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Prednisolone-α-cyclodextrin-star poly(ethylene glycol) polypseudorotaxane with delayed pH-sensitivity as a targeted drug delivery system

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ABSTRACT

The acylation of prednisolone 20-hydrazone with star poly(ethylene glycol) tetracarboxylic acid (M = 20,000) has been used to prepare the corresponding pH-sensitive conjugate. With α-cyclodextrin, this conjugate forms a polypseudorotaxane, which was characterised by means of ^{1}H NMR spectra, powder X-ray diffraction patterns and STM microscopy. The rate of acid-catalysed hydrolysis of the conjugate was studied under *in vitro* conditions in model media of hydrochloric acid solutions, phosphate and acetate buffers (pH 2–5.8). The acid-catalysed hydrolysis (at pH 2) of the polypseudorotaxane was ca 3.5 times slower than that of the original conjugate. After 1 h in this medium, 86% of the covalently attached prednisolone remained unchanged. The prepared polypseudorotaxane represents a promising peroral transport system of prednisolone with a pH-sensitive linker with delayed acid-catalysed hydrolysis thanks to protection at the molecular level using α-cyclodextrin.

1. Introduction

Glucocorticoids such as prednisolone (PS) belong among medical drugs with high therapeutic potential, and are used for the treatment of a number of autoimmune diseases connected with inflammatory processes. Prednisolone and other anti-inflammatory and immunosuppressive steroids elicit unpleasant side effects, such as diabetes, hypertension, Cushing syndrome and osteoporosis (Robert and Ferid, 1996). In order to restrict these side effects, new drug forms and conjugates of prednisolone and methylprednisolone are continuously being developed (Bílková et al., submitted for publication). The design of the chemical structure of targeted types of conjugates often makes use of the finding that certain enzymes exhibit higher activity specifically in certain organ (liver – carboxyesterases E.C.3.1.1.1) or are present in the pathologically affected tissue (β-glucosidases (E.C.3.2.1.21)). Such enzymes enable selective splitting of a certain kind of covalent bond with a subsequent targeted release of the given drug at the required place (Bílková et al., 2010; Hiyarama and Uekama, 2007a,b). Our previous paper (Bílková et al., 2010) dealt with the preparation and characterisation of conjugates and polypseudorotaxanes of PS with esterase-sensitive linkers, designed for targeted release of the drug in the liver. A similar principle of the release of the drug from its carrier is also used in pH-sensitive conjugates (Chytil et al., 2010; Ulbrich and Šubr, 2004). In these cases, the approach makes use of the finding that a number of pathological processes, such as local tumour diseases, infection or autoimmune diseases, for example, are accompanied by inflammatory processes connected with a local decrease in pH values (Hiyarama and Uekama, 2007a,b; Ulbrich and Šubr, 2004). This idea was utilised in our previous works (Sedlák et al., 2007a,b) that dealt with the preparation and *in vitro* tests of pH-sensitive conjugates of amphotericin B. However, the pH-sensitive systems that have been described so far are only intended for use in intravenous drug forms. Any direct peroral applications of such conjugates are prevented by the acidic milieu in the stomach. The time lag needed for the delayed release of pH-sensitive drugs was achieved using suitably coated pills (Reddy et al., 2010; Schultz and Kleinebudde, 1997; Schultz et al., 1997; Siepmann et al., 2008).

The objective of this work was to suggest, prepare and characterise a potential peroral pH-sensitive conjugate of prednisolone, protected at molecular level, which, with high selectivity, would release PS only in the tissue with a pathologically decreased pH value. The aim was to prepare such a system that would resist the acidic medium during passage through the stomach (HCl; pH ≈ 2) to such an extent that a significant amount of PS would later be released, but only in the affected tissue. Star poly(ethylene glycol) tetracarboxylic acid (sPEG–COOH; M = 20 kDa) was chosen as the polymeric carrier because of its biocompatibility and bonding capacity (Harris, 1992; Sedlák, 2005, 2009). The chosen

