Heliyon 6 (2020) e03096

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The effectiveness of plant hydrocolloids at maintaining the quality characteristics of the encapsulated form of L-phenylalanine-ammonia-lyase

Olga Babich [a,b], Lyubov Dyshlyuk [c], Svetlana Noskova [b], Alexander Prosekov [a], Svetlana Ivanova [c,d,*], Valery Pavsky [c,d]

[a] Laboratory of Biocatalysis, Kemerovo State University, Krasnaya Street 6, Kemerovo, 650043, Russia
[b] Institute of Living Systems, Immanuel Kant Baltic Federal University, A. Nevskogo Street 14, Kaliningrad, 236016, Russia
[c] Research Institute of Biotechnology, Kemerovo State University, Krasnaya Street 6, Kemerovo, 650043, Russia
[d] Department of General Mathematics and Informatics, Kemerovo State University, Krasnaya Street 6, Kemerovo 650043, Russia

ARTICLE INFO

Keywords:
Pharmaceutical chemistry
Biotechnology
Pharmaceutical science
Capsules
Natural polysaccharides
Enzyme preparation
Phenylalanine ammonia-lyase
Stability
Phenylketonuria

ABSTRACT

The effect of three types of polysaccharides (agar-agar, carrageenan, hydroxypropyl methylcellulose) on the activity and stability during storage at given temperature conditions of the enzyme preparation L-phenylalanine ammonia-lyase was studied. It was found that the most suitable storage temperature for encapsulated L-phenylalanine-ammonia-lyase is room temperature up to 25 °C for all samples of capsules from plant polysaccharides. Samples of capsules with agar-agar and hydroxypropyl methylcellulose under different temperature conditions inhibited the decrease in enzyme activity, which in other samples of capsules reached 90% in 6 months of storage. In samples of capsules with carrageenan at temperatures of 4 °C and 30 °C, there was a significant decrease in the activity of the enzyme preparation. Selection of capsule samples from plant polysaccharides suitable for L-phenylalanine-ammonia-lyase replacement therapy is done after studying the mechanisms of capsule destruction under conditions close to the conditions of the gastrointestinal tract, to which the next stage of our research will be devoted.

1. Introduction

Hereditary metabolic diseases associated with impaired amino acid metabolism, form a special group. To date, there are about 90 hereditary amino acid metabolism defects (primary aminoacidopathy) known, including phenylketonuria (PKU) [1]. The most promising is the use of enzyme replacement therapy with L-phenylalanine-ammonium-lyase (PAL), both per iniectio and per os, which cleaves phenylalanine into safer products [2, 3]. The greatest attention to this problem is paid by the company Biomarin Pharmaceutical Inc (www.BMRN.com) in the framework of the US national program [4, 5].

With first attempts of PKU therapy with PAL, the need to protect PAL from prophyliotic destruction by digestive enzymes was found [3]. Coating the drugs, including the enzyme, allows protecting them from the conditions under which their quality characteristics deteriorate, both during direct consumption and during passage through the gastrointestinal tract (GIT). The effectiveness of encapsulation depends on the used materials of matrix capsules, on the method of manufacture and production technology [6]. The use of alginate microcapsules [7, 8] is widespread; several polysaccharide and protein coatings (chitosan, gelatin, glucomannan, whey proteins, etc.) have also been studied to enhance protection, since encapsulation does not guarantee the achievement of proper GI tract segments in proper quality [9, 10].

The widespread therapeutic use of PAL is hampered by the absence of the tested technologies for purification and stabilization, as well as a stable form that ensures the preservation of the enzyme to a direct reaction with phenylalanine, especially in the acidic environment of the stomach. Significant results in the development of technologies for obtaining PAL were achieved by BioMarin Pharmaceutical Inc., which in 2013 started the third stage of clinical trials of PEG-PAL (pegylated recombinant L-phenylalanine ammonia-lyase) [3]. In 2017 and 2018, the FDA issued licenses for both the drug and therapy with PAL per iniectio, respectively [5]. Clinical trials for the drug PEG-PAL and the therapy with it continue. However, the time and money spent on the development of this drug do not imply its affordable price. The availability of alternative PAL therapy requires the availability of alternative technologies for obtaining an affordable drug.

* Corresponding author.
E-mail address: pavvm2000@mail.ru (S. Ivanova).

https://doi.org/10.1016/j.heliyon.2019.e03096
Received 11 January 2019; Received in revised form 13 November 2019; Accepted 18 December 2019

Polysaccharides are a popular basis for targeted drug delivery systems due to their naturalness, availability, relatively low cost, high biocompatibility, bioavailability and low toxicity [11, 12, 13, 14, 15, 16].

The use of polysaccharides can increase the solubility of drugs in water and increase the stability of drugs including enzymes [12, 17].

