RESEARCH STUDIES ON IN VITRO AND EX VIVO YIELD OF THE MICONAZOLE NITRATE FROM ORAL BIOMUCOADHESIVE TABLETS

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RESEARCH STUDIES ON IN VITRO AND EX VIVO YIELD OF THE MICONAZOLE NITRATE FROM ORAL BIOMUCOADHESIVE TABLETS (Abstract): Among the various routes of drug administration, the oral mucosa is perhaps the most often preferred by patients and medical staff. However, oral administration of drugs has disadvantages, which may limit or prevent oral administration of some drugs, especially peptides and proteins, little when they are inserted in special administration systems for the colon. The disaggregation of some oral biomucoadhesive tablets and the in vitro yield of the miconazole nitrate was evaluated and in parallel with this, the evaluation of the in vivo yield of the antifungal from the pharmaceutical form. Thus, for a clear determination of the oral mucobioadhesive tablets’ disintegration with miconazole nitrate, it was necessary to implement a method to simulate the conditions of the oral cavity at a flow of solution (artificial saliva) similar to that of the human one. Materials and methods: materials: miconazole nitrate. Methods: The determination of disintegration time according to method A (FRX); the disaggregation of oral biomucoadhesive tablets with miconazole nitrate by means of simulation methods of in vitro conditions; the quantitative determination of the miconazole nitrate by means of HPLC method, after the in vitro dissolution test; the study of miconazole nitrate’s yield in dynamic condition from biomucoadhesive tablets in the presence of artificial saliva (AFNOR). Results: The yield profile of the miconazole nitrate in the disintegration solutions by means of classical method from FR X, by HPLC dosage was researched. The release of miconazole nitrate from the oral mucobioadhesive tablets was determined, that varies in time, depending on the type and relation of matrix forming polymers; a low yield speed of the miconazole nitrate from the tablets was determined; the yield profile of miconazole nitrate in disintegration solutions by means of the new suggested method was researched. Conclusions: The release of miconazole nitrate from the formulated biomucoadhesive tablets is of swelling and erosion. Keywords: MICONAZOLE NITRATE, MUCO-BIOADHESIVE TABLETS, IN VITRO YIELD.

The influence of some formulation factors on the release of miconazole nitrate from biomucoadhesive oral tablets was studied. Biomucoadhesive oral tablets with miconazole nitrate by the method of direct compression as a result of the completion of the mixture of suitable excipients (sodium carboxymethylcellulose and lutrol 6000 excipients used for the bioadhesion, mannitol used as a sweetening agent and aerosil as lubricant) (1).

The bioadhesive systems ensure an in-
timate contact between the dose and the absorbent tissue, which can lead to high local concentrations and thus a strong flow through the tissue. The efficiency of bioadhesive systems for oral administration of the active substance is influenced by the environment and biological properties of the polymer and of the drug (2, 3).

The researches on bioadhesive administration systems continue at a rapid pace, aiming at an efficient treatment of commonly encountered lesions (sores, ulcers, etc.) of periodontitis, gingivitis and caries. Optimizing the systemic treatment of disease by transmucosal drug administration in the oral cavity continues to be investigated using a variety of dosage forms containing new bioadhesive polymers (4).

Determining the disintegration time of tablets during tests was an old requirement in most pharmacopoeias. Currently, there are severe limitations of the disintegration test, this being applied especially to conventional tablets. The dissolution test was included as a consequence also for other solid forms with extended release. Both the disintegration time and the *in vitro* yield (or the dissolution tests) are determined by official methods (5, 6).

The mucobioadhesive tablets as pharmaceutical form and as a release system of the active substance requires the implementation of a new method of disaggregation method that better simulates the conditions of the oral cavity (7). Thus, for a clearer determination of the oral mucobioadhesive tablets’ disintegration with miconazole nitrate it was necessary to implement a new method for determining the flow rate of solution (artificial saliva) similar to that of the human one.

### MATERIALS AND METHODS

**Materials:** Miconazole nitrate (Sigma Aldrich, Germany); *Sodium carboxymethyl cellulose* (Lubrizol, U.S.A); *Mannitol* (East Chemical, Weifang City, China); *Polyethylene glycol, PEG 6000 microcrystalline powder* (Lutrol, BASF Pharma, Germany); *Aerosil* (Degussa, Germany). All the materials used had the purity degree in conformity with the normatives in force. The miconazole nitrate, the antifungal was used as active substance with 99% purity in oral mucobioadhesive tablets (1) (tab. I).

