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Influence of physicochemical and biological parameters on drug release from microspheres adhered on vesical and intestinal mucosa

M. Bogataj [a,*], A. Mrhar [a], L. Korošec [b]

[a] *Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia*
[b] *Department of Pharmacology, Medical Faculty, University of Ljubljana, Korytkova 2, 1000 Ljubljana, Slovenia*

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Abstract

The object of our work is to develop mucoadhesive microspheres to be applied into the urinary bladder. In the present study the microspheres were prepared and the release of a model drug after their adhesion to mucosa was evaluated. The microspheres were prepared by solvent evaporation method using Eudragit RL or hydroxypropylcellulose as matrix polymers and one out of five different polymers as mucoadhesives or non-mucoadhesive references. A method for the evaluation of the drug release from microspheres adhered on guinea pig urinary bladder and small intestine mucosa was developed and the influence of the following parameters on this process was followed: mucoadhesion strength of polymeric films, swelling of polymers and the drug release from microspheres. The results showed that the detachment forces were decreasing in the following order: CMCNa > Carbopol 934P > HPC > EE.HCl = PVP/VA. Carbopol swelled to the largest volume among all polymers and the drug release from microspheres was more retarded when Eudragit RL was used as matrix polymer. When comparing the results of pipemidic acid release from microspheres adhered on intestinal mucosa with detachment forces, similar ratios among the mucoadhesive polymers can be seen. On the other hand, differences between two mucosae were observed. These differences are due to the amount of mucus on mucosa and might also be influenced by the charge of mucus. The goal of our work at this point of investigation was achieved by microspheres containing carboxymethylcellulose as mucoadhesive and Eudragit RL as matrix polymer because they provide the longest release time from microspheres adhered on vesical mucosa and sufficient high strength of mucoadhesion.

Keywords: Mucoadhesion; Microspheres; Vesical mucosa; Urinary bladder application; Detachment force; Release from adhered microspheres

* Corresponding author. Tel.: +386-61-1769-510; fax: +386-61-1258-031; e-mail: marija.bogataj@ffa.uni-lj.si.

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1. Introduction

The mucoadhesion phenomenon became very important for the development of drug delivery systems. The mucoadhesive interactions depend on both components which form the contact: the mucoadhesive polymer, as a component of the drug delivery system, and the mucus, which covers the surface of mucosae. The structure of both components and the mechanisms of their interactions are studied in many research works, which are reviewed by Peppas and Buri (1985), Mikos and Peppas (1986), Duchêne et al. (1988), Junginger (1991) and others.

The structure and composition of the mucus layer depends on the where the mucosa occurs. The structure of intestinal mucus is very frequently and extensively described in literature (Allen, 1978, 1983; Hase et al., 1992). Glycoproteins constructed of protein core and sugar side chains are the main component of the intestinal mucus. The protein core is represented by a long polypeptide chain which is constructed of a strongly glycosylated part and a region without carbohydrates. The oligosaccharide chains which are attached to the protein core contain five different monosaccharides which carry negative charge, due to sialic acid and sulfates. The glycoprotein chains are connected by disulfide bridges to form large glycoprotein macromolecules. The structure of the layer covering urinary bladder mucosa is not known as well as that of the intestinal mucus. Different authors studied vesical mucus on different animals, and carbohydrates–glycosaminoglycans (GAG) carrying sulfate groups and high negative charge were determined (Parsons et al., 1977; Callahan et al., 1985; Ruggieri et al. 1992). Hurst and Zebrowski (1994) proposed a model for the bladder surface layer. Approximately 85% of total surface GAG are covalently attached to core proteins which are intercalated into the cell membrane and about 55% of protein-bound GAG is heparan sulfate and 29% chondroitin sulfate. Also on the surface there is a large amount of glycoproteins and some GAG molecules which are both only loosely adhered. The carbohydrates are present at very high densities on the bladder surface and they are responsible for the very high negative charge. Hurst and Zebrowski (1994) also found out that human and bovine bladders have similar amounts of carbohydrates of similar composition on their mucosal surface.

