In Vitro Evaluation of Mucoadhesive Vaginal Tablets of Antifungal Drugs Prepared with Thiolated Polymer and Development of a New Dissolution Technique for Vaginal Formulations

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The main objective of this work was to develop antifungal matrix tablet for vaginal applications using mucoadhesive thiolated polymer. Econazole nitrate (EN) and miconazole nitrate (MN) were used as antifungal drugs to prepare the vaginal tablet formulations. Thiolated poly(acrylic acid)–cysteine (PAA-Cys) conjugate was synthesized by the covalent attachment of cysteine to PAA with the formation of amide bonds between the primary amino group of cysteine and the carboxylic acid group of the polymer. Vaginal mucoadhesive matrix tablets were prepared by direct compression technique. The investigation focused on the influence of modified polymer on water uptake behavior, mucoadhesive property and release rate of drug. Thiolated polymer increased the water uptake ratio and mucoadhesive property of the formulations. A new simple dissolution technique was developed to simulate the vaginal environment for the evaluation of release behavior of vaginal tablets. In this technique, daily production amount and rate of the vaginal fluid was used without any rotational movement. The drug release was found to be slower from PAA-Cys compared to that from PAA formulations. The similarity study results confirmed that the difference in particle size of EN and MN did not affect their release profile. The release process was described by plotting the fraction released drug versus time and fitting data to the simple exponential model: \( M_t/M_s = k t^n \). The release kinetics were determined as Super Case II for all the formulations prepared with PAA or PAA-Cys. According to these results the mucoadhesive vaginal tablet formulations prepared with PAA-Cys represent good example for delivery systems which prolong the residence time of drugs at the vaginal mucosal surface.

Key words thiomer; mucoadhesion; tablet; candidiosis; econazole nitrate; miconazole nitrate

In recent studies, considerable attention has been paid on novel and controlled release systems to provide a long-term therapeutic concentration of drug following single administration. Many drug delivery systems are based on mucoadhesive polymers which are able to adhere to the mucosal tissue and swell rapidly in aqueous environmental conditions. Presumptive new generation of mucoadhesive polymers are thiolated polymers—designated as thiomers. In contrast to well-established mucoadhesive polymers, these novel polymers are capable of forming disulfide bonds with secreted mucus layer. They provide strong mucoadhesion and guarantee the localization of a drug delivery system at the application site. It is also known that due to the in situ cross-linking properties of thiomers by forming intra- and interchain disulfide bonds within the polymeric network the cohesiveness and subsequently the stability of the swollen carrier matrix can be guaranteed. Additionally, matrix tablets prepared with a thiolomer offer the advantage to control the drug release. Therefore, polyacrylic acid polymer–cysteine conjugate which is well-established and promising polymer, was synthesized to prepare the mucoadhesive vaginal tablet formulations of antifungal drugs.

Vulvovaginal candidiasis is the most frequent gynecologic diagnosis encountered by physicians and approximately 75% of women experience at least one episode during their lifetime. Candida albicans is recognized as the most frequent aetiologic agent of vulvavaginal candidiasis. For many years, imidazole derivatives have been used for the fungal infections caused by Candida species. Econazole nitrate (EN) and miconazole nitrate (MN) are commonly used imidazole compounds effective in the treatment of vaginal candidiosis.

The objective of this study was to design mucoadhesive vaginal tablet formulations with a thiolated polymer in order to provide a strongly prolonged residence time at the application site for the treatment of vaginal candidiosis and to evaluate their properties under in vitro conditions. In addition, a new apparatus for release studies from mucoadhesive vaginal tablets was designed, since to the best of our knowledge no existing drug release technique takes the amount of vaginal fluid and its turnover into account. Although certain methods are designed to mimic the general conditions encountered in the physiological environment of the vagina, large volume of simulated vaginal fluids was used with rotational movements in these methods. In order to mimic in vivo conditions for vaginal administration, the various parameters that must be considered when designing a dissolution apparatus include volume and composition of the dissolution medium, environmental conditions of the absorption site and surface exposure of the tablet. Taking these parameters into account, a new dissolution apparatus was designed that could simulate the vaginal environment for mucoadhesive vaginal tablets.

Experimental

Materials
EN and poly(acrylic acid) (PAA) (average molecular weight 450 kDa) were purchased from Sigma-Aldrich (Germany). MN was donated by Ilisan-Ittas Drug Company (Turkey). All other chemicals were of analytical grade.

