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Optimization of Chitosan Microspheres Spray Drying via 3^2 Full Factorial Design

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Background: Generally, the preparation of spray-dried microspheres is strongly affected by the process parameters. Particle size and production yield are mainly influenced by the spraying solution concentration and the pump rate of the spray dryer.

Aim: The aim of this study was to assess optimum spray drying parameters - polymer concentration and pump rate required for the production of chitosan microspheres with high production yield and targeted for nasal administration particle size.

Materials and methods: Full 3^2 factorial design was used to study the investigated parameters. Three different concentrations of the chitosan solution were selected: a low concentration of 1%, average concentration of 1.5% and high concentration of 2%. The rate of the peristaltic pump was also varied at three levels: low rate of 10%, medium rate of 14% and high rate of 18%.

Results: Nine models of chitosan microspheres were formulated and characterized in terms of shape, surface morphology, size, particle size distribution and production yield. The particles obtained from 2% chitosan solutions, sprayed at 10% pump rate were of the highest yield (64.33%) and appropriate for nasal administration median diameter (3,434 μm).

Conclusion: The two investigated spray-drying parameters interact with each other and their influence on the production yield and the size of the chitosan microspheres should be evaluated together, instead of one at a time. The assessed process parameters allow the production of chitosan microparticles with high yield and desirable characteristics (size, size distribution and shape) for intranasal delivery.

BACKGROUND

Nowadays micro- and nanotechnologies have emerged as one of the most promising strategies in the pharmaceutical practice to achieve site-specific and controlled drug delivery.[1] Biodegradable microparticles have been extensively utilised for oral and parenteral administration, and recently they have also been considered as an approach to deliver drugs nasally.[2,3] For the production of polymer microparticles spray drying is amongst the most widely used methods.[4] Although a lot of fundamental investigations on spray drying technique have been undertaken, the process still has some uncertainties and difficulties to handle. The impossibility for accurate prediction of the complex interactions between spray drying parameters and their influence on the product quality is undoubtedly of significant importance. Varying the technological parameters can seriously affect the formulated microparticles - their shape, size and yield. The process parameters of spray drying – sample concentration, inlet temperature, gas flow rate, pump rate and aspiration should be carefully selected for the reliable formulation of a product with desirable characteristics.[5]

The correct approach to dealing with several factors is to conduct a factorial experiment. Factorial designs in general are defined as most efficient for studying the impact of two or more parameters.

Their concept is to vary at different levels two or more factors using a factorial grid and thus to investigate all possible combinations of the levels of the factors. Compared with the traditional altering of one factor at a time, the factorial design enables scaling up production processes more correctly with fewer individual experimental runs, evaluating the combined influence of the investigated factors.[6,7]

AIM

The aim of this study was to optimize the process parameters - polymer concentration and pump rate via 3^2 factorial design in order to formulate spray-dried chitosan microspheres with high production yield and relevant for nasal administration particle size.

MATERIALS AND METHODS

Chemicals and reagents

Chitosan (from shrimp shells, low-viscosity, degree of deacetylation >70%), and acetic acid (analytical grade, purity > 99.8%) were purchased form Sigma Aldrich, USA.

Experimental design

For evaluating the influence of both process parameters – polymer concentration and pump rate on the particles characteristics, a 3^2 full factorial design was applied.[7] Three different concentrations of the chitosan solution were selected: a low concentration of 1%, average concentration of 1.5% and high concentration of 2%. The rate of the peristaltic pump was also varied at three levels: low rate of 10%, medium rate of 14% and high rate of 18%. Varying the two factors at three different levels resulted in nine possible combinations.

