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ORIGINAL ARTICLE

The pH-dependent complexation between risperidone and hydroxypropyl-β-cyclodextrin

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Abstract The complexation between risperidone and hydroxypropyl-β-cyclodextrin (HP-β-CD) was studied in buffered cyclodextrin solutions with the aim to improve the aqueous solubility of the drug, which may allow the formulation of a suitable nasal preparation. The acid-base ionization constants of risperidone and its inclusion complex with HP-β-CD were determined by potentiometric titration. The solubility studies indicated the formation of soluble inclusion complex with equimolar stoichiometry at all pH values tested. The pH value had significant influence on the interaction mode between the risperidone and HP-β-CD, indicating the different affinity of neutral and monoprotonated drug form for the inclusion complex formation. Although the drug ionization has resulted in decrease of the complex stability constant, the overall risperidone solubility in cyclodextrin solution with pH value of 6.0 was the highest. These results may lead to novel pharmaceutical formulation of risperidone, suitable for nasal application.

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Keywords Risperidone · Hydroxypropyl-β-cyclodextrin · Complex formation · Acid-base ionization constants · Aqueous solubility

Introduction

The nasal route is one of the most permeable and highly vascularised sites for drug administration, ensuring rapid absorption and onset of therapeutical action, comparable to that of parenteral drug administration. Thus, the nasal drug delivery has generated widespread interest among scientific community as an alternative route for the administration of drugs and biomolecules that are susceptible to enzymatic or acidic degradation in the gut and first pass hepatic metabolism [1]. The possibility of direct nose to brain transport of drug molecules via the olfactory region of the nasal cavity may be an additional advantage of the nasal drug application [2]. Hence, nasal drug application could be a suitable alternative to oral application of psychoactive drugs such as risperidone.

There are many drug delivery systems aimed for nasal drug application. Among them, intranasal aerosols have attracted widespread attraction. Their advantages include the delivery of a metered dose of the drug, reduced droplet or particle size and excellent deposition inside the nasal cavity with minimal inadvertent delivery into lungs. Furthermore, it is possible to maintain the dose-to-dose sterility, reduce mucosal irritation and these devices are suitable to self-medication, which increase patient's compliance [3].

The lack of adequate aqueous solubility of the drug is often a problem in development of a suitable formulation for the nasal application. The entire drug dose must be applied in a volume of 25–200 µl which requires quite high aqueous drug solubility [4]. There are several approaches

that may increase the solubility of poorly soluble compounds for nasal administration and one among them is the use of cyclodextrins. Cyclodextrins are a family of structurally related cyclic oligosaccharides composed of α-(1,4) linked glucopiranose units that are capable of altering undesirable biopharmaceutical drug properties by the inclusion complex formation [5]. The formation of dynamic molecular inclusion complexes increases the aqueous solubility and chemical stability of many drugs. The inclusion complex formation between prostaglandin E_1 and hydroxypropy-β-cyclodextrin has significantly enhanced solubility and stability of the drug [6], allowing the formulation of a suitable nasal preparation. After its nasal application to rats, the pharmacodynamic effect was about 25% of that obtained by intravenous injection with T_{max} less than 1 min. Enhanced aqueous solubility and stability was obtained by complexation of the anti-rhinovirus drug disoxaril with dimethyl-β-cyclodextrin. In vivo studies on rabbits evidenced that cyclodextrin based disoxaril formulation was well tolerated, having no observable effect on the nasal mucosa following repeated administration [7]. Solubilizing effects of cyclodextrins may be further upgraded by addition of a water soluble polymer, resulting in a formation of the ternary complexes. Loftsson et al. [8] have prepared midazolam nasal formulation by dissolving the drug (17 mg/mL) in aqueous solution containing 14% (w/v) sulphobutylether-β-cyclodextrin, 0.1% (w/v) hydroxypropyl methylcellulose, preservatives and buffer salt (pH 4.3). The obtained formulation showed remarkable chemical stability (t_{95} of over 2 years at room temperature) even after autoclaving. The nasal absorption of midazolam in humans was rapid and the absolute drug availability was 73 ± 7%. The midazolam/cyclodextrin formulation was well tolerated without causing notable nasal irritation.

Numerous studies have demonstrated that cyclodextrins are efficient nasal permeation enhancers for different drugs, including hormones and peptides [9]. The possible mechanism of the permeation enhancing effect of cyclodextrins may be explained by the opening of the tight junctions in the nasal mucosa which triggers the paracellular drug absorption [10].