Methods. Synthesis of PAA-Cys
The poly(acrylic acid)–cysteine con-
jugates (PAA–Cys) were synthesized according to a method described previously.11 Briefly, PAA was hydrated in demineralized water (1% solution) and the pH value of the solution was adjusted to 6 by the addition of 5 m NaOH. Then, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (EDAC, Sigma) was added to activate the carboxylic acid moieties of the hydrated polymers. After 20 min incubation under stirring at room temperature, L-cysteine (Sigma) was added and the pH was readjusted to 6. The resulting conjugates were isolated by dialysis in the dark at 10 °C. Polymers were dialyzed two times against 0.1 M NaCl and then two times against 0.2 M HCl. After dialysis the polymer solution was freeze-dried at −30 °C and 0.01 mbar (Benchtop 2K, VirTis, NY, U.S.A.) and stored at 4 °C until further use.

**Determination of the Thiol Group Content** The amount of free thiol groups immobilized on polymer backbones was determined photometrically with Ellman’s reagent [DTNB, 5,5'-dithiobis(2-nitrobenzoic acid)]. In addition, the oxidized thiol moieties available in form of disulfide bonds were quantified after reduction with NaBH₄ and addition of Ellman’s reagent as described previously.9

**Particle Size Determination of the Active Substances** Particle size of EN and MN was determined using a laser diffraction particle size analyzer (FRITSCH Analysette22 Particle Sizer, Idar-Oberstein, Germany). The size of the particles was determined in water as a non-dissolving medium. The particle suspensions were prepared with an ultrasonic stick (DR. HIELSCHER GmbH, UP200H) prior to analysis for 1 min. In the measurement cell, propeller mixer (dispersion equipment, Fritsch GmbH, Idar Oberstein) was used to facilitate the continuous flux of particles. For calculations of particle size determinations the Fraunhofer model was used.

**Determination of Minimum Inhibitory Concentration and Minimum Fungicidal Concentration** Minimum inhibitory concentration (MIC, μg·ml⁻¹) and minimum fungicidal concentration (MFC, μg·ml⁻¹) were determined by the reference broth microdilution (BMD) method based on the CLSI (Clinical and Laboratory Standards Institute) document M27A210 against the following strains: Candida albicans ATCC 10231, Candida albicans ATCC 9028 strains and one clinical strain which was isolated from infected women complaining vaginal candidiasis. Prior to initiation of the study, these strains were subcultured on Sabouraud dextrose agar at 35 °C for 24 h to ensure viability and purity.

Inoculum was prepared according to CLSI M27A2 and the final concentration of fungal suspension in RPMI 1640 medium buffered to pH 7.0 with 0.165 M MOPS, was 10⁵ colony forming unit (CFU)/ml. Stock solutions EN/MN were prepared in dimethyl sulfoxide (DMSO). Further dilutions of antifungal agents were prepared with twofold concentrated in the test medium. The final concentrations ranged from 0.03 to 16 μg/ml for EN/MN.

Each isolate was tested in duplicate. Plates were incubated at 35 °C for 24—48 h. MIC endpoints were visually defined as the lowest concentration that produces prominent (50%) decrease in turbidity compared with that of drug-free control wells.

The MFCs were determined by subcuturing 20 μl aliquots from each well with no visible growth onto SDA plates.

**Preparation of Tablets** For each tablet, 40 mg of the PAA-Cys conjugate and PAA were hydrated in demineralized water until homogenous gels were formed. After addition of 5 mg of EN or MN for each tablet, these gels were homogenized and lyophilized. The 45 mg of polymer—drug conjugates were compressed into 5.0 mm diameter flat faced tablets (Hanseaten Type V, Idar Oberstein) was used to facilitate the continuous flux of particles. For calculations of particle size determinations the Fraunhofer model was used.

**Evaluation of the Water Uptake Capacity** The water uptake capacity of the tablets was determined by a gravimetric method as described previously.12 Tablets were fixed to a needle and immersed in a beaker containing simulated vaginal fluid at 37 °C. At scheduled time intervals, the swollen tablets were taken out of the incubation medium, excess water was removed, and the amount of water uptake was determined gravimetrically. The water uptake ratio was then calculated according to the following equation:\(^{(1)}\)

\[
\text{water uptake ratio} = \frac{W_{t}}{W_0}
\]

where \(W_t\) is the weight of uptaken water at time \(t\) and \(W_0\) is the initial weight of the dry tablet.

