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Credit authorship contribution

Ezequiel Vidal: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization.

Anabela S. Lorenzetti: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization.

Federico J.V. Gomez: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization.

Maria Fernanda Silva: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration.

Claudia E. Domini: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration.
Brand New Dual Absorption and Emission Smartphone-Based Spectrophotometer (DAESS) for the Study of the Role of Water in the Preparation of Natural Deep Eutectic Solvents

Ezequiel Vidal¹, Anabela S. Lorenzetti²,³, Federico J.V. Gomez², María F. Silva²* and Claudia E. Domini¹*

¹INQUISUR, Departamento de Química, Universidad Nacional del Sur (UNS)-CONICET, Av. Alem 1253, 8000 Bahía Blanca, Argentina.

²Instituto de Biología Agrícola de Mendoza (IBAM-CONICET) Facultad de Ciencias Agrarias Universidad Nacional de Cuyo Almirante Brown 500, Chacras de Coria, Mendoza, Argentina

³Departamento de Química, Universidad Nacional del Sur (UNS)-CONICET, Av. Alem 1253, 8000 Bahía Blanca, Argentina.

Abstract

Natural Deep Eutectic Solvents (NADES) are highly important for Green Chemistry principles and can be used instead of harmful organic solvents. Indeed, nowadays smartphone-based analytical devices can replace some traditional laboratory equipment. In the present work, a smartphone based dual spectrophotometer and spectrofluorometer device was designed, 3D manufactured, and validated. A resolution of 0.241 ± 0.010 pixel nm⁻¹ and a stability comparable with commercial instruments were obtained. Using the proposed device it was possible, for the first time, to study the role of water in NADES (fructose:urea:water) preparation, by testing the influence of structural and dilution water. In this sense, it was observed that when water was added before NADES preparation (integrated into the superstructure of the solvent), fluorescence and absorbance intensities sharply decayed (up to 90% and 95%, respectively). In contrast, dilution water had minor effects on spectroscopic features of the eutectic system, which was expressed as 29% and 23% of diminution of signal intensities for both techniques. The obtained results suggest that the moment the water is added plays a significant role in NADES properties.

Keywords: DUAL ABSORPTION AND EMISSION SMARTPHONE-BASED SPECTROPHOTOMETER; NADES; ROLE OF WATER.

*Corresponding author: mfsilvamb@gmail.com; claudia.domini@uns.edu.ar
Introduction

It is well known that human activity around the world has been gradually, constantly and favorably changing in terms of environmental preservation to counteract past behaviors that have had a significant impact on climate change. Caring for the environment requires a more sustainable and less polluting Analytical Chemistry, guided by the principles of Green Chemistry [1]. From the Analytical Chemistry point of view, de la Guardia and co-workers have introduced the concepts of Green Analytical Chemistry (GAC) and Democratic Analytical Chemistry (DAC), with the aim of raising awareness and motivating the scientific community to develop greener and eco-friendly analytical methods [1]. Lately, we have witnessed a considerable evolution of GAC with more energy-efficient and sensitive instruments, together with miniaturization already adopted by researchers. Nevertheless, solvents still have a great impact on method sustainability [2].

One of the key aspects in Green Chemistry is to design new green solvents to substitute hazardous ones. In this sense, Natural Deep Eutectic Solvents (NADES) are a new generation of solvents that have emerged in the last decade as promising green means. They are constituted by metabolites that are present in most types of cells and organisms [3,4]. The most common components are sugar (glucose, sacarose, fructose, etc.); organic acids (lactic, malic, citric acids, etc.); urea and choline chloride. NADES are of great interest in several areas of research and industry [5]. Readily available with low cost components, easy preparation, biodegradability and biocompatibility are some of the advantages that NADES present. Indeed, they show excellent physicochemical properties: a wide range of liquid state, high solubilisation capacity, adjustable viscosity and polarity. There is scarce information concerning the spectroscopic features of NADES [6]. Recently, the fluorescent properties of NADES have been reported by our group [7] opening interesting perspectives for NADES applications.

The addition of water in NADES can be used to optimize their selectivity, and adjust their polarity [8]. However, in most cases, water is added to decrease the high viscosities and improve their solubilization skills [9]. For this reason, several authors have studied the effect of the addition of water in NADES preparation, where it could be added either as a component or after preparation as a diluent [10,11].