The above data indicate that the mucus layers in urinary bladder and intestine are different regarding the structure and the thickness, but yet certain similarities exist. They both contain sugar chains, completely or partly attached to proteins and they both carry negative charge which is probably not equal. It is therefore expected that polymers which are good mucoadhesives on intestinal mucosa, will exhibit some mucoadhesivness also on vesical mucosa. But for exact evaluation, polymers and drug delivery systems must be experimentally tested also on vesical mucosa.

The purpose of our work is the preparation of microspheres for urinary bladder application through a catheter. Inside the bladder microspheres are expected to adhere on mucus/mucosa and release the drug over a certain period of time. Such a drug delivery system would be most suitable for treatment of superficial bladder cancer due to the high toxicity of anticancer drugs after systemic application and for treatment of urinary bladder infections in patients which permanently use a catheter.

The superficial bladder cancer is usually treated by transurethral resection followed by intravesical chemotherapy or immunotherapy (Witjes, 1997). In the case of intravesical chemotherapy, a low volume (20–60 ml) of a high concentrated drug solution is instilled into the bladder and urine should not be voided for 1–2 h which may represent a great problem to the patients. This limitation could be avoided by the use of mucoadhesive microspheres which are expected to adhere on mucosa and maintain the adequate drug concentration on its surface during a prolonged period of time. If the release profile from the microspheres is suitable, the drug concentration on the mucosa surface is maintained constant and is not significantly influenced by voiding.

Additionally, Ueda et al. (1992) discovered that the anticancer drug remained longer within the urinary bladder tissue when administered together with hydroxypropylcellulose and that higher concentrations of drug were achieved in tumorous

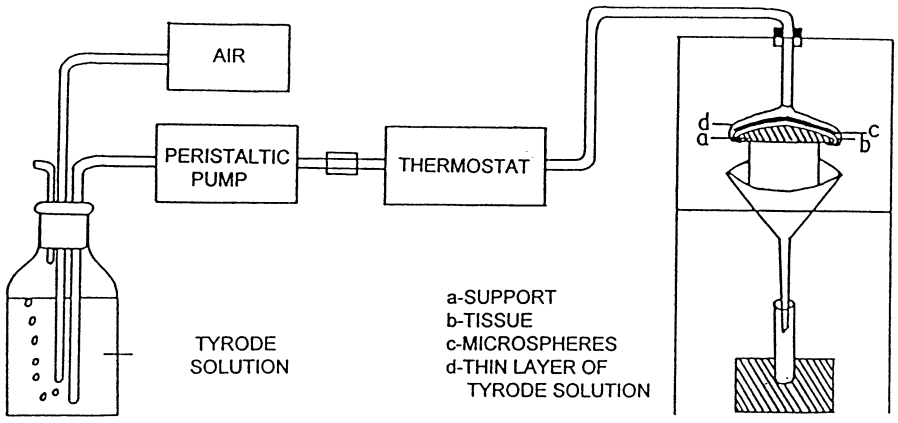

Fig. 1. The apparatus for the evaluation of the drug release from microspheres adhered on guinea pig urinary bladder or small intestine mucosa.

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 other polymers.

During the present study the microspheres were prepared and their mucoadhesive properties were evaluated on guinea pig urinary bladder mucosa. Pipemidic acid was used as a model drug, but its application in such systems could represent an alternative to the treatment of urinary bladder infections after systemic application. An in vitro method was developed to evaluate the drug release from microspheres after their adhesion on mucosa. The experiments were also performed on the mucosa of a small intestine to compare the obtained results with those on urinary bladder mucosa.

2. Materials and methods

2.1. Materials

Sodium carboxymethylcellulose (viscosity of 2% aqueous solution 2500 mPa·s) was produced by Hercules, VI, hydroxypropylcellulose (Klucel-LF, NF) by Aqualon, VI, Carbopol 934P by Goodrich, OH, vinylpyrrolidone/vinyl acetate copolymer (60/40, S-630) by GAF, Italy and Eudragit E100 and Eudragit RL100 by Röhm Pharma, Germany. Pipemidic acid and magnesium stearate were supplied by Lek, Ljubljana, Slovenia.

All substances used for the preparation of Tyrode solution were of analytical grade.

