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The effect of different polymers on the solubility, permeability and distribution of poor soluble 1,2,4-thiadiazole derivative

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ABSTRACT

Solubility and permeability are the main parameters determining the bioavailability of drugs. In this study the increased solubility of novel 1,2,4-thiadiazole derivative (TDZ) proposed for the prevention and treatment of Alzheimer's disease was achieved in the solutions of polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), and pluronic F127 (F127). It was found that solubilizing power of polymers follows the order F127 > PVP > PEG. The mechanism of TDZ solubilization was proposed on the basis of ^{1}H NMR and UV-spectroscopy studies. It was suggested that PEG enhances the TDZ solubility by acting mainly as cosolvent, whereas PVP can be considered as cosolvent and complexing agent. In case of F127, the insertion of TDZ into micelles was detected. The solubilization capacity of pluronic was quantified in terms of average number of TDZ and F127 per micelle and binding constant. In order to reveal the effect of polymers on the TDZ membrane permeability, the distribution coefficients in the 1-octanol/buffer system and permeability coefficients through the novel Permeapad™ barrier were determined. The solubility-permeability and solubility-distribution relationships were discussed.

1. Introduction

Alzheimer's disease is a widely-spread neurodegenerative disease in the elderly age [1], and the number of cases is growing steadily throughout the world, which is associated with an increase of longevity. In the case of Alzheimer's disease, effective and timely treatment is extremely necessary. Considering these factors, great attention is being paid all over the world to the creation of new effective anti-Alzheimer's drugs and pharmaceutical formulations on their basis [2]. Thiadiazole derivatives belong to the class of compounds which have biological activity to specific targets of various pathologies [3–7].

A huge amount of new drugs have been produced over the past few decades, most of which have low aqueous solubility and/or inadequate permeability which limit the oral bioavailability [8,9]. Oral administration prevails over others due to its obvious advantages, such as ease of use, the presence of natural barriers that make the administration of the drugs safer, minimal discomfort for the patient, and others. Overcoming the problem of low bioavailability of drug compounds during oral administration requires for the research aimed at increasing the solubility and membrane permeability [10]. For this purpose, various approaches to enhance these characteristics are used, such as particle size reduction of [11–13], nanoparticle systems [13,14], solid dispersions [15,16], polymorphic modification [16,17], formation of host-guest complexes [18], lipid-based formulations [19] and so on. Different biopolymers including polyethylene glycols with different molecular weights and polyvinylpyrrolidone are widely used as excipients for improving the solubility [20–22]. To date, a large number of works which evidences an increase in the bioavailability of drugs with the help of polymers forming the micelles in solution have been appeared in literature [23,24]. Micelles of polymers are nano-self-assemblies with a hydrophobic core and a hydrophilic corona. Usually, these structures solubilize poorly soluble drugs by incorporating them into the hydrophobic core, while the hydrophilic part provides the protection against micelle-protein interactions that contribute to longer-lasting action and stability. In addition, these surfactants improve wettability and prevent precipitation of the drug in an aqueous medium. Moreover, they can prolong the release of the drug from the formulation which allows to maintain the required therapeutic level of the drug in the blood [25]. One of the most commonly used surfactants in pharmaceutical formulations are pluronics (poloxamers) – nonionic triblock copolymers PEO_x-PPO_y-PEO_x built from chains of poly(ethyleneoxide) (PEO), representing the hydrophilic corona, and a poly(propyleneoxide) (PPO) block constituting the hydrophobic core. The applications of pluronics as solubilizing agents as well as the stabilizing agents and drug delivery agents are described in the literature [26].

It is known that the next step after solubilization is the interaction of drug with biological membrane. This process plays an important role in

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transport of the drugs to the target organ, distribution, accumulation in the tissues and, consequently, provides stability and therapeutic effect. The distribution of compounds in membranes depends on their lipophilicity. This property is widely used in pharmaceutical chemistry to predict and interpret permeability across the membrane. The distribution coefficient of the drug substance in the 1-octanol/water system, in which 1-octanol models the lipid layer of the biological membrane and water phase imitates the blood flow, is commonly used as a characteristic of substance lipophilicity. Due to the fact that most drug compounds have high lipophilicity and poor solubility in aqueous media, the solubilizers are employed to obtain water-soluble dosage forms. It is logical to assume that the presence of solubilizers can cause a change in the lipophilicity (distribution coefficient), as it was shown by the authors [27] for systems with hydroxypropyl-β-cyclodextrin which forms supramolecular complexes with drugs and acts as solubilizer. It should be noted that the formation of aggregates of the solute in an aqueous phase can occur not only with cyclodextrins, but also with the micelle-forming polymers [28]. The hydrophobic molecule interacts with the solubilizer molecules and its total concentration in the aqueous layer increases, respectively, the concentration in the octanol phase decreases. Investigations of the distribution processes in the presence of solubilizers not only help to detect the effect of solubilizers on the interaction with the biological membranes, but also to determine the stability/association constants of the drug compound with solubilizer using the phase-distribution method quickly and with minimal amounts of drug samples [27]. It is noted in the literature [29] that permeability of drugs can be reduced in the presence of solubilizing agents. In this connection, the control of permeability of the designed dosage forms is required. For this purpose, various types of synthetic and artificial membrane barriers are widely used. One of them is Permeapad™ barrier currently produced by Labtastic (www.labtastic.shop) for in vitro permeability studies. This barrier being a new and innovative artificial barrier, has proven to be a powerful tool for a fast and reliable determination of drugs passive permeation. It has been proposed and tested on a number of compounds [30] and solvents [31].

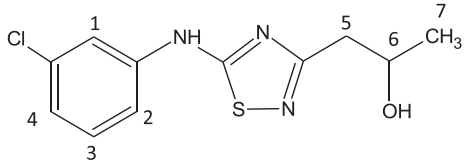
This work is aimed to increase the solubility of the 1,2,4-thiadiazole derivative (TDZ) which was proposed as a novel drug compound for the prevention and treatment of Alzheimer's disease [32,33]. The structure of TDZ under study is represented in Scheme 1.

