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**SYNTHESIS OF METALLIC NANOPARTICLES IN DEEP
EUTECTIC SOLVENTS FOR ELECTROANALYTICAL
APPLICATIONS**

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I dedicate this work,

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List of Abbreviations and Symbols

a	Potential pulse amplitude
A	Ampère (current unit)
A	Electroactive area (cm ²)
[]	Concentration
cm	Centimetre
BRB	Britton-Robinson buffer
C	Concentration (mol cm ⁻³)
C_s	Specific faradaic capacitance (F g ⁻¹)
CB	Carbon black
CV	Cyclic voltammetry
CPE	Carbon paste electrode
D	Diffusion coefficient (cm ² s ⁻¹)
DA	Dopamine
D_{app}	Apparent diffusion coefficient (cm ² s ⁻¹)
DES	Deep eutectic solventes
DMF	N, N-dimethylformamide
DMSO- d_6	Deuterated dimethylsulfoxide
DPV	Differential pulse voltammetry
EDS	Energy dispersive spectrum
EIS	Electrochemical Impedance Spectroscopy
E_{ap}	Anodic peak potential
E_{cp}	Cathodic peak potential
E	Potential
ΔE	Potential difference
ΔE_p	Peak potential difference
ΔE_s	Potential increment
f	Pulse Frequency
F	Faraday constant (96,485 J mol ⁻¹ K ⁻¹)
FT-IR	Fourier transform infrared
GCE	Glassy carbon electrode
g	Grams
H ₂ Q	Hydroquinone
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
I	Current
I_p	Peak current
I_{ap}	Anodic peak current
I_{cp}	Cathodic peak current
I_C	Catalytic current
I_L	Diffusion-limited current in the absence of analyte
IL	Ionic liquids

k^0	Heterogeneous electron transfer constant
k_{app}	Apparent heterogeneous electron-transfer rate constant
k_{cat}	Catalytic rate constant
LOD	Limit of detection
LOQ	Limit of quantification
MWCNT	Multi-walled carbon nanotubes
n	Number of electrons
NMR	Nuclear magnetic resonance
PBS	Phosphate buffer solution
pH	Logarithmic value of hydrogen ion concentration
R^2	Correlation coefficient
R	Molar gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$)
R_{ct}	Charge transfer resistance (Ω)
RB	Riboflavin
RSD	Relative standard deviation
RTIL	Room temperature ionic liquids
SD	Standard deviation
SAED	Selected area electron diffraction
SEM	Scanning electron microscopy
SWV	Square-wave voltammetry
t	Time (s)
T	Thermodynamic temperature (K)
T_f	Freezing temperature
T_g	Glass transition temperature
T_m	Melting temperature
TEM	Transmission electron microscopy
v	Potential scan rate
vs.	<i>Versus</i>
XRD	X-ray diffraction

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Resumo

SÍNTESES DE NANOPATÍCULAS METÁLICAS EM SOLVENTES EUTÉTICOS PROFUNDOS PARA APLICAÇÕES EM ELETROANALÍTICA. Neste trabalho foram desenvolvidas sínteses de nanopartículas metálicas (MNPs) em solvente eutético profundo (DES, *Deep Eutectic Solvents*) para posteriores aplicações em sensores eletroquímicos à base de dois materiais de carbono: *carbon black* (CB) (negro de fumo) e nanotubos de carbono de multiplas paredes (MWCNT, *Multiwalled Carbon Nanotubes*). Primeiramente, foi utilizado um DES a base de cloreto de colina (ChCl) e uréia (U) na razão molar 1:2 (*mol:mol*) como meio estabilizante na síntese de nanopartículas ultrapequenas (USNPs, *Ultrasmall nanoparticles*) (tamanho $< 1,0$ nm) usando uma abordagem *bottom-up* baseada em conceitos solvotérmicos em condições brandas de temperatura (< 100 °C). USNPs de platina e óxido de cério com tamanhos médios de $0,73 (\pm 0,07)$ nm e $0,204 (\pm 0,008)$ nm, respectivamente, foram obtidas utilizando DES. Microscopias eletrônica de varredura (SEM, *scanning electron microscopy*) e eletrônica de transmissão (TEM, *transmission electron microscopy*) foram utilizadas para caracterizar morfologicamente as USPtNPs (nanopartículas ultrapequenas de platina) e USCeONPs (nanopartículas ultrapequenas de óxido de cério). Os resultados revelaram formas aproximadamente esféricas com linhas de emissão características tanto da platina quanto do cério no espectro de energia dispersiva (EDS). O DES a base de ChCl:U foi caracterizado por espectroscopias de infravermelho (FT-IR, *Fourier transform infrared*) e ressonância magnética nuclear (NMR, *nuclear magnetic resonance*), o que indicou sua formação por meio de ligações de hidrogênio entre os precursores. Os estudos de calorimetria exploratória diferencial (DSC, *differential scanning calorimetry*) desmonstraram eventos térmicos de transições vítreas e fusões para o DES e seus precursores. Além disso, sensores eletroquímicos foram desenvolvidos: (i) para determinação de riboflavina (RB) em bebidas energéticas comerciais e fluidos biológicos utilizando as USPtNPs junto com MWCNT; (ii) para determinação de dopamina (DA) em fluidos biológicos usando as USCeONPs com CB. Na determinação voltamétrica da RB, o sensor USPtNPs-DES/MWCNT/GCE alcançou respostas lineares na faixa de $0,02 - 1,2 \mu\text{mol L}^{-1}$, com limite de detecção de $1,8 \text{ nmol L}^{-1}$, bom desempenho para repetibilidade e anti-interferência. O sensor USCeONPs-DES/CB/GCE apresentou uma resposta linear para DA na faixa de $5,0 \times 10^{-7} \text{ mol L}^{-1}$ a $3,2 \times 10^{-4} \text{ mol L}^{-1}$, com limite de detecção de 80 nmol L^{-1} , bons desempenhos de repetibilidade e anti-

interferência. Ambos os sensores foram aplicados com sucesso para determinar os analitos alvos nas amostras de bebidas energéticas e fluídos biológicos. Nesta primeira parte, tanto a síntese das USNPs em DES quanto suas aplicações nos sensores foram bem sucedidas. Em sequência, foi realizada a elaboração de um novo DES hidrofílico à base de ChCl e dietanolamina (DEA), que incluíram as caracterizações físico-químicas, moleculares e térmicas. A partir das análises por DSC, um diagrama de fases foi elaborado para determinar a composição eutética no conjunto das frações, sendo que os resultados mostraram que o ponto eutético do DES estava na razão molar 1:3 (*mol:mol*) com temperatura de fusão de 285,8 K para ChCl:DEA. Caracterizações realizadas por FT-IR e RMN possibilitaram identificar interações entre os precursores utilizados. O DES no ponto eutético foi utilizado para sintetizar AgNPs (nanopartículas de prata) sem a adição de um agente redutor convencional e em condições brandas ($< 100\text{ }^{\circ}\text{C}$). Os resultados das caracterizações morfológicas das AgNPs obtidas por TEM, EDS, difração de elétrons de área selecionada (SAED, *Selected area electron diffraction*) e difração de raio-x (XRD, *X-ray diffraction*), revelaram formatos variados com linhas de emissão características da prata de natureza policristalina e planos cristalográficos de estruturas cúbicas de face centrada. Adicionalmente, as AgNPs foram incorporadas a um filme a base de MWCNT, dimetilformamida e Nafion™. O sensor AgNPs-DES/MWCNT/GCE apresentou aumento significativo nas atividades eletrocatalíticas referentes ao comportamento redox da hidroquinona, boa estabilidade nas respostas voltamétricas, especialmente devido as AgNPs que possibilitaram alto valor de constante heterogênea aparente de taxa de transferência de elétrons (k_{app}) e baixa resistência à transferência de carga (R_{ct}). O coeficiente de difusão (D_{app}) e a constante catalítica (k_{cat}) foram determinados em $(4,1 \pm 0,5) \times 10^{-5}\text{ cm}^2\text{ s}^{-1}$ e $2,8 \times 10^6\text{ mol}^{-1}\text{ L s}^{-1}$, respectivamente. Os resultados apresentados neste trabalho demonstram a importância de caracterizar os DES e que suas aplicações em sínteses ecológicas podem gerar nanomateriais promissores em aplicações de sensores eletroquímicos.