**Evaluation of the Mucoadhesive Strength of Formulations** The mucoadhesive strength of the formulations under investigation was evaluated by measuring the force required to detach the formulation from a mucin disc using a TA-XTplus Texture Analyzer (Stable Micro Systems) in tension mode. Mucin discs were prepared by compression of a known weight of crude porcine mucin (300 mg) using a tablet machine with a 10-mm diameter die. Tablets were then horizontally attached to the lower end of the cylindrical probe (length 4 cm, diameter 1 cm) using double-sided adhesive tape. Prior to mucoadhesion testing, the mucin disc was hydrated by submersion in a 5% solution of mucin for 30 s. Excess surface liquid was removed by gentle blotting. At room temperature, mucin disc was placed under the analytical probe which was then lowered until the tablet was in contact with the surface of the mucin disc. Without delay, a downward force of 2 N was applied for a predefined time (180 s) to ensure intimate contact between the mucin disc and the tablet sample. The probe was then moved upwards at a constant speed of 1.0 mm·s⁻¹ and the force required to detach the mucin disc from the surface of each tablet was determined from the resulting force—distance plot. The area under the curve (AUC) was calculated from force—distance plot as the mucoadhesion. In addition, the equation given below was used to calculate the work of mucoadhesion (N·mm² cm⁻³).

\[
\text{work of mucoadhesion} = \frac{AUC}{\pi r^2}
\]

where, \(\pi r^2\) is the surface of the tablet being in contact with mucin disc.

**Ex Vivo Mucoadhesion Studies via Rotating Cylinder** Time of adhesion of compressed discs to the bovine vaginal mucosa was established by the rotating cylinder as follows. Tablets were attached to freshly excised mucosa which was provided by the local slaughterhouse and fixed on a stainless-steel cylinder (diameter 4.4 cm, height 5.1 cm; apparatus 4-cylinder, USP XXX) using a cyanoacrylate adhesive. Thereafter, the cylinder was placed into the dissolution apparatus according to USP and completely immersed into the simulated vaginal fluid at 37 °C. The cylinder was rotated at a speed of 100 rpm. Every 30 min the changes in the test system were observed visually and registered until all of the discs were either disintegrated or detached from the mucosa. Mucoadhesive times of the formulation were determined performing at least five times for each tablet.12

**Release Studies** A new simple technique which mimics the vaginal environment was developed to investigate the release behavior of the vaginal tablet formulations. The apparatus mainly consists of a perfusor and syringe which were connected with a thin latex connector and a sample collection vessel as it was illustrated in Fig. 1. The vaginal physiology was simulated with a syringe that has the inner diameter of 20 mm and total length of 75 mm. Tablets were placed at the bottom of the syringe without needle and the assembly dipped into a water bath at 37 ± 0.5 °C. The daily production of vaginal fluid is approximately 6 ml/d and 0.5—0.75 ml continually present in the vagina.13 Therefore, a perfusor (Perfusor Fm, Braun) was connected with the upper site of the syringe to give 6 ml of vaginal fluid to tablets in 24 h. The same amount of samples was collected concurrently from the bot-
tom. The collected samples were diluted with methanol (1:1) to increase the solubility of EN or MN.

The samples were assayed for EN or MN content by HPLC using a Prontosil 120-5 C18 column (5.0 μm, 250×4.6 mm), LaChrom Elite L2130 pump and L2200 autosampler with a L2450 diode array detector (VWR, Vienna, Austria). For all peak reports the EZChrom software (VWR, Vienna, Austria) was applied. 230 nm was chosen as detection wavelength. A flow rate of 0.5 ml/min was maintained, using solvents A (0.5% acetic acid solution in water) and B (0.5% acetic acid solution in methanol). The following gradient was used: 0—11 min (80—20% A), 11—12 min (20—0% A), 12—18 min (0% A), 18—19 min (0—80% A) and 19—30 min (80% A). Analysis of Release Data The mechanism of drug release from the prepared formulations was analysed by using Korsmeyer–Peppas model. Polymer water uptake, gel layer formation and polymeric chain relaxation are currently regarded as primilary involved in the modulation of drug release process. Korsmeyer et al. described drug release from a polymeric system using Eq. 3. To find out the mechanism of drug release, first 60% drug release data was fitted in Korsmeyer–Peppas model.\(^{16,17}\)

\[
\frac{M_t}{M_\infty} = k_t^n
\]

