FORMULAS FOR DEATH AND LIFE: CHEMICAL COMPOSITION AND BIOTHERMODYNAMIC PROPERTIES OF MONKEYPOX (MPV, MPXV, HMPXV) AND VACCINIA (VACV) VIRUSES

Marko POPOVIC [*1]

[1]School of Life Sciences, Technical University of Munich, Freising, Germany

[*] Corresponding author; E-mail: marko.popovic@tum.de; marko.popovic.td@gmail.com

"The scientist understands nothing except the reduction to the least and simplest basic laws possible; From them, however, he deduces the phenomena absolutely completely as necessary."
Carl Fridrich Gauss, Titan of Science 2004

Today, the World Health Organization has declared a global health emergency, caused by the Monkeypox outbreak. In the monthly analysis for June, 3500 cases have been reported in 50 countries around the world. In the analysis for July, more than 30 000 cases have been reported in 75 countries. Thus, in the circumstances of the continuing COVID-19 pandemic, the appearance and dynamics of spreading of Monkeypox is alarming. In this paper, for the first time, elemental composition of Poxvirus, Monkeypox virus and Vaccinia virus have been reported. Additionally, thermodynamic properties have been reported for nucleic acids, nucleocapsids and entire virus particles. The similarity in chemical composition and thermodynamic properties of the analyzed viruses has been used to explain the crossed immunity to Poxviruses. Finally, binding thermodynamic properties have been reported for the Vaccinia virus.
Key words*: Gibbs energy; Enthalpy; Entropy; Biosynthesis; Empirical formula; Antigen-receptor binding*

1. Introduction

Since early May 2022, cases of Monkeypox have been reported from countries where the disease is not endemic, and continue to be reported in several endemic countries [1]. Since 1 January and as of 22 June 2022, 31000 laboratory confirmed cases and one death have been reported to WHO from 50 countries/territories in five WHO Regions [2, 3]. With 3000 cases in Germany, 9500 in the US, 2900 in the UK and 25 in Serbia, it is obvious that Monkeypox is beginning to represent a treat for the health system [2]. Thus, it is necessary to perform fundamental research on chemical, biological and biothermodynamic nature of the Monkeypox virus, as well as interactions of the virus with host cells.

Viruses represent biological agents [4, 5]. However, viruses also represent chemical systems [5-7] and biothermodynamic systems [8-10]. Viruses interact with host cells [11-16]. The interaction

is performed at the cell membrane (antigen-receptor binding) [11-14, 17]. Virus-host interactions also occur in the cytoplasm, when the virus hijacks the host cell metabolism [15].

Thermodynamics of virus-host interactions is very important [18]. During its life cycle, a virus performs various chemical processes. Two of them are the most important: binding of the virus antigen to the host cell receptor and multiplication of the virus inside the cell. Binding represents a process similar to the protein-ligand interaction. On the other hand, virus multiplication represents a polymerization reaction of nucleotides into nucleic acids and amino acids into proteins [9]. The driving force for the binding reaction is Gibbs energy of binding [7]. The driving force for growth of microorganisms is Gibbs energy of growth [19-24]. The driving force for multiplication of viruses is Gibbs energy of biosynthesis of the virus components [9, 15]. The driving force of growth of host cells is Gibbs energy of growth [19, 20, 25]. Since growth and binding are nonequilibrium processes, in their analysis it is necessary to use nonequilibrium thermodynamics [26, 27].

During the last several years, a great effort has been made to chemically and thermodynamically describe SARS-CoV-2 and other viruses [6, 11-16, 28-31], as well as the biothermodynamic background of their interactions [32]. Until now, Monkeypox and Vaccinia viruses have not yet been thermodynamically characterized.

The aim of this paper is to determine the elemental composition, empirical formula and biothermodynamic properties of binding and biosynthesis for Vaccinia and Monkeypox viruses. Moreover, the calculated values will be used to quantitatively describe virus-host interactions with the point of entry tissue.

2. Methods

2.1. Data sources

Genetic sequences of the Monkeypox and Vaccinia viruses were obtained from the NCBI database [33]. The genetic sequence of the Monkeypox virus was found under the accession number ON983168. The genetic sequence of the Vaccinia (Buffalopox) virus was found under the accession number OK422496. The molecular composition of Poxviridae virions was taken from [4]. The molecular composition of the Vaccinia virus was taken from [34]. The dissociation constant of the Vaccinia virus was taken from [35]. Standard thermodynamic properties of human host tissues were taken from [25].

2.2. Elemental composition of viral nucleic acid

Based on the genetic sequences, elemental compositions of virus nucleic acids were calculated, using the atom counting method [10]. The atom counting method calculates the number of atoms of each element in a virus particle or its part, based on its genetic sequence, protein sequences, protein copy numbers and virus size [10]. The elemental composition of viral nucleic acids was determined from genetic sequences. The atom counting method was applied, using a custom-made computer program. More details on the atom counting method can be found in [10].

2.3. Elemental composition of entire virions and nucleocapsids

Elemental composition of entire virions and nucleocapsids was calculated using the molecular composition method [10]. Viruses consist of four main kinds of molecules: nucleic acids, proteins,

lipids and carbohydrates [4, 34]. Each of the four kinds of molecules has a different chemical composition, which is known [10]. Thus, the elemental composition of the virus particle can be found if the amounts of the four kinds of molecules are known [10]. Molecular composition of viruses is usually reported as mass fractions [4, 34]. Thus, molecular composition is first converted from mass fraction into mole fraction through the equation

$$x_X(VIR) = \frac{w_X(VIR)/M_r(X)}{\sum_Y w_Y(VIR)/M_r(Y)} \quad (1)$$

where $M_r(X)$ is the molar mass of the empirical formula of molecular component X, $x_X(VIR)$ and $w_X(VIR)$ are the mole fraction and mass fraction of molecular component X in the virion, respectively [10]. The summation is over all molecular components. Mole fractions of molecular components are then used to find the empirical formula of viruses using the equation

$$n_J = \sum_X \left[x_X \cdot n_J(X)\right] \quad (2)$$

where n_J is the number of moles of element J in the virus empirical formula and $n_J(X)$ is the number of moles of element J in the empirical formula of molecular component X [10]. The sum is over all molecular components of the virus.