### TABLE I

<table>
<thead>
<tr>
<th>Pharmaceutical substances mg/tablet</th>
<th>Formula I</th>
<th>Formula II</th>
<th>Formula III</th>
<th>Formula IV</th>
<th>Formula V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miconazole nitrate</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Sodium carboxymethylcellulose (CMC-Na)</td>
<td>20</td>
<td>60</td>
<td>100</td>
<td>100</td>
<td>140</td>
</tr>
<tr>
<td>Mannitol</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>PEG 6000(Lutrol)</td>
<td>80</td>
<td>40</td>
<td>-</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Aerosil</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total (mg)</td>
<td>219</td>
<td>219</td>
<td>219</td>
<td>219</td>
<td>219</td>
</tr>
</tbody>
</table>

**Methods:** 1. The assay of disintegration time according to method A (FRX). For the disintegration of mucobioadhesive tablets with miconazole nitrate one has used 6 bioadhesive tablets from every formulation and as a device. DISINTEGRATION TESTER QC-21 (4). 2.B. The disintegration of mucobioadhesive tablets with miconazole nitrate by means of simulation method of in vivo conditions. The equip-
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...ment used in the disintegration of mucobioadhesive tablets and in the desorption of miconazole nitrate – a personal conception using a burette provided with a dropper, which regulates the artificial saliva flow that goes under dynamic condition on the tested tablet (0.5 mL /minute), an adjustment system of the artificial saliva flow, a support vial of the tablet, a vial for collecting the mixed solution (artificial saliva with active substance), muco-bioadhesive tablets with miconazole nitrate (1 tablet from each formula), 6 porcelain capsules, 10 mL graduated vials, thermostat bath GRANTw6 and artificial saliva solution with pH 7.24 AFNOR (0.26 g of disodium phosphate, 6.7 g sodium chloride, 0.33 g of potassium sulfocyanide, 0.2 g of monopotassium dihydrogen phosphate, potassium chloride 1.2g, sodium bicarbonate 1.5 g, distilled water up to 1000g).

The human saliva is released into the oral cavity with a flow of 1000mL up to 1500mL/day, resp. 0.5-1mL / minute, a flow rate that is regarded by the equipment. A tablet from each formula was placed in the equipment for the desorption of the miconazole nitrate and the equipment was started so that 0,5mL AFNOR artificial solution per minute falls on the tablet. The tablet is kept in this installation until it totally disintegrates. The artificial saliva solution that has disintegrated the table is being collected every 20 minutes, at a quantity of 10 mL.

3. The quantitative assay of miconazole nitrate by means of HPLC method, after performing the *in vitro* dissolution test. The dosage method HPLC was confirmed, which constitutes the objective of this paper, of the miconazole nitrate from oral mucobioadhesive tablets with long yield following the specificity, the exactness (accuracy), the retrieval capacity and also the precision (fig.1).

![Dose linearity by HPLC for MICONAZOLE NITRATE](image)

Fig. 1 Calibration curve of the miconazole nitrate by means of HPLC method

The gradual yield of the active substance from mucoadhesive tablets was studied using the current USP dissolution equipment. For the quantitative assay of the antifungal and of the HPLC method’s validity one has used an equipment no. 2 (with paddles), dissolution agent respectively phosphate buffer solution to pH = 6.8 (the agent’s volume of 500 ml), the rotation speed of 50 revolutions /minute, the temperature of the dissolution agent of 37 °C ± 0.5 °C, a standard solution consisting of 25.0 mg of miconazole nitrate was dissolved in methanol in a 25ml graduated flask. Subsequently, 0.5ml was diluted with 5ml methanol and 5ml phosphate...
buffer pH=6.8 in a 10ml graduated flask. 2.5ml of this solution was diluted with phosphate buffer to pH=6.8 in a graduated flask of 10ml (concentration of 0.0125 mg/ml miconazole nitrate). The principle of the method: the dosage of the miconazole nitrate is performed by liquids’ chromatography under pressure (HPLC).

