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MIXTURE DESIGN AND PHYSICOCHEMICAL CHARACTERIZATION OF AMINO ACID-BASED DEEP EUTECTIC SOLVENTS (AADES) FOR SAMPLE PREPARATION PRIOR TO ELEMENTAL ANALYSIS

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Abstract:	Amino acid-based deep eutectic solvents (AADES) represent a new subclass of deep eutectic solvents (DES) in which at least one of the components must be an amino acid, offering advantages such as low toxicity, biodegradability and low cost. In this work, β-alanine was used as hydrogen bond acceptor (HBA) in the preparation of a total of 30 AADES mixtures, with the hydrogen bond donor (HBD) being malic acid (AADES 1), citric acid (AADES 2), or xylitol (AADES 3), together with the addition of water. A restricted mixture design was employed to optimize the ideal proportions of the AADES components, which were determined as (% m m-1) 12.50 for β-alanine, 43.75 for the HBD component, and 43.75 for water, with lower values of density and viscosity being the desired responses. Solvents that have low density and viscosity provide greater efficiency in sample preparation procedures, due to faster mass transfer. The highest density and viscosity values were found for AADES 2, due to the greater presence of carboxyl groups in the molecular structure of citric acid, allowing the formation of more hydrogen interactions. The Herschel-Bulkley model provided the best fit to the rheological behavior of the AADES, with AADES 2 showing the highest consistency index. Solvatochromic analyses showed that these solvents had high polarity. Fourier transform infrared (FTIR) spectroscopy analysis revealed hydrogen interactions between the precursor components, confirming formation of the AADES. Thermal analysis revealed the ideal working temperature ranges for applying these solvents in sample preparation, with thermogravimetry (TGA) indicating maximum temperatures of 130 °C for AADES 1 and 150 °C for AADES 2 and AADES 3. Differential scanning calorimetry (DSC) revealed the minimum temperatures at which the solvents remained liquids, which were -13 °C for AADES 1, -22 °C for AADES 2, and -21 °C for AADES 3. Therefore, these AADES were shown to be promising solvents for application in sample preparation, being suitable for the extraction of polar compounds, as well as metals and semimetals. An EcoScale study was carried out, which confirmed that the preparation of the solvents could be considered an excellent green synthesis.
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São José do Rio Preto, 03th, June, 2021.

Editor in Chief
W. Schröer - University of Bremen Faculty 2 Biology Chemistry, Leobener Str. NW2, 28359, Bremen, Germany

Dear Professor W. Schröer,

We are submitting the manuscript entitled "*Mixture design and physicochemical characterization of amino acid-based deep eutectic solvents (AADES) for sample preparation prior to elemental analysis"* for your evaluation and possible publication in **Journal of Molecular Liquids**. The mixture design was applied for the synthesis of β-alanine used as hydrogen bond acceptor (HBA) for the preparation of a total of 30 AADES mixtures, employing as hydrogen bond donor (HBD) malic acid (AADES 1), citric acid (AADES 2), or xylitol (AADES 3), together with the addition of water. The best AADES proportion was selected based on density and viscosity values. The physicochemical properties of the solvents were also evaluated by rheological and solvatochromic analysis, Fourier transform infrared (FTIR), and thermal techniques. The AADES showed to be a very promising solvent for the extraction of inorganic elements in several types of samples, being remarkably compatible with the plasma-analytical techniques even improving its analytical performance. An *EcoScale* study was carried out, which confirmed that the preparation of the solvents could be considered an excellent green synthesis.

Thank you in advance for your attention, time and editorial assistance.

We are looking forward to hearing from you at your earliest convenience.

Yours sincerely,

Mario Henrique Gonzalez

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Highlights

- The best proportion of AADES components based on of β-alanine as HBA was obtained using a mixture design.
- The variation of the HBD component directly influences the physicochemical properties.
- AADES with high polarities were obtained, promising for the extraction of inorganic analytes.

MIXTURE DESIGN AND PHYSICOCHEMICAL CHARACTERIZATION OF AMINO ACID-BASED DEEP EUTECTIC SOLVENTS (AADES) FOR SAMPLE PREPARATION PRIOR TO ELEMENTAL ANALYSIS

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Abstract

Amino acid-based deep eutectic solvents (AADES) represent a new subclass of deep eutectic solvents (DES) in which at least one of the components must be an amino acid, offering advantages such as low toxicity, biodegradability and low cost. In this work, β-alanine was used as hydrogen bond acceptor (HBA) in the preparation of a total of 30 AADES mixtures, with the hydrogen bond donor (HBD) being malic acid (AADES 1), citric acid (AADES 2), or xylitol (AADES 3), together with the addition of water. A restricted mixture design was employed to optimize the ideal proportions of the AADES components, which were determined as (% m m^{-1}) 12.50 for β-alanine, 43.75 for the HBD component, and 43.75 for water, with lower values of density and viscosity being the desired responses. Solvents that have low density and viscosity provide greater efficiency in sample preparation procedures, due to faster mass transfer. The highest density and viscosity values were found for AADES 2, due to the greater presence of carboxyl groups in the molecular structure of citric acid, allowing the formation of more hydrogen interactions. The Herschel-Bulkley model provided the best fit to the rheological behavior of the AADES, with AADES 2 showing the highest consistency index. Solvatochromic analyses showed that these solvents had high polarity. Fourier transform infrared (FTIR) spectroscopy analysis revealed hydrogen interactions between the precursor components, confirming formation of the AADES. Thermal analysis revealed the ideal working temperature ranges for applying these solvents in sample preparation, with thermogravimetry (TGA) indicating maximum temperatures of 130 °C for AADES 1 and 150 °C for AADES 2 and AADES 3. Differential scanning calorimetry (DSC) revealed the minimum temperatures at which the solvents remained liquids, which were -13 °C for AADES 1, -22 °C for AADES 2, and -21 °C for AADES 3. Therefore, these AADES were shown to be promising solvents for application in sample preparation, being suitable for the extraction of polar compounds, as well as metals and semimetals. An *EcoScale* study was carried out, which confirmed that the preparation of the solvents could be considered an excellent green synthesis.

Keywords: Mixture design, Sample treatment, Physicochemical properties, ICP-MS, ICP-OES.

1. Introduction

In the last decade, the incorporation of the principles of sustainability in Chemistry, aiming at the reduction of environmental impacts, less risk to humans, and savings of costs and energy, with the implementation of simple and fast synthesis methods, has stimulated research into new sustainable solvents [1]. Scientific interest in the development of sustainable solvents is driven by the still unavoidable use of solvents in chemical analysis, notably in sample preparation, so this has become one of the main lines of research in green analytical chemistry [2].

Ionic liquids (ILs) are non-molecular solvents formed by the combination of an organic cation and an organic or inorganic anion, whose main characteristic is a melting point below 100 °C [3]. They are non-volatile and non-flammable and have been considered sustainable solvents that are ideal candidates to replace potentially toxic traditional solvents [3]. However, the current literature reports that this designation may be erroneous, because in some cases, many steps are necessary to synthesize ILs, using high- cost precursors, in addition to presenting degradation products with high toxicity, low biodegradability, and long lifetimes in the environment [4].