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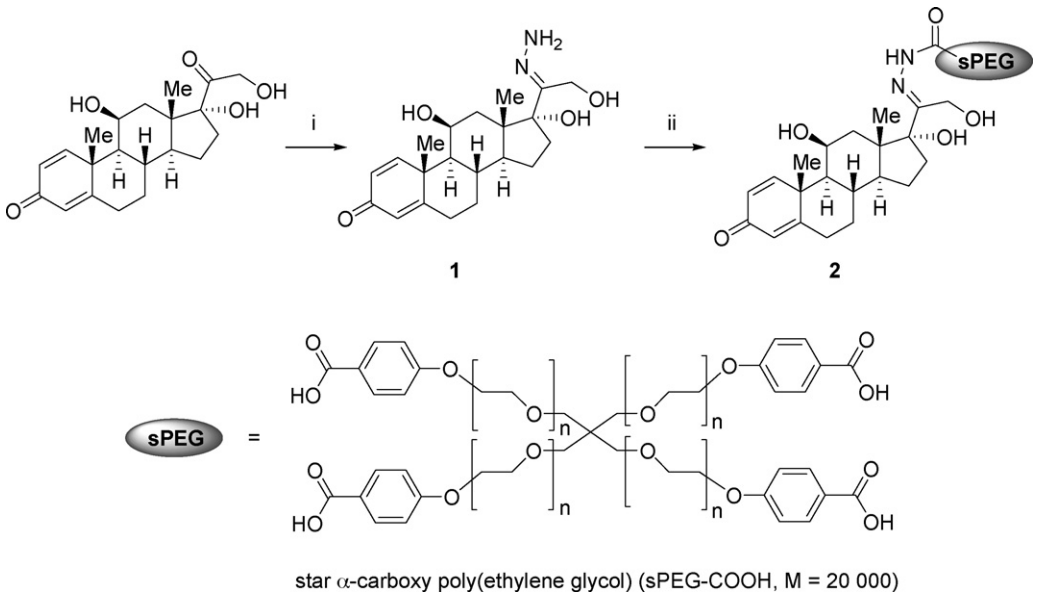


Scheme 1. Synthesis of conjugate **2**; reagents and conditions; (i) $NH_2NH_2{\cdot}H_2O$/EtOH, 45 °C/2 h; (ii) sPEG–COOH/DIC/4-DMAP/CH_2Cl_2, 25 °C/120 h.

pH-sensitive linker was PS hydrazone (Ulbrich and Šubr, 2004) attached to the carboxylic group of sPEG–COOH (Scheme 1).

Our earlier paper (Bílková et al., 2010) showed that it would be possible to use the complex formation of the conjugate with α-cyclodextrin (α-CD), giving polypseudorotaxane (Harada and Kamachi, 1990, 1992, 1994; Harada et al., 2009; Higashi et al., 2007, 2009; Loethen et al., 2007; Ooya and Yui, 1997, 1999; Topicheva et al., 2004; Wenz et al., 2006; Yui et al., 1998) as a molecular tool for the time-limited protection of the pH-sensitive hydrazone linkage. The same paper (Bílková et al., 2010) describes a fivefold deceleration of the enzymatic hydrolysis of the ester linkage of PS released from polypseudorotaxane as compared with the rate of release of prednisolone from the original conjugate. The transformation of the conjugates into polypseudorotaxanes can also lead to a distinct deceleration in the excretion of the conjugates from the organism (Harada and Kamachi, 1990; Higashi et al., 2007, 2009; Ooya and Yui, 1997, 1999; Yui et al., 1998).

2. Methods and materials

Unless otherwise stated pentaerythritol poly(ethylene glycol)ether (M = 20 kDa) was obtained from JenKem Technology, USA. Prednisolone and other chemicals and solvents were obtained from Fluka or Aldrich and used without further purification.

2.1. NMR

The ^{1}H NMR spectra were calibrated with respect to the middle signal in the multiplet of solvent (δ = 2.50; DMSO-d_6) or (δ = 3.31; CD_3OD). The ^{1}H NMR spectra of polymer conjugates (Sedlák et al., 2008) were measured with a relaxation delay of 6 s and an acquisition time of 4 s. The ^{13}C NMR spectra were measured in standard way using broad-band proton decoupling and/or pulse sequence APT. The ^{13}C NMR spectra were calibrated with respect to the middle signal in the multiplet of solvent (δ = 39.5). 2D NOESY measurements were performed using the standard Bruker pulse sequence library with the experimental conditions as follows: the spectral width of 4807 Hz, 128 transients per increment for the 128 increments, with 400 ms mixing time duration and 2048 data points in the F2 domain. The spectra were processed with a sine-bell squared in both dimensions.

