Indian Journal of Chemistry
Vol. 62, October 2023, pp. 1056-1060
DOI: 10.56042/ijc.v62i10.6184

Levodopa nanoencapsulation in nanostarch as anti-Parkinsonian drugs candidate

Ratnaningsih Eko Sardjono*[a], Dhea Salsabila[a], Ramdhan Gunawan[a], Asep Kadarohman[a], Budiman Anwar[a], Vidia Afina Nuraini[a], Erdiwansyah[b], Rizalman Mamat[c], Fitri Dara[d] & Melati Khairudean[e]

[a] Study Program of Chemistry, Department of Chemistry Education, Universitas Pendidikan Indonesia, Setiabudi 229 Bandung 40154 Indonesia

[b] Universitas Serambi Mekah, Aceh, Indonesia, [c] Universiti Malaysia Pahang, Pahang, Malaysia

[d] Badan Risetdan Inovasi Nasional, [e] Universiti Sains Malaysia, Penang, Malaysia

E-mail: ratnaeko@upi.edu

Received 1 December 2022; accepted(revised) 21 September 2023

Parkinson's disease is caused by damage to the dopaminergic nerves in brain. Levodopa has been widely used to treat Parkinson's disease. The levodopa nano encapsulation aims to improve drug-delivery efficiency thereby increasing the concentration of released levodopa in the brain. This study aims to obtain the optimum conditions for levodopa nano encapsulation in nanostarch, the characteristics of levodopa–starch nanocapsules, encapsulation efficiency, and its drug-release capabilities. Levodopa nano encapsulation has been carried out by ultrasonication method. Characterization has been carried out by FTIR, SEM, and TEM. The encapsulation efficiency is measured through changes in levodopa concentrations using a UV-Vis spectrometer. The drug release profile has been measured at *p*H 1.2 and 7.4 for 5 hours. The optimum conditions for levodopa nano encapsulation in nanostarch have been obtained at a mass ratio of nanostarch, maltodextrin and levodopa of 1:3:1. The SEM and TEM characterization show that levodopa–starch nanocapsules have a spherical morphology with the particle size ranged from 17.77-49.43 nm. The FTIR characterization show the presence of new peaks for C=O stretch and N-H bend. The highest encapsulation efficiency has been obtained at 46.11%. Drug release capability has been obtained by 40.93% in *p*H 1.2 media and 48.28% in *p*H 7.4 media for 5 hours of released time.

Keywords: Drug release, Levodopa, Nanoencapsulation, Nanostarch, Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease[1]. Currently Parkinson's sufferers are about 5 million and it is estimated that the number of people with Parkinson's disease will increase up to 10 million. Parkinson's disease is caused by dopaminergic nerve damage in the substantia nigra pars compacta (SNC) of the brain. This can lead to a significant decrease in dopamine production. This disease is usually suffered by the elderly but can also occur at a young age[2]. Dopamine is a neurotransmitter that plays an important role in regulating the motoric, cognitive, and mood systems[3,4]. Low levels of dopamine can cause the onset of several symptoms such as bradykinesia, tremors, slow movements, and rigidity[5–7].

One of the commonly used treatments for Parkinson's disease is therapy using a dopamine precursor, levodopa[8]. The use of dopamine directly to treat Parkinson's disease is not possible because dopamine does not have the ability to penetrate through the blood brain barrier[9], while levodopa can penetrate through the blood brain barrier by LAT-1 transporter[10,11] and converted to dopamine by aromatic L-amino acid decarboxylase[12]. However, the oral use of levodopa has faced a number of limitations in its delivery system. Levodopa can be oxidized before it reaches the brain, hence its bioavailability in the body is low.

Nanoencapsulation of levodopa can be carried out to overcome this limitation. Nanoencapsulation is a process in which drug compounds are coated by encapsulants to form particles on a size scale of 1 – 1000 nm. Nanoencapsulation can prevent the degradation of levodopa, enhance its stability, and improving the regulation of their release at physiologically active sites[13,14]. In the form of nanoparticles, starch has a good potential to be used as a drug vehicle due to its stability and it can protect the active compounds from degradation due to the influence of *p*H and enzymes. Nanostarch preparation can be done through several methods such as: (1) acid hydrolysis, (2) enzyme hydrolysis, (3) high-pressure homogenization, (4) ultrasonication, (5) extrusion, (6) nanoprecipitation, and (7) cross-emulsion (16). Nanostarch has been widely

used to encapsulate various active compounds such as curcumin[17–19], piperine[20], and catechin[21,22].

