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Microcapsules made of sodium alginate for the prolonged release of phenibut

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ABSTRACT: A prolonged system of the pharmaceutical substance delivery with nootropic effect was developed; it was phenibut in the form of microcapsules. The polymers used were sodium alginate as polyanion and Calcium chloride as polycation. It was realized by spraying a solution of sodium alginate in a solution of Calcium chloride, which induces the gelation. The features of the surface for the obtained microcapsules with the different ratio of nucleus/substance were determined in the work. Identification of phenibut in microcapsules was performed by IR-spectroscopy. Dependence of the microencapsulation efficiency on the concentration of film-forming material solution and the ratio of nucleus/substance was found. The process of phenibut release from microcapsules in the medium of 0.1 M solution of hydrochloric acid was investigated.

KEYWORDS: Microencapsulation; extrusion; IR-spectroscopy; microencapsulation efficiency; release kinetics; morphology; phenibut.

1. INTRODUCTION

Over the past few decades, the rise of modern pharmaceutical technology and the amazing growth of the biotechnology industry have revolutionized the approach to drug discovery and development.

The most common method of administration of drugs is in the form of pills or injections. These methods of administration meet the requirements of efficacy for several drugs. However, these methods are inadequate for many new drugs. To overcome these difficulties, new technologies, like the microencapsulation, have been developed [1,2] .

These technologies are based on the use of polymers [3-5]. Polysaccharides, such as alginate, have been widely used in the microencapsulation technology.

Probably, the most important property of alginates is their ability to form gels by reaction with divalent cations such as calcium or barium by binding between guluronic acid blocks in alginate and the divalent cations. These gels are similar to solids because they retain their shape and resist stress. This process of gelation is an almost instantaneous and irreversible process, which is governed by the relative rate of diffusion of barium ions and polymer molecules into the gelling zone [6-8].

A lot of microencapsulation methods involve one of two heavy-load conditions (contact with organic solvent and/or thermal contact during treatment) that is usually a problem, especially during a treatment of biomaterials [9-15].

Vascular encephalopathy takes the second place in the structure of mortality as a result of circulatory system diseases [16,17]. Annual death rate from the stroke is one of the highest in the world. It should be noted an important physiological role of gamma aminobutyric acid (GABA) in the regulation of the functional activity of central nervous system for these kinds of diseases [18,19].

At present, the establishment of the new drug formulations for such derivative of GABA as γ-amino-β-phenylbutyric acid hydrochloride (phenibut) characterized by a prolonged action is quite actual [20,21].

The aim of the work is the development of a system for prolonged release of nootropic effect. It is based on the use of sodium alginate microcapsules charged with phenibut.

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2. RESULTS AND DISCUSSION

2.1. Morphology of microcapsules

Using extrusion technique microcapsules of sodium alginate were obtained charged with phenibut.

Porosity of the microcapsules structure is known to be likely influenced by the composition and the ratio of extracted complexes. Therefore, an influence of the ratio of nucleus/polymer on morphology of the obtained samples was studied in the work.

Rough surface with a lot of tension bars can be well observed in the micrographs of the obtained polymer microcapsules (Figures 1 and 2).