Generally, the nasal application of cyclodextrins to humans is well tolerated, with only minor adverse effects [9]. Hydroxypropyl-β-cyclodextrin (HP-β-CD) showed superior tolerability compared to other cyclodextrin derivates, since the long-term nasal application of 20% HP-β-CD dose had no influence on the integrity of the mucosa in rats [11]. Also, studies involving human cell cultures have shown that HP-β-CD has no significant cilio-inhibitory effect [12]. Thus HP-β-CD may be considered to be safe excipients for nasal application.

The aim of this work was to increase the aqueous solubility of risperidone through inclusion complex formation, thus overcoming the problems connected with the formulation of a suitable nasal formulation. The complexation of risperidone with different cyclodextrin derivates has been already reported [13, 14]. The results of our previous study [14] have indicated no formation of ternary complexes between risperidone, HP-β-CD and HPMC. Thus, in this paper we have investigated the possibility of increasing the drug aqueous solubility by cyclodextrin complexation accompanied with modulation of the formulation pH value.

Materials and methods

Materials

Risperidone (3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido [1,2-a] pyrimidin-4-one; Fig. 1a) was kindly donated by Pliva, a member of the Barr Group (Zagreb, Croatia). Hydroxypropyl-β-cyclodextrin (HP-β-CD; Fig. 1b) with the average substitution degree per anhydroglucose unit of 0.9 was used as received (Wacker Chemie GmbH, Munich, Germany). Risperidone/HP-β-CD cyclodextrin inclusion complex was prepared according to the previously described method [14]. Perchloric acid ($HClO_4$, Fluka), sodium perchlorate ($NaClO_4$, Sigma-Aldrich) and all other solvents and reagents were of analytical grade and were used without further purification. All solutions were prepared using water twice distilled from alkaline $KMnO_4$ in an all-glass apparatus.

Potentiometric titration

Potentiometric titrations were performed using Mettler Toledo DL55 automatic titrator equipped with Mettler DG-111SC combined glass pH electrode and Mettler DV910 10 mL glass burette with 0.3% accuracy. Temperature was kept constant using a Haake DC10-K10 water bath with 0.02 °C accuracy.

Stock solutions of risperidone (0.0025 M risperidone, 0.01 M $HClO_4$, 0.14 M $NaClO_4$, 5% C_2H_5OH) and risperidone cyclodextrine complex (0.00196 M risperidone cyclodextrine complex, 0.01 M $HClO_4$, 0.14 M $NaClO_4$, 5% C_2H_5OH) were prepared by weighing appropriate amounts of risperidone, risperidone cyclodextrine complex, ethanol perchloric acid and sodium perchlorate. The substances were dissolved in CO_2-free water and filled to volume. 25.00 ± 0.03 mL of the stock solution was placed in a water jacketed cell connected to the thermostat and titrated against 0.2 M standard CO_2-free NaOH solution in an argon saturated atmosphere. All solutions were mixed for 5 min for temperature equalization before titration. Addition of NaOH was made in 0.02 mL increments and the

