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Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. **00(0)** 1-11 (2024)
JSCS–12431
Original scientific paper
Published DD MM, 2024

The influence of conversion creatine and guanidinoacetic acid from zwitterionic to cationic form on their solubility in water - a thermodynamic study

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(Received 10 June 2023; revised 19 July 2023; accepted 4 April 2024)

Abstract: In this work, the solubility of creatine, creatinine, guanidinoacetic acid, and their hydrochlorides in water at atmospheric pressure and in the temperature range T = (293.15 - 313.15) K was determined by the gravimetric method. The thermodynamic parameters of dissolution in water for the mentioned compounds were calculated. The solubility increases significantly by converting the zwitterionic structures of creatine and guanidinoacetic acid into a cationic form, i.e. hydrochloride salt. The effect of increasing solubility is more pronounced for guanidinoacetic acid and decreases with temperature for both compounds. A simple process of transforming electrically neutral zwitterionic structures into cations represents a good way to increase the solubility in water and bioavailability of biologically active compounds.

Keywords: solubility; creatine hydrochloride; guanidinoacetate hydrochloride; creatinine.

INTRODUCTION

Creatine (CR) is an endogenous amino acid that the human body naturally produces as a source of muscle energy. It promotes the renewal of ATP used during muscle work, and with its supplementation, "extended work" is achieved, i.e. greater strength and better endurance.[1] There are various forms of creatine, but the most studied and commonly used form is creatine monohydrate. Due to its disadvantages, such as low solubility in water, spontaneous cyclization into creatinine, and the possibility of entering the cell only through specific transporters,[2] it is necessary to find new forms that will try to overcome these drawbacks.

Guanidinoacetic acid (GAA) is a natural metabolic precursor of creatine. GAA is biologically converted into creatine in the liver through a methylation

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https://doi.org/10.2298/JSC230610041V

reaction. Many recent scientific papers indicate that the combination of creatine and GAA is more effective in increasing the amount of creatine in serum, muscle tissue and brain compared to pure creatine.[3-5] However, both creatine and GAA are relatively poorly soluble in water, so their conversion to more soluble hydrochloride salts is desirable. Several formulations on the sports supplement market, described as advanced, contain creatine hydrochloride salts. However, the solubility of creatine, as well as creatine and GAA hydrochloride salts, has not been systematically investigated. In contrast, the solubility of GAA in water was determined in our previous work.[6]

Therefore, this work aims first to convert creatine and GAA into hydrochloride salts and then determine the solubility of creatine, creatinine, creatine hydrochloride and GAA hydrochloride in water at atmospheric pressure and in the temperature range T = (293.15-313.15) K. From the solubility results, the thermodynamics of the dissolution of the studied compounds will be analysed.

EXPERIMENTAL

Materials, apparatus and procedure

Commercial creatine monohydrate (Scitech Nutrition), anhydrous creatine (Sigma-Aldrich, purity ≥ 0.99), anhydrous creatinine (Sigma-Aldrich, purity ≥ 0.98), guanidinoacetic acid (Sigma-Aldrich, purity ≥ 0.99), 37% water solution of hydrochloric acid (Sigma Aldrich) and ultrapure water were used in the experimental work.

To synthesize the hydrochloride salts of creatine and guanidinoacetic acid (Figure 1), the appropriate mass of CR or GAA was measured and dissolved in distilled water. Then, an equimolar amount of standardized hydrochloric acid was added. The solutions were mixed at room temperature for 30 min. Water was removed using a rotary vacuum evaporator at T = 353.15 K. Both salts, creatine hydrochloride (CR-HCl) and guanidinoacetate hydrochloride (GAA-HCl) remained as a white powder at room temperature. The synthesized salts are stored in a desiccator over P_2O_5 before use. The structures were confirmed by IR spectroscopy (Figure S1). Chloride content was also checked by potentiometric titration with silver nitrate solution and purity of both salts were higher than 99%. Thermogravimetric (TG) and differential scanning calorimetric (DSC) curves of creatine hydrochloride and GAA hydrochloride are given in the Supplementary Material as Figure S2.