In this study, nanoencapsulation of levodopa using nanostarch was carried out through the ultrasonication method by optimization of mass composition variations. Characterization of levodopa–starch nanocapsules was carried out using SEM, TEM, and FTIR. The efficiency of encapsulation and drug release profile was evaluated using UV-Vis spectrometry. This study is expected to provide information regarding the role of nanostarch as an encapsulant matrix, the optimum conditions of levodopa nanoencapsulation using starch, the characteristics of levodopa–starch nanocapsules, the encapsulation efficiency, and the release profile of levodopa–starch nanocapsules.

Materials and Methods

Levodopa, water, etanol 96%, soluble starch (Merk), demineralization water, sulfuric acid (H_2SO_4), and sodium hydroxide (NaOH).

Synthesis of Nanostarch

The synthesis of nanostarch was carried out by acid hydrolysis method adapted from Saeng-on and Aht-Ong[23]. Briefly, 15g starch was dispersed in 100 mL H_2SO_4 3.16 M solution and stirred at 40°C for 10 hours. NaOH 1 M solution was added to neutralize the suspension then centrifuged at 3500 rpm for 30 minutes. The nanostarch suspension was washed with water and ethanol then re-centrifuged for 30 minutes. Dried nanostarch was obtained through oven drying at 50°C. The nanostarch was stored at 4°C for further use.

Nanoencapsulation of Levodopa

Nanoencapsulation of levodopa using nanostarch was adapted from Sunarti et al.,[24] using maltodextrin as a stabilizing agent. Briefly, the nanostarch was mixed with maltodextrin with composition variations of (1:3, 1:1, and 1:0.3), then dispersed in demineralized water and sonicated for 15 minutes. Levodopa solution was added to the mixture then sonicated for 10 minutes. The mixture was centrifuged at 3500 rpm for 30 minutes. The suspension was oven-dried at 50°C. Then, levodopa–starch nanocapsules were characterized using FTIR, SEM, and TEM.

Characterization

Characterization was carried out using several instruments such as the Fourier Transform Infra-Red (FTIR), Scanning Electron Microscope (SEM), and Transmission Electron Microscope (TEM). Characterization using FTIR was carried out to determine the functional groups of levodopa–starch nanocapsules and the interaction between levodopa and nanostarch. Characterization using SEM (Jeol JSM-IT3300) was performed to determine the surface morphology of nanostarch and levodopa–starch nanocapsules. Characterization using TEM (HT7700) was performed to determine the shape and the size of the levodopa–starch nanocapsules.

Encapsulation Efficiency

The encapsulation efficiency of levodopa in nanostarch was evaluated using UV-Vis spectrometry. Levodopa concentrations before and after encapsulation were obtained through measurements at wavelengths of 280 nm[25]. Encapsulation Efficiency is calculated using the following equation[19]:

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Co}-\text{Ct}}{\text{Co}} \times 100\ \%$$

Co = Concentration levodopa before encapsulation
Ct = Concentration levodopa after encapsulation

Drug Release Profile

Drug Release profile was evaluated by UV-Vis spectrometry adapted from Morales et al[26]. Levodopa–starch nanocapsule (2 mg) was suspended to 3 ml of solution (*p*H 7.4 and *p*H 1.2) in a dialysis bag and dialyzed against 30 ml of *p*H solution at 37°C. To determine the diffused levodopa through a dialysis bag, then about 3.5 ml of the receiving solution is taken. Then, the levodopa in the receiving solution was measured using a UV spectrophotometer at 280 nm.

