UDC 678.06:615.45

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POLYMERIC WATER SOLUBLE FORMS OF CLOPHELINE BASED ON BLOOD SUBSTITUTES

The new polymeric forms of clopheline based on blood substitutes have been developed. By means equilibrium dialysis method the interaction of clopheline with polyvinylpyrrolidone. polyvinyl alcohol and dextran was studied. The dynamic of drug release into physiological solution was investigated. It was concluded the possibility of polymeric blood substitutes application for prolongation action of clopheline.

One of the most widespread diseases of the cardiovascular system is arterial hypertension. The most effective drug for hypertension treatment is clopheline, representing hydrochloride of 2-(2,6-dichlorphenilamino)-imidazoline [1,2]. The main pharmacological feature of clopheline is strongly pronounced hypothensive action at very small doses (0,000075 g). Clopheline is not toxic and has extraordinary big therapeutic breadth. Clopheline has found wide application in clinical practice for treatment of various forms of arterial hypertension. Favorable hypothensive effect was achieved at 50-80 % of patients.

However, alongside with a number of positive properties, clopheline has certain drawback. It is low-molecular substance and its pharmacological effect is kept quickly. With the purpose of elimination these drawbacks, the researches on development of new highly effective medicinal forms of clopheline with long hypertensive action are widely conducted. Such medicinal forms allow to provide constant concentration of drug at the therapeutic level during a long time [3–5].

One of perspective directions in the field of drug delivery systems is development of complexes of drugs with various water-soluble polymers. Rigid requirements of medicine (biocompatibility, solubility in water or physiological solution, ability to completely remove from organism, etc.) sharply narrow circle of the polymers used as drug carriers. For these purposes it is considered the most expedient application of polymers which have properties of blood substitutions. Among the high-molecular compounds having such properties, the wide spreading have found dextran, polyvinylpyrrolidone and polyvinyl alcohol [6,7].

In the present study the polymeric water soluble forms of clopheline based on blood substitutes such

as polyvinylpyrrolidone. polyvinyl alcohol and dextran are described.

EXPERIMENTAL PART

Polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA) and dextran were purchased from Sigma Chemicals, St.louis, USA. Clopheline was used pharmaceutical grade.

For detailed understanding of character and nature of binding, the interaction between drug and macromolecules was studied by means equilibrium dialysis method. The release behaviour of clopheline from SPU polymeric solutions was examined by dialysis method in a modelling biological medium at 37°C. The amount of drug released was determined spectrophotometrically by measuring the absorbance maximum at 278 nm. UV spectra were recorded on a Jasco UV-VIS (Japan) spectrophotometer.

RESULTS AND DISCUSSION

Polyvinylpyrrolidone has found wide application in medicine as blood substitute and deintoxicator, filler for tablets and dragees, bases for ointments, prolonger of action of many medicinal substances. The basic advantages of this polymer is solubility in water and other solvents, hydrophylity, absence of toxic and allergenic action, high ability to complexation. Two commercial preparations are issued on the basis of PVP: plasma substitute «Gemovinyl» and deintoxicator «Gemodez». The first is 3,5 % solution of mean-molecular weight polymer, and the second - 6 % solution of low-molecular polymer. To the purposes of prolongation, the polymer with molecular weight 15000-40000 was applied. That provides long stay of polymer and binding drug in living organism.

The determining role in binding of PVP with various low-molecular substances is played hydrogen bonds and hydrophilic interactions. Presence in structure of drug the appropriate groups capable form weak complexes with PVP, gives the basis to use this polymer for prolongation of therapeutic action of drug. Interaction of drug with PVP was studied by means of various physical and chemical methods. UV-spectroscopic researches are shown that at mixing water solutions of drug and polymer precise change in spectral characteristics clopheline at 202 nm as increase of the maximum of absorption (hyperkhromic effect). This process proceeded in time, increase of optical density occur within 6-8 hours. The given effect is caused complexation of molecules clopheline with lactam ring of polymer due to formation of hydrogen bonds. Viscosimetric measurements also testified about complexation of drug and polymer. So, the intrinsic viscosity of 0,5 % solution of PVP at dilution water changes linearly, and at dilution the same solution of 0,5 % solution clopheline occurs sharp increase of the given viscosity in the field of small concentration of polymer, characteristic for polyelectrolites. The given effect testifies to increase of the linear sizes of macromolecules result of its association with clopheline. Measurement of viscosity of solutions at various mole ratio of components has shown, that at the ratio close to equimolar (0,8-0,9 moles clopheline on 1 part PVP), the sharp increase of viscosity testifying to formation of a complex of structure 1:1.