PAL enzyme preparation that is used to treat phenylketonuria is produced either in the form of a solution for injection, or in pegylated form [3], which ensures preservation of the enzyme in the gastrointestinal tract. However, there may be other lower-cost polymers providing same conditions for preserving the qualitative characteristics of the enzyme for the targeted drug delivery. This study assessed the effect of various plant polysaccharides, as the basis of capsules, on the stability of the effectiveness of L-phenylalanine ammonia-lyase during storage.

2. Materials and methods

2.1. Materials and chemicals

The enzyme preparation of the L-phenylalanine-ammonia-lyase (powder, activity 3.2 U mg^{-1}, density 1192 kg m^{-3}, thermal conductivity 3.36 W (m K)$^{-1}$) was obtained by the pigment yeast cultivating technology and the purification and stabilization technology developed by a team of researchers from the Research Institute of Biotechnology of the Kemerovo State University [18, 19, 20, 21, 22, 23]. The study used agar-agar (Helicon, United States); iota-carrageenan (Newgreen Pharmchem Co., China); carboxymethyl cellulose (Acros, Belgium). L-phenylalanine ((*S*)-2-Amino-3-phenylpropionic acid, ≥98%, P2126), Potassium phosphate monobasic (Monopotassium phosphate, Potassium dihydrogen phosphate, prim.-Potassium phosphate, ≥99.0%, P5655), Sodium hydroxide (Caustic soda, ≥98%, S8045) were purchased from Fluka/Sigma-Aldrich (Sigma-Aldrich Rus, Moscow, Russia). All other chemicals (analytical or better grade) used in this study were purchased from Fluka/Sigma-Aldrich (Sigma-Aldrich Rus, Moscow, Russia) or were obtained from the Institute of Biotechnology of Kemerovo State University (Kemerovo, Russia).

2.2. Determination of L-phenylalanine-ammonium-lyase activity

The amount of PAL which catalyzes the conversion of 1 μmol of L-phenylalanine into trans-cinnamic acid and NH_3 at pH 8.5 and temperature of 30 °C per minute was considered as unit of activity. The composition of the standard incubation mixture (1 mL): 0.2 M Tris-HCl (pH 8.5, 0.5 mL); 0.05 M L-phenylalanine (0.04 mL); deionized water (0.42 mL). After stirring and preincubation (at least 5 min at (30 ± 0.1) °C), the reaction was started by adding 0.04 mL of diluted PAL enzyme (0.025–0.125 U mL^{-1}). In control, 0.04 mL of water was added instead of the enzyme. The reaction was continuously recorded in the time interval from 2 to 7 min at 270 nm on a Shimadzu UV-1800 spectrophotometer, equipped with a thermostat, (Shimadzu, Japan), in quartz cuvettes with 1 cm light path. Data collection and analysis of data was carried out by computer software UV-probe V (Shimadzu, Japan). PAL activity was calculated using the formula with a millimolar extinction coefficient of trans-cinnamic acid equal to 19.73 (Sigma technique):

$$Activity\left(\frac{U}{ml}\right) = (\Delta A_{270\ nm} Test - \Delta A_{270\ nm}\ Blank) \cdot V_{rm} \cdot \frac{f}{19.73 \cdot V},$$

where $\Delta A_{270\ nm}$ – recorded indicators in the reaction mixture and control per unit time of the time interval in question;

V_{rm} – reaction mixture volume, mL;
f – the dilution ratio of the original solution of the PAL preparation;
V – sample volume, mL.

2.3. Capsule preparation

The mixture for the production of capsules was prepared from plant hydrocolloids (carrageenan, agar-agar, hydroxypropylmethylcellulose (HPC)) in accordance with the developed formulations (Table 1). With constant stirring, carrageenan and/or agar-agar and/or HPC were gradually added to the measured volume of cold water in quantities corresponding to the recipes, the mixture was heated to 75 °C and stirring was continued for 1.0 h. After dissolving the plant hydrocolloids, glycerol was added to the mixture, while stirring for 0.5 h, to raise flexibility and increase stability time of the capsule matrix [24, 25].

After the agitator was turned off and the mixture was heated, the mixture was left in the reactor for 1.5–2.0 h with vacuum connected to remove air bubbles from the mass. The prepared mass was transferred for stabilization into a thermostatting tank with a controlled temperature and kept at a temperature of 45–50 °C for 2.5–3.0 h.

The production of capsules based on plant hydrocolloids continued in a capsule production machine (LLC "ASK", Russia). The container with the encapsulation mixture was connected to the machine, the solution was fed through the hoses to the boxes (temperature of the boxes was 60°C), from the boxes the solution got to the drums (temperature varied from 18 to 22°C depending on the settings), where the mixture was gelled and a tape of a certain thickness was formed. Next, the tape passed through the lubricating rollers (Vaseline oil was applied to the entire surface of the tape), then through the mold, where capsules were cut and seamed. Filler was fed into the formed capsule from above through the heating element (the filler temperature did not exceed 28°C).