The study of miconazole nitrate’s yield in dynamic condition from mucobuccal-adhesive tablets in the presence of artificial saliva (AFNOR). In order to achieve the yield in the miconazole nitrate from oral mucobioadhesive tablets one has used an equipment, which requires the use of as proof of mucobioadhesive tablets with miconazole nitrate dissolved in a solution of artificial saliva AFNOR at pH 7.24 and pure substance, miconazole nitrate, in order to carry out the calibration curves and as an auxiliary system one has used thermostat bath GRANTw6, colorimeter type 252SHERVEDWOOD, spectrophotometer MICROLAB 300- high-performance semi-automatic equipment.

The reagents used for the performance of miconazole nitrate’s yield are: reagents for elution from the capsule (0.5ml methanol + 0.5 ml of chloroform), update rate reagent of 10 mL (1,2 dichloroethane), the complexation reaction reagent(solution 0.01M iodine in 1,2-dichloroethane-volume 1mL). Benchmark for spectrophotometer MICROLAB 300: 0.25 mg miconazole nitrate+ 1 mL mixture (chloroform, methanol, 1:1 relation). Over the homogenized mixture add solution of 1 mL iodine 0.001 M in 1,2 dichloroethane it is brought to a graduated flask of 10 mL with 1,2-dichloroethane preserving it at 25° C temperature for 50 minutes.

The study of miconazole nitrate’s yield under dynamic conditions was approached in order to simulate the almost similar conditions of the oral cavity. The study under dynamic conditions was carried out based on the traditional methodology in the literature, at a value of pH 7.24, taking into account also the average salivary flow rate (0.5ml/min) (1.b).

A tablet from each formula was placed in the equipment for the desorption of the miconazole nitrate and the equipment was started so that 0.5mL AFNOR artificial solution per minute was dropping on the tablet. The tablet is kept in this installation until it totally disintegrates. The artificial saliva solution that has disintegrated the tablet is being collected every 20 minutes. Therefore, every 20 minutes one has collected 10mL from the sample, which was introduced in porcelain capsules, being evaporated on dry, on a water bath.

The residue of the porcelain cap was eluted with 1 mL of solution of methanol with chloroform. Also, in the porcelain capsule one has added 8 mL 1,2 dichloroethane and 1 mL iodine solution 0.01M. From the homogenized sample one has collected 500μL for the spectrophotometric method performed with the device MICROLAB 300 and for the colorimetric method one has collected 1 mL sample.

RESULTS AND DISCUSSION

By determining the disintegration time according to method A from FR X (5) above mentioned, one has been achieved for the first formulation a disintegration time of 25 minutes, for the second one a disintegration time of 45 minute, for the IIIrd formula 60 minutes, formula IV 65 minutes and for the final formulation a 90 minutes time (fig. 2).

The decomposition of mucobioadhesive tablets with miconazole nitrate by means of
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the new method leads to the following results: the first formulation disintegrates in 30 minutes, the second in 80 minutes, the third in 340 minutes, the fourth in 360 minutes and the last formula, respectively the fifth formula in 380 minutes (fig. 3).

![Fig. 2](image1.png) Decomposition time of oral biomucoadhesive tablets with miconazole nitrate by method A (FR X)

![Fig. 3](image2.png) Decomposition time of oral biomucoadhesive tablets with miconazole nitrate by simulating in vivo conditions.

From the above results we can see a prolonged disintegration time when we simulate the conditions at the level of oral mucosa. Thus, the pH, the saliva flow and also the temperature are important factors which influence the disintegration time. Formulae III, IV and V have a much higher disintegration time compared to formulae I and II, a phenomenon explained by: the presence of sodium carboxymethyl cellulose in a higher quantity for the last three formulae(100 mg-F3; 100 mg-F4; 140 mg-F5) and also the presence of polyethylene glycol in admixture with CMC-Na added in a higher quantity for the last two formulae (100 mg CMC-Na / 40 mg PEG /cpr; 140 mg CMC-Na / 20 mg PEG /cpr). Formula III contains CMC-Na but not PEG, the substance added in the highest quantity (140 mg/cpr) in formula V.

The yield profile of the miconazole nitrate in the solutions from disintegration by the classical method from FR X (method A) by HPLC dosage (tab.II, fig. 4) was researched.