Results and Discussion

Synthesis and Characterization of Nanostarch

Nanostarch was produced by acid hydrolysis using H_2SO_4 3.16 M at 35°C – 45°C for 10 hours. The hydrolysis of starch is initiated by the reaction of acid protons with oxygen from the starch glycosidic bond and followed by the cleavage of the glycosidic C-O bond[27]. The formation of nanostarch occurs when starch undergoes high temperature treatment during the gelatinization process and the presence of mechanical agitation which results in the destruction of covalent and hydrogen bonds in the helical structure of amylopectin, resulting in starch with a shorter chain length and having a smaller particle size[24].

The FTIR spectra in Fig. 1 shows the presence of O-H stretching of hydroxyl groups at 3416.05 cm^{-1} –

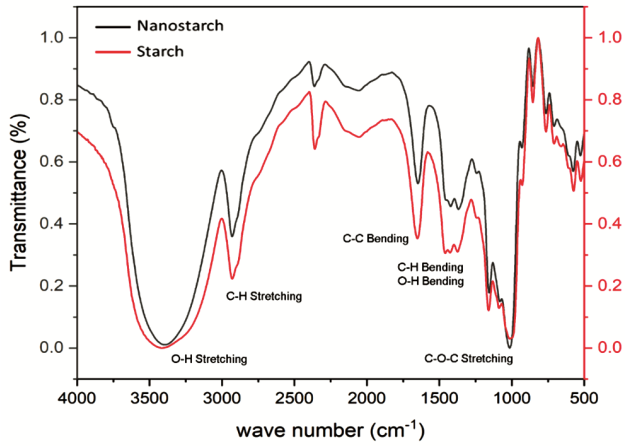

Fig. 1 — FTIR spectra of starch and nanostarch

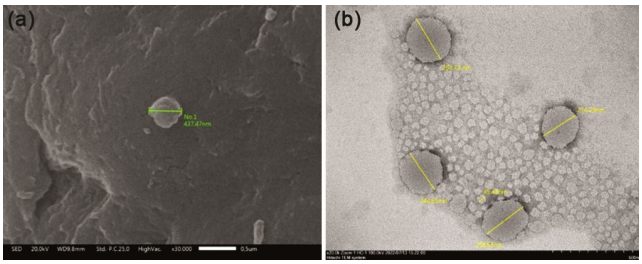

Fig. 2 — SEM (A) and TEM (B) image of nanostarch

3394.83 cm^{-1}, C-H stretching at 2929.97 cm^{-1} – 2928.04 cm^{-1}, C-C bending at 1651.12 cm^{-1} – 1647.26 cm^{-1}, C-H bending at 1458.23 cm^{-1} – 1452.45 cm^{-1}, O-H bending at 1373.36 cm^{-1} – 1367.58 cm^{-1}, and C-O-C stretching of glycosidic bonds at 1159.26 cm^{-1} – 1157.33 cm^{-1}[23]. In addition, Fig. 1 shows that the FTIR spectrum of nanostarch is similar to starch. This suggests that the hydrolysis process doesn't significantly change the chemical composition and doesn't turn the starch polymer into its monomer or oligomer.

The surface morphology, shape, and size of nanostarch were observed using SEM and TEM (Fig. 2). Based on the results of the SEM analysis (Fig. 2A), nanostarch shows the morphology of the non-spherical surface and based on TEM analysis (Fig. 3B), nanostarch is spherical in shape and has a size of 45.43 nm – 264.73 nm.

Nanoencapsulation and Characterization of Levodopa–Starch Nanocapsules

Nanoencapsulation of levodopa was carried out by mixing levodopa solution with nanostarch and maltodextrin. The mixture was further sonicated using the ultrasonicator UCD-250 with a power rate of 40%