Infrared spectra

Infrared spectra were recorded as neat samples from (4000 to 650) cm^{-1} on a Thermo Nicolet Nexus 670 spectrometer fitted with a Universal ATR Sampling Accessory. The measurements were performed with a total of 60 scans, at T = 298.15 K, and a spectral resolution of 2 cm^{-1} in a range of wave number from (4000 to 650) cm^{-1}. The software package Omnic version 6.2 was used in the data acquisition and spectral analysis.

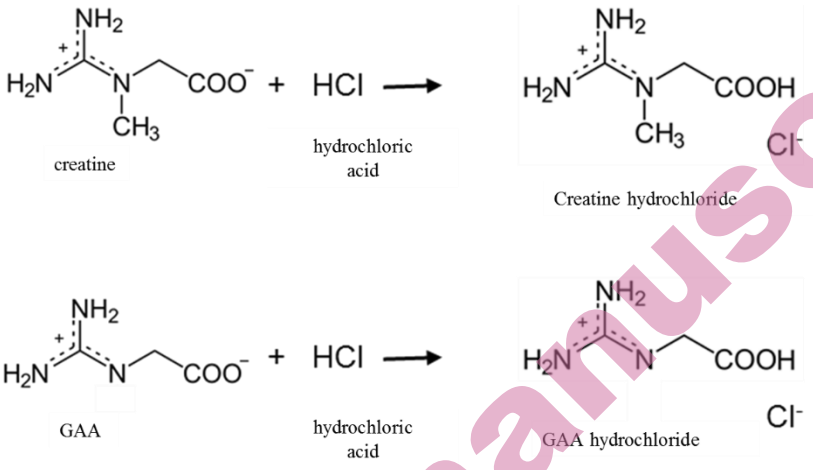

Figure 1. Synthesis scheme of creatine hydrochloride and GAA hydrochloride.

Solubility measurements

The solubility determination was performed at five temperatures T = (293.15, 298.15, 303.15, 308.15, 313.15) K, using the gravimetric method already described in work by *Romero & Oviedo*.[7] An excess amount of investigated compound was added to the double-layer glass flask containing 40 cm^3 of ultrapure water, and a small magnetic stirrer bar was capped tightly. Then, the suspensions were stirred for 12 h to saturation with temperature control of ±0.01 K (Lauda E-100 circulator) at selected temperatures. After, the samples stirrer was turned off to allow the undissolved solute to precipitate for the next 12 h at each temperature. Then, the solution was tested on creatinine content using Jaffe's method,[8] and it was determined the amount of formed creatinine after 12 hours of mixing was negligible.

Finally, after separating the liquid layer and excess solute, the upper phase (solute-saturated aqueous solution) was carefully taken using a 3 cm^3 syringe and a needle. To prevent undissolved solute from causing inaccurate results, taken solution was filtered off in 10 cm^3 pre-weighed glass flasks through a 0.45 μm pore size mixed cellulose esters membrane. The mass was measured with an accuracy of $\pm 1 \cdot 10^{-5}$ g in the lower range. The samples were placed in the dryer at 423.15 K, evaporated to dryness, and then in a vacuum desiccator to recover the solid compound. The mass of the solute was determined gravimetrically. Each obtained value represents the average of at least five independent measurements. The uncertainty in the mass fraction solubility is ±0.001. Reproducibility was found to be better than ±0.002.

Thermal analysis

A TA Instruments SDT Q600 TG/DSC thermal analyzer was used to test the thermal stability of creatine, CR-HCl and GAA-HCl. This device was used to register the change in mass of the sample in the temperature interval from room temperature to 773 K. In addition to the change in mass of the sample as a function of temperature, the change in mass as a function of time can also be monitored on the mentioned device. Sample (≈ 3.0 mg) was placed in an open platinum pan. The measurements were carried out in an argon atmosphere (flow rate 50 $cm^3 \cdot min^{-1}$) up to 723 K with a heating rate of 20 $K \cdot min^{-1}$.

