Screening of Mucoadhesive Microparticles Containing Hydroxypropyl-Beta-Cyclodextrin for the Nasal Delivery of Risperidone

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Abstract: Interaction of the antipsychotic drug risperidone with hydroxypropyl-beta-cyclodextrin (HPBCD) in solution and in the solid state was studied with the aim of overcoming the limitations associated with nasal administration of low solubility drugs. Risperidone solubility studies revealed inclusion complex formation with a 1:1 stoichiometry. Low concentrations (0.1 w/v %) of hydroxypropylmethyl cellulose (HPMC) and carbomer affected risperidone solubility in water. No formation of a ternary complex was detected. The solid inclusion complex was prepared by spray drying and was characterised by thermal (DSC) and spectral (FTIR) analyses. Risperidone and the inclusion complex were loaded into microparticles by spray drying using HPMC, carbomer and HPMC/carbomer interpolymer complex (IPC) as mucoadhesive components. The microparticles were characterised with respect to drug loading, particle size distribution, thermal analysis, and zeta potential measurements. Mucoadhesive properties of the microparticles were studied by measuring the work of adhesion. Carbomer and IPC based microparticles revealed superior mucoadhesive microparticles compared to HPMC based microparticles. Drug incorporation into microparticles reduced their mucoadhesive properties, while incorporation of the cyclodextrin complex caused no additional reduction in mucoadhesion. The *in vitro* dissolution studies showed that formation of the inclusion complex significantly increased the risperidone dissolution rate from the microparticles, thus providing sustained drug release.

Keywords: Risperidone, hydroxypropyl-beta-cyclodextrin, nasal administration, mucoadhesive microparticles, hydroxypropylmethyl cellulose, carbomer, interpolymer complex.

1. INTRODUCTION

Nasal administration for systemic drug delivery has been extensively investigated, and many formulations have been marketed [1]. The nasal cavity offers a large, highly vascularised subepithelial layer for efficient drug absorption into the systemic circulation, allowing fast onset of drug pharmacological effect and avoidance of liver first pass metabolism. Numerous studies in animal models and critical evaluation of clinical studies have indicated the possibility of direct nose to brain drug delivery *via* the olfactory region of the nasal cavity [2]. Nasal administration of small lipophilic molecules such as dihydroergotamine, cocaine, lidocaine, and cefalexin has resulted in very fast drug appearance in cerebrospinal fluid and in the brain, with the same or higher drug concentrations compared to intravenous application [2, 3]. Easy accessibility of the nasal route facilitates self-medication, thereby improving patient compliance compared to parenteral routes. Hence, nasal drug application could be a suitable alternative to oral application of psychoactive drugs such as risperidone.

However, nasal drug delivery has certain limitations due to the mucociliary clearance of the applied drug formulation from the site of deposition, resulting in a short residence time for absorption. The use of bioadhesive drug delivery systems increases the residence time of formulations in the nasal cavity, thus allowing for longer absorption [1]. By using mucoadhesive drug delivery systems, it is possible to achieve a more intimate contact with the nasal mucosa, which results in a higher concentration gradient of the drug and subsequent increased absorption. Mucoadhesive microparticles are among the most studied nasal drug delivery systems [4]. Polymers used in the formulation of mucoadhesive microparticles are usually acrylic acid derivates (carbomers), different cellulose esters and chitosan [1,4]. Polymers such as carbomers and chitosan may act as drug absorption enhancers since they trigger paracellular drug transport [5].

Incorporation of poorly water-soluble drugs, such as risperidone, into mucoadhesive microparticles may result in incomplete drug delivery because of the limited drug solubility and dissolution rate within the matrix. This problem can be overcome by the use of drug carrier systems such as drug-cyclodextrin complexes. Mucoadhesive microparticles containing cyclodextrins can provide controlled and complete *in vitro* drug release. Cyclodextrins have been demonstrated to be safe nasal absorption enhancers of different drugs, including macromolecular and polypeptide drugs [6]. Histological and cell-culture based studies [7] have shown that cyclodextrins do not induce mucosal damage and have no significant influence on the nasal ciliary movement [8]. Further, clinical data [6] have shown no significant adverse effects, indicating that cyclodextrins are effective and safe excipients for nasal drug delivery.

Therefore, the aim of the present work was to study the complexation of risperidone with hydroxypropyl-β-cyclodextrin, and to develop cyclodextrin based mucoadhesive microparticles for risperidone nasal delivery.

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2. MATERIALS AND METHODS

2.1. Materials

Risperidone was kindly donated by Pliva, a member of the Barr Group (Zagreb, Croatia). Hydroxypropyl-β-cyclodextrin (HPBCD) with the average substitution degree per anhydroglucose unit of 0.9 was used as received (Wacker Chemie GmbH, Munich, Germany). Hydroxypropylmethyl cellulose (HPMC, Metolose® 90 SH; η=4000 mPa s; Shin-Etsu Chemical. Tokyo, Japan) and carbomer (Carbopol® 941, η=4000 – 10 000 mPa s; Noveon, Cleveland, USA) were used as mucoadhesive polymers. All other materials and solvents used were of analytical reagent grade.

2.2. Phase Solubility Studies

Phase solubility studies were performed according to the method described by Higuchi and Connors [10]. An excess amount of risperidone (100 mg) was added to 20 mL of aqueous solution containing HPBCD in concentrations ranging from 0 to 40 mmol L^{-1} (binary systems). The suspensions were vigorously shaken at 25 ± 1°C for 3 days until solubility equilibrium was reached. The samples were filtered through a 0.20 μm Millipore® membrane filter and drug concentrations in the samples were determined spectrophotometrically at a wavelength of 280 nm (Ultrospec Plus, LKB, Pharmacia, Sweden). Preliminary studies showed that the presence of HPBCD did not interfere with risperidone absorbance at 280 nm.

To establish the effects of HPMC and carbomer on the risperidone/HPBCD interaction in solution, the polymer was added (0.1% w/v) to the samples (ternary systems). The suspensions were sonicated in an ultrasonic bath for 1 h, at 70°C, and were then allowed to equilibrate at 25 ± 1°C for 3 days. After equilibrium was attained, undissolved drug was removed from the samples by centrifugation (3500 rpm, 15 min) and the risperidone content was determined by the previously described method.

The apparent stability constant Ks was calculated from the phase solubility diagram using the equation:

$$K_s = \frac{slope}{s_0(1 - slope)} \quad (1)$$

where s_0 is the solubility of the drug in water (intercept).