2.2. HPLC

The HPLC analysis was performed using a Shimadzu (Kyoto, Japan) HPLC system consisting of two Model LC-10ADvp pumps, a Model SPD-M10Avp UV/vis spectrophotometric detector, a DGU-14A degasser, a CTO-10ASvp column oven and a SCL-10Avp system controller at the temperature of 25 °C. Separation was achieved using a guard column Security Guard, 4 mm × 3 mm, C18 (Phenomenex, Torrance, CA, USA). Elution was carried out with a gradient of methanol:water at a flow rate of 1 mL/min. The mobile phase was filtered through a 0.45 μm Hydrophilic Polypropylene Membrane Filter (Pall Corporation, Ann Arbor, MI, USA). The injection volume was set at 20 μL. The detection wavelength was 245 nm. The purity of conjugate **2** was estimated by means of HPLC using LiChroCART® 125 mm × 4 mm column packed with LiChrospher® 100 RP-18e 5 μm (Merck, Darmstadt, Germany) and eluted with mobile phase of acetonitrile containing 20 mM chelaton II.

2.3. GPC

The gel permeation chromatography was used for the estimation of M_w of the conjugate **2**. The HPLC device was identical with that used for purity determination except for the following parameters: HEMA-BIO columns (hydrophilic modification HEMAGel, particle size 10 μm, porosity 40/100/300/1000) at the temperature of 25 °C using an RI detector and UV/vis detector. Redistilled water (pH 7.1) was used as the eluent. The columns were calibrated with a series of standard PEGs of various molecular weight values (JenKem Technology, USA).

2.4. FT-IR spectroscopy

The IR spectra were measured in solid state on an FT-IR-Perkin Elmer Spectrum BX instrument with horizontal ATR module with ZnSe crystal in the range from 650 to 4000 cm^{-1}.

2.5. Microanalyses

The microanalyses were performed on an apparatus of FISONS Instruments, EA 1108 CHN.

2.6. X-ray diffraction

The powder X-ray diffraction data (Cu Kα, λ = 1.5418 Å) were collected on a D8 Advance diffractometer (Bruker AXS, Germany) with Bragg-Brentano Θ-Θ goniometer (radius 217.5 mm) equipped with a secondary beam curved graphite monochromator and Na(Tl)I scintillation detector. The generator was operated at 40 kV and 30 mA. The scan was performed at room temperature from 2 to 65° (2Θ) in 0.02° steps with a counting time of 10 s per step.

2.7. Scanning tunnelling microscopy (STM)

The STM was performed for characterisation of particle topology/location. Aqueous solution of **3** (0.5 wt.%) was dropped onto a fresh, highly ordered pyrolytic graphite (HOPG) surface at room temperature, and then the samples were dried at the temperature of 60 °C for one week in vacuum. The STM experiment was performed under ambient conditions by using Solver ProM, Nt-MDT (Russia) with an Pt/Ir tip with the calibration based on atomic scale resolution. The measurement was carried out in constant height mode with a sample bias voltage of +200 mV, with resolution 256 × 256 or 512 × 256 pxs^2.

2.8. Stability and kinetics measurements

Methanolic solution of the studied conjugate **2** (20 μL; 30 mg mL^{-1}) was injected into solutions (1 mL) of buffers with pH values: 7.4, 5.8 (phosphate, 1×10^{-1} mol L^{-1}), 5.0, 4.0, 3.0 (acetate, 2×10^{-1} mol L^{-1}) and into the solution of hydrochloric acid (1×10^{-1} mol L^{-1}; I = 1 mol L^{-1}; KCl). Polypseudorotaxane **3** (2 mg) was dissolved in the solution of buffers or hydrochloric acid (1 mL) under the same conditions as mentioned above. The increase in the PS concentration was monitored by means of HPLC at the temperature of 37 °C.