In order to solve the problem of TDZ poor solubility, the solubilization in the aqueous solutions of polymers such as polyvinylpyrrolidone K29–32 (PVP), polyethylene glycol 6000 (PEG) and triblock copolymer pluronic F127 (F127) was investigated by phase solubility method. The influence of the polymer structure on the manifestation of the solubilizing effect was analyzed. The TDZ–polymer intermolecular interactions determining the enhancement of TDZ solubility was examined using ^{1}H NMR and UV-spectroscopy. The influence of the polymers on the TDZ permeation across the Permeapad™ barrier and distribution in the 1-octanol/buffer pH 7.4 system was evaluated to reveal the solubility-permeability and solubility-distribution interplays.

2. Material and methods

2.1. Materials

1,2,4-Thiadiazole derivative ((*R*,*S*)-1-[5-(3-chlorophenylamino)-1,2,4-thiadiazol-3-yl]-propan-2-ol) was synthesized in the Institute of Physiologically Active Compounds of the Russian Academy of Sciences based on the method of Vivona et al. [34]. Synthetic approaches and scheme were described by us before [35]. TDZ – yield 73%; mp 115–117 °C; ^{1}H NMR [200 MHz, $CDCl_3$] δ: 1.34 (3H, d, J = 6.2, CH_3), 2.87 (1H, dd, J = 6.2, 15.5 Hz, CH), 3.05 (1H, dd, J = 3.0, 15.5 Hz, CH), 4.20 (1H, br s, OH), 4.31 (1H, m, CH), 7.12–7.44 (4H, m, ArH), 8.59 (1H, br s, NH). Anal. ($C_{11}H_{12}ClN_3OS$) C,H,N. The purity of the compound was 98%.



Scheme 1. The structure of TDZ.

Polyvinylpyrrolidone K29–32 (M_w = 58,000 Da) was purchased from Acros Organics, polyethylene glycol 6000 (M_w = 6000 Da) and pluronic F127 (M_w = 12,600 Da) were from Sigma-Aldrich. Buffer solution pH 1.2 was prepared on the basis of HCl (0.06 mol/L) and phosphate buffer pH 7.4 – on the basis of KH_2PO_4 (16.5 ± 0.1 mmol/L) and $Na_2HPO_4 \cdot 12H_2O$ (53 ± 0.2 mmol/L). Buffer solution pH 7.4 for the permeability studies was prepared accordingly to [30]. All chemicals for buffers preparation were of analytical grade. The pH of the solutions was monitored using the Mettler Toledo Five Easy pH-meter. The osmolality of the buffers was controlled by means of Semi-Micro Osmometer K-7400 (Herbert Knauer GmbH, Berlin, Germany). Bidistilled water was used throughout the experimental work.

2.2. Equilibrium solubility in buffers of physiological pH

The solubility study was carried out using the shake-flask method reported by Higuchi and Connors [36]. The dissolution medium – 12 mL of the buffered solutions of different concentrations of PVP (0–15% w/w), PEG (0–15% w/w) or F127 (0–15% w/w) was added to an excess amount of TDZ in screw-capped vials. Suspensions were shaken in a thermostated chamber for 72 h until equilibrium was reached. Then the suspensions were centrifuged (Biofuge pico, Thermo Electron LED GmbH, Germany) at 6000 rpm for 20 min at 25 °C. Concentrations of TDZ in the samples were determined spectrophotometrically (Shimadzu 1800, Japan) at λ = 284 nm. The experiments were performed in triplicate with the accuracy of 2–4%.

2.3. ^{1}H NMR spectroscopy

^{1}H NMR experiments were performed on a Bruker-AV-500 spectrometer at 500 MHz at 25 °C. Deuterated water (D_2O of 99.9% isotopic purity) was used as solvent. The ^{1}H NMR spectra of TDZ were recorded at different polymer concentrations. Changes in the chemical shift Δδ were calculated as follows:

$$\Delta\delta = \delta_{polymer} - \delta_{free} \quad (1)$$

where $\delta_{polymer}$ and δ_{free} are the chemical shifts of TDZ protons with and without polymer in solution, respectively.

The stability constants of TDZ/polymer complexes were obtained from the concentration dependences of Δδ using the non-linear least-squares fitting.

2.4. UV-spectroscopy

The UV-spectra of TDZ solutions were recorded on Shimadzu 1800 spectrometer (Japan) using the 1.0 cm optical path length quartz cuvettes. Concentration of TDZ was constant ($6 \cdot 10^{-5}$ M), whereas the polymer concentration was changed from 0 to 10% w/w.

2.5. In vitro permeability assay

Vertical type Franz diffusion cell (PermeGear, Inc., PA, USA) of 5 mL volume was applied to permeability study. An artificial phospholipid Permeapad™ barrier of 0,64 cm^2 effective surface was used as a membrane. The donor compartment (bottom) was filled with 5 mL of the thiadiazole solution of the predetermined concentration in the phosphate buffer pH 7.4. The system was thermostated at 37.0 ± 0.1 °C,

and the donor solution was stirred vigorously. The receptor compartment (upper) was filled with 1 mL of fresh buffer. Thus, the permeation process followed the reverse dialysis [30]. An aliquot of 0.5 mL was withdrawn from the receptor compartment at 30 min intervals, and replaced with an equal amount of fresh buffer at each respective time point. The samples of the solution were analyzed via spectrophotometer (Shimadzu UV-1800, Japan) at $\lambda = 284$ nm. 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:

$$J = \frac{dQ}{A \times dt} \tag{2}$$

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

$$P_{app} = \frac{J}{C_0} \tag{3}$$

Each permeability experiment was repeated at least 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.

