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Influence of Microencapsulation Technology on The Morphological and Biopharmaceutical Characteristics of Phenibut Microcapsules.

Yuliya Polkovnikova[1*], Alexey Slivkin[1], Kseniya Koryanova[2], and Ekaterina Tretyakova[3].

[1]Federal state budgetary educational institution of higher professional education "Voronezh state University» Universitetskaya p.1, Voronezh, 394006 Russia
[2]Pyatigorsk Medical and Pharmaceutical Institute - branch of Volgograd State Medical University of the Ministry of Health of Russia, Kalinina ave., 11, Pyatigorsk, 357532 Russia [3]Russian University of friendship of peoplesMiklukho-Maklaya, 6, Moscow, 117198 Russia

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. The obtained microcapsules were exposed in chitosan solution. Application of scanning electron microscopy allowed to determine the features of the structure for the obtained materials in a dependence of their technology. A dependence of microencapsulation efficiency on the concentration of film-forming material and technology of microcapsules fabrication was derived.

Keywords: Microencapsulation, sodium alginate, chitosan, extrusion, release kinetics, morphology, phenibut

Corresponding author:

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INTRODUCTION

Encapsulation of pharmaceuticals and biologically active supplements is widely spread now in medical practice and special alimentation as one of the most effective means to reach the determined site in human organism in determined time and by control [[1]-[3]]. Encapsulation has many advantages in comparison of traditional forms of pharmaceuticals and biologically active food supplements and allows to create new molecular structures with new useful properties for different branches such as material study, biotechnology, cell therapy, dietology and so on [[4]-[6]].

Application of such systems based on the use of biopolymer microparticles can eliminate a majority of the conventional medications: high toxicity, inefficiency of the active principle, instability of a substance, inconvenience of introduction and so on.

A lot of microencapsulation techniques involve at least one of two severe conditions (contact with an organic solvent and/or thermal treatment while advancing the process) that is usually a problem, especially when processing biomaterials [[7]-[9]].

Sodium alginate is characterized by unique colloid-chemical properties – it is non-toxic, biodegradable and thus it can be successfully applied as a film-forming material in the production of microcapsules [[10]].

At the same time polysaccharide chitosan related to the biopolymers is regarded as the most perspective one for production of biomedical materials [[11]-[12]]. Chitosan is characterized by unique biological properties including biocompatibility, non-toxicity, capability of biodegradation [[13]].

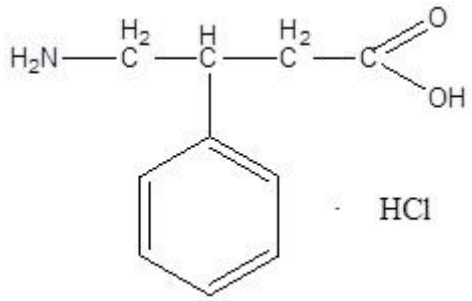
Vascular encephalopathy takes the second place in the structure of mortality as a result of circulatory system diseases. Annual death rate from the stroke is one of the highest in the world. It should be noted an important physiological role of gamma aminobutyric acid (GABA) in the regulation of the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

At present, the establishment of the new drug formulations for such derivative of GABA as phenibut characterized by a prolonged action is quite actual [[14]].

The aim of the work is elaboration of technique for obtaining of microcapsules with sodium alginate shell and next their treatment in chitosan solution; the study of technological and biopharmaceutical properties of microcapsules.

MATERIALS AND METHODS

Substance of γ-amino-β-phenylbutyric acid hydrochloride (figure 1), corresponding to the requirements ND 42-14132-06, sodium alginate FOODALGA of 500 grade, «NevaReaktiv», chitosan used as bioactive additive produced by "Pharmacon Production LLC (St. Petersburg).