2.3. Preparation of the Solid Complex

Risperidone (0.821 g) and an equimolar amount of HPBCD (3.0 g) were separately dissolved in 50 mL of 96% ethanol and 50 mL of water, respectively. The solutions were mixed together and stirred (600 rpm) for 24 h at ambient temperature to obtain complexation equilibrium. The obtained solution was subjected to spray drying using a Büchi 190 Mini Spray Dryer (Flawil, Switzerland) with a standard 0.5 mm nozzle. The drying conditions were as follows: flow rate 0.25 l h^{-1}, inlet temperature 150°C, outlet temperature 100°C and air flow rate 700 NL h^{-1}.

The ethanol/water solution of the drug without HPBCD was also prepared by the same procedure, and the solid product was isolated by spray drying as described above.

2.4. Preparation of Mucoadhesive Microparticles

The microparticles were prepared by spray drying using HPMC, carbomer and HPMC/carbomer interpolymer complex (IPC) as mucoadhesive components. IPC was previously prepared by mixing 1% (w/v) aqueous solutions of HPMC and carbomer in a 1:1 volume ratio. The obtained solution was stirred uniformly for 1 h and subjected to spray drying to prepare IPC in the solid state. The drying conditions were as described in section 2.3.

For the preparation of microparticles, the polymer (0.5 g) was dissolved in 50 mL of water. Risperidone (0.5 g) or the cyclodextrin complex containing the same amount of the drug was dissolved in 50 mL of 96% ethanol and mixed with the polymer solution. The obtained solution was uniformly stirred (600 rpm) at room temperature for 1 h and subjected to spray drying under the conditions as described in section 2.3. Compositions of the microparticles are given in Table **1**. The mass ratio of risperidone, free or in complex form, and mucoadhesive polymer was 1:1 in all the prepared microparticles. Drug-free (blank) microparticles were prepared according to the same procedure, omitting the drug (free or as cyclodextrin inclusion complex) from the preparation.

Table 1. Effect of the Addition of HPMC and Carbomer to Aqueous HPBCD Solution on Risperidone Solubility, Correlation Coefficient of the Solubility Isotherm (r^2) and Apparent Stability Constants of the Complexes (K_s)

System	s_p/s_0	s_{CD}/s_0	r^2	$K_s/(M^{-1})$
no polymer	-	18.32	0.9952	456.61 ± 12.77
HPMC	1.94	19.13	0.9870	491.84 ± 11.67
carbomer	0.96	15.72	0.9855	198.81 ± 6.62

s_0 – risperidone solubility in water.
s_p – risperidone solubility in aqueous solution of the polymer (HPMC or carbomer) without HPBCD.
s_{CD} – risperidone solubility in aqueous solution of the polymer (HPMC or carbomer) with 40 mmol L^{-1} HPBCD.

2.5. Determination of the Drug Content in Solid Products

For determination of the drug content in solid products, 10 mg of the samples was extracted with 96% ethanol under sonication in an ultrasonic bath (Branson B1210E-DTH, Danbury, USA) until a clear solution was obtained. After filtration through a 0.20 μm Millipore® membrane filter, the risperidone concentration in the samples was determined spectrophotometrically at 280 nm. Drug loading was calculated as the ratio between the actual and theoretical drug content in the microparticles, expressed as percentage.

2.6. Particle Size Determination

A microscopical imaging analysis technique was applied for the determination of particle size distribution using an Olympus BH-2 microscope, equipped with a computer-controlled image analysis system (Optomax V, Cambridge, UK).

2.7. Differential Scanning Calorimetry (DSC)

DSC thermograms of the solid products were recorded on a Perkin Elmer DSC 7 (Wellesley, USA). The instrument was calibrated with indium and zinc prior to the analysis of samples under nitrogen purge. All accurately weighed samples (2 mg) were placed in sealed aluminium pans, and

scanned at a heating rate of 10°C min^{-1} over the temperature range of 20-220°C.

2.8. FTIR Spectroscopic Studies

FTIR spectra of the solid products were recorded on a Perkin-Elmer spectrum GX spectrometer. The samples were prepared by the potassium bromide disc method (2 mg sample in 200 mg KBr) and scanned for absorbance in the range of 4000– 500 cm^{-1} at 1 cm^{-1} resolution.

2.9. Zeta Potential Measurement

The zeta-potential of spray-dried components and prepared microparticles was determined by photon-correlation spectroscopy (Zetasizer 3000 HSA, Malvern Instruments, UK) in 10 mM NaCl solution (pH 6.7) at 25°C.

2.10. *In Vitro* Mucoadhesion Test

To evaluate the microparticles mucoadhesive properties, a tensile study was performed using bovine nasal mucosa. Prepared microparticles (100 mg) as well as drug-free microparticles (HPMC, carbomer, IPC) were compressed into discs of 1 cm diameter by means of a hydraulic press (Glenrothes, UK), applying a compression pressure of 160 kPa cm^{-2}. The discs were attached to the stainless steel support connected to a precise balance (Sartorius BP 221S, Goettingen, Germany) using cyanoacrylate glue. The bovine nasal mucosa were fixed to the glass dish mounted on the mobile support. The mucosal surface was wetted with 0.1 mL of simulated nasal fluid (8.77 g NaCl, 2.98 g KCl and 0.59 g $CaCl_2$ per 1000 mL of demineralised water; pH 6.4) and brought in contact with the sample. The sample and the tissue were left in contact for 5 min, allowing the formation of a mucoadhesive bond. The force of detachment was measured as a function of displacement, by lowering the mobile support at a constant rate of 5 mm min^{-1} until total separation of the components was achieved. The work of bioadhesion (W) was calculated as the area under the force/distance curve.

2.11. *In Vitro* Drug Release Studies

The drug release experiments employed a standard Franz diffusion cell (PermeGear, USA) with a diffusion area of 10.18 cm^2 and an acceptor compartment volume of 16 mL. Risperidone, inclusion complex or prepared microparticles containing 5 mg of the drug were applied evenly across the pre-hydrated semipermeable membrane (Medicell Dialysis Tubing, MWCO 600 Da) clamped between the donor and acceptor compartments. The membrane was wetted with 0.05 mL of simulated nasal fluid containing 1% of mucine. After the sample was applied, the donor compartment of the Franz-diffusion cells was closed with Parafilm® "M" sealing film (American National Can Company, Chicago, IL, USA) to avoid evaporation of the release medium and to allow the establishment of constant relative humidity around the sample. Phosphate buffer (pH 7.4) in the acceptor compartment was continuously stirred at 600 rpm using a magnetic stirrer. The cells were thermostated at 37ºC. At set time intervals, aliquots of the acceptor phase were removed and assayed for the drug content. The removed samples were immediately replaced with an equal quantity of prewarmed receptor medium. Cumulative corrections were made for previously removed samples.