Formulation of chitosan microparticles

Chitosan microparticles were prepared by spray drying technique using co-current flow type B-290 Mini Spray Dryer (Büchi Labortechnik AG, Flawil, Switzerland). Chitosan solutions (100 mL) of different concentration (1% w/v, 1.5% w/v, 2% w/v) were prepared by dissolving the polymer into an aqueous solution of acetic acid (2% v/v). Compressed (5 bar) nitrogen was used to disperse the chitosan solutions into fine droplets through a 0.7 mm nozzle. The droplets were consequently dried in the cylinder and the solid particles were collected in the cyclone at aspiration rate of 95%. The inlet temperature was kept at 140°C in order to preserve the polymer stability and to limit the excessive material loss during drying. The process was performed at 600 L/h compressed nitrogen flow rate, which was experimentally determined as optimum for the achievement of a production yield from the chitosan solutions.

Shape and surface morphology of the particles

For particles visualization an optical microscope (Leica DM2000 LED, Leica Microsystems, Germany) was used, equipped with digital camera (Leica DMC 2900) and software for processing images (Leica Application Suite, LAS). The samples were observed dry at 400x magnification. For studying the particles surface morphology, a scanning electron microscope with secondary electrons detection was used (Philips SEM 515, Eindhoven, The Netherlands). The micrographs were generated at 25 kV accelerating voltage and 5000x magnification.

Size and particle size distribution

The size of the formulated particles and their size distribution were determined by laser diffraction analyzer (LS 13 320, Beckman Coulter, USA), equipped with tornado system for powdered samples (Tornado Dry Powder System, DPS). For each measurement accurately weighed, pre-homogenized in an agate mortar samples of 100 mg were used.

Particle production yield

The yield of the formulated particles was calculated on the basis of the obtained particle mass (W1) and the total mass of the used polymer (W2), using the following equation: Yield (%) = (W1/W2)100.

Statistical analysis

All experiments were repeated three times and the results were expressed as mean values ± SD. Univariate analysis of variance (two-way ANOVA) was used for estimating the statistical significance of the individual studied factors and their combinations on the response.[8]

RESULTS

Microparticle models

Polymer concentration and peristaltic pump rate were varied at three different levels according to the applied 3^2 full factorial design resulting in the formulation of nine microspheres models **(Table 1A)**.

The models were obtained from chitosan solutions with concentration 1%, 1.5% and 2% at 10%, 14% and 18% pump rate **(Table 1B)**. The other parameters of the process were kept constant as described in Materials and methods section. The resulting particles from all of the models were

Table 1. Models of spray-dried chitosan microparticles, obtained by varying the process parameters polymer concentration and pump rate at three levels – low (-1), medium (0) and high (+1), using 3^2 factorial design

	A	A	B	B
Model	**Polymer concen-tration (level)**	**Pump rate (level)**	**Polymer concen-tration (%)**	**Pump rate (%)**
$MP_{1/10}$	-1	-1	1	10
$MP_{1/14}$	-1	0	1	14
$MP_{1/18}$	-1	+1	1	18
$MP_{1.5/10}$	0	-1	1.5	10
$MP_{1.5/14}$	0	0	1.5	14
$MP_{1.5/18}$	0	+1	1.5	18
$MP_{2/10}$	+1	-1	2	10
$MP_{2/14}$	+1	0	2	14
$MP_{2/18}$	+1	+1	2	18

characterized by their shape, size, size distribution and yield.

Shape and surface morphology of the particles

The light microscopy showed that the particles from all models were spherical in shape which was also confirmed by SEM. The obtained light and scanning electron micrographs of the microspheres are shown in **Fig. 1**. SEM revealed that the particles surface was not smooth and presence of multiple folds and grooves was clearly observed.

Size and particle size distribution of the models

The median particle diameter of the chitosan spheres was in the range from 2.585 µm to 3.646 µm **(Fig. 2A)**. Particles with such dimensions are considered appropriate for nasal administration.[9] Increase in the median particle diameter was established when the spray drying was carried out at a higher pump rate. The same tendency was observed when chitosan solutions with higher concentration were used. Respectively, particles of the smallest median size were obtained from 1% chitosan solution sprayed at 10% pump rate (model $MP_{1/10}$) and the largest median diameter was registered with 2% polymer solution and 18% pump rate (model $MP_{2/18}$). The combined influence of the two factors – polymer concentration and pump rate on the particle size is shown in **Fig. 2B**.