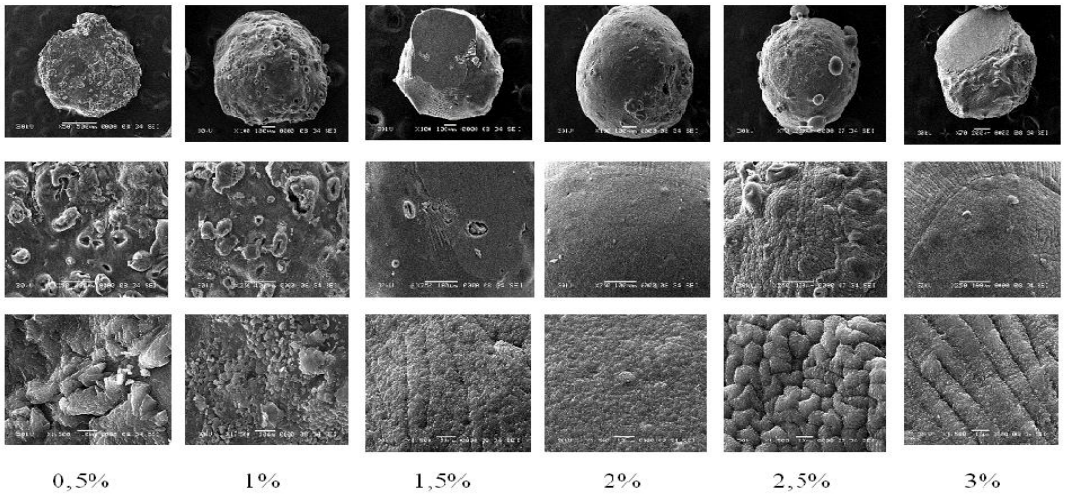

Figure 1. Microphotographs of the microcapsules surface of sodium alginate with phenibut (ratio 1:1).

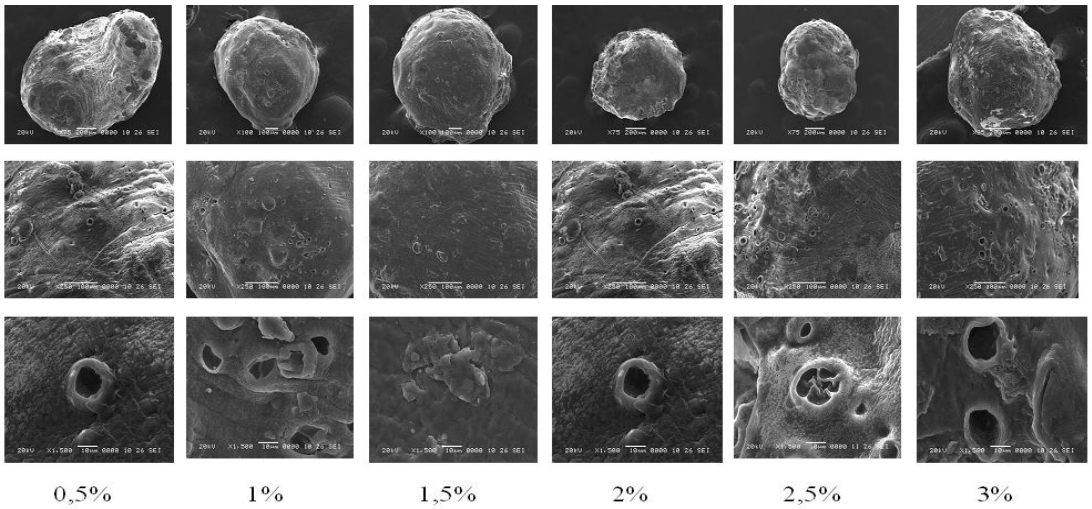

Figure 2. Microphotographs of the microcapsules surface of sodium alginate with phenibut (ratio 0.5:1).

The obtained microcapsules are characterized by relief, porous surface and the presence of the bulk «halls». Surface porosity and formation of «halls» depend on the ratio of nucleus/polymer. For example, if thee ratio of nucleus/polymer is of 0,5:1 more even surface of microcapsules can be noticed with little characteristic «folds». At the same time microcapsules surface with the ratio of nucleus/polymer equal to 1:1 is presented with deep folds and tension bars.

2.2. Phenibut identification

Results of IR-spectroscopy study for a substance of phenibut, microcapsules-placebo and microcapsules with phenibut are presented in Figure 3.

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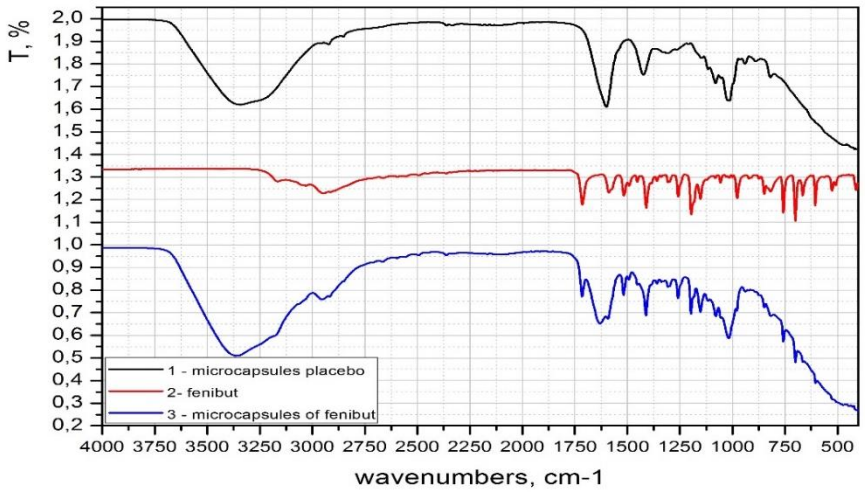