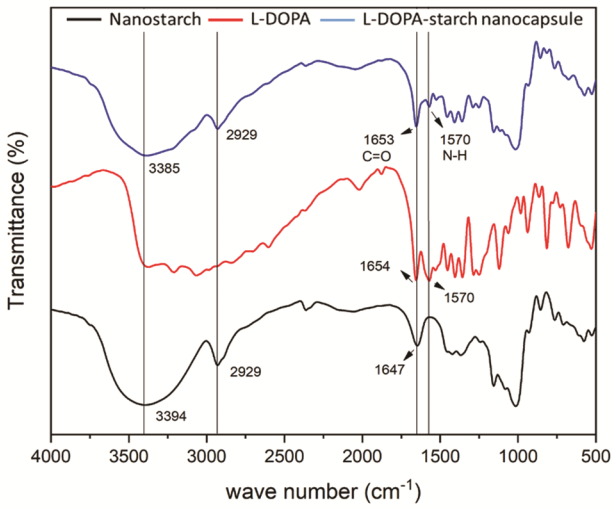

Fig. 3 — FTIR spectra of nanostarch, levodopa, and levodopa nanocapsules

Table 1 — Optimization of levodopa nanoencapsulation

Nanostarch : maltodextrin : levodopa	Levodopa–starch nanocapsules (g)
1 : 3 : 0.2	0.0750
1 : 3 : 0.25	0.0852
1 : 3 : 0.33	0.0876
1 : 3 : 0.5	0.0902
1 : 3 : 1	0.0917

for 10 minutes. During the sonication, acoustic cavitation occurs which can lead to the degradation of the nanostarch polymer resulting in smaller particles. In addition, acoustic cavitation can also cause the formation of radical species that can trigger interactions between levodopa and nanostarch polymer[28–30]. The optimization of nanoencapsulation was carried out by varying the mass composition of the reagent. Optimization was carried out to determine the optimal conditions of the levodopa nanoencapsulation process using nanostarch that can produce the most levodopa–starch nanocapsules. The results of the optimization of levodopa nanoencapsulations in Table 1 show that nanostarch, maltodextrin, and levodopa with a composition of 1: 3: 1 produce more levodopa–starch nanocapsules.

FTIR analysis was performed to determine the functional groups contained in levodopa–starch nanocapsules and see the interaction between levodopa and nanostarch after the nanoencapsulation process was carried out. The results of the FTIR analysis are shown in Fig. 3. Based on the results of FTIR analysis, it can be seen that levodopa nanocapsules show C = O stretching peak at a 1653

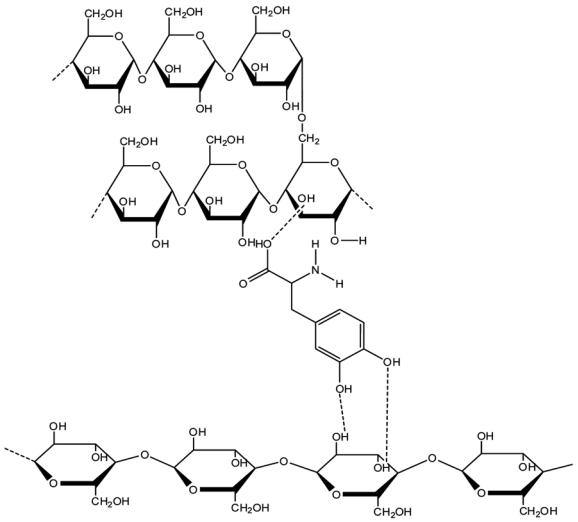

Fig. 4 — Prediction of the interaction of nanostarch and levodopa

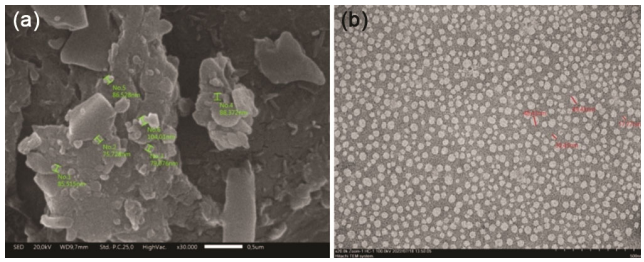

Fig. 5 — SEM (A) and TEM (B) image of levodopa–starch nanocapsules

cm^{-1} and N-H bending at 1570 cm^{-1} which is a typical absorption peak of levodopa. This suggests that levodopa has been encapsulated in nanostarch. In addition, peak shift in FTIR spectra indicate an interaction between levodopa and nanostarch. The O-H stretch was shifted from 3394 cm^{-1} to 3385 cm^{-1}. Based on this analysis, it is suspected that there is an interaction between levodopa and nanostarch in the form of hydrogen bonds as shown in Fig. 4.

