AN IN VITRO STUDY OF CLOTRIMAZOLE MICROSPHERES

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Abstract: *This paper aims at presenting the formulation and preparation of a dosage form with slow release (microsphere ovules) with chloramphenicol as an active substance, useful in the treatment of vaginal infections with Candida albicans and Trichomonas vaginalis. Microspheres are solid dosage forms of the reservoir type, formed of the particles of the active substance, in liquid or solid state, covered in a polymeric film, which represents the microcapsule wall and in whose compositions one or more natural or synthetic polymers are included. These microspheres have been incorporated into suppositories through enclavation and casting. We produced three microsphere types: with sodium alginate, chitosan, and ultrasonic produced chitosan. We then pursued the study of the release of chloramphenicol from microcapsules incorporated in vaginal suppositories at various pH levels. The slowest release was proved to be that of the chitosan microspheres obtained by gelification with tripolyphosphate and syringe extrusion, as a result of the modern preparation technique.*

INTRODUCTION

At world level, approx. 5-10 million patients presented to the gynecologist are reported per year, most of them associated with vaginitis (lower genital infections) of many types: mycotic vaginitis, also known as vaginal mycoses or fungal vaginitis, bacterial vaginitis, mixed vaginitis or sexually transmitted infections. Mycoses produce discomfort, and their high incidence has determined us to consider the study for obtaining vaginal suppositories containing clotrimazole microcapsules, which can be used in treating infections with *Candida albicans* and *Trichomonas vaginalis*.

PURPOSE

The paper aims at preparing chloramphenicol microspheres with sodium alginate, chitosan and ultrasonic produced chitosan, and at studying the chloramphenicol release from ovules. The preparation of the ovules was followed by qualitative assessment of the pharmaceutical product obtained in accordance with the provisions of the quality norms in force and of Romanian Pharmacopeia, 10th edition. We intended to obtain slow release dosage forms. The study conducted could provide pharmaceutical formulae applicable at the level of vaginal mucous membrane, with good pharmaceutical, pharmacological and pharmacokinetic properties.

MATERIALS AND METHODS

We applied the complex coacervation method (1) in view of obtaining the microcapsules and the method of microspheres enclavation in the suppositories mass.(2)

The materials used were: chloramphenicol powder, having the properties provided by Romanian Pharmacopeia, 10th edition, chitosan, glacial acetic acid 1%, sodium alginate, calcium chloride, Tween, gelatin mass.

We used the suppository forms of the pharmaceutical technology laboratory at the Faculty of Pharmaceutical Medicine and Bandelin ultrasonic system Sonoplus at 35% amplitude of the Physics-Chemistry laboratory.

The use of microcapsule-based therapy allows the release of the medicine to be carefully targeted, through selection and formulation of various polymer-medicine combinations.

The medicine total dose and the release kinetic are variable, and subject to modifications in view of attaining the expected results. By using innovative microencapsulation technologies (2) and by varying the relations of copolymer, the molecular weight of the polymer etc., microcapsules may be developed in an optimal system of medicine formulation, meant to ensure the desired release profile. As they are small-sized, microspheres have a wide surface of volume relations and may be used for the controlled release of insoluble medicine.

Chitosan microspheres are used to ensure the controlled release for many medicines and to improve the bioavailability of degradable substances, such as proteins, or to increase the absorption of hydrophilic substances from the epithelial layers.(3)

Chitosan has also been used as a potential medicine transporter with slow release and macromolecules. Chitosan is a biodegradable natural polymer with a great potential for pharmaceutical applications due to its biocompatibility, high density, non-toxicity and adherence to mucous membrane. It has been proven that not only does it improve the dissolving of soluble medicines, but also has a significant effect in the lipid metabolism of the organism. Gel formation may be obtained by interaction of chitosan with low molecular counterions, such as polyphosphates, sulfates, and glutaraldehyde reticulation.(1) This gelification property of chitosan allows a wide range of applications, such as pharmaceutical products coating, gelification of biochemical, embryonic products, whole cells, microorganisms and algae.

Chitosan is a weak base and is insoluble in water and organic solvents, while being soluble in aqueous diluted acid solution (pH < 6.5), which can convert the glucosamine units in a soluble form R-NH_3^+. It precipitates in alkaline solution or at contact with polyanions, forming a gel with lower pH. Properties such as biodegradability, low toxicity and good biocompatibility deem it suitable for use in biomedical and

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pharmaceutical formulae.