The laser diffraction analysis revealed bimodal size distribution of all models, expressed in a small fraction of particles with median size ranging from 0.4 µm to 1 µm, followed by a larger fraction with diameter varying between 1 µm and 15 µm.

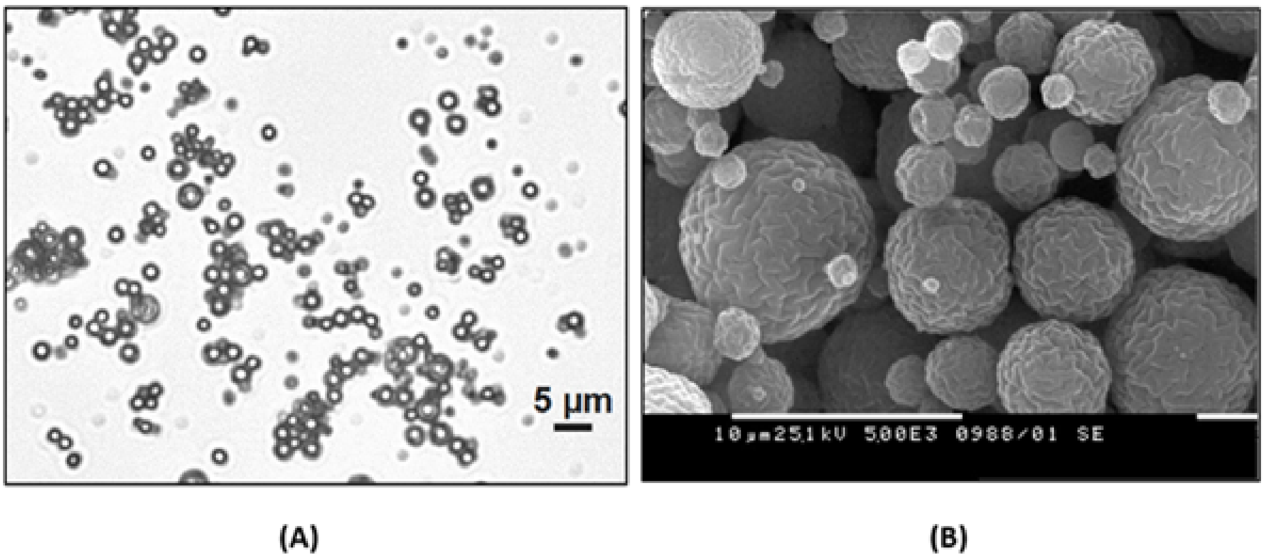


Figure 1. Micrographs of spray-dried chitosan microparticles, obtained by: A) light microscopy (400x) and B) scanning electron microscopy (5000x).

