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# The study of drug release from microspheres adhered on pig vesical mucosa

M. Burjak, M. Bogataj *, M. Velnar, I. Grabnar, A. Mrhar

*Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia*

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**Abstract**

The object of our work is the preparation of a mucoadhesive drug delivery system intended for intravesical application. In the present work, microspheres with Eudragit RS matrix polymer and different mucoadhesive polymers, i.e. chitosan hydrochloride (Ch), sodium salt of carboxymethyl cellulose (CMC) and polycarbophil (PC) were prepared to evaluate their influence on the mucoadhesive properties of microspheres. Different parameters were determined and their influence on pipemidic acid release from microspheres adhered on intact and damaged pig vesical mucosa was evaluated: swelling of polymers, mucoadhesion strength of polymeric films and drug dissolution according to USP XXIV method. The dissolution rate from microspheres containing different mucoadhesive polymers decreases as follows: PC > Ch > CMC. PC swelled to the largest volume among all polymers and as a result the fastest release of the drug from PC microspheres was obtained. The release rate of pipemidic acid from microspheres adhered on intact mucosa followed the order PC > CMC > Ch. These results show that both drug dissolution and mucoadhesion strength strongly influence drug release from adhered microspheres. The slowest release from Ch microspheres could be interpreted by the largest mucoadhesion strength of Ch polymeric films. The release rate of pipemidic acid from microspheres adhered on damaged mucosa followed the order PC = Ch > CMC. The results obtained on pathologically changed mucosa model support the indication of the role of glycosaminoglycans and polymer charge in the mucoadhesion process on vesical mucosa. Analysis of release data shows that the drug dissolution profiles follow the Higuchi kinetics better than the release profiles from adhered microspheres and different kinetics might be a consequence of different release mechanisms. 

*Keywords:* Chitosan; Polycarbophil; Carboxymethyl cellulose; Mucoadhesive microspheres; Pig vesical mucosa; Release from adhered microspheres

## 1. Introduction

Mucoadhesive drug delivery systems for applications on different mucosae have been intensively investigated in the last two decades but there are very few descriptions of such application

* Corresponding author. Tel.: +386-1-4769-510; fax: +386-1-4258-031.

*E-mail address:* marija.bogataj@ffa.uni-lj.si (M. Bogataj).

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in the urinary bladder. A delivery system for application in the urinary bladder is expected to be in the form of micro- or nanoparticles which have to be suspended in a suitable solvent and instilled in the urinary bladder through a catheter. After application, the drug delivery system is expected to adhere on urinary bladder mucosa. The composition of mucus, which covers urinary bladder mucosa, seems to be very important for mucoadhesive bond formation with the drug delivery system components and is assumed to influence drug release.

The mucus layer is frequently described by the term glycosaminoglycan layer. Hurst and Zebrowski (1994) proposed a model for the bladder surface layer. They reported that 80–90% of total surface glycosaminoglycans are covalently attached to core proteins which are intercalated into the cell membrane. On the surface, there is also a large amount of glycoproteins and some glycosaminoglycan molecules which are both loosely adhered. The carbohydrates are present at very high densities on the bladder surface and they produce a very high negative charge. Mucus protects the surface of urinary bladder mucosa against the adhesion of bacteria and different substances present in urine. It may also protect the bladder against carcinogenesis by preventing the adhesion of carcinogens and cocarcinogens excreted in urine (Kaufman et al., 1987).