Where \(M_t/M_\infty\) is fraction of drug released at time \(t\), \(k\) is the rate constant and \(n\) is the release exponent. The \(n\) value is used to characterize different release mechanisms. \(n=0.45\) indicates Fickian release, and \(0.45 < n < 0.89\) indicates anomalous behaviour (non-Fickian kinetics corresponding to coupled diffusion/polymer relaxation). Values of \(n=0.89\) for release from cylinders which has been regarded Super Case II kinetics which has been observed occasionally.\(^{18}\)

Similarity Factor \((f_2)\) Analysis The similarity factor between the PAA-Cys and PAA tablets was determined using the data obtained from the drug release studies.\(^{19}\) The data was analyzed by the formula shown in Eq. 4.

\[
=50 \log \left[1 + \left(1/N \right) \sum \left(R_i - T_i \right)^2 \right]^{-0.5} \times 100
\]

\(N\) is the number of time points, \(R_i\) and \(T_i\) are dissolution of reference and test products at time \(i\), respectively. If \(f_2\) is greater than 50 it is considered that 2 products share similar drug release behaviors.

Results and Discussion

Determination of the Thiol Group Content The amount of thiol content of the thiomer has a great impact. First of all, the formation of the interpolymer disulfide bonds leads to decreased flexibility of the polymeric chains and consequently improved cohesive properties. In addition, mucoadhesive properties can be improved due to the interaction between the thiol groups of the polymer and cysteine-rich subdomains of mucus glycoproteins.\(^{19}\) Therefore, the amount of free thiol groups and oxidized thiol groups in the form of disulfide bonds were determined and listed in Table 1. The total amount of sulfhydryl groups immobilized on polymer is represented by the summation of free thiol groups and oxidized thiol groups available in form of disulfide bonds.

Particle Size Determination of the Active Substances One of the formulation variables which influences the drug release rate from the swellable matrix tablets is the particle size of the drug. It is expected that the small particle size of drug will dissolve more rapidly due to their larger surface area.\(^{20}\) Ford et al. demonstrated that the particle size is in particular of importance for the dissolution of almost insoluble drugs.\(^{21}\) Hence, in the present study, the mean size of EN and MN, sparingly soluble drugs, was determined and found to be 34.245±5.852 μm and 19.327±4.396 μm, respectively. The size distribution of these drugs is shown in Fig. 2.

<table>
<thead>
<tr>
<th>C. albicans ATCC 10231 (μg/ml)</th>
<th>C. albicans ATCC 90028 (μg/ml)</th>
<th>Clinical C. albicans (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;EN&lt;/sub&gt;</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;MN&lt;/sub&gt;</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>MFC&lt;sub&gt;EN&lt;/sub&gt;</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>MFC&lt;sub&gt;MN&lt;/sub&gt;</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 1. The Amount of Thiol Groups and Disulfide Bonds Immobilized on PAA

<table>
<thead>
<tr>
<th></th>
<th>–SH (μmol/g polymer±S.D.)</th>
<th>–S–S (μmol/g polymer±S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA-Cys</td>
<td>172.80±30.33</td>
<td>377.93±85.10</td>
</tr>
</tbody>
</table>
were found to be 2.677 ± 0.081, 2.698 ± 0.088, 2.721 ± 0.0870 and 2.723 ± 0.055, respectively. The hardness of the formulations was found higher than 16 kg/mm² likely due to the typical shape of vaginal tablets which exhibit diameter/thickness ratio <4.

**In Vitro Disintegration Studies** Tablet formulations showed pronounced difference in disintegration times in spite of the similar hardness values. As shown in Fig. 3, EN and MN tablets prepared with PAA showed a complete disintegration within 5.40 ± 1.14 h and 5.16 ± 0.87 h in simulated vaginal fluid, respectively. On the other hand, the disintegration time of PAA-Cys tablets was found to be more than 120 h and it was 24-fold compared to PAA.

The reason of the long disintegration time of PAA-Cys combinations was the presence of disulfide bonds which were formed within the thiomier itself. Crosslinking of polymeric chains as a result of formation of inter and intramolecular disulfide bonds results in high cohesive property due to great stability of the tightened polymeric network and cohesive property. Cohesion of the delivery system over the intended period of drug liberation is also an important requirement for achieving controlled release. Besides, high cohesive property of the polymeric carrier matrix is advantageous to minimize irritating vaginal outflow of eroded fragments.