2.9. Synthesis

2.9.1. Prednisolone 20-hydrazone (**1**):*

The solution of prednisolone (800 mg; 2.22 mmol) in ethanol (15 mL) was added dropwise to the solution of hydrazine hydrate (111 mg; 2.22 mmol) in ethanol (3 mL). The reaction mixture was stirred at the temperature of 40 °C for 2 h, then allowed to cool to r.t. and stirred overnight. The reaction course was monitored by means of TLC (Silica gel Merck, $CHCl_3$:MeOH:NH_4OH = 13:1:0.1), R_F = 0.06; detection with the Ehrlich reagent). The solution was poured onto a mixture of ice and water (150 g), the precipitated product was filtered off and recrystallized from water:ethanol (5:1). Yield 500 mg (60%); m.p. 154–158 °C. Mixture of isomers *E*/*Z* (80%); prednisolone (20%). ^{1}H NMR (500.13 MHz, DMSO-d_6) δ: 0.76 (s, 0.6H); 0.78 (s, 3H); 0.88 (m, 1.2H); 1.00 (m, 1.2H); 1.16–1.30 (m, 2.4H); 1.39 (s, 3H); 1.61 (m, 3.6H); 1.82 (dd„ J = 12.6 Hz„ J = 1.3 Hz, 1.2H); 2.02 (m, 2.4H); 2.29 (d, J = 12.9 Hz, 1H); 2.51 (m, 1.2H); 2.79 (m, 0.2H); 4.05 (d, J = 5.6 Hz, 0.2H); 4.11 (m, 1.2H); 4.26 (m, 1.2 H); 4.40 (d, J = 14.0 Hz, 1H); 4.47 (d, J = 5.9 Hz, 0.2H); 4.53 (m, 0.2H); 4.67 (m, 1.2H); 4.85 (m, 1H); 5.18 (d, J = 13.3 Hz, 1H), 5.30 (d, J = 7.2 Hz, 0.2H); 5.91 (s, 1H); 5.98 (s, 0.2H); 6.16 (d, J = 10.1 Hz, 1H); 6.21 (m, 0.2H); 6.27 (s, 1H); 7.32 (d, J = 10.1 Hz, 1.2H); 8.39 (s, 0.2H). *^{13}C NMR (125.76 MHz, DMSO-d_6) δ*: 16.8, 17.8, 20.7, 20.8, 23.2, 23.5, 30.9, 31.1, 31.3, 31.4, 32.3, 32.9, 33.9, 34.0, 43.7, 43.8, 46.4, 46.5, 50.5, 51.0, 55.3, 55.6, 65.8, 68.4, 68.6, 85.5, 88.4, 121.4, 121.5, 126.9, 127.0, 150.1, 150.3, 156.7, 156.9, 170.5, 170.8, 185.1, 185.2, 211.5. IR: ν_{max}/cm^{-1} 2932, 2864, 1656s (CO), 1612, 1439. Anal. calcd for $(C_{21}H_{30}N_2O_4)_4$: ($C_{21}H_{28}O_5$) (%): C, 67.86; H, 8.03; N, 6.03. Found: C, 67.68; H, 7.97; N, 6.31.

2.9.2. Star poly(ethylene glycol)hydrazide-prednisolone-20-hydrazone (**2**)

The solution of 4-dimethylaminopyridine (3 mg; 0.025 mmol) with a mixture of prednisolone 20-hydrazone (**1**) (80%; 114 mg; 0.25 mmol) with non-reacted prednisolone (20%) in CH_2Cl_2 (5 mL) was added to a cooled solution of star α-carboxy poly(ethylene glycol) (sPEG–COOH) (500 mg; 0.025 mmol; M_w = 20,000)[30] and dicyclohexylcarbodiimide (DCC) (3 mg; 0.025 mmol) in CH_2Cl_2 (10 mL), and the reaction mixture was stirred at room temperature. After 5 days, the mixture was filtered and poured into diethyl ether (200 mL). The crystals formed were collected by filtration, and the crude product was recrystallized from propan-2-ol. Yield 450 mg (85%). *^{1}H NMR (500.13 MHz, CD_3OD) δ*: 0.79 (s, 3H); 0.89 (m, 2H); 1.16 (m, 2H); 1.36 (s, 3H); 1.49 (m, 3H); 1.99 (m, 1H); 2.16 (m, 2H); 2.40 (m, 2H); 2.69 (m, 2H); 3.64 (m, 316H); 3.81 (t, J = 4.6 Hz, 2H); 3.87 (t, J = 4.5 Hz, 2H), 4.21 (t, J = 4.5 Hz, 2H); 6.01 (s, 1H); 6.26 (d, J = 8.7 Hz, 1H); 7.02 (d, J = 8.8 Hz, 2H); 7.53 (d, J = 8.7 Hz, 1H); 7.97 (d, J = 8.8 Hz, 2H); 8.14 (s, 1H). IR: ν_{max}/cm^{-1} 2884, 2360, 2336, 1653 (CO), 1604, 1107s (C–O–C). M_w/M_n = 1.13.

