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RESEARCH PAPER

Development of a Mucoadhesive Dosage Form for Vaginal Administration

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ABSTRACT

The antimycotic imidazole derivative clotrimazole is employed locally for the treatment of genitourinary tract mycotic infections and is formulated as creams, foams, tablets, gels, irrigations, or pessaries. In this study, a new dosage form was developed by including bioadhesive polymers (polycarbophyl, hydroxypropylmethylcellulose, and hyaluronic sodium salt) into pessaries made of semisynthetic solid triglycerides. These polymers hold the delivery systems in the vaginal tract for a few days without any toxic effects or important physiological modifications, prolonging the permanence of the drug on the vaginal mucosa. Technological controls (compatibility with differential scanning calorimetry [DSC] studies, particle size analysis, and liquefaction time test) and biopharmaceutics studies for the evaluation of the release of the drug from the dosage form and of the bioadhesive properties were carried out. Moreover, a new test for the evaluation of the permanence of the drug in a simulated application site was developed from a modification of the Satnikar and Fantelli method for the evaluation of the liquefaction time of rectal suppositories. This test simulates the physiological vaginal condition and verifies the efficiency of the polymers in prolonging the permanence of the dosage form in the location where it is applied. The technological controls demonstrated that the presence of the polymers did not have an influence on the characteristics of the pessaries. On the other hand, there was an improvement in adhesivity of the pessaries in the in vitro adhesion test and prolonging of the liquefaction time in the liquefaction time test in the presence of mucoadhesive polymers, which increased with increasing polymer

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concentration. The presence of the mucoadhesive had a large influence on the permanence of the drug in the simulated application site because it modified the distribution of the drug along the simulated application site. In conclusion, the developed new formulations showed good technological and adhesion properties and the capacity of hold the dosage form in the target site. Among the employed bioadhesive polymers, the best behavior in the performed test was by polycarbophyl at its maximum concentration.

INTRODUCTION

Genitourinary tract mycotic infections are commonly treated with antimycotic imidazole derivatives, such as clotrimazole, which are locally effective and present no major side effects (1). Present conventional delivery systems, such as creams, foams, tablets, gels, irrigations, or pessaries, are limited by residence time because they are removed in a relatively short period of time by the self-cleansing action of the vaginal tract (2,3). Moreover, multiple daily doses are often required, leading to systemic adverse effects and poor patient compliance. Delivery systems that prolong the permanence of the drug on the vaginal mucosa could improve therapeutic efficacy and patient compliance. Such a goal can be reached through the addition into conventional dosage forms of bioadhesive polymers that, interacting with the mucosa, hold the delivery systems in the vaginal tract for a few days without any toxic effects or important physiological modifications (4–7).

The purpose of this work was to develop new bioadhesive pessaries able to prolong the permanence of the drug in the application site. For the developed pessaries, we made technological controls (such as studies of compatibility, particle size analysis, and liquefaction time test) and biopharmaceutics studies for the evaluation of the release of the drug from the dosage form and of the bioadhesive properties (in vitro adhesion test).

Moreover, to verify the efficiency in prolonging the permanence of the drug in the location where the pessary is applied, we developed a new test resulting from a modification of the Setnikar and Fantelli method for the evaluation of the liquefaction time of rectal suppositories. This test simulates the physiological vaginal condition by means of a cellophane tube placed in a thermostated bath containing a simulated vaginal fluid; the pessary is introduced in the tube, and after a fixed period of time, the distribution of drug in the simulated application site, in the melted pessary inside the tube, and the drug lost outside the tube are determined.

In developing the pessaries, we used polycarbophyl (Noveon AA1), hydroxypropylmethylcellulose (HPMC) (Methocel K4MCR), and hyaluronic sodium salt as bioadhesive polymers at three different concentrations with clotrimazole as the model drug.

EXPERIMENTAL

Materials

The following materials were used: clotrimazole (Farchemia S.r.l.), polycarbophyl PCP (Noveon AA1®, B. F. Goodrich Chemical Italy S.r.l.), HPMC (Methocel K4MCR®, Eigenmann and Veronelli S.p.A.), hyaluronic acid sodium salt (NaHA) (Res Pharma S.r.l.), semisynthetic solid triglycerides (Suppocire BS 2X®, Gattefossè Italia S.r.l.), purified porcine gastric mucin type III (Sigma Chimica).