**Evaluation of the Water Uptake Behavior** The water uptake behavior of a mucoadhesive formulation is a main parameter influencing both its adhesive and cohesive properties and drug release.

Mucoadhesive polymers are supposed to take water from the underlying mucosal tissue by absorbing, swelling, and capillary effects, leading to a considerably stronger adhesion. To improve the hydration ability of a formulation, a prolonged adhesion is required and strategies such as crosslinking and introduction of hydrophobic entities are applied. Thiomiers are appropriate polymers to prepare mucoadhesive and controlled release drug delivery systems preventing inopportune tablet disintegration.

The water uptake of the formulations consisting of PAA-Cys conjugate or PAA is shown in Fig. 4. Tablets prepared with PAA-Cys conjugate started to uptake water immediately after being immersed in simulated vaginal fluid and continued to uptake water at approximately constant rate. The covalent attachment of cysteine had significant influence on the water uptake behavior of PAA. The weight of EN+PAA-Cys and MN+PAA-Cys tablets increased continuously over 24 h with the highest water uptake ratios of 7.53 ± 0.28 and 8.19 ± 0.64, respectively. On the contrary, EN+PAA and MN+PAA tablets reached the highest water uptake ratio within 2 h and showed just a 2.38 ± 0.68 and 1.99 ± 0.43-fold weight gain, respectively. EN+PAA and MN+PAA tablets progressively dissolved within 7 h whereas the swollen thiomier tablets retained their integrity for 24 h.

**Evaluation of the Mucoadhesive Strength of Formulations** Defining the mucoadhesive characteristics is of great importance to prolong residence time and to prevent a decrease the detachment of the tablet formulation from the application site with the mucosal secretion are required. Therefore, it is important to quantify the interaction between the dosage form and the mucosal surface in the development of mucoadhesive dosage forms. Mucoadhesive property was evaluated by means of a tensile test. This was used to find the measurements of maximum detachment force, mucoadhesion and work of adhesion required to detach mucin disc from tablet formulations. The highest work of adhesion was determined with thiomier formulations as it is presented in Table 4. It is known that, work of adhesion, provides wider evaluation of the detachment phenomena, representing the sum of all established bonds, not only the maximum force of detachment which corresponds to F evaluation. Hence, it was thought that work of adhesion is a more valid parameter to evaluate the mucoadhesive property. The mucoadhesive properties of the tablet formulations are shown in Table 4.

**Table 3. Physical Properties of Tablets**

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Weight (mg)</th>
<th>Friability (%)</th>
<th>Hardness (kg/mm²)</th>
<th>Diameter (mm)</th>
<th>Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN+PAA</td>
<td>47.420±1.581</td>
<td>0.525</td>
<td>&gt;16</td>
<td>4.952±0.017</td>
<td>1.837±0.059</td>
</tr>
<tr>
<td>EN+PAA-Cys</td>
<td>46.850±2.076</td>
<td>0.426</td>
<td>&gt;16</td>
<td>4.987±0.008</td>
<td>1.834±0.0533</td>
</tr>
<tr>
<td>MN+PAA-Cys</td>
<td>47.010±1.701</td>
<td>0.489</td>
<td>&gt;16</td>
<td>4.961±0.025</td>
<td>1.822±0.040</td>
</tr>
</tbody>
</table>

Fig. 3. Disintegration Time of Tablets in Simulated Vaginal Fluid at 37 °C

The black bars indicate the disintegration of PAA-Cys tablets and the white bars indicate PAA tablets (indicated values are the means of three experiments ± S.D.).

Fig. 4. Water Uptake Behavior of EN+PAA Tablets (△), EN+PAA-Cys Tablets (●), MN+PAA Tablets (○) and MN+PAA-Cys Tablets (▲) in Simulated Vaginal Fluid at 37 °C.