2.2. Preparation of polymeric films

The polymeric films were prepared by pouring the aqueous solution of polymer on the limited surface of glass plate (2 cm × 2 cm) and dried overnight at room temperature. Dry films weighed 10 mg, i.e. 2.5 mg/cm^2.

2.3. Preparation of microspheres

The microspheres were prepared by solvent evaporation method. Eudragit RL (ERL) and hydroxypropylcellulose (HPC) were used to form the matrix of microspheres and sodium salt of carboxymethylcellulose (CMCNa), Carbopol 934P (Carbopol), HPC, salt of Eudragit E with hydrochloric acid (EE.HCl) and vinylpyrrolidone/vinyl acetate copolymer (PVP/VA) as mucoadhesive polymers or non-mucoadhesive references. ERL or HPC was dissolved in acetone and the second polymer was added as a powder. The suspensions of magnesium stearate and pipemidic acid in acetone were prepared separately and added to the dispersion of polymers. The whole

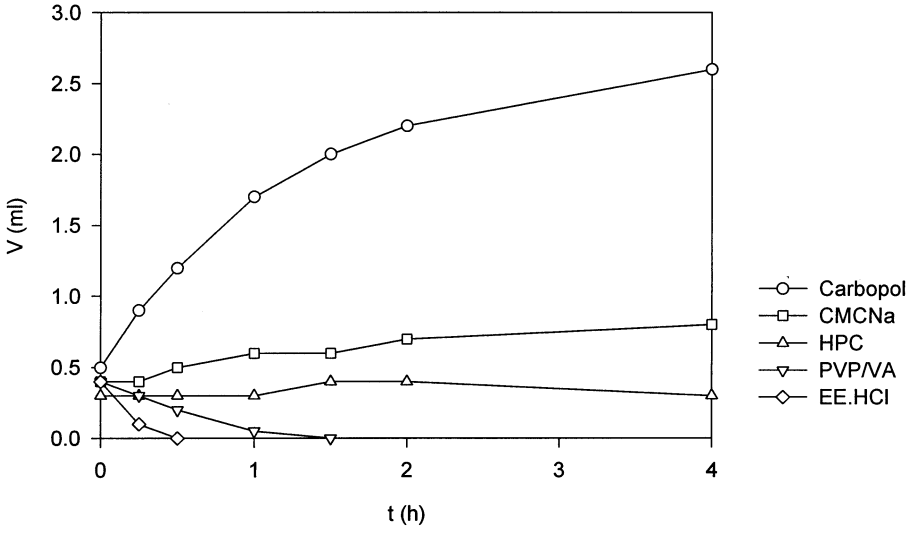

Fig. 2. The change of polymer powders volume in water due to the swelling or dissolution of polymers. V, volume of swollen or partly dissolved polymer.

volume of acetone was 23 ml. The homogeneous final dispersion was cooled to 5°C and poured slowly by stirring (750 rpm) into 80 ml of liquid paraffin, which was previously also cooled to 5°C. Acetone was removed by evaporation during stirring at 40°C. The microspheres were separated, washed with *n*-hexane and dried overnight at room temperature under reduced pressure. All samples of microspheres were sieved and fraction 200–250 μm was used for further testing.

For the preparation of microspheres the following quantities of substances were used: 1.5 g of polymers, 0.5 g of magnesium stearate and 0.5 g of pipemidic acid. The ratios of polymers in the samples are shown in Table 1.

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

Sample	Polymers	Ratio	Drug content (%)
A	HPC:CMCNa	1:3	18.4 ± 0.5
B	HPC:Carbopol	1:3	20.0 ± 1.0
C	HPC		16.6 ± 1.8
D	HPC:EE.HCl	1:3	24.3 ± 2.0
E	HPC:PVP/VA	1:3	13.3 ± 2.1
F	ERL:CMCNa	1:3	25.2 ± 2.7
G	ERL:Carbopol	1:3	21.4 ± 0.6
H	ERL:HPC	1:3	25.2 ± 3.0

2.4. Drug content determination

Microspheres (10 mg) were dispersed in 50 ml of methanol or 0.1 M NaOH in the case of Carbopol microspheres and stirred for 1 h. The dispersion was filtered and the pipemidic acid concentration was determined spectrophotometrically.