Palavras-chave: Solvente eutético profundo. Diagrama de fases. Nanopartículas metálicas. Sensores eletroquímicos.

Abstract

SYNTHESIS OF METALLIC NANOPARTICLES IN DEEP EUTECTIC SOLVENTS FOR ELECTROANALYTICAL APPLICATIONS. In this work, the synthesis of metal nanoparticles (MNPs) in DES were developed for subsequent applications in electrochemical sensors based on two carbon materials: carbon black (CB) and multiwalled carbon nanotubes (MWCNT). First, a DES-based on choline chloride (ChCl) and urea (U) in a molar ratio of 1:2 (*mol:mol*) was used as a stabilizing medium in the synthesis of ultrasmall nanoparticles (USNPs) (size < 1.0 nm) using a bottom-up approach based on solvothermal concepts, under mild temperature conditions (< 100 °C). Platinum and cerium oxide USNPs with average sizes of 0.73 ($\pm$ 0.07) nm and 0.204 ($\pm$ 0.008) nm, respectively, were obtained using DES. SEM and TEM were used to morphologically characterize the USPtNPs and USCeOxNPs, revealing approximately spherical shapes with characteristic emission lines of platinum and cerium in the EDS spectrum. The ChCl:U-based DES was characterized by FT-IR and NMR spectroscopy, which indicated its formation through hydrogen bonds between the precursors. DSC studies demonstrated thermal glass transition and melting events for DES and its precursors. Furthermore, electrochemical sensors were developed: (i) for determination of riboflavin (RB) in commercial energy drinks and biological fluids using USPtNPs together with MWCNT; (ii) for determination of dopamine (DA) in biological fluids using USCeOxNPs with CB. In the voltammetric determination of RB, the USPtNPs-DES/MWCNT/GCE sensor achieved linear responses in the range of 0.02 – 1.2 $\mu\text{mol L}^{-1}$, with a detection limit of 1.8 nmol L^{-1} , and good repeatability and anti-interference characteristics. The USCeOxNPs-DES/CB/GCE sensor showed a linear response for DA in the range from $5.0 \times 10^{-7} \text{ mol L}^{-1}$ to $3.2 \times 10^{-4} \text{ mol L}^{-1}$, with a detection limit of 80 nmol L^{-1} , good repeatability and anti-interference performances. Furthermore, both sensors were successfully applied to determine the target analytes in energy drinks and biological fluid samples. In this first part, both the synthesis of USNPs in DES and their applications in the sensors were successful. Subsequently, a new hydrophilic DES based on ChCl and diethanolamine (DEA) was developed, which included physicochemical, molecular and thermal characterizations. From the DSC analyses, a phase diagram was elaborated to determine the eutectic composition in the set of fractions, and the results showed that the eutectic point of the DES was in the molar ratio 1:3 (*mol:mol*) with a melting temperature of 285.8 K for ChCl:DEA. Additionally, characterizations performed

by FT-IR and NMR allowed the identification of interactions between the DES precursors. The DES at the eutectic point was then used to synthesize AgNPs without adding a conventional reducing agent and under mild conditions ($< 100\text{ }^{\circ}\text{C}$). The results of the morphological characterizations of AgNPs obtained by TEM, EDS, SAED, and XRD revealed varied shapes with emission lines characteristic of polycrystalline silver and crystallographic planes of face-centred cubic structures. Additionally, AgNPs were incorporated into a film based on MWCNT, dimethylformamide, and NafionTM. AgNPs-DES/MWCNT/GCE sensor showed a significant increase in the electrocatalytic activities related to the redox behavior of hydroquinone, good stability in the voltammetric responses, especially due to the AgNPs, that enabled a high value of apparent heterogeneous electron transfer rate constant (k_{app}) and low charge transfer resistance (R_{ct}). The diffusion coefficient (D_{app}) and catalytic constant (k_{cat}) were estimated to be $(4.1 \pm 0.5) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and $2.8 \times 10^6 \text{ mol}^{-1} \text{ L s}^{-1}$, respectively. The results presented in this work show the importance of characterizing DES and that their applications in green syntheses can generate promising nanomaterials in electrochemical sensor applications.

Keywords: Deep eutectic solvent. Phase diagram. Metallic nanoparticles. Electrochemical sensors.

Summary

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Chapter I

General Introduction

Chapter I || General Introduction

1.1 – Deep eutectic solvents: general aspects

The starting point for developing deep eutectic solvents (DES) goes back to another class of solvents well established in the literature, the ionic liquids (ILs). ILs are composed exclusively of ions, usually derived from molten salts or oxides, with high melting points (*e.g.*, 501.6 K for IL based on 1-benzyl-2-(2-phenyl-2-oxoethyl)-3-methylimidazolium and bromide, [BMPOEI][Br]). In 1914, Walden synthesized the first low-melting temperature IL, ethylammonium nitrate (12 °C), based on concentrated nitric acid and ethylamine [1]. However, only in the 1990s molten salts with melting temperatures below 100 °C started to be explored as “*Room Temperature Ionic Liquids*” (RTIL) due to their stability at room temperature, which made them adaptable to applications such as organic synthesis, electrochemistry and materials chemistry [1,2].

With the rise of Green Chemistry promoted by Anastas and Warner [3], there was a need to research ILs free of metals and compounds based on pyridines and benzenes. These compounds are known for their high toxicity and low biodegradability, raising doubts about the sustainability of ILs. In this sense, DES emerge as an alternative to these types of ILs as a more ecological and economically viable alternative.

Considered a class analogous to ILs, DESs are commonly described as a mixture resulting from the combination of two or more components that, at specific molar ratios, form a fluid characterized by a deep decay of the melting point, when compared to the initial precursors. The various combinations of DES can produce totally, partially, or nonionic liquids. As a result, these solvents have a wide range of compositions compared to ILs [4].

DES was first described in 2001 by Abbot et al. [5]. In this study, varieties of quaternary ammonium salts were heated with zinc and tin chlorides so that the lowest melting points were obtained when choline chloride (ChCl) was used as the ammonium salt. This initial work stimulated the development of a series of new eutectic solvents intended for various applications. Subsequently, the same group (Abbot et al. [6]), in 2003, conducted a research that led to the publication of the first work using deep eutectic solvents formed from eutectic mixtures between amides (*i.e.* urea and thiourea) and ChCl, which presented a deep decay in the freezing point when compared to the melting

temperatures (T_m) of their precursors ($T_m(\text{urea}) = 134\text{ }^{\circ}\text{C}$ and $T_m(\text{ChCl}) = 302\text{ }^{\circ}\text{C}$). Urea (U) in the composition of 2 moles to 1 mole of ChCl resulted in a liquid at room temperature with a freezing temperature (T_f) of $12\text{ }^{\circ}\text{C}$ (Figure 1). Among the amides studied, this was the combination that presented the greatest decay in the T_f , and for this reason, it became one of the most classic systems of this class of solvents, as it is also credited with the term “deep” due to the drastic reduction of $122\text{ }^{\circ}\text{C}$ in T_f .