RESULTS AND DISCUSSION

Thermal analysis and solubility results

The gravimetric procedure for determining the solubility of compounds involves drying the sample to a constant mass. From aqueous solutions, creatine crystallizes in the form of creatine monohydrate. Thermogravimetric measurements (Figure 2a) indicate that creatine monohydrate begins to lose a water molecule at 341 K and that complete conversion to anhydrous creatine ends at 398 K. The next mass loss starts at 503 K. At that temperature, the creatine molecule converts into creatinine, releasing one water molecule. Thermal decomposition of creatinine molecules occurs in the temperature range of 553 - 593 K. The thermal analysis results indicate that after evaporating the water, the obtained creatine monohydrate needs to be dried in the temperature range of 398 - 503 K, because, in this way, the presence of creatine monohydrate and creatinine is avoided. The drying temperature in this research was between 423 - 433 K. The obtained onset temperatures of the beginning of decomposition (T_{onset}) and endothermic peak of samples phase transition are shown on the thermogravimetric and DSC curves in Figure 2b.

The obtained data for solubility in water of creatine, creatinine, creatine hydrochloride and GAA hydrochloride determined by the gravimetric method are tabulated in Table 1, together with solubility values of GAA from our previous work.[6]

Literature data on the water solubility of creatine at different temperatures were found. The data showed that at 293.15 K, 1.4 g of creatine dissolves in 100 ml of water,[2,9] while at 298.15 K, 1.7 g of creatine dissolves in 100 ml of water.[9] Our experimental findings also agree with these literature water solubility data.