Compatibility Studies: Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was used to assess the compatibility of clotrimazole with each excipient and of the excipients with each other (8). The experiments were performed on the drug and on the excipients separately, on binary mixtures of the drug with each excipient, and on Suppocire with the bioadhesive polymers. The experiments were performed in a range of temperatures, from 100°C to 330°C, chosen on the basis of the melting point of clotrimazole (146°C); samples containing semisynthetic solid triglycerides were analyzed in a range of temperatures from 25°C to 330°C. A scan rate of 20.0°C/min was used.

Methods of Preparation of the Pessaries

The semisynthetic triglyceride was melted at 40°C–45°C; the mucoadhesive polymer and the clotrimazole were added to the melted mass. The product was homogenized with a turbine homogenizer at 4000 rpm for 5 min at a constant temperature of 40°C. Pessaries were then

Table 1

Composition of the Developed Clotrimazole 100-mg Pessaries

Formulation	Composition			
	Hyaluronic Sodium Salt (NaHA)	Hydroxylpropylmethyl-cellulose (HPMC)	Polycarbophyl (PCP)	Semisynthetic Triglyceride
Blank	—	—	—	2950 mg
NaHA 25	25 mg	—	—	2925 mg
NaHA 50	50 mg	—	—	2900 mg
NaHA 100	100 mg	—	—	2850 mg
HPMC 25	—	25 mg	—	2925 mg
HPMC 50	—	50 mg	—	2900 mg
HPMC 100	—	100 mg	—	2850 mg
PCP 25	—	—	25 mg	2925 mg
PCP 50	—	—	50 mg	2900 mg
PCP 100	—	—	100 mg	2850 mg

dosed into the polyvinylchloride (PVC) molds to obtain pessaries of 3.0 ± 0.1 g each. The compositions of the pessaries are reported in Table 1.

Particle Size Analysis

The particle size analysis was performed on the drug powder before use and on the drug powder suspended in the pessary. The drug powder was suspended in a drop of mineral oil on a slide and observed under a optic microscope at a magnification of 400×. A sample of the pessary was then collected on a slide, melted on a hot plate, and observed under the same optic microscope at 400×. The percentage of particles having a size less than 2.5 μm (including those between 2.5 and 5 μm and those larger than 5 μm) was calculated. The test was carried out five times.

Drug Assay

The determination of the quantity of clotrimazole was performed by high-performance liquid chromatography (HPLC) using a Waters (Alliance 2690 model) device equipped with a Waters Nova Pack C18 3.9 × 150 mm column. Elution was carried out at 25°C. $(NH_4)_2HPO_3$ 0.5 M buffer adjusted to pH 8 and methanol (25:75 v/v) was used as the mobile phase; the injecting volume was 20 μl. The flow rate was 1 ml/min, and detection was made at 260 nm. In this condition, the retention time of clotrimazole was 5.5 min.

Release Test

The pessaries were tested for dissolution in 900 ml of a 0.1 M KH_2PO_3 buffer at pH 4 using the USP 23 paddle dissolution method at 100 rpm and 37°C ± 1°C. Samples were withdrawn at intervals of 10, 20, 30, 40, 50, 60, 120, 240, 280, 300, 360, 420, and 480 min; they were filtered and assayed for clotrimazole.

The release kinetic data (up to 60% release) were treated by the following equation:

$$\ln M_t/M_\infty = \ln K + n \ln t$$

where M_t/M_∞ is the fraction of drug released at time t (in hours), n is the diffusional exponent, and K is the constant apparent release rate ($\%min^{-1}$) (9). Three experiments were carried out for each formulation.

In Vitro Adhesion Tests

The mucoadhesive properties of the formulations were assessed by means of a tensile stress tester (10). The apparatus employed is described in Fig. 1. Of each formulation, 300 mg were melted at 40°C into the Plexiglas® cylindrical support (F), which had a cavity 6 mm deep to contain the sample. Of pH 4.9 NaH_2PO_4/NaOH buffer, 150 μl thermostated at the same temperature (40°C) were added to the sample to allow polymer hydration.