2.5. Dissolution studies

The apparatus was the same as described in USP XXIII under apparatus 2, i.e. apparatus with paddle stirring element (Farmatester 31, Tehtnica, Železniki, Slovenia). The following are the conditions the tests were carried out under: 200 mg of microspheres were dispersed in 1 l of Tyrode solution and stirred with 100 rpm at 37°C. The samples were filtered and the amount of released pipemidic acid was determined by measuring absorbances at 328 nm.

2.6. Swelling studies

Polymer powder (100 mg) was put into the graduated glass cylinder and 10 ml of distilled water were added. The volumes of hydrated polymer were measured at fixed time intervals.

Table 2
The weights of wet and dry mucus and water content on the surface of 1 cm^2 of mucosa

Animal	Urinary bladder			Small intestine		
	Wet mucus (mg)	Dry mucus (mg)	Water (mg)	Wet mucus (mg)	Dry mucus (mg)	Water (mg)
a	1.4	0.45	0.95	30.0	4.1	25.9
b	3.8	0.45	3.35	48.7	4.2	44.5

2.7. Determination of the amounts of mucus and water on guinea pig urinary bladder and small intestine mucosae

The mucosa was attached on a rubber support and the mucus was scraped gently off. The wet mucus was weighed, dried at reduced pressure and room temperature and the weight of the dry mucus was determined. The water content was also calculated.

2.8. Measurement of detachment force

The detachment force between polymeric film and animal mucosa was measured by the modified precision balance. The polymeric film was prepared on the glass plate, suitable amount of Tyrode solution was dispersed over its surface, left for 2 h in a humid environment to hydrate and then the glass plate was mounted to the upper clamp of the apparatus. The upper part of small intestine (jejunum) or urinary bladder of male guinea pigs (300–400 g) was used. The guinea pigs were sacrificed, small intestine and urinary bladder were removed and kept in aerated Tyrode solution at room temperature until usage. All experiments were performed within the period of 5 h. The 3-cm long segments of the small intestine or whole urinary bladder were washed by Tyrode solution and cut longitudinally. The tissue was mounted to the lower support of the apparatus, then the clamp with the tissue was slowly raised and the contact with the hydrated film was formed. The detachment force needed for the separation of the two surfaces was determined 2 min after the formation of the contact. Results are means of five to seven experiments.

2.9. Determination of pipemidic acid release from adhered microspheres

The in vitro method similar to that described in literature (Rao and Buri, 1989) was developed to evaluate the release rate of pipemidic acid from adhered microspheres. The guinea pigs' small intestine and urinary bladder were isolated as described above and used as follows. At the beginning of the experiment 2 cm segments of the small intestine were washed by Tyrode solution and cut longitudinally. The urinary bladder was also washed by Tyrode solution and cut on the front side longitudinally. The prepared tissue was attached to a rubber support. The microspheres were placed on the mucosa and wet by spraying the mucosa by the Tyrode solution. After 2 min hydration of the microspheres, the support was inserted into the apparatus and the mucosa was washed by Tyrode solution at 37°C. The washings were collected and the concentrations of released pipemidic acid were spectrophotometrically determined by measuring the minimum of the second derivative of the UV spectrum at 270 nm. Results are means of duplicate or triplicate experiments.

The apparatus for the determination of pipemidic acid release from adhered microspheres is shown schematically in Fig. 1.

3. Results and discussion

The microspheres were prepared by a solvent evaporation method, which was developed earlier (Bogataj et al., 1991). The different polymers, of which the microspheres were composed, were chosen with regard to their structure and mucoadhesive properties. Since most of mucoadhesive