2.4. Standard thermodynamic properties of live matter

Elemental composition of live matter was used to find standard thermodynamic properties of the Monkeypox and Vaccinia nucleic acids, as well as Poxviridae and Vaccinia entire virus particles (virions) and nucleocapsids. Standard enthalpy of formation of virus live matter was calculated using the Patel-Erickson equation, also known as Thornton's rule. Elemental composition of live matter can be used to find the number of electrons transferred to oxygen during its complete combustion, E, using the equation

$$E = 4n_C + n_H - 2n_O - 0\ n_N + 5n_P + 6n_S \quad (3)$$

where n_C, n_H, n_O, n_N, n_P and n_S represent the number of carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur atoms in the empirical formula of live matter, respectively [9, 24, 36, 37]. The number of electrons E can be used to calculate standard enthalpy of combustion of live matter, $\Delta_C H^0(bio)$, using the Patel-Erickson equation [9, 24, 36-38]

$$\Delta_C H^0(bio) = -111.14 \frac{kJ}{C-mol} \cdot E \quad (4)$$

$\Delta_C H^0(bio)$ is the enthalpy change of the combustion reaction of live matter

$$C_{nC}H_{nH}O_{nO}N_{nN}P_{nP}S_{nS} + (n_C + ¼\ n_H + 1¼\ n_P + 1½\ n_S - ½\ n_O)\ O_2 \rightarrow n_C\ CO_2 + ½\ n_H\ H_2O + ½\ n_N\ N_2 + ¼\ n_P\ P_4O_{10} + n_S\ SO_3 \quad (5)$$

Thus, Hess's law can be used to convert standard enthalpy of combustion of live matter, $\Delta_C H^0(bio)$, into standard enthalpy of formation of live matter, $\Delta_f H^0(bio)$ [39, 40].

$$\Delta_f H^0(bio) = n_C\ \Delta_f H^0(CO_2) + \frac{n_H}{2}\ \Delta_f H^0(H_2O) + \frac{n_P}{4}\ \Delta_f H^0(P_4O_{10}) + n_S\ \Delta_f H^0(SO_3) - \Delta_C H^0(bio) \quad (6)$$

A similar procedure can be used to find standard molar entropy of virus live matter, using the Battley equation. The Battley equation relates elemental composition of live matter to its standard molar entropy, $S_m^0(bio)$,

$$S_m^0(bio) = 0.187\ \sum_J \frac{S_m^0(J)}{a_J} n_J \quad (7)$$

where $S_m^0(J)$ is standard molar entropy of element *J*, a_J number of atoms of element *J* in its standard state form and n_J is the number of atoms of element *J* in the empirical formula of the virus [9, 24, 41]. The Battley equation can be modified to give standard entropy of formation of live matter, $\Delta_f S^o(bio)$. This is done by replacing the coefficient +0.187 with -0.813 [41]

$$\Delta_f S^0(bio) = -0.813 \sum_J \frac{S_m^0(J)}{a_J} n_J \quad (8)$$

Finally, $\Delta_f S^o(bio)$ can be combined with $\Delta_f H^o(bio)$, to find standard Gibbs energy of formation of live matter, $\Delta_f G^o(bio)$, using the equation [9, 24, 36]

$$\Delta_f G^0(bio) = \Delta_f H^0(bio) - T\,\Delta_f S^0(bio) \quad (9)$$

2.5. Biosynthesis reactions

Elemental composition of virus particles was used to construct biosynthesis reactions of the Monkeypox and Vaccinia nucleic acids, as well as Poxviridae and Vaccinia entire virus particles (virions) and nucleocapsids. Biosynthesis reactions are macrochemical equations that quantify growth of organisms, describing conversion of nutrients into new live matter and other metabolic products [19, 20, 36, 42]. Biosynthesis reactions have been used to study a wide range of organisms, including bacteria [37], fungi [36, 42], algae [43], plants [44] and viruses [15-17]. Biosynthesis reactions for the analyzed viruses have the general form [9, 15-17]

$$\text{(Amino acid)} + O_2 + HPO_4^{2-} + HCO_3^- \rightarrow \text{(Bio)} + SO_4^{2-} + H_2O + H_2CO_3 \quad (10)$$

Amino acids represent the carbon and energy source, and the nitrogen source [9, 15, 16, 20]. Oxygen is the electron acceptor [9, 15, 16, 20]. The hydrogenphosphate ion is the phosphorus source, while the hydrogencarbonate ion is a part of the bicarbonate buffer that takes the produced H^+ ions [9, 15, 16, 20]. (Bio) denotes newly synthetized virus live matter [9, 15, 16, 20]. The sulfate ion takes excess sulfur, while H_2CO_3 takes oxidized carbon and excess H^+ ions, as a part of the bicarbonate buffer [9, 15, 16, 20]. The stoichiometric coefficients for the biosynthesis reactions of the analyzed viruses are given in Table 7.

2.6. Standard thermodynamic properties of biosynthesis

Standard thermodynamic properties of virus live matter can be combined with biosynthesis reactions, to find standard thermodynamic properties of biosynthesis [9, 19, 20]. Standard thermodynamic properties of biosynthesis are thermodynamic property changes accompanying biosynthesis reactions [9, 19, 20]. They include standard enthalpy of biosynthesis, $\Delta_{bs}H^o$, standard entropy of biosynthesis, $\Delta_{bs}S^o$, and standard Gibbs energy of biosynthesis, $\Delta_{bs}G^o$ [9, 19, 20]. These properties can be found using the Hess's law

$$\Delta_{bs}H^0 = \sum_{products} \nu\,\Delta_f H^0 - \sum_{reactants} \nu\,\Delta_f H^0 \quad (11)$$

$$\Delta_{bs}S^0 = \sum_{products} \nu\,S_m^o - \sum_{reactants} \nu\,S_m^o \quad (12)$$

$$\Delta_{bs}G^0 = \sum_{products} \nu\,\Delta_f G^0 - \sum_{reactants} \nu\,\Delta_f G^0 \quad (13)$$

where ν represents a stoichiometric coefficient [39, 40]. Standard Gibbs energy of biosynthesis, $\Delta_g G^o$, is of particular importance, since it represents the driving force of biosynthesis and is related to biosynthesis rate [9, 19, 20].

2.7. Standard thermodynamic properties of binding

The dissociation process is the opposite of binding [9, 45]. Thus, dissociation equilibrium constants are reciprocal of binding equilibrium constants [9, 45]. Thus, dissociation equilibrium constants were used to calculate binding equilibrium constants, K_B, using the equation [9, 45]

$$K_B = \frac{1}{K_D} \tag{14}$$

The binding constants were used to find standard Gibbs energy of binding, $\Delta_B G^{\,o}$, using the equation [9, 45]

$$\Delta_B G^0 = -R_g T \ln K_B \tag{15}$$

3. Results

Elemental compositions of nucleic acids of the Monkeypox and Vaccinia viruses have been determined for the first time and are given in Table 1. The empirical formula of the nucleic acid of the Monkeypox virus is $CH_{1.2542}O_{0.5759}N_{0.3726}P_{0.1017}$, while that of the Vaccinia virus nucleic acid is $CH_{1.2542}O_{0.5763}N_{0.3729}P_{0.1017}$.

Table 1: Elemental composition of nucleic acids of Monkeypox and Vaccinia viruses.