For more detailed investigation of interaction clopheline with PVP the method of equilibrium dialysis was used, allowing to establish degree of binding between components in solution not only qualitative, but also quantitatively. Experiments with dialysis carried out in water solution at various temperatures, using acetylcellulose dividing membrane. Clopheline, diffused from one cell through membrane, contacted with polymer which is taking place in other cell. Changing concentration of drug in various experiments with constant concentration of PVP, quantitative characteristics of process of interaction (coefficient of distribution, binding constant, thermodynamic parameters) were determined. The coefficient of distribution clopheline at equilibrium dialysis characterizes itself the relationship of drug amount in dialysed cell and outside solution. Constant of binding determined according to Klotz equation [8] by the diagram of dependence 1/a from 1/C, where a - parameter describing the share of macromolecules, formed the complex, C - concentration free or unbound drug (fig.1). This dependence represented a straight line which corner of inclination corresponded 1/Kc. Values of thermodynamic parameters of interaction at various temperatures are presented in table 1.

The data indicate clearly that with increase of temperature parameters of drug the binding with PVP decrease. It is shown that process of complex formation has exothermic character, negative values of change testify to it enthalpy and free energy, and also positive change of entropy. Low absolute values of thermodynamic parameters indicates on prevailing value of hydrogen bonds in complexation process. Alongside

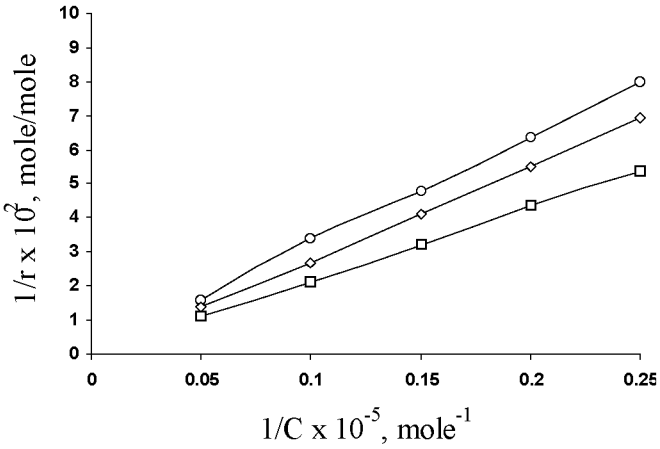

Fig.1. Klotz dependence for interaction of clopheline with PVP at different temperature.
□ – 25°C, ◊ – 37 °C, ■ – 50 °C

Table 1. **Thermodynamic parameters of interaction of clopheline with PVP and PVA**

Temperature, °C	$K_в$x10^{-2}, L/mole	Free Energy, kG/mole	Enthalpy, kG/mole	Entropy, E.u.
		PVP		
25	41,6	- 9,24	- 5,46	12,68
37	38,4	- 9,39	- 4,12	15,32
50	35,7	- 9,48	- 3,32	18,71
		PVA		
25	57,2	- 11,52	- 7,05	17,2
37	48,1	- 11,59	- 6,12	20,4
50	46,1	- 11,76	- 5,48	22,5

Table 2. **Dynamic of clopheline release from polymers into physiological solution**

Polymer	Ratio	Quantity of released drug, %				
---	Drug:Polymer	1 h	2 h	4 h	6 h	8 h
		23	46	72	84	96
	z					
PVP	1:1	18	36	61	76	78
	1:2	16	33	56	71	74
	1:3	14	30	51	70	72
	1:4	13	27	47	64	68
PVA	1:1	21	43	69	80	88
	1:3	19	37	62	78	82
	1:5	15	32	52	66	72
Dextran	1:1	22	44	70	82	92
	1:3	20	39	64	80	84
	1:5	18	36	60	75	78

with them existence of hydrophobic interactions between components of complex is possible.

For an estimation of prolongation time the release of drug from polymeric solutions was investigated. Experiments carried out at various ratio polymer:drug - from 1:1 up to 4:1. For comparison the amount of drug, released through membrane in absence of polymer was determined. Results of investigation are presented in table 2.

Received data indicate that at the presence of polymer the diffusion clopheline through membrane is reduced. So, for 8 hours at molar ratio polymer: drug = 1:1 preparation is diffused on 78 %, while from water solution clopheline is released on 96 %. On the basis of received data the diagrams of logarithmic dependence of amount released clopheline in time were drawn and constants of rate of drug diffusion through membrane are calculated.

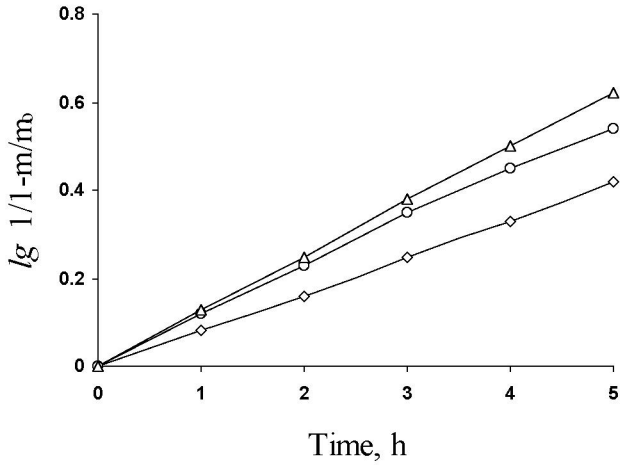

Fig.2. Dynamic of clopheline release from PVA solution at different ratio polymer:drug. ◊ – 5:1, ■ – 3:1, Δ – 1:1.

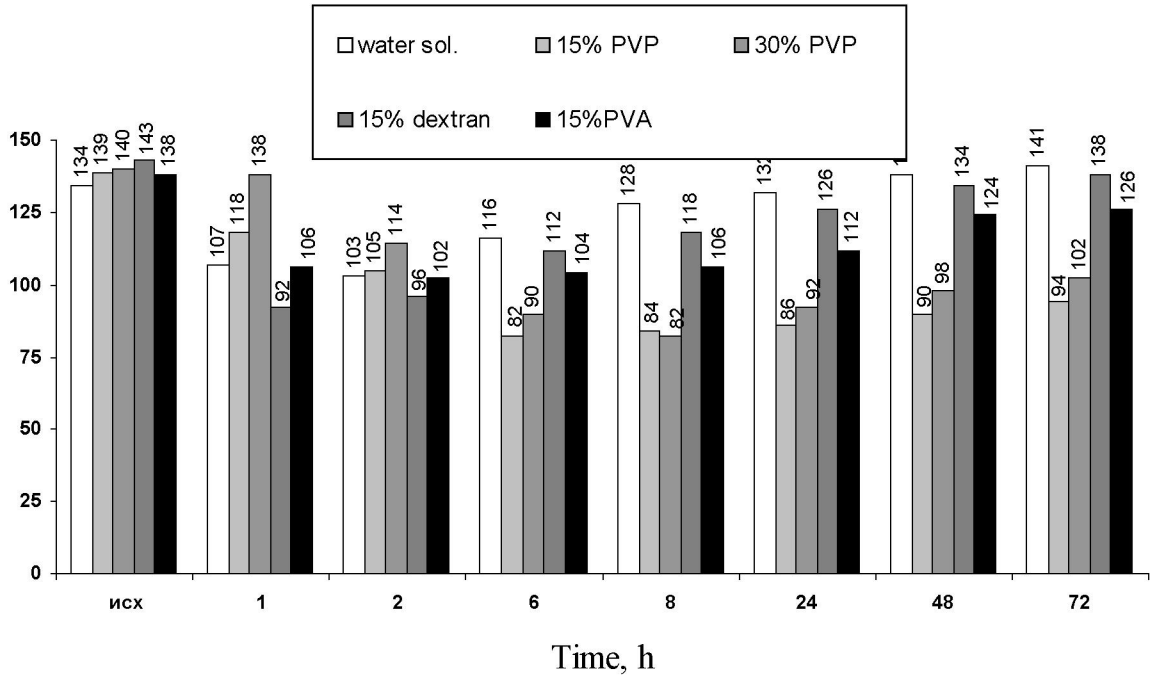

Fig.3. Changes of arterial pressure at animals after single injection of polymeric forms of clopheline (dose 50 mcg/1 kg)

It is shown that with increase molar ratio of reagents from 1:1 up to 1:4, the value of constant of diffusion decreases and makes 5,93; 5,61; 5,33 and 5,06 x 10-5 s-1, accordingly.

For prolongation of action of various medicinal substances alongside with PVP it is widely used other polymers having blood substitute properties - polyvinyl alcohol («Polydes») and dextran («Polyglyukin»). These high-molecular compounds were used in our works for increase of duration of action clopheline in injectable forms. Viscosimetric researches have shown, that PVA is nonionogenic polymer and dependence of the viscosity versus concentration represents a direct line. At titration PVA by a clopheline solution there is an increase of viscosity of the system caused by increase of the linear sizes macromolecules PVA as a result of association with a molecule of drug.

Thermodynamic parameters of binding of clopheline with PVA, received of Klotz equation, testify about exothermic character of process. Negative values of change of free energy and enthalpy show on existence in researched systems of hydrogen bonds (tab. 1) and hydrophobic interactions. At research of prolonging properties of the received complexes it is established, that the association clopheline with PVA results in insignificant delay of drug release (tabl.2). Kinetic curve of diffusions

show, that the greatest prolonging effect is achieved at a ratio PVA:drug, equal 1:5 (fig. 2).

Widely used as blood substitute the natural polysaccharide dextran only in an insignificant degree binds with clopheline molecules. In formation of associates in this case, apparently, the dominant role is played the hydrophobic interactions. It Is shown, that polymeric complexes clopheline with dextran have no long prolonging action (tab. 2).

For realization medical and biologic tests the concentrated solutions of polymers (15 %) containing clopheline were prepared. At research of dynamics of drug release from such solutions it was established, that the greatest prolonging action has PVP. Medical and biologic tests have shown, that water-soluble forms clopheline on the basis of the used blood substitutes provide long-term reduction of the level of arterial pressure at hypothensive animals (till 48-72 against 6-8 hours of action free clopheline). The greatest prolonging action was observed for the PVP solution (fig.3). At polymeric solutions of dextran the sharply expressed decrease of the level of arterial pressure in an initial stage of time was observed.

Thus, the carried out investigations have shown that hypothensive drug clopheline in water solutions forms with polymers – blood plasma substitutes (polyvinylpyrrolidone, polyvinyl alcohol, dextran) complexes due to hydrogen bonds and hydrophobic interactions. Long therapeutic action of polymeric water-soluble complexes was established. The opportunity of creation on their basis injected medicinal forms of clopheline with prolonged hypothensive actions was shown.

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Резюме

Қаналмастырғыш негізіндегі клофелин препаратының және суда еритін полимерлік дәрілік түрлері алынды. Теңестіру диализ әдісімен клофелиннің поливинилпирролидонмен, поливинил спиртпен және декстранмен әрекеттесуі зерттелді. Препараттың физиологиялық ерітіндісіне бөлініп шығу динамикасы зерттелді. Полимерлік қаналмастырғыштар клофелиннің әсер ету уақытысын создыру үшін колдануға болатындығы көрсетілді.

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Поступила 7.11.2007 г.