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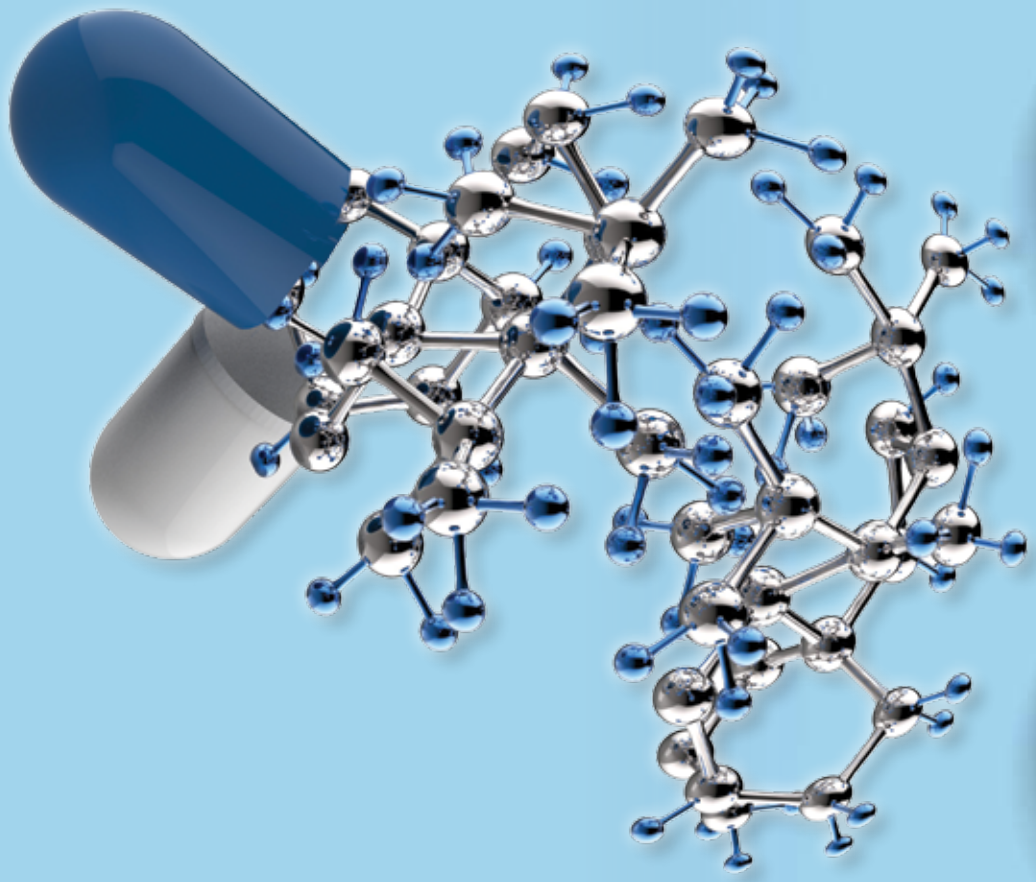
ΤΡΙΜΗΝΙΑΙΑ ΕΚΔΟΣΗ ΜΕ ΘΕΜΑΤΑ ΦΑΡΜΑΚΕΥΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ
A QUARTERLY EDITION ON PHARMACEUTICAL SCIENCES' TOPICS



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Study of biopharmaceutical solubility of thioctic acid

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KEYWORDS:
biopharmaceutical solubility; thioctic acid; solid dispersion; spectrophotometric determination.

SUMMARY

Thioctic acid is an endogenous vitamin-like substance that acts as a coenzyme and participates in the oxidative decarboxylation of α-ketoacids. It is widely used in the treatment of type II diabetes mellitus . According to literature, the relative bioavailability of thioctic acid in the oral use of solid dosage forms is more than 60% in proportion to the drinking solution. The establishment of biopharmaceutical solubility is a necessary step in the development of the composition and technology of medicines.

The purpose of the work was to study the biopharmaceutical solubility of the thioctic acid and its solid dispersions with the Macrogol-6000.

