the solid inclusion complexes TDZ/CD. The obtained permeation profiles (Fig. S4) were used for calculation of the TDZ fluxes (J) across the membrane. Values of J are summarized in a diagram (Fig. 6). As one can see from Fig. 6, the flux is maximal for freeze-dried TDZ/HP-β-CD

and TDZ/β-CD complexes displaying high solubility in both media (Fig. 2). The higher flux across a membrane occurs due to a larger concentration gradient between the donor and acceptor solutions. In comparison with blank buffer pH 6.5, the difference between *J* of all samples is not so significant in FaSSIF (Fig. 6). As it was mentioned above, taurocholate interacts with CDs and displaces the TDZ molecule from the inclusion complexes. Moreover, TDZ participates in binding with the micelles of taurocholate and lecithin. Therefore, the solubilizing effect of CDs is not so pronounces compared with the micellar solubilization. This evidences for the importance of evaluating the permeability formulations of drugs with CDs in biorelevant media.

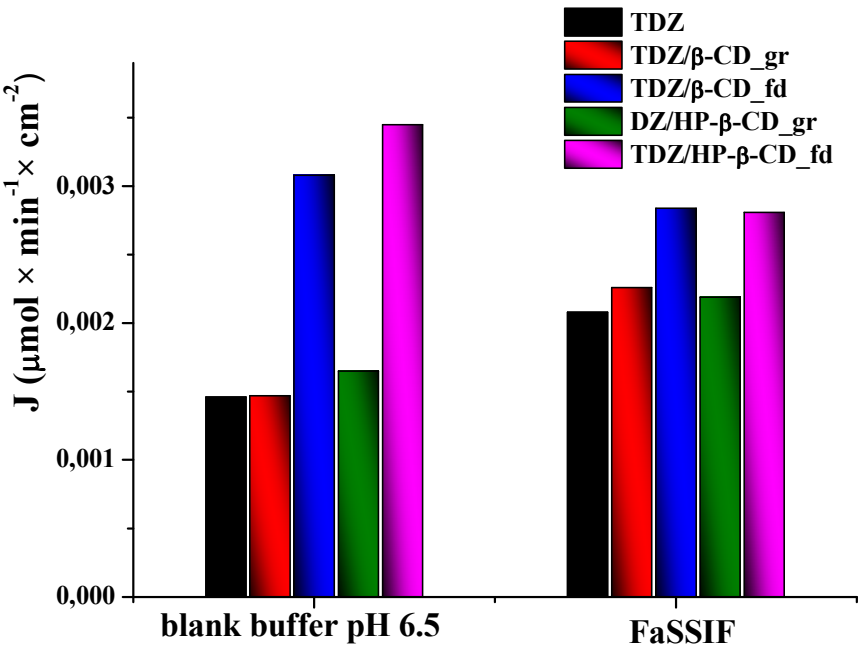

Fig. 6. Fluxes of TDZ and TDZ/CD complexes measured in blank buffer pH 6.5 and FaSSIF at 37 °C.

3.5. Pharmacokinetic study in rats

The pharmacokinetic study was performed for pure TDZ and TDZ/HP-β-CD formulations. The choice of the sample for *in vivo* experiments was determined by the improved solubility and dissolution behavior of these complexes in all media under study (see Fig. 4 and Table 2). The concentration of TDZ in rat plasma was registered when pure TDZ and TDZ/HP-β-CD complexes prepared by grinding and freeze-drying procedures were orally administered. Plasma concentration – time profiles are given in Fig. 7. The main pharmacokinetic parameters of TDZ for pure and complexed formulations are summarized in Table 3.

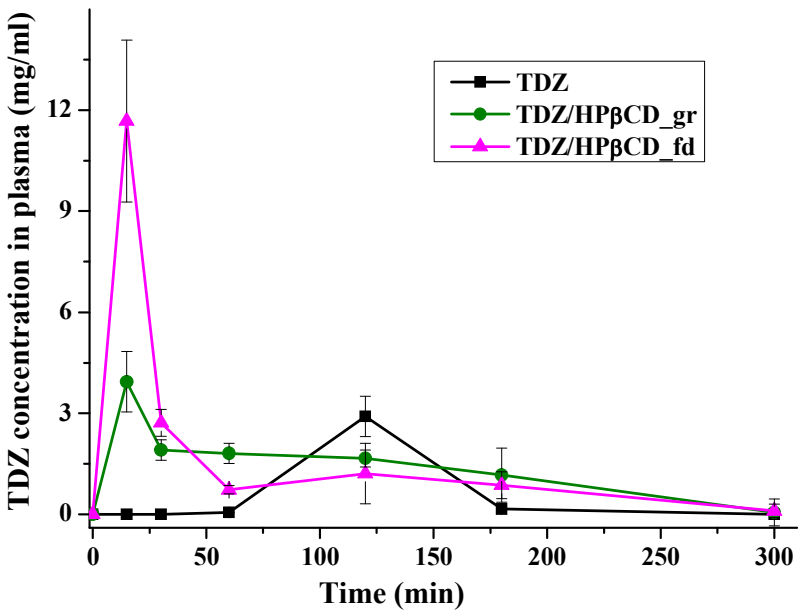

Fig. 7. Plasma concentration – time profiles of 1,2,4-thiadiazole derivative after oral administration of different TDZ formulations to rats (each value represents the mean ± S.D., n = 4).