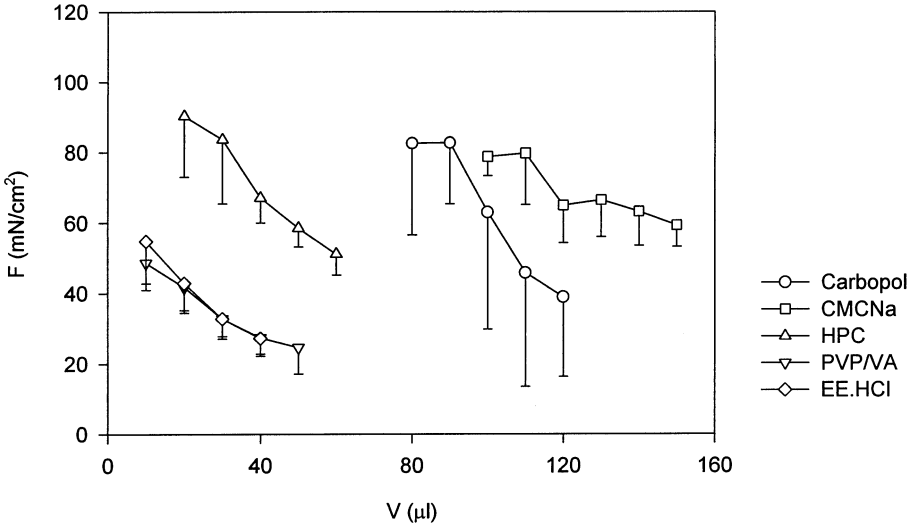

Fig. 3. Detachment force (F) as a function of increasing volume of Tyrode solution (V) used for the hydration of different polymeric films which weighed 2.5 mg/cm^2. The detachment forces were determined on guinea pig small intestine mucosa.

polymers are very hydrophilic and are not soluble in acetone (CMCNa, Carbopol), another acetone-soluble polymer was used to form the matrix of microspheres and connected all other components. For that reason ERL and HPC were chosen as matrix polymers and CMCNa, Carbopol, HPC, EE.HCl and PVP/VA as potential mucoadhesives or as reference polymers, which were not expected to produce mucoadhesion.

Pipemidic acid content in microspheres was determined and is shown in Table 1. The obtained values are influenced greatly by the polymers used for the microspheres preparation. These differences are probably due to different losses of different bioadhesive polymers and the drug during the preparation procedure.

The drug dissolution, swelling of polymers and mucoadhesion strength were evaluated in order to study their influence on the release of pipemidic acid from microspheres adhered on mucosa.

The results of dissolution studies indicate that the dissolution of pipemidic acid from all microspheres containing HPC is very fast, whereas the release from ERL microspheres is retarded markedly. This difference was expected and is in accordance with the physical properties and the solubilities of polymers under the experimental conditions.

Results of polymer powders swelling are shown in Fig. 2.

It can be seen that Carbopol swells to the largest volume. The swelling is observed also at CMCNa, whereas in the case of HPC only a small enlargement of volume is noticed due to its good water solubility. PVP/VA and EE.HCl are also very soluble in water and they dissolve completely in a very short time.

We also determined the amount of wet and dry mucus on both mucosae and the results are shown in Table 2. The obtained results are in accordance with the literature data where a very thin layer of mucus on human vesical mucosa was reported (Cornish et al., 1990).

In the course of the present study the mucoadhesion strength of polymeric films on guinea pig intestinal mucosa was evaluated by the determination of detachment forces. Films were composed of only one polymer, either bioadhesive one or non-bioadhesive reference to estimate separately the contribution of each polymer to the bioadhesion strength of microspheres. Different volumes of Tyrode solution were used as the different polymeric films need different amounts of water to hydrate. The experiments were not feasible when volumes of Tyrode solution used for hydra-

Table 3
Mean values and standard deviations of detachment forces measured on guinea pig vesical and intestinal mucosae

Polymer	V^a (μl)	F^b (mN/cm^2), bladder	F^b (mN/cm^2), intestine
CMCNa	120	77.89 ± 21.36	79.68 ± 14.60
Carbopol	100	45.36 ± 10.42	72.21 ± 15.45
HPC	40	73.91 ± 6.50	77.97 ± 25.39
PVP/VA	20	45.60 ± 7.80	40.16 ± 6.31
Eudragit E.HCl	30	52.87 ± 25.23	37.53 ± 6.46

[a] V, volume of Tyrode solution used for the hydration of the polymeric film.
[b] F, detachment force.

tion were smaller or larger than the limit values of intervals shown in Fig. 3.