The objects of the study were the substance of thioctic acid, its solid dispersion with macrogol-6000. Samples of solid dispersions were obtained by liquid phase method. The API and carrier ratios were taken as 1:1. Biopharmaceutical solubility of the samples was determined by the method of shaking in a flask at a constant temperature (37 ± 1°C) for 24 hours in a medium of buffer solutions with a physiological pH value (1.2; 4.5; 6.8) prepared according to the formulation given in SPU 5.17.1.

Spectrophotometrically, by the standard method equilibrium biopharmaceutical solubility of thioctic acid and its solid dispersions with macrogol -6000 at a maximum dosage of 600 mg has been established. According to the results of research, biopharmaceutical solubility of thioctic acid can be defined as "low" at different values of pH, and its solid dispersion as "high" at pH 1.2, 4.5 and low at pH 6.8.

The results of the studies can be considered in further studies in the development of solid dosage forms with thioctic acid.

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1. Introduction

The behavior of an active pharmaceutical ingredient (API) can be predicted if its class has been determined by the biopharmaceutical classification system (BCS). According to the indices of solubility in an aqueous medium with different pH value and degree of permeability through the intestinal membrane, APIs are divided into 4 classes: I - high solubility, high permeability; II - low solubility, high permeability; III - high solubility, low permeability; IV - low solubility, low permeability[1].

The value of the biopharmaceutical solubility (BPS) of an API, is not constant and depends on the maximum dose, which is determined experimentally, by the method of shaking in a thermostated flask. This method is the "gold standard" for solubility determination according to the data of the US Food

and Drug Administration (FDA). The characteristics of BPS are the ratio of the maximum dose to the solubility index and the dose number. The following factors influence the BPS value: polymorphism, dose, crystal form and size, method of obtaining, etc[2].

Thus, the establishment of biopharmaceutical solubility is a necessary step in the development of the composition and technology of drugs.

The purpose of the work was to study the biopharmaceutical solubility of the thioctic acid and its solid dispersions with the macrogol-6000.

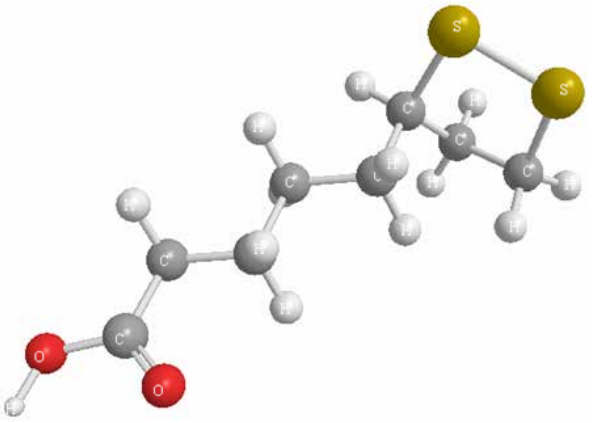

Figure 1. *Structural formula of thioctic acid*

2. Materials and Methods

2.1 Materials

The objects of the study were the substance of thioctic acid, its solid dispersions with macrogol-6000. Thioctic acid ($C_8H_{14}O_2S_2$) - 1,2-dithiolane-3-pentanoic acid or d, 1 -α-5- (1,2-dithiolan-3-yl) valeric acid (CAS 62-46-4) is a light yellow powder with a weak specific odor, bitter to taste[3].

Practically insoluble in water, readily soluble in 96% ethyl alcohol. The melting point is 58.5 - 60.0 °C. In the structure of thioctic acid, there are no traditional chromophores, such as aromatic or heteroaromatic cycles, systems of conjugated double bonds, however, the presence of a carboxyl group and a 1,2-dithiolane ring containing undivided pairs of electrons suggests that this compound will be opaque for ultraviolet light, and this makes possible determination of its amount in solution by spectrophotometric method (**Fig.1**).

2.2 Preparation of solid dispersions

Samples of solid dispersions were obtained by liquid phase method[4]. API and carrier ratio was as 1 : 1.