2.6. Distribution coefficient determination

The distribution coefficients of TDZ in 1-octanol/buffer system were measured at 25 °C by standard shake-flask method according to the literature papers [37–39]; the experimental procedure was followed by some specific features described in [27,28] for the solutions containing the solubilizing additives. Taking into account a poor solubility of TDZ in buffer solution, two phase ratios were applied: 0.75 mL octanol phase: 10 mL (buffer pH 7.4 + F127 of different concentrations), and 0.75 mL octanol phase: 15 mL (buffer pH 7.4 + F127 of different concentrations). The concentrations of F127 were: 0.05; 0.7; 1; 2; 3; 5; 10% w/w. Solution of TDZ was prepared in 1-octanol, which previously had been saturated with buffer during 24 h. The initial concentration of TDZ in 1-octanol before partitioning was $1.95 \cdot 10^{-2}$ M. The predetermined volumes of TDZ in 1-octanol solution and buffer solution of the predetermined concentration of F127 were transferred to 20 mL vials. The vials were mixed during 24 h at 25 °C to equilibrate the phase-distribution. After that, the phases were left to separate at least 24 h at 25 °C. The samples from both phases were centrifuged for 20 min at 14000 rpm and analyzed by UV-spectroscopy (spectrophotometer Cary-50, USA). At least three parallel experiments were carried out. The distribution coefficients (D) were calculated from the absorbance of the molecules before and after partitioning according to the following equation:

$$D = \frac{(C_0 - C_{oct})V_{oct}}{C_{oct}V_{buf}} \tag{4}$$

where C_0 and C_{oct} are the TDZ concentrations in 1-octanol phase before and after partition experiment, respectively; V_{oct} and V_{buf} are the volumes of 1-octanol and aqueous phases, respectively.

The accuracy of the distribution coefficient value was verified by checking the mass balance of the starting amount of compound i compared to the total amount of the compound partitioned between two phases.

2.7. Determination of micelle aggregation number

Static light scattering (Zetasizer Nano ZS, Malvern Instruments) was applied to determine the aggregation number of the F127 micelles. The principle of proportionality of the intensity of scattered light that a particle produces to the weight-average molecular weight (M_w) and the concentration of the particle lies in the basis of the measurement procedure. The intensity of scattered light (K/CR_Θ) of various concentrations of F127 (10–50 g/L) at one angle was measured and compared with the scattering produced from a standard (toluene was used as a standard). A Debye plot was then constructed and absolute molecular weight was determined from the intercept point on the x-axis ($K/CR_\Theta = 1/M_w$ in Daltons). The Debye plot uses the following equation:

$$Hc/\Delta R(\Delta, c) = 1/M_w + 2A_2c \tag{5}$$

$$H = \frac{4\pi^2 n_0^2 (dn/dc)^2}{\left(N_0 \lambda_0^4\right)} \tag{6}$$

where H is an optical constant; n_0 is equal to the refractive index of the solvent; dn/dc is the specific refractive index increment of the solution; N_0 is Avogadro's number; λ_0 is the wave length of the laser, which is equal to 633 nm; c is the polymer concentration; ΔR is the Rayleigh ratio which is calculated at different concentrations and it is proportional to the corrected scattered light intensity (the proportionality constants determined by calibration against a substance with a known Rayleigh ratio); Θ is the scattering angle in degrees, which is fixed at 90°; M_w is the molar mass; A_2 is the second virial coefficient. The solutions of F127 at different concentrations were prepared by dissolving appropriate amounts of F127 in buffer pH 7.4 under vigorous stirring and filtered with 0.22 μm polycarbonate membrane filters (Millipore) directly into the light scattering cell. The aggregation number of a micelle was determined from the molecular weights of the micelle and the surfactant monomer.

2.8. Measurement of apparent viscosity

The apparent viscosities of PVP, PEG and F127 solutions were measured using a rotation Brookfield viscometer with measuring system "cone-plate" (Brookfield Engineering Laboratories, USA, model DV2TLV + CP) with a small sample volume adapter and spindle CPA-40Z. The spindle was rotated at 40 rpm. The viscosity experiments were made in triplicate for each sample. The uncertainty of the viscosity measurements was estimated to be (±0.08) cPs.

3. Results and discussion

3.1. Phase solubility study

Solubility is an important parameter determining the bioavailability of drugs, so the study of solubility is a prior task in the characterization of new drug candidates. Previously, we obtained the TDZ solubility in buffer solutions with physiological pH (pH 1.2 and 7.4) [40]. On the basis of these data, it has been shown that TDZ solubility is rather low in both acidic and alkaline media (0.17 mg/mL and 0.14 mg/mL, respectively) and it is slightly dependent on pH. The TDZ molecule has two ionization centers – the nitrogen atom in the heterocycle (H^+-acceptor) and the hydroxyl group (H^+-donor). The dissociation constants were calculated using ACD/ChemSketch program as $pKa_1 = 2.33$ and $pKa_2 = 14.03$. Taking into account these values one can conclude that the cationic (93%) and neutral (7%) forms of TDZ are present in the buffer solution pH 1.2, while there is only a neutral form of TDZ molecule exists at pH 7.4.

The possibility of increasing the TDZ solubility by means of water-soluble polymers which are often used in the pharmaceutical industry as solubilizers [21,22,41] was investigated in this work. The effect of polymers on the TDZ solubility in buffer solutions pH 1.2 and pH 7.4 is depicted in Fig. 1. As one can see from Fig. 1, the TDZ solubility increases with the rise of polymer concentration. Among the polymers under