Table 3
Pharmacokinetic parameters of TDZ after oral administration of pure TDZ and TDZ/HP-β-CD complexes (mean ± S.D., n=4)

	C_{max} mg/ml	t_{max} min	AUC_{0-t} mg·min/ml
TDZ	2.9 ± 0.4	120 ± 15	191 ± 19
TDZ/HP-β-CD_gr	3.9 ± 0.5	15 ± 2	390 ± 41
TDZ/HP-β-CD_fd	11.7± 1.8	15 ± 2	425 ± 49

The pharmacokinetic study revealed that plasma concentration of pure TDZ reached the maximal value at 2 hours after oral administration. When comparing the t_{max} for pure TDZ and TDZ/HP-β-CD complexes, the considerably lower values were detected for complexes (Table 3). The high AUC obtained after administration of TDZ/HP-β-CD complexes indicated a significant improvement in the extent of absorption of these formulations. The freeze-dried TDZ/HP-β-CD inclusion complex displays highest C_{max} and AUC as well as shorter t_{max} when it was compared with corresponding parameters for pure TDZ (Table 3). These pharmacokinetic data are in accordance to the results of *in vitro* experiments. Namely, the highest solubility (Fig. 2) and dissolution rate (Fig. 4) of freeze-dried TDZ/HP-β-CD complexes could explain the observed enhancement of pharmacokinetic parameters.

Conclusions

To improve the biovailability of 1,2,4-thiadiazole derivative, a novel drug-like compound with neuroprotective action, its solid complexes with cyclodextrins were suggested as dosage forms for oral administration. Complexes of thiadiazole with β- and hydroxypropyl-β-cyclodextrins were prepared by grinding and freeze-drying methods. The complexation was confirmed by ^{13}C solid-state NMR experiments. Solubility, dissolution and permeability of the complexes in biorelevant media simulating the human gastrointestinal tract fluids were evaluated.

It was found that complex formation with CDs significantly improves the solubility, dissolution rate and absorption of TDZ. The CD type and the preparation method influence the biopharmaceutical properties of the inclusion complexes. In particular, the freeze-dried complexes of 1,2,4-thiadiazole derivative with hydroxypropylated β-cyclodextrin showed significantly better solubility, dissolution rate and absorption. This can be caused by better aqueous solubility and wettability of HP-β-CD as well as by the amorphous state of the complexes.

It was revealed that the medium pH has no influence on the solubility and dissolution process of TDZ and TDZ/CD inclusion complexes, whereas the effect of the biorelevant media content is visible. In SGF and FaSSIF, the micellar solubilization of TDZ and the TDZ solubilization by CDs coexist. However, the solubilizing effect of CDs towards TDZ becomes less pronounced in SGF and FaSSIF due to stronger binding of macrocycles with monomers of sodium dodecyl sulfate and taurocholic acid present in these media, respectively.

The results of *in vivo* experiments being in accordance with the data obtained *in vitro* demonstrate the improved pharmacokinetic parameters of the freeze-dried inclusion complexes TDZ/HP-β-CD. Thus, the *in vitro* experiments carried out in biorelevant media can adequately predict the *in vivo* performance of the inclusion complexes of 1,2,4-thiadiazole derivative with CDs.

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Supporting Information

Results of DSC, powder X-ray diffraction and hot-stage microscopy studies, permeation profiles and values of difference and similarity factors are available at http://pubs.acs.org/.

