Study of the in vitro degradation dynamics of the encapsulated PAL enzyme preparation in model biological fluids

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Abstract. The use of PAL in the phenylketonuria treatment is hindered by the lack of well-studied mechanisms and systems of targeted transport of enzyme preparations to certain organs and tissues (brain, liver, skin, muscle and connective tissues, blood cells). It is known that as a result of the incorporation of any pharmaceutical composition into the composition of the carrier, the pharmacokinetics and pharmacodynamics of the drug change. The essence of the enzyme stabilization process is to increase and maintain the catalytic activity of proteins, increase the duration of the catalytic action of enzymes on the substrate, change the optimal temperature and active acidity, increase productivity, as well as change the temporary effect of enzymes on the substrate in order to transform into an end product of the reaction. When stabilizing enzymes, encapsulation is preferred due to the fact that this method provides a high stability of enzymes under the influence of low temperature and low acidity of the environment, as well as the effect of harmful microflora and oxygen. It was proven that the degradation degree of capsules with PAL reaches 55–65% after 120 minutes of the experiment in distilled water. The maximum degradation of the encapsulated form of the PAL enzyme preparation (the proportion of capsule weight loss is 90–98%) occurs in model biorelevant media that mimic intestinal juice: SIF and FaSSIF. It should be noted that the rate of capsule degradation is higher in the simulated intestinal fluid (SIF) without pancreatin (the proportion of capsule weight loss is 92–98% after 15 minutes of the experiment) compared to the fasted state simulated intestinal fluid (FaSSIF) (the proportion of capsule weight loss is 90-97 % after 30 min of experiment).

1. Introduction
The widespread use of PAL in the phenylketonuria treatment is hindered by the lack of well-studied mechanisms and systems of targeted transport of enzyme preparations to certain organs and tissues (brain, liver, skin, muscle and connective tissues, blood cells). It is known that as a result of the incorporation of any pharmaceutical composition into the composition of the carrier, the pharmacokinetics and pharmacodynamics of the drug change. At the same time, there is an increase in the effectiveness of the action and a decrease in the side effects of the medicinal substance both by increasing the bioavailability and by the selective concentration of the substance in the affected tissues and organs [1].

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temperature and active acidity, increase productivity, as well as change the temporary effect of enzymes on the substrate in order to transform into an end product of the reaction [2].

When stabilizing enzymes, encapsulation is preferred due to the fact that this method provides a high stability of enzymes under the influence of low temperature and low acidity of the environment, as well as the effect of harmful microflora and oxygen. An important advantage of encapsulated preparations is the ability to ensure uniform dosage of the enzyme [3].

It is reasonable to develop a capsule shell for PAL create a stable form of the enzyme that guarantees its preservation before direct reaction with phenylalanine, especially in the acidic environment of the stomach.

In recent years, the greatest preference in the development of new drugs has been given to encapsulated drugs, which have certain advantages. At the beginning of the 20th century, gelatin was widely used for creating capsules, which showed good physicochemical properties suitable for use in the pharmaceutical industry. By the beginning of the 1970s, the number of the produced gelatin-based capsules reached more than 22 billion pieces. Nowadays gelatin capsules take second place, second only to tablets. Such popularity and demand for encapsulated dosage forms is explained by the following advantages of capsules:

- the capsule delivers the active substance directly to the intestines;
- the most convenient form of drugs that have an unpleasant odor and taste;
- the reduced impact of external environmental factors on the active substance of the drug.

Like 100 years ago, gelatin is still one of the most common capsule compound. Gelatin is a protein compound of animal origin. Today a wide variety of capsules that differ in their sizes, shapes and established properties can be found. A special gelatin is used, which is produced by a small number of enterprises located in Europe and China, is used for their production. The wholesale price of this gelatin is approximately 600 thousand rubles per ton. However, the price is not sustainable and rises annually [4].

In Russia, there is not enough production capacity for the production of high-quality gelatin and the collection of raw materials is not organized over a large area. Besides every year more and more territories are closed by veterinary services for various veterinary diseases, including BSE (mad cow disease). Gelatin can transfer prions - small infectious particles that are resistant to inactivating influences that can modify nucleic acids and cause Creutzfeldt-Jakob disease, vector-borne spongiform encephalopathies. The use of raw materials from such territories is impossible.

The result of the raw materials shortage and the growing demand is a constant rise in prices for gelatin and long delivery periods (2–3 months) from abroad [5]. All of the above factors determine the relevance of developing a technology for producing capsules based on non-traditional raw materials, such as plant raw materials (compositions of plant hydrocolloids).

Since the essence of encapsulation is to create a kind of shell around the enzyme, an important step is the selection of a suitable material. The effectiveness of protecting enzymes from negative environmental factors depends on its properties. Currently, there is a wide range of polymers used for encapsulation, the selection of suitable polymers for this research was performed according to the following criteria:

1) the material must be non-toxic, free of chemicals and additives that can affect the enzyme and the human body;
2) the material must be degraded by the gastrointestinal tract microbiota to allow sustained release of PAL;
3) the material must be rehydrated properly to ensure the enzyme stability.

