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Experimental and computational study of guanidinoacetic acid self-aggregation in aqueous solution

Milan Vraneš [a,*], Sergej Ostojić [b], Aleksandar Tot [a], Snežana Papović [a], Slobodan Gadžurić [a]

[a] Faculty of Science, Department of Chemistry, Biochemistry and Environmental Protection, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia
[b] Faculty of Sport and Physical Education, University of Novi Sad, Lovćenska 16, 21000 Novi Sad, Serbia

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ABSTRACT

In this work for the first time the physicochemical and thermal properties of guanidinoacetic acid (GAA) and its aqueous solutions have been performed to test for its viability as a potential dietary supplement. Thermal stability, viscosity, solubility and experimental density are determined. From measured densities the volumetric properties were estimated and discussed in the scope of GAA self-aggregation in aqueous solutions using experimental and computational results. Based on thermal stability and solubility measurements, it is found that GAA is more thermally stable but less soluble comparing to creatine due to a self-aggregation process that occurs at GAA concentrations higher than 0.013 mol · dm^{-3}. Existence of self-aggregation influences the macroscopic properties of aqueous GAA solutions, but also its bioavailability.

1. Introduction

Guanidinoacetic acid (GAA, also known as glycocyamine or guanidinoacetate) is an amino acid derivative and a natural precursor of creatine, a key molecule in cellular bioenergetics. GAA has been recently recognized by EUR-Lex (Official Journal of the European Union, L270/4, 5.10.2016) and the Food and Drug Administration (Federal Register, 81, 30.11.2016.) as a feed substance for animals. As an experimental food additive in human nutrition, supplemental GAA enhances cellular bioenergetics in energy demanding tissues, such as the brain and skeletal muscle (Ostojić, Ostojić, Drid, Vraneš, & Jovanov, 2017; Ostojić, 2017; Ostojić, Drid, & Ostojić, 2016; Ostojić, Ostojić, Drid, & Vraneš, 2016). In addition, oral GAA seems to be superior to creatine in facilitating creatine levels in healthy men (Ostojić et al., 2016), with GAA being considered as a preferred alternative to creatine. However, while food chemistry of creatine has been comprehensively detailed (Dash, Mo, & Pyne, 2002; Ganguly, Jayappa, & Dash, 2003), GAA remains poorly described concerning its fundamental chemistry as a nutritional additive.

No information is currently available concerning GAA water solubility, thermal stability and viscosity, nor its chemical characterization. In this study, fundamental physicochemical properties of GAA will be described, which is highly important information in establishing GAA as a nutritional additive.

GAA crystallizes from aqueous solution as transparent monoclinic prisms (Guha, 1973). By using X-ray crystallography it was determined that molecules of GAA exist in the zwitterionic form (Fig. 1), where the proton from the carboxyl group is transferred to the guanidino group. These two groups interact electrostatically through strong Coulomb interactions, but formation of hydrogen bonds also takes place (Guha, 1973). Those interactions between are the reason for its low solubility in polar solvents, such as water. Water solutions of GAA are practically pH-neutral, while the acidic constants are: pK_1 = 2.90 and pK_2 = 10.91 (Felcman & de Miranda, 1994). The first dissociation constant is very close to the value of creatine (p$K_{1(cre)}$ = 2.79, Eadie & Hunter, 1926), but the second dissociation constant is higher than the constant obtained for creatine (p$K_{2(cre)}$ = 12.1) which is a consequence of the positive inductive effect of methyl group in the creatine molecule thus increasing the electron density on the guanidino group. The isoelectric point (pI) of GAA is the mean value of these two constants and its value is pI = 6.91.

Depending on pH of the solution, GAA may occur in three different forms. When pH is lower than 2.90 it occurs primarily in the cationic form (Fig. S1), while at pH values above 10.91 it has the anionic form. The zwitterionic form is present at all other pH values (including pH of biological fluids). Solvent pH and subse-

* Corresponding author.
E-mail addresses: milan.vranes@dh.uns.ac.rs (M. Vraneš), sergej.ostojic@chess.edu.rs (S. Ostojić), aleksandar.tot@dh.uns.ac.rs (A. Tot), snezana.papovic@dh.uns.ac.rs (S. Papović), slobodan.gadzuric@dh.uns.ac.rs (S. Gadžurić).

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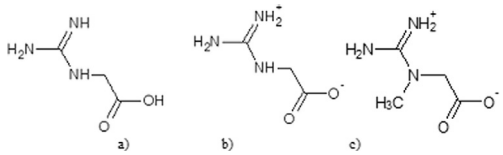

Fig. 1. a) Neutral form, b) zwitterionic form of GAA and c) zwitterionic form of creatine molecule.

quently the form in which GAA is found in the solution are the main determinants of its physicochemical and biological properties.

2. Materials and methods

2.1. Materials

GAA was purchased from G-Power 3, Heinrich Heine University, Düsseldorf, Germany, with final mass fraction $\omega \geq 0.99$. Ultra pure water was obtained using Arium® pro B Ultrapure Water System equipment.

2.2. Solubility determination

The determination of solubility using the gravimetric method has been already described (Romero & Oviedo, 2013). For this purpose a double layer glass flask with a volume of 60 cm^3 was used. Small magnetic stirrers were added to each flask with stirring speed of 60 rpm. Saturated solutions of GAA were prepared by adding 0.5g of GAA to 40 cm^3 of ultrapure water and stirring the flasks containing the samples with temperature control of ±0.01 K.