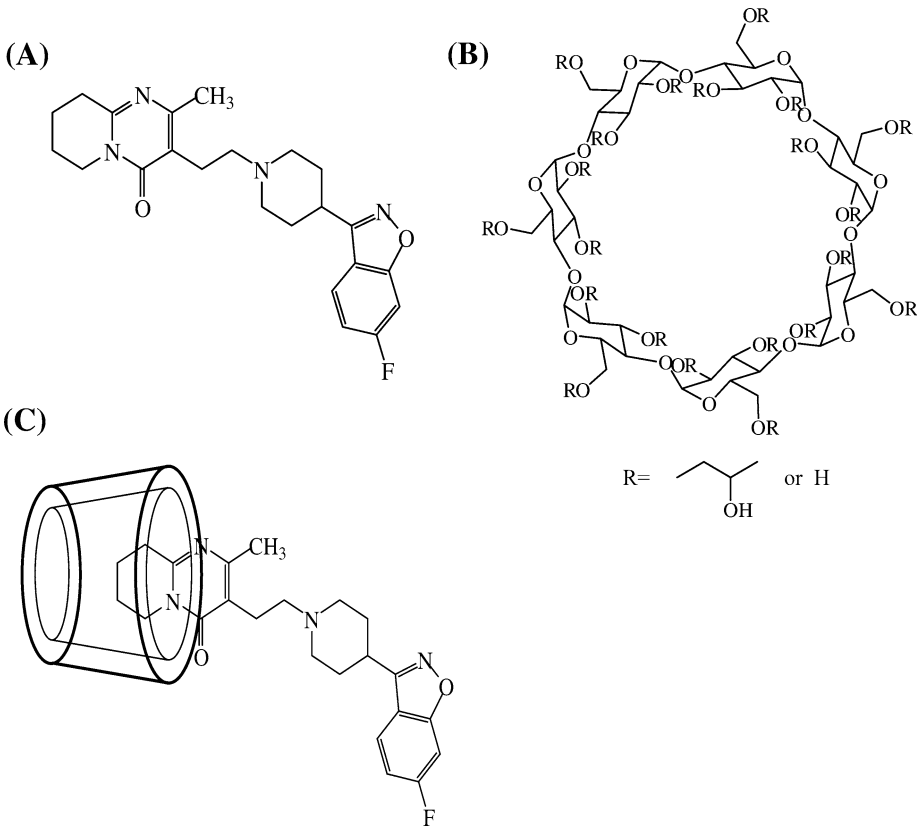

Fig. 1 The chemical structure of rispereidone (**a**), HP-β-CD (**b**) and assumed structure of risperidone/HP-β-CD inclusion complex

resulting electrode potential values recorded. The glass electrode was standardized wit titration of 0.01 M $HClO_4$ with 0.2 M NaOH. Obtained values were used in evaluation of pK_a values using SUPERQUAD software package [15].

Phase solubility studies

Phase solubility studies of risperidone with HP-β-CD were carried out in buffered cyclodextrin solutions (20 mmol L^{-1} phosphate buffer) with pH values of 6.0, 7.4, 8.0, 9.0 and 10.4, according to the method described by Higuchy and Connors [16]. An excess amount of risperidone (100 mg) was added to flasks containing 20 mL of buffered HP-β-CD solutions, with cyclodextrin concentrations ranging from 0 to 40 mmol L^{-1}. The flasks were hermetically sealed and magnetically stirred (600 rpm) at 25 °C for 3 days until the complexation equilibrium was reached. Complexation equilibrium was confirmed in all cases by the preliminary studies. At the equilibrium, the pH value of the samples was checked (Mettler-Toledo, SevenMulti S47 pH-meter equipped with the Mettler Toledo InLab 413 open junction combination pH electrode; UK). In all cases, pH value of the samples remained at the initial values. The aliquots of the samples were filtered through a 0.20 μm Millipore® membrane filter and risperidone concentration in the samples was determined spectrophotometrically at a wavelength of 280 nm (Ultrospec Plus, LKB, Pharmacia, Sweden). Preliminary studies showed that the presence of HP-β-CD and pH value of the samples did not interfere with risperidone absorbance at 280 nm.

The apparent stability constants K_s were calculated from the phase solubility diagram according to:

$$K_s = \frac{tg\alpha}{s(1 - tg\alpha)} \quad (1)$$

where $tg\alpha$ is the slope of the solubility diagram and s is the solubility of the drug in corresponding buffer solution without HP-β-CD (intercept). The complexation efficiency (CE) was calculated according to the equation proposed by Loftsson et al. (1999):

$$CE = \frac{tg\alpha}{(1 - tg\alpha)} \quad (2)$$

Statistical analysis

All values are expressed as a mean ± SD of n separate experiments. Data were compared by one-way ANOVA, followed by Tukey multiple comparison test. Values of $p < 0.05$ were considered significant. Calculations were performed using the GraphPad Prism program (GraphPad Software, Inc., San Diego, CA; www.graphpad.com).

Results and discussion

Influence of the inclusion complex formation on the risperidone acid-base ionization

Risperidone is a basic drug, thus the knowledge of its acid-base ionization constants is of great importance. The pK_a value of the drug can be decisive in determining the passage of the drug across membranes within the body, tissue distribution and elimination [17]. The influence of cyclodextrin complexation on the pK_a values of risperidone was not investigated till now, therefore potentiometric titrations were conducted. The low solubility of the free drug at high pH values was the major difficulty in obtaining reliable values for the ionization constant of risperidone by potentiometry. Therefore, a hydroalcoholic medium (5% ethanol) was used to enhance the drug solubility.

The obtained results of the potentiometric titrations are presented in Table 1. As it is depicted in Fig. 2, risperidone may exits as a diprotonated (RH_2^{2+}), monoprotonated (RH^+) or neutral moiety (R), depending on the pH value of the solution. The pK_{a1} value may be attributed to the deprotonation of the conjugate acid formed at N1 atom in the pyrido[1,2-a]pyrimidin-4-one ring, while pK_{a2} probably belongs to the deprotonation of the conjugate acid formed at nitrogen atom in piperidine ring of the drug molecule. In case of risperidone/HP-β-CD inclusion complex pK_a values are changed compared to that of the free drug, indicating that the inclusion complex formation affected the ionization of the drug molecule. The positive shift in pK_a value upon complexation which is given by $\Delta pK_a = pK_{a\ complex} - pK_{a\ freedrug}$ is more pronounced in case of pK_{a1}. This clearly indicates a lowering in acidity of pyrimidine-4-on nitrogen upon complexation. The similar effect of inclusion complex formation on pK_a value has been previously reported in case of diclophenac/β-cyclodextrin [18]. The change in the pK_{a1} value observed in case of risperidone/HP-β-CD is suggesting partial or complete inclusion of the part of the drug molecule responsible to the ionization into HP-β-CD cavity. The pK_{a2} in case of inclusion complex is only slightly higher than that of free drug, which may be attributed to weak interaction between conjugated acid formed at piperidine nitrogen and an external hydroxyl group of the cyclodextrin molecule [19].

