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Original Contribution

STATIC SORPTION CAPACITY OF LYOPHILIZED BLOOD PRODUCTS AFTER GRANULATION

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ABSTRACT

Purpose: The present publication is to analyse the impact of initial treatment (modification) with C_2H_5OH and the process of granulation of lyophilised blood products on their CCl_4 sorption capacity and to compare these sorption capacities with the CCl_4 sorption capacity of the initial, non-treated granulated lyophilised blood products.
Method: the static sorption of newly offered sorbents has been studied - lyophilised erythrocyte mass and lyophilised plasma proteins by exsiccator method before and after granulation.
Result: The newly offered absorbents have high sorption capacity.
Conclusion: The sorption capacity decreases significantly after granulation.

Key Words: sorbent, gas mask, lyophilisation

INTRODUCTION

The importance of gas masks for protection against weapons of mass destruction is significant The number of people who died from poisonous gases only, during the First World War was 1 300 000 [1]. The poisonous gases, used in the military nowadays are far more toxic, which means that modern gas masks must meet far greater requirements. The use of chemical weapons for military actions has also been observed during a number of recent wars [2].

Gas masks are also used in overcoming the aftermath of industrial accidents, fires and natural disasters. The example of the Indian city of Bopal alone is enough to illustrate that [3, 4]. Examples of toxic gas leakage during transportation have been described [5].

Different means of protection against inhaling poisonous gases are used when extinguishing fires [6, 7, 8, 9]. The release of highly poisonous gases depends on the burning material and the temperature interval of the burning process [10, 11].

During the last years a new type of sorbent was proposed for protection from the vapours of toxic compounds based on blood products.

Lyophilised blood products can substitute the presently used carbon catalysts as sorbents in gas masks [12].

In this paper static sorption capacity of lyophilized erythrocyte mass and lyophilized plasma proteins has been investigated by exsiccator method before and after granulation.

MATERIALS AND METHODS

Reagents

- glucose (Himsnab), Bulgaria
- sodium citrate (Himtex), Bulgaria
- sodium chloride (Himtex), Bulgaria
- carbon tetrachloride (Merk), Germany
- ethanol 96% (Merk), Germany

1. Methods for receiving sorbents

Sheep blood from vena jugularis externa, provided by the Institute of Communicable and Parasitic Diseases, Sofia was used.

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1.1. Preparation of blood products

Erythrocyte mass and plasma

Alsewer`s solution, modified by Bucantz should be used. It contains: 4.1 g glucose (Himsnab), 1.6 g sodium citrate (Himtex), 0,84 g sodium chloride (Himtex) and condensed distilled water, up to 200 ml. The pH of 6.1 was obtained using citric acid.
The solution and the blood from vena jugularis, ratio 1:1, were poured into a bank and then centrifuged at a speed of 2000 revolutions per min for 20 min (300 *g*). The two phases were separated.

1.2. Refrigeration and vacuum sublimation dehydration of blood products.

Refrigeration was done at – 45 ºC ± 5 ºC (solution was rotated while freezing). After that the banks were placed in a sublimation chamber of the vacuum sublimation installation. When the temperatures of the products and the refrigerating chamber equalized the equipment was turned on to the heating and vacuuming. The process of lyophilisation goes off automatically, at maximal working vacuum pressure of 10^{-1} Pa and final temperature of the substance of 36 ºC, ± 2 ºC. Depending on the quantity of the substance and the thickness of the frozen layer, the duration of sublimation dehydration is between 30 and 48 h.

T 616 Hochvacuum, GDR equipment was used.

At the end of the process, the vacuum process in the sublimation chamber was stopped by the introduction of dry inert gas (nitrogen). The lyophilised blood products were placed in airtight containers and labelled.

1.3.Granulation method

The lyophilised product was wetted by adding ethanol (96%). The wetted mass was pressed through a sieve with a size of the openings 2 mm. The granules were dried in thermostatically controlled drying oven at 30 ºC for half an hour. After drying the granules were passed through a sieve with size of the openings 0.8 mm.

2. Testing of static sorption capacities of lyophilised blood products

Sorbtive tested: carbon tetrachloride-Merk

The preparation of a mixture of air and evaporations of the tested substance were done directly in an exsiccator, as 50 ml of the tested substance was put into a Beherov`s glass, which was then put into an exsiccator. The temperature of the exsiccator was brought to 20 °C by putting it into a constant temperature water bath. When the liquid and the evaporations were in balance, weight measuring bottles, containing the sorbents that were being tested, were put into the exsiccator. Their mass should have previously been measured by Sartorius scales. At regular intervals they were weighed and by calculating the difference in mass weight, the quantity of sorbed substance was found. Results should then be recalculated in mg of sorbed substance per 1g of sorbent.

The tests were carried out in triplicates.

3. Statistic data processing

Variation analysis was used for statistic processing of quantity measurable indices. Data were presented as an average arithmetic quantity of the values, measured in three separate experiments [13]. Results were taken into account only if the difference between the highest and the lowest value was 10% lower than the lowest value. Since all experiments were carried out in vitro, repetitiveness was very high.