Figure 3. IR-transmission spectra of phenibut substance, microcapsules-placebo and microcapsules with phenibut.

Comparison of IR-spectra made it possible to identify phenibut substance in the microcapsues. IR-spectra of the substance and microcapsules with phenibut within the range of 4000 – 400 cm^{-1} show absorption bands at 3050-2800 cm^{-1}, meaning the presence of the primary aliphatic aminogroup in the samples; while the bands at 1712, 1656, 1668, 1620 indicate at the presence of carboxylic group in the same samples and thus allowing to state that chemical interaction between the chosen components of the mixture is absent.

2.3. Microencapsulation efficiency

Calibration plot for the dependence of the optical density on the amount of phenibut in a solution appears as a straight line (Figure 4).

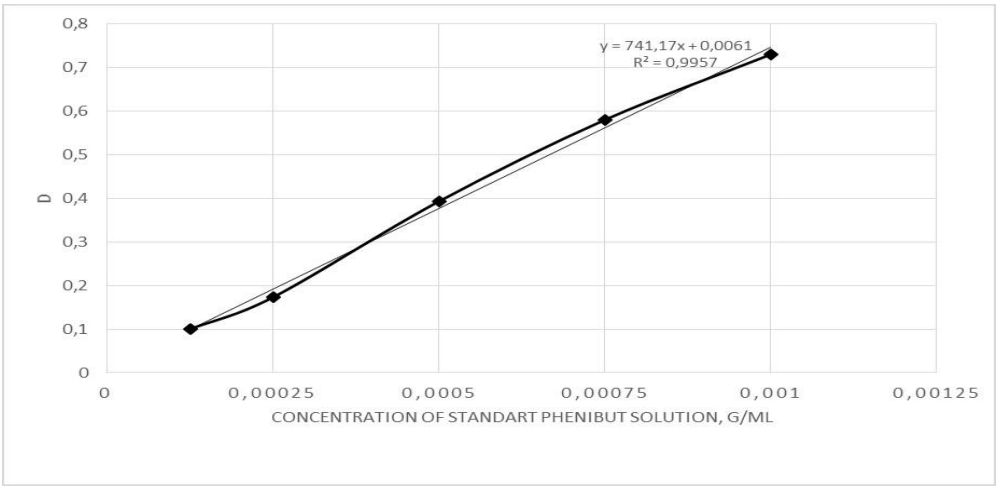

Figure 4. Calibration curve for the dependence of the optical density on phenibut concentration in a solution.

Efficiency of phenibut microencapsulation in a dependence on the concentration of film-forming material solution and the ratio of nucleus/polymer is presented in Table 1.

From Table 1 it follows that the efficiency of microencapsulation is considerably effected as by the concentration of film-forming material as by the ratio of nucleus/polymer. For example with an increase of concentration of sodium alginate solution efficiency of microencapsulation is also enhanced and attains 28.1 % at the concentration of sodium alginate solution of 2 % and the ratio of nucleus/polymer 1:1. For the concentration of sodium alginate solution more than 2% a considerable decrease of efficiency is noticeable as for the ratio of nucleus/polymer 1:1, as for that one of 0.5:1.

Table 1. Efficiency of phenibut microcapsulation.

Concentration of sodium alginate, %	Microcapsulation efficiency, %	
	0.5:1	1:1
0.5	17.5	12.0
1.0	23.1	12.5
1.5	26.9	25.2
2.0	18.8	28.1
2.5	22.5	15.6
3.0	21.3	10.1

2.4. Examination of phenibut release from polymer microstructures *in vitro*

In order to make a comparative estimation of a release degree of the reactant from pharmaceutical substances investigations of the release degree of phenibut into the medium of 0.1 M solution of hydrochloric acid were performed.