$C_{10}H_{13}NO_2$
hydrochloride of γ-amino-β-phenylbutyric acid

Fig 1. Structural formula of phenibut

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 elementary chain link in chitosan (poly [(1-4)-2- amino -2-deoxy-β-D-glucopyranose]) is shown in fig. 2.

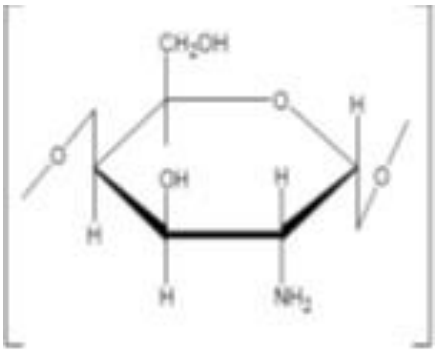


Fig. 2. Structure of the alternating constituent link in chitosan

Mean molecular weight of chitosan is 250-300 kDa, deacetylation degree is 89.6%, humidity – 7.5%, pK = 6.3. In a dependence of pH solution chitosan can be either in ammonium ionic or molecular form.

Obtaining of microcapsules modified with chitosan

Dipping of microcapsules A in 0,1% chitosan solution at the volumetric content of 1:2, providing a capability for their interaction during 30 minutes period in order to obtain microcapsules of sodium alginate-chitosan, washing-out of the obtained microspheres with physiologic saline. Optimum time of exposure for microcapsules in chitosan solution was of 30 minutes; this time was sufficient for the formation of homogeneous layer of chitosan-alginate without the ruptures. Lesser time would not provide homogeneity of chitosan-alginate layer on the surface of microcapsules. An increase of exposure time did not result in the formation of chitosan-alginate layer with better parameters.

Preparation of chitosan solution in the buffer solvent of acetic acid-sodium acetate with pH from 5,5 to 7,0 was performed using exact samples of the dry polymer. Dissolution was made in magnetic stirrer at 900 rpm in the flasks of appropriate size.

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.

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.

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:

$$E=\frac{m_{caps}}{m_{inform}} * 100\%$$

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 □m 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.

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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,%).

RESULTS AND DISCUSSION

Morphology of microcapsules.

Using extrusion technique microcapsules of sodium alginate were obtained charged with phenibut. Composition and the ratio of nucleus/polymer are known to have an effect on the porosity of microcapsules structure [[15],[16]]. Consequently, the influence of the ratio of nucleus/polymer on morphology of the obtained samples treated in a chitosan solution.

One can easily see a rough surface with a lot of tension bars in the microphotographs of the obtained polymer microcapsules (figs. 3,4).