2.9.3. Polypseudorotaxane of α-cyclodextrin-star poly(ethylene glycol)hydrazide-prednisolone-20-hydrazone (**3**)

A mixture of aqueous solutions of α-cyclodextrin (443 mg; 0.46 mmol) (4 mL) and conjugate **2** (50 mg; 2.3 μmol) (3 mL) was stirred in a closed vial at room temperature. Turbidity began to appear after 24 h, and the conjugate that separated after 10 days standing was centrifuged, washed with small amount of water, centrifuged again and dried in vacuum at room temperature. Yield: 220 mg (40%). *^{1}H NMR (500.13 MHz, DMSO-d_6) δ*: 0.70 (m, 1H); 0.86 (m, 3H); 1.13 (m, 3H); 1.21 (s, 3H); 1.37 (m, 4H); 1.62 (m, 2H); 1.90 (s, 1H); 2.00 (m, 2H); 2.08 (s, 1H); 2.33 (m, 2H); 3.27 (m, 140H); 3.41 (t, J = 9.1 Hz, the signal is partially overlapped by the water signal); 3.49 (m, 351H); 3.54–3.68 (m, 420H); 3.76 (t, J = 9.1 Hz, 140H); 4.15 (t, J = 4.3 Hz, 4H), 4.31 (s, 1H); 4.51 (brs, 140H); 4.79 (d, J = 2.6 Hz, 140H); 5.45 (brs, 140H); 5.52 (d, J = 6.2 Hz, 140H); 5.91 (s, 1H); 6.15 (d, J = 10 Hz, 1H); 7.01 (d, J = 8.6 Hz, 2H); 7.30 (m, 1H); 7.87 (d, J = 8.6 Hz, 2H); 8.17 (s, 1H).

3. Results and discussion

Similar to the hydrazone of doxorubicin (Chytil et al., 2010; Ulbrich and Šubr, 2004) prednisolone 20-hydrazone (**1**) represents a suitable derivative for the preparation of a pH-sensitive conjugate. However, so far hydrazone **1** has not been described in the literature as being a chemically pure compound. The first step was to optimise the reaction of prednisolone with hydrazine hydrate in order to prepare hydrazone **1**. Various ratios of the two reactants and various reaction temperatures and times were tested, but all the experiments gave mixtures that were inseparable by chromatography or crystallisation. These mixtures contained either the starting prednisolone together with the required hydrazone **1**, or the required hydrazone **1** together with prednisolone 3,20-dihydrazone. The reaction product of prednisolone with hydrazine hydrate in the molar ratio of 1:1, after recrystallisation, represented a mixture containing ca 80% of prednisolone 20-hydrazone (**1**) and 20% of non-reacted starting prednisolone. The ^{1}H NMR spectra of the mixture showed the proportions of hydrazone **1** (hydrogen signals of the diastereotopic methylene group H_2N–N=C–$\underline{CH_2}$–OH 4.40 ppm and 5.18 ppm) and of free prednisolone (O=C–$\underline{CH_2}$–OH 4.47 ppm and 5.30 ppm). Due to the formation of *E*/*Z* isomers of prednisolone 20-hydrazone (**1**), several signals in the ^{1}H NMR spectrum were doubled, which was also reflected by the signals in the ^{13}C NMR spectra (H_2N–N=$\underline{C}$–CH_2–OH 150.1 ppm and 150.3 ppm, and O=$\underline{C}$–CH_2–OH 211.5 ppm, respectively). The proportions of the two components were also confirmed by microanalysis (determina-