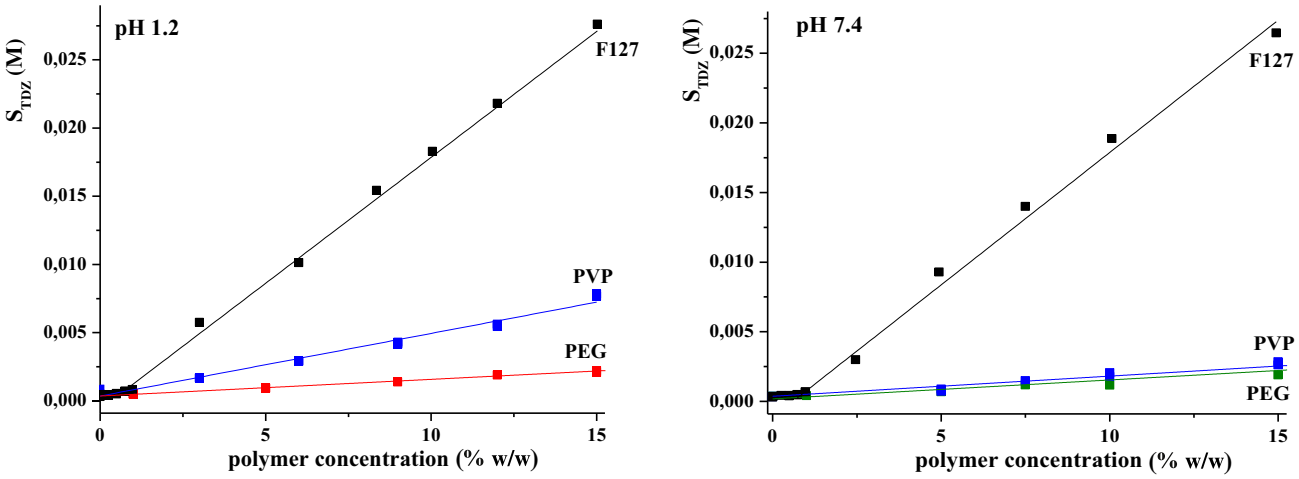


Fig. 1. Solubility of 1,2,4-thiadiazole derivative in solutions of different polymers (25 °C).

study, the solubilizing effect of F127 was found to be more pronounced. For example, the TDZ solubility in buffer pH 1.2 showed a 14.8-fold increase in aqueous F127 (5%) whereas it exhibited a 4.2-fold and 1.4-fold increase in solutions of PVP (5%) and PEG (5%), respectively. Thus, the solubilizing effect of polymers changes in the following order: F127 ≫ PVP > PEG.

Considerable difference in the solubilizing power of polymers under consideration is explained by their nature and different state in solution. Solubilization by PVP and PEG is mainly caused by the ability of these polymers to change the hydration state of solubilizate and to form water-soluble complexes with solubilizate [21]. In this case, van der Waals interactions and hydrogen bonding are responsible for the complexation and solubility increase [20,42]. On the contrary, F127 forms micelles in the aqueous solutions and micellar solubilization occurs [43].

In order to describe the effectiveness of the solubilization of TDZ by PVP and PEG the Setschenow constants were calculated using the following equation [44]:

$$\log\frac{S_0}{S} = K_S m \tag{7}$$

where K_S is the Setschenow constant, m is the molality of the buffered polymeric solution, and S_0 and S are the TDZ solubility in pure buffer solution and in buffered polymeric solution of a given molality, respectively. The Setschenow constants were calculated as the slope of a plot correlating $\log\frac{S_0}{S}$ and m expressing in molality (plots not shown). The Setschenow constant values were obtained to be -22 ± 1 kg·mol^{-1} and -24 ± 1 kg·mol^{-1} for PEG; -255 ± 16 and -301 ± 28 kg·mol^{-1} for PVP at pH 1.2 and 7.4, respectively. The obtained values of the Setschenow constant confirm the more visible enhancing effect of PVP as compared to PEG on the TDZ solubility behavior.

Binding of TDZ with polymers was studied by means of UV-spectroscopy and ^{1}H NMR. To this end, the UV-spectra of TDZ solutions were recorded in the presence of variable concentration of polymers in buffer pH 1.2. Fig. 2 shows the concentration dependences of the absorbance change. As one can see, the absorbance is slightly changed with an increase of PEG concentration and the binding isotherm is linear. These facts point out the weak intermolecular interactions of TDZ with PEG. Probably, PEG possesses the cosolvent properties and does not display the complexing capability towards to TDZ. It can be assumed that PEG reduces the polarity of water [21] and, consequently, this results in an enhancement of TDZ aqueous solubility. On the contrary, concentration dependences are not linear in the case of PVP and F127 and it confirms the binding of these polymers with TDZ. It is interesting to note that absorbance increases under addition of F127, while it decreases in the presence of PVP. The observed difference can be caused by the different binding modes and driving forces of complexation as well as by the change of TDZ chemical environment. More probable, complex formation of TDZ with PVP is realized thought the H-bonding, whereas the TDZ incorporation into F127 micelle core takes place.

To reveal the binding mode of TDZ with the polymers, the ^{1}H NMR experiments were carried out. The ^{1}H NMR spectra of TDZ were recorded at variable concentrations of polymers. Dependences of the chemical shift changes of TDZ protons on polymer concentration are given in Fig. 3. Comparative analysis of the chemical shift changes shows that less pronounced chemical shift changes ($\Delta\delta < 0.1$ ppm) were observed for binding of TDZ with PEG. We tried to calculate the binding constant of TDZ with the monomeric unit of PEG. The nonlinear fitting of the binding isotherms (Fig. 3) applied for this purpose and described in detail in our previous work [45] gave $K < 1$ M^{-1}. This result confirms the low binding affinity of PEG to TDZ. For system with PVP, the significant shifting of the signal from the H-1 proton placed near to the TDZ polar groups (see Scheme) was detected. This can correspond to the participation of TDZ in the hydrogen bonding with PVP. As consequence, more stable complexes are formed between TDZ and PVP. Binding constant of TDZ with PVP monomers was found to be 8 M^{-1}. As follows from Fig. 3, the signals of the TDZ protons H-1, H-2,

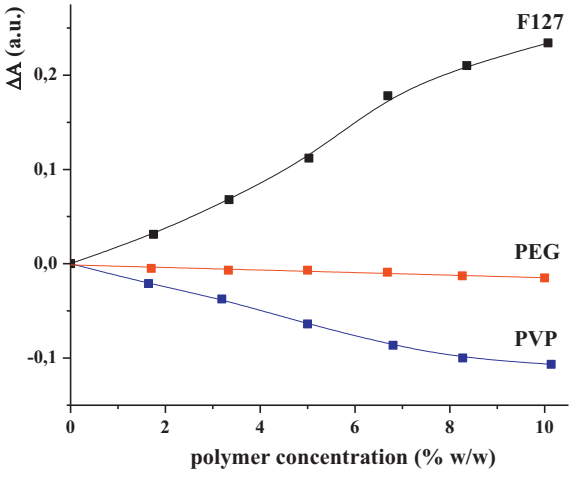


Fig. 2. Dependences of the TDZ absorbance change on the polymer concentration in buffer solution pH 1.2.