Thus, since this pioneering work was established in the literature, different DES have been developed as a potential substitute for conventional solvents used in various applications. This is because DES is easy to prepare with 100% atomic efficiency, low cost, low toxicity, liquid at room temperature (in most compositions), low volatility, high thermal stability, tunable, and biodegradable [4,7]. These characteristics align with the green chemistry principles established by Anastas and Eghbali [8], making it a safer, less hazardous, and designed-for-degradation solvent.

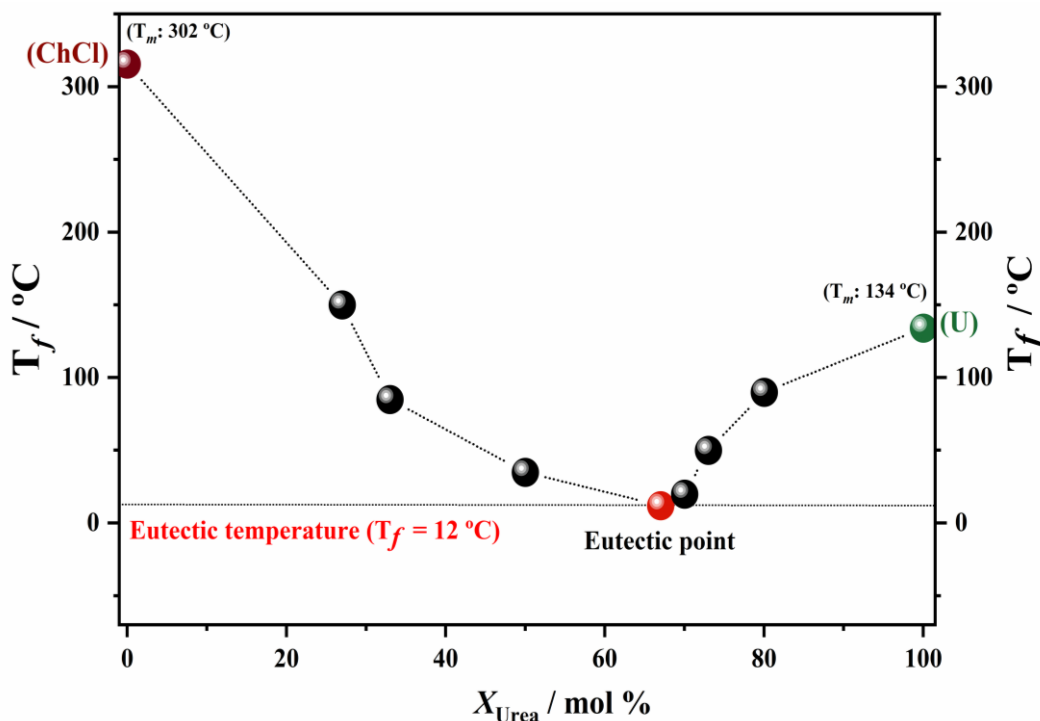


Figure 1. Phase diagram with a freezing point for the mixture of ChCl:U in molar ratio 1:2 (mol:mol). Adapted from Abbot et al. [3].

In general, DES is represented by the following general formula: $\text{Cat}^+ - \text{X}^- \text{zY}$, where Cat^+ is usually an ammonium, phosphonium, or sulfonic cation, and X^- is a Lewis base, usually a halide anion. Complex anionic species are formed between X^- and Y (Lewis or Brønsted acid), and z refers to the number of Y molecules interacting with the

anion [7]. Based on this general formula, these solvents are divided into four main types, as described in Table 1.

Table 1. General classification of DES using a metal chlorine halide (MCl_x) as a base (adapted from Smith et al. [7]).

Type	Formula	Example
I	$\text{Cat}^+ \text{Y}^- z\text{MCl}_x$	M = Zn, Fe, Sn, Al
II	$\text{Cat}^+ \text{Y}^- z\text{MCl}_x \cdot y\text{H}_2\text{O}$	M = Cr, Co, Cu, Ni
III	$\text{Cat}^+ \text{Y}^- z\text{R}^*\text{Y}$	Y = CONH ₂ , COOH, OH
IV	$\text{MCl}_x + \text{RY}$	M = Al, Fe, Zn; Y = COOH e OH

*R = radical with hydrogen bond proton-donating groups.

In some cases, type I, II, and IV DES are similar to ILs due to imidazolium salts with metal halides and chloroaluminates in these groups [7,9]. In contrast, type III DES is formed by the mixture of components known as HBA (Hydrogen bond acceptor), a species that accepts protons from the hydrogen bond, and another called HBD (Hydrogen bond donor), which acts as a proton donor for the hydrogen bond. In short, the most commonly used HBAs include quaternary ammonium salts (choline chloride and tetrabutylammonium salts), and among the HBDs, fatty acids, amides, and alcohols (decanoic acid, urea, and ethylene glycol) stand out [10].

Recently, special classes of type III DES have been described in the literature with classifications according to their constituents, such as Natural Deep Eutectic Solvents (NADES), defined by Choi et al.[11], that are based on metabolites widely found in cells and could form a third type of liquid separate from water and lipids. NADES are naturally occurring cellular constituents that include, for example, sugars, alcohols, amino acids, organic acids, and choline derivatives [12]. Another class called Therapeutic Deep Eutectic Solvents (THEDES) was specified in studies involving bioactive eutectic systems composed of an active drug as one of the constituents [13]. Although the term was not used in the work proposed by Stott et al.[14] in 1998, who prepared a mixture based on menthol and ibuprofen, aiming at increasing skin permeation and developing a transdermal administration system – the literature credits this work as the first application of a THEDES [15]. Finally, the Hydrophobic Deep Eutectic Solvents (HDES) are synthesized through hydrophobic precursors, such as fatty acids, long-chain alcohols, and quaternary ammonium salts. HDES emerged through the need for application in extraction processes in the work proposed by van Osch et al.[16].