Molded capsules were served in a tumble dryer for primary drying of capsules at a temperature of 25 °C for 20 h and the removal of vaseline oil residues from the capsule surface.

The finished capsules were sorted on a capsule screening machine (JET, Russia), laid out on special pallets and stacked. Stacks were rolled into drying tunnels (temperature from 20 to 25°C, relative humidity of air from 20 to 30%).

Table 1. Capsule shell formulations.

Formulation number	The amount of component, weight %				
	Carrageenan	Agar-agar	HPC	Glycerol	Water
1	5.0	-	2.5	5.0	87.5
2	10.0	-	2.5	5.0	82.5
3	5.0	-	5.0	5.0	85.0
4	10.0	-	5.0	5.0	80.0
5	5.0	-	10.0	5.0	80.0
6	10.0	-	10.0	5.0	75.0
7	-	5.0	2.5	5.0	87.5
8	-	10.0	2.5	5.0	82.5
9	-	5.0	5.0	5.0	85.0
10	-	10.0	5.0	5.0	80.0
11	-	5.0	10.0	5.0	80.0
12	-	10.0	10.0	5.0	75.0
13	5.0	2.5	-	5.0	87.5
14	10.0	2.5	-	5.0	82.5
15	5.0	5.0	-	5.0	85.0
16	10.0	5.0	-	5.0	80.0
17	5.0	10.0	-	5.0	80.0
18	10.0	10.0	-	5.0	75.0
19	5.0	2.5	2.5	5.0	85.0
20	10.0	2.5	2.5	5.0	80.0
21	5.0	5.0	5.0	5.0	80.0
22	10.0	5.0	5.0	5.0	75.0
23	5.0	10.0	10.0	5.0	70.0
24	10.0	10.0	10.0	5.0	65.0

Figure 1. Capsules based on vegetable hydrocolloids: 1 – formulation No. 5; 2 – formulation No. 18.

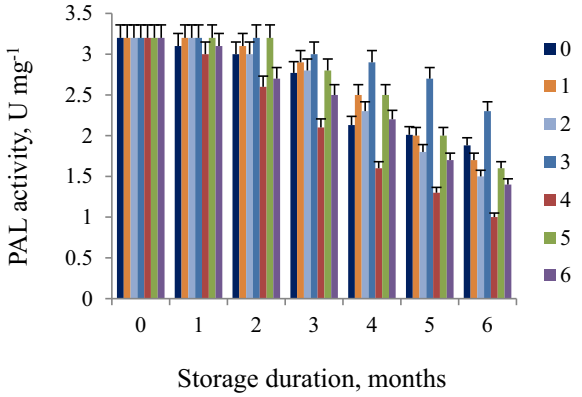

Figure 2. Changes in the activity of PAL enzyme preparation when stored in different encapsulated dosage forms: 0 – native PAL preparation without capsule shell; 1 – capsule sample No. 4; 2 – sample capsule No. 5; 3 – capsule sample No. 10; 4 – capsule sample No. 15; 5 – capsule sample No. 18; 6 – capsule sample No. 24. The data are expressed as mean ± standard deviation ($n = 3$).

It took from 2 to 5 days on a drum set to dry the capsules (Sigma, USA) until the capsules reached the required parameters for hardness, elasticity, weight and moisture of the shell (Figure 1).

2.4. Capsule quality analysis

To determine the mass and diameter of the capsules, 100 capsules were examined. Weighing was carried out on a torsion balance (VT-500, MASSA-K, Russia) with an accuracy of 0.001 g. The capsule diameter was measured using an electronic digital micrometer MCC 25 (LLC "PKP Elbor", Russia) with an accuracy of 0.001 mm. Each measurement was repeated three times.

The mass fraction of moisture in the capsules was determined by the mass loss dynamics during drying. To do this, the capsules were ground, weighed and placed on filter paper. The samples on filter paper were placed in a loss-on-drying oven (SNOL 120/300, AB UMEGA GROUP, Lithuania) and dried at 100–110 °C for 1 h, cooled down for about 20 min in a desiccator and weighed. The mass fraction was calculated by the difference in weight converted into percent.

The solubility of the capsules in water was investigated using a solubility tester (DT 820/1420i, ERWEKA, Germany). The capsules were kept in purified water for 2 h with their condition being constantly monitored.

The flowability of capsules as the average rate of material flow through the funnel hole of a certain diameter was determined on a vibration device (VP-12A, MNPO "Minmedbiospetstekhoborudovanie", Russia, funnel with a cone angle of 60°, the spout was cut at a right angle 3 mm from the end of the funnel cone). 100.0 g of granulate was placed in a funnel and the flow rate was determined. The experiment was repeated 10 times.