The purpose of our work is the preparation of mucoadhesive microspheres intended for intravesical application for the treatment of superficial urinary bladder cancer or urinary bladder infections (Bogataj et al., 1999). Current therapy of human superficial urinary bladder cancer includes the intravesical administration of small volumes of highly concentrated solutions of antitumor drugs or immunomodulators, which is often limited by drug toxicity and needed reduction of frequency of urine voiding. The success of intravesical therapy depends on direct contact between the drug and the abnormal urothelium and whichever mechanism that prolongs the exposure of the urothelium to the drug is expected to increase its efficacy (Frangos et al., 1990). Very few literature data were reported regarding drug delivery systems for intravesical application. Peppas described the development of bioadhesive microparticles for intravesical application (Peppas et al., 1984). A liposome-based drug delivery system containing interferon alpha was prepared and produced significantly higher levels of cytostasis against human bladder cancer cells than free drug (Frangos et al., 1990). Additionally, Ueda et al. (1992) discovered that the anticancer drug remained longer within the urinary bladder tissue when administered together with hydroxypropylcellulose and higher concentrations of drug were achieved in tumorous tissues than in normal tissues. Therefore, it is expected that the transport of anticancer drugs in tumorous tissue will also be enhanced by the use of microspheres containing hydroxypropylcellulose or some other polymers.

In the present study, the influence of drug dissolution from microspheres, swelling of polymers and mucoadhesion strength on the release of pipemidic acid from microspheres, adhered on pig vesical mucosa, was evaluated. We have chosen cationic and anionic polymers which are known as the best mucoadhesives in order to confirm the influence of polymer charge on adhesion on vesical mucosa as we indicated in a previous article (Bogataj et al., 1999). Additionally, with the aid of a damaged mucosa model, the involvement of mucus in the adhesion process was studied as well.

## 2. Materials and methods

### 2.1. Materials

Eudragit® RS 100 (ERS) was supplied by Röhm GmbH, Darmstadt, Germany and polycarbophil (PC), Noveon AA1, by Goodrich, Cleveland, OH; chitosan hydrochloride (Ch), Protasan CL 210, was purchased from Pronova®, Oslo, Norway and sodium salt of carboxymethyl cellulose (CMC), medium viscosity, from Fluka BioChemica, Buchs, Switzerland. Pipemidic acid and magnesium stearate were provided by Lek, Ljubljana, Slovenia.

Solvents and substances, used for the preparation of Tyrode solution, were all of analytical grade.

## 2.2. Methods

### 2.2.1. Preparation of polymeric films

Dispersions of Ch, PC and CMC in purified water were prepared, poured on a limited surface of a glass plate and dried at room temperature. The weight of dry films, which adhered on the glass plate, varied between 1.2 and 1.4 mg/cm$^2$.

### 2.2.2. Preparation of microspheres

Microspheres were prepared by a solvent evaporation method. The solvent system acetone/liquid paraffin was used. Agglomeration of microspheres was prevented by magnesium stearate. ERS was used to form a matrix of microspheres and different mucoadhesive polymers were chosen to produce mucoadhesion: Ch, PC and CMC. Pipemidic acid was used as a model drug.

ERS was dissolved in acetone and mucoadhesive polymer was added as a powder. Suspensions of magnesium stearate and pipemidic acid in acetone were prepared separately and added to the dispersion of polymers. The total volume of acetone was 12 ml. The homogeneous final dispersion was cooled to 5 °C and poured slowly with stirring (1000 rpm) into 80 ml of liquid paraffin, which was previously also cooled to 5 °C. The obtained emulsion was stirred at 40 °C for 40 min. The suspension of microspheres in liquid paraffin was filtered, microspheres were washed by *n*-hexane and dried in vacuum at room temperature overnight.

For the preparation of microspheres the following quantities of substances were used: 0.9 g of matrix polymer and 0.6 g of mucoadhesive polymer, 0.3 g of magnesium stearate and 0.5 g of pipemidic acid. The weight ratios of polymers in the samples are shown in Table 1. Pipemidic acid content in the microspheres was determined as described in Section 2.2.3 and the results are also shown in Table 1.

All samples of microspheres were sieved and the fraction 80–100 μm was used for further testing.

### 2.2.3. Drug content determination

Drug content was evaluated by dispersing microspheres in different solvents depending on the mucoadhesive polymer which was incorporated in the microspheres. Ch microspheres were dispersed in 0.1 mol/l HCl and stirred for 24 h, PC and CMC microspheres were dispersed in 0.1 mol/l NaOH and stirred for 40 min and pipemidic acid concentrations were determined spectrophotometrically. Results are means of triplicate experiments.