The kinetics of drug release was determined by fitting the best fit (r^2>0.98) of the dissolution data to distinct models: zero – order (2) and Higuchi kinetic model (3) defined by the following equations:

$$Q_t = k_0 t \quad (2)$$

$$Q_t = k_H t^{1/2} \quad (3)$$

where Q_t is the amount of the drug released at time t, k_0 and k_H are zero-order and Higuchi rate constants, respectively. Dissolution efficiency (*DE*) was calculated according to equation (4):

$$DE = \frac{\int_0^t Q_t dt}{Q_{100} t} \times 100\% \quad (4)$$

2.12. Statistical Analysis

All values are expressed as mean ± SD of N separate experiments. Data were compared for single comparison by the Student's t test and for multiple comparisons by one-way ANOVA, followed by the Bonferroni's multiple comparison test. Values of $p < 0.05$ were considered significant. Calculations were performed using the GraphPad Prism program (GraphPad Software, San Diego, CA; www.graphpad.com).

3. RESULTS

3.1. Influence of HPMC and Carbomer on Risperidone Solubility and Inclusion Complex Formation in Solution

The phase solubility studies were intended to investigate the influence of HPMC and carbomer on the risperidone inclusion complex formation in solution. The obtained phase solubility diagrams are shown in Fig. **1**. In the binary system, risperidone solubility increased linearly as a function of increased HPBCD concentration with the slope < 1, indicating formation of the soluble inclusion complex with a 1:1 molar stoichiometry. The corresponding stability constant of the risperidone/HPBCD inclusion complex is given in Table **1**.

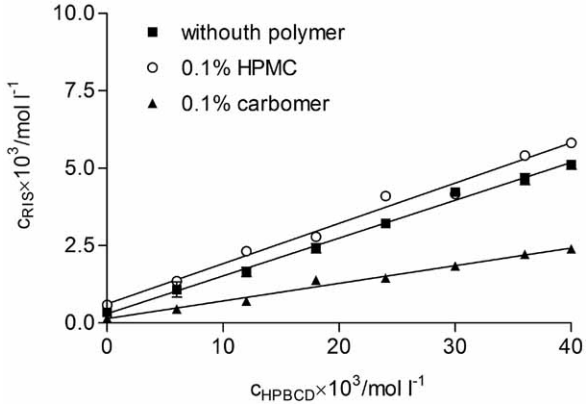


Fig. (1). Phase solubility diagrams of risperidone and HPBCD in water, HPMC and carbomer solution at 25°C (mean ± SD, N = 5).

The presence of HPMC and carbomer affected risperidone solubility in water. While HPMC increased drug solubility and the stability constant value, the presence of carbomer slightly reduced risperidone solubility in water and decreased the inclusion stability constant value (Table **1**).

Addition of polymers to the solution did not influence the type of the solubility isotherm; hence, it could be deducted that the complex stoichiometry remained the same as in the binary system.

3.2. Solid State Studies of the Risperidone/HPBCD Inclusion Complex

Risperidone/HPBCD inclusion complex in solid state was prepared in a 1:1 molar ratio by spray drying, based on the results of the phase solubility studies. The prepared complex (R_{HPBCD}) was characterised by drug content determination and particle size distribution. The results were compared to the spray-dried drug (R_0). Particle size determination indicated a narrow log-normal distribution, with more than 90% of particles having spherical diameters ranging from 1-2.5 μm (Table **2**). The inclusion complex formation did not significantly affect the mean spherical diameter of the drug microparticles ($p > 0.05$).

Evidence for the complex formation in the solid state was obtained by thermal (DSC) and spectral (FTIR) analyses. DSC thermograms of the samples are shown in Fig. **2**. Thermogram of the crystalline risperidone showed an endothermic fusion peak at 168.5°C (ΔH=101.6 Jg^{-1}). In the thermogram of the spray-dried risperidone, the position and intensity of the fusion peak did not differ significantly (169.6°C; ΔH=101.8 Jg^{-1}) compared to that of the crystalline drug, indicating that the spray drying procedure did not influence the drug crystalline state. The HPBCD thermogram exhibited a very broad endothermic peak from 45.7 to 140°C (ΔH=156.7 Jg^{-1}), which could correspond to the loss of water molecules from the cyclodextrin cavity. The endothermic fusion peak corresponding to drug melting was still evident in the thermogram of the risperidone/HPBCD physical mixture (162.2ºC, ΔH=56.82 J/g). In the thermogram of the spray-dried inclusion complex, the risperidone fusion peak was not present.

The FTIR spectra that were obtained are shown in Fig. **3**. The absorption bands position and intensity in the FTIR spectra of spray-dried risperidone were the same as in the crystalline drug spectra, indicating the same crystal lattice formation and thereby confirming the observations based on the DSC results. In the FTIR spectra of the spray-dried inclusion complex, the majority of risperidone absorption bands disappeared and a shift of amide carbonyl-stretching band to lower values could be observed. Also, the intensity of amide carbonyl-stretching band in the case of the spray-dried inclusion complex was significantly reduced.

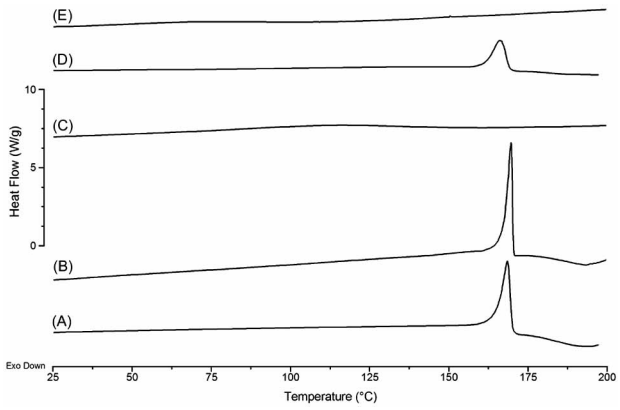


Fig. (2). DSC curves of **A**) crystalline risperidone; **B**) spray-dried risperidone; **C**) HPBCD; **D**) equimolar risperidone/HPBCD physical mixture; and **E**) spray-dried inclusion complex.