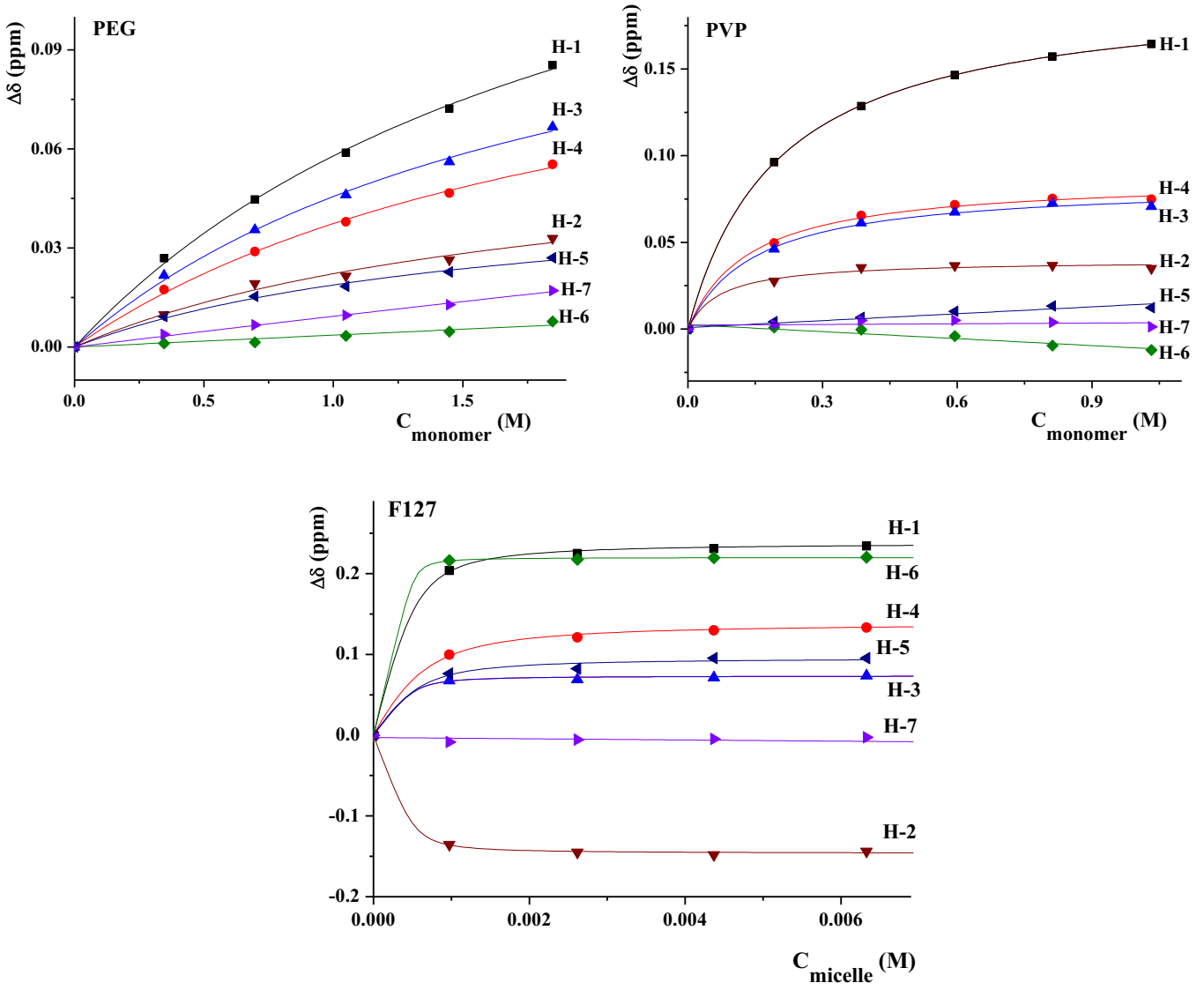


Fig. 3. Dependences of the chemical shift changes of TDZ protons on the polymer concentration (D_2O, 25 °C).

H-4 and H-6 exhibited the considerable shifting in the presence of F127 at concentration above CMC. Such behavior is linked to the penetration of TDZ into micelles. We suggest that benzene ring of TDZ molecule is preferably inserted in the hydrophobic core of the micelles while the TDZ remaining part is located in the hydrophilic corona. The binding constant of TDZ with F127 micelles was estimated as K = 19,240 M^{-1}.

Taking into account the results of ^1H NMR and UV-spectroscopy we can conclude that more pronounced enhancement of TDZ solubility in the presence of F127 (Fig. 1) is due to the micellar solubilization of this polymer. To confirm this fact we measured the TDZ solubility in the premicellar and postmicellar regions of F127. The slow increase of TDZ solubility up to CMC followed by abrupt increase in the postmicellar region was observed. The CMC of F127 in TDZ solution determined from these dependences was equal to 0.67 mM and 0.65 mM at pH 1.2 and 7.4, respectively. As can be seen, these values are not sensitive to pH. A good agreement between CMC obtained herein and available in the literature was detected. Particularly, CMC values of 0.69 mM (at pH 7.4), 0.56 mM and 0.72 mM (25 °C) have been reported for pure F127 by Sezgin et al. [46], Alexandridis et al. [47] and Croy & Know [48], respectively.

Solubilization of TDZ by F127 can be evaluated by the molar solubilization capacity (χ) which represents the amount of compound that can be solubilized by one mole of micellar surfactant. It is given by the following equation [49,50]:

$$\chi = (S_t - S_{CMC})/(C_t - CMC) \quad (8)$$

where S_{CMC} and S_t are the TDZ solubility at CMC and TDZ total solubility, respectively; C_t is the total surfactant concentration. The χ value can be obtained from the slope of the dependences $(S_t\text{-}S_{CMC}) = f(C_t - CMC)$. The χ is equal to 1.95 and 1.97 for buffers with pH 1.2 and 7.4, respectively. It is evident that solubilization power of F127 is not pH dependent.