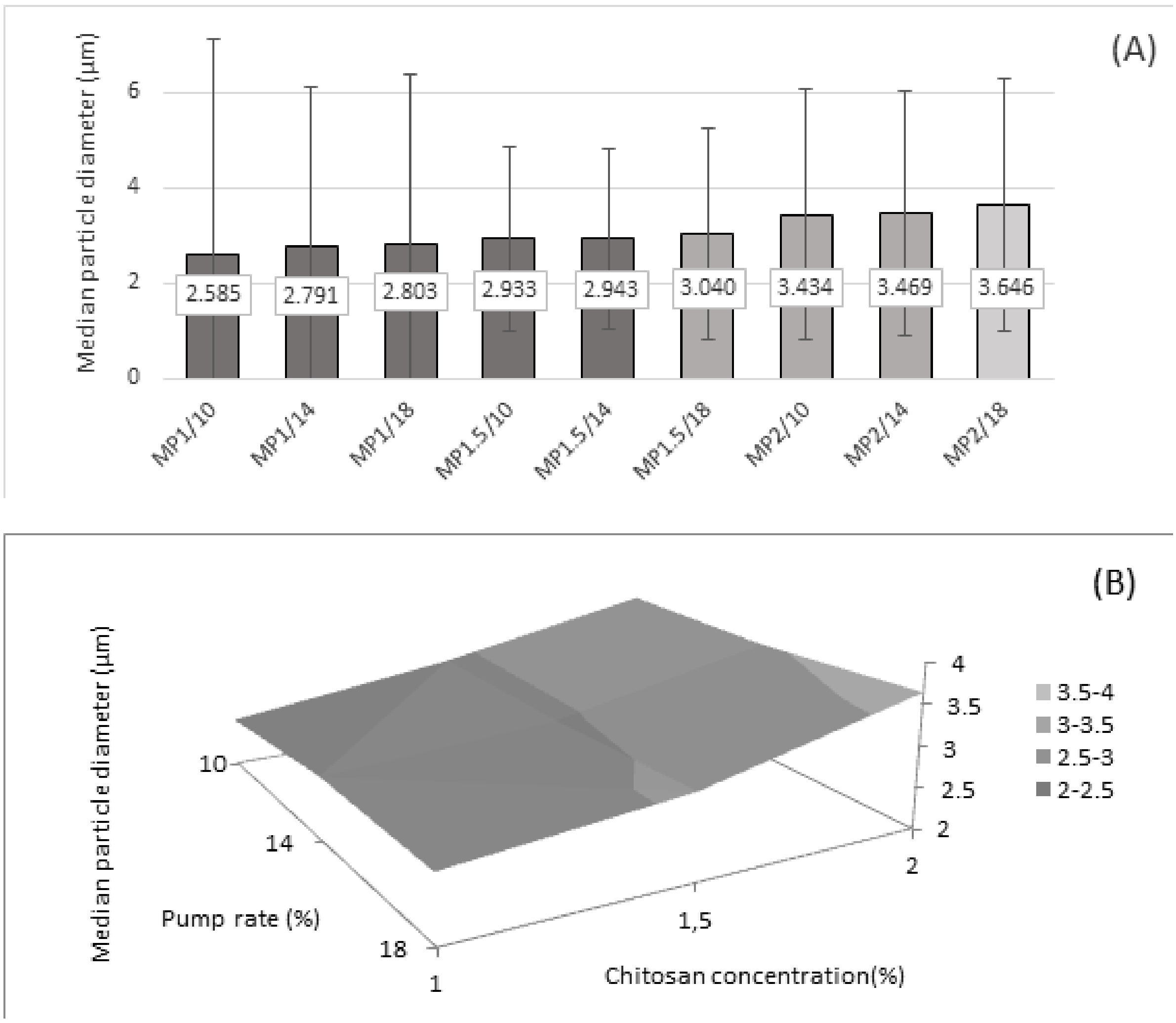


Figure 2. Particle diameter of the spray-dried chitosan models (A) and 3d plot of the combined influence of pump rate and chitosan concentration on particle size (B), (n=3).