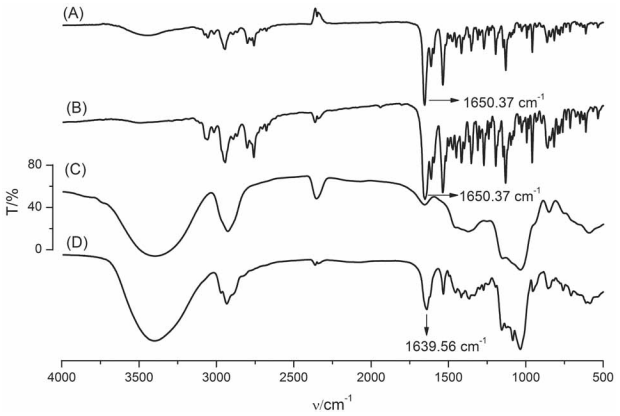


Fig. (3). FTIR spectra of **A**) crystalline risperidone; **B**) spray-dried risperidone; **C**) HPBCD; and **D**) spray-dried inclusion complex.

Table 2. Drug Loading and Mean Spherical Diameter (d) of Prepared Microparticles

Microparticle	Microparticle Composition		Drug Loading / (%)	d(μm)
	Mucoadhesive Polymer	Drug		
R_0	-	risperidone	-	2.45±0.76
R_{HPBCD}	-	inclusion complex	-	2.58±1.23
MR1	HPMC	risperidone	84.78 ± 0.79	2.90 ± 1.44
MR2		inclusion complex	95.08 ± 0.82	3.20 ± 1.47
MR3	carbomer	risperidone	88.34 ± 0.85	2.83 ± 1.28
MR4		inclusion complex	102.09 ± 5.40	3.41 ± 1.68
MR5	IPC[a]	risperidone	89.18 ±1.18	2.86 ± 1.86
MR6		inclusion complex	104.24 ± 2.40	3.19 ± 1.47

[a] HPMC/carbomer interpolymer complex.

3.3. Preparation and Characterisation of Mucoadhesive Microparticles

Risperidone and the inclusion complex were loaded into mucoadhesive microparticles by means of spray drying. This method is much less dependent on the solubility characteristics of the drug and polymer; it is simple, reproducible and easy to scale up. Prepared microparticles were characterised by drug content determination, particle size distribution, DSC analysis and zeta potential measurement. Also, mucoadhesive properties of the prepared microparticles were evaluated.

Drug content determination revealed drug loading into microparticles with average spherical diameters approaching 3.0 μm (Table **2**). The presence of the risperidone inclusion complex increased the average spherical diameter of the microparticles compared to cyclodextrin-free microparticles (Table **2**), but the difference was not statistically significant ($p > 0.05$). The particle size distribution of all prepared microparticles followed a narrow log-normal distribution, with less than 10% of microparticles > 5 μm. The presence of larger particles could be attributed to aggregation of microparticles during the spray-drying procedure.

DSC thermograms of the prepared microparticles are shown in Fig. **4**, and the results are compared to the thermogram of spray-dried risperidone. In all thermograms, a broad endothermic peak could be observed in the range of 35 to 110°C, corresponding to the evaporation of absorbed water. In the thermogram of HPMC based microparticles (MR1), an endothermic peak corresponding to the drug fusion was observed (159.9°C, ΔH=19.3 J/g), but the peak intensity decreased. The peak was shifted to lower temperature compared to spray-dried risperidone (169.6°C, ΔH=110.8 J/g). In the thermograms of MR2-MR6 microparticles, the drug fusion peak was absent.

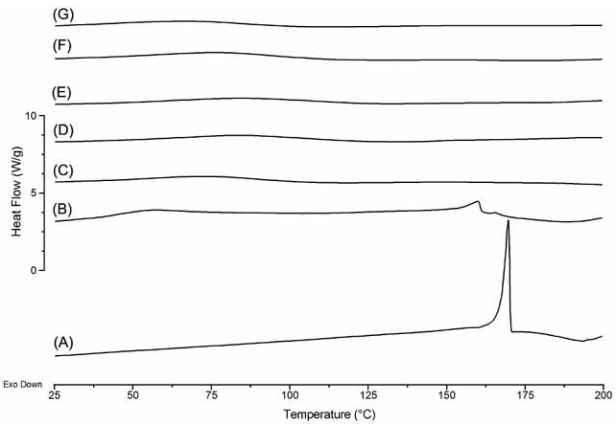


Fig. (4). DSC thermograms of **A**) spray-dried risperidone; **B**) the prepared microparticles MR1; **C**) MR2; **D**) MR3; **E**) MR4; **F**) MR5; and **G**) MR6.

The results of zeta potential determination are presented in Fig. **5**. Zeta potentials of the microparticles were compared to the zeta potential of spray-dried risperidone (R_0) and drug-free microparticles (HPMC, carbomer and IPC, respectively). Zeta potential of drug-free HPMC microparticles was close to zero due to the non-ionic nature of the polymer. The presence of charged carboxyl groups on the particle surface gave rise to negative zeta potential values of drug-free carbomer microparticles. In the case of drug-free IPC microparticles, the zeta potential was lower compared to carbomer drug-free microparticles (Fig. **5**).