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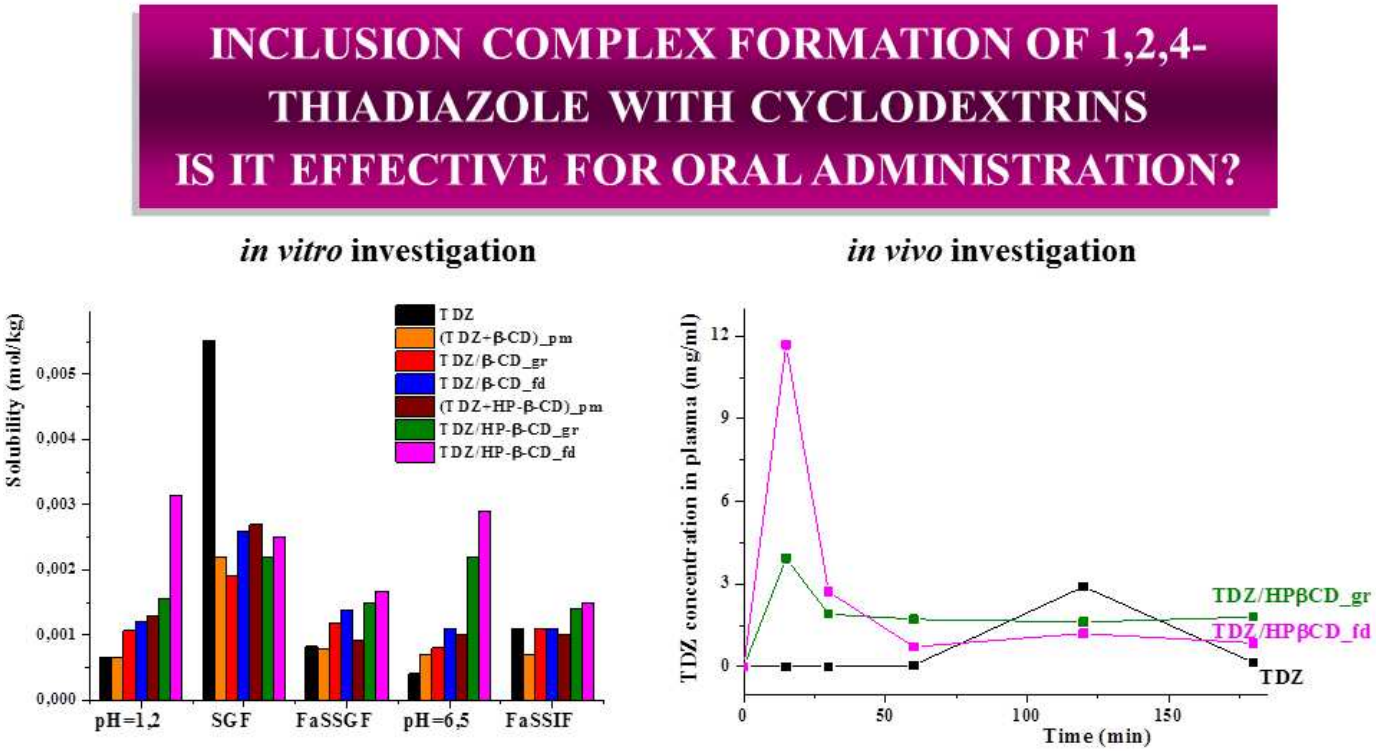
TOC Graphic

Improved biopharmaceutical properties of oral formulations of 1,2,4-thiadiazole derivative with cyclodextrins: *in vitro* and *in vivo* evaluation

Maria Promzeleva, Tatyana Volkova, Alexey Proshin, Oleg Siluykov, Anton Mazur, Peter Tolstoy, Sergey Ivanov, Felix Kamilov, Irina Terekhova



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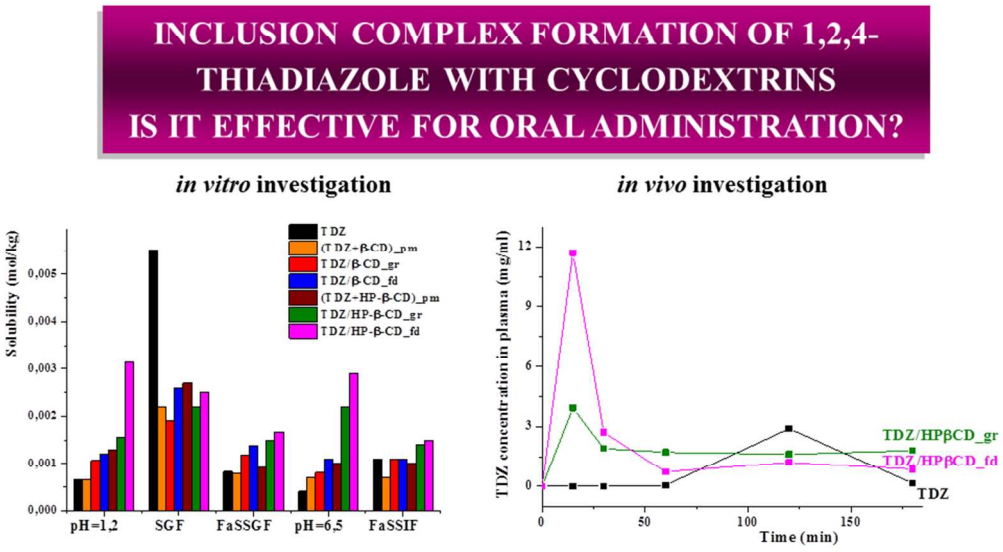

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