### 2.2.4. Swelling studies

Polymer powder (100 mg) was put into the graduated glass cylinder and 10 ml of Tyrode solution were added. The volumes of hydrated polymer were measured at fixed time intervals. Results are means of triplicate experiments.

### 2.2.5. Dissolution studies

Drug release tests were carried out according to the USP XXIV method (Apparatus 2) in 1 l of Tyrode solution at 37 °C and 100 rpm. During the experiments, microspheres floated irrespective of the mucoadhesive polymer used. Results are means of triplicate experiments.

### 2.2.6. Measurement of detachment force

Isolated pig urinary bladders from freshly slaughtered 7 month old pigs of both sexes, weighing 90–110 kg, were obtained from a local slaughterhouse and represented biological component in our experiments.

Table 1
The weight ratios of polymers and determined pipemidic acid content in microspheres

| Sample | Polymers | Ratio | Drug content (w/w %) |
|---|---|---|---|
| Ch | Eudragit RS: chitosan hydrochloride | 3:2 | $15.73 \pm 0.56$ |
| CMC | Eudragit RS: carboxymethyl cellulose | 3:2 | $17.12 \pm 0.52$ |
| PC | Eudragit RS: polycarbophil | 3:2 | $20.96 \pm 0.78$ |

The detachment force between polymeric film and intact pig urinary bladder mucosa or mucosa washed by diluted HCl (0.6 mol/l HCl for 5 min) was measured by a modified precision balance. The appropriate amount of Tyrode solution was dispersed over the surface of polymeric film (15 μl/cm$^2$) and left for 2 h in a humid environment to hydrate. A glass plate with hydrated polymeric film was mounted on the upper clamp of the apparatus. The mucosal layer of pig urinary bladder wall was separated from the underlying muscle layer and mounted on the lower support of the apparatus, then the clamp with tissue was slowly raised and contact with the hydrated film was formed. The detachment force needed for the separation of two surfaces was determined 2 min after the formation of the contact. Results are means of five to seven experiments.

*2.2.7. Drug release from microspheres adhered on vesical mucosa*

Mucoadhesion properties of microspheres were evaluated by an in vitro method as developed earlier (Bogataj et al., 1999). Urinary bladder mucosa was attached on a rubber support. Microspheres were placed on the mucosa and wetted by spraying the mucosa with Tyrode solution. After the hydration of microspheres, the support was inserted in the apparatus and mucosa was washed with aerated Tyrode solution at 37 °C. The flow rate of Tyrode solution was 0.5 ml/min. The pipemidic acid concentration in washings was determined spectrophotometrically. The experiments were performed on intact mucosa and on mucosa washed by 0.6 mol/l HCl. Results are means of triplicate experiments.

## 3. Results and discussion

The majority of mucoadhesion testing described in the literature was performed on gastrointestinal tract mucosa (Lehr et al., 1992). In the present study, polymers with different mucoadhesive properties and charge were incorporated in microspheres in order to investigate their influence on adhesion on vesical mucosa. For this purpose, a cationic polymer, Ch, and two anionic polymers, PC and CMC, were used.

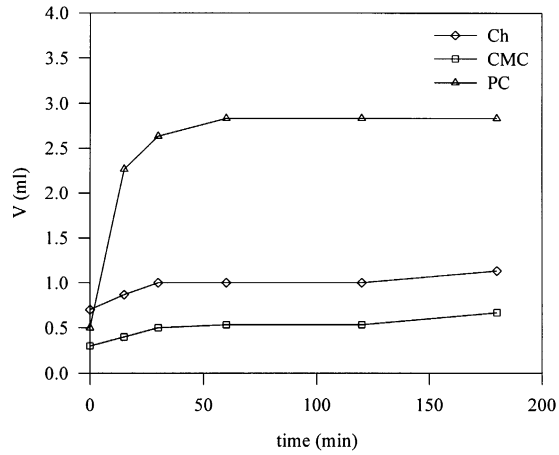


Fig. 1. The change of polymer powder volume in water due to the swelling and/or dissolution of polymers. *V*, volume of swollen polymer.