Samples of 5 cm^3 were withdrawn at 2, 4, 6, 8 and 12 h by means of a syringe filter (33 mm diameter sterile Millex-HA syringe filter with a 0.45 μm pore size mixed cellulose esters membrane) fitted with a long needle. Before transferring the samples, the syringe was immersed in the flask and the stirrer was turned off for 2 h to allow the undissolved solute to precipitate. Samples were transferred to 10 cm^3 pre-weighed glass flasks and their mass was measured with accuracy of $\pm 1 \cdot 10^{-5}$ g in the lower range. The samples were placed in a vacuum oven at 343.15 K and evaporated to dryness to recover the solid compound. The mass of the solute was determined gravimetrically.

Solubility measurements were made at temperatures between 293.15 and 318.15 K. Each obtained value represents the average of at least six independent measurements. The relative standard uncertainty in the mass fraction solubility is ±0.015%. Reproducibility was found to be better than ±0.002.

2.3. Thermal analysis

The thermal stability of GAA is verified by thermogravimetric analysis (TG) and differential scanning calorimetry (DSC). The TG and DSC measurements were performed using simultaneous TG/DSC thermal analyser SDT Q600 (TA Instruments, USA). Sample (≈2.5 mg) was placed in an open platinum pan. Measurements were carried out in air atmosphere (flow rate: 100 $cm^3 \cdot min^{-1}$) to 500 °C (773.15 K) with a heating rate of 10 °C $\cdot min^{-1}$.

2.4. Densimetry

The densities of aqueous solutions of GAA were measured at atmospheric pressure (0.1 MPa) using a vibrating tube Anton Paar DMA 5000 densimeter with a declared reproducibility of $1 \cdot 10^{-6}$ - $g \cdot cm^{-3}$. Before each series of measurements calibration of the instrument was performed at the atmospheric pressure using triple distilled ultra pure water in the temperature range from 293.15 to 313.15 K. The instrument was thermostated within ±0.001 K and viscosity related errors in the density were automatically corrected over full viscosity range. To avoid gas bubbles entrapped in the measuring cell filled with a sample, the cell was filled carefully to minimize the probability of such error. The total volume of the sample used for density measurements was approximately 1 cm^3. The densimeter already has incorporated moisture adsorbent. Standard uncertainty of determining the density is less than $6.4 \cdot 10^{-4}$ $g \cdot cm^{-3}$.

2.5. Viscosity

The viscosity of the GAA + H_2O binary mixtures was measured using Ubbelohde viscosimeter (SI Analytics GmbH, Mainz, Germany, type No. 525 03) by measuring the flow rate of the liquid. The viscosimeter was filled with experimental liquid and placed vertically in glass sided thermostat maintained constant to ±0.01 K, with standard uncertainty of controlled temperature of ±0.02 K. After thermal equilibrium is attained, the flow time of liquids was recorded with a digital stopwatch with an accuracy of ±0.001 s. Presented results were obtained as the mean value of at least ten viscosity measurements. The kinematic viscosity of solutions, v ($m^2 \cdot s^{-1}$), was calculated from the equation $v = K \cdot t$, where t (in seconds) is the flow time and K is the constant characteristic for the viscometer. The absolute (dynamic) viscosity, η (Pa·s = $kg \cdot m^{-1} \cdot s^{-1}$), was obtained from the relation $\eta = v \cdot d$, where d (in $kg \cdot m^{-3}$) is density of the investigated solution. Viscosity of the studied binary mixtures was measured in the molality range up to 0.0302 $mol \cdot kg^{-1}$ of GAA. Relative standard uncertainty of determining the viscosity with Ubbelohde viscosimeter was found to be less than 1%.

2.6. Computational details

For theoretical investigation of GAA self-aggregation, molecular dynamic (MD) simulations were employed using Yasara structure version 10.2.1 (Krieger, Koraimann, & Vriend, 2002). The Amber14 force field was used for all simulations within NPT ensemble class and long range cutoff was set to 10 Å. The size of the box cell was set to 100 Å, temperature to 303.15 K at atmospheric pressure, while pH was set to 7.00. Simulations were performed for three different GAA concentrations, with 4, 8 and 16 molecules of GAA, which refer to concentration of 0.005, 0.015 and 0.030 $mol \cdot kg^{-1}$. Overall simulation time for aqueous solutions of GAA was 30 ns.

3. Results and discussion

3.1. Solubility and thermal stability

Experimental data in this work for the solubility of GAA in water are compared with literature data for solubility of creatine in water (Vraneš & Papović, 2015) in Table 1. It can be noted that GAA has four times less solubility at 298.15 K. The solubility value depends on solvation ability of solvent and/or strength of intramolecular interactions in solute molecule.

In order to determine the thermal stability of GAA the thermogravimetric analysis was performed. The results of thermal stability are presented in Fig. S2. As can be seen from Fig. S2 thermal decomposition (T_{onset}) of GAA molecule starts at 281 °C (554.15 K). Good thermal stability for potential food additives is very important, since it indicates steadiness of the food additive even after suitable thermal processing.

Comparing to creatine thermal stability (T = 230 °C), guanidinoacetic acid is more stable (Dash et al., 2002). This leads to the