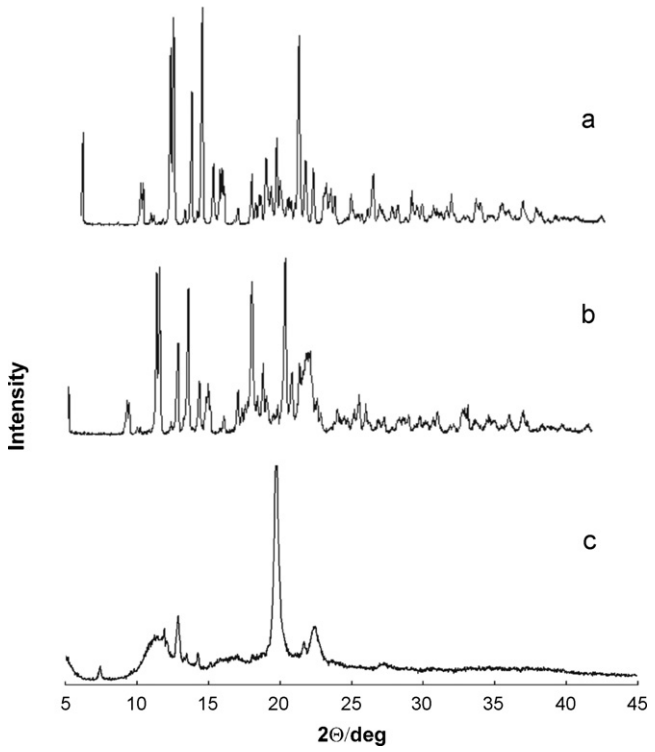


Fig. 1. Diagram of powder X-ray diffraction: (a) α-CD alone; (b) physical mixture of α-CD with conjugate **2**; (c) polypseudorotaxane **3**.

tion of nitrogen) showing the above-mentioned composition of the mixture. However, the preparation of a pH-sensitive conjugate of the hydrazide-hydrazone type (–CONH–N=C) does not necessarily require pure hydrazone **1**. After the activation of star poly(ethylene glycol) tetracarboxylic acid (sPEG–COOH; M = 20,000) with dicyclohexylcarbodiimide, the hydrazide of the acid formed much faster than the corresponding prednisolone ester. After the reaction was finished, the conjugate was precipitated, collected by filtration, and washed with diethyl ether (in which the free prednisolone was soluble washed away). The re-crystallised and dried conjugate **2** (yield: 85%) was characterised by means of ^{1}H NMR spectroscopy and GPC. In the ^{1}H NMR spectra, it was apparent that the molar ratio of PS and sPEG in the prepared conjugate **2** was 4:1. The ^{1}H NMR spectra were also used for determination of the M_n value, and the GPC analysis for determination of the M_w value, which enabled determination of the M_w/M_n ratio. The formation and decomposition of PEG/α-CD polypseudorotaxanes was an equilibrium process (Harada and Kamachi, 1990, 1992, 1994; Harada et al., 2009; Loethen et al., 2007; Topicheva et al., 2004; Wenz et al., 2006); therefore, the complex formation of conjugate **2** was performed with an excess amount of α-CD. After the mixture of the saturated aqueous solution of α-CD and the saturated aqueous solution of conjugate **2** was left for 10 days, polypseudorotaxane **3** precipitated, whereupon it was isolated by centrifugation, purified by repeated decantation with distilled water, again separated by centrifugation, and vacuum dried at room temperature. In this way, the prepared polypseudorotaxane **3** (yield: 40%) was characterised by means of powder X-ray diffraction and ^{1}H, 2D-NOESY NMR spectroscopy and by means of scanning tunnelling microscopy (STM).

Fig. 1 presents a diagram of the powder X-ray diffraction of α-CD alone (a) in comparison with the physical mixture of the α-CD and conjugate **2** (b) and polypseudorotaxane **3** (c). The diffraction diagram of polypseudorotaxane **3** differed from that of the physical mixture and that of α-CD alone. In comparison with earlier works (Harada and Kamachi, 1994; Harada et al., 2009; Loethen et al., 2007; Topicheva et al., 2004; Wenz et al., 2006), these results indicate that the PEG chain formed a polypseudorotaxane arrangement in which the PEG chain passed through the α-CD molecules as a guest. The diffraction peaks at 2Θ = 12.9, 19.7 and 22.5° indicate the arrangement of the α-CD molecules in the order "head-to-head/tail-to-tail" (Topicheva et al., 2004). The stoichiometry of polypseudorotaxane **3** was determined by integrating the peak areas in the ^{1}H NMR spectrum of the anomeric proton of α-CD