According to DAC perspective, portability will become one of the new means of fast and widespread on-site data production, without the use of sophisticated and expensive laboratory instruments. Additionally, with the advancement of 5G technology, we are getting closer to being able
technology [13–15]. The use of smartphones and 3D devices has allowed the adaptation of classical photometric techniques to this perspective although, adapting these versatile instruments to the wide range of smartphone models remains a challenge.

Spectrophotometers and smartphone-based fluorometers have previously been reported [16]. The ability of CMOS (Complementary Metal–Oxide–Semiconductor) smartphone sensors to adequate sensitivity represents the main advantage in order to join spectrophotometry and spectrofluorometry in a single portable analytical instrument. The combination of these techniques simplifies the acquisition of data, not only for isolated determinations, but also for merging the results obtained by both techniques.

Aiming to develop a low-cost, portable and versatile dual spectrometer (spectrophotometer and spectrofluorometer), this manuscript describes the design, fabrication, evaluation and application of a 3D printed universal dual instrument, based on smartphone sensing. Once the device was validated, it was used to measure fluorescence emission and molecular absorption in order to understand the main role of water in NADES preparation and its properties. The features of this outstanding device led to obtain spectroscopic profiles of different eutectic systems prepared by adding the same amount of water before and after NADES preparation.

Experimental
Reagents and solutions
Rhodamine B (Pro Analysis) and methylene blue (Pro Analysis) were purchased from Merck (Germany). Urea (≥99.5%) and fructose (≥99.0%) were acquired from Anedra (Buenos Aires, Argentina). All aqueous solutions were prepared using ultrapure water (18.0 MΩ.cm), obtained from a Milli-Q system (Millipore, Bedford, USA). All chemicals used were of analytical grade. Rhodamine B and methylene blue validation solutions were 50 mg.L⁻¹. The rhodamine B solution was prepared by dissolving 0.0025 g of solid drug in 50.00 mL of ethanol. Methylene blue solution was prepared with 0.0050 g of the dye and adding water to 100.0 mL.

NADES preparation
The composition of NADES (FUH) was fructose:urea:water at 1:1:2 molar ratio [17]. FUH was selected due to its strong native fluorescence. For this purpose, 5.40 g of fructose, 1.80 g of urea and 1.08 mL of water were placed in a 50 mL conical tube. The preparation was carried out using an ultrasonic probe, applying ultrasonic cycles (20 s on- 10 s off) at 70% of amplitude (91 W of
In the case of the dilution study, different amounts of water were added (after preparation) to FUH. In order to obtain D 1.25, 0.27 mL were incorporated, and then vortexed during 1 minute. In the same way 0.54 mL, 0.81 mL and 1.08 mL were added to obtain D 1.5, D 1.75 and D 2, respectively (See S1 for a complete description).

**Instrumentation**

The Dual Absorption and Emission Smartphone-Based Spectrophotometer (DAESS) was designed using Autodesk 123D Design software (San Rafael, CA, USA). For the manufacture, a Geeetech Prusa I3 Pro b 3D printer was used (Shenzhen Getech Technology Co. Shenzhen, China) for printing the designed parts, employing a Grilon3D’s 1.75 mm PLA (Polylactic acid) printing filament (NTH, Chivilcoy, Buenos Aires, Argentina). Ultimaker Cura v4.5.0 (Gelndermalsen, The Netherlands) was used as printing software.

The proposed instrument was equipped with a white LED (0.1 W, 5 mm, Patagoniatic, Buenos Aires, Argentina), a UV LED (390 nm, source 5v x 2000 mA, series resistance 5.3 ohm, 3 W, voltage 3.6 V, 250 mA, Epileds, Buenos Aires, Argentina), a glass microscope slide and a Rainbow Symphony diffraction grating with 1000 lines per millimeter (Reseda, CA, USA). A 10 mm optical path plastic (Poly (methyl methacrylate), PMMA) cuvette was also used. The calibration of the developed spectrophotometer was carried out using two lasers, a green and a red one, both from the same supplier (Zhang Kun, Model TYLaser 303, 532 nm and 650 nm, 100 mW), and a violet led (390 nm, 0.06 W, Patagoniatic, Buenos Aires, Argentina). A Motorola Moto G4 Plus, equipped with a 16 megapixel camera was used for image capturing.