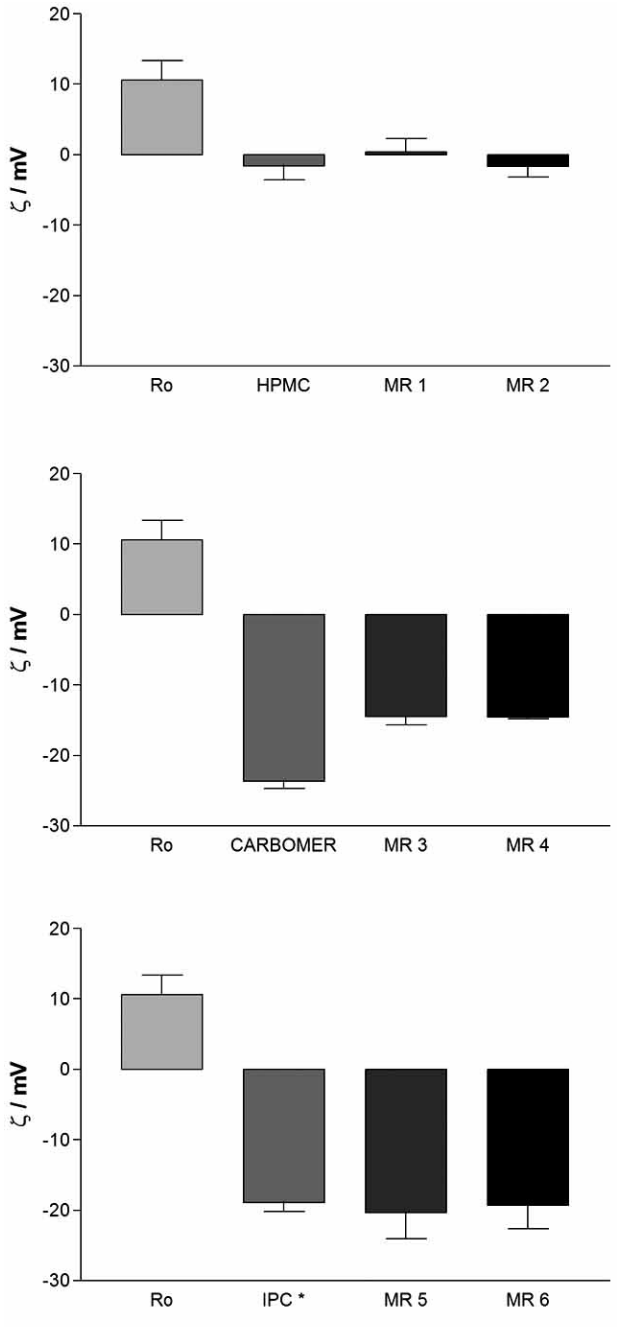
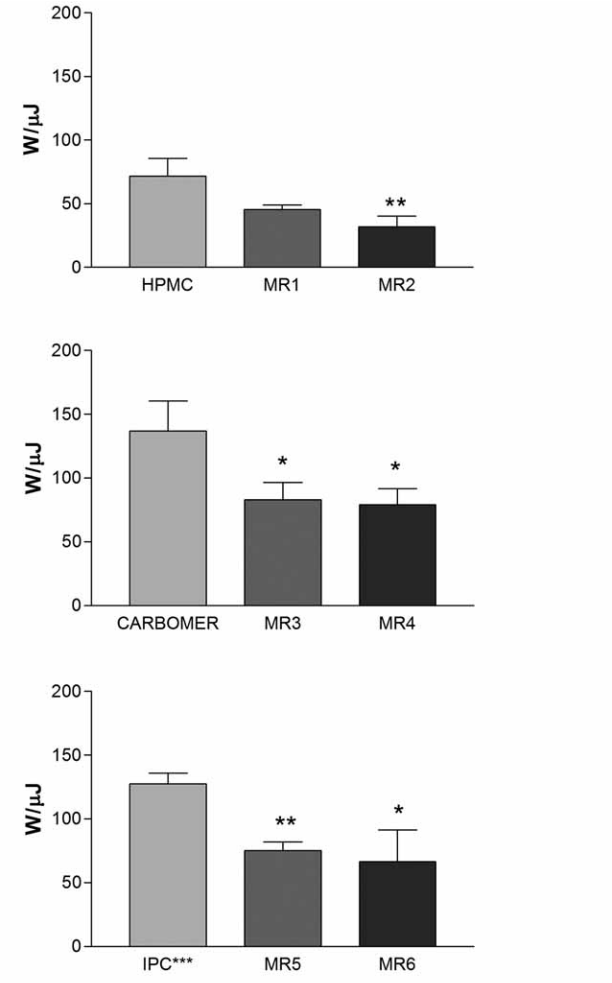


Fig. (5). Zeta potential values of spray-dried components (drug, HPMC, carbomer and IPC) and prepared microparticles.

Zeta potential of spray-dried risperidone (R_0) was positive due to partial drug ionisation under the experimental



Statistically significant differences.
* $P < 0.05$ compared to the drug free microparticles based on the same polymer
** $P < 0.001$ compared to the drug free microparticles based on the same polymer
*** HPMC/carbomer interpolymer complex

Fig. (6). *In vitro* mucoadhesion of the polymers and prepared microparticles expressed as the work of adhesion (W; mean ± S.D.; n=5).

conditions. In the case of drug-loaded microparticles based on HPMC (MR1 and MR2), the zeta potential value was similar to drug-free microparticles based on the same polymer, indicating that the polymer was the dominant component on the surface of microparticles (Fig. **5**). Negative zeta potential values of drug-free and drug-loaded microparticles based on carbomer (MR3 and MR4) and IPC (MR5 and MR6) pointed to the same conclusion. Zeta potential of carbomer based drug loaded microparticles was somewhat lower compared to the zeta potential of drug-free carbomer microparticles. Drug loading did not significantly affect the zeta potential of IPC based microparticles. The influence of HPBCD on the zeta potential of the microparticles was negligible.

The work of adhesion measurement was performed with the aim to evaluate the influence of the drug and polymers on the mucoadhesive properties of the prepared microparticles. Work of adhesion values for obtained microparticles (MR1-MR6) was compared to the work of adhesion value for the corresponding polymer (HPMC, carbomer or IPC). The results of the *in vitro* mucoadhesion investigation are shown in Fig. **6**.

Mucoadhesive properties of the drug-free HPMC based microparticles were less pronounced compared to the drug-free carbomer and IPC based microparticles (Fig. **6**). Risperidone incorporation into microparticles (MR1, MR3 and MR5) reduced their mucoadhesive properties ~1.7 fold compared to the drug-free microparticles based on the same polymer. Incorporation of the drug in the form of cyclodextrin inclusion complex into microparticles (MR2, MR4 and MR6) caused no additional significant reduction of their mucoadhesive properties ($p > 0.05$).

3.4. *In Vitro* Drug Release Studies

In vitro release studies were performed using the Franz-diffusion cell. This model allowed slow hydratation of the samples in a humid environment, conditions designed to be similar to those encountered in the nasal cavity [11]. The release of the drug from the prepared solid products (R_0, R_{HPBCD} and microparticles MR1-MR6) included several steps, such as dissolution/release of the drug into the hydrodynamic layer on the membrane surface, followed by drug diffusion across the semipermeable membrane to the acceptor compartment of the Franz-diffusion cell. Both processes made a contribution to the overall release rate. The semipermeable membrane had a molecular weight cut-off value of 600 Da, so risperidone could pass through freely. The obtained drug release profiles are represented in Fig. **7**.

