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Development of composition and some technology aspects of frozen-dried medicine on the basis of bee venom

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Abstract
Proceeding from the literary data and physical and chemical properties of substance of bee venom and auxiliary substances the rational composition of frozen-dried medicine on the basis of bee venom and its optimal pH level has been experimentally confirmed. Auxiliary substances increasing the mass of frozen-dried medicine and its compactness, providing the isotonicity of the solution and comfort at intramuscular introduction of the medicine have been chosen. The optimal filling volume of ampoules and bottles that provides the quality of frozen-dried medicine satisfying to the requirements of specification has been recommended.

Keywords: frozen-dried, medicine, bee venom.

1. Introduction
In folk medicine bee venom is used for a long time; however in official medicine it has not almost found an application.
At present the fact of change of situation of bee venom application in medicine is traced. Probably, the accumulated experience and practical knowledge in the field of study of venom composition and technology of medicines creation on its basis are the reason of this phenomenon. Lately large amount of medicines in different dosage forms on basis of bee venom has appeared [1, 2]. These medicines can be applied at different diseases; they are exactly dosed and have high-efficiency. At present the medicines on the basis of bee venom are produced by pharmaceutical industry and specialized firms. These medicines are applied as injections, inhalations, liniments for external use and for oral administration [1 – 4].
Bee venom is the secretion liberated by the threadlike gland of stinging mechanism of worker bee. Chemical composition of bee venom is very complex. The complex of lipoid and mineral substances, amino acid and proteins is entered into its composition. The protein fraction forms the bulk of dry substance. Up to date successful application of bee venom as a medicine is conditioned mainly by properties of mellitine. It amounts up to 90% of active peptide complex of venom in therapeutic medicines, and mellitine content in dry venom amounts 40 – 50% of venom mass. 26 amino acid are entered into its composition with dominance of such basic amino acids as lysine and arginine [3, 4].
The extremely high sensitiveness of bee venom to high temperature (thermolability), light (photolability), presence of oxygen in environment [3, 4] condition the necessity of application of the special approaches for stabilizing its solutions in the process of storage, for example, application of the method of lyophilization (freeze-drying). Application of such method when producing parenteral medicines allows to obtain standard and stable medicines of thermolabile and hydrolytically unstable substances.
The aim of our paper is to develop the composition of frozen-dried medicine on the basis of bee venom for parenteral application and to study the influence of layer thickness of solution in an ampoule (bottle) on quality of frozen-dried medicine.

2. Materials and Methods
The objects of our research are the substance of bee venom, the solution of bee venom, the frozen-dried medicine on the basis of bee venom.

In the course of experiment the qualitative and quantitative control of the medicine samples has been carried out. The quality indexes (see in the text) and the methods of its determination are described in State Pharmacopoeia of Ukraine (SPhU) [5] and in the specification project developed by us for the medicine [unpublished data].

3. Results and Discussion

The physical and chemical properties of the active and auxiliary substances have been studied for choosing the optimal composition and obtaining the stable dosage form of bee venom.

Bee venom is a transparent and aromatic liquid of yellowish colour. The taste is bitter, pungent; density is 1.085 – 1.131 (on the average is 1.11 g/cm^3). Bee venom dries out quickly on air. Dry venom is hygroscopic, freely soluble in water and water-glycerine mixtures, sparingly soluble in aqueous-alcoholic mixtures and acids, for example, in formic acid. In aqueous solution, and also under influence of digestive enzymes and oxidants, bee venom is quickly inactivated. Low temperatures and freezing do not influence on the components of bee venom, but higher temperatures, light, air oxygen inactivate and destroy them [3, 4]. Such properties of bee venom make obtaining its stable aqueous solution difficult, but condition the possibility of obtaining the frozen-dried medicine on its basis using different auxiliary substances.

Based on the data of scientific literature sources and preliminary pharmacological researches the therapeutic concentration of bee venom has been chosen as 1 mg/cm^3 of mellitine for preparation of medicine. This concentration corresponds to the therapeutic concentration of active substance in analogous medicines on the basis of bee venom [3, 4].

One of the main physical parameters influencing on stability of parenteral medicines is the level of pH. To provide the necessary value of pH of medicines when preparation of solutions different buffer systems, mainly, acetate, citrate, phosphate and their combinations, and also organic and inorganic acids, their salts, alkaline agents are used [6]. Therefore the important work direction when creating the medicine is research of pH influence on stability of the solution of bee venom in the prescribed limits.

