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A Greener Approach to Prepare Natural Deep Eutectic Solvents

Federico J. V. Gomez,* Magdalena Espino, María A. Fernández, and María F. Silva*[a]

Natural deep eutectic solvents (NADES) is one of the hottest issues in the field of green chemistry. However, it is possible to make them greener? Herein we propose a new microwave (MW) assisted preparation demanding only seconds. The MW-NADES were challenged with those obtained by the classical Heating & Stirring procedure in terms of green analytical metrics and physical parameters. Indeed, the following analytical applications were tested: a) extraction media for phenolics and alkaloids from plant, b) enhancers for electrochemical detection of phenolic compounds. Our results demonstrate outstanding environmental advantages as the dramatic decrease of synthesis time (the shortest time to date, 60 minutes to 20 seconds) and energy consumption (650 times lower). The analytical performance and physicochemical parameters were comparable with the traditional NADES.

The replacement of conventional toxic solvents by sustainable solvents is one of the major concerns in green chemistry.[1] In this context, ionic liquids (ILs) were postulated in the last decades as a green alternative to traditional hazardous solvents. However, their sustainability is often argued, due to their poor biodegradability, biocompatibility, and high cost.[2] Later, novel solvents based on the eutectic behavior of their counterparts, were presented as an alternative to ILs. Deep eutectic solvents (DES) were introduced by Abbott[3] et al, showing promising solvent properties. An eutectic system, from the Greek "ευ" (eu = easy) and "Τήξις" (teksis = melting) refers to a mixture of components forming a joint super-lattice that melts and freezes at a temperature that is lower than the melting points of the separate constituents. The applications of DES have so far been involved to organic reactions, extraction techniques and electrochemistry.[4] Taking into account that the synthetic components of DESs can be toxic, their possible use for applications in food and pharmacology industries is limited.[5]

Inspired by nature, a new generation of solvents has emerged in the last decade as promising green media.[2,6,7] In 2011, Verpoorte and co-workers7 coined the term "Natural Deep Eutectic Solvents" (NADES). NADES, are composed by naturally occurring molecules (sugars, alcohols, amino acids, organic acids, and choline derivatives) and fully represent green chemistry principles. NADES are typically obtained by mixing a hydrogen-bond acceptor (HBA), with a hydrogen bond donor (HBD) molecule, leading to a significant depression of the melting point. Hydrogen bonding and Van der Walls interactions are the main driving force of this phenomenon. Hydrogen bonding interactions lead to highly structured liquids.[8] These solvents offer many remarkable advantages including biodegradability, low toxicity, solute stabilization, sustainability and low costs,[2,8] ease of preparation, large number of possible combinations (around 10^6) and "tunable" physicochemical properties.[9]

To date there are three methods available for preparing NADES:[4]

a) Heating and Stirring method (HS):[8] the components are placed in a bottle with a stirring bar and cap and heated in a water bath (50-90 °C) with agitation till a clear liquid is formed (about 30–90 min).
b) evaporating method:[8] components are dissolved in water and evaporated at 50 °C with a rotary evaporator. The liquid obtained is placed in a desiccator with silica gel till constant weight (total time was not informed).
c) freeze drying method[10] based on the freeze-drying of aqueous solutions of the individual counterparts (about 2–24 hours).

Although these procedures have been successfully applied for NADES preparation and several interesting chemical applications have been proposed, the time and energy that these processes demand could be controversial concerning the greenness of these solvents. Therefore, the development of novel and efficient strategies for NADES preparation of is of utmost importance.