Table 1 pK_a values for deprotonation of risperidone and risperidone/HP-β-CD inclusion complex at 20°

	pK_{a1}	pK_{a2}
Risperidone	3.47 ± 0.03	8.18 ± 0.06
Risperidone/HP-β-CD inclusion complex	3.81 ± 0.02	8.36 ± 0.04

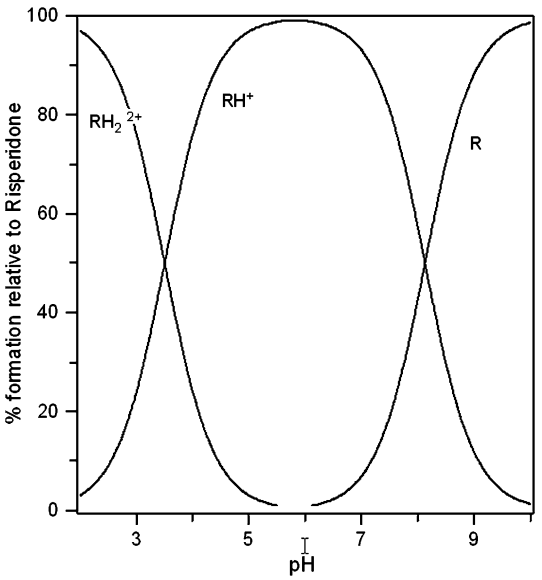

Fig. 2 The pH speciation of risperidone in aqueous solution in pH range between 2 and 11 at $t = 25$ °C

According to these results an assumption about the structure of the formed inclusion complex can be made. If the pyrido[1,2-a]pyrimidin-4-one ring is inserted into central cavity of the HP-β-CD, as suggested in Fig. 1c, than the amide carbonyl group would be available for the hydrogen bond formation with the hydroxyl groups of the cyclodextrin molecule. Our previously published results obtained by FTIR analysis showed a shift of amide carbonyl-stretching band to lower wavenumbers in case of inclusion complex while the intensity of this band was significantly reduced compared to that of free drug [14]. This change in FTIR spectra of risperidone inclusion complex confirmed the hydrogen bond formation between amide carbonyl group and hydrogen groups of the cyclodextrin molecule, thus indirectly confirming our assumptions about the structure of the inclusion complex. To reveal more information about the orientation of the drug into cyclodextrin cavity, a NMR study must be done, what is above the scope of presented investigation.

To further analyze the influence of cyclodextrin complexation on risperidone acid-base ionization, the thermodynamic parameters were determined. The thermodynamic parameters $\Delta_r H$ and $\Delta_r S$ have been calculated according to the van't Hoff model on the basis of pK_a values for risperidone and risperidone/HP-β-CD inclusion complex obtained by potentiometric titration at 20, 25, 30, 37 and 45 °C. The van't Hoff plot of risperidone and risperidone inclusion complex deprotonation is presented at Fig. 3, while the calculated enthalpy and entropy values of deprotonation are listed in Table 2. Inclusion complex formation increased the deprotonation entropy of nitrogen in pyrido[1,2a]pyrimidine-4-one ring for 2.3 kJ/mol. The observed effect may be explained by restriction offered by inclusion complex formation to deprotonation of the

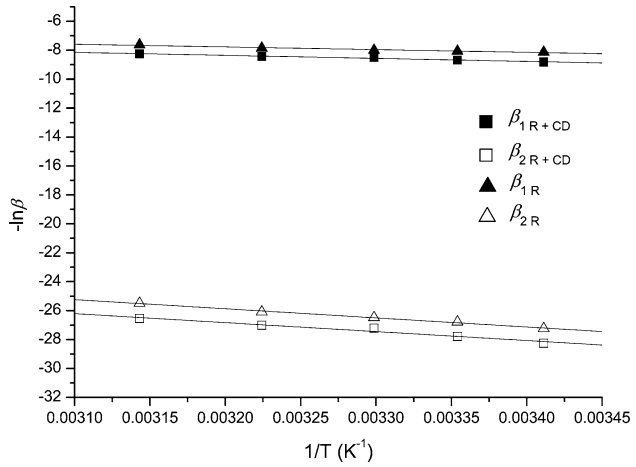

Fig. 3 Van't Hoff plot for deprotonation constants of risperidone and risperidone cyclodextrin complex

nitrogen in pyrido[1,2a]pyrimidine-4-one ring, which is probably inside of cyclodextrin cavity, as depicted in Fig. 1c. The deprotonation enthalpy of nitrogen atom in piperidine ring of the drug molecule is not significantly changed by inclusion complex formation, probably because it is linked to external hydroxyl group of the cyclodextrin molecule via weak hydrogen bonds. Entropy of both deprotonation steps remain at the similar level (Table 2).