Deep eutectic solvents (DES) are a promising class of sustainable solvents that offer an alternative to ILs [5]. When the DES precursors are naturally occurring substances such as primary metabolites and/or cellular components including amino acids, organic acids, sugars, and choline derivatives, natural deep eutectic solvents (NADES) are formed [6-8]. More recently, NADES based on amino acids received a new classification and a more restricted name, becoming known as amino acids-based deep eutectic solvents (AADES), where at least one of the components of the formulation must be an amino acid [9].

The AADES, as well as DES and NADES, are formed by different interactions (electrostatic, dipole-dipole, van der Waals, and especially hydrogen bond interactions) between an HBA component (hydrogen bond acceptor) and an HBD component (hydrogen bond donor), resulting in a mixture that has a lower melting point than those of its individual constituents [6,10]. The advantages of the DES in general are mainly related to their low toxicity, biodegradability, low cost, high water solubility, low vapor pressure, and high degree of solubilization of various compounds, in addition to their ease of preparation, without the need to use other solvents and without formation of byproducts [11,12].

Variations in the physicochemical properties and stabilities of these solvents are directly dependent on variations in the compositions and molar ratios, producing solvents with different properties, resulting from the displacement of charges caused by intermolecular interactions between the precursor compounds [6,11]. Hence, it is possible to adjust the properties of these solvents, such as viscosity and density, which has led to them being denominated *design solvents* [6,13].

DES and NADES have been used in several areas of chemistry, such as catalysis processes, organic synthesis, electrochemistry, and chemistry of materials [14]. Another application that is gaining prominence is their use in extraction methods for sample preparation, mainly for extraction of organic compounds, while there are still few studies reporting the use of NADES for extraction of chemical elements [11,15-20]. Studies concerning the applications of AADES are scarce, although these solvents have shown promise in areas including cosmetics, food, medicines, animal feed, and polymer production [9]. They can be used for drug solubilization and transport, biosynthesis, capture of radioactive iodine, oil separation, extraction of bioactive compounds for use in foods and herbal supplements, enzymatic hydrolysis of biomass, and synthesis of bioactive compounds, such as indoles and their derivatives [9]. However, there are still no reports of application of AADES in extraction procedures for inorganic analysis [9]. The amino acids most used for the synthesis and applications of AADES include betaine, lysine, glycine, glutamine, arginine, cysteine, methionine, tryptophan, valine, leucine, and proline [9].

An interesting and promising potential application of these solvents is their use in sample preparation, for which they must be present in the form of a homogeneous and stable mixture [21]. Their viscosity is an important consideration, since most studies reported in the literature describe solvents with high viscosities (>100 mPa.s), due to their applications that require this characteristic, such as in studies of gas solubility [14]. However, when solvents with low viscosity are required, there are several strategies that can be adopted. Among these, increase of the temperature reduces the viscosity, due to the greater kinetic energy of the molecules and, consequently, their vibration, which increases the fluidity of the solvent [14]. In addition, variation of the components (mainly the HBD) and the addition of water during preparation of the solvent can be used to produce solvents with lower viscosities.

In addition to the importance of low viscosity solvents in mass transfer processes, they are also required in analyses employing plasma techniques, such as ICP-MS (Inductively Coupled Plasma Mass Spectrometry) and ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry), where the viscosity influences the processes of formation of the sample aerosol and its transport to the plasma [22]. More viscous solvents form larger and fewer droplets, affecting the formation of spray in the nebulizer and the separation in the nebulization chamber, reducing the sensitivity of the analytical method [20].

In this work, a restricted mixture design was used to optimize the proportions of the AADES components, with β-alanine as the HBA and varying the HBD between organic acids (malic acid and citric acid) and sugar (xylitol), together with the addition of water. The desired responses in this mixture design were lower density and viscosity values. The experimental design considered three components constituting the final product, with the response (the physical properties of the mixture) depending only on the proportions of the components present [23]. A graphical representation of the mixture design is given by an equilateral triangle, where the vertices correspond to the pure components and the central points represent ternary mixtures [23]. In this way, it is possible to evaluate the effect of the proportion of each component used during the preparation and its influence on the properties of the AADES, using a minimum number of experiments. The AADES categorized as ideal were then characterized by rheological measurements, polarity analyses, infrared spectroscopy (FTIR), thermogravimetry (TGA), and differential scanning calorimetry (DSC).

To the best of our knowledge, there have been no previous studies reported in the literature concerning the preparation of AADES using a mixture design, with broad characterization based on variation of the HBD component. These solvents have excellent potential for application in sample preparation procedures and the extraction of inorganic analytes.

2. Experimental procedures

2.1. Materials and reagents

The materials employed in the experiments were previously decontaminated for 48 h in acid baths containing 10% v v^{-1} HNO_3, followed by washing three times with ultrapure deionized water obtained from a Milli-Q system (ICW-3000, Merck KGaA, Germany).

The AADES studied were prepared using β-alanine, DL-malic acid, citric acid, and xylitol (all from Sigma-Aldrich, MO, USA), together with ultrapure water. Information about the reagents, their molecular structures, and their physicochemical properties is provided in Table 1.

[Table 1]

2.2. Preparation of AADES using a mixture design

Three different AADES were prepared using combinations of the amino acid β-alanine and water with malic acid (AADES 1: Ala-MA), citric acid (AADES 2: Ala-CA), and xylitol (AADES 3: Ala-Xyl). The preparation conditions were optimized using a mixture design (Figure 1), with the proportions of the components restricted by lower and upper limits. In this case, the mixture optimization required the presence of all the components to produce suitable AADES, with the minimum (0%) and maximum (100%) proportions being restricted (see Figure 1).

[Figure 1]

Table 2 shows the combinations and proportions (% w w^{-1}) of each component in the mixtures used to prepare the AADES, with a total of 30 experiments performed (10 experiments for each AADES). The resulting mixtures were heated for 2 h in a water bath at 50 °C, under magnetic stirring at 220 rpm (AccuPlate HotPlate Stirrer, LAbNet, USA), according to the methodology proposed by Dai et al. [6]. The AADES obtained were stored in a desiccator to prevent moisture absorption before their subsequent application.