In addition to molar solubilization capacity, the partition coefficient between the micellar and aqueous phases (K_M) characterizing the effectiveness of the solubilization of hydrophobic drug in micellar solutions can be derived using the following equation [51]:

$$K_M = \frac{\chi}{(S_{CMC} V_{water})(1 + \chi)} \quad (9)$$

where K_M is the micelle partition coefficient, χ is the molar solubilization capacity, S_{CMC} – the solubility at the CMC, V_{water} – the molar volume of water (V_{water} = 0.01805 L·mol^{-1}). In the present study, the partition

coefficients between the micelle and aqueous phases were determined ($\lg K_M = 4.80$ and $\lg K_M = 4.86$ at pH 1.2 and 7.4, respectively). As one can see, the obtained K_M is practically pH independent.

The binding constant (K) serves as the interaction parameter between the solubilizate and surfactant. It can be obtained from the following equation [52]:

$$(S_t - S_{CMC})/S_{CMC} = (C_t - CMC) \cdot K/N_{F127} \quad (10)$$

where N_{F127} is aggregation number of *F*127. The value of K/N_{F127} is obtained from the slope of the dependence $((S_t - S_{CMC})/S_{CMC}) = f(C_t - CMC)$. By knowing the aggregation number of a surfactant the binding constant K can be easily evaluated.

The aggregation numbers were measured in phosphate buffer pH 7.4 by means of the static light scattering and applying the Debye equation [53]. The slope of the Debye plots (α) depicted in Fig. 4 is connected with the molecular weight of the micelle ($M_{micelle}$) via the following equation:

$$\alpha = 1/M_{micelle} \quad (11)$$

For pure F127, the aggregation number is equal to:

$$N_{F127} = M_{micelle}/M_{monomer} \quad (12)$$

where $M_{monomer}$ is the molecular weight of F127 monomer. The aggregation number of F127 in the presence of TDZ was estimated taking into account the number of thiadiazole molecules loaded into the micelle:

$$N_{F127} = M_{micelle}/(M_{monomer} + \chi \cdot M_{TDZ}) \quad (13)$$

where χ is the solubilizing capacity, M_{TDZ} is TDZ molecular weight. The N_{F127} values were estimated as 4 and 6 for pure F127 in phosphate buffer pH 7.4 and in the presence of TDZ, respectively. The aggregation number of F127 alone has been reported in the literature and it was ranged from 3.7 [54] to 72 [55]. The observed variability in N_{F127} determination is explained in the literature by the differences in experimental methods [56] as well as by the incompleteness of the micellar models applied [57]. Our results are more close the data reported by Attwood et al. ($N_{F127} = 3.7$ in water at 35 °C; light scattering) [54].

In comparison to pure F127, it appears that the presence of TDZ results in an enhancement of micellar assembly. As it was obtained, the number of F127 monomers forming the micelle increases under TDZ addition. It was also possible to calculate the number of TDZ molecules inserted into micelle using the following equation:

$$N_{TDZ} = \chi \cdot N_{F127} \quad (14)$$

The N_{TDZ} indicating the number of TDZ molecules per micelle was found to be 12. The binding constant of TDZ with the surfactant micelle was obtained using Eq. (14). This value ($K = 2.4 \cdot 10^4\ M^{-1}$) is consistent with those obtained from ^{1}H NMR experiments. It should be noted that above considered values are useful parameters which can be used for quantitative description of the solubilizing efficiency of F127.

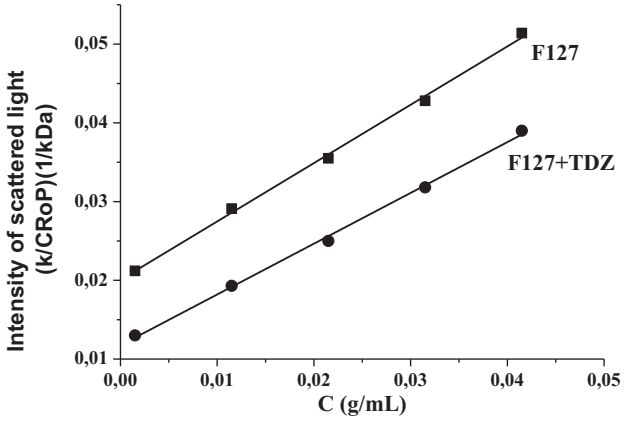


Fig. 4. Dependence of the light scattering intensity on the F127 micelle concentration in the absence (■) and in the presence (●) of TDZ at temperature of 25 °C (for pure F127, C is the concentration of F127 micelles; for binary system F127 + TDZ, C is the sum concentration of micelles and 1,2,4-thiadiazole derivative).

3.2. In vitro permeability study

It was revealed that the polymers used in the present study promote the solubilization of TDZ. However, as it was shown by Miller et al. [29], the enhancement of the solubility in polymer solutions can be accompanied by a dramatic reduction of the permeability, thereby, decreasing the free, bioavailable fraction of drug compound in gastrointestinal tract [58,59]. In the present study the in vitro determination of the TDZ permeability across the Permeapad™ barrier (a thin dry layer of phosphatidylcholine (S-100) on a support sheet) mimicking the model biological membrane was carried out in pure buffer pH 7.4 with and without polymers under consideration. The experiments performed using Franz diffusion cell. Fig. 5 shows the primary experimental data of the permeated amount of TDZ in the presence of 2.5% w/w polymer solutions (Fig. 5a) and apparent permeability coefficients (P_{app}) (Fig. 5b) calculated using Eqs. (2), (3).

It was obtained that TDZ permeability coefficients decrease in polymer solutions. The comparative analysis revealed that F127 displays more significant influence on the TDZ permeability. In particular, 6.5-fold decrease of P_{app} was observed in the presence of F127. Effect of PEG on the TDZ permeability is minimal (1.9-fold reduction). Thus, the permeability coefficients are changed depending on the polymer nature in the following order F127 > PVP > PEG (Fig. 2b). The results of ^{1}H NMR and solubility studies confirmed stronger binding of TDZ with the micelles of F127 than PVP and PEG. Complexation affinity of TDZ to polymers is changed in the order F127 ≫ PVP > PEG. Due to the intermolecular interactions of TDZ with polymers the fraction of free TDZ capable to cross the membrane will be reduced. Besides this, the viscosity of the donor solutions can play an essential role in the process of the diffusion across a membrane, as it was observed by Beig et al. [60] for the system carbamazepine/PEG-400, for which the decreased carbamazepine diffusivity was attributed primarily to the increased solution viscosity with increasing PEG-400 concentration. In order to reveal the factors influencing the TDZ permeability reduction when the polymer was presented in the donor solution, the viscosities of 2.5% w/w solutions of the polymers in phosphate buffer pH 7.4 were measured. The order of the viscosity values obtained was as follows: PVP (1.95 cPs) ≥ F127 (1.90 cPs) > PEG (1.73 cPs). Comparative analysis of complexation ability of polymer and viscosity of polymer solution shows that the TDZ binding with the polymers plays an important role in the TDZ permeability variations. It is especially evident from the comparison of TDZ/PVP and TDZ/F127 systems, viscosity of which are similar, but a stronger binding of TDZ with the micelles of F127 leads to the maximal permeability reduction.