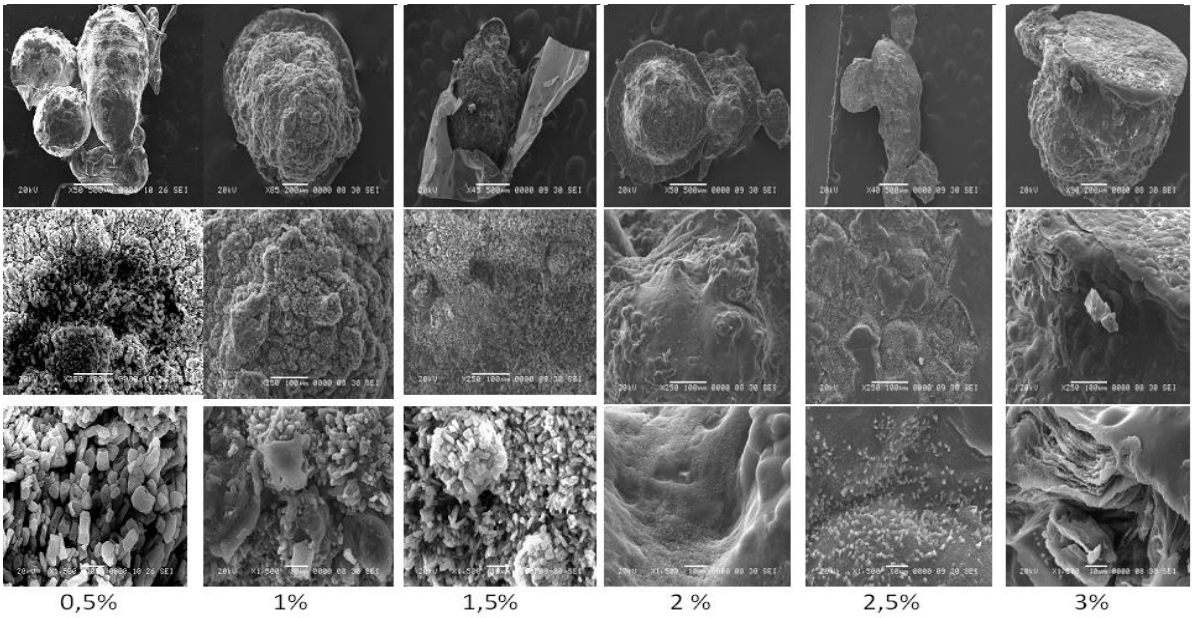

Figure 3. Microphotographs of the microcapsules surface of sodium alginate with phenibut (ratio 1:1)

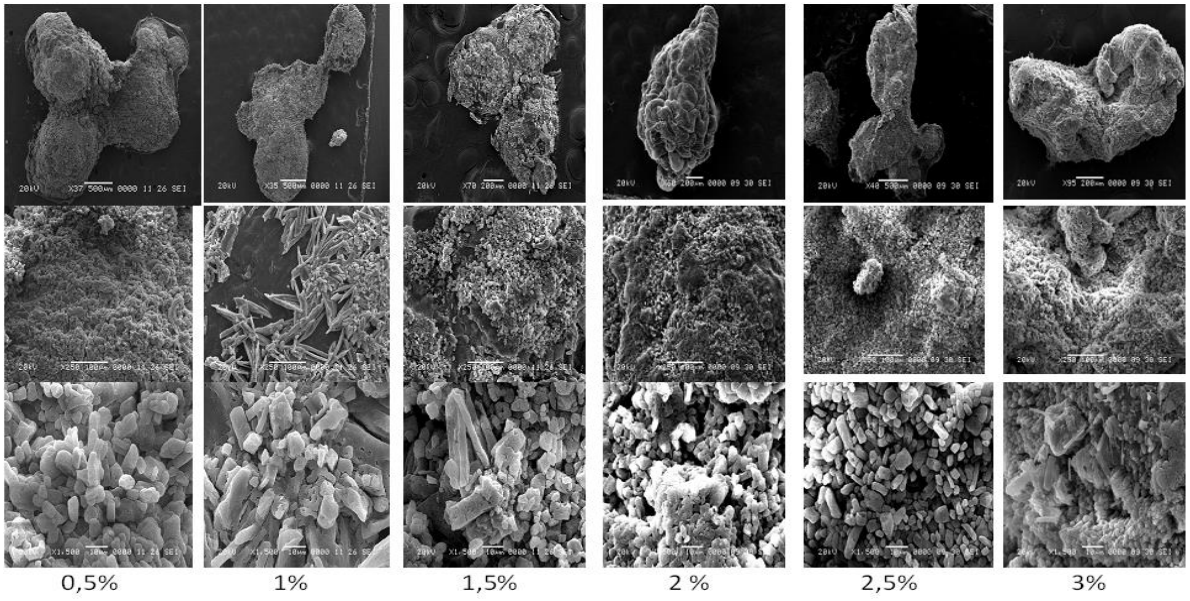

Figure 4. Microphotographs of the microcapsules surface of sodium alginate with phenibut (ratio 0,5:1:)

ISSN: 0975-8585

The obtained microcapsules represent particles of irregular shape, non-uniform in size that is a proper for microcapsules and solid substances almost completely covered with a polymer film. Moreover, each of the particles in the obtained product represents a conglomerate consisting of separate microcapsules with the length of about 1 μm and width of less than 1 μm.