It is noted that the release of miconazole nitrate from oral biomuco-adhesive tablets varies in time, depending on the type and ratio of the matrix forming polymers. The antifungal’s yield depends on the amount of polymers. The most important factor influencing the rate of release of the miconazole nitrate is the ratio between it and the polymers: CMC-Na and PEG 6000.

An increase in concentration of CMC-Na polymers increases the viscosity of the gel formed with the dissolution agent and also the forming of a gel layer around the
tablet, which reduces the rate of diffusion of the active substance. The release mechanism appears to be a swelling or erosion one of the tablets, which is observed during the yield study and also at the dynamic dissolution test.

TABLE II

In vitro yield of the miconazole nitrate from mucobioadhesive tablets (HPLC dosage)

<table>
<thead>
<tr>
<th>Time/Miconazole nitrate %</th>
<th>30’</th>
<th>60’</th>
<th>90’</th>
<th>120’</th>
<th>150’</th>
<th>180’</th>
<th>210’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula I</td>
<td>8.32</td>
<td>9.75</td>
<td>10.0</td>
<td>10.52</td>
<td>13.53</td>
<td>14.00</td>
<td>14.61</td>
</tr>
<tr>
<td>Formula II</td>
<td>5.80</td>
<td>6.15</td>
<td>8.35</td>
<td>10.30</td>
<td>12.16</td>
<td>12.36</td>
<td>14.48</td>
</tr>
<tr>
<td>Formula III</td>
<td>3.88</td>
<td>6.43</td>
<td>7.60</td>
<td>7.80</td>
<td>10.20</td>
<td>10.50</td>
<td>10.70</td>
</tr>
<tr>
<td>Formula IV</td>
<td>4.25</td>
<td>6.00</td>
<td>10.00</td>
<td>10.20</td>
<td>10.50</td>
<td>11.50</td>
<td>12.40</td>
</tr>
<tr>
<td>Formula V</td>
<td>3.50</td>
<td>6.00</td>
<td>9.70</td>
<td>9.90</td>
<td>10.00</td>
<td>10.40</td>
<td>11.50</td>
</tr>
</tbody>
</table>

Fig. 4 The yield profiles of miconazole nitrate from I-V formulae after decomposition according to A FRX decomposition

Also, there was a low yield rate of the miconazole nitrate from mucobioadhesive tablets during the research time: the largest yield of 14.51% within 3h and 30’ represents formula I and 14.48% represents formula II followed by formula IV with 12.4%, formula V with 11.5% and formula III with 10.7%.

The yield profile of miconazole nitrate in solutions of disaggregation by the new method, by colorimetric and spectrophotometric dosage was studied. Experimental results are processed in Figure 5. A more effective yield was registered for tablets of formulas I and II, which are noted as having a lower concentration of CMC-Na.

Further one presents the method used in determining the concentration of miconazole nitrate involved in AFNOR saliva solution to pH = 7.24 and D = 0.5 ml/min. The total percent of miconazole nitrate released from the tablet of formula I, in the presence of AFNOR saliva is of 83.49%, in formula II - 79 943%, in formula III - 717%, formula IV - 49.392% and the formula V is of 52.264%.
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It is important to keep in mind this fact, so that during drug administration, one maintains the required concentration for the destruction of fungi in a certain time, so that they do not form as in the case of the antibiotic, drug-resistant strains (fig. 5).

**CONCLUSIONS**

From the results of this research regarding the tablets’ disintegration, it is noted that formula V has the longest disintegration time, that is of 90 minutes, as determined by the method of FRX and of 380 minutes by means of dynamic method, which simulates *in vivo* conditions. Validation study performed on a dosage method adapted for the miconazole nitrate by means of HPLC shows that the method is linear, selective, accurate, precise and robust. The release of *in vitro* miconazole nitrate from oral biomucoadhesive tablets varies in time depending on the type, concentration and the ratio of polymers: CMC-Na/PEG.

In the study performed, one has observed both *in vitro* and *ex vivo* yield. These results explain the role of yield conditions (pH, temperature, flow of saliva, etc). Basically, I have noticed a substance release up to 16% in *in vitro* yield and up to 83.49% in *ex vivo* yield. The release of miconazole nitrate from formulated and studied biomucoadhesive tablets, concerning swelling and erosion.

**REFERENCES**