A Plexiglas plate (E) was fixed, face to the sample, on the holder (D). The carriage (B) was then moved until contact between the sample and the Plexiglas plate was reached. Afterward, a preload of 3000 mN was applied.

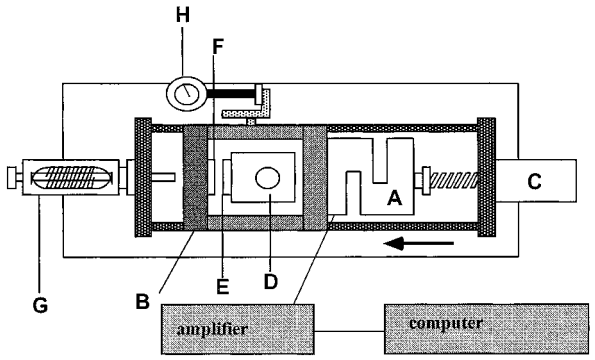

Figure 1. Schematic drawing of the tensile stress tester: A, load cell; B, movable carriage; C, motor; D, fixed holder; E, Plexiglas plate; F, Plexiglas container; G, preload device; H, micrometric device.

The preload value was chosen as the minimum force capable of ensuring the highest and most reproducible results. After a 3-min rest, the preload was removed, and the movable carriage was moved forward at a constant speed of 2.5 mm/min up to the complete separation of the two surfaces. Force of detachment values were recorded every 0.2 s. Eight replicates were performed on each sample. A validation was carried out before each pessary was tested. Force-versus-time curves were subsequently analyzed to evaluate the maximum force of detachment and to verify the reproducibility of the measurements.

Liquefaction Time Test

The liquefaction time test measures the liquefaction time of the pessaries according to the method of Setnikar and Fantelli (11). A cellophane tube was used in a 50-cm condenser with circulating water at 37°C. There were 10 replicates carried out for each sample.

Permanence of the Drug in a Simulated Application Site

The evaluation of the permanence of the drug in a simulated application site was a new test resulting from a modification of the Setnikar and Fantelli method for the liquefaction time of rectal suppositories (Fig. 2). The vaginal physiology was recreated by means of a 2.5 cm diameter cellophane tube in which was inserted a solution of partially purified porcine gastric mucin type III corrected to pH 4.5 with an 85% w/v solution of lactic acid. The tube was tied to both ends of the condenser, and each end of the tube was open. Water at 37°C was circulated

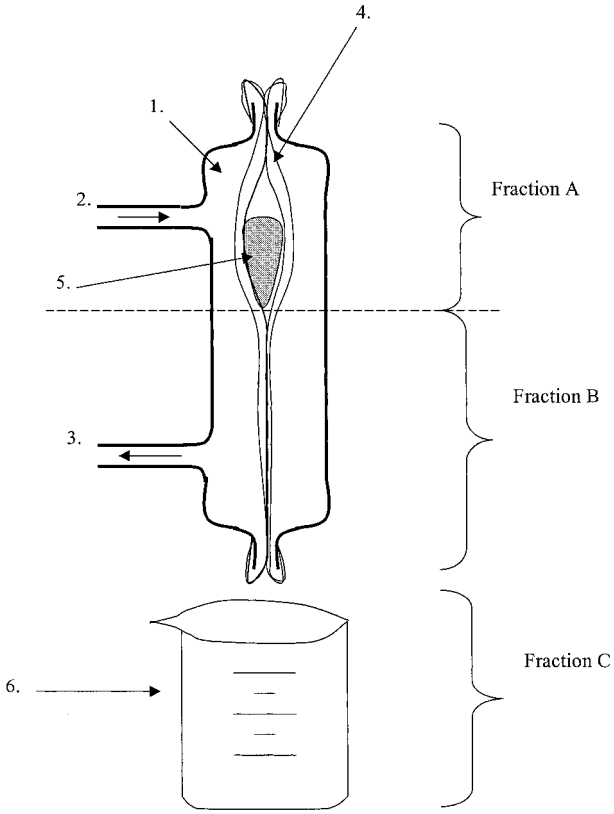

Figure 2. Setnikar and Fantelli apparatus and description of the distribution fractions of the drug: 1, thermostated bath; 2, incoming direction of the thermostated water; 3, exit direction of the thermostated water; 4, cellophane tube; 5, pessary; 6, beaker.

through the condenser at such a rate that the lower half of the cellophane tube collapsed, and the upper half gaped. When the water temperature was stabilized at 37°C, 5 ml of the porcine gastric mucin were dropped into the cellophane tube; after 10 min, the pessaries were dropped in as well.