RESULTS AND DISCUSSION

Results are presented on **Table 1**.

Table 1. *Carbon tetrachloride sorption, milligrams of sorbed substance per 1g of sorbent*

Lyophilised sorbent				***Time/hours***
non granulated		*granulated*		
erythrocytes	*plasma*	*erythrocytes*	*plasma*	
41.237	129.032	13.348	32.544	3
49.464	145.152	15.022	33.900	6
63.891	169.344	15.540	35.256	9
74.196	177.408	18.130	36.612	12
113.355	217.728	19.684	37.968	24
129.843	225.792	21.238	42.036	36
146.331	241.920	21.238	43.392	48
164.948	241.920	22.792	46.104	72

Our laboratory test results show that the CCl_4 sorption capacity of materials treated initially with C_2H_5OH and granulated lyophilised blood products is substantially lower than the CCl_4 sorption capacity of the initial, non-treated granulated lyophilised blood products. The analysis of our test results, with regard to the mechanism of absorption of substance evaporations by lyophilised blood products, shows that the reasons for the decreased sorption capacity of the granulated samples are different but that they all result from the initial treatment of added lyophilised blood products with C_2H_5OH. We have also taken into account the fact that one of the reasons for that may be the partial protein precipitation in lyophilised blood products, as a result of their initial treatment with C_2H_5OH. In this sense, the initial treatment with C_2H_5OH leads to solvation of the granulated samples-free sorption centres (irrespectively of their nature and disposition). As a result of this, their total number decreases, which causes further decrease in the CCl_4 sorption capacity of the granulated lyophilised blood products.

A very important factor is the fact that C_2H_5OH mainly reacts with the lyophilised blood products' retaining moisture, uncombined or combined. In both cases, no matter that the action mechanisms of C_2H_5OH are different, the observed result is compression of the carcass of the lyophilised blood products in the granulates. In combined water the C_2H_5OH molecules displace the water molecules of combined water (14) from the solvate wrapping around the polarized functional groups disrupting the solvate cover. The latter plays an important role in sustaining the reciprocal positioning of supra-molecular structures (the macro-structural elements of lyophilised blood products), through hydrogen bonds, forming flexible carcass. The disruption of the solvate cover leading to carcass shrinkage, as well as shrinkage of the space between the macro-structural elements of the lyophilised blood products lead to increase in the genuine density of blood sorbents. Likewise, because of the two-way dissolving process going on between C_2H_5OH and the present uncombined water in the inner space between the supra-molecular structures during the process of drying, dehydration occurs, along with destruction of structural elements, and respectively, inner-structural contraction is observed.

As a result, apart from the decrease in the CCl_4 sorption capacity of the granulated lyophilised blood products, the kinetics of the process of swelling also change.

A confirmation of the assumption for the operational mechanisms of the advance treatment of lyophilised blood products with C_2H_5OH B are the results of researching the process of water steam obtained by lyophilised blood products, by applying the excicator method, with different values of relative moisture (φ), sustained in the excicator. For the interval of relative moisture of 66-75 %, the volume of obtained waters in previously treated lyophilised blood products with C_2H_5OH is about two times lower, compared to initial values of non-granulated lyophilised blood products. The volumes of obtained water are equalized not until reaching the maximal values of relative moisture of $\varphi \approx 0.9 - 1.0$.

CONCLUSIONS

The research carried out on the impact of preliminary treatment (modification) with C_2H_5OH and the subsequent granulation of lyophilised blood products on their CCl_4 sorption capacity support our assumption (14) that the mechanism of substance evaporations absorption (CCl_4 evaporations in that particular case) by lyophilised blood products is not a simple mechanical penetration of those evaporations into the empty space between the separate macro-structural elements of the lyophilised blood products of physical adsorption but it is due to inter-molecular interaction, mainly in the form of solvation of the relevant macro-molecules by the supra-molecular structures of the lyophilised blood products and their subsequent swelling. As a result of this, the use of C_2H_5OH in the process of granulation leads to preliminary partial solvation of the free sorption centres of the lyophilised blood products (irrespectively of their nature and positioning), as a result of which their total number decreases, as well as to "congestion of the carcass" of the lyophilised blood products in the granules, through a process of irreversible dehydration, and respectively, to destruction of the structural elements, and possibly, to partial protein precipitation. All these lead to signify changes in the nature of the process of swelling and process of toxic substances evaporation absorption. The latter supports results of our study. The common result is a substantial decrease in the CCl_4 sorption capacity of the granulated lyophilised blood products, compared to the CCl_4 sorption

capacity of the initial, untreated lyophilised blood products.

Notwithstanding the fact that the use of C_2H_5OH is not advisable and thus it required preliminary treatment to be done with a different substance, granulation of the lyophilised blood products is an indispensable condition for their practical application in the future.

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