2.5. Storage stability

Capsules with PAL enzyme preparation were made according to the procedure described above at room temperature and pH 7.0 and stored at the indicated temperature regimes. PAL activity was monitored over time. The initial activity of the enzyme preparation was considered as a standard for assessing its stability.

Capsules containing PAL were placed in a model biorelevant medium SIF without pepsin, pH 7.5 (potassium dihydrogen phosphate – 50 mm, sodium hydroxide – up to pH 7.5) and kept for 20 min, after which the pH of the resulting solution was adjusted to 8.5 with sodium hydroxide and PAL activity was measured using the spectrophotometric method (as described above).

2.6. Statistical analysis

Each experiment was repeated three times and data were expressed as means ± standard deviation. Data processing was carried out by standard methods of mathematical statistics. Homogeneity of the sampling effects was checked using the Student's t-test. The data were subjected to the analysis of variance (ANOVA) using Statistica 10.0 (StatSoft Inc., 2007, USA). Differences between means were considered significant when the confidence interval was under 5% ($P \leq 0.05$).

3. Results and discussion

At the preliminary stage of research, it was established that capsules based on plant hydrocolloids (agar-agar, carrageenan and carboxymethylcellulose) satisfy the requirements of microbiological purity

Table 2. Physico-chemical properties of capsules based on plant hydrocolloids.

Indicator	Indicator value for samples					
	4	5	10	15	18	24
Capsule diameter (mm)	6.5 ± 0.3^a	6.2 ± 0.3^a	6.0 ± 0.3^a	6.7 ± 0.3^a	6.9 ± 0.3^a	6.4 ± 0.3^a
Capsule length (mm)	14.8 ± 0.7^a	15.0 ± 0.8^a	14.7 ± 0.7^a	14.5 ± 0.7^a	14.4 ± 0.7^a	14.6 ± 0.7^a
Useful capacity (mL)	0.25 ± 0.01^a	0.24 ± 0.01^a	0.27 ± 0.01^a	0.26 ± 0.01^a	0.23 ± 0.01^a	0.24 ± 0.01^a
Capsule weight (mg)	42.0 ± 2.1^a	40.0 ± 2.0^a	45.0 ± 2.3^a	43.0 ± 2.2^a	44.0 ± 2.2^a	41.0 ± 2.1^a
Moisture content (%)	12.8 ± 0.6^a	13.5 ± 0.7^b	12.5 ± 0.6^a	13.0 ± 0.7^a	14.2 ± 0.7^b	13.7 ± 0.7^b
Capsule disintegration (min)	16.0 ± 0.8^a	15.5 ± 0.8^a	14.0 ± 0.7^a	15.0 ± 0.7^a	17.0 ± 0.9^b	15.0 ± 0.7^a
Flowability ($g\ s^{-1}$)	8.5 ± 0.4^a	7.7 ± 0.4^b	8.0 ± 0.4^b	8.4 ± 0.4^a	7.2 ± 0.4^b	7.0 ± 0.4^b

The data are expressed as mean ± standard deviation ($n = 3$). Values followed by different letter a and b in a row indicate significant differences between groups ($P < 0.05$) by LSD post-hoc test.