A beaker was placed under the condenser to collect the discharged liquid. After 2 h, the cellophane tube was extracted from the condenser and cut horizontally into two parts (A and B). The cut was made just under the point at which the pessary was placed.

The drug was extracted from the two parts of the cellophane tube into a beaker with methanol and titrated. Also, the drug in the discharged liquid collected in the beaker under the condenser was titrated.

In this manner, the drug was distributed into three fractions: Fraction A was the drug that remained in the higher part of the cellophane tube (this fraction represents

the drug that remained in the simulated application site); fraction B was the drug that remained in the lower part of the cellophane tube (this fraction represents the drug that remained in the simulated application site after the melting of the pessary); fraction C was the drug that was recovered in the beaker placed under the condenser (this fraction represents the drug washed from the vagina by the vaginal fluid). The test was repeated five times for each pessary.

RESULTS AND DISCUSSION

Study of Compatibility

The DSC thermal curve of clotrimazole showed a single sharp endothermic peak at its melting point of 146.26°C. In all curves of the binary mixture, the peaks due to the clotrimazole and to the semisynthetic solid triglycerides were identifiable, showing that there was no interaction between the clotrimazole and the excipients.

Particle Size Analysis

In the drug powder analysis results, 94% of the particles were less than 2.5 μm, 5% were between 2.5 and 5 μm, and 1% were larger than 5 μm. Otherwise, all the particles in the drug powder suspended in the pessary were less than 2.5 μm, meaning that the method of preparation did not allow the formation of aggregates. Any other visual changes have occurred during the study period.

Release Test

The diffusional exponents n and the apparent release rate K of the different pessaries are given in Table 2. For all the curves, a linear regression analysis was performed, and a correlation coefficient $r^2 \geq 0.99$ was obtained. For all the formulations, the n values were between 0.452 and 0.588, which is indicative of Fickian diffusion. In this case, the drug diffusion through the matrix is the governing step for drug release.

Moreover, n and K are comparable in all the pessaries, meaning that the presence of the mucoadhesive polymer did not influence the kinetic release profile.

In Vitro Adhesion Test

The results of the adhesion tests are shown in Table 3. An improvement in the adhesivity of the pessaries was observed in the presence of the mucoadhesive polymers. The maximum force of detachment increased with increasing polymer concentration. The polymer that showed the highest adhesivity was polycarbophyl, followed by hyaluronic sodium salt and HPMC.

Table 2

Diffusional Exponents n *and Apparent Release Rate* K *of the Release Test*

	$n \pm$ SD	K (%min^{-1})
Blank	0.550 ± 0.021	0.677
NaHA 25	0.452 ± 0.014	0.601
NaHA 50	0.534 ± 0.016	0.674
NaHA 100	0.564 ± 0.018	0.596
HPMC 25	0.588 ± 0.016	0.580
HPMC 50	0.526 ± 0.020	0.643
HPMC 100	0.558 ± 0.019	0.673
PCP 25	0.556 ± 0.017	0.667
PCP 50	0.502 ± 0.018	0.571
PCP 100	0.482 ± 0.016	0.624

HPMC, hydroxypropylmethylcellulose; NaHA, hyaluronic sodium salt; PCP, polycarbophyl.