[Table 2]

2.3. Characterization of the AADES

2.3.1. Density and viscosity measurements

The responses evaluated for the restricted mixture design were density and viscosity, which were determined by analyses performed in triplicate for all the prepared AADES. The densities of the solvents were analyzed volumetrically, using a pycnometer previously calibrated with water at 25 °C and an analytical balance with accuracy of ±0.0001 g (Model AG200, Gehaka, Brazil). The values were obtained using a simple calculation for density, as shown in Eq. 1, where *m* (g) is the mass and *V* (mL) is the volume of the AADES.

$$Density\ (\rho) = \frac{m}{V} \quad (1)$$

The viscosity measurements of the AADES were performed using a Cannon-Fenske viscometer calibrated with water, at a controlled temperature of 25 °C. The values were determined using Eq. 2, where *d1* and *t1* are the density and flow time for the AADES, *d2* and *t2* are the density and flow time for water, all at 25 °C, and η is the value of the water viscosity at 25 °C.

$$Viscosity\ (\mu) = \frac{(d1\ .t1)}{(d2\ .t2)}\ .\eta \quad (2)$$

2.3.2. Rheological measurements

The rheological measurements were performed using an AR-2000EX rheometer (TA Instruments, Delaware, USA) using concentric cylinder geometry and gap of 5920 μm. After transfer of the sample to the rheometer, it was allowed to rest during a 5 min equilibration time, before starting the flow test. Tests were performed varying the shear rate from 1 to 1000 s^{-1}, at 25 °C. Flow curves were obtained and the Newton, Ostwald-de-Waele, Bingham, and Herschel-Bulkley models were fitted to the experimental data.

2.3.3. Polarity analysis

Solvatochromic analyses of the AADES were performed using 10 mM Reichardt's dye solution prepared in methanol, which was subsequently removed from the solution at room temperature. The AADES-dye mixtures were prepared by diluting 1 mL of dye solution in 4 mL of AADES, followed by ultrasonication for 20 min at 25 °C to completely solubilize the dye. Ultraviolet-visible scanning spectra were obtained using a UV-Vis spectrophotometer (UV-2600, Shimadzu, Japan), in the range 200-600 nm. The Dimroth-Reichardt parameter was calculated based on the molar energy value, *Et (30)*, in kcal mol^{-1}, as shown in Eq. 3 [24], where λ_{max} is the maximum absorbance wavelength. The analyses were performed in triplicate for each solvent evaluated.

$$Et\ (30) = \frac{28591}{\lambda max} \quad (3)$$

2.3.4. Fourier transform infrared (FTIR) spectroscopy analysis

Fourier transform infrared spectroscopy (FTIR) analyses were performed using an IRPrestige-21 instrument (Shimadzu, Japan) equipped with an attenuated total reflectance accessory, in order to obtain the spectra of the prepared AADES solvents and their precursors. The instrument was operated at spectral resolution of 4 cm^{-1}, in the range from 4000 to 600 cm^{-1}, with each sample being scanned 30 times.

2.3.5. Thermogravimetry (TGA) and differential scanning calorimetry (DSC) analyses

The thermal stabilities of the prepared solvents were evaluated by thermogravimetric analysis (Pyris 1TG, PerkinElmer, USA). A mass of approximately 5 mg of the AADES sample was placed on a platinum support and heated from 25 to 500 °C, at 10 °C min^{-1}, under a flow of N_2 at 100 mL min^{-1}.

The melting points of the AADES were determined using a calorimeter (DSC-60, Shimadzu, Japan). A mass of approximately 10 mg of the AADES sample was placed in an aluminum crucible and heated from -100 to 400 °C, at 10 °C min^{-1}, under a flow of N_2 at 100 mL min^{-1}.

3. Results and discussion

The optimized proportions of the components used to prepare the AADES were selected based on lower values of density and viscosity, in addition to the stability aspects evaluated. According to Espino et al. [11], the solvents should remain as clear and homogeneous liquids, without instantaneous or progressive precipitate formation. These parameters are very important, since solvents with both low density and low viscosity have the advantage of higher extraction efficiency, due to high diffusivity and consequently faster mass transfer [25,26].

To obtain the simultaneous optimization of both responses, the mathematical approach proposed by Derringer and Suich was used [27,28]. This method is based on desirability functions, which is an approach widely used in experiments involving multiple responses. Initially, each response was individually coded from 0 (undesirable response, higher density and viscosity values) to 1 (desirable response, lower density and viscosity values). The individual desirability (d_i) for each response (y_i) was calculated using Eq. 4:

$$d_i = \begin{cases} 1 \; if \; y < T \\ \left(\frac{U-y}{U-T}\right)^t \; if \; T \leq y \leq U \\ 0 \; if \; y > U \end{cases} \quad (4)$$

where, U is the highest acceptable value for the response, T is the target value (the lowest value for the response), and t is the weight for the desirability function (for example, equal to 1 for a linear desirability function).

The overall desirability (D) was calculated, combining the individual desirabilities into a single response, according to Eq. 5:

$$D = \sqrt[m]{d_1 \times d_2 \times \cdots d_m} \quad (5)$$

Finally, three models were calculated using the global desirability for each AADES. In this case, ten coefficients were obtained: constant (b0), linear coefficients (b1, b2, and b3), quadratic coefficients (b11, b22, and b33), and interaction coefficients (b12, b13, and b23). The significance of these coefficients was calculated using one-way analysis of variance (ANOVA, 95% confidence level). Figure 2 shows the contour plots obtained for the model

after optimizing the AADES preparation, where higher desirability values are represented by the blue color.

[Figure 2]

Similar results were observed for the three AADES, with increase of the water content in the preparation mixture resulting in lower density and viscosity values, as reported previously by Santana et al. [26]. A compromise condition for preparation of the three AADES was established at 12.50% w w^{-1} for β-alanine, 43.75% w w^{-1} for the HBD component (malic acid, citric acid, or xylitol), and 43.75% w w^{-1} for water, according to experiment 10 of the mixture design (see Table 2). This proportion was selected based on solvent stability and lower density and viscosity values of the AADES obtained (Table 3). Although the models shown in Figure 2 suggested the maximum contribution of water (75% w w^{-1}), preparation of the AADES using water concentrations above 50% w w^{-1} weakened the interactions between the HBA and HBD molecules, due to competition for hydrogen bonds [6]. As water was introduced into the system, the hydrogen bonds between the DES precursors were broken, resulting in dissociation of the precursor compounds in water, without interaction between them and changing the physicochemical properties of the solvent [28].