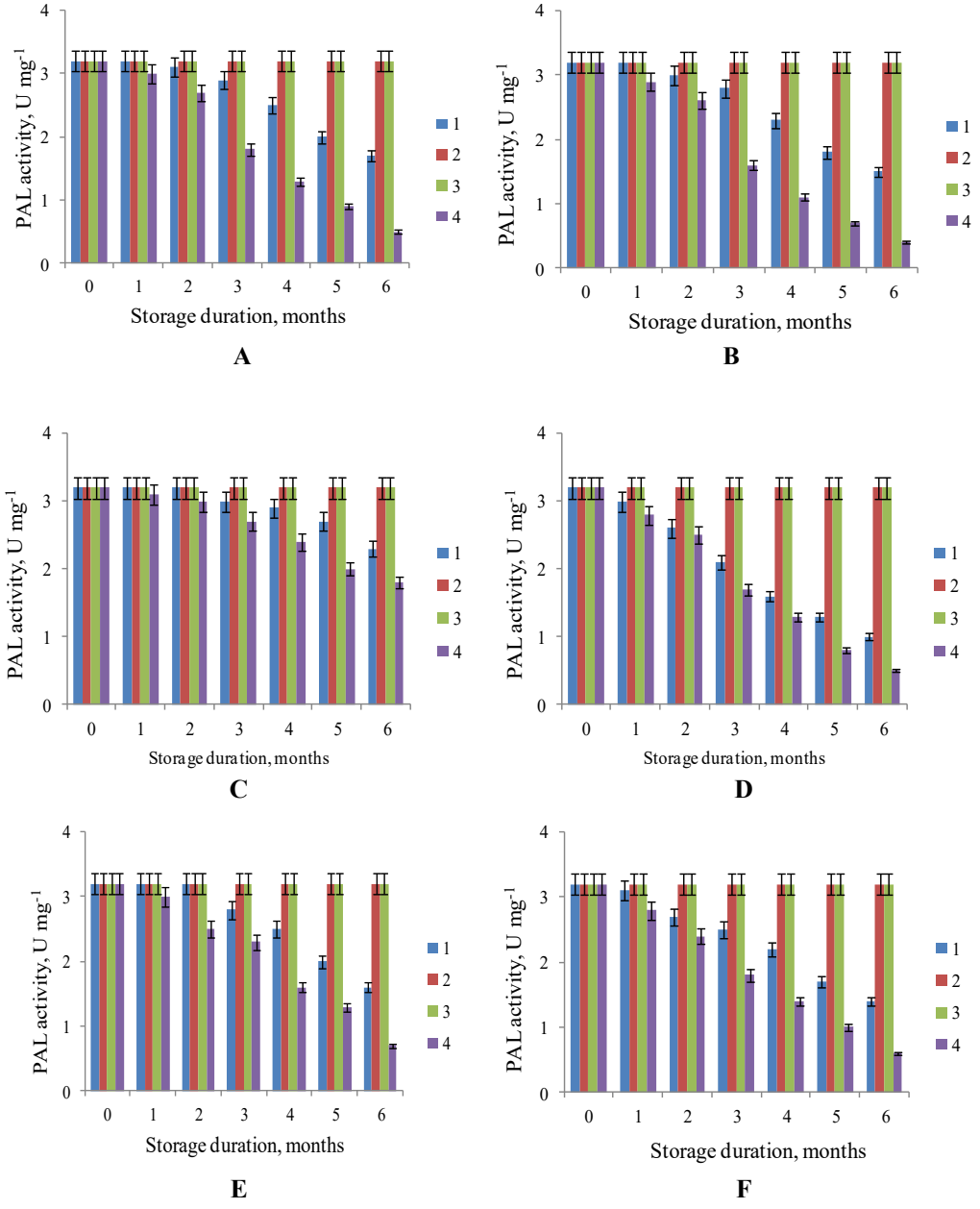

Figure 3. The change in the activity of the PAL enzyme preparation in an encapsulated dosage form (**A**) capsule sample No. 4, (**B**) capsule sample No. 5, (**C**) capsule sample No. 10, (**D**) capsule sample No. 15, (**E**) capsule sample No. 18, (**F**) capsule sample No. 24 during storage at different temperatures: 1–4°C, 2–15°C, 3–25°C, 4–30°C. The data are expressed as mean ± standard deviation ($n = 3$).

according to OFS.2.2.4.0002.15. 24 capsule formulations were studied, by which only cylindrically shaped and having high strength were chosen (Table 2). Testing the solubility of the obtained capsules in water at room temperature for 4 h confirmed the preservation of their structure (swelling of the outer shell with preservation of the capsule structure). The diameter of the obtained caosules varied from 6.0 to 6.9 mm, their length - length from 14.4 to 15.0 mm, useful capacity from 0.23 to 0.27 mL, empty capsule weight from 40.0 to 45.0 mg, moisture content from 12.5 to 14.2%, disintegration from 14.0 to 17.0 min, flowability from 7.0 to 8.5 g s^{-1}.

It is believed that the main reasons for the decrease in the activity of encapsulated PAL is an unfavorable environment inside the capsules, inactivation of the enzyme during encapsulation or a combination of all factors [26]. Initially, the stability of PAL enzyme preparation was tested with or without a capsule shell of natural polysaccharides (temperature 4 °C, humidity 55%). The results are shown in Figure 2.

The results show that within the first month of storage, no statistically significant differences in the activity of the enzyme preparation with different types of capsule shells were observed. Starting from the 2 nd month of storage, inactivation of the enzyme preparation in capsule samples from natural polysaccharides formulations No. 15 and No. 24 was faster than in other capsule shell types (P = 0.9243–0.9318). From the fifth month of storage, a significant decrease in enzyme activity was observed in the No. 5 formulation capsules compared to the capsule-free form (up to 25%).

During the storage period of up to 6 months, the activity of L-phenylalanine ammonia-lyase can be influenced by environmental conditions (materials from which the capsules are made, temperature, humidity, etc.). Apparently the very process of encapsulation of PAL with natural polysaccharides (formulations No. 4, No. 10, No. 18) did not have a deactivating effect on the enzyme preparation in the considered technological conditions and time period. In the capsules of formulations No.