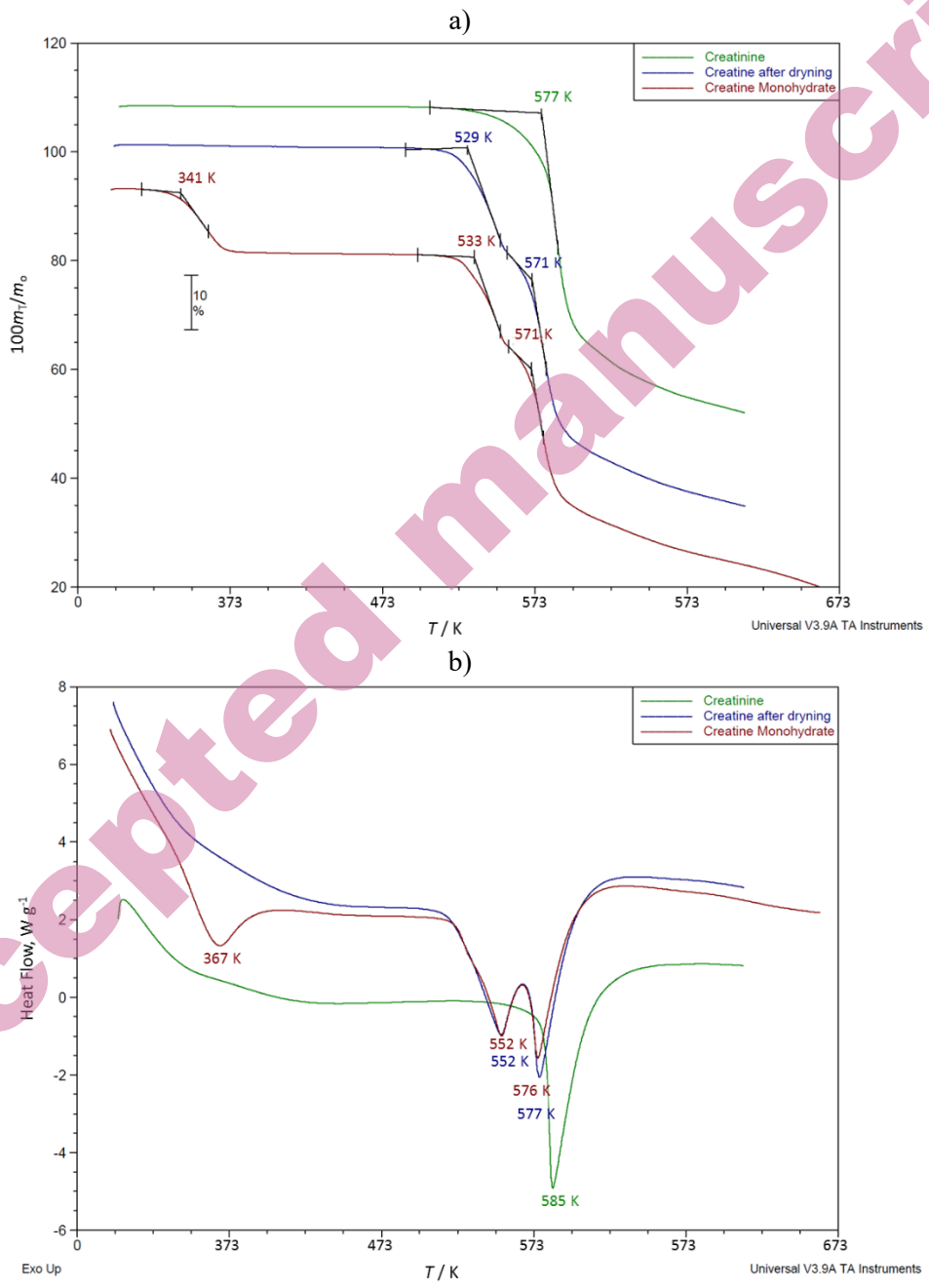

Figure 2. a) Thermogravimetric and b) DSC curves of creatine monohydrate, creatine after drying and creatinine.

Table 1. Water solubility values of creatine, creatinine, GAA,[6] creatine hydrochloride and GAA hydrochloride at atmospheric pressure and in the temperature range T = (293.15 - 313.15) K.

T / K	R / g/100g water				
	Creatine	Creatinine	GAA[6]	CR-HCl	GAA-HCl
293.15	1.3172	9.5831	0.357	112.64	36.036
298.15	1.6288	10.419	0.422	118.36	41.646
303.15	1.8787	11.931	0.499	130.09	47.646
308.15	2.2727	13.325	0.583	138.96	53.169
313.15	2.6358	14.712	0.678	146.82	60.138

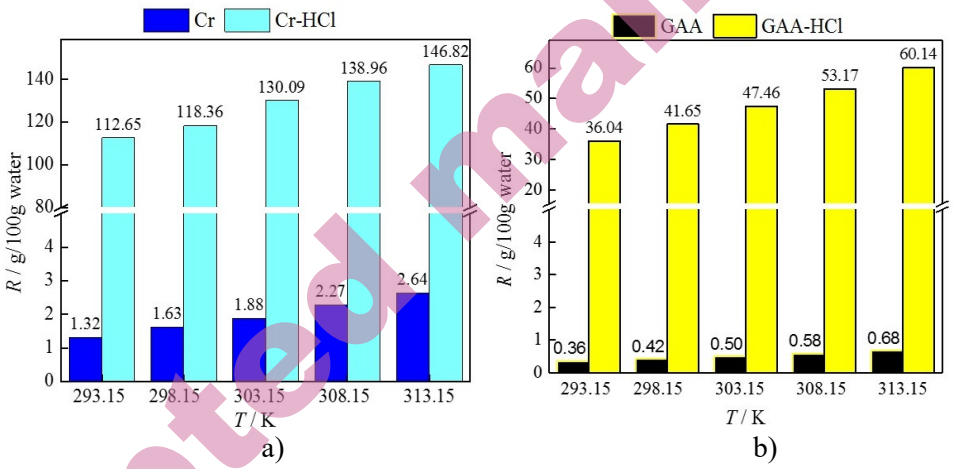

Figure 3. Comparison of water solubility of a) creatine with creatine hydrochloride and b) GAA with GAA hydrochloride.