In order to reveal the effect of the polymer concentration on the TDZ permeability and to retrace the solubility-permeability interplay, the system TDZ/PEG was chosen. The obtained concentration dependence of TDZ permeability coefficient was depicted in Fig. 6 along with the phase solubility diagram. It should be emphasized that the opposed effects of the polymer concentration on the permeability and solubility of TDZ are observed.

As one can see from Fig. 6, permeability coefficients decrease with an increase of PEG concentration. This can be caused by two possible factors. First of them is the reduction of the free fraction of TDZ in the

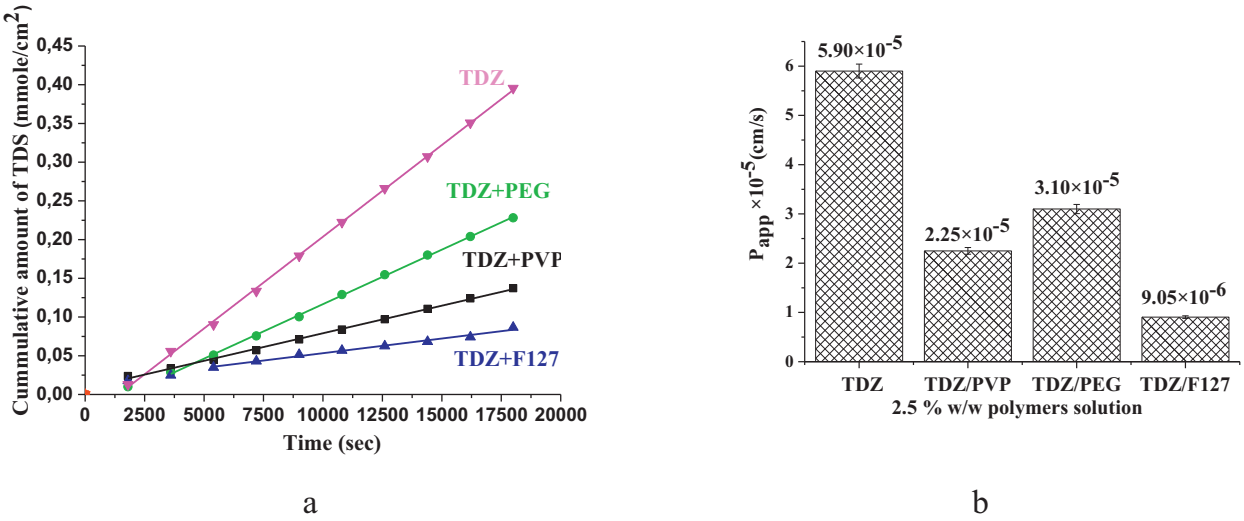


Fig. 5. TDZ permeability across the Permeapad barrier in the presence of 2.5% w/w polymers used: (a) TDZ transport (amount of the permeated TDZ vs. time); (b) permeability coefficients. Data presented as mean ± S.D.; $n = 3$. $T = 37$ °C.

donor solution due to weak interaction with PEG. The second one is the increased viscosity of polymer solution with the PEG concentration growth [61]. To differentiate the influence of the binding of TDZ with PEG and viscosity factor we calculate the values of the permeability coefficient using the following equation [60]:

$$P_{app} = \frac{P^0_{app} \cdot S^0_{buf}}{S_{buf}} \quad (15)$$

where P_{app} is the permeability coefficient of TDZ at given PEG concentration; P^0_{app} is the permeability coefficient of TDZ in the absence of PEG; S_{buf} is the solubility of TDZ at given PEG concentration; S^0_{buf} is the solubility of TDZ in the absence of PEG. It is evident from Fig. 6 that the calculated permeability coefficient values lie higher than the experimental ones. This fact clearly demonstrates that the viscosity factor mainly affects the permeation behavior of TDZ in the PEG solution.

Taking into account poor solubility of TDZ, the maximal increase in solubility using the polymeric formulations would be desirable. On the other hand, an excessive permeability decrease should be avoided. According to the permeability classification proposed by di Cagno et al. [30] for permeability assay based on the Permeapad barrier ($P_{app} = 9.09 \cdot 10^{-5}$–$2.41 \cdot 10^{-5}$ cm·s^{-1} for highly permeable compounds, $P_{app} = 2.01 \cdot 10^{-5}$–$1.92 \cdot 10^{-5}$ cm·s^{-1} for moderately permeable compounds), the concentration of PEG in TDZ/PEG system up to 5% is highly desirable for the successful solubility-permeability interrelation.