The influence of polymer swelling, drug dissolution and mucoadhesion strength on the release of pipemidic acid from microspheres, adhered on pig vesical mucosa was evaluated.

Results of testing of polymer powder swelling are shown in Fig. 1. It can be seen that PC swells to the largest volume and the fastest swelling occurred during the first 15 min of the contact with Tyrode solution. PC is known as a good mucoadhesive polymer and because of its chemical structure, i.e. polyacrylic acid crosslinked with divinyl glycol (BFGoodrich, 1994), it is not soluble in water but it swells very well. Ch and CMC swell as well but to a lesser extent than PC.

These observations can serve as an explanation for the results obtained from dissolution tests (Fig. 2) where the dissolution rate from microspheres containing different mucoadhesive polymers decreases as follows: PC > Ch > CMC. The fastest release of pipemidic acid occurred from PC microspheres where 90% of pipemidic acid was released in 8 min. PC represented a part of the total mass of PC microspheres and because of good swelling properties, relaxation of PC chains occurred and hydrophilic drug easily formed contact with solvent and dissolved. As a result the fastest release of pipemidic acid was obtained. The solubility of Ch, which is a linear polysaccharide obtained by alkaline N-deacetylation of

chitin, in water and at pH higher than about 6.5, depends on the degree of N-acetylation and also on the arrangement of the monomers along the chain (Anthonsen, 1993). Ch with 27% degree of N-acetylation was used in our experiments. Because of its low solubility in Tyrode solution (pH 8), 90% of pipemidic acid was released not earlier than 120 min from Ch microspheres. CMC also exhibits low solubility in Tyrode solution and consequently delayed release of the drug from CMC microspheres (i.e. 90% in 300 min) was observed.

The kinetics of drug release was evaluated with the aid of a Higuchi model which is usually used for the evaluation of drug release from matrix systems (Eq. (1)).

$$\%\ \text{drug released} = k\sqrt{t} \quad (1)$$

where $k$ is a drug release constant. Experimental data between 10–90% of the drug released were used for the linear regression analysis where the percentage of the drug released represented the dependent variable and $\sqrt{t}$ the independent variable. Table 2 shows the results of analysis. Very high Pearson correlation coefficient values show that the Higuchi model is suitable for the evaluation of drug release from all microspheres. Good agreement between the model responses and experimental data can also be seen from Fig. 2.

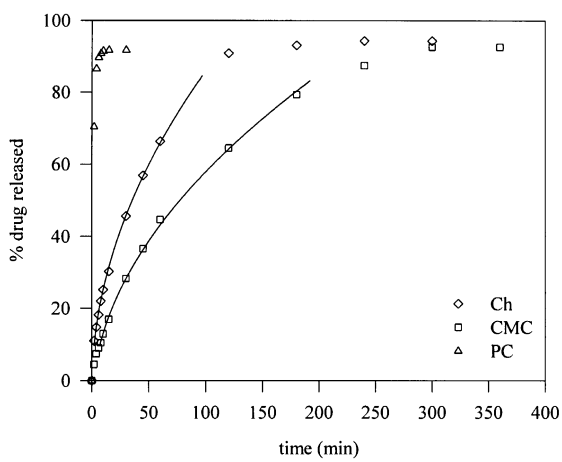


Fig. 2. Average dissolution profiles of pipemidic acid from different microspheres according to the USP test (points) and Higuchi model responses (curves). Coefficients of variation varied between 0.9 and 32%. Abbreviations are presented in Table 1.

