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NEW DOSAGE FORM OF L-ARGININE BASED ON ITS COMPLEX WITH CELLULOSE ACETATE SULFATE AND ACTIVATED CARBON

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Abstract

Introduction: The oral route of L-arginine intake, despite its great comfort, is less effective in comparison with infusions. The development of new pharmaceutical forms for oral preparation is of undoubted interest.

Objectives: To obtain a new dosage form of L-arginine by complexation with sodium salt of cellulose sulfate acetate (Na-CSA) followed by immobilization on activated carbon, and to evaluate its endothelial, cardioprotective and antihypertensive activity.

Methods: UV-, visible spectroscopy, FTIR spectroscopy, viscometry, molecular mechanics, animal experiments with male Wistar albino rats in the modeling of L-NAME-induced endothelium dysfunction.

Results: The physicochemical parameters of complexation, the adsorption activity of activated carbons of various origins with respect to individual L-arginine and its complex with Na-CSA were investigated. The greatest amount of adsorption and its smaller difference between L-arginine and the complex were taken into account when choosing activated carbon as a carrier (AUT-MI: $G_{L\text{-}Arginine}$=320.2±0.1 mg/g, $G_{complex}$=340.2±0.1 mg/g; OU-A: $G_{L\text{-}Arginine}$=210.1±0.1 mg/g, $G_{complex}$=235.1±0.1 mg/g; TH-90G: $G_{L\text{-}Arginine}$=190.1±0.1 mg/g, $G_{complex}$=190.2±0.1 mg/g). Release of L-arginine as a result of its desorption into the model media of the human body was higher (96.3±0.5 wt.%) for the alkaline medium of intestines than for the acidic medium of stomach (10.0±0.1 wt.%). In animal experiments, it was shown that a combined preparation of L-arginine complex immobilized at doses of 30 mg/kg, 70 mg/kg and 200 mg/kg exhibits a pronounced antihypertensive, endothelioprotecive and cardioprotective activity at a dose of 200 mg/kg with L-NAME-induced nitric oxide deficiency ($p<0.05$; 30 mg/kg: $EDC_{L\text{-}Arginine}$=3.6±0.3; $EDC_{complex}$=3.0±0.2; 70 mg/kg: $EDC_{L\text{-}Arginine}$=2.7±0.1; $EDC_{complex}$=2.3±0.2; 200 mg/kg: $EDC_{L\text{-}Arginine}$=2.5±0.1; $EDC_{complex}$=1.9±0.1).

Conclusion: Due to the antihypertensive, endothelial and cardioprotective activity of L-arginine as a complex with Na-CAC immobilized on activated carbon, it is possible to obtain an effective dosage form (tableted or granulated) of L-arginine for oral use.

Key words: L-arginine; L-NAME; nitric oxide deficiency; water-soluble cellulose derivative; activated carbon; immobilized complex; dosage form; antihypertensive, endothelioprotecive and cardioprotective activity.

Introduction

L-arginine is a conditionally indispensable amino acid with a wide spectrum of biological activity, associated primarily with participation in the enzymatic synthesis of nitric oxide and polyamines in the human body [1]. Drugs based on L-arginine have antihypertensive, endothelial and cardioprotective activity, hepatotoxic, antioxidant, antihypoxic, antithrombotic, hypoglycemic, bactericidal and even antitumor properties [2, 3, 4]. Endogenous synthesis of L-arginine is carried out directly in the body, but in certain cases, it must necessarily additionally enter

the body from the outside with food, drinks, and supplements or in the form of infusions. The oral route of L-arginine intake, despite its great comfort, is less effective in comparison with infusions, since this highly polar amino acid is poorly absorbed in the digestive tract, metabolized by the microflora and only partially enters the bloodstream [5].

Therefore, recently, for oral administration of L-arginine, various derivatives thereof are proposed, which are absorbed almost completely into the bloodstream. In this connection, the development of new pharmaceutical forms of preparations containing L-arginine is of undoubted interest.

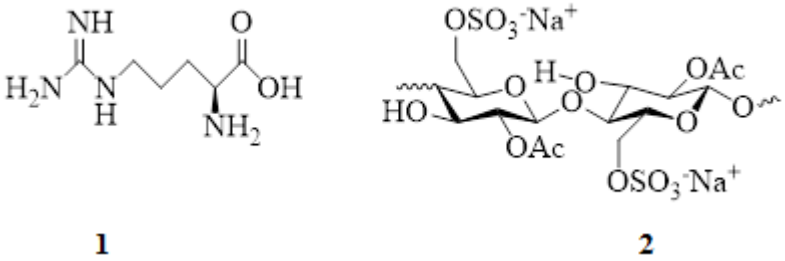
Currently the main pharmaceutical form of L-arginine is a capsule, which upon dissolving in the gastric environment can affect the medical and biological properties of the drug. One of the promising methods for increasing biological activity is a modification of the original substance L-arginine with the natural polymers and the development of new dosage forms based on the product of their interaction [6, 7]. It was previously shown [8, 9, 10], that L-arginine applied both in monotherapy and in combination with antihypertensive agents in the ADMA-(asymmetric dimethylarginine)-like model of L-NAME-(NG-nitro- L-arginine methyl ester)-induced endothelial dysfunction increases effectively the activity of endothelial NO-synthase and the production of nitric oxide, and also prevents the development of endothelial dysfunction in experimental animals.

