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Doxylamine/pyridoxine loaded chitosan microspheres as potential nasal drug delivery systems

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Dedicated to Acad. Bogdan Kurtev on the occasion of his 100th birth anniversary

Chitosan microspheres loaded with doxylamine and pyridoxine were formulated as potential drug delivery systems intended for nasal administration via spray drying technique. The possibility of incorporating the combination of these two drugs into one polymer matrix was studied. X-ray powder diffraction (XRPD) was applied to investigate possible transformations in the solid state of the drugs. A first-derivative ratio UV-spectrophotometric method for the assay of doxylamine in a binary mixture with pyridoxine was developed. The obtained particles were spherical in shape with median diameter ranging from 3.51 μm to 8.27 μm which is considered appropriate for nasal administration. The production yields (48.06%–66.97%) were recognized as optimum in regard to the spray drying method. The drug entrapment efficiency was high (87.36%–95.67%), indicating a tendency to increase with higher drug/polymer ratio. Drug release studies from the microspheres showed initial burst effect within the first 30 min, followed by a sustained release.

Key words: chitosan microspheres; doxylamine; pyridoxine; drug combination

INTRODUCTION

Drug delivery systems such as microspheres have gained serious popularity in the last few years due to the advantages they offer – enhanced drug stability, high carrier capacity and feasibility of variable routes of administration, including nasal application [1]. Different drugs have already been incorporated in microparticulate structures [2–4]. However, no information concerning microspheres loaded with doxylamine succinate and pyridoxine hydrochloride has been reported so far.

Doxylamine succinate (DOX), a first-generation antihistamine drug, and pyridoxine hydrochloride (PYR), a water-soluble vitamin, are two active substances, which have been well-known and used for the treatment of various pathological conditions. Their combination, on the other hand, is currently gaining serious interest because it is one of the few options on the pharmaceutical market, recently approved by FDA, as safe and effective for the treatment of morning sickness during pregnancy [5, 6]. So far, the drug combination is available only as orally administered dosage forms (tablets and capsules), though this route of administration is not generally recommended for symptoms such as nausea and vomiting. Nasal administration, on the other hand, has been considered as suitable alternative for achieving good drug absorption and systemic therapeutic effect [7, 8]. For nasal formulations mucoadhesive polymer carriers are often used to ensure optimum deposition of the drugs in the nasal cavity and hence, a more intimate contact with the lining mucosa, which is a prerequisite for improved bioavailability. Chitosan, due to its great mucoadhesive and permeation enhancing properties, is one of the most promising natural polymers used in the pharmaceutical practice. It is also non-toxic, biocompatible and biodegradable, which defines its high potential as a carrier in drug delivery systems [1, 9].

The aim of this study was to formulate and characterize chitosan microspheres loaded with the drug combination DOX/PYR as drug delivery systems intended for nasal administration.

EXPERIMENTAL

Materials

Chitosan (from shrimp shells, low-viscosity, degree of deacetylation >70%), acetic acid,

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doxylamine succinate and pyridoxine hydrochloride were purchased from Sigma Aldrich, USA. Phosphate-buffered saline (PBS) with pH 6.8 was prepared according to Ph. Eur. 8 [10]. All of the reagents used for the preparation of the PBS were of analytical grade.

Microspheres formulation

Chitosan microspheres were prepared by spray drying technique using co-current flow type B-290 Mini Spray Dryer (Büchi Labortechnik AG, Flawil, Switzerland). Drug/polymer solutions were prepared by dissolving an accurately weighed amount of chitosan, DOX and/or PYR in 2% (*v/v*) acetic acid aqueous solution. Drug/polymer ratio was varied. The solutions were further spray-dried through a 0.7 mm nozzle at 600 L/h compressed nitrogen flow rate, 140 ºC inlet temperature, 10% pump rate and 95% aspiration. These conditions were experimentally determined as optimum for obtaining satisfactory production yields from the spray-dried solutions.