Table 2
Accommodation of the Higuchi model to experimental values of the amounts of pipemidic acid released according to the USP test

| Sample | $n$ | $k$ ($\text{min}^{-1/2}$) | $r$ |
|---|---|---|---|
| Ch | 9 | 8.900 | 0.9993 |
| CMC | 8 | 6.568 | 0.9995 |
| PC | 2 | [a] | [a] |

$n$ number of analyzed experimental data, $k$ drug release constant, $r$ Pearson correlation coefficient obtained with linear regression analysis. Abbreviations are presented in Table 1.

[a] The drug release was too fast and $k$ could not be calculated.

Mucoadhesion strength of polymeric films on intact pig vesical mucosa was evaluated by determination of detachment forces and the results are presented in Fig. 3 (white columns). Statistical evaluation showed that Ch polymeric films exhibit significantly greater mucoadhesion than CMC and PC polymeric films ($P < 0.05$). The results could be interpreted by the fact that Ch, a cationic polymer, forms additional interactions between its amino groups and negatively charged carboxylic and sulfuric groups of urinary bladder mucus components. This resulted in the largest mucoadhesion strength of Ch in comparison with anionic polymers CMC and PC.

Since we postulate that the release of pipemidic acid from adhered microspheres depends strongly

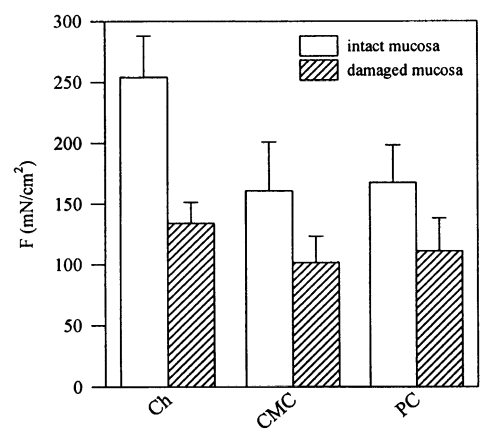


Fig. 3. Mean values and standard deviations of detachment forces ($F$) of different polymeric films measured on intact and on damaged mucosa. Abbreviations are presented in Table 1.

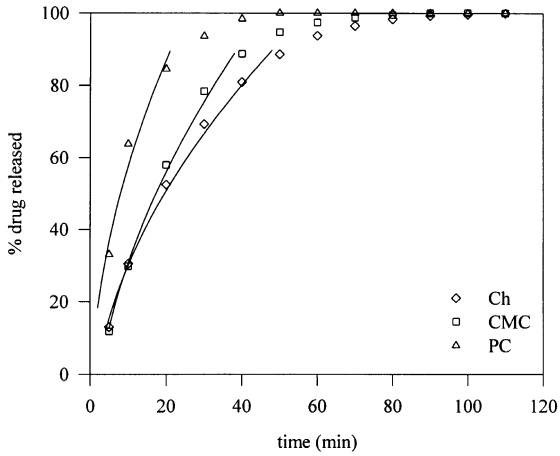


Fig. 4. Average release profiles of pipemidic acid from different microspheres adhered on intact pig vesical mucosa (points) and Higuchi model response (curves). Coefficients of variation varied between 0.6 and 33.4%. Abbreviations are presented in Table 1.

on the type and pathological condition of mucosa, swelling and mucoadhesion strength of polymers, and retardation properties of microspheres, the results obtained from the above mentioned in vitro experimental models can be viewed as a means for explaining the influence of each factor separately on drug release from adhered microspheres.

An experimental model which was developed earlier (Bogataj et al., 1999) was also used in this study to follow drug release from microspheres adhered to vesical mucosa. Experimental conditions which simulate the in vivo situation were chosen to obtain results that could represent the basis for the prediction of microsphere behavior in vivo.