An Agilent 8453 spectrophotometer (Agilent technologies, CA, USA) and a Jasco Spectrofluorometer FP6500 (Maryland, USA) with a xenon discharge light source (150 W), were used for the validation study.

For NADDS preparation, a Sonics Vibra Cell VCX 130 ultrasonic probe (titanium probe tip of 9.5 mm diameter, 20 kHz frequency, 130 W nominal power) and a Vortex Velp Zx3 were employed.

**Device configuration**

The presented DAESS was made up of three functionally differentiated parts (Figure 1): a stand or base, an adjustable holder to adapt to any smartphone model (previously developed by our group [18]), and the spectrometer itself. The stand provided comfort and stability for the use of


Figure 1: Layout diagram of the proposed instrument. A convergent lens (a), a glass slide (b), a diffraction grating (c), a light path (d), a slit (e), a cuvette holder (f), a collimator (g), a white LED (h), an UV LED (i). A complete image of the device can be observed in the inset (j).

The spectrometer was made up of a 1000 lines/mm diffraction grating (Figure 1a), a 1.0 mm slit (Figure 1b), a cuvette holder (Figure 1c) and two windows for radiation entry: one at 180° from the diffraction grating with a 2 mm collimator (Figure 1d) equipped with a white LED (Figure 1e),
slide (Figure 1h) was placed ahead of the diffraction grating to hold and straighten it. Between the glass and the camera, a 25 mm diameter convergent lens with a focal length of 120 mm was placed to improve focus at short distance (Figure 1i). The device measured 170 mm x 120 mm x 110 mm and weighed 110 grams (based on selected print settings).

The spectrometer parts were made of black PLA to avoid external light. To cover the cuvette, a black lid was manufactured.

For the preparation of the device, the smartphone was first positioned placing the camera in front of the diffraction grating. Then, the white LED was connected to the mobile phone with a micro-USB cable provided with alligator clips. With the camera on, the decomposed spectrum was aligned into the frame (Figure S2). The Moto G4 Plus native camera application was set in manual mode in order to obtain reproducible captures. The wide range of configurations provided by the manufacturer allowed many settings with different sensitivities. Once the camera was aligned it was possible to select the light source (frontal white LED or lateral UV LED) for the determinations.

**Image capture**

Capturing images required prior optimization. Due to differences in intensities received by the detector, studies were carried out with different camera sensitivities, set according to the light intensity generated in the phenomenon to be studied (spectrofluorometry or spectrophotometry).

In order to capture the spectral absorption phenomenon once the sample cuvette was placed, the white LED was switched on and the phone camera was set with the focus at infinity, the white balance in cloudy mode, the shutter speed at 1/1500 s and the ISO value at 100 (the lowest value allowed by the smartphone used). Three successive captures were made then, to be averaged.

Subsequently, the white LED was switched off, the UV LED for fluorescence was switched on, and the aforementioned process was completed again. The cell phone camera for fluorescence measurements was set with a shutter speed in 1/6 s and 400 of ISO value. All captured images were saved in JPEG format.

**Image processing**

Once the images were captured, the absorption and emission images were separated. ImageJ and MATLAB were used for extraction and quantification.
(Figure S2) and positioned, according to a previous work [18]. This ROI was centered with respect to the image spectrum and the 25 vertical pixels were averaged to obtain a single value. Then, the intensity results were saved in a text file, and finally they were processed and analyzed using a Microsoft Excel spreadsheet.

Results and Discussion

Instrument performance

Calibration

In order to calibrate the spectrophotometer, a violet LED and green and red laser pointers were used, which were placed in the position of the white LED. For every light source six pictures were captured and averaged. The obtained information was plotted as seen in Figure 2. Taking into account the nominal wavelength of the lasers (provided by the manufacturer) and the distance in pixels between the peaks of each curve (Table 1), a regression model was applied. The resulting linear correlation was Wavelength = (0.241 ± 0.010) pixel number + (360.8 ± 8.2), R²= 0.996, then, a theoretical resolution of 0.241 nm per pixel was obtained (see S3). After that, all the curves could be extrapolated and plotted in relation to their wavelength.
Figure 2. Device calibration. Spectra of each light source can be distinguished, referring to its nominal wavelength. The graphic shows both the pixel number and the wavelength relation.

