3³ factorial design-based optimization of the formulation of nitrofurantoin microcapsules

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Introduction
Nitrofurantoin is a urinary tract antibiotic [1]. Its dissolution rate [2], bioavailability in humans, and incidence of side-effects due to its poor solubility are significantly affected by the size of the nitrofurantoin particles [3-5]. However, smaller particles also increase gastrointestinal irritation, which explains why a macrocrystalline rather than an amorphous form of nitrofurantoin is used in the marketed product [6, 7]. In a recent study, we have shown that the bioavailability of nitrofurantoin is decreased due to the instability of the drug in gastrointestinal fluids [8]. In order to reduce the side-effects and increase the bioavailability of nitrofurantoin, different dosage forms, such as microcapsules and matrix tablets, have been developed [9-13]. It has been found that the blood level and dissolution profiles of slightly soluble powders are affected by the particle size of the drug [14-18]. Monkhouse et al. [19] reported on the effects of pH, wetting agents, and agitation intensity on micronized drug dispersions and Smallenbroek et al. [20] also discussed the effect of particle size of disintegrants on the disintegration of tablets. Some investigators have used factorial design in their experiments to evaluate the effect of various factors, such as particle size, pH, core/wall ratio, dehydration agent, bloom strength of gelatin, hardening time, drying temperature, quantities of binder, and compression forces, on drug bioavailability [21-24].

In this study microcapsules of nitrofurantoin were prepared by a carboxymethylcellulose-aluminium sulfate coacervation technique which was developed in our laboratory [12] and three different variables, namely, particle size of nitrofurantoin powder, size of microcapsule, and pH of the dissolution medium, were compared by 3³ factorial design. The results of the factorial analysis was expected to show the best formulation which meets the United States Pharmacopeia XXII criteria for the solubility of nitrofurantoin tablets.

Methods
Preparation of nitrofurantoin capsules
Nitrofurantoin powder (28 g; Eaton Co., Luzern, Switzerland) was sieved for 30 min at 60 cycles/s with a combined sieving system (>200, 200-400, <400 mesh (>75 μm, 38-75 μm, and <38 μm); Retsch, Haan, Germany) and the powder remaining in each sieve was weighed. Nitrofurantoin microcapsules were prepared as described previously [12] and were then sieved as described above.

Factorial design experiment
27 formulations of nitrofurantoin microcapsules were prepared. Table 1 shows the composition of the formulations. 3³ factorial design was applied to each nitrofurantoin particle size and nitrofurantoin micro-
Table 1  Composition of the formulations studied by 3^3 factorial design

<table>
<thead>
<tr>
<th>Particle size of nitrofurantoin powder (µm)</th>
<th>&gt;75</th>
<th>38-75</th>
<th>&lt;38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size of nitrofurantoin microcapsules (µm)</td>
<td>&gt;180</td>
<td>150-180</td>
<td>&lt;150</td>
</tr>
</tbody>
</table>

pH of dissolution medium

<table>
<thead>
<tr>
<th>pH</th>
<th>Particle Size Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>aab, abc, baa, bab, bac, caa, cab, cac</td>
</tr>
<tr>
<td>5.0</td>
<td>aba, abb, abc, bba, bbb, bbc, cba, cbb, cbc</td>
</tr>
<tr>
<td>7.5</td>
<td>aca, acb, acc, bca, bcb, bcc, cca, ccb, ccc</td>
</tr>
</tbody>
</table>

capsule for three different pH values of the dissolution medium.

Assay of nitrofurantoin

Nitrofurantoin (0.01 g) was put into a 10 ml volumetric flask and the volume was adjusted with dimethylformamide (Merck, Darmstadt, Germany). Then 20-100 µl of this solution (corresponding to 2-10 µg of nitrofurantoin) was transferred to a 10 ml volumetric flask and 1 ml phosphate buffer was added. The buffer contained 6.8 g/l of H₂KPO₄ (Merck) and was adjusted to pH 7.5 with 1 mol/l sodium hydroxide (Merck). The volumes were adjusted with 1% dimethylformamide solution in water.

The absorbance of each concentration of nitrofurantoin solution was measured in a 1 cm quartz cuvette at 375 nm, using a double-beam spectrophotometer (UV-150-02, Shimadzu, Kyoto, Japan), against a blank. Each experiment was carried out in triplicate.

Assay of drug content in microcapsules

Nitrofurantoin microcapsules (0.05 g) were put into a 50 ml volumetric flask and the volume was adjusted with dimethylformamide. The mixture was shaken for 1 h at 200 cycles/min (B. Braun Melsungen AG, Germany), and then 200 µl of this solution was transferred into a 10 ml volumetric flask and assayed as described above.

Kinetic evaluations

The results thus obtained were evaluated kinetically by (Bi)³, first-order, zero-order, Hixson-Crowell, RRSBW, Q/Vt, Higuchi, erodible spherical (Hopfenberg), erodible cylindrical (Hopfenberg), erodible slab (Hopfenberg) equations [25-29]. The release rate constants (k), correlation coefficients (r), and determination coefficients (r²) were calculated by means of a computer program [Ağabeyoğlu Tı, unpublished observations].

Assay of in vitro dissolution of microcapsules

The dissolution rates of the microcapsules were measured by using the United States Pharmacopeia XXII basket method at a stirring rate of 100 rpm at pH 1.2 [simulated gastric medium: 2 g/l sodium chloride (Merck)] and 7 ml/l hydrochloric acid (Merck), pH 5 and pH 7.5 [simulated intestinal medium: 6.8 g/l H₂KPO₄ (Merck), adjusted to pH 7.5 with 0.2 mol/l sodium hydroxide (Merck)] at 37 °C ± 0.5. A 1 ml sample was withdrawn from 900 ml dissolution medium at selected times, using a syringe fitted with a HA 0.45 µm filter (Millipore, Göttingen, Germany) and then 1 ml fresh solution was added to the dissolution medium. The sample was transferred to a 10 ml volumetric flask and assayed as described above. Each dissolution study was carried out in triplicate.

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