The results of detachment forces measurements on small intestine mucosa show that the increasing volume of Tyrode solution decreases the detachment force. This phenomenon is well known, seeing that detachment forces are, besides weak chemical bonds, also a consequence of capillary attractions at low volumes of liquid (Lehr et al., 1992) and are lowered due to increasing thickness of water layer between mucus and polymer at high volumes (Chen and Cyr, 1970).

The curves in Fig. 3 can be extrapolated to the same volume to compare mucoadhesion strength of different polymers. Thus, our results show that mucoadhesive bond strength decreases in the following order: CMCNa > Carbopol 934P > HPC > EE.HCl = PVP/VA. These relations were also statistically proved (t-test, $p < 0.05$) for the volumes where the strength was experimentally determined for the pairs of polymers. The obtained succession of polymers was expected and is in accordance with literature data for polymers that are described in literature as mucoadhesives (Smart et al., 1984).

The detachment forces on urinary bladder mucosa were determined by using only one volume of Tyrode solution for hydration of polymeric films. The comparison of detachment forces determined on vesical and intestinal mucosa using the same volume of Tyrode solution is shown in Table 3. The data were evaluated statistically and significantly lower detachment force on urinary bladder than on intestinal mucosa was determined for Carbopol films. No differences between the two mucosae were found for other polymers.

The process which is important for the efficiency of a drug delivery system intended for the urinary bladder application is the drug release after the adhesion on mucosa. The drug is released in the lumen of the bladder and may also diffuse in the mucosa tissue. In this study only the drug release from adhered microspheres in the lumen of the urinary bladder was followed and the parameters which influence this process were defined. The results obtained by this method on the guinea pig vesical in comparison with intestinal mucosa are shown in Fig. 4.

Fig. 4 shows that the highest release rate was obtained by microspheres containing PVP/VA or EE.HCl in HPC matrix which is due to the structure and very fast dissolution of these two polymers under the experimental conditions. The retardation effect was slightly stronger when HPC was used as the only polymer in microspheres. The strongest effect within the series with HPC matrix was achieved by the use of CMCNa.

From the dissolution experiments, performed according to USP, stronger retardation properties of ERL than HPC were observed and similar influence on release from adhered microspheres was expected. From Fig. 4 it can be seen that in the case of CMCNa microspheres, ERL retards the release more efficiently than HPC, but the situation is just the opposite in the case of Carbopol microspheres. It is obvious that in the case of Carbopol microspheres another influence prevails over the retardation properties of microspheres matrix.

As seen from Fig. 2 Carbopol has a much higher swelling capacity than all other polymers. In Carbopol/HPC microspheres, HPC swells and

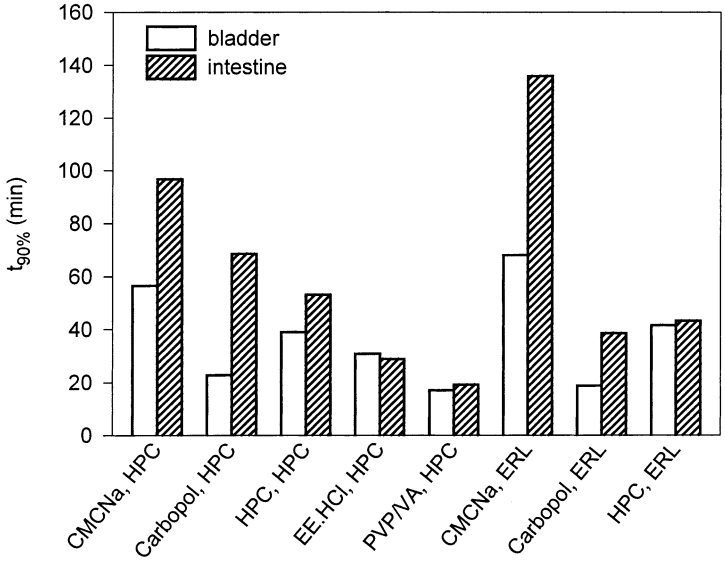

Fig. 4. The values of $t90\%$ release of pipemidic acid from adhered microspheres as a function of polymer composition for urinary bladder and small intestine mucosa.

partly dissolves. Thus it enables Carbopol to swell to its maximum degree and interact with mucus. HPC itself also has mucoadhesive properties which contribute to the strength of mucoadhesive bonds. It is possible that in Carbopol/ERL microspheres, ERL prevents swelling of Carbopol to such a degree that interactions of Carbopol with mucus are hindered and consequently, a lower mucoadhesion and a higher dissolution rate are observed. Therefore, the polymer which retards the pipemidic acid release from microspheres, does not necessarily have the same influence when microspheres are adhered on mucosa.