Porosity of the surface and "hollows" formation depend on the ratio of nucleus/polymer. So, for the ration of nucleus/polymer 0,5:1 more smooth surface of microcapsules with small characteristic "folds can be observed". At the same time the surface of microcapsules with the ratio of nucleus/polymer 0,5:1 is represented with deep folds and tension bars.

Phenibut identification

Results of IR-spectroscopy for the substance of phenibut, microcapsules-placebo and microcapsules with phenibut treated in chitosan solution are presented in fig. 5.

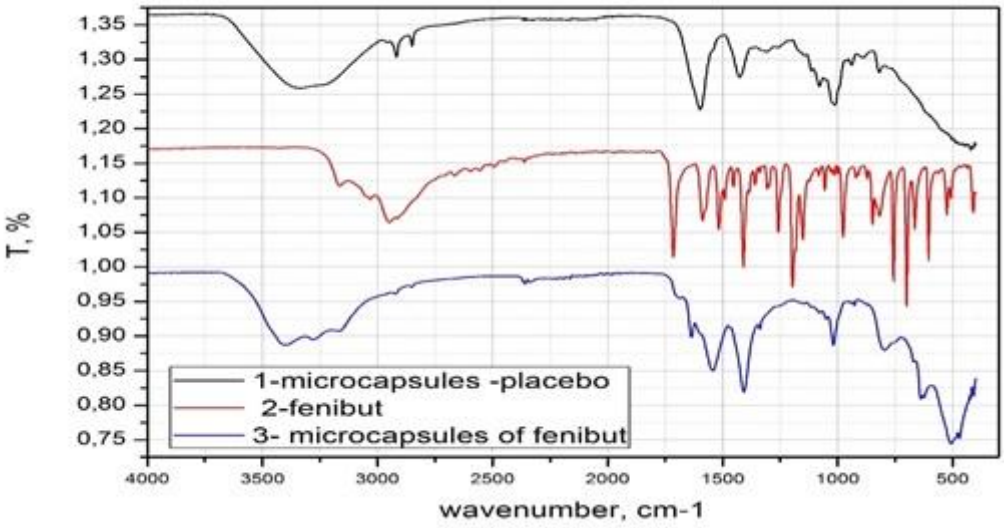

Fig. 5. IR-transmission spectra of phenibut substance, microcapsules-placebo and microcapsules with phenibut

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.

Microencapsulation efficiency

Calibration plot for the dependence of the optical density on the amount of phenibut in a solution appears as a straight line (Fig. 6).

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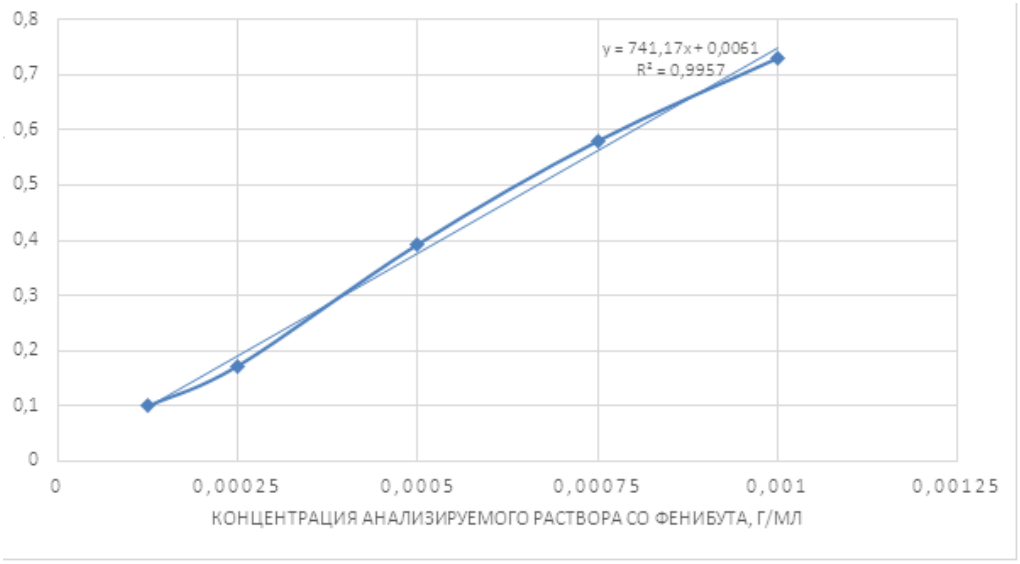