Figures 5 and 6 represent the profiles (or curves) of release of the pharmaceutical substance into dissolution medium (%) in a dependence on time (min) (RSD, %).

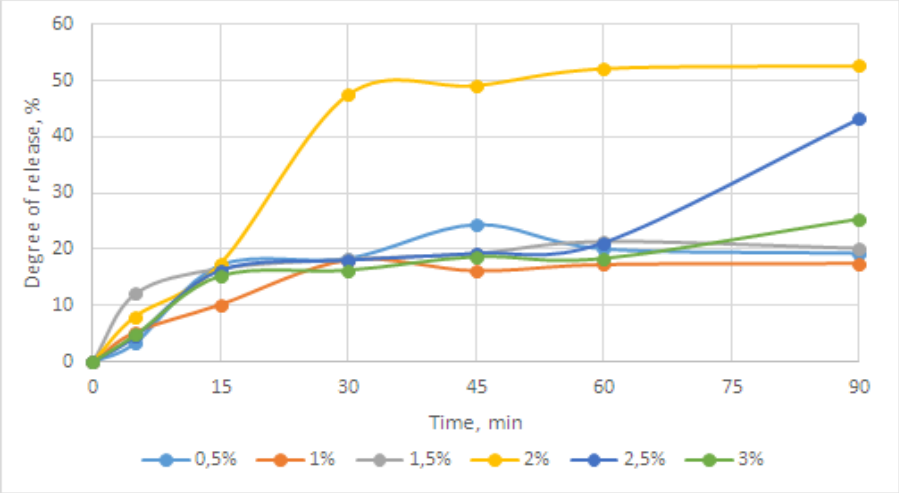

Figure 5. Dynamics of phenibut release from microcapsules (0,5:1).

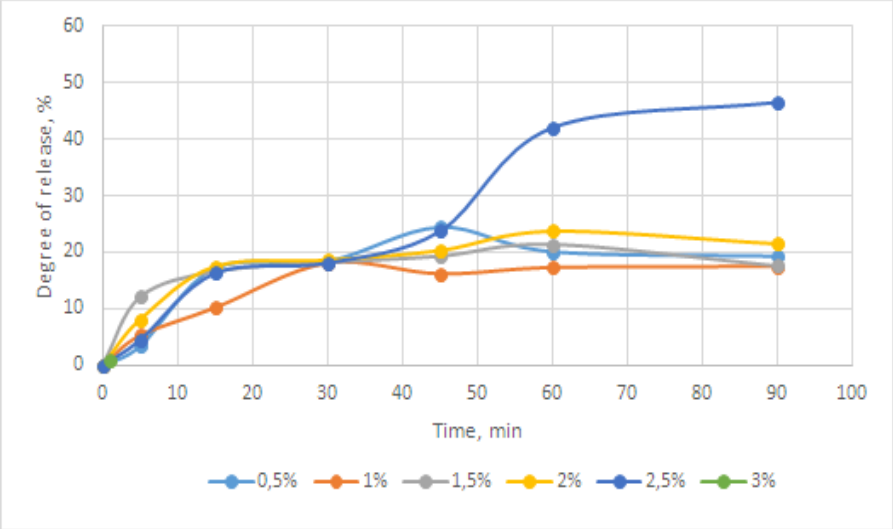

Figure 6. Dynamics of phenibut release from microcapsules (1:1).

As a result of the performed biopharmaceutical investigations it was shown the influence of concentration of the film-forming material solution and the ratio of nucleus/polymer on a release of phenibut into dissolution medium.

2.4.1. Ratio of nucleus/polymer 1:1

The observed homogeneous release of the pharmaceutical substance takes place from microcapsules for all the concentrations of the film-forming material solution. Most complete release of phenibut is observed from the microcapsules with the concentration of the film-forming material solution of 2%; at 90 min of the experiment concentration of the substance is of 52.2 %. Slightly less release of phenibut was observed for the microcapsules with the concentration of the film-forming material solution of 0.5 and 1% and it was of 19.3 and 17.5% , respectively (Figure 5).