[Table 3]

Density, viscosity, and polarity are considered crucial physicochemical properties for understanding possible applications of AADES [29,30]. Density and viscosity are related to the molecular structures of the precursors employed, as well as to the extensive networks of hydrogen bonds that form AADES, which can result in less mobility between free species in the solvent structure, making it denser and more viscous, with the variation of HBD having the greatest influence [30]. The use of precursors with molecular structures rich in hydroxyl groups increases the sites available for formation of hydrogen bonds, resulting in higher density and viscosity values, due to the greater forces of attraction between the molecules [31,32]. The same occurs with the use of organic acids, where the carboxyl groups are

299 responsible for hydrogen interactions [33]. However, the values of these properties can
300 decrease with variation of the molecular structure of the precursors, related to the size and
301 organization of the molecules, or the presence of bulky groups that can cause steric effects
302 and prevent the formation of hydrogen bonds [34].
303 For the AADES selected in this work, the density values increased in the order
304 AADES 3 < AADES 1 < AADES 2, with the density of the solvents being directly reflected
305 by the density of the HBD employed (see Table 1 and Table 3) [35]. The highest viscosity
306 was found for AADES 2, due to the greater quantity of hydrogen bond interactions, resulting
307 in decreased movement of free species. AADES 1 presented the lowest viscosity value,
308 because the HBD had a smaller and more organized molecular structure, with the occurrence
309 of van der Waals interactions prevailing [36]. The value obtained for AADES 3 was close to
310 that for AADES 2, despite the fact that xylitol has five hydroxyl groups in its molecular
311 structure, so more hydrogen interactions would be expected, compared to the use of citric
312 acid, which has three sites for formation of these interactions. This lower viscosity could also
313 be attributed to the high structural organization presented by the xylitol molecule, which
314 favors the formation of van der Waals interactions [36]. The Hole Theory presented by
315 Abbott et al. [37] is used to explain the variation of these properties, where the interaction
316 between the HBA and HBD components causes vacancies in the molecular structure. The
317 use of citric acid as HBD acted to reduce the size of the holes (vacancies) in the solvent
318 structure, resulting in strong cohesive energy due to the formation of a strong intermolecular
319 hydrogen network, contributing to less free space and molecular movement.
320 Application of one-way ANOVA to the density and viscosity values determined for
321 the AADES revealed significant differences in most cases, except between AADES 2 and
322 AADES 3 for viscosity (95% confidence level), showing that changing the HBD component
323 directly influenced the physicochemical properties of the solvents, enabling them to be used
324 for different applications.
325 In another study, NADES based on citric acid and malic acid (CA-MA), malic acid
326 and xylitol (MA-Xyl), and citric acid and xylitol (CA-Xyl) were prepared, where the last
327 showed the highest viscosity, followed by the CA-MA NADES [26], corroborating the data
328 presented here, where the use of citric acid resulted in greater viscosity of the solvent. Shafie
329 et al. [32] and Singh et al. [38] obtained similar results for NADES based on citric acid as

HBD, with increases of density and viscosity with increase of the molar proportion of this organic acid. This was attributed to an abundance of HBD favoring the formation of hydrogen bonds, resulting in less mobility between free groups in the molecules of the components making up the solvent.

The application of DES in extraction processes has been attracting increasing attention, especially focusing on the influence of the solvent viscosity [6,39]. The addition of water during the preparation procedure is one way to reduce viscosity [33]. In this work, the solvents obtained presented lower viscosity than most DES reported in the current literature, due to the 43.75% water content. Huang et al. [40] studied the effect of adding water in NADES based on choline chloride and glycerol, noting that there were reductions in solvent viscosity of 630 mPa.s with 5% water content, 40 mPa.s with 20% water addition, and 30 mPa.s with 30% water addition, for measurements performed at 40 °C. Subsequently, these same NADES were studied as solvents for the extraction of rutin from wheat husk samples, which showed that water addition greater than 20% resulted in lower extraction efficiency [40].

The rheological properties of the AADES were evaluated based on shear stress curves plotted as a function of the shear rate (Figure 3). AADES 2 presented the highest shear stress, followed by AADES 3 and AADES 1. All the rheological models evaluated could be fitted to the experimental data, with high coefficients of determination (R^2 >0.999). The best fit to the data was obtained with the Herschel-Bulkley model. The Bingham model is normally used to describe the rheological behavior of DES and NADES [41,42]. However, for the AADES evaluated here, it provided negative yield stress values, which are meaningless from a physical point of view. Altamash et al. [43] also reported that the Herschel-Bulkley model was suitable for describing the flow behavior of DES based on phenylacetic acid.

[Figure 3]

The parameters of the Herschel-Bulkley model are shown in Table 4. All the fluids presented *n* values slightly greater than 1, indicating weak shear thickening behavior (with the shear stress increasing slightly when the shear rate increases). In this work, all the fluids required a yield stress to start flowing. The yield stress indicates the minimum stress required

to disrupt the networked structure and achieve flow. This parameter can be correlated with the pumping energy required to initiate flow [42,44].

[Table 4]

AADES 2 presented the highest yield stress and consistency coefficient, while AADES 1 showed the lowest values of these parameters. These results were in agreement with Martinetto et al. [45] and Qin et al. [46], who studied the behaviors of ionic liquids based on polyoxometalate and imidazole compounds, respectively. The shear thickening behavior were determined at room temperature for solvents that presented cations and anions with longer alkyl chains in their structures, associated with the lengths of the alkyl chains of the HBDs used, which were greater for citric acid, xylitol, and malic acid. The results obtained here were consistent with the viscosity data, confirming that the molecular structure of the donor component in the AADES directly influenced the fluid consistency index.

Knowing the polarity of AADES is also of fundamental importance for understanding in advance their behavior and capacity for solvation or extraction of different analytes in different sample matrices. However, there have been few studies dedicated to investigating and discussing this physicochemical property [33,47].

The polarity of AADES can be characterized by the spectroscopic responses of solvatochromic absorbance probes, based on changes in the UV-Vis spectrum resulting from the interaction between the probe molecule and the molecules that make up the solvent [48,49]. Reichardt's dye is a probe based on negative solvatochromism, characterized by a shift of the absorption band to shorter wavelengths, as the solvent polarity increases [49]. Dimroth and Reichardt's *Et (30)* scale is normally used to measure solute-AADES interactions, representing the transfer of intermolecular charge of Reichardt's dye and its absorption band, with higher *Et (30)* values indicating greater polarity of the solvent [48-50]. In this work, the values of λ_{max} and *Et (30)* were 290 ± 0 nm and 98.59 ± 0 kcal mol^{-1} for AADES 1, 304 ± 0.7 nm and 93.90 ± 0.22 kcal mol^{-1} for AADES 2, and 305 ± 1 nm and 93.74 ± 0.31 kcal mol^{-1} for AADES 3, reflecting solvents with high polarities. The *Et (30)* value for pure water is 63.1 kcal mol^{-1} [48]. Application of one-way ANOVA to the *Et (30)* values for each solvent revealed significant differences between AADES 1 and AADES 2 or

AADES 3 (95% confidence level), in agreement with data obtained previously concerning changes in solvent properties when using different HBD components.

The nature of the HBD component is one of the main factors influencing the polarity of the solvent [51]. The use of compounds with linear alkyl molecular structures decreases the ability to donate protons, resulting in higher polarity [30,51]. The same behavior is observed when water is added to the mixture. Gabriele et al. [52] found that the addition of between 0 and 30% water in DES mixtures based on choline chloride and glycol linearly increased the polarity of the solvent.