Objective of this study was to obtain polymer complexes of L-arginine with cellulose water soluble anionic polyelectrolyte – cellulose acetate sulfate in the form of sodium salt (Na-CAS) immobilized on activated carbon, and to evaluate their endothelio-, cardioprotectiveand antihypertensive activity in experimental animals under L-NAME-induced nitric oxide deficiency [11, 12, 13].

Methods

L-arginine (2-amino-5-guanidinepentanoic acid) was used in the form of hydrochloride (Fig. 1).

Cellulose acetate sulphate (poly (1β→4)-(2-O-acetyl-6-sulfo-D-glucopyranose) was used as a water-soluble sodium salt with a bound sulfuric acid content of 27.8 wt.%. Bound acetic acid was 21.4 wt.% and a viscosity average molecular weight was of $30 \cdot 10^3$. As a mole-link of Na-CAS was cellobiose unit, which is a structural repeating unit of the cellulose macromolecule, consisting of two glucopyranose cycles (Fig. 1).



1 **2**

Fig. 1. 1 – L-arginine; 2 – Na-SAC

Complexation was carried out by mixing water solutions of the components at the amino acid-to-polymer weight ratio of 5:1 to 1:5. Isolation of the obtained soluble complexes was carried out through precipitation in ethanol with further reprecipitation. Twice reprecipitated complex was dried in a rotary evaporator. L-arginine content in the complex was determined by UV/Visible spectroscopy in the form of a complex with ninhydrin according to the procedure [14, 15]. UV-spectra were recorded with a spectrophotometer Metertech UV/VIS SP 8001 in quartz cuvettes 1 cm thick.

The intrinsic viscosity of the polymer and the complex in aqueous solutions was determined by using an Ubbelodde viscometer in the 0.2 M NaCl solution at 298K. The surface tension at the liquid-air boundary was measured by stalagmometric method [16].

Immobilization of the complex was conducted on AUT-MI activated carbon from an aqueous solutions followed by granulation and drying of the resulting paste. The specific Gibbs adsorption of L-arginine and L-arginine-polymer complex from their aqueous solutions on activated carbon OU-A, TH-90G and AUT-MI was evaluated by the residual concentration of L-arginine in a solution after extraction of coal.

To determine the amount of L-arginine, isolating from the resulting dosage form in the simulated internal environments, the pharmacopeial buffers pH 1.2 (0.1 M HCl), pH 6.2 (phosphate buffer) and pH 7.5 (phosphate buffer) were used.

The package ChemOfficeUltra version 12 (Cambridge Soft, 2010) was used for computer modeling. In the ChemDraw program structural

formulas were drawn. The procedure for finding the conformations with minimal energy (the force field MMFF94) by the molecular mechanics method was used (Calculations-MMFF94-Minimize Energy).

Experiments were performed in male albino Wistar rats weighing 180-200. For simulation of endothelial dysfunction, L-NAME was administered intraperitoneally at a dose of 25 mg/kg/day for seven consecutive days [9, 10]. Complex of L-arginine with polymer immobilized on activated carbon was administered intragastrically by gavage 30 minutes prior to administration of L-NAME at a dose of 30 mg/g, 70 mg/kg and 200 mg/kg based on the active substance once a day for 7 days. Intact animals were intragastrically administered an equivalent volume of saline for 7 days. On day 10 of the experiment, a catheter was inserted under anesthesia (chloral hydrate 300 mg/kg) into the left carotid artery to record blood pressure (BP). Bolus administration of pharmacological agents was into the femoral vein. Hemodynamic parameters: systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured continuously with the use of a TSD104A sensor and the MP150 hardware and software system (BiopacSystem, Inc., USA). In addition to blood pressure measurements, a series of functional tests was performed with subsequent evaluation of changes in hemodynamic parameters (SBP, DBP, HR) in response to endothelium-dependent vasodilatation caused by intravenous administration of solution of acetylcholine (AC) at a dose of 40 mg/kg at the rate of 0.1 ml per 100 g body weight of animal (EDVD), as well as changes in hemodynamic parameters in response to the intravenous administration of sodium nitroprusside (NP) at a dose of 30 mg/kg at the rate of 0.1 ml per 100 g body weight of animal [17, 18]. The degree of endothelial dysfunction in experimental animal, as well as the degree of its correction with the studied medications was assessed by the estimated coefficient of endothelial dysfunction (EDC), which represents the ratio of the area of a triangle above the BP recovery curve in response to the NP administration (EIVD) to the area of a triangle above the BP recovery curve in response to the AH administration (EDVD) [9, 10].

Results

Complexation of L-arginine with Na-CAS is possible by electrostatic interaction of the sulfate groups of the polymer and protonated amine groups of L-arginine with the formation of an amino acid polymeric salt. It was found that the L-arginine with Na-CAS forms a soluble complex of constant composition upon mixing the initial components in any proportions. Complexation is accompanied by a decrease in charge density of a polyelectrolyte chain and, as a consequence, the transition of Na-CAS macromolecules to a more convolute conformation, which is confirmed by a decrease in the value of intrinsic viscosity of the polymer in the solution in the presence of L-arginine: from 0.43 to 0.32 dl/g (Fig. 2) [19].

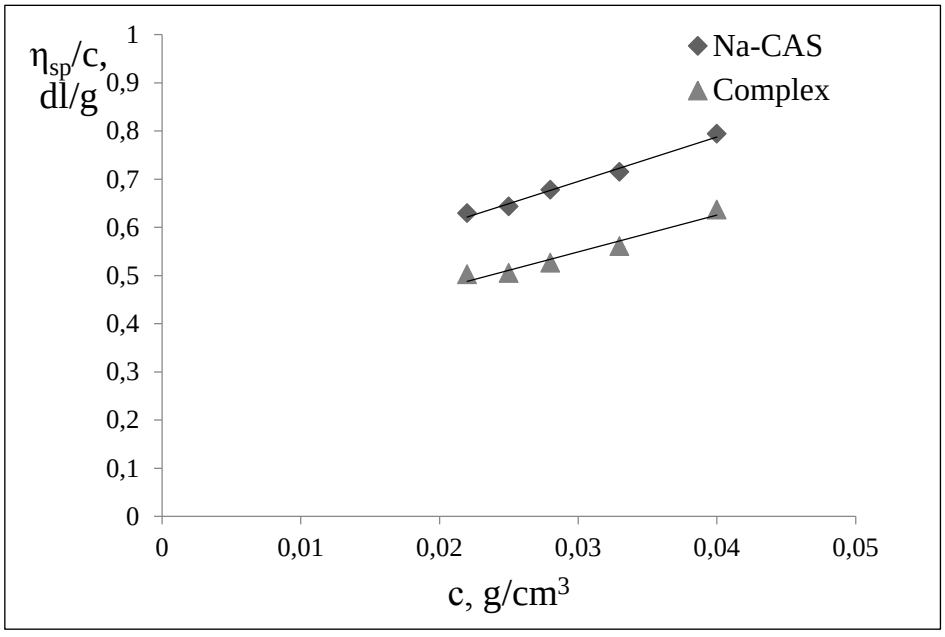


Fig. 2. The variation of reduced viscosity with concentration for Na-CAS and its complex with L-arginine solutions in 0.2M NaCl

The complex formation and its composition has been fixed by FTIR and visible spectroscopy. The composition of the complex corresponds to the molar ratio of Na-CAS cellobiose unit to L-arginine – 1.0:1.0. The polymer salt of L-arginine forms a colored complex in an aqueous solution with ninhydrin, which is characterized in the visible spectra by an absorption band with a maximum at 420. nm. This band is absent in the visible spectrum of mixture of ninhydrin with Na-CAS and presents in the spectrum with L-arginine. The L-arginine content in the desired product was determined by complexation with ninhydrin according to calibration graph.

As follows from the FTIIR-spectroscopic study of the complex films, the main type of interaction between components is electrostatic one (between guanidine group and the protonated amino group of L-arginine and the sulfate group of Na-SAC). The guanidine group in the aqueous solution has a resonant structure and is in protonated form, which corresponds to the presence in the spectrum of three bands in the region of 3358, 3192 and 2953 cm-1 (Fig. 3).

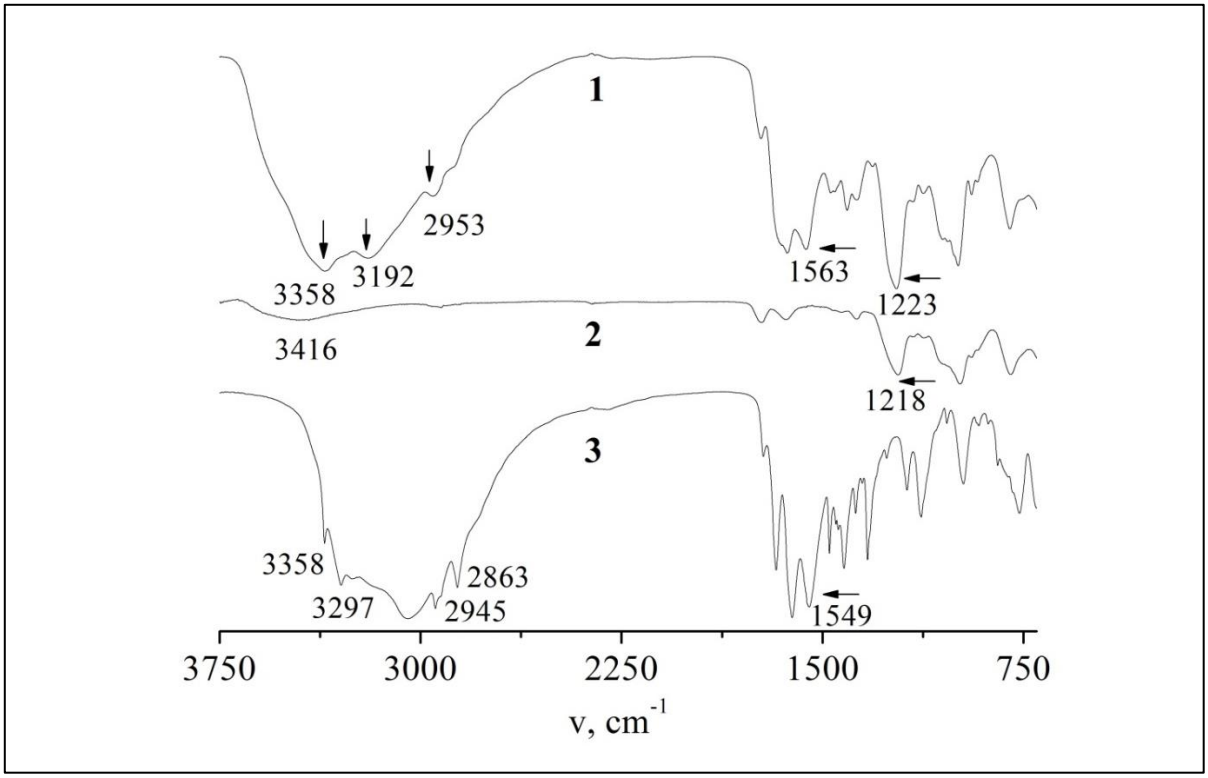


Fig. 3. The FTIR spectra 1-complex Na-CAS-L-arginine; 2- Na-CAS; 3- L-arginine

According to the results of computer modelling, the charge on these nitrogen atoms is respectively -0.85, -0.82 and -0.85. The reduction in the number of bands in this spectral region to two and their shift to a lower frequency region indicate that this group participates in complex formation. The absorption bands in the complex spectrum in the 1632 and 1563 cm^{-1} region can be attributed to the symmetric and asymmetric deformation vibrations of the protonated amino group characteristic for the salt form of amino acids (respectively the I and II amino acid bands). At the same time, for the II amino acid band a bathochromic shift of 14 cm^{-1} is observed in comparison with the spectrum of L-arginine itself. The absorption band of the carbonyl group of 1720 cm^{-1} in the spectrum of the complex is shifted to the high-frequency region up to 1729 cm^{-1}, which can serve as an indication of its participation in the formation of the complex. The 5 cm^{-1} (from 1218 cm^{-1} to 1223 cm^{-1}) bathochromic shift in the spectrum of the complex for Na-CAS-υ_{as} ($-OSO_3^-$) sulfate band, as well as 14 cm^{-1} (from 1549 cm^{-1} to 1563 cm^{-1}) shift for the band of deformation vibrations of the amino group of L-arginine δ (NH) indicates the substitution of sodium cation for a cation with a lower polarizing ability (protonated L-arginine in this case).

Since amino acid molecules exist, as a rule, in the form of dimers [20], a computer modelling of the formation of a polymer complex of L-arginine with two cellobiose units (1.0: 1.0 complex) was carried out. The energy gain was 26.65 kcal / mol. We can assume that when forming the complex, the separation of amino acid dimers and the coordination of the monomeric molecules with respect to the sulfate groups of the polymer take place, as shown in Fig.4.

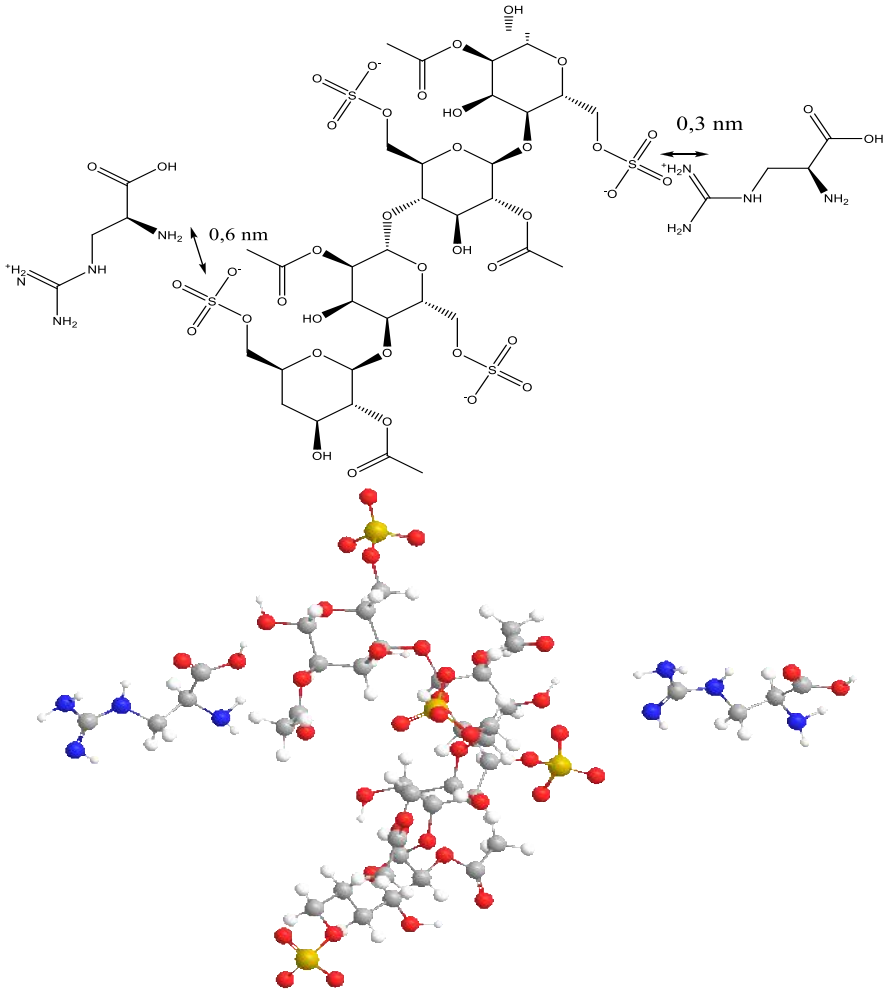


Fig. 4. The fragment of the complex structure for two cellobiose units (2D and 3D models)

Since the resulting complex is water-soluble, a water-insoluble carrier, namely, activated carbon (AC) was used to produce a solid dosage form. We considered that the adsorption of L-arginine on the AC occurs mainly in the micro pores, since the values of maximum adsorption (Fig. 5) for AC with different pore structures correlated with the micro pores volume (Table 1).

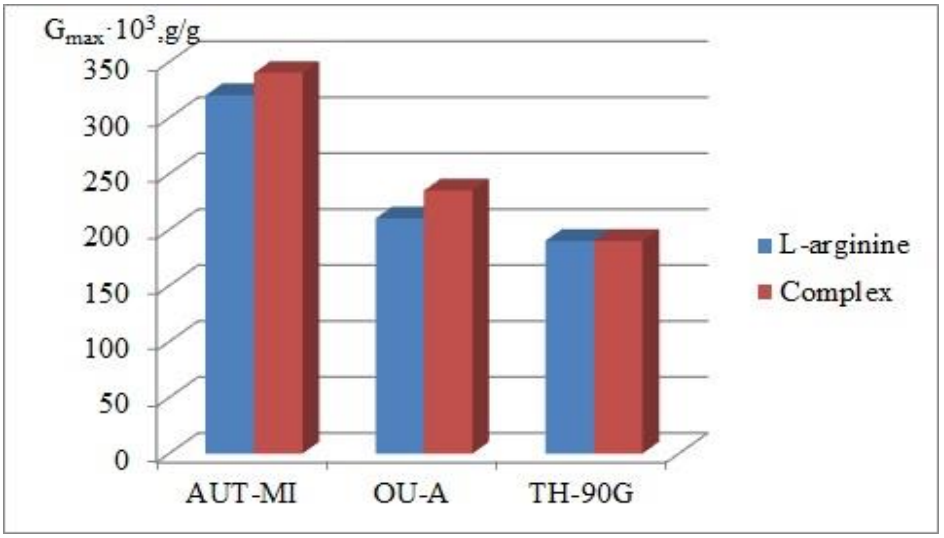


Fig. 5. Maximum Gibbs adsorption value for L-arginine and its complex on AUT-MI, OU-A and TH-90G

Table 1

Specific surface area and pore structure characteristics of the applied activated carbons

Coal	Pore volume, cm3/g			Sspec, m2/g
	Vmicro	Vmeso, macro	Vtotal	
AUT-MI (SvetlogorskKhimvolokno, JSC, Belarus)	0.38	0.18	0.56	920
OU-A (sorbent, Perm, Russia)	0.24	0.31	0.55	750
TH-90G (Silicarbon,Germany)	0.22	0.25	0.47	800

According to the Fig.5 data, the fibrous microporous coal AUT-MI has the maximum adsorption value for both individual (expressed as L-arginine) and complex-bound L- arginine in the comparison with other coals under study. Fig. 6 shows the typical adsorption isotherm for L-arginine and its complex on the carbon material on the example of AUT-MI.

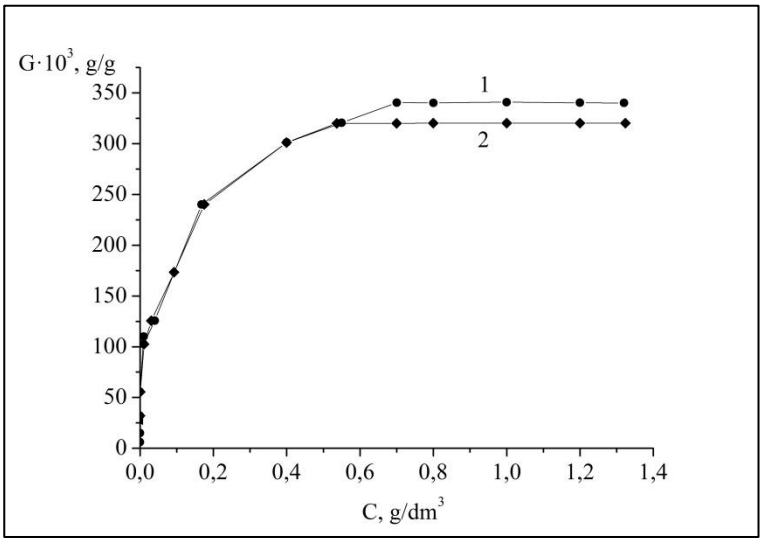


Fig. 6. Adsorption isotherms of L-arginine and L- arginine -Na-CAS on the activated carbon AUT-MI at T = 298 K. 1- complex L-arginine – Na-CAS; 2-L-arginine

As follows from the correlation coefficients the Langmuir equation describes the experimental isotherms rather well (the correlation coefficients > 0,9977). The calculated specific adsorption values G_∞ are less than the experimentally determined ones G_{max} (Fig.7). In this case, both of these values decrease in the rows of coals AUT-MI>OU-A>TH-90G, that correlates with a decrease in the volume of micropores in them, where adsorption of L-arginine molecules predominantly takes place. An increase in the values of G_{max} for polymer complexes in the comparison with an individual amino acid (Fig. 8) may be due to additional adsorption of the complex in mesopores (see Table 1).

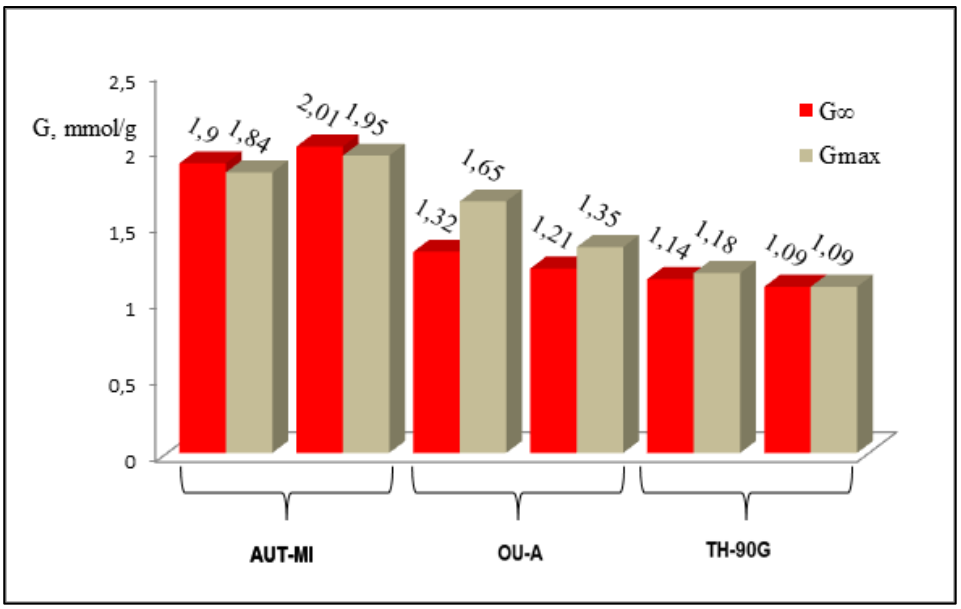


Fig. 7. Limit values of adsorption, determined by the Langmuir equation G_{∞} and experimental values G_{max}, for different carbon materials

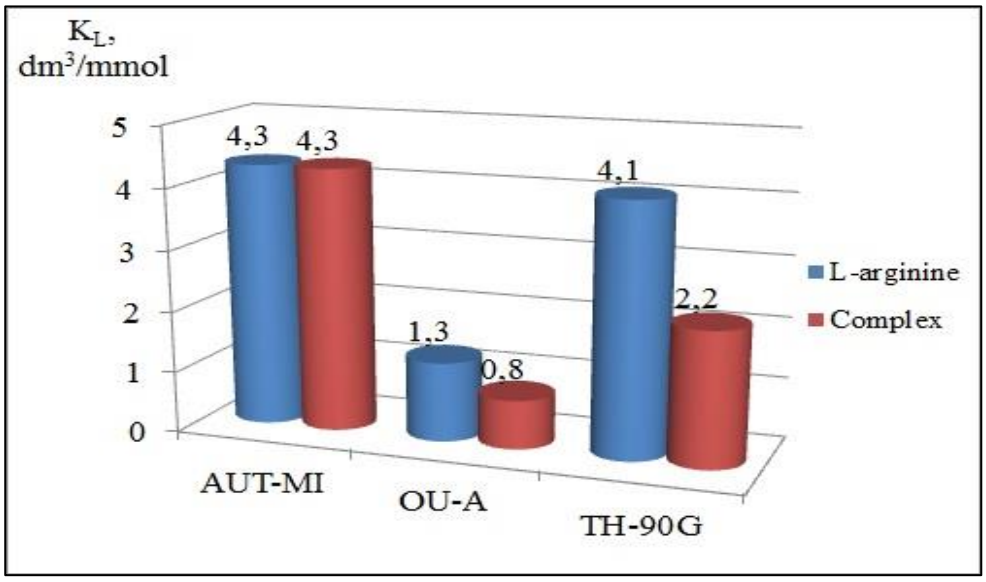


Fig. 8. The Langmuir constant values for different carbon materials

At the same time, complex-bound L-arginine is adsorbed to a greater extent that can be associated with an increase in its hydrophobic property resulting from complexation (Fig. 9). Experimental evidence of hydrophobic properties of the L-arginine-Na-CAS complex is its surface activity manifesting itself on the background of surface-indifferent properties of L-arginine and surface-inactive properties of Na-CAS.

Desorption of L-arginine from the carbon carrier surface into pharmacopeial buffers depends on their pH. 10.0±0.1 wt. % of L-arginine was found to release into acidic gastric medium (pH 1.0). Further increase of the desorption time gives no release of L-arginine observed. In intestinal-simulating alkaline medium, a significantly higher amount of L-arginine is released during the same period of time – 96.3±0.5 wt. %.

The influence of L-arginine in the form of a complex with Na-CAS immobilized on activated carbon at doses of 30 mg/kg, 70 mg/kg and 200 mg/kg on initial values of blood pressure and the coefficient of endothelial dysfunction was assessed in vivo in anesthetized rats upon modeling the L-NAME-induced pathology (Table 2) as compared to pure L-arginine.

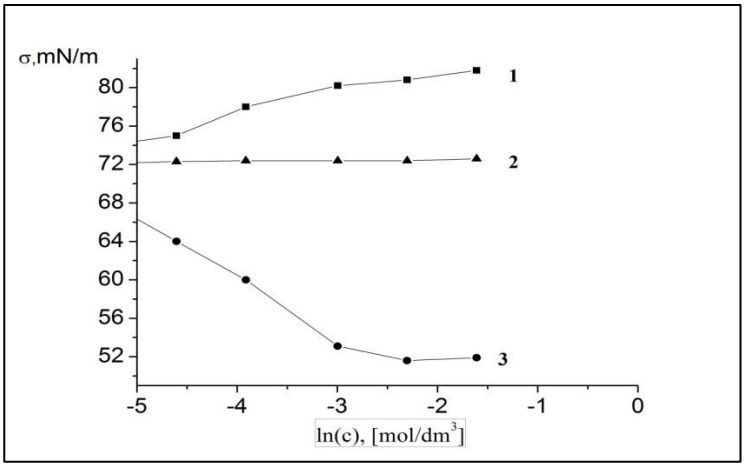


Fig. 9. Surface tension isotherms of aqueous solutions at T = 298 K.
1 – Na-CAS; 2 – L-arginine; 3 – complex L-arginine-Na-CAS

Table 2 data demonstrate that in all dosages of the new drug form of L-arginine, the values of EDC were more close to the level of intact animals in comparison with pure form of L-arginine. Additionally, a new pharmaceutical dosage form of L-arginine at a dose of 200 mg/kg in the largest extent prevented the development of severe systolic hypertension.

Table 2

The effect of new dosage form of L-arginine on hemodynamics values and EDC in modeling of L-NAME-induced endothelium dysfunction (M±m n=10)

Groups of animals	Functional test	SBP, mm Hg	DBP, mm Hg	EDC
Intact	Reference	137.7±3.7	101.9±4.3	1.1±0.1
	AC	84.3±4.5	38.7±2.8	
	NP	83.0±3.7	42.1±4.4	
L-NAME (25 mg/kg)	Reference	190.3±6.7*	145.0±3.9*	5.4±0.6*
	AC	110.6±5.2	82.8±6.6*	
	NP	88.7±4.7	50.8±4.2	
L-NAME + L-arginine-Na-CAS (30 mg/kg)	Reference	180.8±8.8	141.4±9.0	3.0±0.2**X
	AC	112.6±8.5	63.4±7.3	
	NP	89.4±4.3	57.6±5.8	
L-NAME + L-arginine (30 mg/kg)	Reference	188.5 ± 14.8*	137.3 ± 9.9*	3.6±0.3**
	AC	85.3±5.0**	53.1±3.4**	
	NP	97.0±6.2	66.7±4.3	
L-NAME + L-arginine-Na-CAS (70 mg/kg)	Reference	176.2 ± 6.0**X	130.7±8.1**	2.3±0.2**X
	AC	98.7±8.7	53.7±9.8	
	NP	96.5±11.5	50.7±3.4	
L-NAME+ L-arginine (70 mg/kg)	Reference	195.2±13.5	136.5 ± 4.8**	2.7±0.1**
	AC	116.8 ± 4.6*	85.1 ± 4.6*	
	NP	100.3 ± 6.7	58.9 ± 7.8**	
L-NAME + L-arginine-Na-CAS (200 mg/kg)	Reference	141.4±12.2**X	118.7±9.8**	1.9±0.1**X
	AC	86.3±10.3	47.1±8.7**	
	NP	94.6±7.1	42.3±6.4	
L-NAME + L-arginine (200 mg/kg)	Reference	177.6 ± 9.6*	120.1 ± 6.4*	2.5±0.1**
	AC	85.3 ± 5.0	51.3 ± 2.5	
	NP	102.0 ±3.8	44.1 ± 2.9	

Note: *– at $p<0.05$ as compared to control animals; **– at $p<0.05$ as compared to group receiving L-NAME; ^{X} – at $p<0.05$ as compared to group receiving to pure L-arginine in the appropriate dose.

When carrying out a load of heart samples the initial contractility of the left ventricle in animals treated with L-NAME were significantly higher than the intact (table 3). Dosage forms of L-arginine immobilized in the complex form of significantly better prevented the increase in baseline left ventricular pressure, which indicates best negative inotropic action of this form of L-arginine in the simulation of hyperkinetic disorders of the myocardium.

Table 3

The effect of new dosage form of L-arginine on the left ventricle of rats' heart contractility (M±mn=10)

Groups of animals	Initial LVP, mm Hg	Adrenoreactivity, mm Hg	Myocardial reserve level,
Intact	108.6±4.3	199.2±8.3	83.6±4.2
L-NAME (25 mg/kg)	167.8±5.6*	247.3±4.8*	66.0±3.4*
L-NAME + L-arginine- Na-CAS (30 mg/kg)	131.9±11.2**X	222.7±16.6**X	68.5±6.3*
L-NAME + L-arginine (30 mg/kg)	173.7±37.6*	253.2±10.8*	69.0±6.1*
L-NAME + L-arginine- Na-CAS (70 mg/kg)	136.0±5.0**X	229.6±2.2**X	83.1±3.7**X
L-NAME+ L-arginine (70 mg/kg)	160.0±29.8*	250.8±12.3*	70.5±5.8*
L-NAME + L-arginine- Na-CAS (200 mg/kg)	127.9±11.5**X	201.6±12.3**X	84.0±4.7**
L-NAME + L-arginine (200 mg/kg)	142.1±8.3**	223.5±7.3**	84.3±4.5**

Note: *- at $p<0.05$ as compared to control animals; **- at $p<0.05$ as compared to group receiving L-NAME; X – at $p<0.05$ as compared to group receiving to pure L-arginine in the appropriate dose.

The results of the study of the functional state of the myocardium during exercise testing revealed the benefits of cardioprotective action of a new form of L-arginine in all doses, expressed in preventing the increase in adrenoreactivity. A statistically significant advantage in preventing the fall of LVP the tests on the load resistance in the conventional form revealed only by the introduction of a new form of L-arginine at a dose of 200 mg/kg. L-arginine at doses of 30 mg/kg and 70 mg/kg of both forms, showed cardioprotective effect in lesser degree.

Conclusion

In the light of the results of the study L-arginine in the form of complex with water soluble cellulose derivative – Na-CAS – immobilized on the activated carbon shows up as an effective dosage form for L-arginine. Complex is a polymeric salt of the amino acid and the background for its formation is electrostatic interaction between sulfate groups of polymer and protonated amino groups of L-arginine. The composition of the complex corresponds to the molar ratio of Na-CAS cellobiose unit to L-arginine – 1.0:1.0. This was approved by FTIR- and UV-spectroscopy. The participation of very hydrophilic sulfate groups in the complexation process causes the appearance of hydrophobicity for the complex as indicated by the appearance of its surface activity. The key point of the explanation for complexation mechanism is the dissociation of L-arginine dimer to monomers and the participation of namely monomeric form of amino acid in the process of complex formation. The computer models of complexes have the minimum of the formation energy in this case only. The parameters of the adsorption-desorption processes allowed to choose activated carbon AUT-MI as the career for the obtaining of tableted of granulated dosage forms because of its higher adsorption activity value and smaller difference between complex and L-arginine itself in the comparison with other carbon materials. The advantage of the complex form of L-arginine has become the most pronounced antihypertensive and

endothelioprotective activity which has been shown at doses of 30 mg/kg, 70 mg/kg and 200 mg/kg. The most pronounced cardioprotective activity showed L-arginine in the form of a complex with Na-CAS, immobilized on charcoal, in the dose of 200 mg/kg. Thus, the aforementioned complex can be recommended for the development of the effective L-arginine dosage form for oral treatment.

Conflicts of Interest: The authors have no conflict of interest to declare.

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