The pH value of aqueous solution of bee venom (1:100) ranges from 4.0 to 6.0 [3]. With the purpose of confirmation of this pH interval we have carried out the researches of 4 batches of the bee venom solutions with the different pH values, which are reached by adding 1 mole/l sodium hydroxide solution or 1 mole/l hydrochloric acid solution [5].

The results of our researches are presented in Table 1.

Table 1: The quality indices of the solutions of bee venom with the different values of pH

Index (specification project)	Batch number			
	1	2	3	4
pH (4.0 – 6.0)	4.00	4.65	5.25	6.00
Transparency (SPhU, 2.2.1)	satisfied	satisfied	satisfied	satisfied
mechanical inclusions (RD 42-501-98)	absent	absent	absent	absent
chromaticity (SPhU, 2.2.2)	satisfied	satisfied	satisfied	satisfied
quantitative content of bee venom as mellitine, mg/ml (0.95 – 1.05)	0.97	0.98	0.97	0.97

It has been found as a result of conducted researches that the solutions with the critical values of pH do not change their physical and chemical characteristics, such as transparency, chromaticity, quantitative content of active substance, absence of mechanical inclusions in the solution – they correspond to the requirements set in the specification project.

Using buffer systems, acid and alkaline agents for maintenance of pH of the solutions on the basis of bee venom is not actual. Therefore, proceeding from the obtained results and studying the data of scientific literature the range of 4.0 – 6.0 is set as the optimal limits of pH for medicine on the basis of bee venom.

Solvents, solubilizers, fillers, preservatives, stabilizers, cryoprotectants belong to the auxiliary substances used in the production of frozen-dried medicines. They provide the maintenance of the proper therapeutic effect and stability of quality indices of medicine [6].

The content of bee venom in the dosage form is small (1 mg/ml), therefore for obtaining frozen-dried medicine on the basis of bee venom we have applied different shape-generating fillers. Such high molecular compounds as polyvinylpyrrolidone, gum, polysaccharides (saccharose, lactose etc.), pectins, polyols (mannitol, sorbitol) are used mostly as fillers [7 – 9].

For obtaining frozen-dried medicine on the basis of bee venom we have used mannitol in the concentration of 2%. Mannitol increases the mass of frozen-dried medicine, its compactness, has influence on realization speed of the lyophilization process and value of final moisture content [6 – 9].

The injections of medicines on the basis of bee venom are very painful when intramuscular introducing, therefore the topical anaesthetic (lidocaine hydrochloride) has been entered in the concentration of 0.05% into the composition of medicine.

One of the requirements requested to injection medicines is the solution isotonicity that conditions the necessity of reaching the osmolality of the solution to the level of osmolality of biological liquids of organism by addition auxiliary substances, which are able to increase osmoticity [6].

It is suggested by us to use sodium chloride as a isotonicity regulator, it plays the role of filler simultaneously. For determining the necessary amount of sodium chloride providing the isotonicity of the developed medicine we use the calculation by the Vant Hoff law with the help of the Clapeyron equation:

$$P \cdot V = n \cdot R \cdot T \cdot i,$$

Where P– is the osmolality of blood plasma (7.4 atm.);
V– is the volume of solution, l;
n– is the amount of dissolved substance, mole;
R– is the universal gas constant (for this case – 0.082 atm·l/K·mole);
T– is the absolute temperature (310 K);
i– is the isotonic coefficient depending on the number of particles appeared when dissociating.

Taking into account that $n = m/M$, where m – is the amount of substance in g/l, and M – is its molar mass, it is possible to calculate the osmolality of 1 l of the solution created by one substance in such way:

$$P = \frac{m \cdot R \cdot T \cdot i}{V \cdot M} \quad \text{or} \quad P = \frac{m}{M} \cdot 25.42 \cdot i .$$

The osmolality created by substances, which enter into the

composition of medicine (except sodium chloride), is presented in Table 2.