Based on the experimentally obtained water solubility values, it can be observed that solubility values decrease in order: creatine hydrochloride > GAA hydrochloride > creatinine > creatine > GAA. Additionally, the solubility of each compound increases linearly with increasing temperature. From Table 1 it can be observed that the solubility of creatinine is about six-fold higher than the solubility of creatine in water, while creatine is about four-fold more soluble than GAA in water. Also, it can be concluded that the solubility of creatine hydrochloride in water is almost three-fold higher than the solubility of GAA hydrochloride.

In Figure 3, a comparison is made between the solubility values of creatine and GAA and their hydrochloride salts. The diagrams demonstrate that the conversion from zwitterionic to cationic form of both molecules considerably enhances their water solubility. Table 2 shows the ratio of increase in solubility of creatine and GAA after translation into hydrochloride salts. The effect of increasing solubility after the conversion of the molecule from the zwitterionic to

the cationic form is more pronounced in the case of the GAA molecule. For both molecules, the solubility ratio of hydrochloride salts and zwitterionic forms in water decreases with increasing temperature, with the decrease being less pronounced in the case of the GAA molecule.

Table 2. The ratio of increase in water solubility values of creatine, GAA and and their respective hydrochloride salts in the temperature range T = (293.15 - 313.15) K.

T / K	CR-HCl / CR	GAA-HCl / GAA
293.15	84.5	99.9
298.15	71.7	97.7
303.15	68.2	94.5
308.15	60.1	90.2
313.15	54.7	87.7

Thermodynamic model for solubility correlation

When studying the dissolution of compounds in water, thermodynamic functions related to solution and solvation processes can offer valuable information. These functions, the apparent standard dissolution enthalpy ($\Delta_{sol}H^o$), entropy ($\Delta_{sol}S^o$) and Gibbs energy ($\Delta_{sol}G^o$) change of solutes, can be calculated by analyzing the temperature-dependent experimental solubility data. The modified *van't Hoff* equation is a commonly used thermodynamic model to determine these values.[10,11] This equation relates the temperature and solubility in solution, and $\Delta_{sol}H^o$ can be obtained using the following equation:

$$\left(\frac{\partial \ln x_1}{\partial(1/T-1/T_{hm})}\right)_p = -\frac{\Delta_{sol}H^o}{R} \quad (1)$$

Here, x_1 is the mole fraction of solute in a saturated solution, T is the absolute temperature in Kelvin, and R is the universal gas constant with a value of 8.314 $J{\cdot}K^{-1}$ mol^{-1}. T_{hm}, the mean harmonic temperature, is calculated using the formula:

$$T_{hm} = \frac{n}{\sum_{j=1}^{n}\frac{1}{T_j}} \quad (2)$$

where n equals the number of temperature points, T_j is the experimental temperature, and the resulting value is T_{hm} = 302.99 K. $\Delta_{sol}H^o$ represents the apparent standard mole dissolution enthalpy change of solute dissolved in water, obtained from the slope of the fitted line curves $\ln x_1$ versus (1/T-1/T_{hm}) (Figure 4).

Equation (3) can be used with the intercept from the modified *van't Hoff* plot to calculate $\Delta_{sol}G^o$. Equation (4) can then be used to obtain $\Delta_{sol}S^o$.