RSD, % is presented in Table 2.

Table 2. RSD, % (Ratio of nucleus/polymer 1:1).

Concentration of sodium alginate, %	Time, min				
	15	30	45	60	90
0.5	3.8	2.5	2.4	2.5	4.8
1.0	1.8	0.7	1.5	1.6	4.9
1.5	2.9	4.9	2.0	4.3	2.6
2.0	4.6	5.0	3.9	3.7	3.5
2.5	2.8	3.1	4.7	4.3	4.6
3.0	2.0	1.9	4.0	3.0	4.1

2.4.2. Ratio of nucleus/polymer 0.5:1

The observed homogeneous release of the pharmaceutical substance takes place from microcapsules for all the concentrations of the film-forming material solution. Most complete release of phenibut is observed from the microcapsules with the concentration of the film-forming material solution of 2.5%; at 90 min of the experiment concentration of the substance is of 46.8 %. Slightly less release of phenibut was observed for the microcapsules with the concentration of the film-forming material solution of 1 and 1.5% and it was of 17.7 and 18.3%, respectively (Figure 6). RSD, % is presented in Table 3.

Table 3. RSD, % (Ratio of nucleus/polymer 0,5:1).

Concentration of sodium alginate, %	Time, min				
	15	30	45	60	90
0.5	15	1.9	4.4	4.2	4.2
1.0	3.1	3.8	4.5	3.8	4.0
1.5	5.0	5.0	3.9	4.7	2.9
2.0	3.2	4.8	4.3	4.9	2.7
2.5	3.8	4.2	3.7	5.0	3.6
3.0	4.4	3.9	4.5	3.8	3.9

Thus, in the study it was determined that a release proceeds more completely and homogeneously from microcapsules with the ratio of nucleus/polymer 1:1 (concentration of sodium alginate solution of 2%) and it attains 52.2% after 90 minutes of experiment.

3. CONCLUSIONS

As a result of the research, modeling samples of phenibut microcapsules were obtained. It was stated, that with the increasing concentration of sodium alginate solution, the efficiency of microcapsulation increases, and reaches 28.1 % at a concentration of 2% sodium alginate solution (microcapsules with chitosan). In the study of the influence of the concentration of sodium alginate solution on the degree of release of Phenibut from microcapsule samples, it was found that at a concentration of 1%, the degree of release is 17.5 % (core/polymer ratio 1:1) and 17.7% (core/polymer ratio 0.5:1). The results which were obtained in the study

are promising for further detailed study of microcapsulated phenibut in order to create its pharmaceutical form.

4. MATERIALS AND METHODS

Substance of γ-amino-β-phenylbutyric acid hydrochloride (Figure 7), corresponding to the requirements ND 42-14132-06, sodium alginate FOODALGA of 500 grade, «NevaReaktiv».

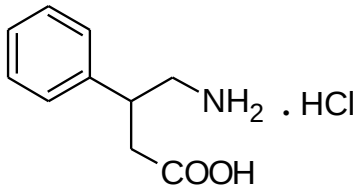

Figure 7. Structural formula of phenibut hydrochloride.

4.1. Obtaining of microcapsules with a shell of sodium alginate

At the first stage the process of cross-linking of alginic acid with calcium ions was examined.

Phenibut immobilization by incorporation was performed according to the following technique: a sample of 1.0 g of phenibut was placed into solution of sodium alginate (concentration of 0.5; 1; 1.5; 2; 2.5; 3%) and dosed with the use of extrusion through a sprayer into 0.2 M solution of calcium chloride. The ratio of volume of the film-forming material solution : calcium chloride solution 1:10. The formed particles of calcium alginate were sustained in a cross-linking agent solution for 20 minutes for the hardening.

Alginic acid is a block-copolymer, formed by polymers of three types: blocks of homopolymers of D-mannuronic and L-guluronic acid and mixed heteropolymeric blocks containing both types of monomeric chains. Residues of uronic acids in alginic acid are bound with β-1,4-glycosidic linkages in the form of linear chain.