Stability of chitosan microcapsules

Some studies have reported the instability of the chitosan microspheres (prepared by precipitation) in acid environment. Adding sodium sulfate in a solution of acetic acid of chitosan leads to chitosan derivatives difficult to dissolve by ionic neutralization of positive charged amino groups. Upon acid adding (increased proton concentration), the balance is displaced to chitosan solubility and the microcapsules are dissolved.(1,3)

Reticulation of chitosan molecules results after the interaction between chitosan and controlled quantities of polyvalent anions. Reticulation may be obtained in acid, neutral or basic environments according to the method applied. This reticulation has been frequently used in the preparation of chitosan microspheres.(3)

Sodium alginate (C6H7O6Na) is extracted from brown algae (alginic acid). It is a colourless or slightly yellowish solid, presenting in filament, granular and powder forms. It forms a colloidal viscous solution with water. The gelification process, the exchange of calcium ions with sodium ions takes place in good conditions. Since the method is based on the availability of guluronic acid residues, molecular permeability does not depend on the immobilization conditions.(5)

2Na (Alginate) + Ca^{+} + Ca (alginate) 2 + 2Na^{+}

Ionic tied gel structure is thermo-stable in the 0 – 100^{o} C interval; therefore heating will not liquefy the gel.

Working technique used for the preparation of chitosan microspheres (2,4)

For obtaining the chitosan solution, 100g solution of glacial acetic acid 1% is prepared, and then a solution of acetic acid 1% adding distilled water. 2g chitosan are weighted then introduced in 98g solution of acetic acid 1%.(2)

The mix is homogenized by electromagnetic stirring at room temperature.

For the preparation of clotrimazole encapsulated material, 10g clotrimazole solution is added on 45g chitosan solution and 1 g emulsifier (Tween 80). The mix obtained is ultrasonned for 1 minute at Bandelin ultrasonic system, Sonoplus at 35% amplitude.(6)

Ultrasonning procedure characteristics: time: 1 minute; interior pulse: 0.5 seconds; exterior pulse: 1 second; Sonda Titan: TT_{13}/FZ.

Emulsion is introduced in a syringe with a needle sized 22G x 1 ½", respectively 0.7 x 40 mm and is placed 15-20 cm above the geliform solution. For chitosan, the geliform solution is sodium tripolyphosphate 5%.

Extrusion was conducted by constant dropping of emulsion drops into the geliform solution during electromagnetic homogenization.(4) In the final stage, the microcapsules were washed and dried at room temperature to form porous chitosan microspheres.

Working technique used for the preparation of sodium alginate microspheres

For obtaining the sodium alginate 2% solution, 2g sodium alginate is weighted, adding 98g distilled water. The mix is homogenized by electromagnetic stirring.(2)

For the preparation of clotrimazole encapsulated material, 10g clotrimazole solution is added on 45g sodium alginate solution and 1g emulsifier (Tween 80). The mix obtained is ultrasonned for 1 minute at Bandelin ultrasonic system, Sonoplus at 35% amplitude Ultrasonning procedure characteristics: time: 1 minute; interior pulse: 0.5 seconds; exterior pulse: 1 second; Sonda Titan: TT13/FZ.

Emulsion is introduced in a syringe with a needle sized 22G x 1 ½", respectively 0.7 x 40 mm and is placed 15-20 cm above the geliform solution

For sodium alginate, the geliform solution is calcium chloride sic 0.05 M.

Extrusion was conducted by constant dropping of emulsion drops into the geliform solution during electromagnetic homogenization.

The microcapsules obtained are filtered and dried at room temperature.

For the preparation of vaginal suppositories with clotrimazole by melting and encapsulation, we used the gelatinous mass, and the required quantity was calculated with the following formula:

$M = F - f' \times S = F - (f \times 0.826) \times S$, where:

- M = quantity of glycerol-gelatinous mass, in grams;
- F = calibration factor (capacity of empty forms);
- f' = factor of dislocation of active substance from glycerol-gelatinous mass (f × 0.826);
- S = quantity of medicine substance for ovule.

Three types of microcapsules were used:

1. Clotrimazole and sodium alginate obtained by extrusion, in a 70.27mg/g concentration.
2. Clotrimazole and chitosan obtained by extrusion, in a 78.74mg/g concentration.
3. Clotrimazole and chitosan obtained by ultrasonning, in a 30mg/g concentration.

We used the method of microcapsule enclavation in the gelatinous mass. For the ovules to have the same active substance concentration, i.e. 10mg/g, calculations were made, resulting that 0.14g Clotrimazole and sodium alginate microcapsules had to be added in the first ovule, 0.12g clotrimazole and chitosan microcapsule had to be added in the second ovule, while in the third ovule, 0.33 g clotrimazole and chitosan microcapsule had to be added.

RESULTS AND DISCUSSIONS

The clotrimazole-loaded chitosan microspheres were prepared by emulsion/internal gelation method, using the sodium tripolyphosphate (TTP) as cross-linking agent and CaCl2 as precipitation agent.