$$\Delta_{sol}G^o = -RT_{hm}Intercept \quad (3)$$

$$\Delta_{sol}S^o = \frac{\Delta_{sol}H^o - \Delta_{sol}G^o}{T_{hm}} \quad (4)$$

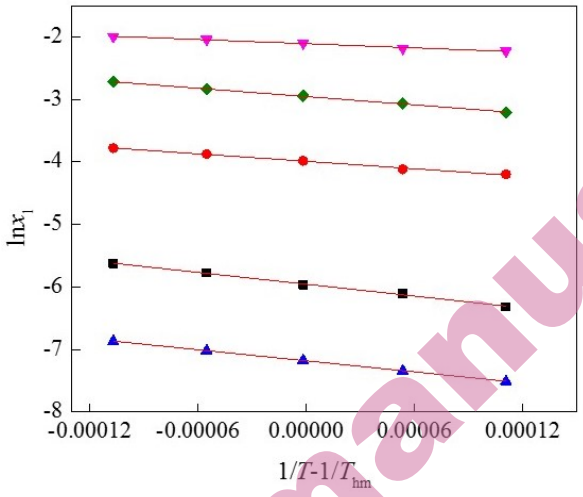

Figure 4. The plot of natural logarithm of the mole fraction of (■) creatine, (●) creatinine, (▲) GAA, (▼) creatine hydrochloride, (◆) GAA hydrochloride in a saturated aqueous solution, $\ln x_1$, against $1/T\text{-}1/T_{hm}$.

To determine the main influence and contribution to $\Delta_{sol}G^o$ in the dissolving process, relative contribution of enthalpy *(ζ_H),* and relative contribution of entropy (ζ_{TS}) were calculated using the following expressions: [10,11]

$$\varsigma_H = \frac{|\Delta_{sol}H^o|}{|\Delta_{sol}H^o|+|T_{hm}\Delta_{sol}S^o|} \quad (5)$$

$$\varsigma_{TS} = \frac{|T_{hm}\Delta_{sol}S^o|}{|\Delta_{sol}H^o|+|T_{hm}\Delta_{sol}S^o|} \quad (6)$$

Table 3 provides the obtained values for the apparent thermodynamic properties of $\triangle_{sol}G°$, $\triangle_{sol}H°$, and $\triangle_{sol}S°$, as well as of ζ_H, ζ_{TS} for creatine, creatinine, GAA, creatine hydrochloride and GAA hydrochloride dissolution in water.

Based on the data in Table 3, it is observed that all $\triangle_{sol}G°$ values for dissolution in water fall within the positive range of (18-75) kJ mol^{-1}. When considering molecular creatine, creatinine, and GAA separately from creatine hydrochloride and GAA hydrochloride as ionic compounds, it is evident that the values of $\triangle_{sol}G°$ for compound dissolution are in reverse order of solubility. The findings in Table 3 indicate that the dissolution process is endothermic in each case due to the positive values of $\triangle_{sol}G°$ and $\triangle_{sol}H°$. This can be attributed to the fact that the new solute-water interactions are weaker than the sum of the solute-solute and water-water interactions they replace. The higher value of $\triangle_{sol}H°$ for creatine and GAA compared to creatinine may be because more energy is required to break the ionic bond between creatine/GAA in the zwitterionic form than to dissolve neutral creatinine. Additionally, the higher value of $\triangle_{sol}H°$ for GAA hydrochloride than for creatine salt may be due to more energy being required to break the ionic

bond of GAA hydrochloride. Positive values for $\triangle_{sol}S°$ indicate that during dissolution, there is an increase in the disorder of the system. Based on the calculated values of the relative contribution of enthalpy and entropy, it can be concluded that during dissolution, enthalpy affects the values of $\triangle_{sol}G°$ the most in all five solutions. Also, it can be seen that converting creatine from zwitterionic to cationic form does not affect the relative contribution of enthalpy and entropy values. However, GAA conversion into salt alters the ratio between enthalpy and entropy contribution.

Table 3. The obtained apparent thermodynamics properties values of $\triangle_{sol}G°$, $\triangle_{sol}H°$, and $\triangle_{sol}S°$, ζ_H, ζ_{TS} for creatine, creatinine, GAA, creatine hydrochloride and GAA hydrochloride dissolution in water.