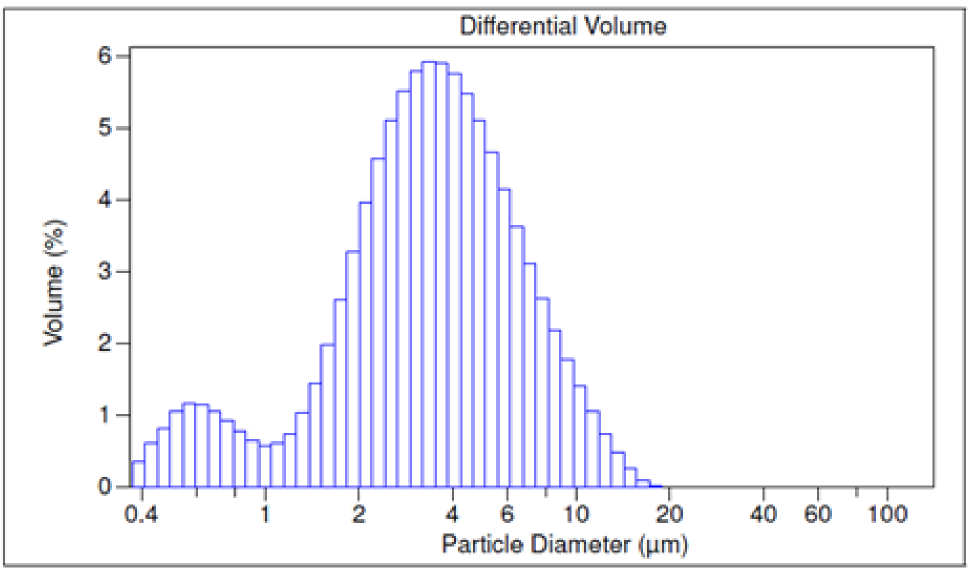


Figure 3. Particle size distribution curve of chitosan microspheres from model $MP_{2/10}$.

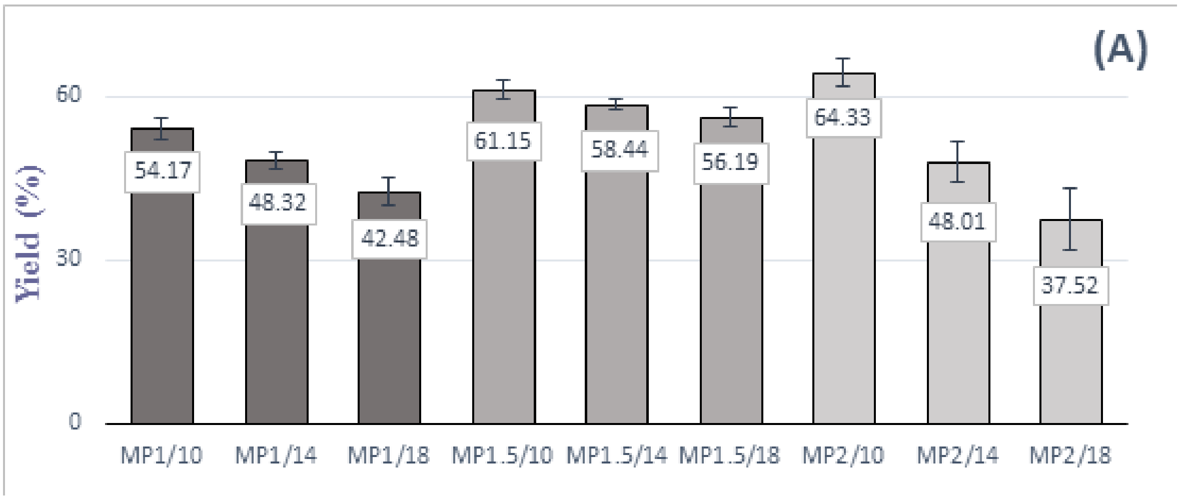
A typical curve of particle size distribution from the obtained microspheres models is shown in **Fig. 3**.

Production yield

The yield of the microspheres models varied between 37.52% and 64.33% **(Fig. 4A)**. Increasing the rate of the peristaltic pump from 10% to 14% and 18% resulted in decreased production yield. Such tendency was observed with all three concentrations of chitosan solutions. When increasing polymer concentrations from 1% to 1.5% and 2% at low pump rate of 10%, an increase of the yield was registered. However, such tendency was not observed at the average (14%) and high (18%) pump rate. The highest particles yield (64.33%) was obtained at low pump rate from 2% chitosan solution (model $MP_{2/10}$). The combined influence of the two factors – polymer concentration and pump rate on the production yield is shown in **Fig. 4B**.

The statistical significance of the two individual factors and their combinations on the particle yield was assessed. ANOVA analysis **(Table 2)** gave F-statistics 34.093 ($p<0.001$); 59.165 ($p<0.001$); 12.003 ($p<0.001$) for the concentration of the chitosan solution, the rate of the peristaltic pump and concentration*rate, respectively.