Microspheres characterization

Shape, size and production yield

The shape and surface morphology of the prepared microspheres were studied using a scanning electron microscope with secondary electrons detection (Philips SEM 515, Eindhoven, the Netherlands). The micrographs were generated at 25 kV accelerating voltage and 5000x magnification. The size of the formulated particles and their size distribution were measured by laser diffraction analyzer (LS 13 320, Beckman Coulter, USA), equipped with a Tornado system for powdered samples. The production yield was calculated on the basis of the obtained particle mass and the total mass of the used polymer and drugs.

Drug loading and entrapment efficiency

Accurately weighed amount of 10 mg microspheres was dispersed into 20 mL acetic acid solution (2% *v/v*) and sonicated until complete dissolution of the polymer and extraction of the incorporated drugs occurred. The solution was then filtered through a membrane filter (Chromafil Xtra RC, 0.45 μm) and diluted with PBS (pH 6.8). The amount of DOX and PYR was determined spectrophotometrically. Drug loading (*DL*) and entrapment efficiency (*EE*) were calculated and expressed as average values ± SD after three determinations [11].

UV spectrophotometric assay

The quantitative determination of DOX and PYR was carried out in PBS (pH 6.8) using UV-visible spectrophotometer Evolution 300 (Thermo Fisher Scientific, USA) at wavelengths of 260 nm and 324 nm for DOX and PYR respectively. Due to spectral overlapping, a UV spectrophotometric first-derivative ratio method [12, 13] was developed for the quantitative analysis of DOX in binary mixtures with PYR. The developed methods were validated using mixtures of DOX and PYR and were characterized in terms of the regression parameters: root mean square error of prediction (*RMSEP*), relative error of prediction (*REP*), recovery and coefficient of determination (R^2).

X-ray powder diffraction

X-ray powder diffraction was used to evaluate the solid state structure of the microspheres. The diffractograms were obtained using X-ray powder diffractometer (D2 Phaser, Bruker AXS GmbH, Germany) under the following conditions: Ni-filtered Cu radiation at 30 kV and 10 mA of intensity. Phase analysis using Powder Diffraction, database, (ICDD) was performed for correct interpretation of the studied samples.

FTIR spectroscopy

Infrared spectroscopic study of the formulated microspheres and evaluation of eventual drug/polymer interactions after spray drying was performed using Nicolet iS 10 FTIR spectrometer (Thermo Fisher Scientific, Pittsburgh, PA, USA), equipped with a diamond ATR accessory in the range 650–4000 cm^{-1}.

In vitro drug release

In vitro drug release study was carried out using USP dissolution apparatus I - rotating basket (AT7 Sotax, Allschwil, Switzerland) containing 400 mL medium of PBS (pH 6.8) maintained at temperature of 37±0.5 °C and rotation speed of 50 rpm. Accurately weighed amount of microspheres, equivalent to 10 mg drug content was dispersed into 1 mL medium and transferred in a dialysis membrane bag with the two ends fixed by thread. The bags were then placed in the baskets and were brought into contact with the acceptor medium.

Samples of 5 mL were withdrawn from the solution at fixed time intervals and were replaced with the same amount of fresh PBS. Samples were filtered through a syringe membrane filter (0.45 μm) and analyzed spectrophotometrically for drug content. The release study for each microsphere model was repeated three times.

RESULTS AND DISCUSSION

Microspheres composition and characteristics

Five models of drug loaded microspheres were developed, varying drug concentration and drug/polymer ratio (Table 1). Models D1 and D2 were formulated with DOX, while models P1 and P2 were formulated with PYR. A single model was developed containing the drug combination DOX/PYR (DP). The aim was to investigate the influence of the varied drug concentration (1%–2% *w/v*) and the drug/polymer ratio (0.5:1 and 1:1 *w/w*) on the drug incorporation and microsphere characteristics.

SEM micrographs of the obtained microspheres are presented in Fig. 1. The particles had spherical shape and relatively smooth surface. However, a tendency for roughness was observed when the drug concentration of the spray-dried solutions decreased from 2% to 1 % *w/v* (D1, P1).