Optimization of energy consumption in chemical processes is mandatory from the point of view of Green Chemistry, since energy generation and consumption are essential for the environment. Non-conventional synthesis techniques have gained much attention from the scientific community. Among them, the microwave irradiation technique is a well known and powerful tool for the development of more environmentally benign processes.[11] The use of microwave radiation (MW) is one of the most promising techniques due to its advantages such as higher yields, milder reaction conditions and shorter reaction times.[12] Despite these advantages, to the best of our

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[a] *Dr. F. J. V. Gomez, Prof. M. Espino, Dr. M. A. Fernández, Dr. M. F. Silva*
Instituto de Biología Agrícola de Mendoza (IBAM-CONICET)
Facultad de Ciencias Agrarias
Universidad Nacional de Cuyo
Almirante Brown 500, Chacras de Coria, Mendoza, Argentina
E-mail: mfsilvamb@gmail.com
fedeg33@gmail.com

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knowledge, there are no reports exploring the use of MW for the preparation of NADES.

With this background, the present communication is aimed to introduce a new preparation technique for NADES by means of a microwave assisted preparation. The suggested microwave method has proven to be faster, easier, cheaper and greener than the traditional procedure. Three different microwave (MW) NADES were prepared and compared with those obtained by the most common HS method and comparable chemical and physical behavior were observed. Indeed, two known analytical applications for NADES were tested:[6,13–17] a) extraction media for phenolics and alkaloids from plant, b) enhancers for electrochemical detection of phenolic compounds. Analytical parameters were compared with those obtained with traditional solvents and conventionally prepared NADES with highly promising results.

Results and Discussion

It has already been established that compounds couple with microwaves and absorb the electromagnetic energy volumetrically, transforming them into heat in the microwave-assisted synthesis. Thus, the thermal/kinetic effect,[18] promotes the collision between the HBA and HBD neighboring compounds. The desired temperature inside the eutectic mixture can be rapidly and homogeneously attained due to extremely rapid kinetics. Reaction efficiency could be improved in the preparation of NADES using microwave irradiation in agreement with previous reports for other preparation schemes.[12]

Several parameters, including the power (200 W to 800 W) and time (5 to 30 s) of MW, were investigated. The different MW powers can provide different hydrogen bond network formation rates. In this sense, we found that 200 W power for 20 seconds of microwave irradiation was enough to reach the eutectic system.

Table 1 shows the comparison of the most commonly applied NADES preparation technique and our approach. The MW method dramatically reduces the preparation time from tens of minutes (30-90 min) to seconds (20 s), and the energy consumption (about 650 times).

Table 1. Green metrics for NADES preparation techniques.

Method	Power (W)	Time (s)	Energy consumption (kW/sample)	Penalty points (PP)[a]
HS	650	3600	0.650	1
MW	200	20	0.001	0

[a]PP According to[20] for energy used.

In order to establish the greenness profile of the proposed methodology, green metrics appears as a meaningful assessment in agreement with the “Twelve Principles of Green Chemistry”.[19] In 2012, Galuszka et al[20] proposed the “Eco-scale”, an objective criteria measurement that includes energy consumption. This scale provides penalty points based on these values: 0 points for energy consumption $\leq$ 0.1 KWh^{-1}, 1 for $\leq$ 1.5 K Wh^{-1} and 2 for $>$ 1.5 K Wh^{-1}. Noticeably, an important reduction on the impact of penalty points based on the eco-scale[20] is observed for the MW approach (Table 1).

In order to compare NADES features obtained by the two techniques, the following physicochemical parameters were tested: operational pH, conductivity and density. As can be seen in Figure 1, similar behavior was observed for both

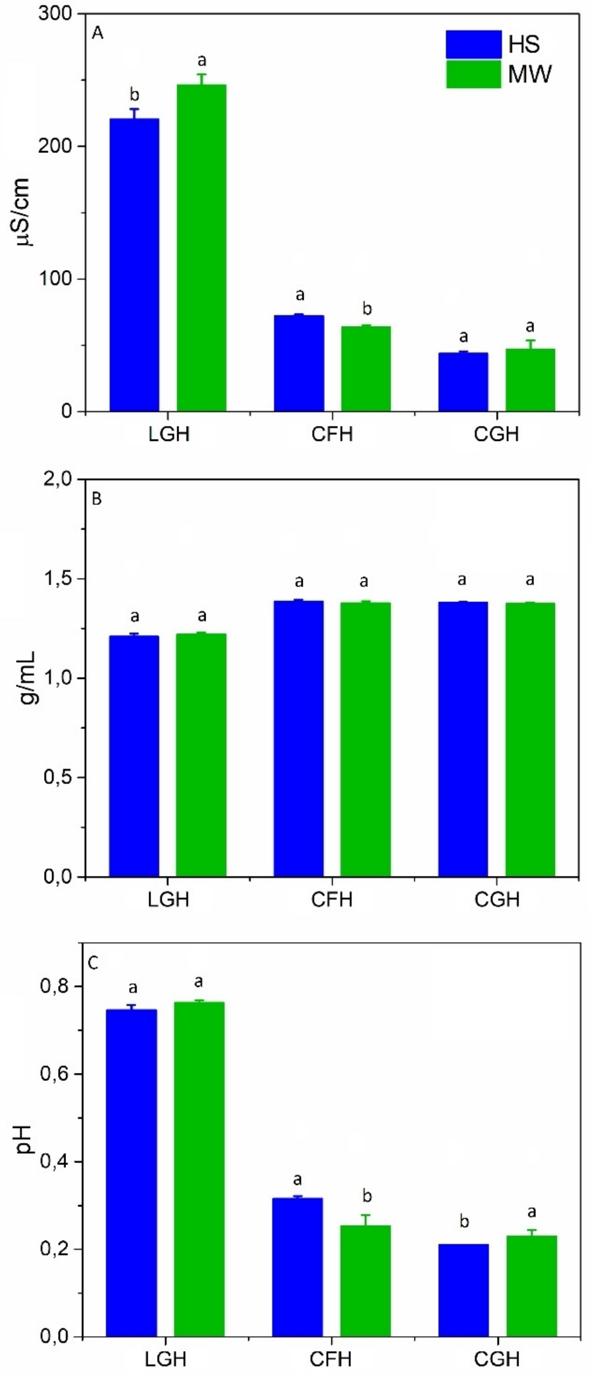

Figure 1. Physical properties comparing the two synthesis methods. Conductivity (A), density (B) and operational pH (C).

Table 2. Extraction performance from *L. divaricata* expressed in peak area.

Extraction solvent	Alkaloids			Phenolic compounds		
	Theophylline	Piperine	Harmaline	Naringenin	Apigenin	Caffeic acid
HS-LGH	2.080 $\pm$ 0.021	2.971 $\pm$ 0.028	1.490 $\pm$ 0.015	0.864 $\pm$ 0.020	7.384 $\pm$ 0.010	0.700 $\pm$ 0.004
MW-LGH	1.621 $\pm$ 0.009	2.180 $\pm$ 0.015	1.420 $\pm$ 0.007	0.860 $\pm$ 0.009	6.761 $\pm$ 0.009	0.602 $\pm$ 0.006
MeOH	0.070 $\pm$ 0.001	2.421 $\pm$ 0.023	0.834 $\pm$ 0.022	0.480 $\pm$ 0.003	5.370 $\pm$ 0.002	0.223 $\pm$ 0.030
H_2O	2.143 $\pm$ 0.025	ND	0.293 $\pm$ 0.006	ND	ND	0.810 $\pm$ 0.020

Values are means $\pm$ SD
ND: non detected
n = 3

synthesis procedures. Nevertheless, significant differences were obtained for the conductivities of LGH and CFH and pH values for CFH and CGH. This could be due to different arrangements formed in the supramolecular structure of the MW-NADES. Structural analysis and theoretical studies should be carried out in order to identify such differences.

Additionally, the stability was evaluated every 24 h. Solvents were considered stable when the mixture remained in the liquid phase. A single liquid phase was observed at least during 120 days at room temperature for both MW-NADES and HS-NADES.

