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IMPACT OF DIFFERENT FACTORS ON VOLUME PROPERTIES OF HUMAN HEMOGLOBIN

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Impact of different factors (temperature, group separation, concentration) on volume properties of human hemoglobin (HHb) has been studied using densitometry method. Investigations were provided at temperatures 301.15, 309.75 and 313.15 *K*. It has been shown, that the densities, apparent partial specific volumes and partial specific volumes of HHb are different for separated and non-separated forms of HHb molecule.

***Keywords*:** human hemoglobin, densitometry, apparent partial specific volume, partial specific volume, density.

Introduction. Human hemoglobin (HHb) is one of the most important and abundant constituent of blood, which is well known as an oxygen carrying protein (Fig. 1) [1]. Nowadays, great interest is represented to the studies, which are considering interactions between HHb and various ligands [2, 3]. Various factors (temperature, protein molecule group separation, pH, presence of different additives) can influence on protein molecule state and reacting possibility [4]. Above mentioned effects can cause structural changes in protein molecule and volume properties, which can be studied using densitometry, viscosimetry and ultrasonic velocimetry methods [5–8].

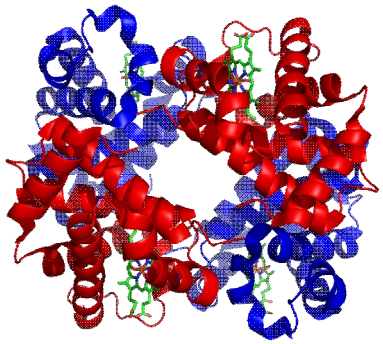

Fig. 1. HHb structure.

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The aim of this study is to gain the effect of group separation on the volume properties of HHb molecule at different temperatures using densitometry method.

Materials and Methods. HHb was purchased from "Sigma Aldrich" (Steinheim, Germany). All materials and reagents were of analytical grade. Solutions were prepared in 0.2 *M* phosphate buffer (pH 7.2). Group separation of HHb was carried out with the help of PD-10 desalting column, which contains Sephadex G-25 Medium. The separation range of Sephadex G-25 Medium is suitable for group separation of low (M_r < 1000) from high (M_r > 5000) molecular weight contaminants [9–11], which in turn can cause changes in volume properties of HHb.

Densitometry Measurements. Densitometry studies were performed using an Anton Paar DMA 4500 (Austria) ($\Delta\rho = \pm 5 \cdot 10^{-5}\ g \cdot cm^{-3}$) vibration densitometer built in water circulating thermostat (ΔT = ± 0.03°*C*). All data were recorded at 301.15, 307.75 and 313.15 *K* temperatures in the concentration range of HHb from $0.989 \cdot 10^{-6}$ to $7.912 \cdot 10^{-6}$ *M*. Using the protein density data, it is possible to calculate the apparent partial specific volume of protein by the following equation [7]:

$$\phi = \frac{1}{\rho_0}\left[1 - \frac{\rho - \rho_0}{c}\right], \qquad (1)$$

where ϕ is the apparent partial specific volume of protein, $cm^3 \cdot g^{-1}$, ρ and ρ_0 are the densities of protein solution and solvent respectively, $g \cdot cm^{-3}$, c is the concentration of protein, $g \cdot mL^{-1}$. The partial specific volume V_ϕ, $cm^3 \cdot g^{-1}$, of protein can be determined by extrapolation of ϕ to zero concentration of protein [6].

Origin 8.0 software was used to construct the graphs.

Results and Discussion. The density data of non-separated and separated forms of HHb are shown in Fig. 2 at different temperatures (301.15, 307.75 and 313.15 *K*).

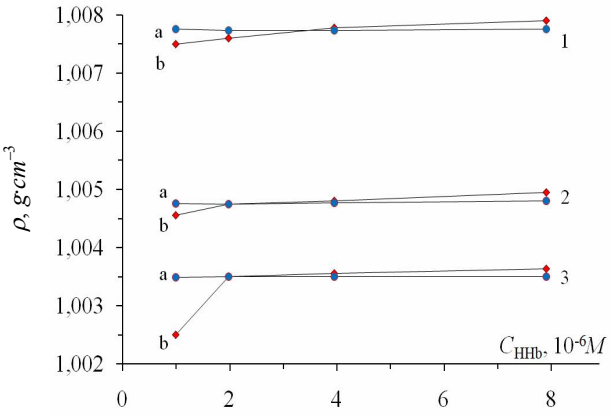

Fig. 2. The values of density for various concentrations (C_{HHb}= $0.989 \cdot 10^{-6}$–$7.912 \cdot 10^{-6}$ *M*) of non-separated (a) and separated (b) HHb at different temperatures, *K*: 1 – 301.15; 2 – 309.75; 3 – 313.15.

Densities of non-separated (a) and separated (b) forms of HHb in all temperatures differ at low concentration range $0.989 \cdot 10^{-6}$–$1.976 \cdot 10^{-6}$ *M*. At higher concentrations these differences are insignificant. Increase in the temperature causes structural changes in HHb molecule as a result of increasing thermal movement of polypeptide chains. The values of ϕ of non-separated and separated forms of HHb at different temperatures are presented in the Table. Increasing the temperature and concentration of HHb leads to changes in ϕ values for non-separated and separated forms. At 313.15 *K* ϕ values change is not typical, because of structural changes of HHb caused by temperature. The data of V_ϕ are 0.811 $cm^3 \cdot g^{-1}$ for non-separated and 0.609 $cm^3 \cdot g^{-1}$ for separated form of HHb at 309.75 *K*. The difference of these values can be explained by the fact that in non-separated HHb arrangement of polypeptide chains is spatial, than in separated HHb, which has a compact structure.

The values of apparent partial specific and partial specific volumes for various concentrations of HHb

ϕ, $cm^3 \cdot g^{-1}$						
C_{HHb}, 10^{-6} *M*	*T*, *K*					
	301.15	309.75	313.15	301.15	309.75	313.15
	Non-separated HHb			Separated HHb		
0.989	0.5291	0.8404	–11.7271	4.5431	0.2515	0.5610
1.976	0.8378	0.8403	–5.8330	0.5580	1.1787	–0.1776
3.956	0.9150	0.7629	–2.5730	1.2945	1.5316	–0.4114
7.912	0.9537	0.8201	–0.8266	1.2312	1.4450	–1.0131

Conclusion. Densitometry method is used to study non-separated and separated forms of HHb at concentration range $0.989 \cdot 10^{-6}$–$7.912 \cdot 10^{-6}$ *M*. It has been shown that group separation causes structural changes in HHb molecule and arrangement of HHb polypeptide chains for non-separated form HHb is spatial, than for separated form.

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