pH-dependent solubility of risperidone

The results of potentiometric titration have revealed the existence of neutral (R) and protonated forms of risperidone $\left(RH^{+} \text{ and } RH_2^{2+}\right)$ at different pH values of the drug solution. The drug ionisation may significantly influence its solubility, therefore the aqueous solubility of risperidone has been evaluated in media with different pH values. The obtained drug solubility values in tested range of pH values are presented in Table 3. Risperidone aqueous solubility is low at high pH values and rises with the decrease of the pH value of the media. The observed behaviour may be contributed to the pH dependent drug ionization. At pH values that is for two units higher than pK_{a2} (pH $\geq$ 10.18), in the solution will dominate the neutral risperidone form (R) with low aqueous solubility (28.13 $\pm$ 1.23 μg mL^{-1}). The low solubility of this drug form may be contributed to the lipophilic nature of the drug. When the pH value of the media is in range $pK_{a2} - 2 \leq pH \leq pK_{a2} + 2$ (6.18 $\leq$ pH $\leq$ 10.18), the neutral and monoprotonated $(RH)^{+}$ drug form will be simultaneously present in the solution and the ratio between each form will be pH dependent. The protonation of risperidone will increase the hydrophilic character of the drug molecule, thus increasing its aqueous solubility. At lower pH values, the portion of diprotonated risperidone form will rise, providing good aqueous solubility of the drug (>13 mg/mL at pH 3.0).

Summarizing all above said, the aqueous solubility of risperidone (s) at different pH values can be expressed by following equation:

$$s = [R] + [RH^{+}] + \left[RH_2^{++}\right] \quad (3)$$

where [R] represents the concentration of neutral risperidone form, $[RH^{+}]$ concentration of monoprotonated risperidone and $[RH_2{}^{++}]$ concentration of diprotonated risperidone form. Since the aqueous drug solubility was monitored in pH range between 6.0 and 10.4, the contribution of the diprotonated risperidone form to the overall drug solubility may be neglected [20, 21], thus the Eq. 3 may be transformed into:

Table 2 Enthalpy ($\Delta_r H$) and entropy ($\Delta_r S$) values for deprotonation of risperidone and risperidone/HP-β-CD inclusion complex

	$\Delta_r H_1$ (kJmol^{-1})	$\Delta_r H_2$ (kJmol^{-1})	$\Delta_r S_1$ (J K^{-1} mol^{-1})	$\Delta_r S_2$ (J K^{-1} mol^{-1})
Risperidone	15.7 $\pm$ 0.2	36.9 $\pm$ 1.1	−13.3 $\pm$ 0.8	−33.1 $\pm$ 3.6
Risperidone/HP-β-CD inclusion complex	18.0 $\pm$ 1.0	35.2 $\pm$ 1.9	−12.5 $\pm$ 3.3	−41.6 $\pm$ 6.3

Table 3 Risperidone aqueous solubility (s_0, s_p), correlation coefficient of the phase solubility diagram (r^2), the inclusion complex stability constant (K_s) and complexation efficiency (CE) at different pH-value of the media

pH	s_0 (mg mL^{-1})[a]	s_p (mg mL^{-1})[b]	r^2	K_s (M^{-1})	CE (%)
6.0	3.735 $\pm$ 0.025	7.332 $\pm$ 0.115	0.9846	31.70 $\pm$ 1.16	29.1
7.4	0.458 $\pm$ 0.002	3.035 $\pm$ 0.171	0.9889	178.84 $\pm$ 6.49	19.9
8.0	0.131 $\pm$ 0.001	2.187 $\pm$ 0.047	0.9942	441.29 $\pm$ 12.10	14.1
9.0	0.043 $\pm$ 0.003	1.987 $\pm$ 0.071	0.9981	1274.07 $\pm$ 20.01	13.3
10.4	0.028 $\pm$ 0.001	1.363 $\pm$ 0.018	0.9954	1308.33 $\pm$ 33.54[c]	9.0