To determine the drug release kinetics, experimental data were fitted to the zero-order and Higuchi kinetic models according to equations (2) and (3). According to the correlation coefficient values (r^2), the drug release from R_0, R_{HPBCD} and HPMC based microparticles (MR1 and MR2) could be described by zero order kinetics, while the release of the drug from carbomer (MR3 and MR4) and IPC based microparticles (MR5 and MR6) followed the Higuchi kinetic model (Fig. **7**). For easier comparison of release profiles described by different kinetic models, dissolution efficiency was calculated according to equation (4). The dissolution efficiency is defined as the area under the dissolution curve up to certain time, expressed as the percentage of the area of the rectangle described by 100% dissolution in the same time. The values of risperidone release kinetic parameters for prepared solid products are shown in Table **3**.

During the experiment, sink conditions in the donor compartment of the Franz-diffusion cell were not achieved, therefore dissolution efficiency in the case of spray-dried risperidone (R_0) was low (Table **3**). Incorporation of risperidone into the mucoadhesive microparticles had a significant influence on the kinetic parameters of drug release (Fig. **7A**). The type of the mucoadhesive polymer used in the formulation affected the drug release rate. Risperidone incorporated into HPMC based microparticles (MR1) led to a 1.3 fold dissolution efficiency increase compared to the spray-dried drug while the dissolution efficiency increase in the case of carbomer (MR3) and IPC based microparticles (MR5) was 5.9 and 6.3 fold, respectively. Inclusion complex formation (R_{HPBCD}) significantly enhanced the risperidone release rate (Fig. **7B**), leading to a 3.2 fold dissolution efficiency increase compared to the spray-dried drug. HPBCD also increased risperidone dissolution efficiency for HPMC (MR2), carbomer (MR4) and IPC

(MR6) based microparticles compared to cyclodextrin-free microparticles prepared with the same polymer.

Table 3. Kinetics Parameters of *In Vitro* Drug Release Studies: Zero Order (k_0) and Higuchi (k_H) Kinetics Constants and Dissolution Efficiency (DE) of Various Solid Products (Mean ± SD; N = 5)

Microparticle	$k_0 \times 10^3$/ (mg min^{-1})	$k_H \times 10^3$/ (mg min$^{-0.5}$)	DE/(%)
R_0	3.66 ± 0.04	-[a]	7.96 ± 0.18
R_{HPBCD}	10.42 ± 0.23	-[a]	25.07 ± 0.12[b]
MR1	4.68 ± 0.03	-[a]	10.06 ± 0.06
MR2	11.97 ± 0.19	-[a]	23.40 ± 0.28[c]
MR3	-[a]	17.23 ± 0.43	33.53 ± 0.36
MR4	-[a]	22.54 ± 0.49	47.14 ± 0.25[d]
MR5	-[a]	22.77 ± 0.30	39.89 ± 0.32
MR6	-[a]	30.05 ± 1.01	49.76 ± 0.26[e]

[a] Correlation coefficients $r^2 < 0.95$.
Statistically significant differences:
[b] $P < 0.001$ compared to spray-dried risperidone.
[c] $P < 0.001$ compared to MR1.
[d] $P < 0.001$ compared to MR3.
[e] $P < 0.001$ compared to MR5.

4. DISCUSSION

4.1. Influence of HPMC and Carbomer on Risperidone Solubility and Inclusion Complex Formation in Solution

Addition of HPMC, a hydrophilic polymer, influenced risperidone solubility, indicating a drug-polymer interaction (Table **1**). Heating and sonication of the samples increased risperidone solubility in water and promoted the drug-polymer interaction. The interaction probably included formation of the van der Waals and hydrogen bonds between the drug and polymer molecules, resulting in aggregate formation [12]. Cooling of the samples led to the formation of a supersaturated drug solution, stabilised due to the drug-polymer interaction. Therefore, risperidone solubility in HPMC solution was slightly higher than in water.

The presence of carbomer reduced only slightly risperidone solubility in water (Table **1**). Different effects of HPMC and carbomer on the risperidone solubility could be associated with the different structure of polymers. The linear structure of HPMC allowed high flexibility of polymer chains, which could facilitate drug-polymer interaction. The cross-linked structure of the carbomer limited the mobility of the polymer chains. Therefore, the interaction of the drug with carbomer could be restrained for sterical reasons. Also, the carbomer is an anionic polymer, and its addition to systems increases the ionic strength of the media. High ionic strength could reduce risperidone solubility in a carbomer solution [13].

Cyclodextrin inclusion complexes are known to interact with water-soluble polymers, resulting in formation of a ternary complex consisting of the drug, cyclodextrin and polymer [14]. The polymer coats the inclusion complex partially or totally, interacting with the drug and CD through hydrogen bonds. This interaction changes significantly the cyclodextrin solubilising effect and the stability constant value of the inclusion complex. Solubility and stability constant data (Table **1**) for the systems containing HPMC indicated that no formation of a ternary complex could be assumed. Higher risperidone solubility could be explained by simultaneous formation of the risperidone/HPBCD inclusion complex and drug-HPMC aggregates. Functional groups important for the interaction of the drug with HPMC were probably obscured by the inclusion complex formation, which reduced the affinity of HPMC for ternary complex formation.

The influence of carbomer on the risperidone/HPBCD inclusion complex formation in solution could be explained by the carbomer-cyclodextrin interaction. This interaction involved hydrogen bonds formation between the carboxyl groups of carbomer and hydroxyl groups of the cyclodextrin molecule [15]. The interaction with HPBCD is especially favoured, because HPBCD has a higher content of pendant hydroxyl groups, which are more accessible to hydrogen bonds establishment. This interaction could restrain the inclusion complex formation, probably for steric reasons.

4.2. Solid State Studies of the Risperidone/HPBCD Inclusion Complex

Based on the results of DSC experiments, a distinction can be made between the drug/cyclodextrin physical mixture and the inclusion complex in the solid state. The disappearance of the risperidone fusion peak in the thermogram of the spray-dried complex indicated an amorphous drug state; and this state may be explained by inclusion complex formation since the spray-drying procedure had no significant influence on risperidone crystalline properties (Fig. **2**). Due to complex formation, crystallisation of the drug during the spray-drying procedure was hampered, probably for steric reasons. The change of the drug fusion peak position and intensity in the risperidone/HPBCD physical mixture could be explained by thermally induced drug-cyclodextrin interaction during the experiment [16].