4 and No. 18, the enzyme preparation stability dynamics did not demonstrate any statistical difference from the capsule-free preparation. Interestingly, after 6 moths of storage, the activity of PAL in the No. 10 formulation capsules significantly (by almost 1.5 times) exceeded the activity of the native preparation. Apparently, the absence of carrageenan in the composition which moisture permeability increases at low temperatures [27], led to the preservation of the enzyme preparation's stability. This observation suggests that the effect of capsule shells on the activity of encapsulated PAL changes dramatically with temperature changes over a wide range. To confirm this assumption, we studied the influence of temperature on the activity of encapsulated PAL during storage.

The effect on the activity of L-phenylalanine-ammonia-lyase (the initial enzyme activity was 3.2 U mg^{-1}) of plant polysaccharides from which capsules were produced, during storage (up to 6 months) at various temperature regimes from 4 °C to 30 °C, was studied (Figure 3). The humidity during the experiment was 55%.

At storage temperatures of 15 °C and 25 °C, L-phenylalanine ammonia-lyase, enclosed in capsules of all studied compounds, remained unchanged after 6 months of storage. At other temperature conditions (4°C and 30°C), destabilization of the qualitative characteristics of the encapsulated enzyme was observed. When stored for 6 months at a temperature of 4 °C the enzyme activity of L-phenylalanine-ammonia-lyase encapsulated in the shell of sample No. 4 decreased by 46.9% compared to the original, when using capsule samples No. 5 - by 53.0%, capsule samples No. 10 - by 28.0%, capsule samples No. 15 - by 68.7%, capsule samples No. 18 - by 50.0%, capsule samples No. 24 - by 56.3%. Earlier [18, 19, 23] it was determined that the native enzyme loses 37% of its activity during storage at 4 °C in 6 months of storage. In this temperature mode, the capsule samples No. 10 slowed down the process of the enzyme deactivation, while all the rest increased.

The maximum decrease in PAL activity was recorded during storage of the encapsulated preparation at a temperature of 30 °C. After 6 months storage of samples of capsules No. 4, the enzyme activity decreases by 84.4% compared to the original, for capsule samples No. 5 - by 87.5%, for capsule samples No. 10 - by 43.8%, for capsule samples No. 15 - by 84.4%, for capsule samples No. 18 - by 78.1%, for capsule samples №24 - by 81.3%. Apparently, the presence of carrageenan in the composition led to an increased loss of enzyme activity, and the combined presence of agar-agar and HPC slowed down these processes.

The authors are not aware of any works that studied the effect of polysaccharides capsules on the stability of encapsulated PAL during storage. Researchers of Biomarin Pharmaceutical Inc used a variety of pegylation recipes to preserve PAL activity in the human gastrointestinal tract [28, 29].

The authors [26] assessed the contribution of incomplete absorption, the internal environment of microcapsules of the nitrate cellulose membrane, the diffusion barrier of the membrane, and the microcapsulation process to a significant decrease in PAL's activity compared to the activity of the free enzyme. In [30], activity of PAL encapsulated in cellulose nitrate microcapsules was similarly evaluated. It was established that the microencapsulation process itself, with incomplete absorption of the enzyme, deactivated a significant proportion of the enzyme.

Pegylation technologies for other enzymes are developed and available. The need to develop new technologies is usually associated with new unique enzymes. There are not so many works on the use of new types of shells, including those with plant polysaccharides. For capsules of other enzyme preparations, cellulose, chitosan, carrageenan etc. are used usually from polysaccharides [31]. The technologies of using cellulose-based polymers and their derivatives in encapsulation have been developed; their application areas are expanding [32].

In [27] capsules from hypromellose with carrageenan as a gelling agent were considered as an alternative to gelatin capsules. The former were more preferable as a transport of therapeutic drugs including enzyme due to their resistance to acidic environments of the stomach, but the stability of the drug in these capsules was not considered.

The authors [33] studied the effect of polysaccharides (negatively charged sodium carboxymethylcellulose, uncharged methylcellulose, and positively charged sodium carboxymethyl chitosan) on the catalytic activity and stability of the model enzyme (glucuronidase). The enzyme activity without polysaccharides dropped sharply by 26% after 10-day storage and continued to decline after that. On the 26th day of storage, only 4% of its initial activity was detected. The enzyme with positively charged sodium carboxymethyl chitosan was characterized by the highest storage stability (94% activity after 26 days of storage). The electrostatic interaction between the polysaccharide and the enzyme is determined by the decisive factor while maintaining the activity of the enzyme. None of our capsule samples resulted in an increase in the activity of the enzyme enclosed in them, but there was not a charged one among them.

4. Conclusions

The use of plant polysaccharides in capsules in combination with temperature regimes significantly affected the enzymatic activity and stability of the PAL enzyme preparation. It is established that the encapsulated form of L-phenylalanine-ammonium-lyase is expedient to be stored in the temperature range from 15 to 25 °C, since under these conditions its activity does not decrease for 6 months. On the basis of the conducted experiments, it was concluded that the encapsulated form of L-phenylalanine-ammonia-lyase should be stored at a room temperature not exceeding 25 °C. The best stabilizing characteristics under other temperature conditions (4 °C and more than 25 °C) were demonstrated by capsule samples No. 10 containing agar-agar and HPC. To develop technologies for the enzyme preparation delivery in the GI tract, the capsule destruction degree is important and will become the subject of our next research stage.