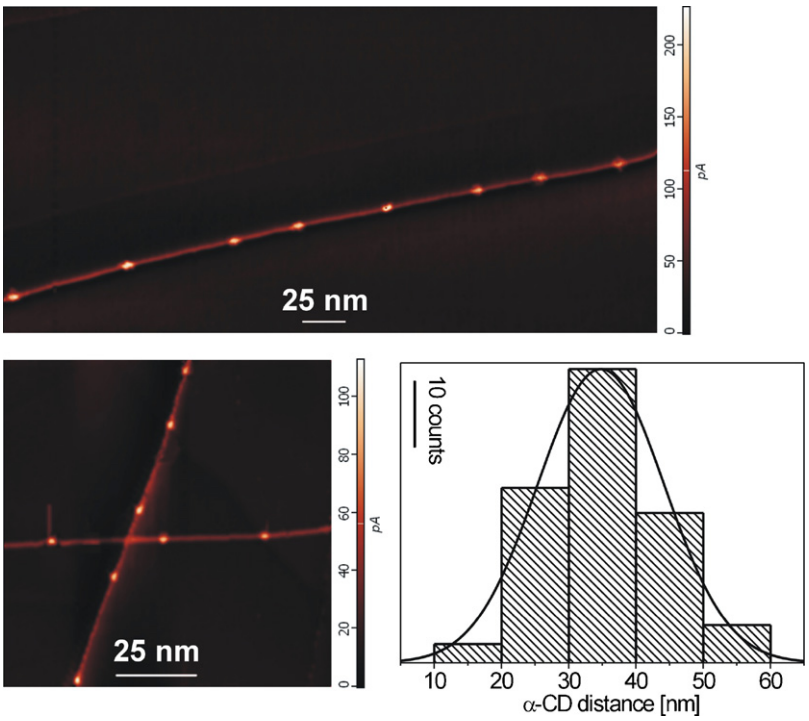


Fig. 2. STM images of polypseudorotaxane **3**: (a) PEG chain with α-CD (100 nm × 200 nm), (b) crossing at star PEG (100 nm × 100 nm), (c) histogram of α-CD distances.

Table 1
Amount of prednisolone (%) released from conjugate **2** and polypseudorotaxane **3** in the phosphate buffer (pH = 5.8), acetate buffers (pH = 5; 4; 3) and solutions of hydrochloric acid (1×10^{-2} mol l^{-1}).

Time (h)	Prednisolone (%) released from conjugate **2**					Prednisolone (%) released from polypseudorotaxane **3**
	pH = 5.8	pH = 5	pH = 4	pH = 3	pH = 2	pH = 2
1	7	8	10	14	51	15
3	8	9	13	19	69	17
5	9	10	18	25	75	19
8	9	11	21	30	87	20

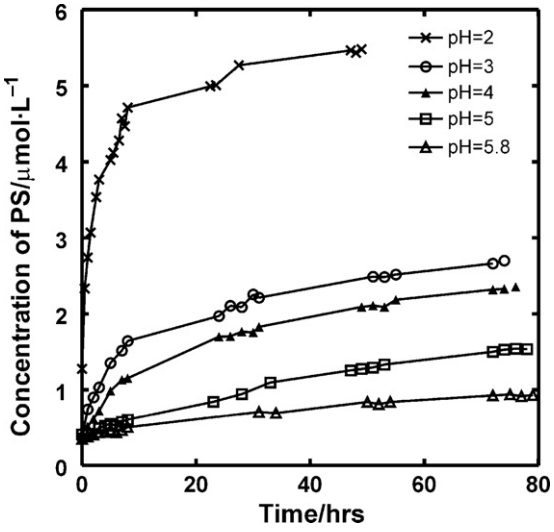


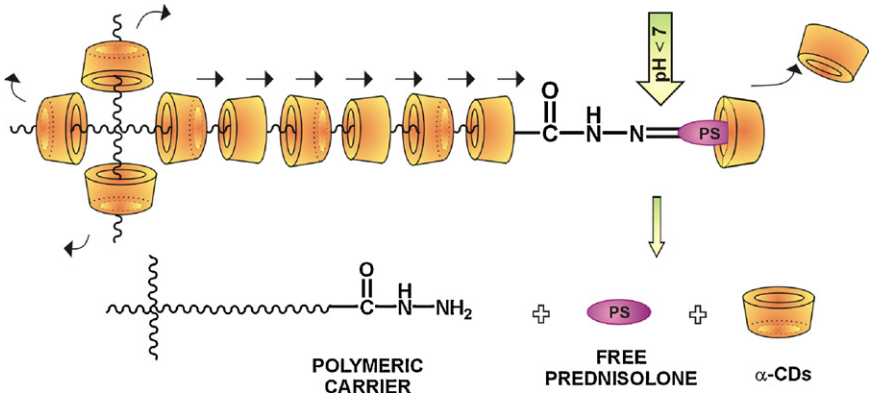
Fig. 3. Concentration–time dependence (μmol l^{-1} *versus* hours) of the prednisolone released from conjugate **2** in the solutions of buffers with pH values: 7.4, 5.8 (phosphate, 1×10^{-1} mol l^{-1}), 5.0, 4.0, 3.0 (acetate, 2×10^{-1} mol l^{-1}) and in the solution of hydrochloric acid (1×10^{-1} mol l^{-1}; $I = 1$ mol l^{-1}; KCl). The points were determined experimentally using HPLC at 245 nm.