When formation of microcapsules a reaction of substitution of Na+ cations with Ca^{2+} cations proceeds with the formation of calcium alginate insoluble in water.

Structure of the surface provides an important information on porosity of microsystems intended for a delivery of the pharmaceutical substances. Scanning electron microscopy (SEM) was applied for the study of morphological features of the obtained samples and it was realized with the use of microscope (JSM-6380LV, JEOL).

Gold was deposited on the samples while performing of morphological investigations with the use of SEM in order to reduce the charging effects.

4.2. Identification of phenibut

IR-spectra were surveyed with Vertex 70 spectrometer (Bruker Optik GmbH, Germany), in the middle part of IR-region in the range of 4000–400 cm^{-1} applying ATR technique (attenuated total reflectance method), using ZnSe attachment with the diamond window; as a result, IR-absorption spectra were obtained for phenibut substance, placebo-microcapsules and microcapsules with phenibut.

4.3. Efficiency of microencapsulation

A key point in obtaining of the microencapsulated product is the efficiency of micro- capsulation, i.e. a part of the encapsulated substance involved inside the capsules related to its reference quantity. Efficiency of micro-capsulation was determined in a «direct» way.

Weighed microcapsules of 50 mg mass were dissolved in 10 ml of 0.1 M of hydrochloric acid solution for 30 minutes under agitation with magnetic stirrer in a closed vessel. Optical density of the obtained solution was determined by spectrophotometry technique with spectrophotometer Hitachi U-1900 at the wavelength of 257±2 nm. Solution of 0.1 M hydrochloric acid was used as a reference one. Concentration of a substance in solution was determined with the use of calibration plot.

To obtain calibration plot for the dependence of the optical density on the concentration of the standard sample of phenibut a precise sampling of 0.25 g phenibut was transferred to the volumetric flask of 250 ml capacity, then added 200 ml of hydrochloric solution and finally brought the volume of solution up to the required label.

To prepare dilutions aliquots of the reference standard were transferred into the volumetric flasks of 25 ml in volume and brought the volume of solution up to the label with 0.1 M of hydrochloric acid solution. Aliquots of standard solution were used, in ml: 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5.

After measuring of the amount of phenibut released under dissolution and knowing its initial concentration it was possible to calculate efficiency of microencapsulation taking into account the amount of substance involved in microcapsules Ccaps., as compared with the amount of the initially dissolved substance Cinit. According to the formula (Eq. 1):

$$E=\frac{m_{caps}}{m_{inform}} * 100\% \quad \text{Eq. 1}$$

4.4. Examination of phenibut release from microstructures *in vitro*

«Dissolution» test was performed with the help of dissolution tester DT 626/1000HH produced by ERWEKA Company (Germany) provided with impeller mixer.

Dissolution medium was as follows: 0,1 M solution of hydrochloric acid, the volume of dissolution medium was of 700 ml, time points for sampling were as follows: 15 min, 30 min, 45 min, 60 min, 90 min, 120 min, 180 min.

1 capsule with the examined microcapsules was placed in each of 6 glasses. The glasses were immersed in the vessels for dissolution with 700 ml of dissolution medium (0.1 M solution of hydrochloric acid), preliminarily temperature-controlled at 37± 0.5 °C. After the above indicated periods of time 5 ml of the medium was sampled. After sampling the specimens were filtered through the membrane filters with a diameter of pores 0,45 μm and the first portions of filtrate were removed.

0.1 M solution of hydrochloric acid was used as a reference solution. Concentration of substance in solution was determined with the help of calibration plot.

Statistical treatment of the experimental results was performed with the use of Microsoft Office Excel 2013 suite calculating the average amount of dissolved substance and the relative standard deviation (RSD,%).

Authorship statement: Author contributions: Concept –Y.P.; Design –Y.P.; Supervision –Y.P., K.K.; Resource Y.P., K.K.; Materials –Y.P., K.K. Data Collection and/or Processing –Y.P.; Analysis and/or Interpretation –Y.P.; Literature Search –Y.P., K.K.; Writing –Y.P., K.K.; Critical Reviews –Y.P., K.K.

Conflict of interest statement: The authors declared no conflict of interest.

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