Declarations

Author Contribution Statement

Svetlana Ivanova, Alexander Prosekov, Valery Pavsky: Analyzed and interpreted the data; Wrote the paper.

Olga Babich: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Lyubov Dyshlyuk, Svetlana Noskova: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Funding Statement

This work was supported by the Russian Foundation for Basic Research [project number 18-08-00444].

Competing Interest Statement

The authors declare no conflict of interest.

Additional Information

No additional information is available for this paper.

References

[1] A. Wessel, F. Rohr, Inborn errors of metabolism, in: K. Sonneville, C. Duggan (Eds.), Manual of Pediatric Nutrition, fifth ed., People's Publishing House-USA, Shelton, Connecticut, 2014, pp. 469–472.

[2] N. Blau, F.J. Van Spronsen, H.L. Levy, Phenylketonuria, Lancet 376 (2010) 1417–1427.

[3] H.L. Levy, C.N. Sarkissian, C.R. Scriver, Phenylalanine ammonia lyase (PAL): from discovery to enzyme substitution therapy for phenylketonuria, Mol. Genet. Metab. 124 (4) (2018) 223–229.
[4] N. Longo, C.O. Harding, B.K. Burton, D.K. Grange, J. Vockley, M. Wasserstein, G.M. Rice, A. Dorenbaum, J.K. Neuenburg, D.G. Musson, Z. Gu, S. Sile, Single-dose, subcutaneous recombinant phenylalanine ammonia lyase conjugated with polyethylene glycol in adult patients with phenylketonuria: an open-label, multicentre, phase 1 dose-escalation trial, Lancet 384 (2014) 37–44.
[5] C.N. Sarkissian, A. Gámez, L. Wang, M. Charbonneau, P. Fitzpatrick, J.F. Lemontt, B. Zhao, M. Vellard, S.M. Bell, C. Henschell, A. Lambert, L. Tsuruda, R.C. Stevens, C.R. Scriver, Preclinical evaluation of multiple species of PEGylated recombinant phenylalanine ammonia lyase for the treatment of phenylketonuria, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 20894–20899.
[6] M. Zhao, F.N. Qu, Z.J. Wu, K. Nishinari, G.O. Phillips, Y.P. Fang, Protection mechanism of alginate microcapsules with different mechanical strength for Lactobacillus plantarum ST-III, Food Hydrocolloids 66 (2017) 396–402.
[7] H.A. Albadran, A. Chatzifragkou, V.V. Khutoryanskiy, D. Charalampopoulos, Development of surfactant-coated alginate capsules containing Lactobacillus plantarum, Food Hydrocolloids 82 (2018) 490–499.
[8] P. de Vos, M.M. Faas, M. Spasojevic, J. Sikkema, Encapsulation for preservation of functionality and targeted delivery of bioactive food components, Int. Dairy J. 20 (4) (2010) 292–302.
[9] N. Joondan, S. Jhaumeer-Laulloo, P. Caumul, A study of the antibacterial activity of L-Phenylalanine and L-Tyrosine esters in relation to their CMCs and their interactions with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DPPC as model membrane, Microbiol. Res. 169 (2014) 675–685.
[10] A. Pinazo, M.A. Manresa, A.M. Marques, M. Bustelo, M.J. Espuny, L. Perez, Amino acid-based surfactants: new antimicrobial agents, Adv. Colloid Interface Sci. 228 (2016) 17–39.
[11] F. Alhaique, M. Pietro, C. Di Meo, T. Coviello, E. Montanari, Polysaccharide-based self-assembling nanohydrogels: an overview on 25-years research on pullulan, J. Drug Deliv. Sci. Technol. 30 (B) (2015) 300–309.
[12] B. Kang, T. Opatz, K. Landfester, F.R. Wurm, Carbohydrate nanocarriers in biomedical applications: functionalization and construction, Chem. Soc. Rev. 44 (22) (2015) 8301–8325.
[13] Y. Xue, X. Xia, B. Yu, X. Luo, N. Cai, S. Long, F. Yu, A green and facile method for the preparation of a pH-responsive alginate nanogel for subcellular delivery of doxorubicin, RSC Adv. 5 (2015) 73416–73423.
[14] X. Yang, H. Du, J. Liu, G. Zhai, Advanced nanocarriers based on heparin and its derivatives for cancer management, Biomacromolecules 16 (2) (2015) 423–436.