Table 2: The osmotic pressure of the medicine components without sodium chloride

Active and auxiliary substances	*m*, g/l	*i*	*M*	*P*
bee venom	1.0	–	–	–
mannitol	20.0	1.00	182.17	2.79
lidocaine hydrochloride	0.5	1.86	288.80	0.08
in all:	–	–	–	2.87

The total osmotic pressure of the solution by the Dalton law consists of osmotic pressure of components, i. e. the total osmotic pressure *P* in the solution without sodium chloride is 2.87 atm. The osmotic pressure of isotonic solution is equal to the blood osmotic pressure of 7.4 atm., therefore it is necessary to make the solution isotonic by adding sodium chloride in the amount, which is able to increase the osmotic pressure on 7.4 – 2.87 = 4.53 atm. This amount *x*(NaCl) is calculated by the formula:

$$x(NaCl) = \frac{(7{,}4 - P) \cdot M(NaCl)}{25.42 \cdot i(NaCl)} = \frac{4.53 \cdot 58.44}{25.42 \cdot 1.86} = 5.59\, g/l\, (mg/ml).$$

I. e. for reaching the solution isotonicity it is necessary to add sodium chloride in amount of 6 mg/ml. On the basis of study of literary data and theoretical calculations the composition of the medicine on the basis of bee venom has been chosen – the data is presented in Table 3.

Table 3: Qualitative and quantitative composition of the medicine on the basis of bee venom

Component	Quantitative content in one ampoule (bottle), mg	Function of the component
bee venom	1.0	active substance
mannitol	20.0	shape-creating filler
lidocaine hydrochloride	0.5	topical anaestetical agent
sodium chloride	6.0	isotonic regulator
water for injection	to 1.0 мл	solvent

With the purpose of confirmation of the composition of medicine chosen in theory on the basis of bee venom 3 batches of medicine of this composition have been obtained and its quality indices have been studied in the process of storage. The results of researches are presented in Table 4.

Table 4: The quality indices of the medicine on the basis of bee venom in the process of storage

Quality index	Storage term, month	Batch number		
		5	6	7
original appearance (dry porous mass of white colour)	0 (initial data)	satisfied	satisfied	satisfied
	12	satisfied	satisfied	satisfied
transparency (transparent as compared to water)	0 (initial data)	transparent	transparent	transparent
	12	transparent	transparent	transparent
pH (4.0 – 6.0)	0 (initial data)	5.50	5.35	5.40
	12	5.45	5.30	5.35
solubility (dissolved freely in 2 ml during 1 min)	0 (initial data)	satisfied	satisfied	satisfied
	12	satisfied	satisfied	satisfied
moisture content (no more than 4.0%)	0 (initial data)	2.30	2.50	2.70
	12	2.30	2.50	2.65
quantitative content of bee venom as mellitine, mg/ml (0.95 – 1.05)	0 (initial data)	0.98	0.97	0.99
	12	0.97	0.97	0.98

As it is obvious from data of Table 4 the chosen composition allows to obtain the frozen-dried medicine on the basis of bee venom, which satisfies the requirements by the quality indices of specification in the moment of producing and in the process of storage.

Simultaneously with development of the composition of the frozen-dried medicine on the basis of bee venom the study of influence of layer thickness of the solution on quality of frozen-dried product has been carried out.

With the purpose of determination of optimal layer thickness for obtaining the medicine of necessary quality the solution on the basis of bee venom has been measured out in bottles with the capacity of 5 ml in 1, 2 and 4 ml and in ampoules with the capacity of 2 ml in 0.5, 1 and 2 ml. The samples of medicine has been dried in sublimation dryer according to the chosen mode and the quality of frozen-dried medicine has been determined by original appearance, solution pH, rest moisture and quantitative content of active substance. The research data are presented on Fig. 1 and 2.

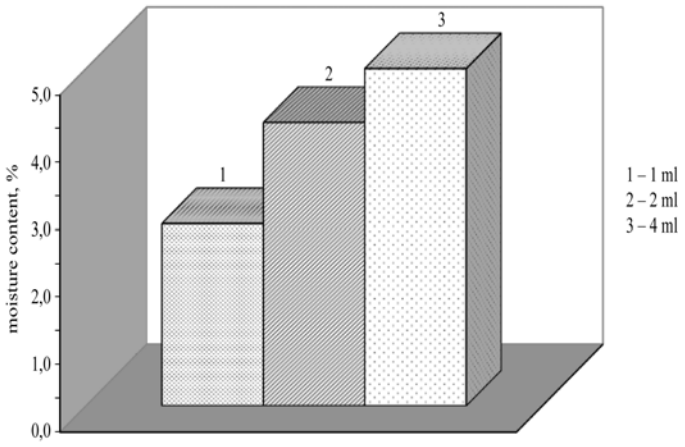

Fig 1: Influence of layer thickness of the solution in a bottle on the index of rest moisture in frozen-dried medicine

