Dr. Szilárd Pál

Optimization of manufacture and examination of micropellets based on pharmaceutical technological and biopharmaceutical parameters

Ph.D. Thesis

Supervisor:

Dr. Attila Dévay

University of Pécs, Faculty of Medicine

Institute of Pharmaceutical Technology and Biopharmacy

Pécs

2013

1. Introduction

Optimization of the drug release of peroral dosage forms and adjusting it to the absorption window of the active ingredient is the fundamental point in the development of a new pharmaceutical product. However, there may be different aspects in cases when local effect is required. In this type of therapy the concept of the dissolution of active ingredients should be revaluated. The enhancement of the bioavailability according to the Biopharmaceutical Classification System (BCS) cannot be applied invariably, since importance of the absorption and the permeability is minor. Conversely, there are other essential parameters which should be taken into account during the development of these preparations, including the adhesivity to the mucous membrane, large contact surface of the drug particles, the profile and the time of drug release. Among several diseases, gastrointestinal candidiasis (GIC) was chosen to formulate and optimize a medicinal product taking into consideration the enhancement of the bioavailability of local effect.

Application of pellets in this therapy offers even more advantages. They are suitable to optimize drug release by their particle size, coating thickness or material.

Pellets among multiparticulate dosage forms offer several advantages generally in the therapy related to safety and effectiveness of the medicinal product such as individual reproducibility of gastric emptying, more regular absorption, increasingly stable, predictable plasma levels and a reduced risk of high concentrations. This basic concept of multiple-unit systems is the fact that the dose of the active ingredient is released by the individual subunits, and the functionality of the entire dose depends on the quality of the subunits. Pellets are suitable for further processing in order to optimize drug release by combining different particle size fractions and coating thickness in capsules or compressing them into tablet.

Manufacturing of pellets requires specialized equipments, technologies and excipients. Pellets are usually prepared by wet agglomeration of fine powders of active ingredient and excipients into spherical units in closed granulating systems, i.e. in rotor-fluid granulators or high-shear mixers. To produce pellets in a high shear mixer, the process involves distinct phases: homogenization of powders, granulation, spheronization and drying. The primer nucleus of future pellets is formed by binder spraying and dispersing during the agitation. Being a multivariate process, it is important to identify and control the process variables, i.e. the appropriate agitation prevents the development of too large particles. Since agglomerates

undergo densification as mixing and spraying, the process time is expected as a critical parameter influencing quality of pellets.

Although various experiments were drawn up to investigate the effect of the formulation variables on the physical characteristics of pellets, only a few reports can be found in the pharmaceutical literature investigating the large number of process variables together during the pelletization concerning both the physical properties of granules and the drug release profile of the dosage form.

Modelling the effect of process variables with factorial designs and analysis of the response surfaces is a powerful, efficient and systematic tool that shortens the time required for the development of pharmaceutical dosage forms and improves research and development work.

The objective of the dissertation is to offer an optimized pharmaceutical preparation for the therapy of GIC containing *nystatin* to achieve a prolonged site-specific antifungal treatment using multiparticulate dosage form and special bioadhesive excipients based on preliminary examinations carried out on micropellets with model drug incorporated.

2. Aims

Aims of this research work are:

- determination of significant process variables and their effect of high-shear pelletization,
- application of the measurement of diffuse reflectance spectra as a possibility for fast in-process control during the pelletization process,
- application of optimized process variables in order to produce micropellets with specific physical characters for an optimized GIC therapy,
- application of Microbiologically Detected Dissolution (MDD) technique based on direct bioautography in order to determine the dissolution kinetics of antifungal substances,
- comparison of the MDD with other analytical method,
- optimizing the biopharmaceutical characters of micropellets for the antifungal action,
- recommendation of the manufacturing method and ingredients for a successful GIC therapy.

3. Materials and Methods

The polyene antifungal antibiotic *nystatin* (Fig. 1.) (BCS class IV.) is one of the most important active agents in the site-specific treatment of the candidiasis, as it was reported by several clinical researches. *Nystatin* is a yellow or slightly brownish hygroscopic substance obtained by fermentation using the ATCC 11455 strain of Streptomyces noursei. *Nystatin* is very poorly absorbed from the gastrointestinal tract, therefore the development of side effects is suppressed. It is excreted almost entirely in the faeces.

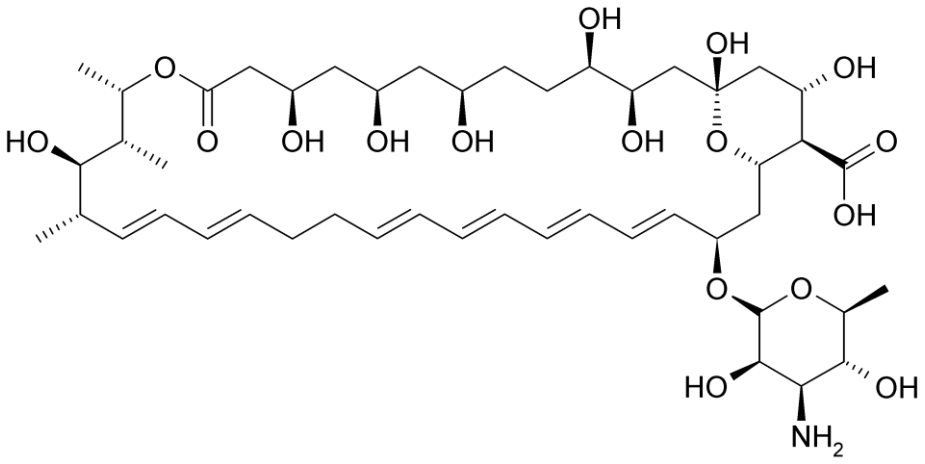

Figure 1. Structure of *nystatin*

3.1. Preliminary studies for high-shear pelletization

Among the available methods of preparation high-shear granulation was chosen to produce pellets. In order to determine significant process parameters of high-shear granulation, pellets were prepared containing anhydrous theophylline as model drug, α-lactose monohydrate, microcrystalline cellulose and ethylcellulose. Quality of all materials used in the experiments was Ph. Eur. 5. The powders were loaded into the bowl and premixed for 3 min.

During the pelletization the amount of the purified water used as granulation liquid was the amount of 45% calculated on the loaded mass. The binder flow time was kept constant at 5 minutes.

After production, pellets were dried to constant weight at 35°C, and stored at 25 °C, 60% RH in closed containers until their evaluation.

The high shear pelletization process was performed in a Pro-C-epT 4M8 granulator (Zelzate, Belgium). During the production two process variables were chosen to be investigated defined as factors**Hiba! A hivatkozási forrás nem található.**:

– impeller speed,
– binder flow rate.

Chopper speed was constant kept at 2000 rpm. An experimental design was carried out to reduce the number of experiments needed to obtain the highest amount of information on product and the effect of manufacturing process variables. The trials were carried out in triplicate in a randomized order.

3.2. Manufacture of micropellets containing nystatin

Pellets were produced in a high-shear mixer (Pro-C-epT 4M8 Granulator, Belgium, Zelzate) with a three-blade impeller and a chopper. Each batch contained 5% *nystatin*, 45% microcrystalline cellulose, hydroxyethylcellulose and carbomer in different amounts (0-5%) according to an experimental design, α-lactose-monohydrate added as diluent so that the total dry mass was 100.0 g. Powders were loaded into a 1000 ml bowl and premixed for 3 minutes. Purified water was used as binder liquid.

Pellets of 15 samples were prepared according to a central composite factorial design (Table I. and II.) in triplicate. Experimental design of full type with five centre points had three numeric factors including average pellet size (x_1), carbomer (CPL) (x_2) and hydroxyethylcellulose (HEC) (x_3) content and five coded levels (-1.414, -1, 0, +1, +1.414). Different average pellet sizes with various CPL and HEC contents were produced in the trials in order to examine different preparations. During statistical evaluations Design Expert v.7.0 (Stat-Ease Inc., Minneapolis, USA) was used to calculate the relative effect of factors, the confidence interval was 95%, significance was determined if $p<0.05$. TableCurve® 3D v. 4.0 (Systat Software Inc., London, UK) was used to reveal the response surface from the polynomial equation calculated using the experimental design.

Table I. Central composite factorial design with two level factorial design points (-1,1), axial points (-1.414, 1.414) and a centre point (0)

Trial	x_1	x_2	x_3
1	1	1	-1
2	1	-1	1
3	-1	1	1
4	-1	-1	-1
5	-1.414	0	0
6	1.414	0	0
7	0	-1.414	0
8	0	1.414	0
9	0	0	-1.414
10	0	0	1.414
11	0	0	0
12	0	0	0
13	0	0	0
14	0	0	0
15	0	0	0

Table II. Process parameters of trials

Factors with coded levels	**-1.414**	**-1**	**0**	**1**	**1.414**
x_1, average pellet size (μm)	217.2	300.00	500.00	700.00	782.8
x_2, CPL content (g)	0.00	0.73	2.50	4.27	5.00
x_3, HEC content (g)	0.00	0.73	2.50	4.27	5.00

4. Results and discussion

The fundamental point of an effective therapy may be a locally acting antifungal agent incorporated in a multiparticulate dosage form. As the active pharmaceutical ingredient, *nystatin* was chosen due to its advantageous properties of having contact antimicrobial effect with negligible absorption. As the dosage form micropellets were produced due to their good physical properties and large contact surface uniformly spreading through the GI tract. For production method among several possibilities high-shear pelletization was chosen, which is a reliable and quick method offering numerous facilities via adjustment of process parameters affecting the properties of the end-product. Optimized GIC therapy also requires long retention time on the surface of action which can be ensured by application of bioadhesive excipients, such as CPL and HEC. Summarizing the facts and observations, by production of *nystatin* based micropellets containing bioadhesive materials optimized GIC therapy can be reached.

In order to achieve the aims, preliminary examinations were carried out on a high-shear granulator to determine significant process parameters affecting the physical properties of micropellets focusing on the particle size distribution. These examinations were made according to a 3-level face-centred central composite experimental design examining the effect of impeller speed and binder flow rate on micropellet parameters.

After evaluation of the experiments a second order polynomial model was set up, which pointed to both factors as significant process parameters representing their effect as a saddle shape function. This experiment represented, that by applying extremities of the impeller speed (500 rpm and 1000 rpm) and liquid addition at speed of 10-12 ml/min together resulted relatively small particle size (500-550 μm). End-point of pelletization was determined by the measurement of the impeller torque. According to the experiments detection of NIR spectra after appropriate transformation could also serve important information of the process, since close (R=0.9842) correlation was observed between the particle size and the diffuse reflectance of the samples.

Results of these preliminary examinations were applied in the preparation of *nystatin* containing micropellets, adjusting process variables according to the previous experiences.

In the second production experimental design of full type with five centre points was set up. Examined factors were the following:

– average pellet size,
– CPL content and
– HEC content.

Investigated biopharmaceutical and pharmaceutical technological quality parameters of micropellets were:

– particle size distribution,
– flowability,
– hardness,
– surface characteristics,
– swelling,
– bioadhesion property and
– dissolution of nystatin determined by spectrophotometer and microbiological assay.

Evaluating the results important observation was the applicability of microbiologically detected dissolution based on direct bioautography, which had close correlation with the drug release determined by sphetrophotometric method regarding both the mean dissolution time (MDT) and the shape factor (β) of the *Weibull* distribution applied as a model for the dissolution studies. Extremities of the micropellet size (~200 μm and ~800 μm), CPL and HEC content (at both excipients 0 and 5%) short dissolution time could be achieved. According to the model established low value of shape factor, which was also the aim of the optimization, could be reached by reducing the pellet size and HEC content, but increasing CPL content. In case of swelling, particle size and CPL content was significant. Increasing CPL content and particle size swelling ratio also increased. Surface characteristics by SEM clearly represented the matrix formation of CPL and HEC when applied in the same amount, which was also confirmed by the diffuse reflectance spectra, since in this experiment it was not the indicator of the particle size, but the surface roughness of micropellets.

4.1. Optimization of GIC therapy

Gathering all experimental data and determining the optimization aims (short dissolution time and low β values with long retention time with appropriate swelling) software Design Expert v.7.0 (Stat-Ease Inc., Minneapolis, USA) was used to calculate the recommended parameters for the optimized GIC therapy by micropellet based drug delivery system.

Result of the optimization is production of *nystatin* containing micropellets with average size of 550μm, containing about 4.25% CPL and 0.75% HEC. To confirm the optimization result, a test sample was produced in triplicate.

Pellets production was carried out according to the previous experiences in a high-shear mixer (Pro-C-epT 4M8 Granulator, Belgium, Zelzate) with a three-blade impeller and a chopper. Powders were loaded into a 1000 ml bowl and premixed for 3 minutes. Ingredients and excipients were *5% nystatin* (Merck, Darmstadt, Germany), 45% α-lactose-monohydrate (DC, BDI, Zwolle, Netherlands), 45% microcrystalline cellulose (Avicel PH 105, FMC, Philadelphia, USA), 0.75% hydroxyethylcellulose (Hercules, Wilmington, USA) and 4.25% carbomer (Carbopol 934P, Hercules, Wilmington, USA). Quality of all materials used in the experiments was Ph. Eur. 7. The total dry mass was 100.0 g. 50 ml of purified water was used as granulation liquid at speed of 10 ml/min. Production was carried out at 25°C with 5 min net pelletization time applying 500 rpm impeller and 2000 rpm chopper speed.

Examinations carried out were the following: average particle size, mean dissolution time, *Weibull* shape factor, flowability, Carr index, Hausner factor and friability.

Before examinations pellets were kept in a closed container for 24 h. Examination of these pellets were carried out in triplicate to test the optimization results (Table III.).

Table III. Results of examinations of optimized micropellets

Property	Result	Desired value
Average particle size	564.7±23.3 μm	550 μm
τ_D	5.7±2.1 min	5-10 min
β	0.3872±0.032	0.3-0.5
Bioadhesion retention	55.3±2.7 %	50-55%
Swelling	50.7±4.2 μm (8.8%±0.7%)	>5%
Flowability (angle of repose)	24.40°±3.18°	<25°
Carr index	10.71±0,23	<10
Hausner factor	1.12±0.05	<1.25
Friability	< 0.5%	<1%

The particle size distribution of the optimized micropellets is monodiperse. According to the RRSB diagram the characteristic particle size parameter (d) at 36.8% is 434.7±15.3 μm and the uniformity factor: n = 4.378 (Fig. 2.). The dissolution curve of these samples is in Fig. 3.

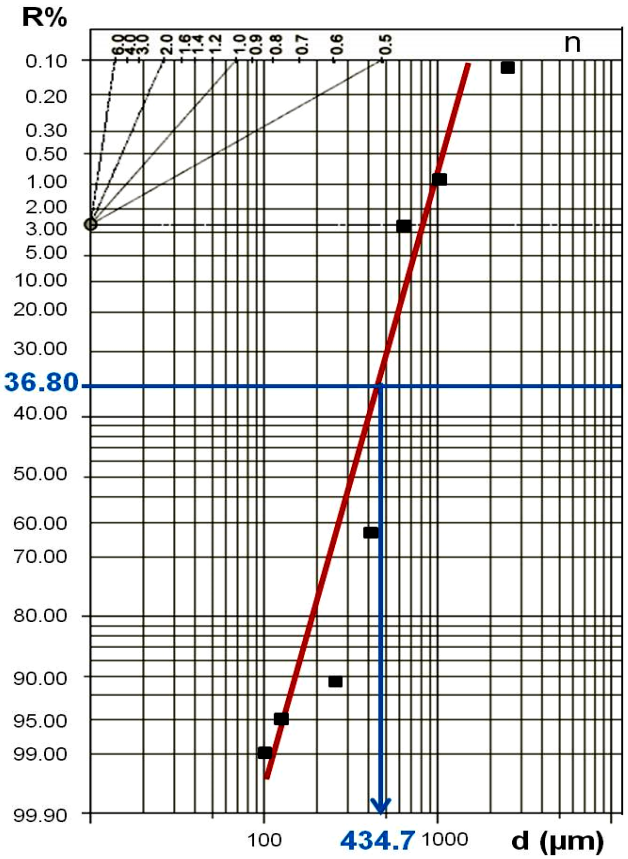

Figure 2. RRSB particle size distribution function of optimized micropellets

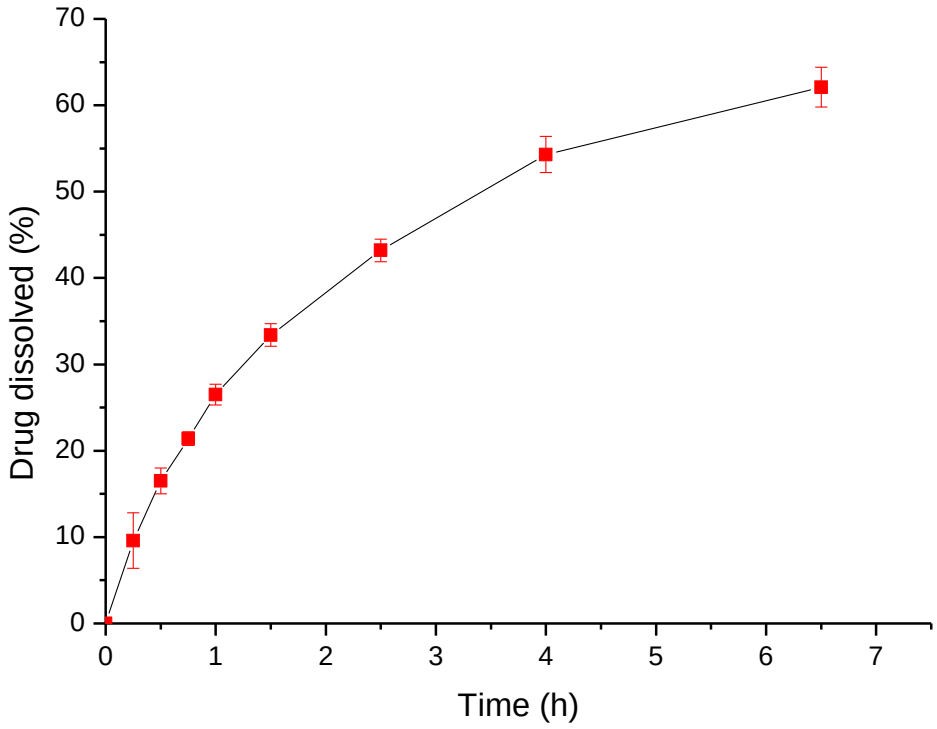

Figure 3. Dissolution curve of the optimized micropellets

According to the examinations the optimized test samples fulfilled all the pharmaceutical technological and biopharmaceutical criteria specified before, in order to facilitate an optimized GIC therapy.

5. Summary of the new results

According to my preliminary and optimizational examinations new results of my research work are the following:

1. In my experiments impeller speed and binder flow rate were found as significant process parameters influencing the pellet size during high-shear pelletization.
2. It can be declared, that impeller torque and diffuse reflectance measurement can both be used in in-process control of high-shear pelletization including the determination of the end-point of the manufacturing process.
3. Measurement of diffuse reflectance was found to be an indicator of the surface characteristics of micropellets which was confirmed by SEM examinations.
4. According to the results application of Microbiologicaly Detected Dissolution (MDD) studies based on direct bioautography were able to modelize the antifungal effect of the *nystatin*.
5. Close correlation was found between spectrophotometric investigations and MDD studies.
6. Experiments verified that by incorporating bioadhesive materials, such as CPL and HEC in the pellet matrix, the residence time significantly increases on the surface of the mucous membranes.
7. According to the optimizational results of my research work optimal ratio of CPL:HEC was determined to be 17:3 to reach 50-55% bioadhesion retention and relatively quick dissolution (MDT=5-10min).
8. Optimal size of pellets in my experiments aiming GIC therapy is found to be 550μm.
9. Summarizing my results recommendation for the ingredients and their proportions in an optimized GIC therapy by production of micropellets with 550μm average particle size may be:
    - *nystatin* (5%)
    - *microcrystalline cellulose* (45%)
    - *α-lactose-monohydrate* (45%)
    - *carbomer* (4.25%)
    - *hydroxyethylcellulose* (0.75%)

10. Optimal process parameter recommendations in laboratory scale are:

- 1000 ml glass pelletizing bowl
- Initial temperature: 25°C
- 100.0 g net weight of powders before liquid addition
- 3 min premixing time
- 5 min net pelletizing time
- 1 min mixing after binder addition
- 24 h drying of samples before examinations
- 500 rpm impeller speed
- 2000 rpm chopper speed
- 10ml/min binder flow rate (purified water)
- 50 ml total amount of binder liquid

Publications and presentations related to the thesis

1. **Sz. Pál**, S. Nagy, T. Bozó, B. Kocsis, A. Dévay:
   Technological and biopharmaceutical optimization of nystatin release from a multiparticulate based bioadhesive drug delivery system
   European Journal of Pharmaceutical Sciences 49.2: 258-264.2013.
   IF: 3.212

2. S. Nagy, B. Kocsis, **Sz. Pál**, T. Bozó, K. Mayer, A. Dévay:
   Antifungális hatóanyagtartalmú bioadhezív mikropelletek vizsgálati tapasztalatai
   XVI. Gyógyszertechnológiai Konferencia és VIII. Gyógyszer az ezredfordulón Konferencia, Siófok, 2010.

3. A. Dévay, B. Kocsis, **Sz. Pál**, K. Mayer, S. Nagy:
   Quick detection of *Nystatin* from sustained release dosage forms using Microbiologically Detected Dissolution (MDD)
   The 2nd BBBB Conference on Pharmaceutical Sciences, Tartu, 2007.

4. **Sz. Pál**, K. Mayer, I. Antal, A. Dévay:
   Comparison of evaluation on pharmaceutical and biopharmaceutical properties of multiparticular dosage forms using factorial design and artificial neural network
   The 2nd BBBB Conference on Pharmaceutical Sciences, Tartu, 2007.

5. A. Dévay, K. Mayer, **Sz. Pál**, I. Antal:
   Investigation on drug dissolution and particle characteristics of pellets related to manufacturing process variables of high-shear granulation
   Journal of Biochemical and Biophysical Methods 69: 197-205.2006.
   IF: 1.302

6. A. Dévay, S. Nagy, **Sz. Pál**:
   MDD, új eljárás antibiotikumok kioldódásának vizsgálatára
   Congressus Pharmaceuticus Hungaricus XIII., Budapest, 2006.

7. A. Dévay, S. Nagy, **Sz. Pál**:
   Comparison of dissolution and microbiological thin layer chromatography detection of drugs containing antimicrobial drugs
   5th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Geneva, 2006.

8. A. Dévay, **Sz. Pál**, S. Nagy:
   A mikrobiológiailag detektált kioldódási (MDD) módszer alkalmazása antifungális hatóanyagtartalom érzékeny és gyors vizsgálatára
   Gyógyszerkutatási Szimpózium, Debrecen, 2006.

9. A. Dévay, I. Antal, **Sz. Pál**:
   Gyógyszeres mikropelletek formulálásásának szempontjai
   Gyógyszerkutatási Szimpózium, Debrecen, 2006.

10. A. Dévay, K. Mayer, **Sz. Pál**:
    Investigation of dissolution of active ingredient from micropellets
    8th Symposium on Instrumental Analysis, Graz, 2005.

11. A. Dévay, B. Kocsis, **Sz. Pál**, A. Bodor, K. Mayer, S. Nagy:
    Application of a microbiological detection for investigation of dissolution of antibiotic delivery systems
    8th Symposium on Instrumental Analysis, Graz, 2005.

12. A. Dévay, **Sz. Pál**, I. Antal:
    Effect of process parameters on characteristics of *theophylline* containing pellets prepared in high shear granulator
    6th Central European Symposium on pharmaceutical technology and biotechnology, Siófok, 2005.

Acknowledgements

I would like to thank to my PhD advisor

Dr. Attila Dévay

for his support and continuous help encouraging me during hard times of my research work, introducing me to the wonderful world of pharmaceutical technology and biopharmacy.

I would like to thank to my family and all my co-authors for their support in my work.

I thank all members of Institute of Pharmaceutical Technology and Biopharmacy for their help.