The different microsphere models had median particle diameter from 3.51 to 8.27 μm (Table 1). Such size was considered appropriate for nasal administration [14]. Particle size distribution curves of the models are shown in Fig. 2. The presence of structures larger than 20 μm was probably due to particle aggregation.

Production yields varied between 48.06% and 66.97% (Table 1) and were considered as optimum in regard to the typical for the production method extensive losses at laboratory scale [15]. The drug/polymer ratio did not influence the yield of DOX-loaded microspheres (48.06% and 48.12% production yields at ratios 0.5:1 and 1:1 *w/w* respectively). With the PYR models, however, much higher yield was obtained at higher drug/polymer ratio (1:1 *w/w*), respectively at higher PYR concentration. When more concentrated solutions were spray dried, larger particles were formed. Larger microspheres were more easily separated and collected as a final product, which diminished losses and resulted in a greater yield. The larger particle size of PYR microspheres (5.12 μm for P1 and 8.27 μm for P2) was probably the reason for the higher production yield of these models in comparison to DOX-loaded microspheres which were much smaller (4.54 μm for D1 and 3.51 μm for D2). The larger size of the PYR microspheres, on the other hand, was probably due to drug crystallization in the particles, which was confirmed by the XRPD analysis.

Table 1. Composition and physicochemical properties of DOX and PYR-loaded chitosan microspheres.

Formulation code	Concentration (%, w/v)			Drug/polymer ratio (*w/w*)	Median particle size (μm±SD)*	Yield (%±SD)*
	DOX	PYR	Chitosan			
D1	1	-	2	0.5:1	4.54 ± 0.48	48.06 ± 3.27
D2	2	-	2	1:1	3.51 ± 0.15	48.12 ± 4.30
P1	-	1	2	0.5:1	5.12 ± 0.67	60.29 ± 1.93
P2	-	2	2	1:1	8.27 ± 1.19	66.97 ± 1.79
DP	1	1	2	0.5:0.5:1	4.70 ± 0.72	53.63 ± 3.42

*n=3

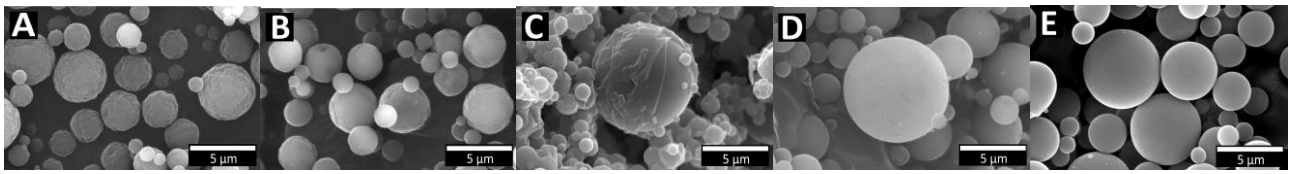

Fig. 1. SEM images of microspheres models: D1 (A), D2 (B), P1 (C), P2 (D), DP (E) (5000x).

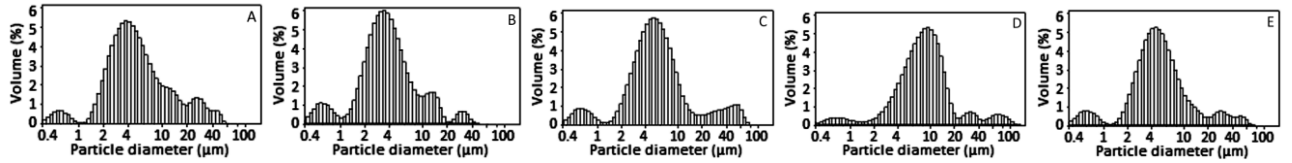

Fig. 2. Particle size distribution of microspheres models D1 (A), D2 (B), P1 (C), P2 (D), DP (E).

Table 2. Drug loading (*DL*) and entrapment efficiency (*EE*) of DOX-, PYR- and DOX/PYR-loaded chitosan microspheres.

Models	Drugs	Theoretical drug content (%)	DL ± SD (%)*	EE ± SD (%)*
D1	DOX	33.33	29.48 ± 0.50	88.43 ± 1.50
D2	DOX	50.00	43.68 ± 0.36	87.36 ± 0.73
P1	PYR	33.33	30.02 ± 0.92	90.09 ± 2.76
P2	PYR	50.00	44.71 ± 0.90	89.41 ± 1.79
DP	DOX + PYR	50.00 (25.00; 25.00)	47.83 ± 0.99 (23.40 ± 0.61;24.43 ± 0.40)	95.67 ± 1.97 (93.60 ± 2.43; 97.73 ± 1.62)

*n=3;

Drug loading and entrapment efficiency

The obtained microspheres models showed *DL* from 29.48% to 47.83% and *EE* from 87.36% to 95.67% (Table 2). *DL* was strongly influenced by drug/polymer ratio. Increase in that ratio in DOX-loaded microspheres resulted in higher *DL* (from 29.48% in D1 to 43.68% in D2). However, *EE* at different drug/polymer ratios were commensurable (88.43% and 87.36%). Such tendency was also observed for the PYR-loaded microspheres. *DL* increased from 30.02% (P1) to 44.71% (P2) and *EE* was again high (90.09% and 89.41%, respectively). Therefore, for the formulation of DOX/PYR-loaded microspheres drug/polymer ratio of 1:1 rather than 0.5:1 *w/w* was preferred, because it led to higher *DL* for both DOX and PYR.

Beside achieving high *DL*, another challenging issue was to optimize DOX/PYR ratio in solution so that equal amounts of both drugs could be incorporated in the obtained DOX/PYR-loaded microspheres (DP), corresponding to the equal therapeutic doses of the drugs (10 mg DOX and 10 mg PYR) [5, 6]. The models loaded with DOX and PYR separately showed similar *DL* at a particular drug/polymer ratio (29.48% DOX and 30.02% PYR at 0.5:1 *w/w* ratio; 43.68% DOX and 44.71% PYR at 1:1 *w/w* ratio). For that reason, a 1:1 *w/w* DOX/PYR ratio was selected for the preparation of model DP. According to the obtained results for DP model, both drugs were incorporated in the microspheres at equal amounts (23.40% *DL* for DOX and 24.43% *DL* for PYR) which conformed to the primary aim.

As a result, an optimum DOX/PYR/chitosan ratio of 0.5:0.5:1 *w/w/w* was determined for the simultaneous incorporation of DOX and PYR in chitosan microspheres.

UV-spectroscopic quantitative analysis

In binary mixtures of the two drugs, PYR was successfully determined at a wavelength of 324 nm (PYR absorption maximum) without any interference from DOX, using absorption spectra according to the following equation:

$$Y_1 = 0.0352 \times C_1 + 0.0020$$

where Y_1 is the absorption value at 324 nm and C_1 is PYR concentration in μg mL^{-1}.

However, due to intense overlapping of DOX and PYR spectra at 260 nm (DOX absorption maximum), a quantitative method for DOX analysis in binary mixtures with PYR was developed, using first derivative of ratio spectra technique. The recorded spectra of DOX were divided by the normalized absorption spectrum of PYR and first derivative of the ratio spectra were obtained. The amplitudes of the derivative ratio values at 271 nm for DOX were selected and plotted against the corresponding concentration of the standard solutions (Fig. 3).

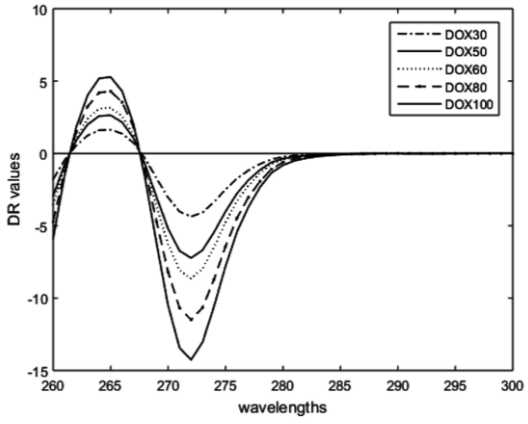

Fig. 3. First derivative ratio spectra of DOX in the range of 30–100 μg mL^{-1} using PYR (10 μg mL^{-1}) as divisor.

The normalized spectrum of 10 μg mL^{-1} PYR solution was determined as an optimum divisor in the proposed method. The following calibration equation for DOX was calculated:

$$Y_2 = 0.0753 \times C_2 + 0.1338$$

where Y_2 is the peak amplitude at 271 nm and C_2 is DOX concentration in μg mL^{-1}.

The proposed quantitative spectrophotometric methods were validated using laboratory-prepared mixtures of DOX and PYR with different concentrations within the determined linearity range of 30–100 μg mL^{-1} for DOX and 10–30 μg mL^{-1} for PYR. The obtained regression parameters were: 1.1679 *RMSEP*, 3.07% *REP*, 101.54±2.41 recovery, 0.9986 R^2 for DOX and 0.1312 *RMSEP*, 0.97% *REP*, 100.57±1.22 recovery, 0.9998 R^2 for PYR.

X-ray powder diffraction analysis

The physical state of DOX and PYR in drug loaded microspheres was evaluated by X-ray powder diffraction. Diffractograms of the pure substances and the formulated microspheres are presented on Fig. 4.

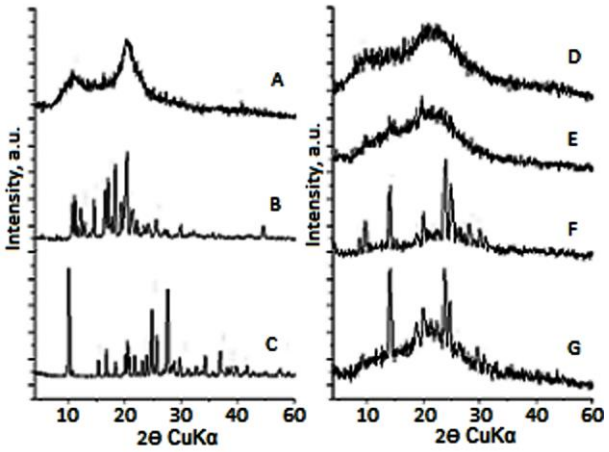

Fig. 4. X-ray powder diffractograms of chitosan (A), DOX (B), PYR (C), chitosan microspheres (D), DOX-loaded chitosan microspheres (E), PYR-loaded chitosan microspheres (F) and DOX-PYR-loaded chitosan microspheres (G).

DOX and PYR diffractograms showed a few characteristic peaks, which proved their crystalline structure. No distinctive peaks were registered in chitosan diffractogram, which indicated its amorphous nature. The microspheres diffractograms revealed that chitosan retained its amorphous structure after spray drying. For the active substances on the other hand, a transition from crystalline into amorphous state was registered. DOX-loaded microspheres diffractograms showed just a few small peaks and amorphous phase corresponding to that of chitosan. PYR-loaded microspheres diffractograms were of a similar pattern. However, a phase corresponding to crystalline PYR and PYR hydrochloride was registered. Such a phase was also noted in the diffraction spectra of DOX/PYR-loaded microspheres. In the latter model, DOX was amorphous. Therefore, both crystalline drugs underwent transformation into amorphous state after spray drying with PYR partially retaining its crystalline structure.

FTIR spectroscopy

The spectra of the chitosan microspheres loaded with DOX or PYR are shown in Fig. 5. In the spectrum of DOX/PYR-loaded microspheres increased intensity of the absorption peaks at 3000–3600 cm^{-1} was observed due to the overlapping of stretching vibrations of the O-H groups of chitosan and pyridoxine, N-H bonds, and C-H bonds of the aromatic rings. Increased intensity was observed also in the region of 1620–1500 cm^{-1} and 1450–1400 cm^{-1} due to the overlapped vibrations of the aromatic rings of pyridine and benzene with the vibrations of the amide N-H bonds and protonated amino groups of chitosan. In the range 1100–1025 cm^{-1} peaks of symmetric and asymmetric stretching vibrations of C=O, C-O-C and CH_2-OH from the monosaccharide ring were observed, as well as wagging C-H vibrations of the monosaccharide ring of chitosan.

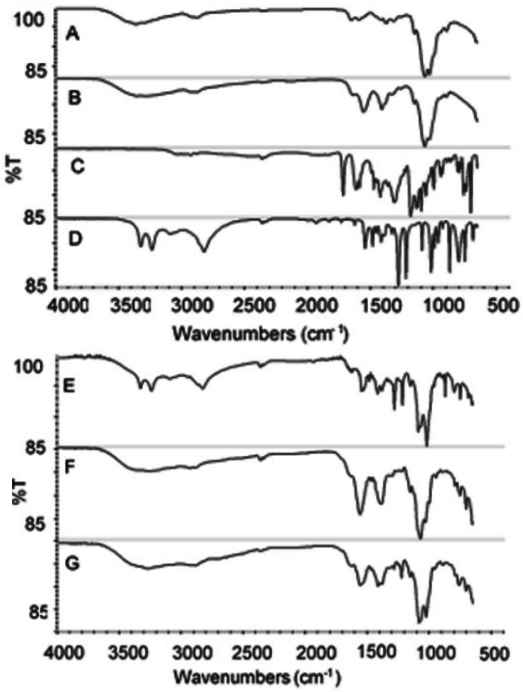

Fig. 5. FTIR-ATR spectra of chitosan (A), chitosan microspheres (B), DOX (C), PYR (D), PYR-loaded chitosan microspheres (E), DOX-loaded chitosan microspheres (F), and DOX-PYR-loaded chitosan microspheres (G).

The observed spectra showed that there were no covalent interactions either between chitosan and DOX or PYR, or between DOX and PYR.

In vitro release study

Dissolution profiles of the microspheres showed that DOX and PYR were released from all of the formulated models in a biphasic way, with initial rapid drug release (burst effect) followed by slower release (Fig. 6). Within the first 30 min 53.32% to 68.96% of the loaded DOX and PYR were released, which was probably due to the immediate dissolution of the molecules incorporated in the particles' periphery. After the initial burst release, prolonged drug release was observed, which was probably due to formation of a gel layer from the swollen chitosan and diffusion of the drugs from the particles core through it [16]. The total drug amount incorporated in the microspheres was completely released within 180 minutes in all investigated formulations. That could be explained with the high aqueous solubility of DOX and PYR [10] and their facilitated diffusion through the swollen polymer matrix and its pores, formed after the initial drug release.

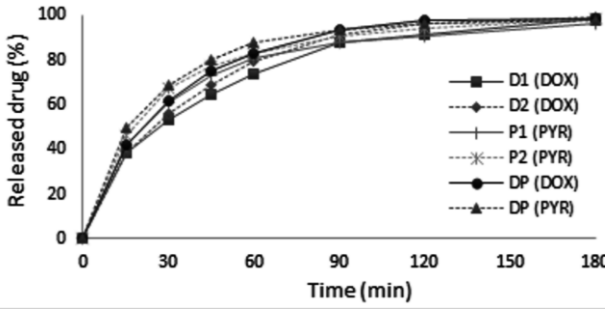

Fig. 6. Dissolution profiles of DOX and PYR from microspheres models D1, D2, P1, P2 and DP. (n=3).

CONCLUSION

Doxylamine and pyridoxine loaded chitosan microspheres of appropriate for nasal administration size were prepared by a conventional spray drying method. The two drugs were incorporated into the obtained particles with high entrapment efficiency. The influence of drug/polymer ratio on the particles drug loading was studied and an optimum model, loaded both with DOX and PYR was proposed as a potential drug delivery system for nasal administration. The registered burst release from the microspheres could be considered as positive, assuring sufficient initial therapeutic drug concentration to alleviate the treated symptoms. On the other hand, further delay of the drug release should be accomplished, which would be a matter of future studies, in order to reduce the dosage frequency.

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REFERENCES

1. A. Mitra, B. Dey, *Indian J. Pharm. Sci.*, **73**, 355 (2011).
2. Y. Jiao, X. Pang, M. Liua, B. Zhanga, L. Li, G. Zhai, *Colloids Surf., B*, **140**, 361 (2016).
3. T. K. Giri, C. Choudhary, Ajazuddin, A. Alexander, H. Badwaik, D. K. Tripathi, *Saudi Pharm. J.*, **21**, 125 (2013).
4. S. B. Patil, K. K. Sawant, *Curr. Drug Deliv*., **5**, 312 (2008).
5. S. R. Slaughter, R. Hearns-Stokes, T. van der Vlugt, H. V. Joffe, *N. Engl. J. Med*., **370**, 1081 (2014).
6. N. Nuangchamnong, J. Niebyl, *Int. J. Womens Health*, **6**, 401 (2014).
7. A. Fortuna, G. Alves, A. Serralheiro, J. Sousa, A. Falcão, *Eur. J. Pharm. Biopharm.*, **88**, 8 (2014).
8. M. Alagusundaram, B. Chengaiah, K. Gnanaprakash, S. Ramkanth, C. M. Chetty, D. Dhachinamoorthi, *Int. J. Res. Pharm. Sci.*, **1**, 454 (2010).
9. V. R. Sinha, A. K. Singla, S. Wadhawan, R. Kaushik, R. Kumria, K. Bansal, S. Dhawan, *Int. J. Pharm.*, **274**, 1 (2004).
10. European Pharmacopoeia Commission, European Pharmacopoeia 8th ed., Council of Europe, Strasbourg, 2014.
11. A. Saigal, W. K. Ng, R. B. Tan, S. Y. Chan, *Int. J. Pharm*., **450**, 114 (2013).
12. H. A. Merey, *Bull. Fac. Pharm. Cairo Univ.*, **54**, 181 (2016).
13. H. W. Darwish, S. A. Hassan, M. Y. Salem, B. A. El-Zeiny, *Spectrochim. Acta Mol. Biomol. Spectrosc.*, **83**, 140 (2011).
14. B. Pilicheva, P. Zagorchev, Y. Uzunova, M. Kassarova, *Int. J. Drug Delivery*, **5**, 389 (2013).
15. M. Maurya, K. Murphyb, S. Kumarb, L. Shib, G. Leea, *Eur. J. Pharm. Biopharm.*, **59**, 565 (2005).
16. M. Jelvehgari, D. Hassanzadeh, F. Kiafar, B. D. Loveymi, S. Amiri, *Iran J. Pharm. Res.*, **10**, 457 (2011).

МИКРОСФЕРИ ОТ ХИТОЗАН С ДОКСИЛАМИН И ПИРИДОКСИН – ПОТЕНЦИАЛНИ ЛЕКАРСТВО-ДОСТАВЯЩИ СИСТЕМИ ЗА НАЗАЛНО ПРИЛОЖЕНИЕ

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(Резюме)

Целта на настоящата работа е получаване и охарактеризиране на микросфери от хитозан, натоварени с доксиламин и пиридоксин, като потенциални лекарство-доставящи системи за назално приложение. Проучена е възможността за едновременното включване на двете лекарствени вещества (ЛВ) в обща полимерна матрица. Микросферите са получени по метода разпръсквателно сушене при вариране концентрацията на ЛВ и съотношението ЛВ/хитозан. Съвместимостта и физичното състояние на лекарствените вещества и полимера са проучени чрез инфрачервена спектроскопия и прахова рентгенова дифракция. За определяне количественото съдържание на доксиламин и пиридоксин в смес е разработен спектрофотометричен метод чрез производна спектроскопия. Получените микрочастици имат сферична форма. Средният диаметър е в границите 3.51 µm–8.27 µm и е подходящ за назално въвеждане. Добивът (48.06%–66.97%) се счита за оптимален предвид особеностите на приложената техника на получаване. Ефективността на включване на ЛВ в микросферите е висока (87.36%–95.67%), като се отчита тенденция към повишаване с увеличаване съотношението ЛВ/полимер. Профилите на освобождаване показват „бърст“ ефект през първите 30 мин, последван от забавено освобождаване.