The surface morphology, shape, and size of levodopa–starch nanocapsules were observed using SEM and TEM shown in Fig. 5. Based on the results of the SEM analysis (Fig. 6A), the surface morphology of the levodopa–starch nanocapsules is irregular spherical in shape. TEM analysis (Fig. 6B) showed that the levodopa–starch nanocapsules is spherical in shape with a particle size of 17.77 nm – 49.43 nm.

Encapsulation Efficiency

Encapsulation efficiency was evaluated to determine how much levodopa was adsorbed in nanostarch.

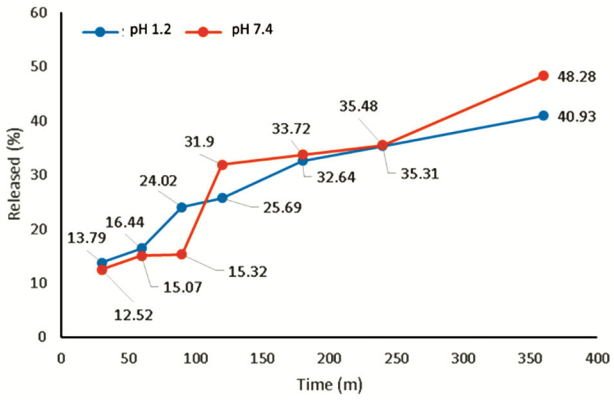

Fig. 6 — Drug release profile of levodopa–starch nanocapsules

Table 2 — Encapsulation efficiency of levodopa–starch nanocapsules

Nanostarch : maltodextrin : levodopa	Encapsulation efficiency (%)
1 : 3 : 0.33	36.31
1 : 3 : 0.5	38.69
1 : 3 : 1	46.11

Encapsulation efficiency was determined using UV-Vis spectrometer at a 280 nm. The results of encapsulation efficiency of levodopa–starch nanocapsules are presented in Table 2. The highest encapsulation efficiency was obtained at composition of nanostarch, maltodextrin, and levodopa 1: 3: 1. Thus we can conclude that the efficiency of encapsulation depends on its composition. The more levodopa used, the more encapsulation efficiency it has.

Drug Release Profile

Drug release evaluation was carried out to see how much levodopa that are released into the target organ. In this study, the target organ that was intended was the brain. The test was carried out at two different media, (*p*H 1.2 and 7.4). The selection of this two *p*Hs is because *p*H 1.2 is a simulation of the stomach condition. Due to the oral delivery of drugs, on its way to the target organ will go through the digestive organs such as the stomach. In such acidic conditions in the stomach, it is desirable that the release of medicinal compounds lasts a minimum. For *p*H 7.4 is a simulation of the brain condition. In this condition, a maximum release of medicinal compounds is expected. Fig. 6 shows the ability of drug release at *p*H 1.2 was 40.93% in 5 hours and at *p*H 7.4 of 48.28% in 5 hours. This indicates levodopa in levodopa-starch nanocapsules is released more on brain condition simulation media as expected.

Conclusions

Based on this study, the optimum conditions for the levodopa nanoencapsulation in nanostarch were obtained at a mass ratio of 1: 3: 1. The results of FTIR characterization showed the presence of new peaks at wave numbers 1653 cm^{-1} for C=O stretch, and 1570 cm^{-1} for N-H bend indicating an interaction between levodopa and nanostarch. The results of SEM and TEM characterization show that levodopa–starch nanocapsules have spherical shape with a size of 17.77-49.43 nm. The highest encapsulation efficiency level was obtained at 46.11% with drug release capability obtained by 40.93% for *p*H 1.2 media and 48.28% for *p*H 7.4 media for 5 hours of released time.

References

1 Dick F D, Palma G D, Ahmadi A, Scott N W, Prescott G J, Bennett J, Semple S, Dick S, Councell C, Mozzoni P, Haites N, Wettinger S B, Mutti A, Otelea M, Seaton A, Soderkvist P, Felice A & Geoparkinson Study Group, *Occup Environ Med*, 64 (2007) 666.