Considering the characteristics of each class of DES, various forms of preparation are established in the literature, including heating and stirring, freeze-drying, vacuum evaporation, grinding, twin-screw extrusion, microwave irradiation, and ultrasound-assisted preparation methods [17].

i) Heating and stirring method: this is the most commonly used method for preparing DES. Basically, the component with the lowest T_m is melted first, followed by the addition of the other component with the highest T_m – when both components have high T_m (*e.g.*, higher than 300 °C), they are mixed and heated, resulting in the formation of the solvent [4,10]. However, control of the temperature, heating time, and stirring must be considered since crystal formation over time can be observed in DES under reduced conditions of these variables [18]. On the other hand, under high-temperature conditions, for example, the formation of by-products from the partial or total decomposition of the components was reported [17,18]. In this method, establishing the appropriate stirring time and temperature is essential to ensure the homogeneity of all components in the preparation of DES.

ii) Freeze-drying method: involves adding stoichiometric amounts of the precursors followed by dilution with distilled water to achieve approximately 5% aqueous solution. This solution is frozen at a very low temperature (77 K or 253 K) and then freeze-dried to obtain a viscous and clear liquid [19,20]. The freeze-drying process allows the incorporation of protein-based polymers and complex unilamellar structures of liposomes and microorganisms into the DES. In addition, the freeze-drying method also allows the incorporation of micelles and vesicles that can act as nanoreactors and capsules, allowing for various biological and pharmaceutical applications of DES [17,19,20].

iii) Vacuum evaporation method: it is based on dissolving the precursors in water and then subjecting the system to a rotary evaporation process at 50 °C to eliminate the water. The resulting liquid consists of DES, which is usually dried in a desiccator with silica gel until the mass remains constant [21–23]. This method can be appropriate for preparing sugar-based NADES since the heating and stirring can cause Maillard reactions and/or caramelization, causing the NADES to darken, which may be undesirable. In addition, it is important to consider that to employ this method, the precursors must be soluble in water, and in cases where they have high melting temperatures (*i.e.* amino acids, choline derivatives, etc.), this approach may be a good alternative. On the other hand, the negative

point is the time required to completely remove the water from the system at a temperature of 50 °C, which can be time-consuming [17,22].

iv) Grinding method: mixing the precursors and grinding them in a mortar and pestle under room temperature conditions until an aqueous mixture is obtained. In cases where one of the precursors is hygroscopic (*e.g.*, ChCl), it is recommended to dry it in a vacuum oven at 40 °C and keep it in a Schlenk under vacuum for 4 days [24,25]. In comparative terms in the preparation of DES, studies report the absence of esterification when the grinding method is adopted, while using heating and stirring results in levels of 5 – 30 wt% esterification reported for a DES-based on ChCl and glutaric acid [17,24,25].

v) Twin screw extrusion method: this approach was initially used by Crawford et al. [26] for large-scale DES production to overcome the limitations of the heating and stirring method. The twin-screw extruder (TSE) used in this method consists of two co-rotating or counter screws encased in a stainless-steel barrel, which constitute multiple transport sections that move the materials forward with a kneading section that applies high shear and compression forces as the screw passes. The DES precursors are mixed manually or using planetary ball mills when the quantities are large. In practice, in the DES preparation process, all sections of the TSE are preheated, followed by the addition of the precursors (HBA and HBD) through a feed port. After passing through the entire section, the DES is collected at the end of the TSE and stored under suitable conditions [17,26].

vi) Microwave irradiation method: this technique reduces the time required to prepare DES and, consequently, low energy consumption, making it highly environmentally friendly [12,27]. In simplified terms, the precursors are added at a given molar ratio to a 20 mL vial and irradiated by microwaves for 20 seconds. It is worth noting that the combinations of HBA and HBD must be carefully selected, and variables such as heating time and power must be optimized to avoid side reactions [12].

vii) Ultrasound-assisted method: DES can be prepared using ultrasonic waves [28]. The precursors are mixed in a glass vial, then sealed and placed in an ultrasonic bath for a time varying from 1 to 5 h at a temperature of 60 °C. After this step, the DES is kept for 24 h in the same vial at room temperature to ensure the homogeneity of the mixture. The stability of DES from this method is maintained for several days without crystallization effects in the solvent [17,28].

Given the methods presented, it is important to highlight the need to control the moisture content of the DES after the synthesis process in all approaches. The presence of water can change the physicochemical properties, such as viscosity and conductivity of the prepared eutectic solvent. This moisture-related control is more important for hydrophilic DES since, unlike the hydrophobics (HDES), these solvents mix easily with water [7,10]. However, there are cases in which water is intentionally added to the DES composition. Studies report that introducing small molar fractions of water does not affect ionic diffusion as long as the water molecules are adsorbed in the DES ionic matrix, an important factor in electrochemical applications [29].

In general, DES has been used in several applications due to its tunable characteristics that allow their use in several research areas, as shown in Figure 2. The graph in Figure 2 shows the results of the database from the Web of Science (WoS) for the search term "*Deep eutectic solvent*", categorising 25 distinct areas according to the number of articles published in indexed journals. In quantitative terms, the 20-year time frame (2004 to 2024) accounts for 14,360 articles up to the date this thesis was written (November 2024).

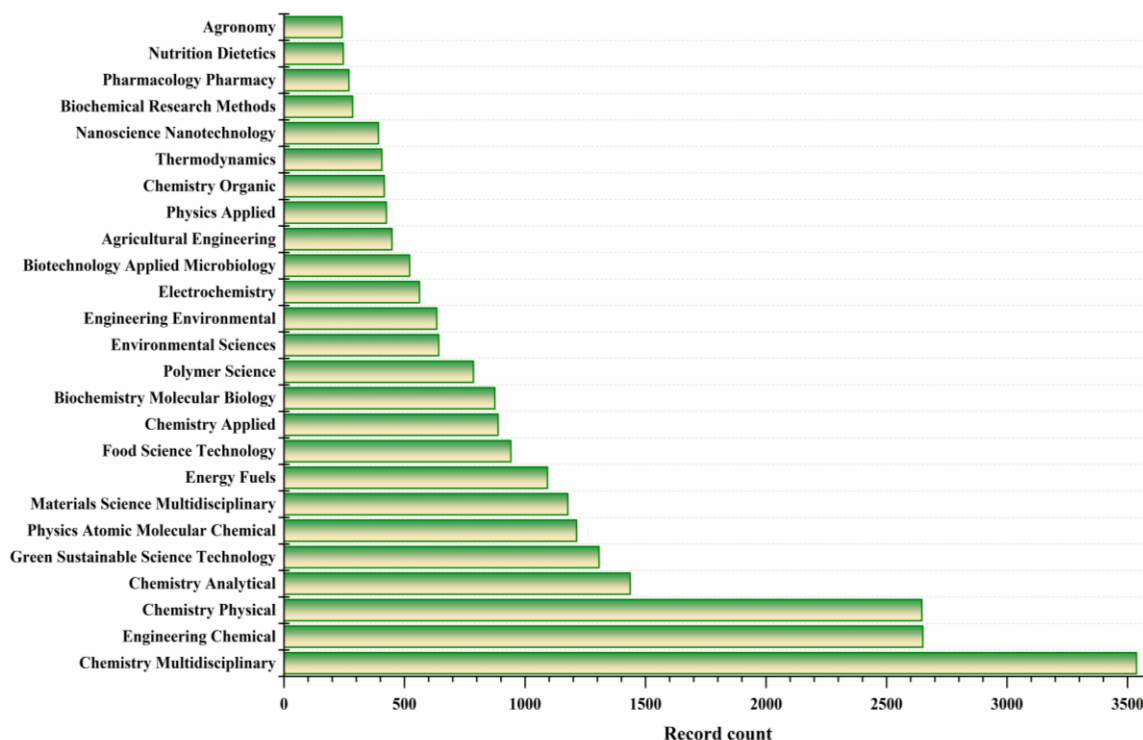


Figure 2. Record of papers using research term “Deep eutectic solvents” in journals in the Web of Science database (period from 2004 to 2024).

It is important to highlight that in 2004, Abbott et al. [30] published the first article using “Deep eutectic solvent”. In August of that year, the work presented a proposal to

develop eutectic systems based on ChCl and carboxylic acids (phenylpropionic, phenylacetic, oxalic, malonic, and succinic) to use them in the solubilization of metal oxides (ZnO , CuO , and Fe_3O_4) and subsequent applications of extraction of the same [30].