2.3. Assay of thioctic acid

Quantitative determination was carried out spectrophotometrically using the standard method. Absorption spectra of the resulting solutions of thioctic acid were taken on Evolution 60-S spectrophotometer in a cell with a layer thickness of 10 mm. As the control solution, an appropriate buffer solution (pH 1.2, 4.5, 6.8) was used. The weight of of thioctic acid sample was 600 mg. The amount of thioctic acid, which passed to solution in grams, was calculated by the formula 1:

$$S = \frac{A \cdot m_{st} \cdot V_{2st}}{A_{st} \cdot V_{1st} \cdot V_{3st}} \quad (1)$$

where: A- optical density of the investigated solution;

A_{st-} optical density of the solution of comparison;

m_{st}- weight of the SS (standard sample) of thioctic acid, mg;

V_{1st} - volume of the volumetric flask for the first dilution of thioctic acid SS (50 ml);

V_{2st} - the volume of aliquot of thioctic acid SS;

V_{3st} - volume of the volumetric flask for the second dilution (10 ml)[5].

2.4. Determination of biopharmaceutical solubility

Biopharmaceutical solubility of the samples was determined by the method of shaking in a flask at a constant temperature (37 ± 1 ° C) for 24 hours in a medium of buffer solutions with a physiological pH values (1,2; 4,5; 6,8) prepared according to the formulation given in SPU 5.17.1 - "Manual for conduct-

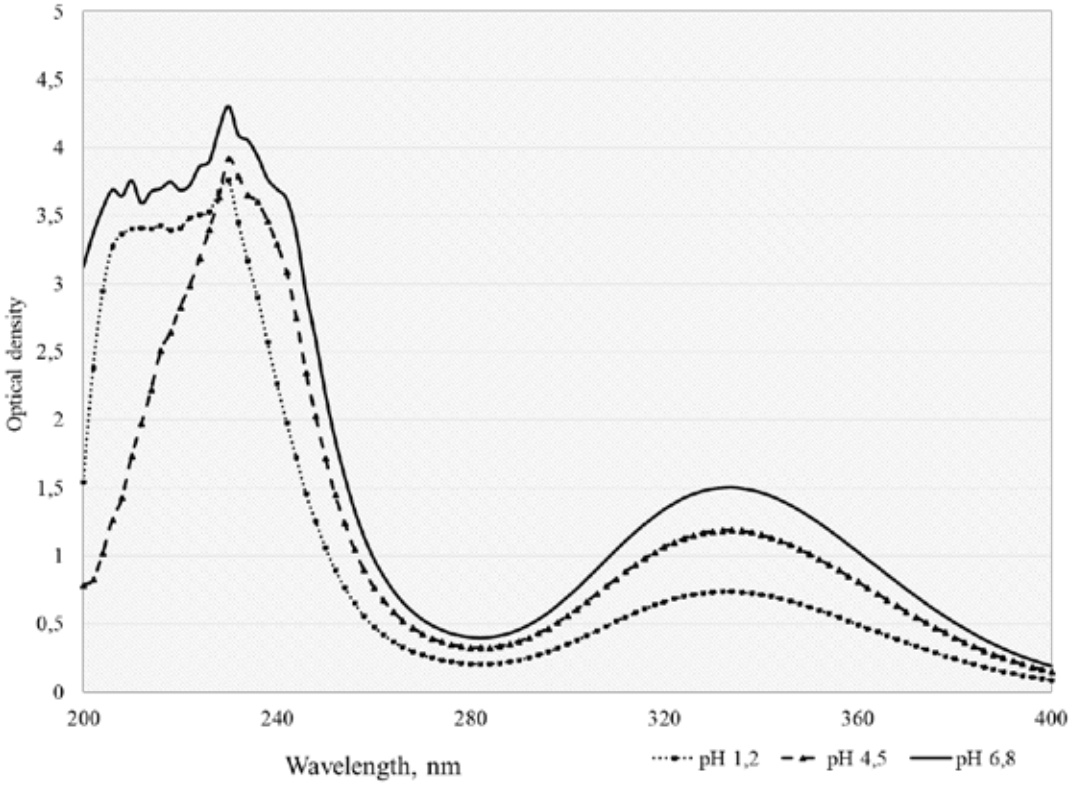

Figure 2. *Absorption spectrum of thioctic acid solution in various buffer solutions*

ing the" dissolution" test"[3].