<table>
<thead>
<tr>
<th>Table 1. Relation between wavelength in nanometers and pixel number.</th>
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<tbody>
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<td><strong>Wavelength (nm)</strong></td>
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<td>Violet LED</td>
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<tr>
<td>Green laser</td>
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<tr>
<td>Red Laser</td>
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</table>

**Validation**

Using the aforementioned information, the DAESS was validated separately for both techniques with two commercial instruments: an Agilent UV-VIS spectrophotometer and a Jasco Spectrofluorometer. The experiments were performed using two dyes: methylene blue, used as a comparative model for spectrophotometer validation, and rhodamine B for fluorometry.

The absorbance graphics were constructed with the data obtained with the spectrophotometer. A calibration curve of methylene blue was prepared, measured, and built (Figure S4). The absorbance at 635 nm was graphicated in a calibration curve obtaining the equation \( A = (0.0501 \pm 0.0018) [\text{methylene blue conc.}] + (0.165 \pm 0.005) \), the determination coefficient was 0.993 with a linear range from 0.39 to 6.25 mg.L\(^{-1}\) (Figure S5).

The emission spectra were obtained using a UV LED (390 nm) for their excitation (Figure S6). The calibration curve obtained for fluorescence (Figure S7) was an equation of the form \( F = (1.08 \pm 0.04) [\text{rhodamine B Conc.}] + (28.54 \pm 2.10) \), with a coefficient of determination \( R^2 = 0.993 \), and with a linear range between 11 and 110 mg.L\(^{-1}\).

The \( t \) test was performed comparing the analytical signals of five different standard solutions (rhodamine B for fluorescence and methylene blue for spectrophotometry) within the ranges 0.08 to 2 mg.L\(^{-1}\) for fluorescence and 0.2 to 2 mg.L\(^{-1}\) for UV-VIS, over a DAESS data acquisition.
were no significant differences between the slopes, neither for molecular absorption nor fluorescent emission.

Stability
The intensity of the light source was studied in function of time lapse to evaluate its stability. For this purpose, a cuvette with deionized water was introduced into the device, the white LED was switched on and every 5 minutes 3 pictures were captured during 70 minutes. The results can be observed in Figure S8 and Figure S9. The relative standard deviation (%RSD) in the range from 390 nm to 640 nm was 0.068% (corresponding to 500 nm). This test represented a variation of 0.0030 A in one hour.

Similar studies were carried out with the UV fluorescence LED. Taking into account the heat given off by the LED (even with a heat sink) the studies were performed during 25 minutes, capturing a picture every 2 minutes. The graphs are shown in Figure S10 and Figure S11, where it can be seen that the %RSD was 0.179% (corresponding to 570 nm).

Both spectrophotometric and fluorometric stability of DAAES can be compared with commercial instruments (See Electronic Supplementary Information). These results confirmed that the use of the DAESS is suitable for accurate analytical results.

Application study: influence of structural and dilution water in NADES
Several attempts have been carried out in order to correlate the amount of water in NADES with their physio-chemical properties [19–21]. Water content acts as an additional H bond donor and acceptor, modifying interactions between DES components, and strongly affecting crucial physicochemical properties and phase behavior.[22]

A high correlation between the content of water and properties such as polarity, viscosity and extraction capacity have been demonstrated. Nevertheless, there are two ways to add water to NADES, one as a structural component (added before preparation) and the other one as dilution water (added after NADES synthesis). It has already been demonstrated that when water is added as a structural component, it plays the role of an additional HBD or HBA [20,23]. However, the effect of water on the optical properties of NADES has been poorly studied. So, as first approximation, its influence was analyzed by means of fluorescence and absorption signals as response.

Figure 3A shows the fluorescence spectra and Figure 4A the absorption spectra for the original amounts of water in furan monomer and NADES (FHU, 1:1:2). Both figures exhibit the increasing...
original (S 2, 1:1:4). In the case of the dilutions (Figure 3B and Figure 4B), the same criterion was adopted, being D 1.25, D 1.5, D 1.75 and D 2 the amount of water added after the preparation step. As it can be seen (Figure 3C, Figure 3D, Figure 4C and Figure 4D), structural water has a notorious effect on fluorescence and absorption signals in comparison with the dilution one. The percentages of decrease of the fluorescence and absorption signals when increasing the structural and dilution water were calculated, showing decays of 90% (Figure 3C), 29% (Figure 3D), 95% (Figure 4C) and 23% (Figure 4D). Thus, demonstrating that water plays a very important role in the structure of NADES. These results are complementary to previous ones [23]; where the role of water on the super molecular lattice of NADES should be considered whenever NADES is prepared. Additionally, a control experiment (Figure S12) was performed in order to demonstrate that the energy employed (ultrasound or vortex) after water addition (dilution water) does not affect the spectra obtained.