The variation in the polarity of AADES influences their ability to extract target analytes, in accordance with the *like dissolves like* theory [53]. Solvents with high polarities and AADES-water mixtures provide greater efficiency in the extraction of polar compounds, as well as metals and semimetals, due to the ability to form hydrogen bonds with the analytes [48]. A NADES based on choline chloride and maltose, with the addition of water, was studied as solvent for the extraction of phenolic compounds of different polarities from *Canajus canjan* leaves (pea species) [54]. The results showed that increasing the proportion of water to 20% increased the polarity of the NADES, enabling efficient extraction of compounds of higher polarity [54]. The extraction of phenolic compounds has also been evaluated using NADES based on choline chloride and different alcohols as HBD, with higher extraction efficiency observed when a greater amount of water was used [55].

FTIR spectra of the starting materials and the prepared solvents were obtained for characterization and evaluation of formation of the solvents (Figure 4). The FTIR technique is sensitive to structural changes, enabling its use to identify functional groups and the formation of hydrogen bonds [32]. The FTIR analyses could confirm the formation of AADES by observing the maintenance of characteristic bands of the starting materials used, with small shifts to smaller wavenumbers in the spectra of the prepared solvents. Archer et al. [56] confirmed the formation of NADES based on choline chloride and (R)-3-hydroxyacids by observing shifts of wavenumbers and variations in the stretching vibrations of COO-H and O-H bonds.

[Figure 4]

The β-alanine spectrum showed peaks at 2873 and 2632 cm^{-1}, characteristic of the various hydrogen bonds present in amino acids [57]. In addition, peaks were observed at 1568 cm^{-1}, attributed to the carbonyl group (C=O), 846 cm^{-1}, corresponding to the amine group (N-H), and 651 cm^{-1}, attributed to the carboxyl group (-COO). The FTIR spectrum for malic acid showed absorption bands at 3487 cm^{-1}, for the free hydroxyl group (-OH) in the molecule, 1745 cm^{-1}, corresponding to C-O stretching, 1675 cm^{-1} (C=O), and 1180 cm^{-1} (-CH_2 stretching) [58,59]. The citric acid spectrum showed absorption bands at 3440 cm^{-1} (free -OH groups), 1683 cm^{-1} (C=O), and 1742 cm^{-1} (C-O) [54,56]. For xylitol, absorption bands were observed at 3426, 3367, and 3229 cm^{-1}, corresponding to free -OH groups in the molecule, in addition to a peak at 1431 cm^{-1}, corresponding to in-plane bending of -OH, and a peak at 743 cm^{-1}, corresponding to out-of-plane bending of -OH [58,59].

For the prepared AADES, the FTIR spectra showed broad bands in the region between 3550 and 3000 cm^{-1}, corresponding to free -OH groups in the molecules of the starting materials and in water molecules [60]. The AADES 1 spectrum presented a peak shift from 1675 to 1629 cm^{-1}, while the AADES 2 spectrum showed a peak shift from 1683 to 1635 cm^{-1}, corresponding to shifts of the C=O group signals to smaller wavenumbers, due to increased electron density in the oxygen atom, indicative of the occurrence of hydrogen bonding [60]. Other wavenumber shifts were also observed in the spectra for AADES 1 (1745 to 1728 cm^{-1}, 1675 to 1629 cm^{-1}, and 1180 to 1112 cm^{-1}), AADES 2 (1742 to 1726 cm^{-1} and 1683 to 1635 cm^{-1}), and AADES 3 (1431 to 1411 cm^{-1} and 743 to 700 cm^{-1}), all attributed to the HBD components employed.

In addition, shifts of the peaks corresponding to the amino acid β-alanine were also observed, providing further evidence of hydrogen bond formation between the initial reagents. For all the solvents, no bands corresponding to new functional groups appeared, indicating that there were only physical interactions between the AADES precursors, without any evidence of chemical reactions [61].

The possible applications of AADES are also dependent on the physical conditions under which they remain as homogeneous liquids, without becoming thermally degraded or solidified [62]. Therefore, thermogravimetric (TG) and derivative thermogravimetric (DTG) analyses were performed, together with determination of the melting points of the solvents using differential scanning calorimetry (DSC) (Figure 5).

[Figure 5]

All the AADES showed mass losses of approximately 40% at around 100 °C (Figure 5A), corresponding to the proportion of water added during the preparation procedure [63]. Thermal decomposition of AADES 1 occurred at around 130 °C, while AADES 2 and AADES 3 showed thermal decomposition temperatures close to 150 °C (Figure 5B). In all cases, the decomposition was complete at around 310 °C, with a 50% mass loss. In previous work by our research group [58], it was found that the HBDs employed here showed thermal decomposition at 234 °C (malic acid), 279 °C (citric acid), and 286 °C (xylitol), while β-alanine degrades at around 200 °C [42]. These decomposition temperatures indicated that the solvents formed were less stable than their precursors. This could be attributed to the high proportion of water employed, which weakened the hydrogen interactions between the components forming the solvent, in agreement with the studies carried out by Paveglio et al. [62].

The nature of the HBA and HBD components, the amount of water in the solvent, the formation of intermolecular interactions, and the alkyl chain size of the precursors are some of the factors that can influence the thermal stability of DES [63]. The use of HBD components with larger alkyl chains increases solvent stability, due to the greater possibility of formation of intermolecular interactions between the molecules of the precursors, with greater energy required for them to be broken [64]. The same occurs when using compounds that enable formation of a greater number of hydrogen bonds, such as compounds rich in hydroxyl and carboxyl groups in their molecular structures [65].

Figure 5C shows the DSC curves for the AADES. The melting point corresponds to the temperature at which the hydrogen interactions between the HBA and HBD components are disrupted, with lower molecular weight of the HBD compound being associated with a lower melting point of the prepared solvent [32,66]. The values obtained here were -13 °C (AADES 1), -22 °C (AADES 2), and -21 °C (AADES 3), showing that the melting temperatures of the solvents formed were lower than the melting temperatures of the precursors (Table 1).

The values indicated that stronger hydrogen interactions occurred when citric acid was used as the HBD component, since a higher temperature was required for its thermal degradation, together with a lower temperature for solidification of the solvent. It could be concluded from the thermal analysis data that the AADES could be employed in working temperature ranges between -13 and 130 °C, for the AADES produced using malic acid, and between -22 and 150 °C, for the AADES produced using citric acid or xylitol.

The classification of solvents as environmentally friendly is relative, where in addition to their main sustainability characteristics, the energy efficiency of their preparation must also be considered [67,68]. The *EcoScale* was applied to evaluate the ecological character of the AADES preparation methodology employed here, considering yield, initial reagent price, safety for the operator and the environment, setting of the magnetic stirrer technique, temperature, and reaction time. The scores obtained for the preparation procedures were 97% (AADES 1 and AADES 3) and 92% (AADES 2), corresponding to classification as excellent green synthesis methods, since the values were above 75% [69].