The release rate of pipemidic acid from microspheres adhered on intact mucosa decreased in the order PC > CMC > Ch (Fig. 4). After microspheres were placed on mucosa, they hydrated and swelled. The majority of Ch microspheres adhered on mucosa and pipemidic acid was released from swelled and adhered particles. However, microspheres with PC and partly microspheres with CMC adhered to a lesser extent and were slowly washed from the surface of mucosa in the form of particles during the experiment. Since a certain amount of the drug could also be released from the particles in the washings before and during the filtration process, the drug release was very likely the result of two processes, i.e. drug release from adhered microspheres and drug release from particles washed off. Therefore the differences in release can be attributed not only to the release rate from adhered particles but also to the extent of microsphere adhesion.

The average values of the times at which 90% of the drug ($t_{90}$) is released from microspheres adhered on intact mucosa were calculated and the results are presented in Fig. 5 (white columns).

Comparison of the results obtained from the experiments where drug release from microspheres adhered on intact mucosa was followed and dissolution tests showed that in both cases the fastest release of the drug was exhibited by PC microspheres which is probably the consequence of very good swelling of PC. The slowest release was obtained from Ch microspheres adhered on intact mucosa regardless of the fact that dissolution according to USP was faster for Ch than for CMC microspheres. These results could be explained by the highest mucoadhesion strength obtained for Ch polymeric films and are in accordance with previous work (Bogataj et al., 1999), where the resolution on the decisive influence of polymer mucoadhesive properties on drug

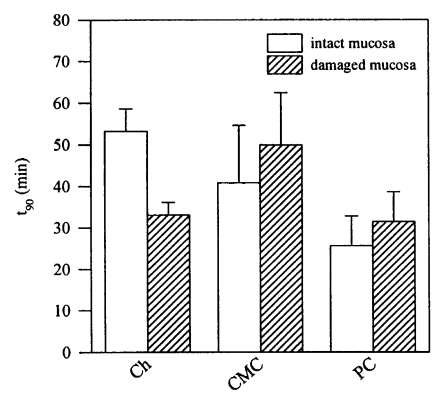


Fig. 5. Release of pipemidic acid from microspheres with different mucoadhesive polymers adhered on intact and on damaged mucosa. Mean values and standard deviations of the times at which 90% of the drug ($t_{90}$) is released are presented. Abbreviations are presented in Table 1.

Table 3
Accommodation of the Higuchi model to experimental values of released amounts of pipemidic acid from different microspheres adhered on intact or on damaged vesical mucosa

| Sample | Intact mucosa | | | Damaged mucosa | | |
|---|---|---|---|---|---|---|
| | $n$ | $k$ ($min^{-1/2}$) | $r$ | $n$ | $k$ ($min^{-1/2}$) | $r$ |
| Ch | 6 | 15.858 | 0.9968 | 4 | 20.409 | 0.9954 |
| CMC | 5 | 19.393 | 0.9971 | 5 | 14.886 | 0.9912 |
| PC | 3 | 22.522 | 0.9778 | 4 | 17.729 | 0.9772 |

$n$ number of analyzed experimental data, $k$ drug release constant, $r$ Pearson correlation coefficient obtained with linear regression analysis. Abbreviations are presented in Table 1.

release from adhered microspheres was made. The results from this study show that positively charged Ch could form additional interactions with mucus components and confirm the influence of polymer charge on adhesion on vesical mucosa.

On the basis of what has been claimed above, mucoadhesive polymers in contact with aqueous medium hydrate, swell and adhere. We can hypothesize that as a result of these processes a gel is formed on the surface of mucosa. Stronger mucoadhesives both increase the extent of microsphere adhesion and retain the gel layer on the surface of mucosa for a prolonged period of time. Consequently they extend the release of the drug which could otherwise be washed off together with the dissolved polymer.

Accommodation of the Higuchi model to experimental values of released amounts of pipemidic acid from adhered microspheres was also performed and the results are presented in Fig. 4. Lower Pearson correlation coefficient values (Table 3) show that the release profiles follow the Higuchi kinetics worse than the profiles of drug dissolution from microspheres according to the USP test. These results could be explained by a different release mechanism which is a consequence of microsphere adhesion.