From the obtained results, it can be observed that when water is part of the structure of NADES, both fluorescence and absorbance change depending on the amount of water, since it is integrated into the superstructure of the solvent [20]. On the other hand, when water is added as a diluent, both the fluorescence and the absorbance are almost unchanged because the structure of NADES remains stable and the weak effect observed is only due to dilution. Taking this into consideration, water becomes part of the supramolecular structure of NADES only when it is added as a component (before preparation), thus changing the physical properties of the solvent depending on the proportion. When water is added after the solvent preparation it acts only as a diluting agent and thus the properties do not change considerably. It has already been demonstrated the potential of NADES as mobile phase component in HPLC [24] and CE [25]. The possibility of tailoring the viscosity of NADES by adding water could have a great impact on the use of eutectic solvents in separation science.

Although additional studies are necessary, it is important to highlight that with a simple and inexpensive approach, it is possible to make a solvent screening, searching for specific properties. For example, to obtain a solvent with less viscosity that maintains its fluorescent properties, it is convenient to perform a dilution instead of preparing a solvent with more water as base component.
Figure 3: Fluorescence spectra of structural (A) and dilution (B) water study. Fluorescence intensity comparison at 570 nm for structural (C) and dilution (D) water.
Conclusion

Democratic Analytical Chemistry demands new and accessible approaches that could be within everyone’s reach. In this context, the design, calibration and validation of a 3D printed device for fluorimetric and spectrophotometric determinations based on image detection with a smartphone was successfully achieved. The proposed device can function both as a fluorometer and a spectrophotometer. DAESS exhibited remarkable stability and much better resolution than previously reported devices (0.2411 nm.pixel⁻¹ to 0.42 nm.pixel⁻¹)[16,26] representing an ultra-low cost (less than U$S 2.5, without the smartphone) option for spectrometric measures. These results, added to the advantages of iterative manufacturing and portability make it an extremely attractive low-end device to be used in developing countries. The functionality of the device was demonstrated by determining the influence of water as a structural component or a dilution solvent in NADES. To the best of our knowledge, this is the
fluorescence and absorbance signals. However, if water is added as a dilution agent, both fluorescence and absorbance are almost unchanged.

The systematic study of the optical properties of NADES provides information on the degree of influence of water inside the structure of the solvent. These properties are impacted depending on when water is added. It is of great importance to highlight once again, that this new device represents an excellent option as a low cost platform for an initial characterization of NADES, avoiding the use of more expensive instruments in the everyday decision making at the laboratory. For all the aforementioned advantages, the proposed device constitutes a novelty option, which would highly benefit low-income countries.

Conflicts of interest

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.

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Absorption and fluorescence spectroscopy on a smartphone, Fifth Asia-Pacific

Microwave-assisted extraction of soluble sugars from banana puree with natural
https://doi.org/10.1016/j.lwt.2019.02.052.

smartphone spectrophotometer to develop a time-based analysis for


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<table>
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<th>Wavelength (nm)</th>
<th>Pixel number peak ± SD</th>
<th>Ratio</th>
<th>Relation nm/pixel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Violet LED</td>
<td>390</td>
<td>134 ± 4.1</td>
<td>Violet to green</td>
<td>0.26</td>
</tr>
<tr>
<td>Green laser</td>
<td>532</td>
<td>684 ± 5.5</td>
<td>Violet to red</td>
<td>0.22</td>
</tr>
<tr>
<td>Red Laser</td>
<td>650</td>
<td>1213 ± 3.5</td>
<td>Green to red</td>
<td>0.22</td>
</tr>
</tbody>
</table>
Highlights

- Fully-functional smartphone-based dual absorption and emission spectrometer was built
- The device validation was carried out with conventional laboratory instrumental
- The instrument was fabricated using a 3D printer
- Versatile configuration of the device allows simple fit to any brand and mobile model
- Water role in NADES synthesis was evaluated by fluorescent and absorption spectra
DECLARATION OF COMPETING INTEREST

- There are no conflicts to declare.