The research areas, including *Multidisciplinary chemistry*, *Chemistry physical*, *Chemistry analytical*, *Chemistry applied*, *Electrochemistry*, and *Chemistry organic*, correspond to 66.5% (9,552 records) of the published works (Figure 2). These areas are considered elementary bases for the development of this doctoral thesis together with the application of DES in synthesising nanomaterials, which in this frame represented only 2.75% (395 records) with the term "*Nanoscience nanotechnology*".

Using this same time frame and applying the search terms "*Synthesis of nanoparticles in DES*", the results of the WoS database account for only 1,854 publications, of which 55.6% correspond to *Materials Science Multidisciplinary* with 1,031 articles (Figure 3). As can be seen in Figure 3, DES has been used in several areas for the elaboration of nanomaterials in various applications, and this indicates that there is great interest from the scientific community in developing the synthesis of nanoparticles (NPs) in these solvents.

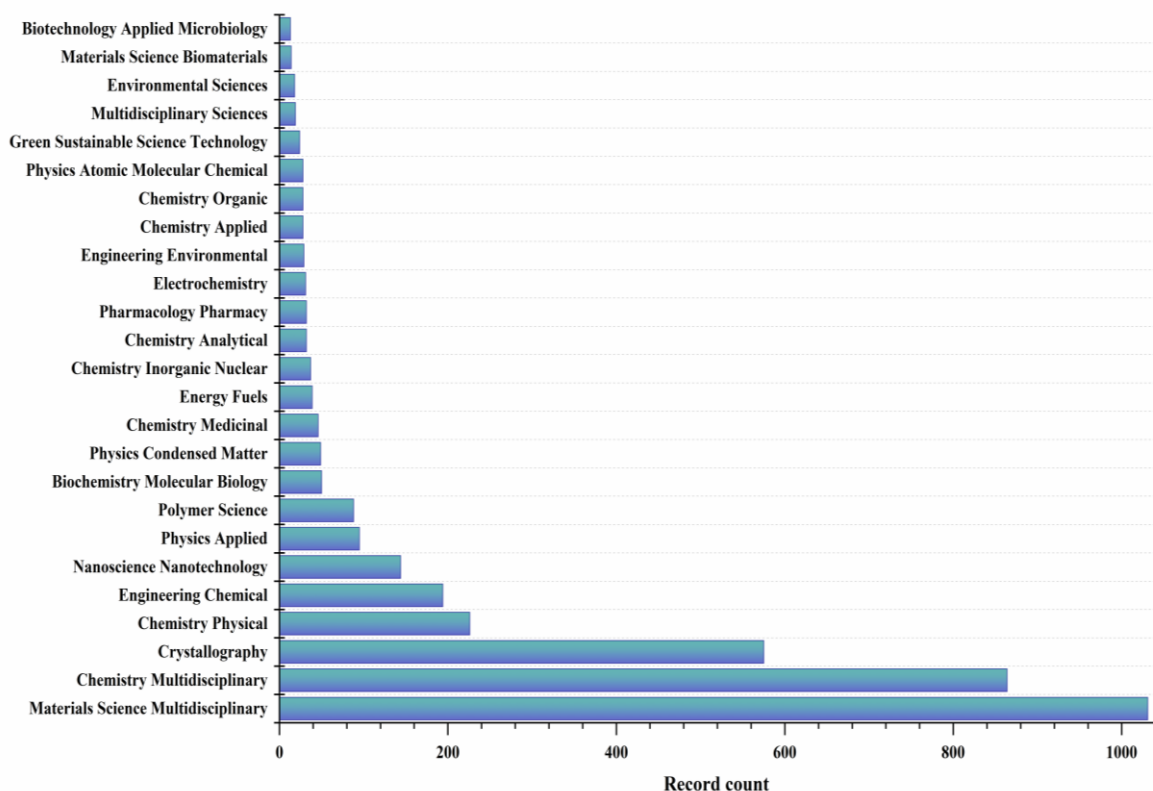


Figure 3. Record of papers on nanoparticle synthesis using DES in journals in the Web of Science database (period from 2004 to 2024).

Analyzing the part involving NPs synthesis and *Electrochemistry* in the selected terms, only 31 records are accounted for in the WoS database, indicating that developing nanomaterials for applications in this area is still little explored. In fact, the first work reporting the use of DES for NP synthesis was published by Liao et al.[31] in October 2008, a shape-controlled synthesis of gold NPs (AuNPs) was developed, obtaining snowflake-like, nanothorns, and star-shaped AuNPs. Furthermore, AuNPs were used to develop an electrochemical sensor to evaluate their electrocatalytic activities in reducing hydrogen peroxide (H_2O_2), with star-shaped AuNPs showing the best catalytic response [31].

From the brief analysis of search terms associated with DES in WoS, it is clear that these solvents have gained notoriety in various research fields during these two decades. Part of this is due to the appeal of approaching the principles of green chemistry established by Anastas and Eghbali [8] and their physicochemical properties of density, viscosity, electrical conductivity, surface tension, polarity, and water content. In addition to these, it is essential to highlight the thermal properties that allow to attest their eutectic identity and how “deep” their decay can be at the melting point of the individual components in relation to their mixtures at a given molar ratio from thermodynamic data (melting enthalpy, $\Delta_m H$) obtained via differential scanning calorimetry (DSC). Another important thermal feature of DES is related to their stability through mass loss as a function of temperature through thermogravimetric analysis (TGA).

Several studies have thoroughly examined the physicochemical properties of DES, correlating them with the type of HBA and HBD used in its composition and their influence on these properties [7,10,32,33]. In general, these properties can be tailored to suit specific applications. As a result, the properties discussed below have been investigated for DES.

i) Density: density is, a crucial physical property for characterizing solvents, and in the case of DES, it can be influenced by factors such as molecular organization, packing and interaction between the components of the mixture [33]. When we talk about eutectic mixtures, the density is generally higher than that of water due to the greater mass of the constituents compared to conventional solvents. As mentioned, this can be related to molecular packing, where the interactions between the components and the arrangement of the molecules result in different densities [33,34].

The phenomenon described involves the theory of the hole or vacancy. In DES, the interactions between the cation and anion of HBA and the ligand of HBD, for example, form a complex network where the HBD molecules occupy positions that create gaps or holes [32]. Commonly, when HBAs are mixed with HBDs, the average radius of these holes can be decreased, which leads to a greater compaction of the structure and, consequently, to an increase in the density of DES compared to pure HBD.

Hole theory is a useful approach to understanding how the organization of DES components can affect properties such as density [35]. Unlike ILs, which can also have similar structures with holes, DES form a network of complex interactions that influence density and other properties such as viscosity and dissolution capacity. Therefore, the difference in density between DES and their pure HBDs largely reflects the modulation of intermolecular forces and the reorganization of molecules in the DES, which can result in a denser and more compact structure [32–34].