The second part of the experiments was performed on pig vesical mucosa previously washed by diluted HCl to remove the mucus as described in the literature. After the treatment with 0.6 mol/l HCl no gross effects were noted on the bladder and histological studies showed that the mucosa was basically intact, the only consistent finding was the loss of the surface mucus layer (Parsons et al., 1980). In our experiments, no microscopic observations were made but on the basis of visual examination we suspect that the mucosa cells were probably also damaged. However, irrespective of the degree of damage this model can be viewed as an example of pathologically changed urinary bladder mucosa. All polymeric films adhered significantly better on intact mucosa than on mucosa washed by diluted HCl ($P < 0.05$) (Fig. 3). The greatest difference occurred in Ch polymeric films. Obviously the change of mucosa surface lowered the extent of interaction between polymers and mucosa surface and consequently interpenetration of polymeric and glycosaminoglycan chains and the possibility of weak chemical bond formation decreased.

The rank order of release rates of pipemidic acid from adhered microspheres changed in the case of damaged mucosa and was as follows: PC = Ch > CMC (Fig. 6). Moreover, it is evident from $t_{90}$ values (Fig. 5, hatched columns) that drug release from microspheres containing anionic polymers (CMC and PC), adhered on damaged mucosa, was slowed down in comparison with intact mucosa. These results could be explained by the fact that mucosa damage probably changes the charge of mucosa surface and as a consequence drug dissolution prevails over mucoadhesion.

Since the experimental model of pathologically changed urinary bladder mucosa resulted in the loss of mucus layer, the obtained results indicate the involvement of glycosaminoglycans in the mucoadhesion process and the influence of polymer charge on the mucoadhesive properties of microspheres.

## 4. Conclusions

Drug release from microspheres adhered on pig vesical mucosa surface is influenced strongly by at least five factors: the type and pathological condition of mucosa, swelling and mucoadhesion strength of polymers, and retardation properties of microspheres. On the basis of the results obtained in the present study the following conclusions can be made:

- fast and extensive swelling of polymers resulted in the fastest drug release from adhered microspheres,
- high mucoadhesion strength slows down drug release from adhered microspheres,
- mucosa damage influences mucoadhesion strength and drug release from adhered microspheres; this finding supports the indication of the role of glycosaminoglycans and polymer charge in the mucoadhesion process on vesical mucosa,
- on the basis of the above results one can expect differences in the extent of microsphere adhesion and consequently in the drug release from adhered microspheres after the application of microspheres on pathologically changed vesical mucosa.

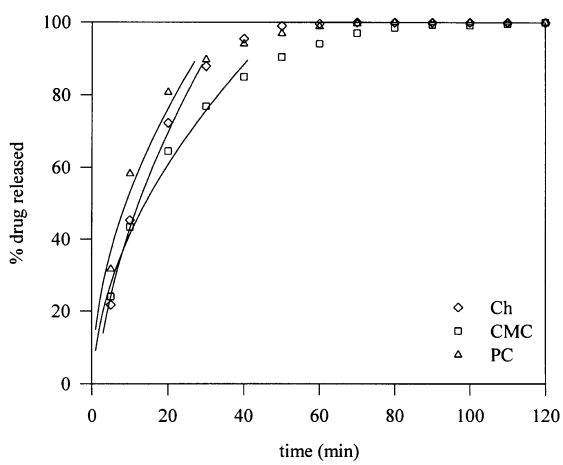


Fig. 6. Average release profiles of pipemidic acid from different microspheres adhered on damaged pig vesical mucosa (points) and Higuchi model response (curves). Coefficients of variation varied between 0.5 and 26.2%. Abbreviations are presented in Table 1.

In addition, a new in vitro whole pig urinary bladder model will be developed to simulate the in vivo situation as near as possible. Furthermore, chitosan/polycarbophil microspheres will be prepared to study the influence of interpolymer interactions on mucoadhesion properties.

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