Virus	n_C	n_H	n_O	n_N	n_P
Monkeypox virus - DNA	1	1.2542	0.5759	0.3726	0.1017
Vaccinia virus (Buffalopox) - DNA	1	1.2542	0.5763	0.3729	0.1017

Elemental compositions of the virus nucleic acids were used to find for the first time standard thermodynamic properties of Monkeypox and Vaccinia virus nucleic acids. The results are given in Table 2. Standard enthalpy of formation, $\Delta_f H^{\,o}$, of the Monkeypox nucleic acid is -136.8 kJ/C-mol, while that of the Vaccinia nucleic acid is -136.9 kJ/C-mol. Standard molar entropies, $S_m{}^o$, of the Monkeypox and Vaccinia nucleic acids are 34.9 J/C-mol K. Standard Gibbs energy of formation, $\Delta_f G^{\,o}$, of the Monkeypox nucleic acid is -91.6 kJ/C-mol, while that of the Vaccinia nucleic acid is -91.7 kJ/C-mol.

Table 2: Standard thermodynamic properties of formation of the nucleic acids of Monkeypox and vaccinia viruses.

Virus	$\Delta_f H^o$ (kJ/C-mol)	$S_m{}^o$ (J/C-mol K)	$\Delta_f G^o$ (kJ/C-mol)
Monkeypox virus - DNA	-136.8	34.9	-91.6
Vaccinia virus (Buffalopox) - DNA	-136.9	34.9	-91.7

Elemental composition of virus live matter was used to construct biosynthesis reactions for the analyzed viruses. The stoichiometric coefficients for the biosynthesis reactions are given in Table 7.

Elemental compositions and standard thermodynamic properties of live matter were combined to calculate for the first time standard thermodynamic properties of biosynthesis of the Monkeypox and Vaccinia virus nucleic acids. Standard enthalpy of biosynthesis, $\Delta_{bs} H^{\,o}$, of the Monkeypox nucleic acid was found to be -412.2 kJ/C-mol, while that of the Vaccinia nucleic acid is -413.2 kJ/C-mol. Standard entropy of biosynthesis, $\Delta_{bs} S^{\,o}$, of the Monkeypox nucleic acid was found to be -78.1 kJ/C-mol, while that of the Vaccinia nucleic acid is -78.3 kJ/C-mol. Standard Gibbs energy of biosynthesis

$\Delta_{bs}G^{o}$ of the Monkeypox nucleic acid is -390.2, while that of the Vaccinia nucleic acid is -391.1 kJ/C-mol. The results are summarized in Table 3.

Table 3: Standard thermodynamic properties of biosynthesis of nucleic acids of the Monkeypox and Vaccinia viruses.

Virus	$\Delta_{bs}H^{o}$ (kJ/C-mol)	$\Delta_{bs}S^{o}$ (J/C-mol K)	$\Delta_{bs}G^{o}$ (kJ/C-mol)
Monkeypox virus - DNA	-412.2	-78.1	-390.2
Vaccinia virus (Buffalopox) - DNA	-413.2	-78.3	-391.1

Similarly, elemental compositions were calculated for the first time, in the form of empirical formulas, for the entire Poxviridae family. The calculation was made for the entire virus particles (virions), including nucleic acids, proteins, lipids and carbohydrates. The calculation was also made for the nucleocapsid, consisting of nucleic acids and proteins. The results are given in Table 4. The entire Poxviridae virion has the empirical formula $CH_{1.5876}O_{0.3008}N_{0.2537}P_{0.00223}S_{0.00553}$. The nucleocapsid of a Poxviridae virion has the formula $CH_{1.5618}O_{0.3149}N_{0.2733}P_{0.00240}S_{0.00596}$. The entire Vaccinia virion has the empirical formula $CH_{1.5877}O_{0.3232}N_{0.2531}P_{0.00371}S_{0.00540}$, while its nucleocapsid has the formula $CH_{1.5675}O_{0.3357}N_{0.2685}P_{0.00394}S_{0.00573}$.

Table 4: Elemental composition of poxvirus and Vaccinia entire virions and nucleocapsids.

Virus	n_C	n_H	n_O	n_N	n_P	n_S
Poxviruses - entire virion	1	1.5876	0.3008	0.2538	0.00223	0.00554
Poxviruses - nucleocapsid	1	1.5618	0.3150	0.2734	0.00241	0.00596
Vaccinia - entire virion	1	1.5877	0.3232	0.2531	0.00371	0.00540
Vaccinia - nucleocapsid	1	1.5675	0.3357	0.2685	0.00394	0.00573

Standard thermodynamic properties of live matter were determined for the first time of Poxviridae entire virions and nucleocapsids. Standard enthalpy of formation of a Poxviridae entire virion is -65.2 kJ/C-mol, while that of the nucleocapsid of Poxviridae is -67.4 kJ/ C-mol. Standard molar entropy of a Poxviridae entire virion is 30.8 J/C-mol K, while that of the nucleocapsid is 31.1 J/C-mol K. Standard Gibbs energy of formation of an entire Poxviridae virion is -25.3 kJ/C-mol, while that of the nucleocapsid is -27.1 kJ/C-mol. The results are summarized in Table 5.

Table 5: Standard thermodynamic properties of formation of poxvirus and Vaccinia entire virions and nucleocapsids.

Virus	$\Delta_f H^{o}$ (kJ/C-mol)	S_m^{o} (J/C-mol K)	$\Delta_f G^{o}$ (kJ/C-mol)
Poxviruses - entire virion	-65.2	30.8	-25.3
Poxviruses - nucleocapsid	-67.4	31.1	-27.1
Vaccinia - entire virion	-70.5	31.2	-30.0
Vaccinia - nucleocapsid	-72.6	31.5	-31.8

Standard thermodynamic properties of biosynthesis were calculated for the first time of Poxviridae entire virions and nucleocapsids. They are given in Table 6. Standard enthalpy of biosynthesis of a Poxviridae entire virion is -71.4 kJ/C-mol, while that of its nucleocapsid is -127.2 kJ/C-mol. Standard entropy of biosynthesis of a Poxviridae entire virion is -6.7 J/C-mol K, while that of its nucleocapsid is -17.5 J/C-mol K. Standard Gibbs energy of biosynthesis of a Poxviridae entire virion is -69.4 kJ/C-mol, while that of its nucleocapsid is -122.0 kJ/C-mol.

The binding constant, K_B, and standard Gibbs energy of binding, $\Delta_B G^o$, of the Vaccinia virus were determined for the first time. The binding constant was found to be $K_B = 5.56 \cdot 10^8$ M^{-1}. The standard Gibbs energy of binding is $\Delta_B G^o$ = -51.92 kJ/mol at 37°C.

Table 6: Standard thermodynamic properties of biosynthesis of poxvirus and Vaccinia entire virions and nucleocapsids.

Virus	$\Delta_{bs}H^o$ (kJ/C-mol)	$\Delta_{bs}S^o$ (J/C-mol K)	$\Delta_{bs}G^o$ (kJ/C-mol)
Poxviruses - entire virion	-71.4	-6.7	-69.4
Poxviruses - nucleocapsid	-127.2	-17.5	-122.0
Vaccinia - entire virion	-73.9	-8.1	-71.5
Vaccinia - nucleocapsid	-117.9	-16.7	-113.0

4. Discussion

Four Orthopox viruses cause infections in humans: Variola, Vaccinia, Cowpox and Monkeypox. Variola has been eradicated [4]. Infections with Vaccinia and Monkeypox viruses appear in animal and human populations (zoonosis) [3, 46, 47]. Zoonoses have large reservoirs of infective agents in nature. Thus, they have a special importance. The morphology of Poxviruses is available in the literature [4]. Poxviruses are large complex enveloped viruses, which contain a double stranded DNA genome [4, 48]. However, elemental composition, empirical formula, thermodynamic properties of formation, biosynthesis and binding, are not available in the literature. Having in mind that Gibbs energy is the driving force for all chemical processes in nature [39, 40, 49], Gibbs energy of biosynthesis is the driving force for multiplication of viruses [9, 15, 16], while Gibbs energy of binding is the driving force for virus attachment and entry into host cells [7-9, 11-14].

Until 2020, elemental composition and empirical formulas have been known only for one virus -the Poliovirus [5, 50]. The reason for this is that most laboratories lack the required biosafety level [10]. During the last years, chemical characterization was made of various viruses [6, 9, 10, 15-17, 31]. Thus, in this paper, the atom counting method [10] and available sequence data were used to perform chemical characterization of Vaccinia and Monkeypox viruses for the first time. The atom counting method has given results that are in good agreement with experimental results [5, 10, 50]. The calculated empirical formulas of nucleic acids of the analyzed viruses differ on the 3rd or 4th decimal, as can be seen from Table 1. This means that elemental compositions of both viruses are very similar. The empirical formula of the Monkeypox nucleic acid is $CH_{1.2542}O_{0.5759}N_{0.3726}P_{0.1017}$, while that of the Vaccinia nucleic acid is $CH_{1.2542}O_{0.5763}N_{0.3729}P_{0.1017}$. Also, the entire Poxviridae virion has the empirical formula $CH_{1.5876}O_{0.3008}N_{0.2537}P_{0.00223}S_{0.00553}$. The nucleocapsid of a Poxviridae virion has the formula $CH_{1.5618}O_{0.3149}N_{0.2733}P_{0.00240}S_{0.00596}$. The entire Vaccinia virion has the empirical formula $CH_{1.5877}O_{0.3232}N_{0.2531}P_{0.00371}S_{0.00540}$, while its nucleocapsid has the formula $CH_{1.5675}O_{0.3357}N_{0.2685}P_{0.00394}S_{0.00573}$. Notice that empirical formulas of the entire virions differ more (at the 2nd decimal). This leads to the conclusion that the nucleic acids are very similar, but that the main difference is in the lipid and polysaccharide components. It is a fact that there is a crossed immune response [4]. It seems that it is a consequence of the similarity in the chemical structure of the viruses.

Table 7: Stoichiometric coefficients of the biosynthesis reactions for the analyzed viruses.

Virus	Reactants						Products					
	Amino acid	O_2	HPO_4^{2-}	HCO_3^-	→	Bio	SO_4^{2-}	H_2O	HCO_3^-	H_2CO_3
Monkeypox virus - DNA	1.6580	0.9059	0.1017	0.0000	→	1	0.0373	0.3205	0.1288	0.5292
Vaccinia virus (Buffalopox) - DNA	1.6596	0.9079	0.1017	0.0000	→	1	0.0373	0.3203	0.1288	0.5307
Poxviruses - entire virion	1.1292	0.1444	0.0022	0.0352	→	1	0.0198	0.0755	0.0000	0.1644
Poxviruses - nucleocapsid	1.2164	0.2653	0.0024	0.0379	→	1	0.0214	0.0783	0.0000	0.2543
Vaccinia - entire virion	1.1265	0.1505	0.0037	0.0324	→	1	0.0199	0.0779	0.0000	0.1589
Vaccinia - nucleocapsid	1.1948	0.2459	0.0039	0.0344	→	1	0.0211	0.0802	0.0000	0.2292

Standard thermodynamic properties of live matter depend on its elemental composition. Since Monkeypox and Vaccinia viruses have very similar elemental composition, their thermodynamic properties are very similar as well, as can be seen from Table 2. For example, standard enthalpy of formation of Monkeypox nucleic acid is -136.8 kJ/C-mol, while that of Vaccinia nucleic acid is -136.9 kJ/C-mol. Both enthalpies of formation are negative, meaning that the energy content of live matter is lower than that of its constituent elements. Standard molar entropy of both Monkeypox and Vaccinia nucleic acids is 34.9 J/C-mol K [39, 40]. Entropies of both organisms are positive, due to the third law of thermodynamics, stating that entropy of any substance can only be positive [39, 40]. Standard Gibbs energy of formation of Monkeypox nucleic acid is -91.6 kJ/C-mol, while that of Vaccinia nucleic acid is -91.7 kJ/C-mol. Notice that both are negative, meaning that hypothetical formation of live matter from elements is a spontaneous process and that live matter does not possess a higher energy content than inanimate matter.

Table 7 shows the stoichiometry of biosynthesis reactions for the analyzed viruses. Multiplication of microorganisms can be represented by a chemical equation [19, 20]. Thus, for virus multiplication, biosynthesis reactions can also be formulated [9, 15]. In that way, biological properties like growth and multiplication can be analyzed through the biosynthesis reactions, which represent the chemical background of biological phenomena.

Replication of nucleic acids represents a chemical and thermodynamic process. The driving force for replication of nucleic acids is Gibbs energy of biosynthesis, since replication is a reaction of polymerization of nucleotides into the nucleic acid macromolecule. Standard thermodynamic properties of the Monkeypox and Vaccinia nucleic acids are very similar (Table 3). Standard enthalpy of biosynthesis of the Monkeypox nucleic acid is -412.1 kJ/C-mol, while that of the Vaccinia nucleic acid is -413.2 kJ/C-mol. Standard entropy of biosynthesis of the Monkeypox nucleic acid is -78.1 J/C-mol K, while for Vaccinia nucleic acid it is -78.3 J/C-mol K. Standard Gibbs energy of biosynthesis of Monkeypox nucleic acid is -390.2 kJ/C-mol, while that of Vaccinia nucleic acid is -391.1 kJ/C-mol. Gibbs energy of biosynthesis give an approximate assessment of the biosynthesis rate, through the biosynthesis phenomenological equation, which belongs to phenomenological equations from

nonequilibrium thermodynamics [9, 19, 22, 23, 49]:

$$r_{bs} = -\frac{L_{bs}}{T}\Delta_{bs}G \tag{16}$$

where r_{bs} is biosynthesis rate (rate of formation of new live matter), L_{bs} biosynthesis phenomenological coefficient, T temperature and $\Delta_{bs}G$ Gibbs energy of biosynthesis. The more negative $\Delta_{bs}G$ leads to greater rate of biosynthesis of nucleic acid. The Vaccinia and Monkeypox viruses have very similar values of $\Delta_{bs}G$ and thus according to equation (16) very similar rate of biosynthesis of nucleic acid. Vaccinia virus was, and still is, being used as a live-virus vaccine against smallpox. A new smallpox vaccine, Imvanex, is based on the modified Vaccinia Ankara strain [51]. Thus, in this paper, the Vaccinia virus has also been analyzed, in addition to the Monkeypox. Even though Vaccinia causes only mild infections, the rate of its multiplication is, according to Table 3, equal to that of the Monkeypox virus. Even though the Vaccinia and Monkeypox viruses have very similar chemical compositions and very similar thermodynamic properties of biosynthesis, the former causes mild symptoms and small damage to tissues, while the latter causes much more severe symptoms and greater tissue damage. Thus, a live vaccine with Vaccinia or modified Vaccinia virus represents a good tool in the fight against Smallpox and Monkeypox. The similar chemical composition enables the crossed immune response, while similar multiplication rate allows the efficiency of protection. From this, we can conclude that the difference in severity of clinical pictures and tissue damage does not arise from virus multiplication rate (permissiveness), but depends on susceptibility. It is obvious that the Vaccinia virus lacks the ability to enter all cells that can be entered by the Monkeypox virus. Thus, the Monkeypox virus can infect a greater variety of cells and tissues, leading to the more severe clinical picture.

In the literature, the dissociation constant (K_d) was available for the Vaccinia virus. Thus, the Gibbs energy of binding was calculated for the Vaccinia virus. The binding constant, K_B, and standard Gibbs energy of binding, $\Delta_B G^o$, of the Vaccinia virus were determined for the first time. The binding constant was found to be $K_B = 5.56 \cdot 10^8$ M^{-1}. The standard Gibbs energy of binding is $\Delta_B G^o$ = -51.92 kJ/mol at 37°C. Unfortunately, K_d, values were not published for the Monkeypox virus. It would be interesting to compare the values of $\Delta_B G^o$ for the Monkeypox and Vaccinia viruses in the future. The value $\Delta_B G^o$ = -51.92 kJ/mol leads to the conclusion that the entry rate into host cells of the Vaccinia virus is great, according to the binding phenomenological equation

$$r_B = -\frac{L_B}{T}\Delta_B G \tag{17}$$

where r_B is the binding rate, L_B binding phenomenological coefficient and $\Delta_B G$ Gibbs energy of binding [7-9]. More negative Gibbs energy of binding leads to greater binding rate [11-14]. Greater binding rate leads to faster entry of the virus into host cells. The result of this is greater infectivity [7-9]. To compare, standard Gibbs energy of binding of the Hu-1 (wild type) strain of SARS-CoV-2 is -43.43 kJ/mol, that of the Delta strain is −43.38 kJ/mol, while that of the Omicron BA.1 strain is −42.82 kJ/mol [9]. Thus, the Vaccinia virus has a more negative Gibbs energy of binding (-51.92 kJ/mol). However, Vaccinia is transmitted by direct or indirect contact, while SARS-CoV-2 is transmitted through Flügge droplets. Thus, it seems that both SARS-CoV-2 and Poxviruses, including Monkeypox, have a similar potential for dissemination, even though they use different paths.

Based on molecular composition published in [4], empirical formulas were calculated for nucleocapsids and entire virions of Poxviruses, using the molecular composition method [10]. Similarly, based on molecular composition published in [34], elemental compositions were found for

the nucleocapsids and entire virions of Vaccinia. They are given in Table 4. The empirical formula of the Poxvirus entire virion is $CH_{1.5876}O_{0.3008}N_{0.2537}P_{0.00223}S_{0.00553}$. The nucleocapsid of a Poxviridae virion has the formula $CH_{1.5618}O_{0.3149}N_{0.2733}P_{0.00240}S_{0.00596}$. The entire Vaccinia virion has the empirical formula $CH_{1.5877}O_{0.3232}N_{0.2531}P_{0.00371}S_{0.00540}$, while its nucleocapsid has the formula $CH_{1.5675}O_{0.3357}N_{0.2685}P_{0.00394}S_{0.00573}$. It seems that empirical formulas of entire Poxvirus and Vaccinia virus particles are very similar. Moreover, the nucleocapsid empirical formulas for the Poxvirus and Vaccinia virus are very similar. This indicates that both belong to the same group Poxviridae. Since the Vaccinia virus has a very similar composition to other Poxviruses and all the viruses in the family have very similar morphology, other Poxviruses should also have a similar elemental composition.

Table 5 shows the standard thermodynamic properties of live matter, of the Poxvirus and Vaccinia virus entire virions and nucleocapsids. Standard enthalpy of formation of Poxvirus entire virions is -65.2 kJ/C-mol, while that of Vaccinia entire virions is -70.5 kJ/C-mol. Standard molar entropy of Poxvirus entire virions is 30.8 J/C-mol K, while that of Vaccinia entire virions is 31.2 J/C-mol K. Standard Gibbs energy of formation of Poxviridae entire virions is -25.3 kJ/C-mol, while that of Vaccinia entire virions is -30.0 kJ/C-mol. Standard enthalpy of formation of Poxvirus nucleocapsids is -67.4 kJ/C-mol, while that of Vaccinia nucleocapsids is -72.6 kJ/C-mol. Standard molar entropy of Poxvirus nucleocapsids is 31.1 J/C-mol K, while that of Vaccinia nucleocapsids is 31.5 kJ/C-mol. Standard Gibbs energy of formation of the Poxvirus nucleocapsids is -27.1 kJ/C-mol, while that of Vaccinia nucleocapsids is -31.8 kJ/C-mol. The thermodynamic properties of Poxviruses are for the entire Poxviridae family, including Variola, Vaccinia, Cowpox and Monkeypox. Thus, they represent a generalization. The small discrepancy of the Vaccinia virus indicates that other group members also deviate very little. This means that all four Poxviruses have very similar elemental composition and thermodynamic properties.

Table 6 presents standard thermodynamic properties of biosynthesis for Poxviruses (generalized) and the Vaccinia virus. Thermodynamic properties of biosynthesis were determined for entire virions and nucleocapsids. Standard enthalpy of biosynthesis of Poxvirus entire virions is -71.4 kJ/C-mol, while that of Vaccinia entire virions is -73.9 kJ/C-mol. Standard entropy of biosynthesis of Poxviridae entire virions is -6.7 J/C-mol K, while that of Vaccinia entire virions is -8.1 J/C-mol K. Standard Gibbs energy of biosynthesis of Poxviridae entire virions is -69.4 kJ/C-mol, while that of Vaccinia entire virions is -71.5 kJ/C-mol. Standard enthalpy of biosynthesis of Poxvirus nucleocapsids is -127.2 kJ/C-mol, while that of Vaccinia nucleocapsids is -117.9 kJ/C-mol. Standard entropy of biosynthesis of Poxvirus nucleocapsids is -17.6 J/C-mol K, while that of Vaccinia nucleocapsids is -16.7 J/C-mol K. Standard Gibbs energy of biosynthesis of Poxvirus nucleocapsids is -122.0 kJ/C-mol, while that of Vaccinia nucleocapsids is -113.0 kJ/C- mol. Notice that there are significant differences in thermodynamic parameters of biosynthesis of Poxviruses and Vaccinia virus.

The portal of entry of Monkeypox virus, just like that of Smallpox, is the mucous membranes of the upper respiratory tract [4]. Primary multiplication appears in the lymphoid tissue draining the site of entry [4]. Thus, at the site of primary infection, the host tissue is the mucosa of the upper respiratory system and lymphoid tissues. Thermodynamic properties of host cells are given in [10]. Respiratory epithelial tissue is characterized by a Gibbs energy of biosynthesis of -49.8 kJ/C-mol [9]. To compare, standard Gibbs energy of biosynthesis of Poxvirus nucleocapids is -122.0 kJ/C-mol. Notice that Gibbs energy, as the driving force for biosynthesis of viral components, is more than two

times more negative than that of host tissues. Thus, permissiveness to Poxviruses is great. This can be measured through permissiveness coefficients [16]. The permissiveness coefficient, *PC*, is given as

$$PC = \frac{r_{bs}(virus)}{r_{bs}(host\ tissue)} = \frac{\Delta_{bs}G^0(virus)}{\Delta_{bs}G^0(host\ tissue)} \quad (18)$$

where r_{bs} is biosynthesis rate and $\Delta_{bs}G^o$ standard Gibbs energy of biosynthesis. Thus, the permissiveness coefficient for the virus-host interaction is 2.5 for Poxviruses and 2.3 for Vaccinia virus. The values of permissiveness coefficients are greater than 1. This means that the virus components are synthetized faster than host cells components, allowing the virus to hijack the host cell metabolism. Multiplication and accumulation of viruses inside host cells and absence of synthesis of host cell components to repair damage lead to damage of cells and tissues, as well as the dispersal of newly produced virions onto neighboring cells, first at the portal of entry and later throughout the organism.

Monkeypox virus contains information written in its RNA. This information should behave according to laws that parallel the laws of thermodynamics [52]. The difference in thermodynamic properties of Monkeypox and Vaccinia viruses appear as a result of different chemical composition and information content.

5. Conclusions

Elemental composition of the nucleic acid was determined for the Monkeypox and Vaccinia viruses. The empirical formula of the Monkeypox nucleic acid is $CH_{1.2542}O_{0.5759}N_{0.3726}P_{0.1017}$, while that of the Vaccinia nucleic acid is $CH_{1.2542}O_{0.5763}N_{0.3729}P_{0.1017}$. Moreover, elemental composition of the entire virus particle (virion) and the nucleocapsid was determined for Poxviridae and Vaccinia. The entire Poxviridae virion has the empirical formula $CH_{1.5876}O_{0.3008}N_{0.2537}P_{0.00223}S_{0.00553}$. The nucleocapsid of a Poxviridae virion has the formula $CH_{1.5618}O_{0.3149}N_{0.2733}P_{0.00240}S_{0.00596}$. The entire Vaccinia virion has the empirical formula $CH_{1.5877}O_{0.3232}N_{0.2531}P_{0.00371}S_{0.00540}$, while its nucleocapsid has the formula $CH_{1.5675}O_{0.3357}N_{0.2685}P_{0.00394}S_{0.00573}$. A similarity can be noticed in elemental compositions of all the members of Poxviridae family. This can explain the crossed immune response between the members of Poxviridae. The Vaccinia virus is thus useful as a vaccine.

Poxviridae are characterized by standard thermodynamic properties, including standard enthalpy of formation, standard molar entropy and standard Gibbs energy of formation of nucleic acid. Standard enthalpy of formation of the Monkeypox nucleic acid is -136.8 kJ/C-mol, its standard molar entropy is 34.9 J/C-mol K, while its standard Gibbs energy of formation is -91.6 kJ/C-mol. Standard enthalpy of formation of the Vaccinia nucleic acid is -136.9 kJ/C-mol, its standard molar entropy is 34.9 J/C-mol K, while its standard Gibbs energy of formation is -91.7 kJ/C-mol. Thus, standard thermodynamic properties of formation of nucleic acids are very similar for the Monkeypox and Vaccinia viruses, which is due to their similar chemical composition.

Biosynthesis reactions of the analyzed viruses have been formulated and their stoichiometric coefficients determined. The stoichiometric coefficients were combined with the thermodynamic properties of live matter, to find standard thermodynamic properties of biosynthesis, including standard enthalpies, entropies and Gibbs energies of biosynthesis. Standard enthalpy of biosynthesis for the Monkeypox nucleic acid is -412.2 kJ/C-mol, while that of the Vaccinia nucleic acid is -413.3 kJ/C-mol. Standard entropy of biosynthesis of the Monkeypox nucleic acid is -78.1 J/C-mol K, while that of the Vaccinia nucleic acid is -78.3 J/C-mol K. Standard Gibbs energy of biosynthesis of the

Monkeypox nucleic acid is -390.2 kJ/C-mol, while that of the Vaccinia nucleic acid is -391.1 kJ/C-mol. Standard enthalpy of biosynthesis of the Poxvirus entire virion is -71.4 kJ/C-mol, while that of Vaccinia entire virion is -73.9 kJ/C-mol. Standard entropy of biosynthesis of the Poxvirus entire virion is -6.7 kJ/C-mol, while that of the Vaccinia entire virion is -8.1 kJ/C-mol. Standard Gibbs energy of biosynthesis of the Poxvirus nucleic acid is -69.4 kJ/C-mol, while that of the Vaccinia entire virion is -71.5 kJ/C-mol. Finally, standard thermodynamic properties of nucleocapsids have been determined. Standard enthalpy of biosynthesis of Poxvirus nucleocapsid is -127.2 kJ/C-mol, while that of the Vaccinia nucleocapsid is -117.9 kJ/C-mol. Standard entropy of biosynthesis of the Poxvirus nucleocapsid is -17.5 J/C-mol K, while that of the Vaccinia nucleocapsid is -16.7 J/C-mol K. Standard Gibbs energy of biosynthesis of the Poxvirus nucleocapsid is -122.0 kJ/C-mol, while that of the Vaccinia nucleocapsid is -113.0 kJ/C-mol.

References

1. WHO (2022a). Monkeypox outbreak 2022 - Global [Online] World Health Organization, Available at: https://www.who.int/emergencies/situations/monkeypox-oubreak-2022 (Accessed on July 23, 2022)
2. CDC (2022). 2022 Monkeypox Outbreak Global Map [Online] Centers for Disease Control and Prevention. Available at: https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.html (Accessed on August 10, 2022).
3. WHO (2022b). Multi-country monkeypox outbreak: situation update 27 June 2022 [Online] World Health Organization, Available at: https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON396 (Accessed on July 23, 2022)
4. Riedel, S., Morse, S., Mietzner, T. & Miller, S. *Jawetz, Melnick & Adelbergs Medical Microbiology, 28th ed*., McGraw-Hill Education, New York, USA, 2019. ISBN: 978-1260012026
5. Wimmer E. The test-tube synthesis of a chemical called poliovirus. The simple synthesis of a virus has far-reaching societal implications. *EMBO reports*, *7* (2006), *Spec No*, S3–S9. https://doi.org/10.1038/sj.embor.7400728
6. Degueldre C. Single virus inductively coupled plasma mass spectroscopy analysis: A comprehensive study. *Talanta*, *228* (2021), 122211. https://doi.org/10.1016/j.talanta.2021.122211
7. Popovic, M. and Popovic, M., Strain Wars: Competitive interactions between SARS-CoV-2 strains are explained by Gibbs energy of antigen-receptor binding. *Microbial Risk Analysis*, *21* (2022), 100202. https://doi.org/10.1016/j.mran.2022.100202
8. Popovic, M., Strain wars 2: Binding constants, enthalpies, entropies, Gibbs energies and rates of binding of SARS-CoV-2 variants. *Virology*, *570* (2022), 35-44. https://doi.org/10.1016/j.virol.2022.03.008
9. Popovic, M., Strain wars 3: Differences in infectivity and pathogenicity between Delta and Omicron strains of SARS-CoV-2 can be explained by thermodynamic and kinetic parameters of binding and growth. *Microbial risk analysis*, (2022), 100217. Advance online publication. https://doi.org/10.1016/j.mran.2022.100217

10. Popovic, M., Atom counting method for determining elemental composition of viruses and its applications in biothermodynamics and environmental science. *Computational biology and chemistry*, *96* (2022), 107621. https://doi.org/10.1016/j.compbiolchem.2022.107621
11. Gale, P., Using thermodynamic equilibrium models to predict the effect of antiviral agents on infectivity: Theoretical application to SARS-CoV-2 and other viruses. *Microbial risk analysis*, *21* (2021), 100198. Advance online publication. https://doi.org/10.1016/j.mran.2021.100198
12. Gale, P., How virus size and attachment parameters affect the temperature sensitivity of virus binding to host cells: Predictions of a thermodynamic model for arboviruses and HIV. *Microbial risk analysis*, *15* (2020), 100104. https://doi.org/10.1016/j.mran.2020.100104
13. Gale, P., Towards a thermodynamic mechanistic model for the effect of temperature on arthropod vector competence for transmission of arboviruses. *Microbial risk analysis*, *12* (2019), 27–43. https://doi.org/10.1016/j.mran.2019.03.001
14. Gale, P., Using thermodynamic parameters to calibrate a mechanistic dose-response for infection of a host by a virus. *Microbial risk analysis*, *8* (2018), 1–13. https://doi.org/10.1016/j.mran.2018.01.002
15. Popovic, M., & Minceva, M., A thermodynamic insight into viral infections: do viruses in a lytic cycle hijack cell metabolism due to their low Gibbs energy?. *Heliyon*, *6* (2020), 5, e03933. https://doi.org/10.1016/j.heliyon.2020.e03933
16. Popovic, M., & Minceva, M., Thermodynamic insight into viral infections 2: empirical formulas, molecular compositions and thermodynamic properties of SARS, MERS and SARS-CoV-2 (COVID-19) viruses. *Heliyon*, *6* (2020), 9, e04943. https://doi.org/10.1016/j.heliyon.2020.e04943
17. Popovic, M., & Minceva, M., Coinfection and Interference Phenomena Are the Results of Multiple Thermodynamic Competitive Interactions. *Microorganisms*, *9* (2021), 10, 2060. https://doi.org/10.3390/microorganisms9102060
18. Head, R. J., Lumbers, E. R., Jarrott, B., Tretter, F., Smith, G., Pringle, K. G., Islam, S., & Martin, J. H., Systems analysis shows that thermodynamic physiological and pharmacological fundamentals drive COVID-19 and response to treatment. *Pharmacology research & perspectives*, *10* (2022), 1, e00922. https://doi.org/10.1002/prp2.922
19. Von Stockar, U., Live cells as open non-equilibrium systems, in: *Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering* (Ed. U. von Stockar), EPFL Press, Lausanne, Switzerland, 2013, pp. 475-534. https://doi.org/10.1201/b15428
20. Von Stockar, U., Biothermodynamics of Live Cells: Energy Dissipation and Heat Generation in Cellular Cultures, in: *Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering* (Ed. U. von Stockar), EPFL Press, Lausanne, Switzerland, 2013, pp. 475-534. https://doi.org/10.1201/b15428
21. von Stockar, U., & Liu, J., Does microbial life always feed on negative entropy? Thermodynamic analysis of microbial growth. *Biochimica et biophysica acta*, *1412* (1999), 3, 191–211. https://doi.org/10.1016/s0005-2728(99)00065-1
22. Hellingwerf, K.J., Lolkema, J.S., Otto, R., Neijssel, O.M., Stouthamer, A.H., Harder, W., van Dam, K. and Westerhoff, H.V., Energetics of microbial growth: an analysis of the

relationship between growth and its mechanistic basis by mosaic non- equilibrium thermodynamics. *FEMS Microbiology Letters*, *15* (1982), 1, 7-17. https://doi.org/10.1111/j.1574-6968.1982.tb00028.x