Some studies were focused on the preparation of chitosan nanoparticles by increasing the ionic strength of chitosan solution at high pH values. Thus, the addition of salts (CH3COONa, NaCl, KCl) and hydroxides (KOH, NaOH) in chitosan solution and allows the formation of chitosan nanoparticles.(7)

TPP is a multivalent anion and its negative charges can form the electrostatic bonds with the positive charges of the protonamed amino groups of chitosan

We prepared:

Samples:

I. Sodium alginate microcapsules (70.27 mg/g), in 10 mg/g concentration - 0.14 g microcapsules/ovule.
II. Chitosan microcapsules (78.74 mg/g), in 10 mg/g concentration - 0.12 g microcapsules/ovule.
III. Ultrasonned chitosan microcapsules (30 mg/g), in 10 mg/g concentration - 0.33 g microcapsules/ovule.

Measurements were performed with SECOMAM spectrophotometer S.250 la λ = 400 nm

Table no. 1. Spectrophotometre measurement

15 min	0.588	0.385	0.477
15 min	0.806	0.417	0.731
30 min	1.102	0.794	0.945
30 min	1.468	0.802	1.082
30 min	1.573	0.814	1.412
30 min	1.587	0.851	0.827

30 min	1.633	0.884	1.940
30 min	1.624	1.012	2
30 min	1.656	1.002	2

After 30 minutes, a slight dissolving of the ovules (gelatinous mass) with the initiation of microcapsules release was observed.

After an hour, a better dissolving of the ovules (gelatinous mass), and an improved microcapsules release was observed.

After two hours, a very good dissolving of the ovules (gelatinous mass), and a much improved microcapsules release was observed.

After two hours and 30 minutes, the gelatinous mass dissolved completely, while the microcapsules dissolved as follows:

- Partial dissolving of the first sample.
- In the second sample, the microcapsules were not dissolved.
- In the third sample, almost complete dissolving.

Dissolving was complete for the three samples after 3 hours and 30 minutes, the third sample reaching balance.

For determining the released clotrimazole quantity, we further aplied the spectrophotometric method. 6 samples of clotrimazole alcoholic solution of concentration according to the table below were prepared.

Table no. 2. Spectrophotometric concentration

Clotrimazole concentration (mg/ml)	Extinction
0	0
5	0.438
10	0.806
15	1.102
20	1.468
25	1.733
30	1.997

For each sample, we determined the extinction at 400 nm wavelength, drawing the curve E= f(c). Concentration was calculated from the equation y = 0.0701x, R^2 = 0.9879.

Figure no. 1. Etalon curve for calculation of clotrimazole concentration

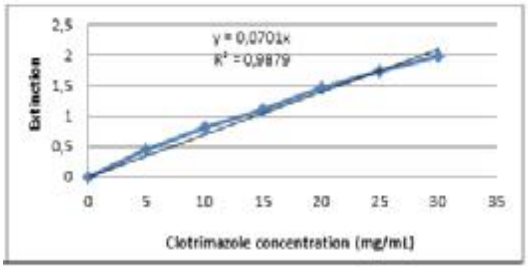
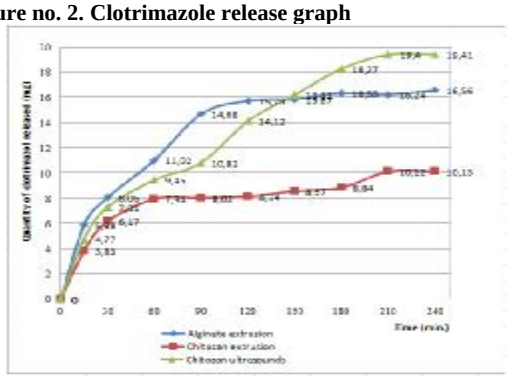


Figure no. 2. Clotrimazole release graph



CONCLUSIONS

In the first 30 minutes, the values of clotrimazole release rate from the three pharmaceutical formulae are close as the clotrimazole molecules have to penetrate two barriers: one represented by microcapsules and the other, by the ovule.

After 30 minutes, the ovule is dissolved and clotrimazole is released only from microcapsules. During the 30-150 minutes interval, the highest release rate is that of alginate microcapsules, and the lowest is that of chitosan microcapsules obtained by extrusion. This is because chemical modifications occur at the level of chitosan macromolecule in the presence of sodium tripolyphosphate, which allow intermolecular interactions which determine the microcapsule rigidization. After 150 minutes, the maximum release rate is that of chitosan microcapsules obtained by ultrasonning. This is because the scission of the macromolecular chain of chitosan occurs at ultrasonning, and also the inflation degree of microcapsules is altered. These microcapsules are initially agglomerated, and the inflation phenomenon is slower, reaching the maximum value after 150 minutes. The microcapsules inflation represents the main mechanism of releasing clotrimazole from microcapsules. The slowest release rate is manifested in chitosan microcapsules obtained by gelification with tripolyphosphate and syringe extrusion.

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