4. Conclusions

Amino acid-based deep eutectic solvents (AADES) were prepared using β-alanine as the HBA component, together with malic acid, citric acid, or xylitol as the HBD component. The proportions of the components were optimized by applying a restricted mixture design. Density and viscosity analyses revealed that the molecular structure of the HBD component had a major influence on the physicochemical properties of the solvents, with the AADES formed using citric acid being denser and more viscous, due to strong hydrogen interactions provided by a structure rich in carboxyl groups. This behavior was corroborated by rheological measurements. Solvatochromic analyses revealed that the solvents presented high polarity and were suitable for the extraction of polar compounds. Infrared spectroscopy analyses confirmed the formation of the solvents, as shown by wavenumber shifts in the solvent spectra, compared to the spectra of the starting materials. Thermal analyses (TGA and DSC) revealed the working temperature ranges in which the solvents could be used, in addition to confirming that stronger interactions with the HBA were exhibited by the citric acid-based AADES. Finally, evaluation using the *EcoScale* confirmed that the AADES preparation procedure could be considered an excellent green synthesis.

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Tables

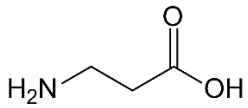
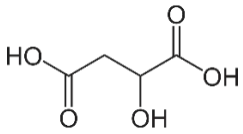
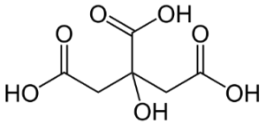
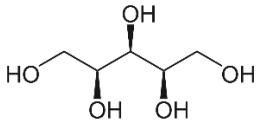
Reagent	CAS number	Purity (%)	Density (g mL^{-1})	Melting point (°C)	Molecular structure
β-alanine	107-95-9	98	1.44	207	
DL-malic acid	6915-15-7	99	1.61	130	
Citric acid	77-92-9	99.5	1.66	153	
Xylitol	87-99-0	99	1.52	92	

Table 1. Molecular structures and physicochemical properties of the reagents used to prepare the AADES.

Mixture	β-alanine (% w w^{-1})	HBD component (% w w^{-1}) [a]	Water (% w w^{-1})
1	50.00	25.00	25.00
2	25.00	50.00	25.00
3	25.00	25.00	50.00
4	33.33	33.33	33.33
5	12.50	12.50	75.00
6	12.50	75.00	12.50
7	75.00	12.50	12.50
8	43.75	12.50	43.75
9	43.75	43.75	12.50
10	12.50	43.75	43.75

[a] Malic acid (AADES 1), citric acid (AADES 2), or xylitol (AADES 3).

Table 2. Experimental conditions for the synthesis of AADES 1 (Ala-MA), AADES 2 (Ala-CA), and AADES 3 (Ala-Xyl).

Mixture	AADES 1		AADES 2		AADES 3	
	Density ($g\ mL^{-1}$) [a]	**Viscosity (mPa s) [a]**	**Density ($g\ mL^{-1}$) [a]**	**Viscosity (mPa s) [a]**	**Density ($g\ mL^{-1}$) [a]**	**Viscosity (mPa s) [a]**
1	1.30 ± 0.01	165.71 ± 2.05	1.31 ± 0.01	237.56 ± 3.00	- [d]	- [d]
2	1.33 ± 0.01	107.56 ± 2.40	1.36 ± 0.01	252.89 ± 4.19	1.29 ± 0.01	112.38 ± 1.18
3	1.21 ± 0.01	5.95 ± 0.01	1.22 ± 0.01	7.47 ± 0.63	1.18 ± 0.01	6.00 ± 0.29
4	1.29 ± 0.01	45.08 ± 1.14	1.29 ± 0.01	50.84 ± 0.67	1.25 ± 0.01	27.34 ± 1.68
5	1.10 ± 0.01	1.33 ± 0.26	1.10 ± 0.01	1.49 ± 0.00	1.09 ± 0.01	1.48 ± 0.01
6	- [b]	- [b]	- [b]	- [b]	- [b]	- [b]
7	- [b]	- [b]	- [b]	- [b]	- [d]	- [d]
8	$1.21 \pm 9.06.10^{-5}$	9.05 ± 0.30	1.23 ± 0.01	17.19 ± 0.32	1.20 ± 0.01	8.59 ± 0.01
9	- [c]	- [c]	- [c]	- [c]	- [b]	- [b]
10	1.25 ± 0.01	7.332 ± 0.005	1.26 ± 0.01	9.56 ± 0.01	1.21 ± 0.01	9.02 ± 0.29

[a] Average ± standard deviation (n=3).
[b] The solvent was unstable, with precipitate formation at room temperature.
[c] The solvent showed high density and viscosity, making it impossible to carry out the analyses.
[d] There was no homogeneous solvent formation.

Table 3. Density and viscosity values determined for AADES 1 (Ala-MA), AADES 2 (Ala-CA), and AADES 3 (Ala-Xyl), at 25 °C.

	Herschel-Bulkley model			
	τ_0 (Pa)	k (Pa.s)	n	R^2
AADES 1	0.01329	0.00848	1.06	0.99996
AADES 2	0.01753	0.01401	1.05	0.99997
AADES 3	0.01537	0.01132	1.05	0.99997

Table 4. Herschel-Bulkley model rheological parameters for AADES 1 (Ala-MA), AADES 2 (Ala-CA), and AADES 3 (Ala-Xyl). τ_0: yield stress; k: consistency coefficient; n: flow behavior index.