[a] Aqueous risperidone solubility in the absence of HP-β-CD

[b] Aqueous risperidone solubility in the 40 mmol L^{-1} HP-β-CD solution

[c] Statistically significant difference compared to K_s at pH 6.0 ($p < 0.001$)

$$s = [\mathrm{R}] + [\mathrm{RH}^+] \tag{4}$$

The protonation of the risperidone molecule may be depicted by following equilibriums:

$$\mathrm{R} + \mathrm{H_2O} \overset{K_b}{\leftrightarrows} \mathrm{RH}^+ + \mathrm{OH}^-$$

and

$$\mathrm{RH}^+ + \mathrm{H_2O} \overset{K_a}{\leftrightarrows} \mathrm{R} + \mathrm{H_3O}^+$$

From above stated equilibrium it follows that the influence of $[H_3O^+]$ concentration i.e. pH value of the media on the concentration of the monoprotonated form may be expressed by the following equations:

$$[\mathrm{RH}^+] = \frac{[\mathrm{R}][\mathrm{H_3O}^+]}{K_a} \tag{5}$$

and

$$[\mathrm{RH}^+] = [\mathrm{R}] \times 10^{\mathrm{p}K_a - \mathrm{pH}} \tag{6}$$

The concentration of the neutral drug form in the solution is equal to the intrinsic drug solubility (s_0), therefore [R] may be substituted by s_0 and pK_a may be substituted by experimentally determined pK_{a2} value. By combining the Eqs. (4) and (6), the following equation may be obtained:

$$s = s_0\left(1 + 10^{\mathrm{p}K_{a2} - \mathrm{pH}}\right) \tag{7}$$

To test the suitability of the proposed mathematical model that describes the influence of pH value on the aqueous solubility of risperidone, the experimental data were fitted to the Eq. 7. The obtained results are presented in Fig. 4. The correlation between experimental and mathematically generated data is high ($r^2 = 0.9804$) indicating that the Eq. 7 may be used to describe pH-dependent risperidone aqueous solubility in tested pH-range.

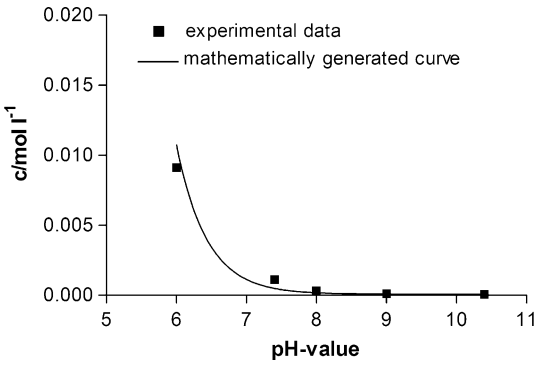

Fig. 4 The correlation between experimentally determined risperidone aqueous solubility at different pH-values and mathematically generated data according to the Eq. 7

pH dependent complexation of risperidone with HP-β-CD

To investigate the influence of risperidone ionization on the drug complexation with HP-β-CD, the phase solubility studies were conducted at different pH values of the media. The obtained solubility diagrams are presented in Fig. 5. At all pH values tested, the risperidone aqueous solubility increased linearly ($r^2 > 0.98$) as a function of the cyclodextrin concentration. This has indicated the formation of an inclusion complex with 1:1 molar stoichiometry [16]. The corresponding stability constants are presented in Table 3. The experimental data have showed that the stability constant value is depended upon the pH value of the media, indicating rather strong influence of the drug ionisation on the risperidone/HP-β-CD interaction. By plotting the stability constant values versus the pH values of the media, the sigmoidal relationship was observed ($r^2 = 0.9968$; Fig. 6) with two extremes and inflexion point at pH value of 8.3, which well corresponds to the pK_{a2} value of the complexed drug determined by potentiometric titration (Table 2).

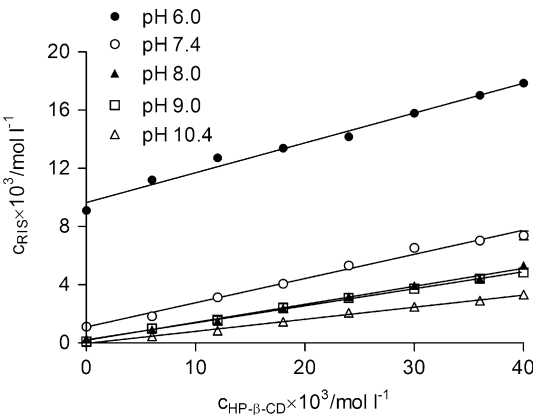

Fig. 5 Phase solubility diagram of risperidone/HP-β-CD in aqueous media of different pH-values at 25 °C