23. Westerhoff, H.V., Lolkema, J.S., Otto, R. and Hellingwerf, K.J., Thermodynamics of growth. Non-equilibrium thermodynamics of bacterial growth: the phenomenological and the Mosaic approach. *Biochimica et Biophysica Acta (BBA) - Reviews on Bioenergetics*, *683* (1982), 3-4, 181-220. https://doi.org/10.1016/0304-4173(82)90001-5
24. Popovic, M., Thermodynamic properties of microorganisms: determination and analysis of enthalpy, entropy, and Gibbs free energy of biomass, cells and colonies of 32 microorganism species. *Heliyon*, *5* (2019), 6, e01950. https://doi.org/10.1016/j.heliyon.2019.e01950
25. Popovic, M. and Minceva, M., Thermodynamic properties of human tissues. *Thermal Science*, *24* (2020), 6B, 4115-4133. https://doi.org/10.2298/TSCI200109151P
26. Popović, M. E., & Minceva, M., Comment on:"A critical review on heat and mass transfer modelling of viral infection and virion evolution: the case of SARS-COV2. Thermal Science, *25* (2021), 6 Part B, 4823-4825. https://doi.org/10.2298/TSCI211021329P
27. Trancossi, M., Pascoa, J. C., & Sharma, S., A critical review on heat and mass transfer modelling of viral infection and virion evolution: the case of SARS-COV2. *Thermal Science*, *25* (2021), 4A, 2831-2843. https://doi.org/10.2298/TSCI210614215T
28. Lucia, U., Grisolia, G., & Deisboeck, T. S., Thermodynamics and SARS-CoV-2: neurological effects in post-Covid 19 syndrome. *Atti della Accademia Peloritana dei Pericolanti*, *99* (2021), 2, A3. https://doi.org/10.1478/AAPP.992A3
29. Lucia, U., Grisolia, G., & Deisboeck, T. S., Seebeck-like effect in SARS-CoV-2 bio-thermodynamics. *Atti della Accademia Peloritana dei Pericolanti-Classe di Scienze Fisiche, Matematiche e Naturali*, *98* (2020), 2, 6. https://doi.org/10.1478/AAPP.982A6
30. Lucia, U., Deisboeck, T. S., & Grisolia, G., Entropy-based pandemics forecasting. *Frontiers in Physics*, *8* (2020), 274. https://doi.org/10.3389/fphy.2020.00274
31. Şimşek, B., Özilgen, M., & Utku, F. Ş., How much energy is stored in SARS- CoV- 2 and its structural elements?. *Energy Storage*, (2021), e298. https://doi.org/10.1002/est2.298
32. Popovic, M., Differences in infectivity and pathogenicity between Delta and Omicron strains of SARS-CoV-2 can be explained by Gibbs energies of binding and growth, *Proceedings, 21st Conference of the International Society for Biological Calorimetry (ISBC 2022)*, Vilnius, Lithuania, 2022, p. 20. ISBN: 978-609-96039-2-6
33. National Center for Biotechnology Information (2022). NCBI database [online] https://www.ncbi.nlm.nih.gov/ (Accessed on July 21, 2022)
34. Knight, C.A., *Chemistry of Viruses*, Springer, Berlin, Germany, 1975. ISBN: 978-3-642-85899-4
35. Lai, C. F., Gong, S. C., & Esteban, M., The 32-kilodalton envelope protein of vaccinia virus synthesized in Escherichia coli binds with specificity to cell surfaces. *Journal of virology*, *65* (1991), 1, 499–504. https://doi.org/10.1128/JVI.65.1.499-504.1991
36. Battley, E.H., The development of direct and indirect methods for the study of the thermodynamics of microbial growth. *Thermochimica Acta*, *309* (1998), 1-2, 17-37. https://doi.org/10.1016/S0040-6031(97)00357-2

37. Battley, E. H., On the enthalpy of formation of Escherichia coli K-12 cells. *Biotechnology and bioengineering*, *39* (1992), 1, 5–12. https://doi.org/10.1002/bit.260390103
38. Patel, S.A. and Erickson, L.E. Estimation of heats of combustion of biomass from elemental analysis using available electron concepts. *Biotechnology and Bioengineering*, *23* (1981), 2051-2067. https://doi.org/10.1002/bit.260230910
39. Atkins, P.W., & de Paula, J., *Physical Chemistry: Thermodynamics, Structure, and Change, 10th Edition*. W. H. Freeman and Company, New York, USA, 2014.
40. Atkins, P. W., & de Paula, J., *Physical Chemistry for the Life Sciences (2nd edition)*, W. H. Freeman and Company, New York, USA, 2011.
41. Battley, E.H., An empirical method for estimating the entropy of formation and the absolute entropy of dried microbial biomass for use in studies on the thermodynamics of microbial growth. *Thermochimica Acta*, *326* (1999), 1-2, 7-15. https://doi.org/10.1016/S0040-6031(98)00584-X
42. Battley, E. H., A theoretical study of the thermodynamics of microbial growth using Saccharomyces cerevisiae and a different free energy equation. *The Quarterly review of biology*, *88* (2013), 2, 69-96. https://doi.org/10.1086/670529
43. Wang, L., Wang, X., Jin, X., Xu, J., Zhang, H., Yu, J., Sun, Q., Gao, C., & Wang, L. Analysis of algae growth mechanism and water bloom prediction under the effect of multi-affecting factor. *Saudi journal of biological sciences*, *24* (2017), 3, 556–562. https://doi.org/10.1016/j.sjbs.2017.01.026
44. Popovic, M., & Minceva, M., Standard Thermodynamic Properties, Biosynthesis Rates, and the Driving Force of Growth of Five Agricultural Plants. *Frontiers in plant science*, *12* (2021), 671868. https://doi.org/10.3389/fpls.2021.671868
45. Du, X., Li, Y., Xia, Y. L., Ai, S. M., Liang, J., Sang, P., Ji, X. L., & Liu, S. Q. Insights into Protein-Ligand Interactions: Mechanisms, Models, and Methods. *International journal of molecular sciences*, *17* (2016), 2, 144. https://doi.org/10.3390/ijms17020144
46. Alakunle, E. F., & Okeke, M. I., Monkeypox virus: a neglected zoonotic pathogen spreads globally. *Nature reviews Microbiology*, 20 (2022), 507–508. https://doi.org/10.1038/s41579-022-00776-z
47. Di Giulio, D. B., & Eckburg, P. B., Human monkeypox: an emerging zoonosis. *The Lancet. Infectious diseases*, *4* (2004), 1, 15–25. https://doi.org/10.1016/s1473-3099(03)00856-9
48. Johnson, L.; Gupta, A. K.; Ghafoor, A.; Akin, D.; Bashir, R. Characterization of vaccinia virus particles using microscale silicon cantilever resonators and atomic force microscopy. *Sensors and Actuators B Chemical*, 115 (2006), 1, 189–197. https://doi.org/10.1016/j.snb.2005.08.047
49. Demirel, Y. *Nonequilibrium Thermodynamics: Transport and Rate Processes in Physical, Chemical and Biological Systems, 3rd ed.* Elsevier, Amsterdam, Netherlands, 2014. ISBN: 9780444595812
50. Molla, A., Paul, A. V., & Wimmer, E., Cell-free, de novo synthesis of poliovirus. *Science*, *254* (1991), 5038, 1647–1651. https://doi.org/10.1126/science.1661029
51. European Medicines Agency (2013). Assessment report: IMVANEX, Common name: Modified Vaccinia Ankara virus, Procedure No. EMEA/H/C/002596. [Online] Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency.

Available at: https://www.ema.europa.eu/en/documents/assessment-report/imvanex-epar-public-assessment-report_en.pdf (Accessed on July 24, 2022)

52. Hansen, L. D., Popovic, M., Tolley, H. D., & Woodfield, B. F., Laws of evolution parallel the laws of thermodynamics. *The Journal of Chemical Thermodynamics*, *124* (2018), 141-148. https://doi.org/10.1016/j.jct.2018.05.005

Submitted: 24.05.2022
Revised: 02.08.2022
Accepted: 15.08.2022