Further evidence of inclusion complex formation was obtained by FTIR analysis. There are three known polymorphs of risperidone (A, B and E). In FTIR spectra of the crystalline drug and spray-dried risperidone, the vibration band at 1650.37 cm^{-1} appeared as a single peak, indicating the presence of the most stabile polymorph A in both samples [17]. Shift of the amide carbonyl absorption band in the spray-dried complex suggested the formation of hydrogen bonding between the amide carbonyl group of the drug and hydroxyl groups of the cyclodextrin molecule during the inclusion complex formation [18]. The inclusion complex formation restricted vibrational motions of an included drug moiety in the cyclodextrin cavity, resulting in decreased absorption band intensity. Therefore, the change of the amide carbonyl-stretching band position and intensity indirectly confirmed the inclusion complex formation.

4.3. Preparation and Characterisation of Mucoadhesive Microparticles

Incorporation of risperidone in the form of inclusion complex into microparticles increased the drug loading (Table **2**). Due to the lipophilic nature of the drug, risperidone affinity for the interaction with the hydrophilic polymer was reduced, resulting in lower drug loading. The inclusion com-

plex formation enhanced the hydrophilic nature of the drug and thereby facilitated its interaction with the hydrophilic polymer, and increased risperidone loading was observed.

To evaluate the internal structure modification, the prepared microparticles were analysed by DSC (Fig. **4**). Reduction in the onset temperature and risperidone fusion peak intensity in thermograms of HPMC based microparticles (MR1) indicated a partial loss of risperidone crystalline properties due to incorporation into the HPMC matrix, but the presence of the drug microcrystalline areas in the polymer matrix could be still assumed. Disappearance of the risperidone fusion peak in carbomer and IPC based microparticles (MR3 and MR5, respectively) indicated formation of the drug molecular dispersion inside the polymer matrix of the microparticles, probably due to the drug-polymer interaction. Risperidone possesses 3 tertiary amino groups in the molecule, and interaction between an amino group of the drug and an carboxyl group of the polymer may be assumed [19]. Absence of the risperidone fusion peak in thermograms of HPMC based microparticles containing HPBCD (MR2) might indicate that incorporation of the inclusion complex into the polymer matrix caused no degradation of the inclusion complex. Disappearance of risperidone fusion peaks in thermograms of carbomer and IPC based microparticles containing HPBCD (MR4 and MR6) may be attributed to the presence of the inclusion complex and/or the drug-polymer interaction.

Zeta potential measurements were carried out to investigate surface characteristics of the prepared microparticles. Changes of the zeta potential might also point to interactions between the components of microparticles [20]. Chemical structure was the main parameter affecting the zeta potential of HPMC and carbomer drug-free microparticles. The interpolymer complex formation involved simultaneous establishment of a large number of intermacromolecular hydrogen bonds between carboxyl groups of the carbomer and hydrogen groups of HPMC [21]. This interaction reduced the number of charged carboxyl groups at the particle surface, thereby reducing negative zeta potential values of drug-free IPC microparticles.

The zeta potential reduction of the carbomer based microparticles containing the drug (MR3) revealed the electrostatic nature of the risperidone/carbomer interaction. A similar reduction of zeta potential was observed when risperidone was loaded in the form of an inclusion complex (MR4). The risperidone/HPBCD inclusion complex probably involved partial insertion of the drug molecule into cyclodextrin cavity and one or two tertiary amide groups were still available for the interaction with the carboxyl groups of the carbomer. Due to this interaction, the number of charged carboxylic groups on the particle surface was reduced and therefore the zeta potential value was lower. Interpolymer complex formation occupied the carboxyl groups of the carbomer and sterically hampered its interaction with the drug molecules. Therefore, the presence of the drug, free or in the form of a cyclodextrin complex, had no significant influence on the zeta potential of IPC based microparticles (MR5 and MR6).

The type of polymer used for preparation of microparticles determined their mucoadhesive properties. HPMC is a neutral polymer with a linear structure, and swelling of the polymer matrix occurred in contact with the mucosa, followed by interpenetration of mucine and HPMC strands. A weak mechanical bond was thereby formed, which seems to be the main mechanism of the mucoadhesive bond formation [22]. Carbomer is a cross-linked polymer, and the large number of carboxyl groups contributed to fast swelling of the polymer matrix due to electrostatic repulsion forces between negatively charged segments of the polymer [23]. Pronounced swelling and the cross-linked structure of the carbomer contributed to the formation of a stabile mechanic mucoadhesive bond that could be further stabilised by hydrogen bonds [24]. IPC was formed by simultaneous establishment of a large number of hydrogen bonds between carbomer and HPMC strands (cooperative phenomena). The cooperativeness of this interaction led to the formation of a sufficiently stable ladder-type structure [21]. Interpenetration of mucine strands into this formed structure could lead to the formation of a strong mucoadhesive bond; hence, the mucoadhesive properties of IPC and carbomer drug-free microparticles did not differ significantly ($P>0.05$).

Reduction in microparticles mucoadhesion after risperidone incorporation could be explained by lower polymer concentration in the matrix. Also, the presence of the drug molecule could prevent formation of electrostatic or hydrogen bonds, which are responsible for stabilisation of mucoadhesive bonds [25]. The presence of cyclodextrin in the polymer matrix did not significantly affect the mucoadhesive properties of microparticles. This may be explained in part by the results of zeta potential measurements, which indicated that the polymer was the main component on the surface of microparticles. Also, it has been demonstrated that HPBCD has a positive effect on the swelling properties of HPMC and carbomer based matrices [26]. The presence of a readily soluble, amorphous inclusion complex in the polymer matrix could promote hydration of the matrix, acting as a channelling or wicking agent. Thus, the polymer matrix containing HPBCD could swell more rapidly compared to the matrix without cyclodextrin. Swelling of the matrix may lead to greater mobility of the polymer chain segments, which could increase the availability of polymer chains for the interaction with mucine and the mucoadhesive bond formation.

4.4. *In Vitro* Drug Release Studies

In vitro release studies were intended to investigate the influence of inclusion complex formation and incorporation of the drug into mucoadhesive microparticles upon the risperidone release properties under the conditions designed to be similar to those of the nasal cavity.