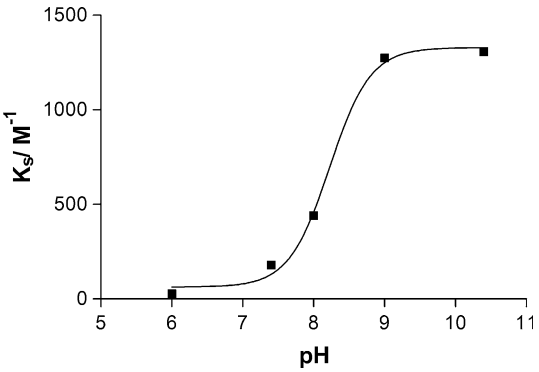

Fig. 6 The correlation between stability constant (K_s) and pH-value of the media

The highest value of the stability constant value was observed for solutions with pH value of 10.4, in which only the neutral drug form (R) was present. Therefore, the stability constant of 1308.33 ± 33.54 M^{-1} at pH 10.4 (K_1) may be attributed to the complex formation between neutral risperidone and HP-β-CD. The lowest value of the stability constant of 31.70 ± 1.16 M^{-1} was observed at pH 6.0. At that pH value, the monoprotonated risperidone (RH^+) was the dominant drug form in the solution. Thus, the stability constant at this pH value may be considered as a stability constant of the inclusion complex between monoprotonated risperidone and HP-β-CD (K_2). The low stability constant value form monoprotonated complex may be explained by the increased drug hydrophilicity upon protonation, which probably reduced the drug affinity for the inclusion into lipophilic central cavity of the cyclodextrin molecule [22]. The neutral risperidone is more lipophilic, thus it has higher affinity for the inclusion complex formation. This result also reflects the significance of the hydrophobic effect contribution to the inclusion complex formation between risperidone and HP-β-CD [23].

Perlovich et al. [24] has showed that the molar enthalpy of an inclusion complex formation decreases with ionization of the drug molecule, indicating weaker interaction of the ionized drug form with cyclodextrin. But, the change of standard entropy is always positive, regardless of the drug ionization. This means that the complexation reaction is accompanied by increasing standard entropy in all cases. In the solution, the drug molecule is surrounded by the salvation shell, consisted of highly ordered water molecules. Upon inclusion complex formation the reorganization of the salvation shell occurred accompanied by the release of the water molecules from the central cavity of the cyclodextrin molecule. All this contributes to the positive change of the standard entropy. Thus, it may be concluded that the entropy change is the main driving force for the inclusion complexation, so even the ionised drugs will form complexes with cyclodextrins.

The risperidone concentration in the solutions containing cyclodextrin at different pH values may be defined by following equation:

$$s = s_0 + [RH^+] + [CD - R] + [CD - RH^+] \quad (8)$$

where $[CD - R]$ and $[CD{-}RH^+]$ represent the concentrations of the neutral and monoprotonated inclusion complex, respectively. Since the phase solubility studies have revealed that the molar ratio between risperidone and HP-β-CD is 1:1 at all pH values tested, the concentration of each complex was defined by the stability constants values of neutral and monoprotonated complex, K_1 and K_2, that are defined by following expressions:

$$K_1 = \frac{[CD - R]}{[R] \times [CD]_{free}} \quad (9)$$

$$K_2 = \frac{[CD - RH^+]}{[RH^+][CD]_{free}} \quad (10)$$

The concentration of the free cyclodextrin molecules, $[CD]_{free}$ that are not involved into inclusion complex formation was represented by following equation:

$$[CD]_{free} = [CD]_{total} - [CD - R] - [CD - RH^+] \quad (11)$$

where $[CD]_{total}$ represents the overall cyclodextrin concentration added to the system. By substituting the equality (6), (9) and (10) into Eq. 11, the concentration of the free cyclodextrins in the media of different pH value may be expressed as:

$$[CD]_{free} = \frac{[CD]_{total}}{1 + K_1 s_0 + K_2 s_0 10^{pK_{a2CD} - pH}} \quad (12)$$

where pK_{a2CD} represents the pK_{a2} value of the risperidone involved into inclusion complex, determined by potentiometric titration (Table 2).