Fig. 6. Calibration curve for the dependence of the optical density on phenibut concentration in a solution.

Efficiency of phenibut microencapsulation in a dependence on the concentration of solution of the film-forming material and process technology is presented in Table 1.

Table 1. Efficiency of phenibut microcapsulation

Concentration of sodium alginate, %	Microcapsulation efficiency, %	
	Microcapsules with chitosan	Microcapsules without chitosan
0,5	12,0	6,4
1,0	12,5	6,6
1,5	25,2	21,7
2,0	28,1	21,0
2,5	15,6	11,8
3,0	10,0	7,8

From Table 1 it is seen that the efficiency of microencapsulation is considerably affected as by the concentration of solution of the film-forming material as by process technology of preparing microcapsules. Thus, with an increase of concentration of sodium alginate solution the efficiency of microencapsulation is enhances and attains 28,1 % for the concentration of sodium alginate solution of 2 % (microcapsules with chitosan). This dependence can be observed for all values of the concentration of sodium alginate solution. Note, that without the treatment involving chitosan the maximum efficiency of microencapsulation was of 21,7% at the concentration of sodium alginate solution equal to 1,5%, that is by 30% less than the efficiency of the treatment involving the use of chitosan solution.

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.

Figs. 7,8 represent the profiles of release for the pharmaceutical substance into dialysis environment (%) in a dependence on time (min).

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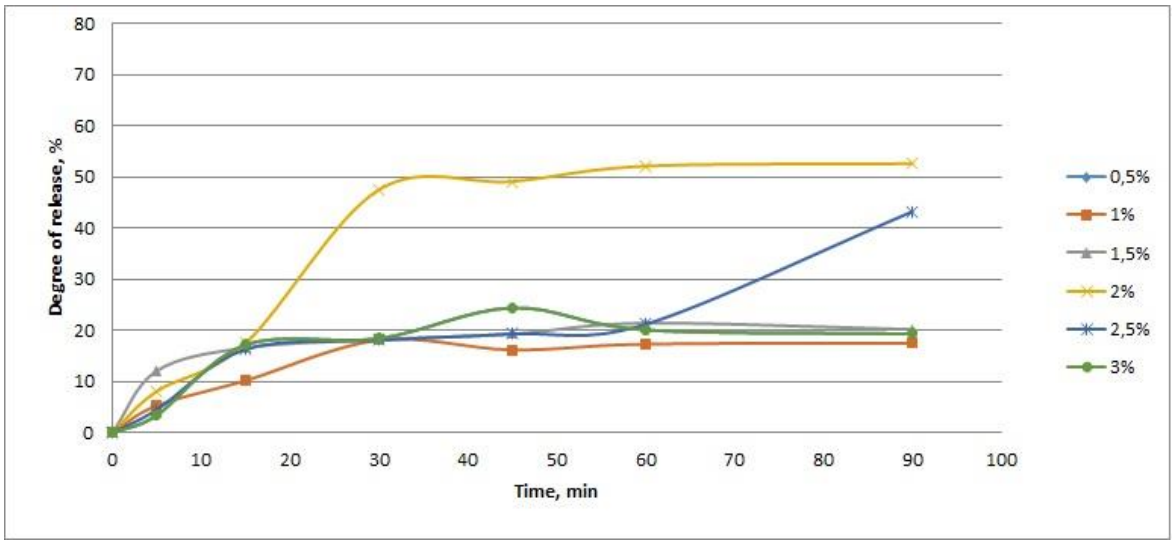

Fig. 7. Dynamics of phenibut release from the microcapsules without chitosan

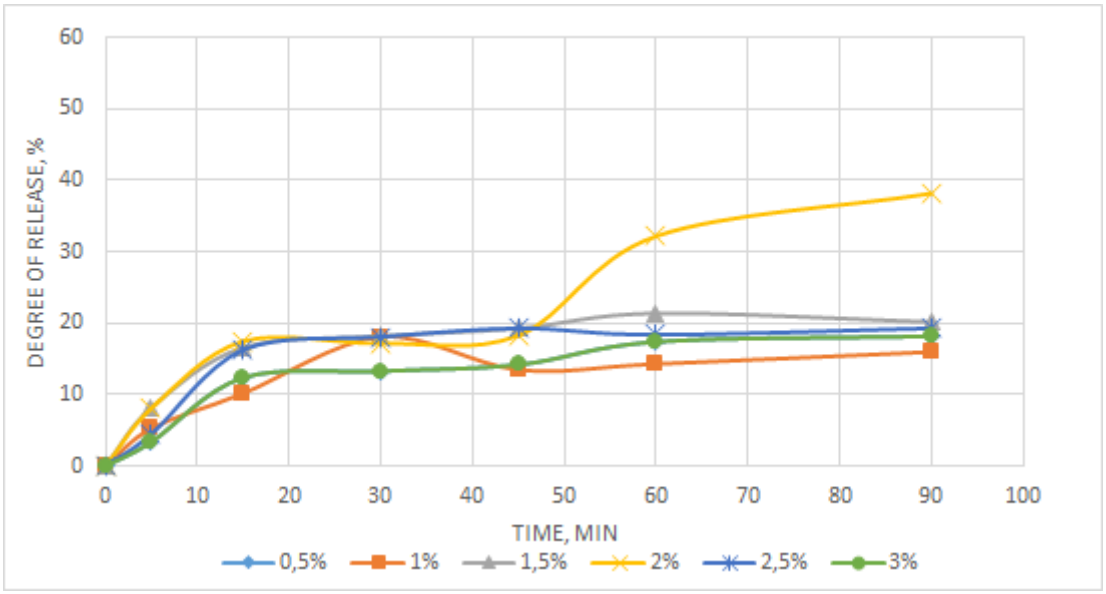

Fig. 8. Dynamics of phenibut release from the microcapsules without treatment in chitosan

As a result of performed biopharmaceutical investigations it was shown the effect of the concentration of the film-forming material solution and microencapsulation technology on release of phenibut into the dialysis environment.

Microcapsules without chitosan

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 (Fig. 7.).

Microcapsules with chitosan

A homogeneous release of the pharmaceutical substance observed in our experiments takes place from the microcapsules for all of the concentrations of the film-forming material solution. It should be noted that the maximum release of phenibut from microcapsules was attained for with the concentration of the film-forming material solution equal to 2%. Phenibut concentration was of about 38,2 % when the experiment lasted for 90 minutes. More prolonged release of phenibut was observed from microcapsules with the concentration of the film-forming material solution equal to 0,5 and 1%, that is 18,2 and 16%, respectively (***Fig. 8.***).

CONCLUSIONS

As a result of the performed investigations microcapsules with phenibut were obtained. The elaborated technique makes it possible to obtain microcapsules with a high technological outcome.

The obtained system of prolonged phenibut release based on the use of a natural biopolymer – sodium alginate used as a material for microcapsules shell can be used as a backbone in creation of the new drug formulations of the nootropic action.

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