The literature data analysis showed that such materials as sodium alginate, pectin, chitosan, carrageenan, gelatin, agarose, xanthan-gelatin mixture, various cellulose derivatives, etc., satisfy these criteria [6, 7].
2. Research objects and methods

The dynamics of *in vitro* degradation of the encapsulated form of the PAL enzyme preparation (samples of capsules No. 4, 5, 10, 15, 18, 24) was studied in distilled water and model biological fluids at 37°C using the laboratory identifier of the degradation process ERWEKA, series ZT 220. As biorelevant media simulating intestinal and gastric juices, we used simulated gastric fluid without pepsin (SGFsp), simulated intestinal juice without pancreatin (SIFsp), fasted state simulated intestinal fluid (FaSSIF), and fasted state simulated gastric fluid (FaSSGF) (Table 1).

| Table 1. Model biorelevant media. |
|----------------------------------|
| Medium                           | Medium composition                  |
| Intestinal                       | Potassium dihydrogen phosphate – 50 mM |
| SIFsp                            | Sodium hydroxide – up to pH 7.5      |
|                                  | Sodium taurocholate – 3 mM           |
|                                  | Lecithin – 0.2 mM                    |
| FaSSIF                           | Maleic acid – 19.12 mM               |
|                                  | Sodium hydroxide – 34.8 mM           |
|                                  | Sodium chloride – 68.62 mM           |
| Gastrointestinal                 |                                          |
| SGFsp                            | Sodium chloride – 30 mM              |
|                                  | Hydrochloric acid - up to pH 1.2     |
|                                  | Sodium taurocholate – 0.08 mM        |
|                                  | Lecithin – 0.02 mM                   |
| FaSSGF                           | Pepsin with an activity of at least 600 U / mg – 0.1 mg / ml |
|                                  | Sodium chloride – 34.2 mM            |
|                                  | Hydrochloric acid – up to pH 1.6     |

3. Results and discussion

The results of studying the *in vitro* degradation dynamics of the encapsulated PAL enzyme preparation in model biological fluids are presented in Figures 1–5.

![Figure 1](image-url) - The results of studying the *in vitro* degradation dynamics of the encapsulated PAL enzyme preparation in SIFsp: 1 – capsule sample No. 4, 2 – capsule sample No. 5, 3 – capsule sample No. 10, 4 – capsule sample No. 15, 5 – capsule sample No. 18, 6 – capsule sample No. 24.
**Figure 2.** The results of studying the *in vitro* degradation dynamics of the encapsulated PAL enzyme preparation in FaSSIF: 1 – capsule sample No. 4, 2 – capsule sample No. 5, 3 – capsule sample No. 10, 4 – capsule sample No. 15, 5 – capsule sample No. 18, 6 – capsule sample No. 24.

**Figure 3.** The results of studying the *in vitro* degradation dynamics of the encapsulated PAL enzyme preparation in SGFsp: 1 – capsule sample No. 4, 2 – capsule sample No. 5, 3 – capsule sample No. 10, 4 – capsule sample No. 15, 5 – capsule sample No. 18, 6 – capsule sample No. 24.
Figure 4. The results of studying the in vitro degradation dynamics of the encapsulated PAL enzyme preparation in FaSSGF: 1 – capsule sample No. 4, 2 – capsule sample No. 5, 3 – capsule sample No. 10, 4 – capsule sample No. 15, 5 – capsule sample No. 18, 6 – capsule sample No. 24.

Figure 5. The results of studying the in vitro degradation dynamics of the encapsulated PAL enzyme preparation in distilled water: 1 – capsule sample No. 4, 2 – capsule sample No. 5, 3 – capsule sample No. 10, 4 – capsule sample No. 15, 5 – capsule sample No. 18, 6 – capsule sample No. 24.

Based on the analysis of Figures 1–5, it was concluded that the degree of degradation of capsules with PAL reaches 55–65% after 120 minutes of the experiment in distilled water.

The maximum degradation of the encapsulated form of the PAL enzyme preparation (the proportion of capsule weight loss is 90–98%) occurs in model biorelevant media that mimic intestinal juice: SIF and FaSSIF. It should be noted that the rate of capsule degradation is higher in the simulated intestinal fluid (SIF) without pancreatin (the proportion of capsule weight loss is 92–98% after 15 minutes of the
experiment) compared to the fasted state simulated intestinal fluid (FaSSIF) (the proportion of capsule weight loss is 90-97 % after 30 min of experiment).

Thus, the dynamics of in vitro degradation of the encapsulated form of the PAL enzyme preparation in model biological fluids has been studied.

4. Conclusion
It was established that the use of PAL in the phenylketonuria treatment is hindered by the lack of well-studied mechanisms and systems of targeted transport of enzyme preparations to certain organs and tissues. The pharmacokinetics and pharmacodynamics of the drug change as a result of the incorporation of any pharmaceutical composition into the composition of the carrier. The encapsulation is preferred when stabilizing enzymes because this method provides a high stability of enzymes under the influence of low temperature and low acidity of the environment, as well as the effect of harmful microflora and oxygen. It was proven that the degradation degree of capsules with PAL reaches 55–65% after 120 minutes of the experiment in distilled water. The maximum degradation of the encapsulated form of the PAL enzyme preparation (the proportion of capsule weight loss is 90–98%) occurs in model biorelevant media that mimic intestinal juice: SIF and FaSSIF. The rate of capsule degradation is higher in the simulated intestinal fluid (SIF) without pancreatin compared to the fasted state simulated intestinal fluid (FaSSIF).

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