Interestingly, NADES can form hydrogen bonds with solutes, which enables the development of different analytical applications.[8] NADES have received growing attention for the extraction and separation of analytes from natural sources.[21–23] The latter is attributed to their high solubilization and stabilization abilities of both non-polar and polar compounds. High viscosity of some NADES causes severe errors during injection step in the HPLC, as well as decreases of extraction efficiency. In this sense, LGH was selected for further experiments due to its lower viscosity. Thus, the efficiency of MW-NADES as extraction media of bioactive compounds from complex plant matrices was evaluated and compared with HS-NADES and traditional solvents (MeOH and H_2O). Representative phenolic compounds and alkaloids from *Larrea divaricata* were extracted using these solvents and determined by HPLC-DAD by the approach described in the experimental section. The results obtained are shown in Table 2. Similar extraction yields were obtained for NADES prepared by both approaches. Also, high extractability for polar as well as weak polar compounds was observed for both MW-LGH and HS-LGH, compared to methanol and water.

In previous works, it has been demonstrated that NADES are enhancers for electrochemical detection of phenolic compound.[13,14] This effect is due to the interaction of the analyte with the supramolecular structure of NADES, providing a suitable environment for electron transference. In order to challenge MW-NADES, electrochemical measurements were performed at room temperature, following the conditions previously reported by Gomez et al.[14] with some modifications. Oleuropein (OLE) and quercetin (QR) were chosen as target analytes for the comparison of the two NADES (HS-LGH and MW-LGH). Results are shown in Figure 2. As can be seen, a NADES enhancement (up to 11.31 times) was achieved. Interestingly, MW-NADES showed higher sensitivity than HS-NADES for the two analytes under study. The latter could be explained by a different super-lattice formed in the MW-LGH from that in the HS-LGH which could be changing the electron rate transference. Further studies are needed to confirm this finding and explore the physicochemical parameters related to this behavior.

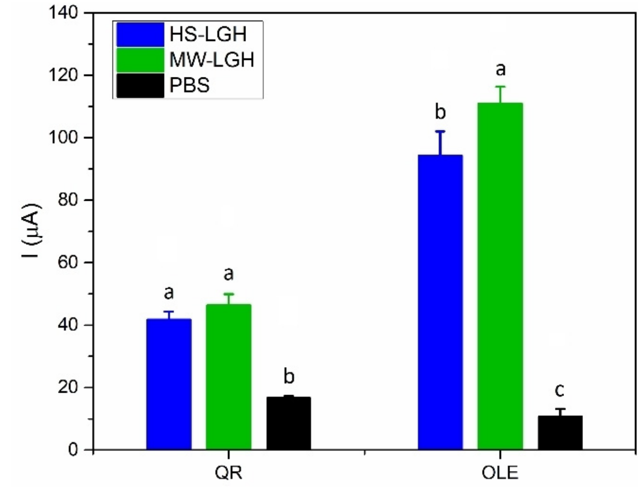

Figure 2. Peak intensity enhancement (µA) for 10 ng mL^{-1} of QR and OLE in Phosphate buffer solution (PBS) at pH 2.5 and 9 respectively. LGH addition was 10%.

The main goal of this work was to develop a greener method for NADES preparation with the purpose to optimize this process according to Green Chemistry principles. An environmental friendly microwave-assisted approach for the preparation of NADES in the shortest time to date is proposed. Comparing to traditional HS process, the MW method has outstanding advantages as the dramatic decrease of synthesis time and energy consumption.

Our results contribute to consolidate the movement of the "solvents of new era" that began at the early 90s with surfactants and ionic liquids. Further studies are needed to test the use of microwave for the preparation of eutectic mixtures containing other NADES components such as choline chlorine and urea, glycerol or amino acids. Indeed, the molecular structure of MW-NADES should be studied to definitely establish Natural Deep Eutectic Solvents for the replacement of conventional toxic solvents.

Supporting Information Summary

Supporting information includes the experimental details of the methodologies proposed in this communication, such as extraction procedure, HPLC and electrochemical detection conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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