2 Lewitt P A, *N Engl J Med*, 359 (2008) 2468.

3 Olguín H J, Guzmán D C, García E H & Mejía G B, *Oxid Med Cell Longev*, 2016 (2016) 9730467.

4 Radwan B, Liu H & Chaudhury D, *Brain Res*, 1713 (2019) 42.

5 Ratlam C, Phanichphant S & Sriwichai S, *J Polym Res*, 27(2020)183.

6 Hayes MT, *Am J Med*, 132 (2019) 802.

7 DeMaagd G, Philip A, *Pathophysiology, Clinical Presentation, and Diagnosis*, 40(2015) 504.

8 Bogetofte H, Alamyar A, Blaabjerg M & Meyer M, *CNS Neurol Disord Drug Targets,* 19 (2020) 572.

9 Vitaliano G, Kim J, Mintzopoulos D, Adam C, Vitaliano F, Lukas S & Kaufman M, *Biol Psychiatry*, 83 (2018) S450.

10 Kageyama T, Nakamura M, Matsuo A, Yamasaki Y, Takakura Y, Hashida M, Kanai Y, Naito M, Tsuruo T, Minato N & Shimohama S, *Brain Res*, 879 (2000) 115.

11 Puris E, Gynther M, Auriola S & Huttunen KM, *Pharm Res*, 37 (2020) 88.

12 Oktariza Y, Amalia L & Sobaryati S, *Indones J Clin Pharm*, 8 (2019) 246.

13 Pateiro M, Gómez B, Munekata PES, Barba FJ, Putnik P, Kovačević D B & Lorenzo J M, *Molecules*, 26 (2021) 1547.

14 Dzakwan M, *J Ilm Farm Farmasyifa*, 2 (2019) 84.

15 Ab'lah N, Wong TW, *Polymer Science and Innovative Applications*, (2020) 287. (https://doi.org/10.1016/B978-0-12-816808-0.00009-3).

16 Maryam M, Kasim A, Emriadi E & Novelina N, *Majalah Ilmiah Teknologi Industri*, 15 (2018) 36.

17 Chin S F, Mohd Yazid S N A & Pang S C, *Int J Polym Sci*, 2014 (2014) 340121. (https://doi.org/10.1155/2014/340121).

18 Maghsoudi A, Yazdian F, Shahmoradi S, Ghaderi L, Hemati M & Amoabediny G, *Mater Sci Eng C Mater Biol Appl*, 75 (2017) 1259.

19 Athira GK & Jyothi AN, *Int J Pharm Pharm Sci*, 2014 (2014) 171. (https://innovareacademics.in/journals/index.php/ijpps/article/view/1359).

20 Wan-Hong C, Chin S, Pang S-C & Kok K-Y, *J Phys Sci*, 31 (2020) 57.

21 Ahmad M, Mudgil P, Gani A, Hamed F, Masoodi F A & Maqsood S, *Food Chem*, 270 (2019) 95.

22 Liu C, Ge S, Yang J, Xu Y, Zhao M, Xiong L & Sun Q, *J Funct Foods*, 26 (2016) 632.

23 Saeng-on J & Aht-Ong D, *Polym from Renew Resour*, 8 (2017) 91.

24 Sunarti T, Pasaribu F J & Winarti C, *Pharm Sci Res*, 7 (2020) 75.

25 Chin S F, Yazid S N A M & Pang S C, *Int J Polym Sci*, 2014 (2014) 1-8. (https://doi.org/10.1155/2014/340121).

26 Morales V, McConnell J, Pérez-Garnes M, Almendro N, Sanz R & García-Muñoz RA, *J Mater Chem B*, 9 (2021) 4178.

27 Sarip H, Hossain M, Azemi M, Allaf K. A Review of the Thermal Pretreatment of Lignocellulosic Biomass towards Glucose Production: Autohydrolysis with DIC Technology. *Bioresources*. 2016 Aug 11;11.

28 Taurozzi J S, Hackley V A & Wiesner M R, *Nanotoxicology*, 5 (2011) 711.

29 Sardjono R, Fauziyah N, Puspitasari M, Musthapa I, Khoerunnisa F, Azzahra G, Mamat R & Erdiwansyah, *IOP Conf Ser Mater Sci Eng*, 856 (2020) 12009.

30 Leong T S H, Martin G J O & Ashokkumar M, *Ultrason Sonochem*, 35 (2017) 605.