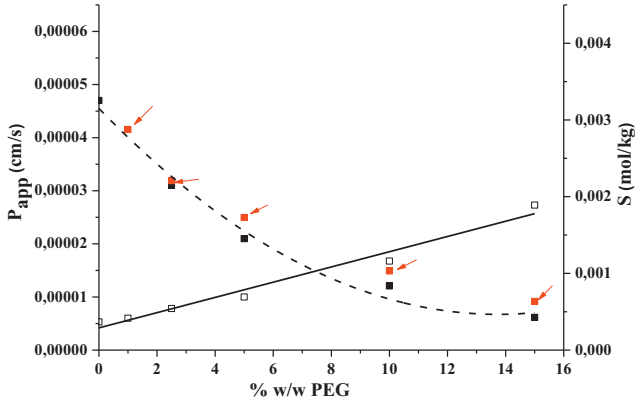


Fig. 6. Solubility-permeability interplay exemplified by the effect of PEG concentration on TDZ permeability coefficients (experimental values – filled black symbols; calculated – filled red symbols indicated by arrows) and solubility (opened black symbols). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Distribution experiments

Distribution coefficient in 1-octanol/water system is an important parameter describing the transport properties of drugs on the way to the target organ, especially the permeability across the biological membranes. To the best of our knowledge, the distribution phenomenon in the presence of solubilizing agents is poorly investigated. At that, like the permeability, the distribution behavior of drug can vary dramatically in the presence of the solubilizing components of the dissolution medium, such as cyclodextrins [27,28], bile salts, phospholipids and polymers, especially if these polymers form micelles in the solution [62–64]. In the present study we investigated the distribution of TDZ in the 1-octanol/buffer pH 7.4 system in the presence of F127 which forms the micelles in solution enhancing the solubility and reducing the permeability of TDZ. The TDZ phase distribution diagram depicted in Fig. 7 shows the linearity ($R > 0.99$) in the postmicellar region of F127.

It should be emphasized that the micelle formation process of F127 affects dramatically the distribution of TDZ in the 1-octanol/buffer system. The TDZ distribution coefficient changes from the value of $D = 1381$ determined in the absence of F127 in our previous paper [65] to $D = 19.8$ measured in micellar region (70-fold decrease). At that, the

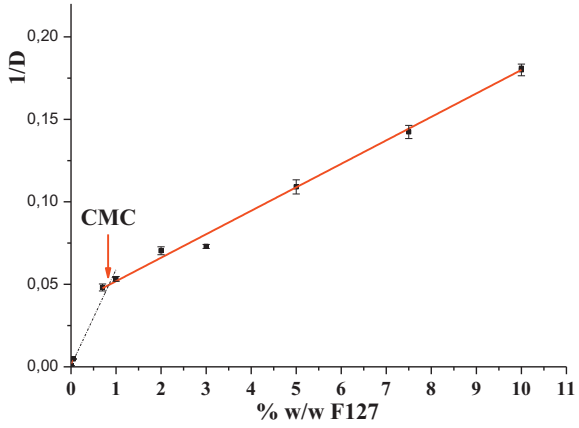


Fig. 7. Octanol/(buffer pH 7.4 + F127) TDZ phase distribution diagram at 25 °C.

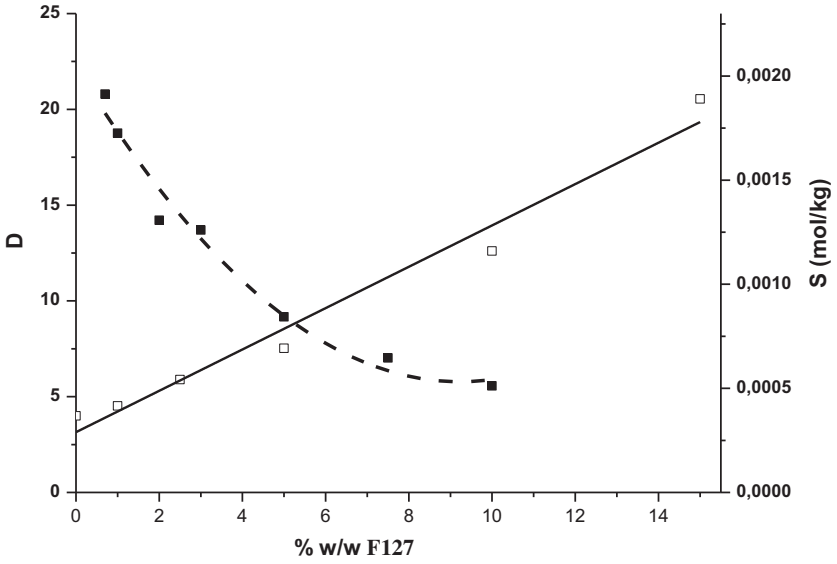


Fig. 8. Solubility-distribution interplay exemplified by the effect of F127 concentration on TDZ distribution coefficients (filled black symbols) and solubility (opened black symbols).

increase of the distribution coefficient in the F127 concentration range from CMC to 10% w/w is only 3.5-fold. In spite of the fact, that the phase-distribution system is more complex than the phase-solubility system due to the presence of the organic solvent (1-octanol in our case), it is possible to determine the CMC by the distribution experiment. The CMC value is in accordance with the value obtained from solubility measurements. When the distribution is studied only small amount of the drug is required in comparison with the solubility making this approach to be advantageous.

As the distribution behavior in 1-octanol/buffer system can predict the permeability of drug across the biological membranes, the next step of the study was to construct the dependence illustrating the solubility-distribution interplay (Fig. 8) similar to the solubility-permeability (Fig. 6).

Fig. 8 shows the tendency of the distribution coefficient to decrease with increasing solubility of TDZ when the polymer concentration grows. Moreover, the distribution-solubility interplay is also useful for estimating the optimal intestinal absorption. As it is reported in the literature [66], an ideal range of the intestinal absorption is $1 < \lg D < 3$. According to these requirements, the concentration of F127 in TDZ/F127 system should be up to 5% (see Fig. 8) for the optimal intestinal absorption.

As a conclusion, the phase distribution approach can be useful for screening the permeability of new drug compounds in solubility enhancing formulations instead of the permeability assay especially if a small amount of the drug is available.

4. Conclusions

In this study the effect of different polymers such as PEG, PVP and pluronic F127 on the biopharmaceutical properties of novel 1,2,4-thiadiazole derivative designed for the prevention and treatment of Alzheimer's disease was investigated. It was demonstrated that increase of thiadiazole solubility in the presence of polymers is accompanied by the decrease of both permeability coefficients through the artificial Permeapad™ barrier and distribution coefficients in 1-octanol/buffer system. It was found that effect of F127 capable to micelle formation in aqueous solutions is more pronounced compared with PVP and PEG. The mechanisms of TDZ solubilization in the presence of polymers were proposed. The impact of the polymer nature on the pharmacologically important properties of TDZ was investigated. The ^{1}H NMR and UV-spectroscopy studies were carried out to reveal the binding mode of TDZ with polymers under consideration.

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