Parameters	Creatine	Creatinine	GAA	CR-HCl	GAA-HCl
$\Delta_{sol}H°$ / kJ mol^{-1}	26.21	16.52	24.50	92.60	183.63
$\Delta_{sol}G°$ / kJ mol^{-1}	15.00	10.05	18.09	53.09	74.41
$\Delta_{sol}S°$ / kJ K^{-1} mol^{-1}	0.037	0.021	0.021	0.13	0.36
ζ_H / %	70	72	79	70	63
ζ_{TS} / %	30	28	21	30	37

The reason for the increased solubility of hydrochloride salts in relation to zwitterionic structures is a change in the type of interactions that occur between molecules.[9] Strong electrostatic interactions and intermolecular hydrogen bonds occur between CR and GAA molecules when they are in the zwitterionic form. Therefore, their solubility in water is low. By converting GAA and CR molecules into cationic forms, attractive electrostatic interactions are replaced by repulsive ones. On the other hand, the interactions between GAA and CR as cations and Cl ions as anions are significantly weaker. Another reason for increasing the solubility of GAA-HCl and CR-HCl in water is the change in the conformation of the molecules themselves. Our previous research[12] showed that in GAA and CR molecules in zwitterionic form, intramolecular hydrogen bonds occur between the oxygen atom from the carboxylate anion and the hydrogen atom from the guanidino group. These intramolecular H-bonds make molecules rigid and less accessible for interactions with water molecules. The possibility of intramolecular H-bond formation in CR-HCl and GAA-HCl molecules disappears by protonating the carboxylate anion. Therefore, GAA-HCl and CR-HCl molecules have a greater possibility of free rotations around single bonds after dissolving in water, which increases the entropy of the system and contributes to better solubility.

CONCLUSION

In summary, hydrochloride salts of creatine and guanidinoacetic acid – creatine hydrochloride and guanidinoacetonium hydrochloride – were successfully synthesized. Further, this study reveals distinct solubility behaviors among

creatine, creatinine, creatine hydrochloride, and GAA hydrochloride in water. Notably, solubility follows a descending order from creatine hydrochloride to GAA, with temperature positively impacting the solubility of each compound. Remarkably, the conversion to hydrochloride salts significantly enhances water solubility, especially for GAA, indicating a pronounced effect of shifting from zwitterionic to cationic form. Additionally, the thermodynamic functions of solution and solvation were calculated using a modified van't Hoff equation. These findings provide a fundamental, high-accuracy set of results, filling an essential gap in the literature but also highlighting differential solubility characteristics and the impact of structural changes, which hold substantial implications for pharmaceutical and biomedical applications where precise solubility control is crucial.

SUPPLEMENTARY MATERIAL

Supplementary Materials are available electronically from https://www.shd-pub.org.rs/index.php/JSCS/article/view/12431, or from the corresponding authors on request.

Acknowledgements: The authors are grateful for the financial support of Provincial Secretariat for Higher Education and Scientific Research, grant number: 142-451-2545/2021-01.

И З В О Д

УТИЦАЈ КОНВЕРЗИЈЕ КРЕАТИНА И ГВАНИДИНОСИРЋЕТНЕ КИСЕЛИНЕ ИЗ ЦВИТЕРЈОНСКОГ У КАТЈОНСКИ ОБЛИК НА ЊИХОВУ РАСТВОРЉИВОСТ У ВОДИ – ТЕРМОДИНАМИЧКА СТУДИЈА

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У овом раду је одређена и упоређена растворљивост креатина, креатинина, гванидиносирћетне киселине и њихових хидрохлорида у води на атмосферском притиску и у температурном опсегу T = (293,15 - 313,15) K гравиметријском методом. Израчунати су термодинамички параметри растварања у води за наведена једињења. Добијени резултати указују да се растворљивост се значајно повећава претварањем цвитерјонских структура креатина и гванидиносирћетне киселине у катјонски облик, тј. хидрохлоридну со. Ефекат повећања растворљивости је израженији за гванидиносирћетну киселину и опада са температуром за оба једињења. Једноставан процес конверзије електро неутралних цвитерјонских структура у катјоне представља добар начин да се повећа растворљивост у води и биодоступност биолошки активних једињења.

(Примљено 10. јуна 2023; ревидирано 19. јула 2023; прихваћено 4. априла 2024.)

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