Generally, the density of type III DES is expressed as a function of temperature and presents values between 1.0 and 1.3 g cm⁻³ at 25°C [36], while DES based on metal salts (type IV) vary from 1.3 to 1.6 cm⁻³ [33]. Obviously, the values are related to the molar ratios of the HBA and HBD used and the temperature at which density is measured. Thus, the values established for DES as a function of temperature follow the linear relationship [36]:

$$\rho / \text{g cm}^{-3} = \alpha + b (T / \text{K}) \quad (\text{Equation 1})$$

where ρ is the density, T is the absolute temperature, α and b are two constants for the DES molar ratio.

ii) Viscosity: another important and extensively studied property of DES. Most of these solvents generally have high viscosity values at room temperature ($\eta > 100$ mPa.s). The main cause is related to the extensive network of hydrogen bonds that occurs between the HBAs and HBDs that make up the system, which can vary with the molar composition, giving them a very wide viscosity range. Viscosities of the order of 37 mPa.s at 25 °C can be observed for DES based on ChCl and ethylene glycol (EG) at a molar ratio of 1:2 (*mol:mol*) [7]. On the other hand, DES based on sugars present high viscosities; in the literature, 12,730 mPa.s are reported for DES based on ChCl and sorbitol (Sor) at a molar ratio of 1:1 (*mol:mol*) at 30 °C [36,37] and 34,400 mPa.s for ChCl and glucose (Gly) at a molar ratio of 1:1 (*mol:mol*) measured at a temperature of 50 °C [33]. DES based on

metal salts can reach viscosities of 85,000 mPa.s at room temperature [32]. In contrast, HDES based on DL-menthol and octanoic acid (OctA) in a molar ratio of 1:3 (*mol:mol*) can present viscosities of the order of 7.61 mPa.s [38]. Thus, it is evident that the viscosity of a DES is affected by the nature of its components, molar ratio, temperature, and water content [30,33,39].

iii) Conductivity: Due to their relatively high viscosities, most DES have low ionic conductivities (less than 2 mS cm^{-1} at room temperature). In general, the conductivity of DES increases considerably with increasing temperature, which occurs due to the reduction in their viscosity. Thus, an equation similar to the Arrhenius equation can be used to predict the conductivity behavior of a DES [32].

The relationship between temperature and conductivity of DESs was first investigated by Abbott et al. in their study on mixtures of ChCl and urea [6]. Later, these same researchers examined the conductivity of other DES and concluded that it depends significantly on the composition of the mixture [40].

The electrical conductivity of a DES is directly related to the ions present, their mobility and electrical charge. Low conductivity values can be attributed to the limited mobility of the ions due to their large size and/or the formation of ion pairs, which results in a reduced number of available charge carriers [41].

iv) Surface tension: this is one of the essential physical properties of DES, reflecting the tendency of the fluid to minimize its surface area [32,38,42]. In terms of industrial applications, it has been observed that surface tension plays a crucial role in processes such as permeability and bubbling, directly impacting the design of systems such as distillation columns, thermal systems, and heat exchangers [42]. Intermolecular attractive forces influence surface tension, and as the molecular kinetic energy of a liquid increases with increasing temperature, these attractive forces decrease, resulting in a reduction in surface tension [38,42]. In the case of DES, in addition to temperature, the molar proportions of the constituent components are key factors affecting surface tension [42]. The surface tension of DES follows a similar trend to viscosity since it directly depends on the strength of the intermolecular interactions that determine the formation of these mixtures. Specifically, it has been observed that the surface tensions of several mixtures based on ChCl as HBA exhibit a linear correlation with temperature [33,42]. Furthermore, surface tension decreases with increasing ChCl concentration, which reinforces the idea that adding ChCl interferes with glycerol's extensive hydrogen bonding network.

v) Polarity: this is an essential property, as it reflects the general solvation capacity of solvents. Although it is of great importance, the polarity of DES has still been poorly studied. This property is generally estimated utilizing solvatochromic parameters, which analyze the hypsochromic (blue) or bathochromic (red) shift of the UV–vis bands in relation to negative (such as Reichardt betaine) or positive (such as Nile red) solvatochromic dyes, respectively, which is related to the polarity of the solvent [43]. The most commonly used scales to measure polarity include the Dimroth and Reichardt (ET and ETN) scales [43] and the Kamlet and Taft multiparameter scale, which takes into account hydrogen bond donating capacity (α), hydrogen bond accepting capacity (β) and dipolarity/polarizability (π^*) [44,45]. Works have reported the solvation properties of DES systems by studying their solvatochromic response to construct the betaine dye scale (ETN). ETN value of 0.36 was obtained for DES based on ChCl with glycolic acid compared to 0.79 for DES with malonic acid [46,47], thus revealing their different solvation characteristics.

vi) Water content: water significantly influences the physicochemical properties of DES, whether present in small or large amounts. Its presence can help overcome certain limitations of DES, such as high viscosity, while maintaining its unique properties, which explains the growing interest in water-DES mixtures for various applications. However, due to water's high polarity and its tendency to interact with the hygroscopic components of the eutectic system, it is important to investigate how water affects the intra- and intermolecular bonds within the DES network [37]. Although this topic is highly relevant, research on the effects of water in DES systems is still limited, with most studies focusing on ChCl-based eutectics. Some research suggests that adding water can cause the DES to shift into an aqueous solution of its individual components, while other studies indicate that a transition occurs from a "water-in-DES" system to a "DES-in-water" system once a certain hydration threshold is reached [24,32,37].

Among the properties mentioned above, the water content in DES affects all other physicochemical and thermal characteristics. In particular, considerations should be made on determining freezing and melting points with their respective temperatures as a function of molar fractions for binary DES. This is a fundamental discussion to establish the eutectic identity of these solvents.

Thermodynamically, the freezing point (transition from liquid to solid) and the melting point (transition from solid to liquid), both denoted as T_m , are equivalent.

However, the freezing point, also known as the crystallization point, is strongly influenced by kinetics and does not always correspond exactly to the melting point. There are two types of crystallization: crystallization during cooling, in which an amorphous liquid transforms into a more organized solid structure, and cold crystallization, which occurs when an amorphous solid transforms into a crystalline form during heating [48].

The crystallization process involves two steps: forming nucleation centres and crystal growth. In high-purity systems, nucleation is more difficult due to the absence of defects or impurities that can serve as nucleation centres, which makes the system kinetically stable. In this case, the liquid can be cooled below its melting point without forming crystals, remaining in a supercooled state. Furthermore, the crystallization temperature depends on the cooling rate [49]. In rapidly cooled systems, the time available to organize the structure is reduced, resulting in the formation of less perfect crystals and a higher crystallization temperature. On the other hand, when cooling is slower, the rearrangement of the molecules occurs over a longer period of time, leading to the formation of more perfect crystals and a lower crystallization temperature [48,49].

The aspects related to the melting point directly depend on the enthalpy of fusion ($\Delta_m H$) and entropy of fusion ($\Delta_m S$). $\Delta_m H$ has an immediate relationship with intermolecular interactions of atoms and constituent groups of the molecules, while $\Delta_m S$ is totally dependent on the arrangements of the groups that constitute the molecule. In entropic terms, the increasing order of the influence of functional groups on the melting point is as follows: $\text{CONH}_2 > \text{COOH} > \text{OH} \sim \text{NH}_2 \sim \text{I} \sim \text{Br} > \text{Cl} > \text{F} > \text{CH}_3$. Carboxylic acids and amides form stronger hydrogen bonds than molecules of amine and hydroxyl groups (comparable strengths). Consequently, these groups with greater strengths have a higher melting point [50]. As mentioned, $\Delta_m S$ depends on the group arrangements and can be defined as the difference between the entropy of the crystal and the liquid at the melting point, conditioned by the probability of a set of molecules conforming to the crystal, which can be affected by rotational, translational and conformational factors [50,51]. Combining these factors can lead to the formation of crystalline networks and, consequently, to melting. In contrast, systems can also arrange amorphous phases that often form transition states from a more rigid form to a less rigid one or vice versa. This phenomenon in thermal analysis is called glass transitions (T_g).