Indicated values are the means of three experiments ± S.D.
Table 4. Results of Mucoadhesion Studies Performed with Texture Analyzer

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Force (N)</th>
<th>Mucoadhesion (N·mm)(^b)</th>
<th>Work (N·mm·cm(^{-2}))(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN+PAA-Cys</td>
<td>3.435±0.007</td>
<td>2.127±0.010</td>
<td>2.710±0.013</td>
</tr>
<tr>
<td>MN+PAA</td>
<td>3.436±0.011</td>
<td>0.935±0.007</td>
<td>1.191±0.009</td>
</tr>
<tr>
<td>EN+PAA-Cys</td>
<td>3.281±0.059</td>
<td>2.115±0.008</td>
<td>2.694±0.011</td>
</tr>
<tr>
<td>EN+PAA</td>
<td>3.245±0.086</td>
<td>0.915±0.007</td>
<td>1.166±0.0090</td>
</tr>
</tbody>
</table>

\(a\) Each value represents the mean (± standard deviation) of at least five replicates.
\(b\) Mucoadhesion and work of adhesion values of MN+PAA-Cys and MN+PAA differ from each other with \(p<0.05\) and mucoadhesion and work of adhesion values of EN+PAA-Cys and EN+PAA differ from each other with \(p<0.05\).

**Ex Vivo Mucoadhesion Studies via Rotating Cylinder**

Thiomers mimic the natural mechanism of secreted mucus glycoproteins, which are also covalently anchored in the mucus layer by the formation of disulfide bonds. The immobilization of thiol groups on polymer strongly improved the mucoadhesive properties. In case of anionic mucoadhesive polymers the PAA-Cys conjugate seems to be a good example for this observation. In this study, our formulations prepared with PAA-Cys showed longer mucoadhesion time than PAA tablets (Fig. 5). The mucoadhesion time of EN+PAA, EN+PAA-Cys, MN+PAA and MN+PAA-Cys tablets were 4.66±0.76, 20±3.46, 5.33±1.04 and 17.83±5.39 h, respectively. The mucoadhesion time of EN and MN tablets prepared with PAA-Cys were found to be 4.29 and 3.34 times longer than PAA tablets. This considerable improvement in the mucoadhesive properties of thiomers is based on the formation of disulfide bonds between thiol-bearing side chains of the polymer and cysteine-rich subdomains of mucus glycoprotein.

The water uptake behavior of the mucoadhesive formulation affects the adhesive property. The extent of hydration is a typical behavior for the thiomer formulations due to the hydration process of the PAA-Cys tablets. It was also thought that, the high water uptake ratio of PAA-Cys tablets which were around 8 (shown in Fig. 4) may also help to prolong the retaining time of the formulation in vagina due to the mechanical fixation in *in vivo* conditions.

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**In the consideration of whole indicators of ex vivo mucoadhesion behavior of the formulations, it was seen that the thiomer formulations displayed higher mucoadhesive property than the formulations consisting unmodified polymer.** These findings were in accordance with the previous studies. 

**Release Studies** Formulations based on the thiolated polymer decreased the release rate drastically and significant difference was determined between the formulations prepared with modified or unmodified polymers. The release from PAA-Cys tablets was found significantly slower than PAA matrix tablets as it can be seen from Fig. 6. A quick release within 2 h was observed and the release rate of both EN and MN reached 100% within 24 h with PAA tablets. EN and MN tablets prepared with PAA-Cys released nearly 50% of the active substance within 5 d with a practically constant rate (zero order).

The release amount at each sampling time was found significantly higher than MIC and MFC values of the drugs which was shown in Table 2 for all the formulations except the first 4 h of PAA-Cys formulations. *In vitro* susceptibility testing as described by the MIC and MFC may be useful when comparing the spectrum and potencies of various agents the species of fungi most likely encountered in selected clinical settings. However, *in vitro* susceptibility testing has not been validated and is not reliable in predicting clinical response in vaginitis. Nevermore, the higher released amounts from the formulation than the MIC values of the antifungal agents are required for the therapeutic efficacy of the formulation. Therefore, it was thought that the PAA-Cys formulations provided sufficient released amount of drug from the vaginal tablet for the antifungal efficacy.

The drug liberation from the tablet formulations was inhibited in one face of the tablet in this study. It has been reported that in the literature, the restriction could affect the drug release when one of the faces of the mucoadhesive layers is inhibited, as may occur when the tablet is adhered to the mucosa.

The relatively lower release rate of the formulation can be explained by the hydration process of the PAA-Cys tablets. It is a typical behavior for the thiomer formulations due to their crosslinking process providing a tightened three-dimensional polymeric network to cause a more controlled release.