The main parameter affecting risperidone release *in vitro* was the drug solubility. Due to low risperidone solubility, only limited amounts of the drug were available for diffusion in the acceptor phase, leading to low values of the kinetic parameters (Table **3**). Incorporation of the drug into microparticles enhanced the drug dissolution rate. The presence of the hydrophilic polymer facilitated to some extent the wetting of drug particles, providing a lower energy pathway for drug dissolution. The effect of HPMC on the drug dissolution rate was inferior compared to that of carbomer and IPC (Table **3**). This could be connected with the presence of the drug microcrystalline regions in the HPMC microparticles (MR1), as shown by DSC studies. On the other hand, in

carbomer and IPC microparticles (MR3 and MR5, respectively), the drug was molecularly dispersed in the polymer matrix due to interactions with the polymer. This could lead to faster risperidone release. Also, the ionic strength of the dissolution medium has a significant impact on the swelling and erosion of HPMC matrices and thereby on drug release [27]. During the experiment, microparticles were in contact with the dissolution medium, and swelling of the polymer matrix occurred, leading to the formation of a gel layer around the dry core. Drug diffusion in the gel layer and erosion of the polymer matrix were the main mechanisms controlling the drug release [28]. Uptake of the dissolution medium into HPMC microparticles (MR1) was followed by partial ionisation of risperidone molecules inside the polymer matrix. Charged drug molecules may compete for available water of hydration with HPMC chains, leading to slower swelling and erosion of the microparticles. This might affect the drug release. Therefore, a lower concentration of the drug was available for diffusion across the semipermeable membrane, leading to lower drug concentration in the acceptor phase. Uptake of the dissolution media into MR3 and MR5 microparticles caused ionisation of the polymer carboxyl groups, leading to the formation of an acidic microenvironment [29]. This could increase drug solubility in the swollen gel layer and thereby facilitate drug release.

Inclusion complex formation significantly enhanced the risperidone (R_{HPBCD}) release rate and increased the dissolution efficiency (Table **3**). Though the complex could not penetrate the membrane, the drug in the complex was in rapid dynamic equilibrium with the "free" drug, thus continuously supplying risperidone molecules to the membrane in a diffusible form. Cyclodextrin complexation increased the drug concentration gradient over the membrane, which led to higher drug concentrations in the acceptor compartment.

Incorporation of risperidone/HPBCD into microparticles additionally increased the drug release rate, leading to higher dissolution efficiency compared to cyclodextrin-free microparticles (Table **3**). The inclusion complex formation enhanced risperidone solubility in the swollen gel layer of the microparticles and thereby increased the concentration of diffusible species within the matrix. This contributed to the overall release rate. Also, the presence of HPBCD in the microparticles could enhance drug release by acting as a channelling or wicking agent [30]. This increased the diffusion of the dissolution medium into microparticles and thereby promoted their swelling and erosion. Enhanced erosion of the microparticles containing HPBCD could also contribute to faster drug release, leading to higher dissolution efficiency.

The presence of carbomer, free or as interpolymer complex, seems to be essential for the kinetic type of drug release from the microparticles, irrespective of HPBCD presence (Fig. **7**). According to the r^2 value, drug release from those microparticles (MR3-MR6) followed the Higuchi square root of time equation. Fast swelling of MR3-MR6 microparticles and the acidic microenvironment of the formed gel layer [23] may increase risperidone solubility. This contributed to fast initial drug release ("burst effect"). During the experiment, the drug concentration in the microparticles decreased causing a decrease of the overall drug release rate. In the case of HPMC based microparticles (MR1-MR2), drug release occurred at a constant rate during the experiment, showing a better fit to the zero-order kinetic model (Table **3**). This behaviour may be attributed to slower HPMC matrix swelling, due to partial drug ionisation, as discussed above.

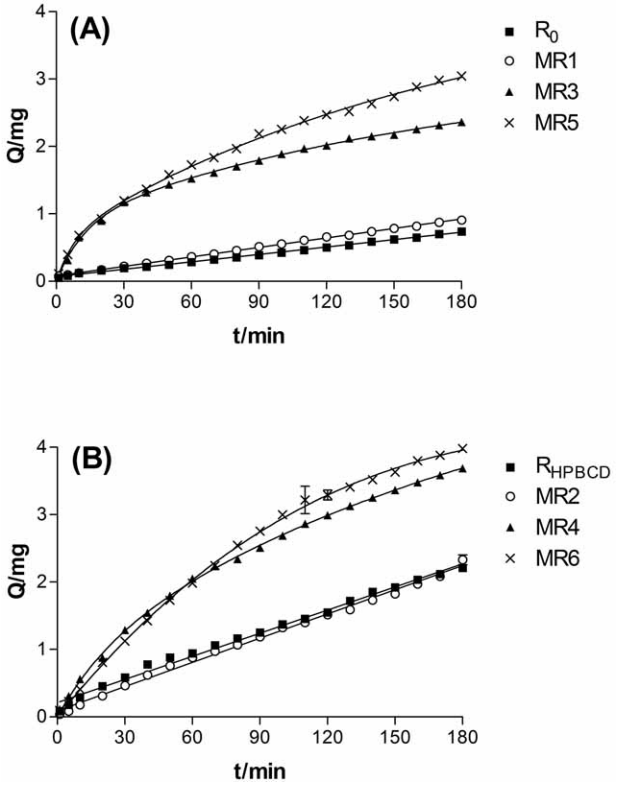


Fig. (7). *In vitro* release of risperidone from prepared microparticles without (**A**) and with HPBCD (**B**).

The influence of HPBCD on risperidone release from mucoadhesive microparticles was studied in order to overcome formulation problems associated with designing a controlled release system intended for nasal administration of low solubility drugs. It has been demonstrated that HPBCD can enhance risperidone release with a minimal influence on mucoadhesive properties of the microparticles. The results suggest that this effect can be attributed to the ability of HPBCD to form an inclusion complex with risperidone, resulting in increased drug solubility. Also, the risperidone/carbomer interaction demonstrated by the DCS analysis and zeta potential measurements additionally enhanced the drug release rate. Polymeric matrices containing the mucoadhesive components and cyclodextrin inclusion complex may be used as a non-invasive alternative to oral drug administration, providing sustained drug release *in vitro*.

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ABBREVIATIONS

DE	=	Dissolution efficiency
DSC	=	Differential scanning calorimetry
FTIR	=	Fourier transform infrared spectroscopy
HPBCD	=	Hydroxypropyl-beta-cyclodextrin
HPMC	=	Hydroxypropylmethyl cellulose
IPC	=	HPMC/carbomer interpolymer complex

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