Finally, we can understand that the formation of a crystal does not necessarily imply the melting of the material, and this explains the divergences in the literature regarding the determination of the eutectic point in DES using freezing point techniques

and the melting point of mixtures. Two of the main works developed by Abbott with DES based on ChCl with amides [6] and carboxylic acids [30] as HBDs present phase diagrams based on the T_f of the mixtures and T_m of the components, providing determinations of the freezing points of the various mixtures, implying the equivalence of T_m and T_f . In contrast, for the same classical system, ChCl:U, in the molar ratio 1:2 (*mol:mol*), other works have reported the absence of thermal events associated with the melting of this composition using DSC analysis [52,53]. In both cases, the amorphous phase's cold crystallization occurs at $-23.1\text{ }^{\circ}\text{C}$ and its melting at $25.3\text{ }^{\circ}\text{C}$. This classical ChCl:U system was used as a stabilizing medium in the synthesis of metallic NPs in this thesis, and in the DSC curves obtained for this DES, no thermal event that characterized a fusion was observed (these results are discussed in the second chapter) [54].

Based on these two classical works, the term “deep” was established as a decay of the order of 100 degrees or more in T_f relative to T_m of the pure compounds when ChCl was mixed with HBDs (urea, thiourea, and oxalic, malonic, and succinic acids). Allegedly, the formation of hydrogen bond complexes between HBA (halogenated ion) and HBD at a specific molar ratio is responsible for the decay [6,30]. However, some works have questioned and presented tangible discussions for the “deep” in DES [55–57]. The authors consider that this term depends on a deviation from ideality that can be calculated based on the molar fractions (x), activity coefficient (γ), T_m , and $\Delta_m H$ of the mixtures of the components [56,57]. Furthermore, in the third chapter of this thesis, a system based on ChCl and diethanolamine (DEA) was elaborated, and all these variables are discussed based on the T_m , $\Delta_m H$, and $\Delta_m S$ obtained experimentally to determine the eutectic nature of the compositions as well as the depth of this system. In addition to elaborating a solid-liquid equilibrium (SLE) phase diagram.

1.2 – Applications of DES in nanoparticle synthesis and electrochemical sensors

The general characteristics of DES previously reported, as well as their appeal as a tunable and more environmentally friendly solvent, make them widely used in various research fields. Above all, they have gained prominence in analytical chemistry with applications involving extraction and microextraction processes of analytes in biological [58], food [59,60], and environmental samples [61]. In addition, they are used in the

modification of electrodes [59], production of electrochemical sensors/biosensors [62,63] and synthesis of metallic nanoparticles [31,64,65].

1.2.1 Nanoparticles synthesis

Nanomaterials have significant applications in different fields, such as environment, food, agriculture, biotechnology, biomedicine, drugs, electrochemistry, among others [66]. The designation “nano” refers to a material (particle) that has extensions of the order of billionths of a meter (10^{-9} m) that can vary in size between 1.0 and 100 nm in two or three dimensions. In Figure 4, the idea of size is illustrated on a scale with images of objects in the order of centimetres (tennis ball) up to the size of atoms in angstroms (Å).

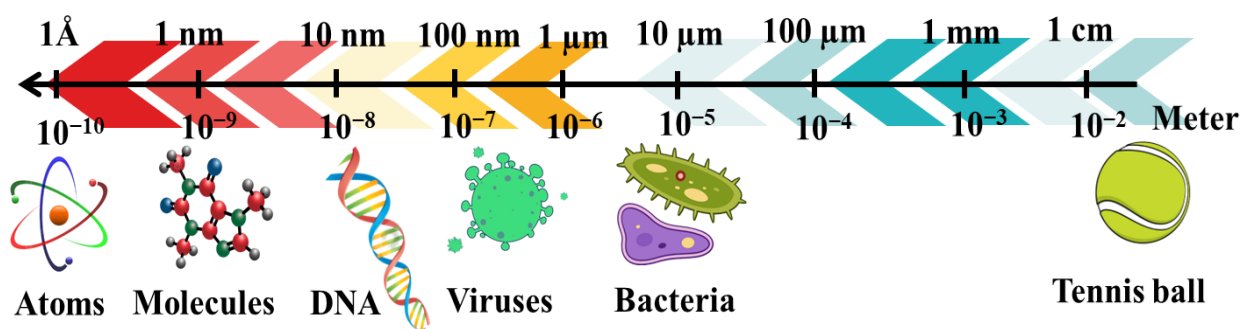


Figure 4. Illustrated scale with metric dimensions (Source: author).

Nanomaterials can be characterized by several parameters, such as dimension, surface chemistry, and crystalline structures [67,68]. Nanoparticles (NPs) can be classified into three types based on their geometry: zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D). 0D-NPs are generally spherical or nearly spherical, with dimensions less than 100 nm. 1D-NPs, such as nanotubes (NTs) and nanowires (NWs), have dimensions greater than 100 nm. 2D-NPs include materials, such as graphene, with atomic thicknesses. In addition to these dimensions, 3D-NPs as polycrystalline structures with dimensions in the microscale (10^{-6} m) [67].

Synthesis methods are fundamental to creating nanostructured particles. Thus, *bottom-up* and *top-down* approaches are already well established in the literature for conventional NP synthesis, with their sub-classifications as illustrated in Figure 5.

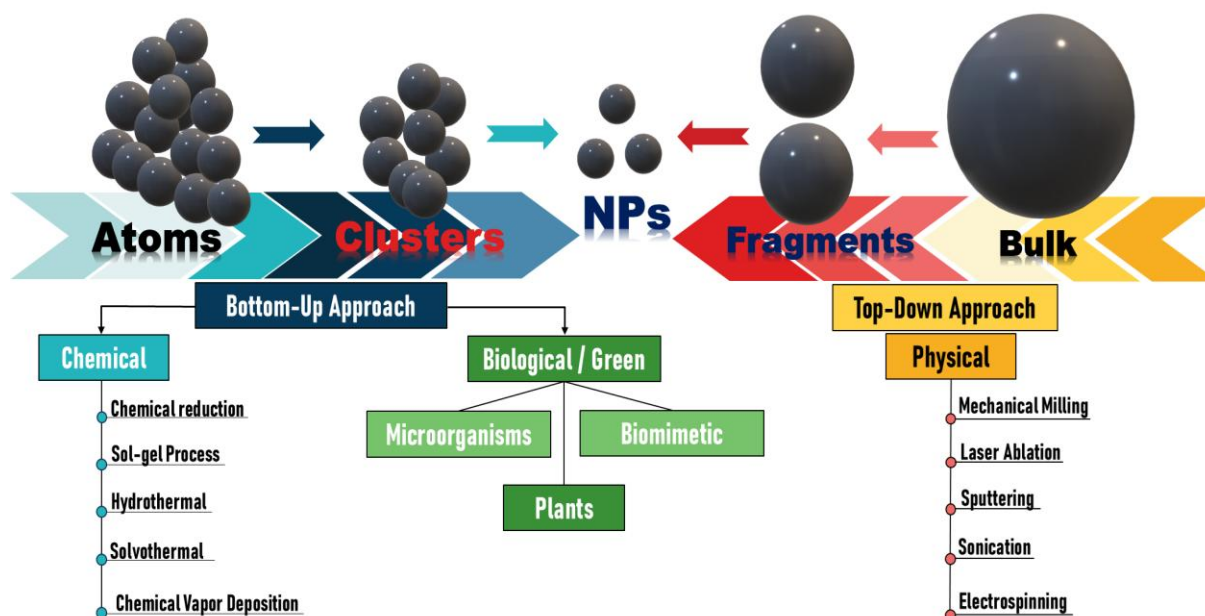


Figure 5. Approaches of NPs synthesis and the difference between top-down vs. bottom-up (Source: author).