The release rate from tablets could also be controlled as long as the cohesiveness of the swollen carrier matrix is
guaranteed, demonstrating the importance of mechanical stability of this type of dosage form.\(^{10}\) The overall drug release process is influenced not only by the drug but also by the physical and mechanical properties of the gel barrier around the tablet. As a result, within this study, a controlled release of the drug from the PAA-Cys tablet formulation was guaranteed and the matrix tablet prepared with a thiomier offer the advantage to control the drug release. Therefore, PAA-Cys could be useful tool in improving the patient compliance with the reduction of dosing frequency for the treatment of vaginal candidiosis. It was also observed that there was no difference of the release rate of EN or MN from the tablet formulations. In spite of different mean particle sizes of the drugs, the reason of the similar release profiles might be due to the physical characteristics of the active substances such as solubility in the polymer or in the release medium.

Additionally, a new dissolution apparatus was designed to mimic the in vivo conditions of the vaginal environment to study the release of vaginal mucoadhesive tablets. In the conventional methods for determining the release behavior of a vaginal formulation, the formulation is hung into a beaker containing dissolution fluid that is stirred with a paddle rotor. The drawbacks of these methods are that the volume of dissolution fluid is large (500—900 ml), there is no control of the mass–medium interface ratio and it cannot simulate the in vivo environment.\(^{15}\) The vaginal discharge is often minimal due to the vaginal candidiasis.\(^{30}\) This newly designed dissolution apparatus was found to be successful in minimizing the volume of the dissolution medium. It also eliminates the risk of exposure of the vaginal formulation to agitation and sampling devices according to the design of the apparatus. Although there is no description characterized the shape of the human vagina and the baseline dimensions are changed due to their age and height,\(^{37}\) the tube in which consists the tablet formulation mimics the shape and the dimensions of the vaginal cavity. In brief, the dissolution apparatus with its small volume of dissolution medium without agitation and similar shape which is used for our study may prove to be of value for carrying out routine quality control tests. However, the results need to be supported with in vivo studies for the in vitro–in vivo correlation.

### Analysis of Release Data

In our results, release rates from the formulations prepared with or without thiomier showed a good fit a Super Case II transport. The values of \(n\) were found as 1.792, 1.741, 1.197 and 1.008 for EN+PAA, MN+PAA, EN+PAA-Cys and MN+PAA-Cys tablets, respectively. Super Case II mechanism could result from an increased plasticization at the relaxing boundary (gel layer).\(^{17}\) In the gel layer, a reduction of attractive forces among the polymeric chains raise the plasticization process and the mobility of the macromolecules increases. Therefore, the chain mobility is decisive for drug transfer kinetic, so diffusion rate increases with increase in relaxation rate of polymeric chains.\(^{18}\)

### Similarity Factor (\(f_2\)) Analysis

The similarity factor analysis was used to compare dissolution profiles of the formulations. The similarity factor analysis between PAA-Cys tablets showed an \(f_2\) factor (\(f_2 = 64.246\)) greater than 50. This result confirms that the release of EN and MN tablets prepared with PAA-Cys were similar to each other.\(^{19}\) In addition, the similarity factor between PAA tablets was found to be 68.180. The similarity study results confirmed that the presence of EN or MN did not affect the release profile of the drug from the formulations. The release rate was significantly changed due to the presence of thiolated polymer in the formulation.

### Conclusion

Within this study, the vaginal mucoadhesive tablet formulations of EN and MN were prepared with PAA-Cys conjugate and compared with PAA tablets. The results demonstrated that PAA-Cys improved the mucoadhesive properties of the vaginal tablets. In addition, water uptake capacity and the disintegration time were increased and controlled drug release was achieved. The results of this study enable us to state that the vaginal tablet formulations of EN or MN prepared with PAA-Cys conjugate provide a good candidate for drug delivery systems which prolong the residence time of the drug at the mucosal surface of vagina and improve the patient compliance with the reduction of dosing frequency for the treatment of vaginal candidiosis.

In addition, a new dissolution apparatus which mimics in vivo vaginal conditions including vaginal discharge volume was designed for the release studies of vaginal mucoadhesive tablets. It has the advantages of minimization of dissolution fluid volume and elimination of exposure to agitation and sampling devices to mimic the vaginal conditions. Thus, it was also concluded that, the newly designed dissolution apparatus is very promising and can be adopted for routine quality control studies in the future.

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### References and Notes