The dose -solubility dependence was determined by the formula 2:

$$S_i = \frac{D_{max}}{S} \quad (2)$$

where: D_{max} - maximum dose of API, allowed for medical use,

S - solubility, mg / ml.

Dosage number shows the amount of API that dissolves in 250 ml of aqueous solution. This indicator was calculated by the formula 3:

$$D_0 = \frac{D}{S \times V_0} \quad (3)$$

where: D_0 – Dosage number

D – dose of API, used

S - solubility, mg / ml,

V_0 - the volume of liquid by which a dose of API is waed down (250 ml).

The solubility is considered to be high if the amount of the substance passed to the solution is within the range of 85 - 100%, the dose number is 250.0 - 225.0, the ratio of the dose to the solubility is close to 1[6].

2.5 Data processing

The statistical processing of the results of the experiment was carried out using the Microsoft Office Excel 2007 package by calculating the average value of the amount of dissolved substance and the relative standard deviation.

3. Results and Discussion

The absorption spectrum of thioctic acid solution in a buffer solution with a pH of 1.2 in the range from 200 to 400 nm is characterized by the presence of

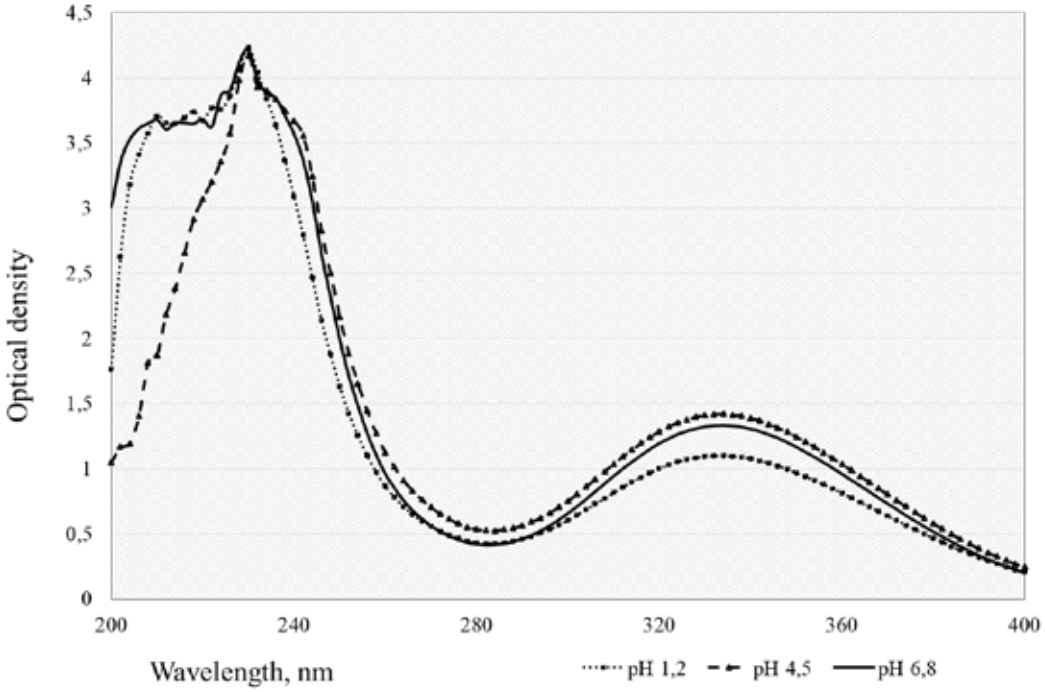

Figure 3. *Absorption spectra of the solutions of thioctic acid solid dispersions with macrogol-6000*

two absorption bands (**Fig.2**).