The coefficient of determination R^2 which showed the proportional variation in the response explained by the independent variables in the linear regression model was 0.929. The adjusted coefficient of determination $R^2_{Adj.}$ was 0.897. The value of R^2, which was close to 1, revealed that there was a considerable linear relationship between the factors



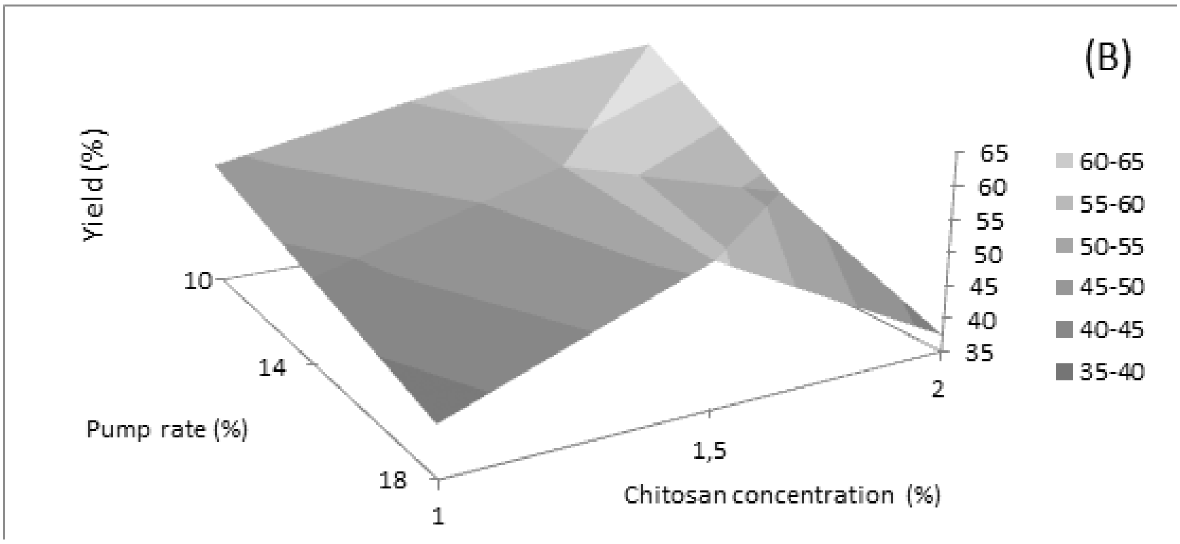


Figure 4. Yields of the spray-dried chitosan microspheres (A) and 3d plot of the combined influence of pump rate and chitosan concentrations on the production yield (B), (n=3).

Table 2. Analysis of the statistical significance of the investigated factors - chitosan concentration, pump rate and their combination on the particle yield (Tests of Between-Subjects Effects, ANOVA)

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (p-value)
Corrected model	1885.813[a]	8	235.727	29.316	.000
Intercept	73823.545	1	73823.545	9181.077	.000
Polymer concentration	548.277	2	274.139	34.093	.000
Pump rate	951.470	2	475.735	59.165	.000
Polymer concentration * Pump rate	386.065	4	96.516	12.003	.000
Error	144.735	18	8.041		
Total	75854.093	27			
Corrected total	2030.548	26			

[a] $R^2 = 0.929$; $R^2_{Adj.} = 0.897$

and the response. The value of $R^2 = 0.929$ showed that with almost 93% confidence, the change in the response could be explained with the variables of the model. Moreover, the slight difference between R^2 and $R^2_{Adj.}$ values showed that some insignificant conditions were included in the model.