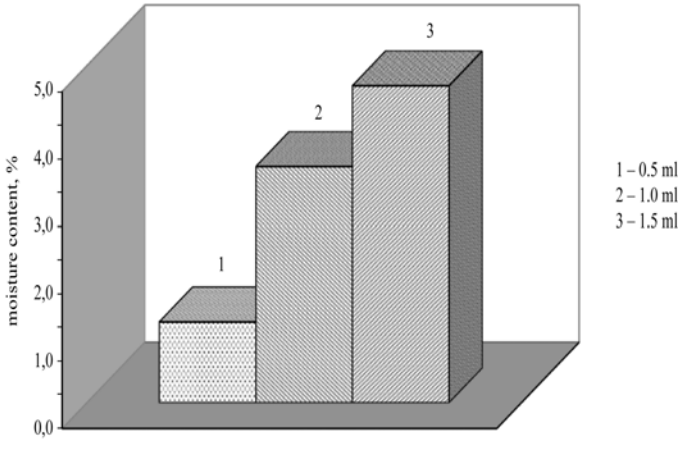

Fig 2: Influence of layer thickness of the solution in an ampoule on the index of rest moisture in frozen-dried medicine

From Fig.1 it is evidence that when freeze-drying the medicine in the bottles with the capacity of 5 ml with the filling volume of 1 ml the rest moisture in the dosage form is 3.2%. The quantitative content of bee venom as mellitine and solution pH satisfy to the requirements of specification. In the bottles with the filling volume of 2 ml and 4 ml the rest moisture is 4.5% and 5.0% respectively that does not satisfy to the requirements of specification. Moreover, in these cases the medicine does not satisfy to the requirements of normative documentation by the index «quantitative content».

From Fig. 2 it is evidence that when freeze-drying the medicine in the ampoules with the capacity of 2 ml with the filling volume of 0.5 ml the rest moisture in the dosage form is 1.5%. Quantitative content of bee venom as mellitine and solution pH satisfy to the requirements of specification. In this case the medicine is dry nonuniform porous mass by appearance that does not satisfy to the requirements of specification by the index «original appearance». In ampoules with the filling volume of 1 ml the rest moisture in medicine is 3.7%, all quality indices satisfy to the requirements of normative documentation, for the filling volume of 2 ml the rest moisture is 4.7% that does not satisfy to the requirements of specification by the index «moisture». The quantitative content of bee venom as mellitine in medicine is 0.93 mg/ml that does not satisfy to the requirements of normative documentation.

Proceeding from the obtained data it is obviously that the optimal filling volume of the solution for carrying out the process of freeze-drying the medicine on the basis of bee venom is 1 ml – both for ampoules and bottles.

The layer thickness of frozen-dried medicine when filling with 1 ml in ampoules is 14 ± 1 mm, in bottles – 9 ± 1 mm, respectively the rest moisture for medicine in bottles is 3.2%, and for medicine in ampoules is 3.7%, by the indices of pH, quantitative content of active substance and original appearance the medicine both in ampoules and in bottles satisfy to the requirements of specification, therefore for the primary packing of frozen-dried medicine on the basis of bee venom it is possible to recommend both ampoules and bottles with the filling volume of 1 ml.

4. Conclusions

Proceeding from the literary data and physical and chemical properties of substance of bee venom and auxiliary substances the rational composition of frozen-dried medicine has been experimentally confirmed on its basis.

The optimal pH level of the solution on the basis of bee venom providing the stability of medicine has been experimentally confirmed.

Auxiliary substances increasing the mass of frozen-dried medicine and its compactness, providing the isotonicity of the solution and comfort at its intramuscular introduction have been chosen.

The influence of layer thickness of the solution in ampoule (bottle) on the index of the rest moisture and other quality indices of frozen-dried medicine on the basis of bee venom has been studied. The optimal filling volume of ampoules and bottles that provides the quality of frozen-dried medicine satisfying to the requirements of specification has been chosen.

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