The wide high-intensity absorption band in the region of 200-240 nm (distant ultraviolet) is characteristic of many organic substances of both natural and synthetic origin, containing functional groups with undivided pairs of electrons in their structure. In buffer solution of pH 1.2, this band has a maximum at 229 nm. We can assume that the basis of absorption is the sum of electronic transitions in the carboxyl group and in the disulfide bond. Thioctic acid solutions have pale yellow-green coloration. In the adsorption spectrum, this is illustrated by the presence of a broad but rather low intensive absorption band in the near ultraviolet with a maximum in the area of 332 - 335 nm (λ max 334 nm), which is due to the presence in its structure of the disulfide bond in the 1,2-dithiolane cycle.

The absorption spectrum of thioctic acid solutions in an acetate buffer solution with pH 4.5, where dimerization of carboxyl groups is possible due to hydrogen bonds, is characterized by the fact that the short-wave band becomes narrower and finds a clear expressed maximum at 230 nm.

Absorption in the near ultraviolet does not change. The less intense, but broad band also has a maximum of 334 nm.

The absorption spectrum of thioctic acid solutions in a phosphate buffer solution at pH 6.8 again has a broad blurred short-wave absorption band with a maximum at 229 nm. The longwave band does not change the nature of the absorption and still has a maximum absorption at 334 nm.

Thus, in the spectra of thioctic acid solutions in buffer solutions with pHs of 1.2, 4.5 and 6.8, the same specific long-wave band with a fairly flat broad maximum at 334 nm is observed, which can be used as an analytical absorption band for determining the concentration of thioctic acid solutions in these buffer solutions.

To test the possibility of using spectrophotometry in determining the concentration of thioctic acid solutions, it was necessary to investigate the influ-

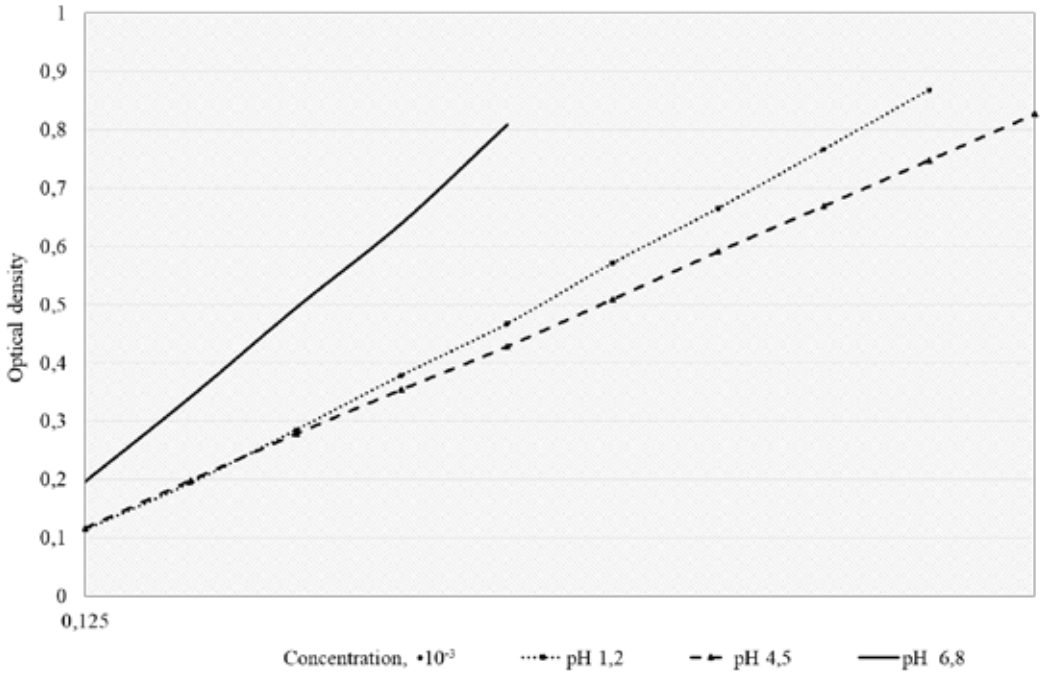

Figure 4. *Calibration graph of the thioctic acid solutions optical density dependence on concentrations in buffer solutions with different pH values*

ence of auxiliary substances on the total spectrum of solutions that are being investigated. To this end, we have studied the absorption spectra of solutions obtained at determination of thioctic acid solid dispersions solubility with an appropriate pH value.

The absorption spectra of the obtained solutions of thioctic acid solid dispersions in the range 200-400 nm in the nature, location of absorption bands and their intensity completely coincide with the nature, location and intensity of absorption of thioctic acid SS solutions in the corresponding buffer solutions, which allows us selecting an analytical band with a maximum at 334 nm and using it to determine the concentration of solutions by adsorption spectrophotometry (**Fig.3**)

One of the main requirements that make it possible to use spectral methods for quantification of a substance is the subordination of light absorption of its solutions to the Bouguer-Lambert-Beer law. The verification of the subordination to the Bouguer-Lambert-Beer law is deduced by plotting the graph of dependence of the optical density (A) to the concentration of the solution. Absorbance of solutions is subject to the Bouguer-Lambert-Beer law only within the concentrations in which the calibration graph is a straight line. Within these limits, the absorption index χ calculated in the form of a specific absorption index should be the same.

The optical density of the resulting solutions of thioctic acid was determined at a wavelength of 334 nm. The obtained results are presented in **Fig. 4** and in **Tables 1 - 3**.

The analysis of the data shows that light absorbance of thioctic acid solutions in a buffer solution with a pH of 1.2 at a maximum at 334 nm is linear and obeys Bouguer-Lambert-Beer law in the entire range of investigated concentrations from 2.50 to 11.25 x 10^{-2} %. Specific absorption index is 7.62 ± 0.04.

The data shown in the table. 2 indicate that light absorbance of thioctic acid solutions in a buffer solution with a pH of 4.5 at a maximum at 334 nm

Table 1. The value of the optical density, depending on the concentration of thioctic acid at pH 1.2

№	1	2	3	4	5	6	7	8	9
C •·10^{-2},%	1.25	2.50	3.75	5.0	6.25	7.50	8.75	10.0	11.25
A	0.114	0.194	0.285	0,379	0.467	0.571	0.665	0.766	0.868
$A_{1\hat{\imath}}^{1\%}$	9.12	7.76	7.60	7.58	7.47	7.61	7.60	7.66	7,72

Table 2. The value of optical density depending on the concentration of thioctic acid at pH 4.5

№	1	2	3	4	5	6	7	8	9	10	11	12
C x·10^{-2}, %	1.25	2.50	3.75	5.0	6.25	7.50	8.75	10.0	11.25	12.50	17.50	25.0
A	0.116	0.198	0.278	0.354	0.428	0.509	0.591	0.669	0.748	0.827	1.149	1.637
$A_{1\hat{\imath}}^{1\%}$	9.28	7.92	7.41	7.08	6.85	6.79	6.75	6.69	6.65	6.62	6.57	6.55

Table 3. The optical density depending on the concentration of thioctic acid at pH 6.8

№	1	2	3	4	5	6
C x·10-2,%	2.50	5.0	7.50	10.0	12.50	16.00
A	0.197	0.342	0.495	0.640	0.809	1.023
$A_{1\hat{\imath}}^{1\%}$	7.88	6.84	6.60	6.40	6.47	6.39

is linear and obeys Beer-Lambert Law in the whole range of investigated concentrations from 3.75 to 25.0 • 10 -2 % . Specific absorption index is only 6.82 ± 0.13.

Analysis of the results presented in Table. 3 shows that light absorbance of thioctic acid solutions in a buffer solution of pH 6.8 at a maximum at 334 nm is linear and obeys Beer-Lambert Law in the entire range of investigated concentrations from 2.50 to 16.0 • 10 -2 %. Specific absorption index is 6.77 ± 0.24.

The presence of a linear dependence between concentration and optical density suggests the possibility of determining the concentration of thioctic acid solutions in buffer solutions with pH of 1.2, 4.5 and 6.8 by the method of one-wave direct spectrophotometry with the subsequent calculation of concentration by the standard method.

The conducted studies have allowed to determine the biopharmaceutical solubility of thioctic acid and its solid dispersions with a macrogol -6000 (**Fig.5**).

Data analysis shows that thioctic acid has poor solubility in a medium with a pH of 1.2 and low in solutions with pH values of 4.5 and 6.8. The dose

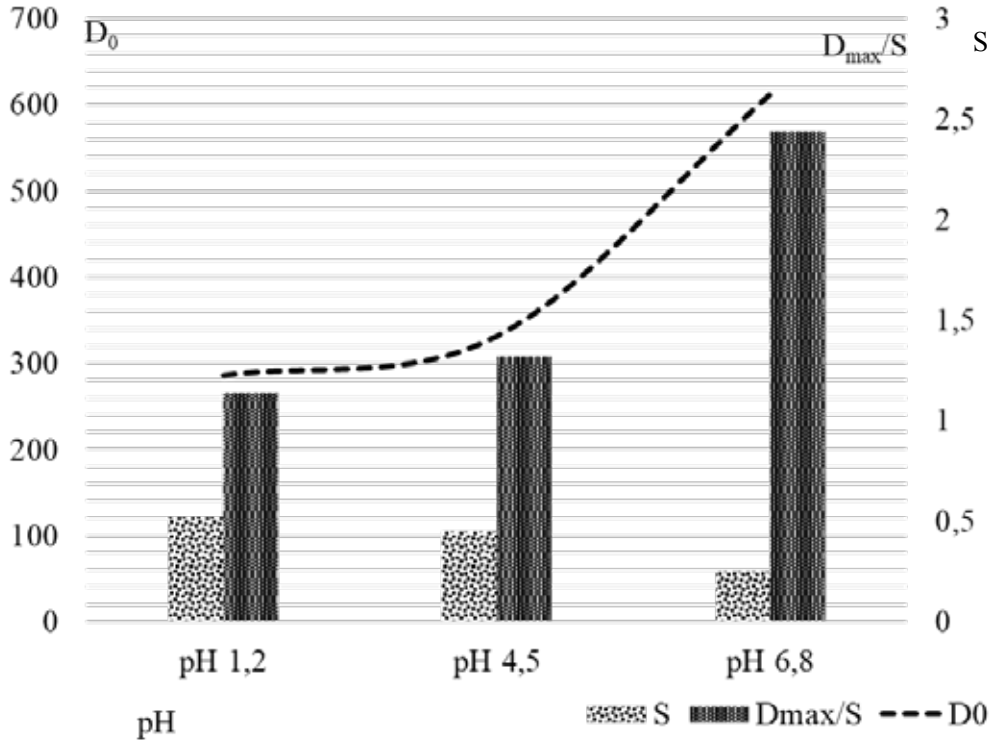

Figure 5. *Indices of solubility, ratio of "dose / solubility", dose number of thioctic acid depending on the pH of solution*

number varies from 286.26 to 612.24 depending on the pH of the solution, which significantly exceeds the required limits. The amount of matter passed to the solution in the neutral medium is 40% in acidic - 80% of the amount taken

The use of a solid dispersion with macrogol -6000 allows increasing the solubility index to high values (**Fig.6**).

In acidic medium, the D / S index of TA solid dispersion is 1.01, D 0 - 254, which indicates the maximum possible solubility level in solution with pH 1.2. When the pH is changed to neutral, the degree of solubility decreases by 60%. But this indicator exceeds the value of solubility of the thioctic acid substance almost 2 times. The amount of API that has passed to solution at pH 1.2 is close to 100% (98,33). The dose number of solid dispersion of TA with macrogol in an acid medium does not, on average, exceed 6% of normal.

Thus, based on the performed studies, it can be concluded that it is expedient to use solid dispersions to increase the bioavailability of thioctic acid.

4. Conclusions

1. In order to determine the possibility of developing a spectrophotometric method for determining the concentration of thioctic acid solutions for studying its biopharmaceutical solubility in the composition of dosage forms based on solid dispersions, the adsorption spectra of its solutions in buffer solutions with pH 1.2; 4.5; and 6.8 have been studied. It has been established that the adsorption spectra of thioctic acid solutions in the indicated buffer solutions contain a wide inclined absorption band with

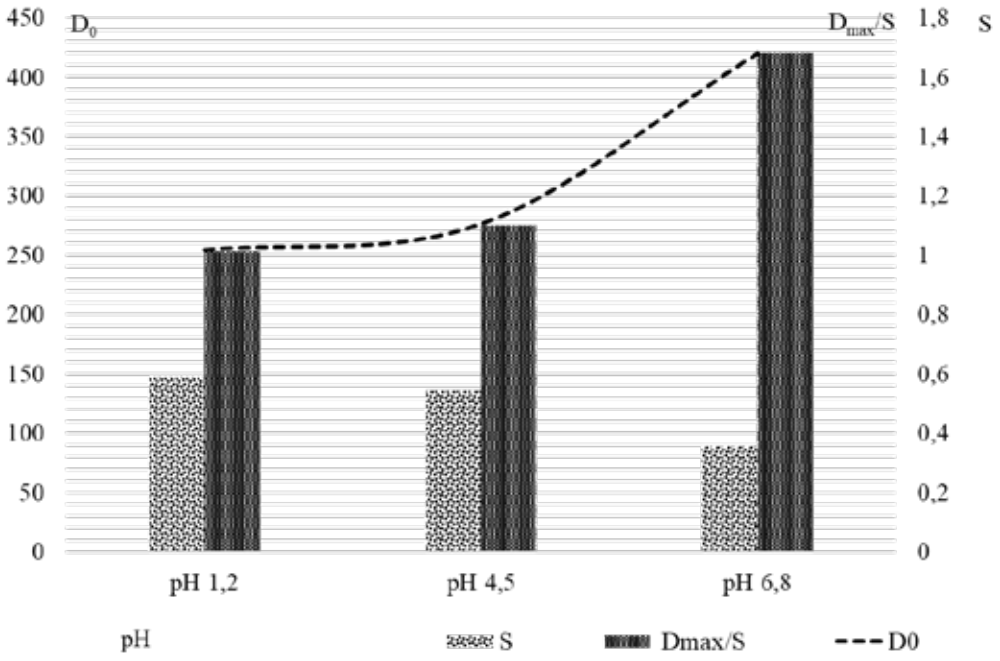

Figure 6. *Solubility indices, "dose / solubility" ratio, dose number of solid dispersions of thioctic acid with macrogol 6000, depending on pH of solution*

a maximum at 334 nm, which can be used to determine the concentration of thioctic acid solutions by the one-component single-wave spectrophotometry method according to the standard.

2. It has been established that light absorbance of thioctic acid solutions at λ 334 nm is linear and obeys the Beer-Lambert Law at pH 1.2 in the concentration range from 2.50 to 11.25 x 10 -2 % at pH 4.5 in the concentration range from 3.75 to 25.0 • 10 -2 % and at pH 6.8 in the concentration range from 2.50 to 16.0 • 10 -2 %.

3. It has been proved that macrogol-6000, used in the preparation of solid dispersions, does not affect the absorption spectrum of thioctic acid solutions in buffer solutions with pHs of 1.2, 4.5, and 6.8. The nature of the spectrum, location, and geometry of the maximum at 334 nm are unchanged, and no additional bands in the spectrum or their overhangs are observed. This indicates the correctness of determining the concentration of solutions by the method of direct one - component single- wave spectrophotometry with the calculation of concentration by the standard method.

4. Spectrophotometrically, biopharmaceutical solubility of thioctic acid and its solid dispersions with macrogol -6000 at a maximum dosage of 600 mg has been established. According to the results of research, biopharmaceutical solubility of thioctic acid can be defined as “low” at different values of pH, and its solid dispersion as “high” at pH 1.2, 4.5 and low at pH 6.8.

5. The results of the studies can be considered in further studies on the development of solid dosage forms with thioctic acid. □