The influence of polymer mucoadhesion strength on release rate was also studied. The times when 90% of the drug is released from microspheres which are adhered on intestinal mucosa and contain the same matrix polymer were compared with detachment forces of polymeric films. The following results were obtained: detachment forces: CMCNa > Carbopol 934P > HPC > EE.HCl = PVP/VA (Fig. 3); $t90\%$ for HPC microspheres: CMCNa > Carbopol 934P > HPC > EE.HCl > PVP/VA; $t90\%$ for ERL microspheres: CMCNa > Carbopol 934P = HPC.

The results of both methods are similar and allow a resolution that the influence of polymers mucoadhesive properties on the drug release from adhered microspheres is decisive.

Finally, the results obtained on small intestine mucosa were compared with those on vesical mucosa. The ratios of the times of 50 and 90% of the drug release were calculated separately for both HPC and ERL microspheres and as the results did not differ significantly they are presented as average values in Table 4. To make a correlation, the ratios of the detachment forces are also shown.

Table 4
The ratios of pipemidic acid release times from HPC and ERL microspheres adhered on urinary bladder and small intestine mucosa and ratios of detachment forces between two mucosae

Polymer	$t_{bladder}/t_{intestine}$	$F_{bladder}/F_{intestine}$
CMCNa	0.55 S[a]	1.00
Carbopol	0.52 S[a]	0.64 S[a]
HPC	0.88	0.94
PVP/VA	0.85	1.17
EudragitE.HCl	1.02	1.42

[a] S, difference in release times (detachment forces) between two mucosae is significant.

It is evident from Table 4 that the ratio of release times has the lowest value for negatively charged polymers and the highest for cationic Eudragit E.HCl. The same situation can be observed for detachment forces ratios with the exception of CMCNa. However, the significant differences between the two mucosae were proved in three cases for negatively charged polymers. The reason lies in the amount, composition and/or the structure of the vesical and intestinal mucus. Lower amount of mucus on vesical mucosa (Table 2) might be the reason for formation of weaker mucoadhesive bonds. Due to the higher amount of mucus and water on intestinal mucosa a higher amount of water from mucus is absorbed by the polymer and more intimate contact with mucus chains is formed. It is expected that this influence will be the most evident for polymers which need the highest amount of water for swelling, i.e. Carbopol and to a lower degree also CMCNa.

The difference between the two mucosae was observed with polymers carrying negative charge. There are no exact data in literature on the charge of intestinal and vesical mucus, but on the basis of their structures, described in literature, a greater negative charge of vesical mucus in comparison with intestinal cannot be excluded. If this difference exists, it may help to explain lower mucoadhesion strength of Carbopol films on vesical than intestinal mucosa (Table 3) and also a higher release rate from CMCNa and Carbopol microspheres adhered on vesical mucosa (Fig. 4) due to the negative charge of these two polymers. The same influence can also be observed in Table 4 where the values of bladder/intestine ratios increase within the series of studied polymers.

4. Conclusions

In the present study the drug release from microspheres adhered on guinea pig vesical and intestinal mucosa was evaluated and on the basis of obtained results the following conclusions can be made:

- the release from adhered microspheres is influenced by at least four parameters: type of mucosa, mucoadhesion strength and swelling of polymers, and retardation properties of microspheres;
- differences in mucoadhesion strength and in drug release after adhesion of microspheres on vesical and intestinal mucosa, which were observed for Carbopol films, and Carbopol and CMCNa microspheres are due to the amount of mucus on mucosa and might also be influenced by the charge of mucus;
- CMCNa/Eudragit RL microspheres will be used in further experiments to achieve the final goal of our work because they provide the longest release time and sufficient high strength of mucoadhesion;
- in addition, positively charged polymer chitosan hydrochloride will be tested to confirm the influence of polymer charge on adhesion on vesical mucosa.

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