Figures

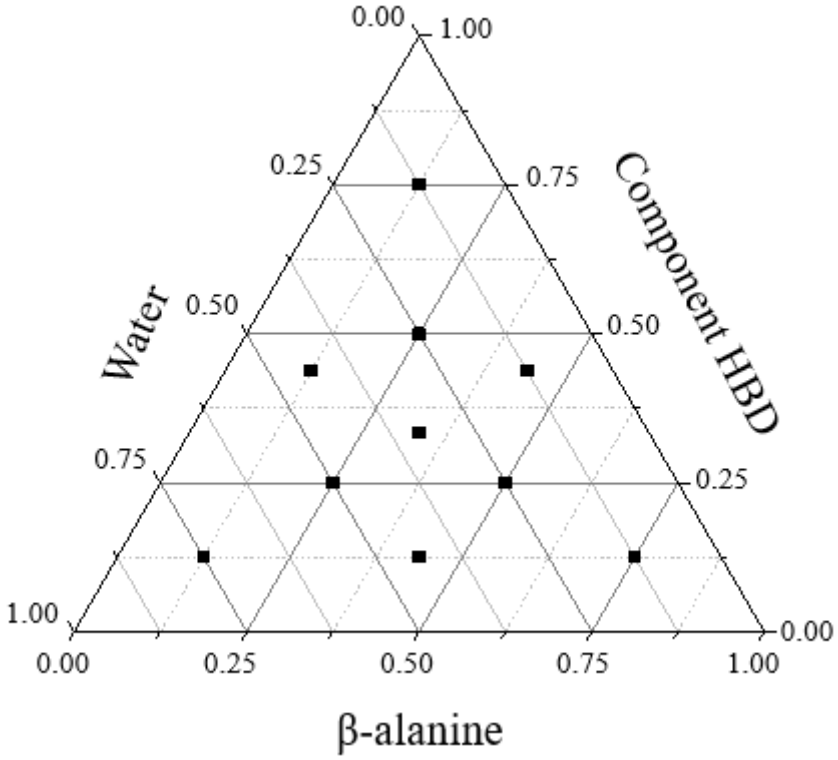
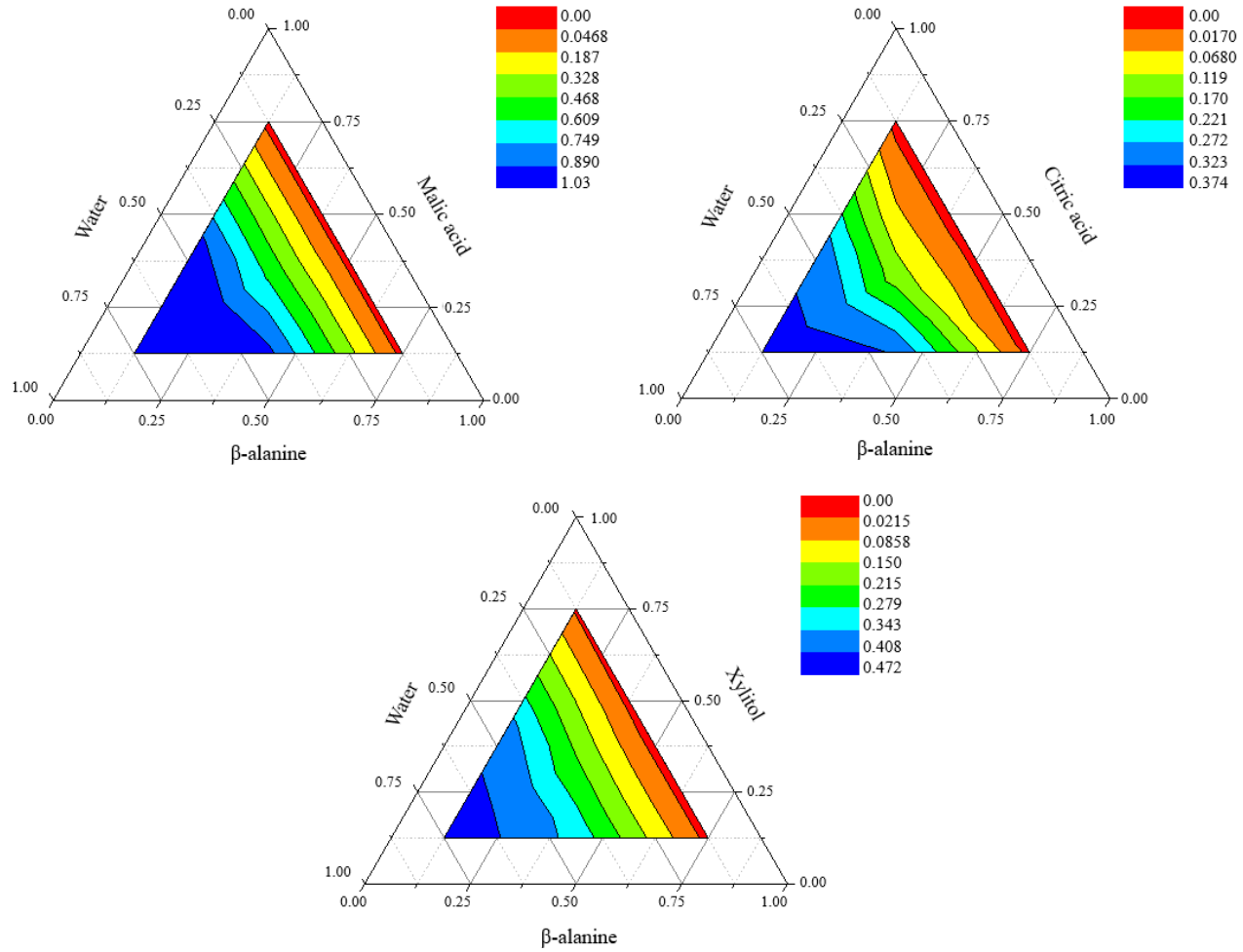


Figure 1. Mixture design delimited by restrictions to optimize synthesis of the AADES.



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794 **Figure 2.** Contour plots for optimization of the mixture components to synthesize AADES 1

795 (Ala-MA), AADES 2 (Ala-CA), and AADES 3 (Ala-Xyl).

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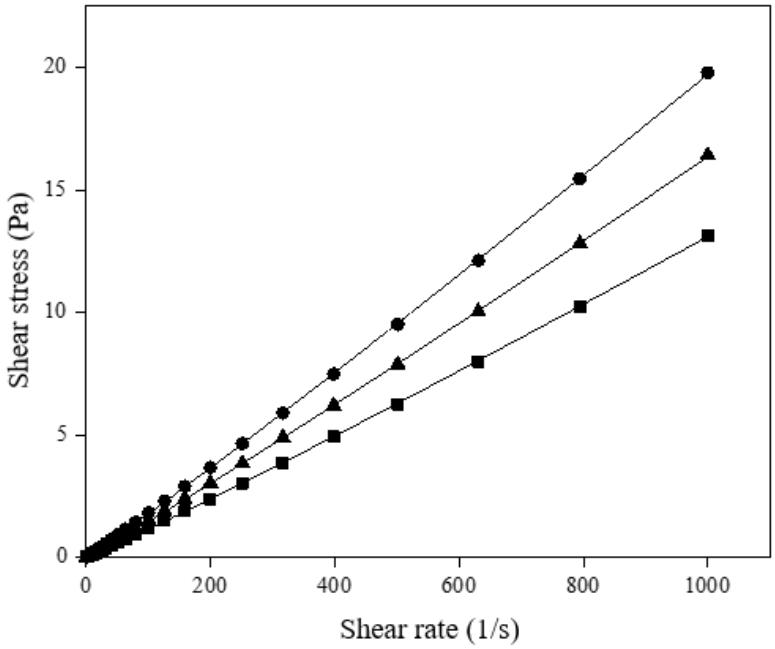

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798 **Figure 3**. Flow curves for ■ AADES 1 (Ala-MA), ● AADES 2 (Ala-CA), and ▲ AADES 3

799 (Ala-Xyl), obtained at 25 °C. Fits obtained using the Herschel-Bulkley model.