[15] T.A. Debele, S.L. Mekuria, H.-C. Tsai, Polysaccharide based nanogels in the drug delivery system: application as the carrier of pharmaceutical agents, Mater. Sci. Eng. C 68 (2016) 964–981.
[16] T.G. Barclay, C.M. Day, N. Petrovsky, S. Garg, Review of polysaccharide particle-based functional drug delivery, Carbohydr. Polym. 221 (2019) 94–112.
[17] A. Zhang, Z. Zhang, F. Shi, J. Ding, C. Xiao, X. Zhuang, C. He, L. Chen, X. Chen, Disulfide crosslinked pegylated starch micelles as efficient intracellular drug delivery platforms, Soft Matter 9 (7) (2013) 2224–2233.
[18] O.O. Babich, Biotehnoloiya L-Fenilalanin-Ammohiy- Liazy [Biotechnology of the L-Phenylalanine-Ammonium-Lyase], pub. Nauka, Novosibirsk, 2013 (in Russian).
[19] O.O. Babich, V.S. Pokrovsky, N.Y. Anisimova, N.N. Sokolov, A.Y. Prosekov, Recombinant L-phenylalanine ammonia lyase from Rhodosporidium toruloides as a potential anticancer agent, Biotechnol. Appl. Biochem. 60 (3) (2013) 316–322.
[20] O.O. Babich, Screening and identification of pigmental yeast producing L-phenylalanine ammonia-lyase and their physiological and biochemical characteristics, Foods Raw Mater. 2 (2) (2014) 75–87.
[21] S.A. Ivanova, V.A. Pavsky, M.A. Poplavskaya, M.V. Novoselova, Studying the biokinetics of pigmented yeast by stochastic methods, Food Raw Mater. 2 (1) (2014) 17–21.
[22] A.Yu. Prosekov, O.O. Babich, V.S. Pokrovsky, S.A. Ivanova, M.V. Novoselova, Efficient expression of recombinant L-phenylalanine ammonia-lyase from rhodosporidium toruloides using Escherichia coli, J. Appl. Biotechnol. 2 (1) (2014) 24–36.
[23] O.O. Babich, A.Yu. Prosekov, L.S. Dyshlyuk, S.A. Ivanova, Investigation of kinetic aspects of L-phenylanine ammonia-lyase production in pigmental yeast Kinetic aspects of L-phenylanine production, Chim. Oggi 33 (6) (2015) 16–20.
[24] P. Khatri, D. Desai, N. Shelke, T. Minko, Role of plasticizer in membrane coated extended release oral drug delivery system, J. Drug Deliv. Sci. Technol. 44 (2018) 231–243.
[25] S.W. Hoag, Capsules dosage form: formulation and manufacturing considerations, in: Y. Qiu, Y. Chen, G.G.Z. Zhang, L. Yu, R.V. Mantri (Eds.), Developing Solid Oral Dosage Forms. Pharmaceutical Theory and Practice, Elsevier Inc, Academic Press, UK, London, 2017, pp. 723–747.
[26] S. Habibi-Moini, A.P. D'mello, Evaluation of possible reasons for the low phenylalanine ammonia lyase activity in cellulose nitrate membrane microcapsules, Int. J. Pharm. 215 (1–2) (2001) 185–196.
[27] B.E. Jones, A.W. Basit, C. Tuleu, The disintegration behaviour of capsules in fed subjects: a comparison of hypromellose (carrageenan) capsules and standard gelatin capsules, Int. J. Pharm. 424 (1–2) (2012) 40–43.
[28] T.S. Kang, L. Wang, C.N. Sarkissian, A. Gamez, C.R. Scriver, R.C. Stevens, Converting an injectable protein therapeutic into an oral form: phenylalanine ammonia lyase for phenylketonuria, Mol. Genet. Metab. 99 (2010) 4–9.
[29] C.N. Sarkissian, T.S. Kang, A. Gámez, C.R. Scriver, R.C. Stevens, Evaluation of orally administered PEGylated phenylalanine ammonia lyase in mice for the treatment of Phenylketonuria, Mol. Genet. Metab. 104 (3) (2011) 249–254.
[30] R.M. Shah, A.P. D'mello, Strategies to maximize the encapsulation efficiency of phenylalanine ammonia lyase in microcapsules, Int. J. Pharm. 356 (1–2) (2008) 61–68.
[31] S. Dumitriu (Ed.), Polysaccharides: Structural Diversity and Functional Versatility, second ed., CRC press, Taylor and Francis Group, 2004.
[32] N. Devi, M. Sarmah, B. Khatun, T.K. Maji, Encapsulation of active ingredients in polysaccharide–protein complex coacervates, Adv. Colloid Interface Sci. 239 (2017) 136–145.
[33] J. Li, Z. Jiang, H. Wu, Y. Liang, Y. Zhang, J. Liu, Enzyme–polysaccharide interaction and its influence on enzyme activity and stability, Carbohydr. Polym. 82 (1) (2010) 160–166.