(4.79 ppm) and the ethylene protons of the PEG chain (~3.49 ppm) in dimethyl sulphoxide solution. The integral intensities show that there was one α-CD unit per ca four units (CH_2CH_2O), this means that the overall molar ratio of α-cyclodextrin and sPEG in the prepared polypseudorotaxane was 114:1. The necklace-like structure of the α-CD units on the PEG chain was directly observed by using STM for polypseudorotaxane **3** (Fig. 2(a, b)). The α-CD units were detected as bright dots and the PEG chains as lines connecting them. The distance between two α-CD units was analysed (Fig. 2(c)) (*L* value according to Miyake et al., 2003). The minimum *L* value found was 6.3 nm and the typical value was 30 nm. No shuttle manipulation, according to Miyake et al. (2003), was observed during the STM experiment due to the higher interaction of HOPG in comparison to the MoS_2 substrate. In the next part of this work, HPLC was used to study and compare the hydrolytic stabilities of conjugate **2** and polypseudorotaxane **3** under *in vitro* conditions in the model media of hydrochloric acid solutions, phosphate and acetate buffers (pH 2–5.8). Table 1 and Fig. 3 present the time dependences of the increments (% and μmol l^{-1}) of PS released from conjugate **2** for individual pH values. The presented dependencies showed an exponential increase in PS; however, they did not correspond to the first-order dependence. At pH = 5.8, conjugate **2** was relatively stable, which means that only approximately 9% of the PS was released in the time interval of 8 h, while at pH = 2 the 87% of PS was released.

A comparison of the effect of protection of conjugate **2** by its transformation into polypseudorotaxane **3** upon the hydrolysis rate at pH = 2 showed that the PS amounts released during 1 h from conjugate **2** and from polypseudorotaxane **3** were 51% and 15%, respectively (Table 1), which means that the protection decelerated the rate of release of PS by about 3.5 times under the given conditions. Hence, a 1 h incubation of polypseudorotaxane **3** in a model milieu of stomach (HCl; pH ≈ 2) left 86% of the attached PS unchanged. In subsequent sections of digestive tracks there were higher pH values (pH 5–6) and, therefore, it can be presumed that the release of PS will be substantially slowed down. After that, the conjugate taken in could only release PS in the affected tissue with a pathologically lowered pH value (Scheme 2).

4. Conclusions

The delayed release of pH-sensitive medical drugs represents a possibility of their application in peroral pharmaceutical dosage forms. For these purposes, application forms are developed which are based on suitably protected pills (coated pellets) (Reddy et al., 2010; Schultz and Kleinebudde, 1997; Schultz et al., 1997; Siepmann et al., 2008). In this work we have suggested and discussed an alternative of delayed releasing of drug based on transient molecular protection of the pH-sensitive bond. Compared



Scheme 2. The principle of releasing of prednisolone from polypseudorotaxane **3** (schematic structure).

with the original conjugate, the protection of pH-sensitive linker with α-cyclodextrin decelerated the acid-catalysed hydrolysis ca 3.5-times. After 1-h stay in a model milieu of stomach (HCl; pH = 2), 86% of attached PS from the original polypseudorotaxane remains unchanged and can be transported further through the digestive track. The pH values in subsequent sections of digestive track are much higher and, therefore, the releasing of PS in this medium is substantially slowed down. After that, the conjugate taken in can only be released in the affected tissue with pathologically lowered pH value. The application of transient protection of pH-sensitive linker with α-cyclodextrin at the molecular level can represent a new alternative enabling peroral administration of pH-sensitive conjugates, which substantially increases the patients' comfort. However, potential clinical utilisation will necessitate realisation of further tests on animal models.

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