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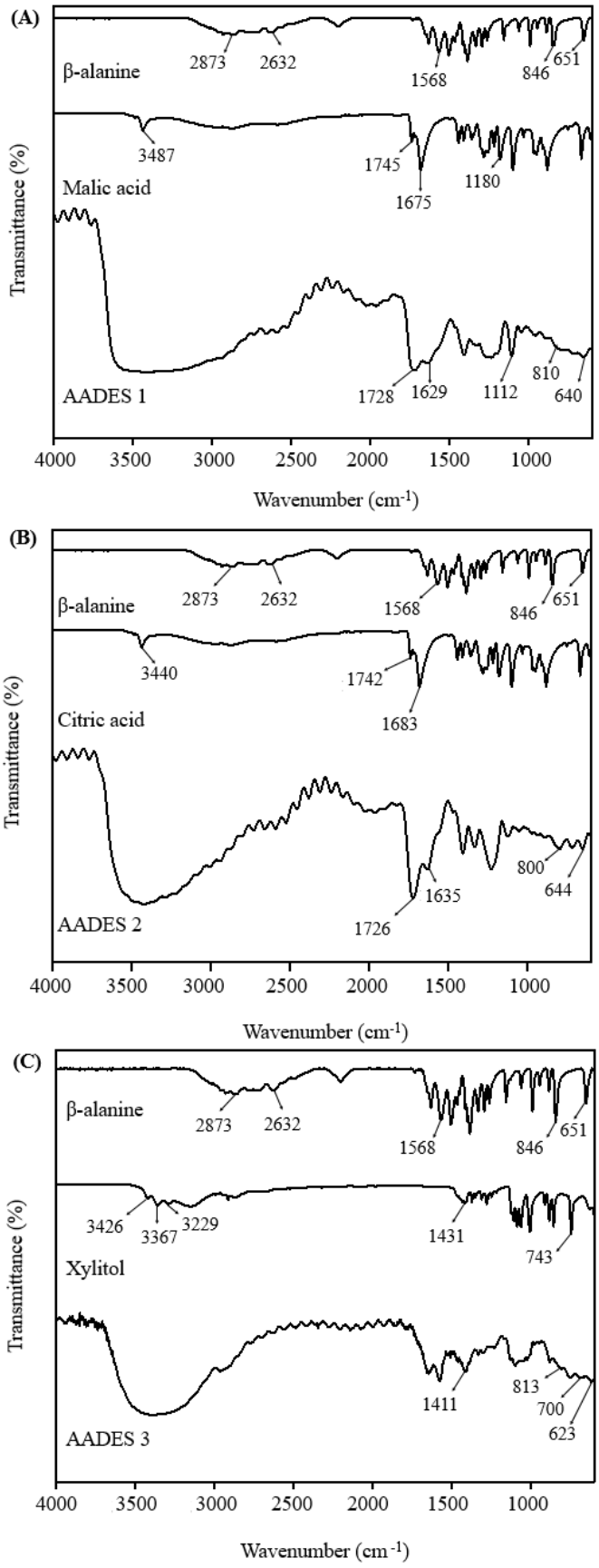
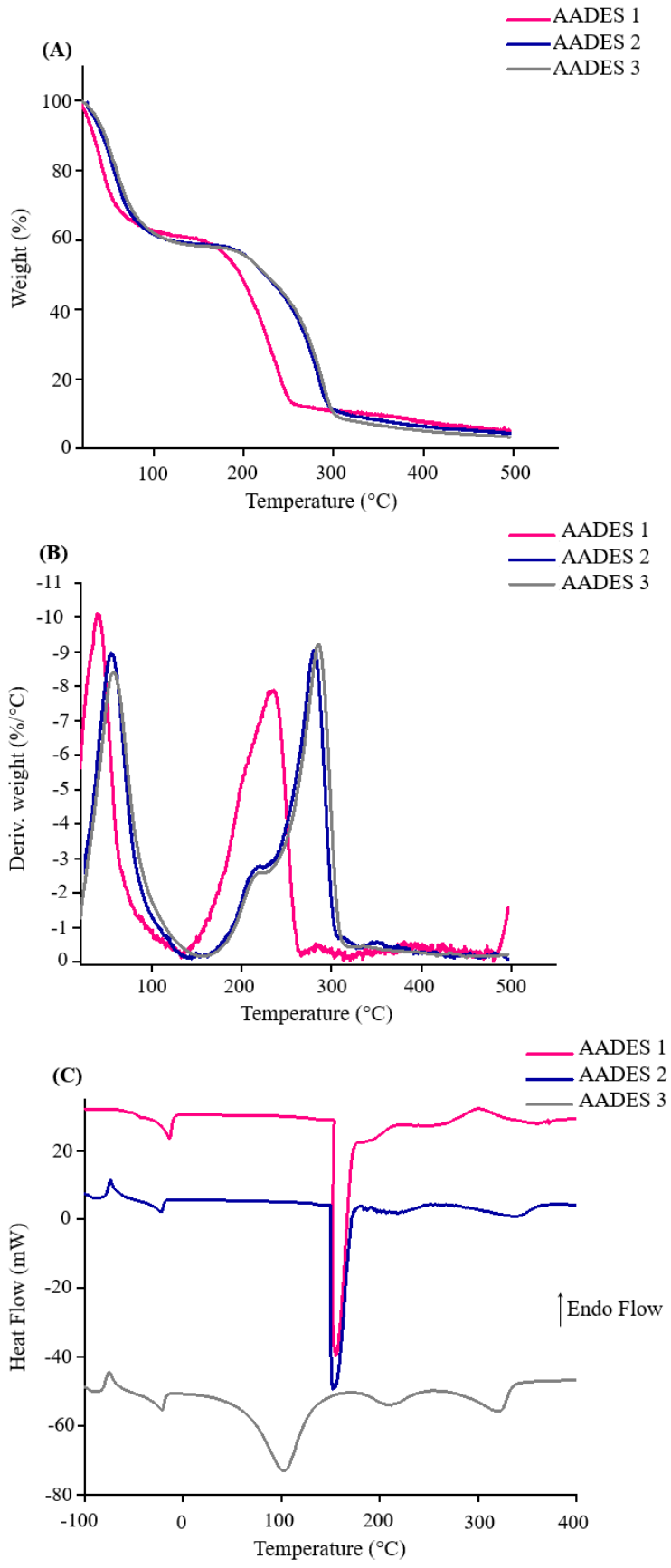

(A)
β-alanine
2873
2632
1568
846
651
3487
1745
1180
Malic acid
1675
Transmittance (%)
AADES 1
1728
1629
1112
810
640
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)
(B)
β-alanine
2873
2632
1568
846
651
3440
1742
Citric acid
1683
Transmittance (%)
800
644
1635
AADES 2
1726
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)
(C)
β-alanine
2873
2632
1568
846
651
3426
3367
3229
1431
743
Xylitol
Transmittance (%)
1411
813
700
623
AADES 3
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

Figure 4. FTIR spectra of the initial reagents and the prepared solvents: (A) AADES 1 (Ala-MA), (B) AADES 2 (Ala-CA), and (C) AADES 3 (Ala-Xyl).


(A)
AADES 1
AADES 2
AADES 3
Weight (%)
Temperature (°C)
(B)
AADES 1
AADES 2
AADES 3
Deriv. weight (%/°C)
Temperature (°C)
(C)
AADES 1
AADES 2
AADES 3
Heat Flow (mW)
Endo Flow
Temperature (°C)

807 **Figure 5.** (A) TGA, (B) DTA, and (C) DSC spectra for the three synthesized AADES.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: