ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in
A. N. Pavlov et al /J. Pharm. Sci. & Res. Vol. 10(6), 2018, 1457-1460

The Research of Physicochemical Properties and Determination of Nano-L-DOPA Quality Attributes Based on PLGA Nanoparticles for the Treatment of Parkinson's Disease

A. N. Pavlov[1], N. V. Pyatigorskaya[1], G. E. Brkich[1], S. A. Kedik[2], A. V. Panov[2]

[1] *I. M. Sechenov First Moscow State Medical University, Trubetskaya Street, 8-2, Moscow, Russian Fed., 119991*
[2] *MIREA -Russian Technological University, Prospekt Vernadskogo, 78, Moscow, Russian Fed., 119454*

Abstract

The experimental study uses a modern approach to the pharmaceutical development of innovative forms of L-Dopa by incorporating nanosomal L-Dopa in biodegradable polymer PLGA nanoparticles and using the promising nasal route of administration alternative to the oral one in the treatment of Parkinson's disease. PLGA nanoparticles provide for the stability of L-Dopa and increase bioavailability as a result of its controlled and sustained release. Nano-L-Dopa based on PLGA nanoparticles contains the following components: L-Dopa, PLGA polymer, D-mannitol, polyvinyl alcohol. The work is devoted to studying of the physicochemical properties, the definition of quality attributes and methods of analysis of Nano-L-Dopa based on PLGA-nanoparticles for nasal treatment of Parkinson's disease. The content of L-Dopa and its impurities in the obtained experimental Nano-L-Dopa samples has been quantified. The identification identificationof PLGA nanoparticles has been established. The obtained results allow to standardize the Nano-L-Dopa and explore its stability during storage.

Keywords: Nano-L-DOPA, Nanoparticles, PLGA, Parkinson's disease, nasal administration, physicochemical properties, drug quality attributes.

INTRODUCTION

Many people in the world suffer from Parkinson's disease (PD), with up to 210 thousand patients registered in Russia. At the same time, the number of cases and the number of patients aged 30 to 40 years is steadily growing [1, 2].

L-DOPA is the drug of choice in the PD treatment. Traditional oral drug formulations provide for low bioavailability of L-DOPA, therefore they are used repeatedly throughout the day to maintain a constant level of the drug in the blood [3, 4].

The creation of innovative forms of L-DOPA is aimed at increasing its bioavailability and efficiency by integrating the pharmaceutical substance in polymeric nanoparticles with the controlled release and using the nasal administration route [5, 6, 7]. The methods for the preparation of nanosomal L-DOPA (Nano-L-DOPA) based on a biodegradable copolymer of lactic and glycolic acids (PLGA) are known [6, 7]. High efficiency and safety of Nano-L-DOPA in the nasal administration have been well studied in the in vivo experiment on the model of induced PD [8, 9].

The study on the physicochemical properties of Nano-L-DOPA with a view to standardizing the innovative medicinal products and further exploring its stability during storage is of undoubted interest.

The scope of this research is to study the physicochemical properties and quality attributes for Nano-L-Dopa.

MATERIALS AND METHODS

The following components were used in the preparation of Nano-L-Dopa: L-DOPA (3,4-dihydroxy-L-phenylalanine, L-DOPA> 98%, USP36, "Sigma-Aldrich", USA); Poly (lactic-co-glycolic acid) PLGA 50/50 (Poly (DL-lactide-co-glycolide) nominal, Ester Terminated, Inherent Viscosity 0.37 dL/g, USP36; LACTEL® Absorbable Polymers International, USA); polyvinyl alcohol (87-90% hydrol., average mol. wt 30,000-70,000, "Sigma-Aldrich", USA); D-Mannit (D-Mannitol> 98%, "Fluka", USA).

The trial series of Nano-L-Dopa were developed in the laboratory by incorporating micronized L-Dopa into biodegradable polymeric PLGA nanoparticles using the modified technology of Zhou Y.Z et al. (2013) and G.G. Barsegyan et al. (2014) [6, 7]. Nano-L-Dopa is a lyophilized powder containing L-Dopa, polyvinyl alcohol and D-mannitol in the PLGA 50/50 polymer matrix.

The visual and organoleptic methods were used when describing the appearance, color, and odor of Nano-L-Dopa. The size of nanoparticles was defined using laser diffractometry, the pH was measured using the potentiometric method, and the water content was defined by the loss in weight when drying. Solubility and sedimentation resistance were investigated using the OFS.1.2.1.0005.15 and OFS.1.4.1.0014.15 official procedures of the State Pharmacopoeia of the Russian Federation, 8th edition.

To determine the identification and quantitative content of L-Dopa, the high-performance liquid chromatography (HPLC) method was used. Chromatographic conditions are as follows: the column - Zorbax Eclipse XDB-C18 (150×4.6 mm; 5 µm); the mobile phase consists of a mixture of A and B solutions (solution A - phosphate buffer, pH = 2.54, solution B - acetonitrile); the column temperature - 30 °C; flow rate - 0.8 ml/min; detector - UV 280 nm; the volume of the injected sample - 10 µl; the sample temperature - 4 °C; the run time - 35 min; and the L-Dopa retention time - about 8 min. The retention time of the main peak in the chromatogram of the test solution should correspond to the retention time of the main peak in the chromatogram of the solution of the L-Dopa standard sample.

The percentage of L-Dopa (X) on anhydrous basis, which does not contain residual organic solvents, is calculated by the following formula:

$$X = \frac{S_1 \times a_0 \times 10 \times P}{S_0 \times 10 \times a_1 \times 100} = \frac{S_1 \times a_0 \times P}{S_0 \times a_1 \times 100}, \quad (1)$$

where: S_1 is the area of the L-Dopa peak on the chromatogram of the test solution;
S_0 is the area of the L-Dopa peak on the chromatogram of the standard L-Dopa solution;
a_1 is the weight of the drug used to prepare the test solution, in mg;
a_0 is the weight of the standard L-Dopa sample, in mg;
P is the content of the active substance in the standard L-Dopa sample, as a percentage.

Foreign impurities were analyzed by HPLC method together with the quantification of L-Dopa. The quantitative content of impurities (X) as a percentage, calculated on an anhydrous substance basis that does not contain residual organic solvents, is calculated by the following formula:

$$X = \frac{S \times C_0 \times 200 \times 100}{S_0 \times 1000 \times a} \quad (2)$$

where:

S is the area of the peak of the impurity to be determined or the sum of the areas of the impurity peaks on the chromatogram of the test solution;

S_0 is the area of the L-Dopa peak on the chromatogram of the standard solution;

C_0 is the concentration of the standard L-Dopa solution, in μg/ml;

a is the test sample of the drug calculated on an anhydrous substance basis that does not contain residual organic solvents, in g.

The copolymer of lactic and glycolic acids (PLGA 50/50) was determined by IR spectroscopy. The solids are analyzed in the solid state, dispersed in a suitable liquid in the form of the suspension or by forming a film from the molten mass between two plates, transparent to infrared radiation. The infrared spectrum of the drug tested according to the position of absorption bands must match the IR spectrum of the standard PLGA 50/50 copolymer sample.

RESULTS

In the laboratory, three trial Nano-L-Dopa series have been developed with the following composition per 100 g, % by weight:

- L-Dopa – 9.5-10.0;
- PLGA 50/50 polymer - 79.0 – 80.0;
- D-mannitol - 8.0 - 8.3; and
- Polyvinyl alcohol -1.7 – 3.5 (the rest, up to 100.0).

The results of the study of the physicochemical properties of Nano-L-Dopa are shown in Table 1. Nano-L-Dopa is an amorphous lyophilized, white or light white, hygroscopic, photosensitive, odorless powder. It is soluble in dimethylsulfoxide and dimethylformamide, and poorly soluble in ether, hexane, 96% alcohol; and it forms a suspension with water. The size of PLGA nanoparticles in the main fraction of the aqueous suspension of Nano-L-Dopa is in the range from 407 to 434 nm. Shaking Nano-L-Dopa in purified water (15 mg/10 ml) forms a homogenous suspension free of agglomerates , which is stable for 17-25 min. The pH value of 1% aqueous suspension of Nano-L-Dopa is in the range of 5.73-5.86. The water content in the Nano-L-Dopa test samples does not exceed 0.22%. The quantitative content of L-Dopa in Nano-L-Dopa is 9.10-9.54%.

The determination of related compounds is an important quality attribute of pharmaceutical substances. The related compounds regulated for L-Dopa are shown in Table 2 and include chemical compounds, such as (2S)-2-amino-3-(2,4,5-trihydroxyphenyl) propanoic acid (impurity A), (2S)-2-amino-3-(4-hydroxyphenyl) propanoic acid (L-tyrosine) (impurity B), and (2RS)-2-amino-3-(4-hydroxy-3-methoxyphenyl) propanoic acid (3-methoxy-L-tyrosine) (impurity C). The quantitative content of related compounds was determined simultaneously with L-Dopa by HPLC, and the results are shown in Table 3.

Table 1. Physicochemical properties of Nano-L-Dopa based on PLGA nanoparticles.

Attribute	Series NLD-3	Series NLD-4	Series NLD-5
Description	An amorphous lyophilized, white or light white, hygroscopic, photosensitive, odorless powder.		
Solubility	It is soluble in dimethylsulfoxide and dimethylformamide, and poorly soluble in ether, hexane, 96% alcohol; it forms a suspension with water.		
Content L-Dopa, %	9.52 (± 0.07)	9.54 (± 0.08)	9.10 (± 0.06)
Particle size, nm	434 (± 10)	407 (± 10)	417 (± 10)
Sedimentation stability, min	25.0 (± 1.0)	17.0 (± 0.5)	21.5 (± 0.8)
pH of 0.1% aqueous suspension	5.73 (± 0.02)	5.78 (± 0.02)	5.86 (± 0.02)
Water content, %	0.22 (± 0.01)	0.180 (± 0.02)	0.20 (± 0.01)

Table 2. Related compoundsof L-Dopa.

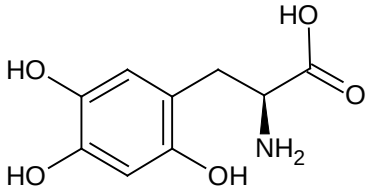
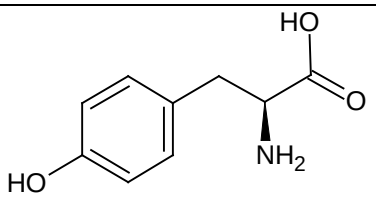
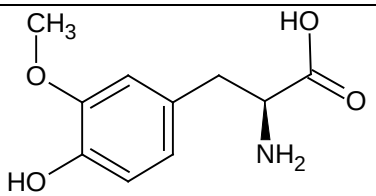
Name	Chemical name	Structural formula
Impurity A	(2S)-2-amino-3-(2,4,5-trihydro-xiphenyl) propanoic acid	
Impurity B	(2S)-2-amino-3-(4-hydroxyphenyl) propanoic acid (L-tyrosine)	
Impurity C	(2RS)-2-amino-3-(4-hydroxy-3-methoxyphenyl) propanoic acid (3-methoxy-L-tyrosine)	

Table 3. Results of quantitative assay of L-Dopa and impurities in Nano-L-Dopa.

Attribute	Nano-L-Dopa Series	Quantitative content, %	Relative standard deviation, $\frac{S}{\overline{x}} \cdot 100$, %	Relative error of mean value, % $\frac{\overline{x} - \mu}{\overline{x}} \cdot 100$
L-Dopa	NLD-3	9.52	1.01	0.69
	NLD-4	9.54	1.15	0.79
	NLD-5	9.10	0.88	0.60
Impurity A	NLD-3	0.132	4.11	2.94
	NLD-4	0.441	2.30	1.64
	NLD-5	0.340	4.24	3.03
Impurity B	NLD-3	0.315	3.64	2.60
	NLD-4	0.088	3.20	2.29
	NLD-5	0.298	5.10	3.65
The amount of impurities	NLD-3	0.792	3.04	1.15
	NLD-4	0.959	2.90	2.07
	NLD-5	1.139	4.05	2.90

Table 4. Quality attributes and analysis methods of Nano-L-Dopa.

Attribute	Method	Quality requirements (standard)
1	2	3
Description	Organoleptic Visual	An amorphous lyophilized, white or light white, hygroscopic, photosensitive, odorless powder.
Solubility	SF XIII, GPA.1.2.1.0005.15	Soluble in dimethylsulfoxide and dimethylformamide, and poorly soluble in ether, hexane, 96% alcohol; forms a suspension with water.
Identification (2S)-2-Amino-3- (3,4-dihydroxyphenyl) propanoic acid 3,4-dihydroxy-L-phenylalanine (L-Dopa)	A. HPLC	The retention time of the main peak on the chromatogram of the test solution should correspond to the retention time of the main peak in the chromatogram of the solution of the standard L-Dopa sample
A copolymer of lactic and glycolic acids 50/50 (PLGA 50/50)	B. IR spectroscopy	The infrared spectrum of the substance tested according to the position of absorption bands must match the IR spectrum of the standard PLGA 50/50 copolymer sample.
Particle size	Method of laser diffractometry	In the suspension (0.2 mg/ml), not less than 90% of the main fraction of the particles should be no larger than 500 nm
Sedimentation resistance	National pharmacopeia XIII, general monographOFS.1.4.1.0014.15	When shaking L-Dopa in water (15 mg/10 ml), a homogeneous, agglomerate-free suspension is formed that is stable for at least 15 minutes
pH	Potentiometrically	from 4.5 to 7.0 (0.1 % suspension)
Foreign impurities	HPLC	Any single impurity - not more than 1.0%; total impurities - not more than 3.0%
Water	Loss in mass upon drying	below 1.0 %
L-Dopa quantification	HPLC	At least 9.0% and below 11.0% of L-Dopa in terms of an anhydrous and organic solvent-free substance

The content of (2S)-2-amino-3-(2,4,5-trihydroxyphenyl) propanoic acid impurity was 0.132-0.441% with a relative standard deviation of 2.30-4.24% and a relative error of the mean value of 1.64-3.03%. The impurity content of (2S)-2-amino-3-(4-hydroxyphenyl) propanoic acid (L-tyrosine) impurity was in the 0.088-0.315 concentration range at a relative standard deviation of 3.20-5.10% and a relative error of the mean value of 2.29-3.65%. The total content of all impurities did not exceed 1.139%.

During the identification of a copolymer of lactic and glycolic acids 50/50, by the position of the most intense absorption bands the IR spectrum corresponded to the spectrum of a standard sample of the PLGA copolymer 50/50 in the range from 1,900 to 800 cm^{-1}.

Based on the results of the conducted studies of the Nano-L-Dopa experimental series, a specification of the main attributes of its quality and analysis methods was developed (Table 4).

DISCUSSION

System analysis of the nomenclature of pharmaceutical substances introduced into drugs for the PD treatment revealed the most widely used ones, in particular, L-Dopa [4]. The main drawback of L-Dopa, associated with the peculiarity of its pharmacokinetics, is its low bioavailability. Only about 1% of the orally administered dose of L-Dopa penetrates the blood-brain barrier (BBB) because of the intensive metabolism in peripheral tissues [3, 4].

The nasal drug delivery is considered to be an actual alternative to oral administration, since the nasal cavity is easily accessible, abundantly vascularized, well permeable, has a large

surface area, which promotes good absorption and increased bioavailability. The drugs enter the bloodstream, avoiding the primary passage through the liver. Moreover, some drugs can be transported from the nasal cavity to the central nervous system, not through the circulatory system of the nasal cavity mucosa, but through the extracellular path along the olfactory nerve to penetrate directly into the brain, bypassing the BBB [10, 11]. Therefore, the nasal delivery route provides new opportunities for the treatment of neurodegenerative diseases of the brain [12].

Low water solubility and high metabolic rate of L-Dopa in peripheral tissues pose certain difficulties in the development of its nasal dosage form. One solution to this problem is the packaging of L-Dopa in biodegradable polymeric nanoparticles. It is known that the brain is one of the least accessible objects for pharmacotherapy because of BBB. V.Yu. Balabanyan (2015) has proposed a completely new concept that nanoparticles can serve as a means of delivery to the brain of the substances that are not able to overcome the BBB in a free form [13].

Among biocompatible biodegradable copolymers widely used in the pharmaceutical field for the production of nanoparticles, aliphatic esters of lactic and glycolic acids are of particular interest. PLGA nanoparticles are capable of controlled release of various pharmaceutical substances, have good tissue compatibility and are nontoxic [14].

The development of Nano-L-Dopa based on polymer nanoparticles with adjustable degradation time, as well as the use of the nasal route of administration, reduce the therapeutic dose of L-Dopa, minimize side effects and increase the effectiveness of therapy through controlled release and optimization of the biodistribution of the pharmaceutical substance.

We developed the technology for producing Nano-L-Dopa based on PLGA nanoparticles by modifying the technology previously proposed by Zhou Y.Z. et al. (2013) and G.G. Barsegyan et al. (2014) [6, 7]. In the laboratory, three experimental Nano-L-Dopa series of prolonged action have been developed for the treatment of the PD by nasal administration. The physicochemical properties of the experimental samples have been investigated. As a result of the systematization of the data obtained, the main quality attributes and methods for analyzing Nano-L-Dopa have been proposed.

Particularly interesting is the subsequent standardization of the innovative form of Nano-L-Dopa based not only on the developed specification containing the main physicochemical quality attributes and analysis methods but also taking into account microbiological and pharmacokinetic ("in vitro" release) pharmacopoeia requirements.

The developed quality attributes and methods for analyzing the innovative form of Nano-L-Dopa allow for the subsequent study of its stability during storage under various temperature regimes and propose optimal packaging in order to ensure a shelf life of at least 24 months.

CONCLUSIONS

Thus, the experimental series of the innovative form of Nano-L-Dopa on the basis of PLGA nanoparticles have been developed, with the following composition per 100 g, % weight:

- L-Dopa – 9.5-10.0;
- PLGA 50/50 polymer - 79.0 – 80.0;
- D-mannitol - 8.0 - 8.3; and
- polyvinyl alcohol - up to 100.0.

The following physicochemical properties of Nano-L-Dopa have been studied: appearance, color and odor, solubility, nanoparticle size, pH, sedimentation stability and water content. The content of L-Dopa and its impurities has been quantified. The identification of PLGA nanoparticles has been established. Physicochemical quality attributes and methods for analyzing Nano-L-Dopa have been proposed.

The modern approach to the pharmaceutical development of innovative forms of Nano-L-Dopa in this experimental work includes the following:

1) the use of polymer PLGA nanoparticles to ensure a prolonged action, increase the bioavailability and stability of L-Dopa;
2) the use of a more promising nasal route of L-Dopa administration alternative to oral one.

REFERENCES

[1] Pugacheva, V. S., Karpov, S. M., Vyslova, I. A., Muravyov, K. A., Bolezn' Parkinsona: Rasprostranennost' zabolevaniya s tochki zreniya meditsinskoy statistiki [Parkinson's disease: The prevalence of the disease in terms of medical statistics], *Bulletin of Science and Practice*, 2016, *5*, 193-204.

[2] Razdorskaya, V. V., Voskresenskaya, O. N., Yudina, G. K., Bolezn' Parkinsona v Rossii: Rasprostranennost' i zabolevayemost' (obzor) [Parkinson's disease in Russia: Prevalence and morbidity (review)], *Saratov Journal of Medical Scientific Research*, 2016, *12(3)*, 379-384.

[3] Illarioshkin, S. N., K 50-letiyu vnedreniya levodopy v klinicheskuyu praktiku. Evolyutsiya form preparata i progress v lechenii bolezni Parkinsona [For the 50th anniversary of the introduction of L-Dopa into clinical practice. Evolution of drug forms and progress in the treatment of Parkinson's disease], *Bulletin of the National Society for the Study of Parkinson's Disease and Movement Disorders*, 2017, *1*, 10-17.

[4] Pyatigorskaya, N. V., Brkich, G. E., Pavlov, A. N., Beregovykh, V. V., Evdokimova, O. V., A Scientific Methodology for Expansion of Anti-Parkinson Drug Product Range, *J. Pharm. Sci. & Res.*, 2017, *9(9)*, 1561-1563.

[5] Kim, T. K., Kang, W., Chun, I. K., Oh, S. Y., Lee, Y. H., Gwak, H. S., Pharmacokinetic evaluation and modeling of formulated levodopa intranasal delivery systems, *Eur. J. Pharm. Sci.*, 2009, *38*, 525-532.

[6] Zhou, Y. Z., Alany, R. G., Chuang, V., Wen, J., Optimization of PLGA nanoparticles formulation containing L-dopa by applying the central composite design, *Drug Development and Industrial Pharmacy*, 2013, *39(2)*, 321-330.

[7] Barsegyan, G. G., Vorontsov, E. A., Gambaryan, P. E., Kamenskiy, A. A., Kondrashova, I. G., Kuznetsov, S. L., Novikova (Naydenova), A. A., Severin, E. S., *Lekarstvennyy preparat dlya lecheniya bolezni Parkinsona [Medicinal preparation for treatment of Parkinson's disease]*, RF Patent No. RU 2 545 734 C1, Publ. 10.04.2015 Bull. No.10.

[8] Kondrasheva, I. G., Antipova, T. A., Barsegyan, G. G., Gambaryan, P. Y., Guseva, Kamensky, A. A., Efficiency and safety of nasal administration of “Nano-L-DOPA” based on PLGA nanoparticles, *Engineering*, 2012, *5*, 27-29.

[9] Gambaryan, P. Y., I.G.Kondrasheva, I. G., E.S. Severin, E. S., A.A. Guseva, A. A., A.A. Kamensky, A. A., Increasing the efficiency of Parkinson’s disease treatment using a poly(lactic-co-glycolic acid) (PLGA) based L-DOPA delivery system, *Experimental Neurobiology*, 2014, *23(3)*, 246-52.

[10] Illum, L., Transport of drugs from the nasal cavity to the central nervous system, *Eur. J. Pharm. Sci.*, 2000, *11*, 1-18.

[11] Chao, O. Y., Mattern, C., Silva, A. M., Wessler, J., Ruocco, L. A., Nikolaus, S., Huston, J. P., Pum, M. E., Intranasally applied l-DOPA alleviates parkinsonian symptoms in rats with unilateral nigro-striatal 6-OHDA lesions, *Brain Research Bulletin*, 2012, *87(2)*, 340-345.

[12] Fazil, M., Shadab, Baboota, S., Sahni, J. K., Ali, J., Nanotherapeutics for Alzheimer's disease (AD): Past, present and future, *Journal of Drug Targeting*, 2012, *20(2)*, 97-113.

[13] Balabanyan, V. Yu., *Farmakologicheskiye i farmatsevticheskiye aspekty sozdaniya nanorazmernykh form faktorov rosta nervnoy tkani, fenazepama i paklitaksela [Pharmacological and pharmaceutical aspects of creating nanoscale forms of growth factors of the nervous tissue, phenazepam and paclitaxel]*, Moscow 2015.

[14] Bose, R. J. C., Lee, S.-H., Park, H., Lipid-based surface engineering of PLGA nanoparticles for drug and gene delivery applications: Review, *Biomaterials Research*, 2016, *20*, 34.