By substituting the Eqs. 6, 9, 10 and 12 into Eq. 8 the mathematical model may be obtained that represents the overall risperidone solubility in HP-β-CD solutions in pH value range between 6.0 and 10.4:

$$s = s_0 + s_0 10^{pK_{a2} - pH} + \frac{K_1 s_0 + K_2 s_0 10^{pK_{a2CD} - pH}}{1 + K_1 s_0 + K_2 s_0 10^{pK_{a2CD} - pH}} [CD]_{total} \quad (13)$$

To test the suitability of the proposed mathematical model that describes the simultaneous influence of the media pH value and cyclodextrin concentration on the aqueous solubility of risperidone, the experimental data were fitted to the Eq. 13. The obtained results are presented in Fig. 7. The theoretically obtained curve has showed some deviation from the experimental data ($r^2 = 0.8972$) especially at pH values ranging from 7.4 and 9.0. In this pH-range,

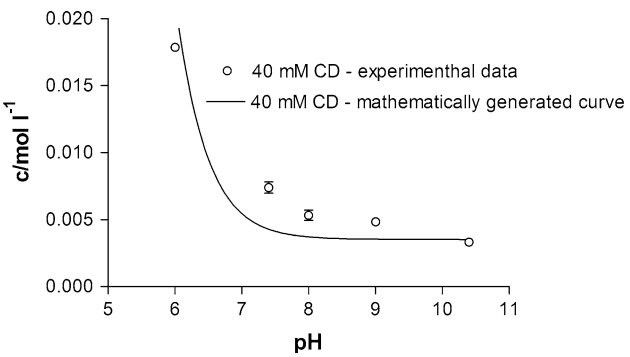

Fig. 7 The correlation between experimentally determined risperidone solubility in 40 mmol L^{-1} HP-β-CD solution at different pH-values and mathematically generated curve according to the Eq. 13

neutral and monoprotonated risperidone as well as their inclusion complexes are simultaneously presented in the solution. The possibilities of the interactions between the components in such systems are numerous. They may include the association between protonated and neutral drug molecules [25], formation of the inclusion complexes, interactions between monoprotonated drug molecules and cyclodextrin inclusion complex and so on [26]. Thus, it may be concluded that in pH-range between 7.4 and 9.0, some other solubilization processes coexist in a non-ideal aqueous cyclodextrin solution that may additionally contribute to the observed drug aqueous solubility.

Despite the fact that risperidone/HP-β-CD complexation has been found to be better with unionized drug form, the total solubility achieved at pH value of 6.0 was the highest. According to these results, it was possible to obtain a greater overall solubility by using a combined approach of pH adjustment and complexation with HP-β-CD. Moreover, the formulation pH value of 6.0 is more preferable, since previous investigations have showed that the formulations with pH value higher than 10 had caused the serious irritation of the nasal mucosa [27]. In other hand, lower values of the complex stability constant are connected with more rapid drug absorption and faster onset of action [28, 29], which is also preferable.

Another parameter that may be used for the describing of drug/cyclodextrin interaction is the complexation efficiency. Complexation efficiency represents the ratio between the concentration of cyclodextrin in the complex and free form, as it can be seen form Eq. 2. For various reasons it is important to use a small amount of cyclodextrin in the dosage form, and thus the complexation efficiency of cyclodextrin in an aqueous vehicle is an important aspect [26]. Also, when selecting the cyclodextrin derivate or complexation conditions during formulation process it can be more convenient to compare complexation efficiency than complex stability constants since the first one is less sensitive to errors related to estimation of intrinsic drug solubility. The corresponding values of the complexation efficiency for the tested pH-range are presented in Table 3. The lowest value of this parameter is determined at pH value of the media of 10.4. At this pH value, the risperidone is in neutral form with limited aqueous solubility. Thus, the concentration of the dissolved drug molecules that are available for the inclusion complex formation is low, leading to the low value of the complexation efficiency. In the other hand, the highest value of the complexation efficiency was determined at pH value 6.0. At this pH value of the media, the drug is manly in the monoprotonated form with increased aqueous solubility. Therefore, higher concentrations of the drug molecules are available for the inclusion complex formation with HP-β-CD. As a consequence, the drug ionization and inclusion complex formation will simultaneously affect the aqueous drug solubility, leading to higher overall drug solubility compared to those of neutral inclusion complex.

Conclusions

The presented study has demonstrated the possibility to improve the risperidone aqueous solubility by simultaneous cyclodextrin complexation and pH value modulation. The pH value had significant influence on the interaction mode between the risperidone and HP-β-CD. At pH 10.4 the formation an inclusion complex between neutral risperidone and HP-β-CD was assumed, while at pH 6.0 the complex between monoprotonated risperidone and cyclodextrin was formed. Although the drug ionization has resulted in decrease of the complex stability constant, indicating the lower affinity of the monoprotonated form for the inclusion complex formation, the overall risperidone solubility in cyclodextrin solution with pH value of 6.0 the highest. This has suggested that cyclodextrin complexation accompanied with pH value modulation may be a suitable strategy to enhance the aqueous solubility of risperidone, thus overcoming the problems connected with the formulations of its nasal preparation.

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