DISCUSSION

The obtained results from varying polymer concentration and pump rate of the spray drying process revealed the impact of these parameters on particle size and production yield of the formulated chitosan microparticles. When enhancing the pump rate from 10% to 18%, a slight increase in particle size was noticed at all of the three varied chitosan concentrations. That could be explained by the greater amount of sprayed material through the nozzle per unit of time and the formation of larger droplets.[10] However, the lack of a significant change in median particle size is a reason to assume that pump rate had negligible influence on microspheres size in the investigated range. According to the published literature, the other evaluated parameter – polymer concentration, has a greater impact on microspheres size[11] and our results confirmed that. When polymer concentration was increased from 1% to 2% at the three different pump rates, particles of a larger size were formed. Very fine particles are generally not preferred for nasal administration because they can be easily inhaled into the lungs. Thus, chitosan solutions of 2% concentration were defined as optimum since the produced microspheres were of largest median diameter. Chitosan solutions with concentration higher than 2% were too viscous and could not be spray-dried due to the risk of clogging the nozzle of the apparatus.

Production yield, on other hand, gradually decreased when the pump rate was enhanced from 10% to 18% at all of the three varied chitosan concentrations. That could be explained by the thermodynamics of the process. The quicker the sample solution is sprayed, the more energy is needed to evaporate the solvent from the droplets and to form dry particles. That also corresponded to the lower outlet temperature of the spray dryer registered at higher pump rates. Therefore, the lowest investigated pump rate (10%) was determined as optimum for the preparation of chitosan microspheres by spray drying. The highest production yield (64.33%, $MP_{2/10}$) at the selected pump rate was obtained from 2% chitosan solution. At higher concentrations larger particles were formed, which were further more easily separated and collected as a final product.

Among all of the formulated chitosan microspheres, it was the model $MP_{2/10}$ (2% polymer concentration, 10% pump rate) that was determined as optimum regarding its greatest production yield and the appropriate for nasal administration particle size.

CONCLUSION

The individual and combined effects of two spray drying factors – polymer concentration and pump rate, on chitosan microspheres size and production yield were studied. A major interaction between the two process parameters was estimated, demonstrating the complexity of the spray drying process. The applied factorial design allowed the obtaining of microparticles at all possible factor combinations and recognizing the most appropriate model for our research. The optimized technology will be applied for the spray drying of drug-loaded chitosan microspheres in our future studies.

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Оптимизация способа получения микросфер хитозана методом распылительной сушки при помощи полного факторного эксперимента типа 3^2

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Введение: Как правило способ получения микросфер методом распылительной сушки сильно зависит от параметров процесса. Размер частиц и выход продукции в основном зависит от концентрации раствора для распыления и от скорости насоса распылительной сушилки.

Цель: Целью настоящего исследования является определение оптимальных параметров распылительной сушки – концентрации полимера и скорости насоса, необходимых для получения микросфер хитозана с высоким выходом продукции и размером частиц, подходящим для назального введения.

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Методы: Полный факторный эксперимент типа 3^2 был использован для изучения исследованных параметров. Были исследованы три различные концентрации хитозана : низкая концентрация (1 %), средняя концентрация (1.5 %) и высокая концентрация (2 %). Скорость перистальтического насоса тоже варьировала в рамках диапазона от 10% до 18 %: низкая скорость (10 %), средняя скорость (14 %) и высокая скорость (18 %).

Результаты: В ходе эксперимента получены девять моделей микросфер хитозана, которые были классифицированы в зависимости от формы, морфологии поверхности, размера, распределения частиц по размерам и выхода продукции. Полученные частицы из 2% раствора хитозана с 10 % скоростью распыления помпы характеризовались самым высоким показателем выхода продукции (64.33%) и подходящим средним диаметром для назального введения (3,434 µm).

Заключение: Оба исследованных параметра распылительной сушки взаимодействуют друг с другом и их воздействие на выход продукции и на размер микросфер хитозана следует рассматривать в совместности, а не в отдельности. Оцениваемые параметры процесса позволяют изготовление микросфер хитозана с высоким уровнем выхода продукции и нужными характеристиками (размер, распределение частиц по размерам и форма) для интраназального применения.