Liquefaction Time Test

The liquefaction times of the pessaries are shown in Table 4. In the pessaries with the mucoadhesive polymer, the liquefaction time was high when compared with the times for the pessaries without the mucoadhesive polymer. Moreover, the liquefaction time increased with polymer concentration. This behavior could be due to the

Table 3

Maximum Force of Detachment F_{max} *(mN) for the Pessaries Containing Increasing Amounts of the Mucoadhesive Polymer (Mean Values ± SE,* n = *8)*

	$F_{max} \pm$ SD (mN)
Blank	1683 ± 72.1
NaHA 25	2188 ± 216.3
NaHA 50	2876 ± 233.8
NaHA 100	3382 ± 144.6
HPMC 25	1835 ± 222.1
HPMC 50	1932 ± 239.3
HPMC 100	3017 ± 230.5
PCP 25	2694 ± 146.0
PCP 50	3071 ± 483.4
PCP 100	3860 ± 104.7

HPMC, hydroxypropylmethylcellulose; NaHA, hyaluronic sodium salt; PCP, polycarbophyl.

Table 4

Liquefaction Time Results of the Liquefaction Time Test

Formulations	Liquefaction Time ± SD
Blank	261.2 ± 1.8
NaHA 25	281.3 ± 2.7
NaHA 50	302.6 ± 3.7
NaHA 100	318.8 ± 2.5
HPMC 25	297.4 ± 1.6
HPMC 50	326.7 ± 1.7
HPMC 100	348.3 ± 2.5
PCP 25	356.1 ± 3.1
PCP 50	373.5 ± 2.8
PCP 100	391.8 ± 3.2

HPMC, hydroxypropylmethylcellulose; NaHA, hyaluronic sodium salt; PCP, polycarbophyl.

formation of a three-dimensional net in the semisynthetic triglyceride matrix or to an increase of the viscosity. The polymer that altered the liquefaction time most was polycarbophyl, followed by HPMC and hyaluronic acid. This characteristic can be profitably used for local controlled-release applications.

Permanence of the Drug in a Simulated Application Site

The distributions of the drug in the three fractions and the sum of drug distributed in the A and B fractions are shown in Table 5. The presence of the mucoadhesive had a large influence on the permanence of the drug in the simulated application site (A and B fractions). In fact, for the pessaries without the mucoadhesive, the drug was located almost exclusively in fraction C, which represents the drug washed away by the vaginal fluid. On the other hand, when the mucoadhesive was used in the pessaries, the amount of drug in A and B fractions increased with increasing concentration of the bioadhesive polymer. The polycarbophyl polymer turned out to be the best because it guaranteed the highest amount of clotrimazole in the A and B fractions and the lowest amount in the C fraction, followed by hyaluronic acid and HPMC.

CONCLUSION

The presence of mucoadhesive polymers greatly influenced the behavior of pessaries in respect to adhesive force, liquefaction time, and permanence of the drug in the simulated application site, but it did not change the release of the drug.

In particular, the developed pessary that guaranteed the greatest permanence in the target area and allowed the least active ingredient to leave the target site is that formulated with polycarbophyl at its highest concentration.

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Table 5

Percentage Distribution of the Drug into the Three Fractions in the Test for the Evaluation of the Permanence of the Drug in the Simulated Application Site

	Percentage Distribution of the Drug (±SD)			
Formulations	Fraction A	Fraction B	Fraction A + B	Fraction C
Blank	13.79 ± 1.02	17.21 ± 1.63	31.00	66.22 ± 4.56
NaHA 25	16.01 ± 1.22	9.13 ± 0.87	25.14	73.66 ± 6.02
NaHA 50	29.01 ± 2.01	12.53 ± 1.10	41.54	61.22 ± 5.31
NaHA 100	61.31 ± 3.01	15.66 ± 2.03	76.97	22.62 ± 1.12
HPMC 25	12.32 ± 1.33	17.59 ± 1.55	29.91	65.01 ± 2.01
HPMC 50	14.59 ± 1.62	21.98 ± 1.12	36.57	63.56 ± 2.21
HPMC 100	59.84 ± 4.56	12.56 ± 1.16	72.40	30.15 ± 2.31
PCP 25	15.45 ± 1.12	12.55 ± 1.32	28.00	72.35 ± 1.98
PCP 50	31.95 ± 1.21	14.52 ± 2.09	46.47	54.66 ± 2.33
PCP 100	63.38 ± 3.66	22.06 ± 2.64	85.44	16.26 ± 1.78

HPMC, hydroxypropylmethylcellulose; NaHA, hyaluronic sodium salt; PCP, polycarbophyl.

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