As can be seen in Figure 5, *bottom-up* approach elaborates NPs from tiny atoms and molecules through chemical and biological methods (green), while *top-down* bulk materials are fragmented through physical methods. Among the various methods with a *bottom-up* approach, chemical reduction, sol-gel, hydrothermal and solvothermal processes have been widely reported in the literature.

In the chemical reduction method, the ionic salt is reduced in a suitable medium with surfactants using different reducing agents. A common example of a reducing agent is sodium borohydride (NaBH_4), used in an aqueous solution to prepare metal NPs. After formation, the nanoparticles are stabilized with trisodium citrate or sodium lauryl sulfate. In some cases, the stabilizing agent is combined with the reducing agent. In addition, other reducing agents such as glucose, ethylene glycol and sodium citrate can also be used. These methods are widely applied in synthesizing silver and other metal nanoparticles. Proper process control ensures that particles with desired sizes and properties are obtained [69].

The sol-gel method is a method that uses metal alkoxides or metal precursors in solution that are hydrolyzed, condensed and thermally decomposed, forming a stable system. The viscosity of the gel increases with hydrolysis or condensation, and the particle size can be adjusted through the concentration of the precursor, temperature and pH. This environmentally friendly technique offers high-quality materials and efficiently produces nanoparticles and composites [70].

Hydrothermal synthesis uses a wide temperature range, from room to high temperatures. Compared to other physical and biological strategies, this approach offers several advantages. However, at higher temperatures, the generated nanomaterials can become unstable. This method is effective, but its efficiency depends on the thermal conditions. The stability of nanomaterials varies depending on the temperature used in the process [71].

The solvothermal method prepares nanophases using water, methanol, ethanol or polyol. The reaction occurs in a pressure vessel, allowing the solvent to be heated beyond its boiling point. This process facilitates the formation of crystals [72]. The kinetics of crystallization can be accelerated by one to two orders of magnitude using microwaves, in a process called microwave-assisted solvothermal.

In addition to the aforementioned methods, chemical vapor deposition (CVD), electrodeposition, and microwave-assisted synthesis deserve to be highlighted. CVD is a process that creates a thin coating on the surface of the substrate using vapor-phase precursors. The method results in high-quality nanomaterials, such as diamond nanocrystal films with surface roughness between 30 and 50 nm, used to develop boron-doped diamond electrodes (BDDE) [73]. Electrodeposition is a method in which NPs are prepared using electrolytic solutions containing metal salts subjected to specific conditions (pH, current density, and temperature) of electrochemical deposition on the surface of a substrate electrode using voltammetric techniques [74]. Finally, microwave-assisted NPs preparation has become preferred over thermal heating. This technique applies a microwave frequency between 300 MHz and 300 GHz, orienting polar molecules, such as water, with the electric field. The reorientation of these molecules causes molecular friction, generating heat due to energy loss [75].

Another aspect of the bottom-up approach that has gained prominence is the synthesis of NPs mediated by biological organisms and plant extracts [68]. These meet the chemistry principles of creating environmentally friendly processes without the environmental impacts caused by using toxic reagents and generating undesirable byproducts [8]. Green methods generally prefer using non-toxic solvents, reactions in closed systems, cleaner energy sources, such as microwaves and ultrasound, and mild temperature and pressure conditions. In general, several microorganisms, including bacteria, fungi, and algae, are used in the biosynthesis of NPs due to their ability to carry out complex chemical reactions [68]. Bacteria, for example, synthesize extracellular NPs of metals such as gold, silver, and cadmium through reductase enzymes. Fungi, known

for their ability to produce monodisperse NPs, are also widely used due to the presence of enzymes in their cells and better handling. Algae, in turn, use their bioactive compounds, such as pigments and antioxidants, to reduce metal ions and form NPs. Nanoparticle biosynthesis can also be performed with plant extracts, where factors such as extract concentration, pH and temperature influence particle formation. In addition, biomimetic synthesis, which mimics natural biological synthesis processes, is an effective approach, using materials such as proteins, enzymes and cells to create NPs with specific characteristics [76].

On the other hand, the *top-down* approach involves reducing the bulk material into small nano-sized particles through mechanical milling, laser ablation, sputtering, sonication, and electrospinning. Although easy to perform, these methods are not ideal for producing irregularly shaped and extremely small-sized particles that may affect the functionality and applications of the generated NPs [66,68].

Mechanical milling, developed by John Benjamin in 1970, is a process that uses high-energy balls to reduce particle size and modify their surface properties. It is divided into low-energy and high-energy grinding. High-energy grinding is particularly effective for producing nanosized particles and is widely used to synthesize intermetallic nanoparticles. In the process, the powder is placed in a container with heavy balls, and the mechanical energy generated by the rotation of the balls fragments the material [77].

Laser ablation uses pulsed laser beams to reduce particles to nanometric dimensions, with the fragmentation of the material creating nanoparticles dispersed in a colloidal solution, with the efficiency depending on parameters such as the duration and energy of the pulse [78]. Sputtering, in turn, uses high-energy plasma or gas particles to eject microparticles from a solid material, forming nanomaterials with a composition similar to the original material and fewer contaminants [79]. Sonication is essential in creating nanofluids and is performed with ultrasonication or homogenizers, being more effective than ultrasonic cleaning baths, and is used in the processing of nanomaterials such as carbon nanotubes and graphene [80]. Electrospinning, a technique used to create polymeric nanofibers, is improved with the coaxial approach, which allows the creation of core-shell type nanoarchitectures, enabling the development of hybrid materials and hollow polymers [81].

The *bottom-up* approaches mentioned above have been widely used for their simplicity and the possibility of creating NPs with specific characteristics, conditioning them for various applications. Among the effects that can be considered in the synthesis

process, solvents play a structural modelling role through interactions with NPs. This is especially true in the steps involving particle nucleation and growth, where interactions between solvent molecules and the surface of the NPs determine the size and morphology of the final particles [82,83].

These effects vary according to the properties of the solvent, such as polarity, dielectric constant, and viscosity. Water, for example, is widely used due to its low cost and ecological features and can dissolve many metallic salts, reducing agents, and surfactants, making it ideal for synthesizing nanomaterials [84]. Polyol solvents, such as ethylene glycol, are also common, serving as solvents and reducing agents thanks to their hydroxyl groups. On the other hand, solvents with high viscosity and boiling point (oleamines) can act as a solvent, surfactant and mild reducer, in addition to being able to interact with metals due to the presence of unsaturation in their structure [83,84].

In general, in media with high polarity, a strong electric layer forms around the NPs, increasing the zeta potential (sum of the initial surface charge and the accumulated layer) and preventing their aggregation by repulsion. The size and shape of the NPs can be manipulated by the polarity of the solvent, affecting the interactions between the particles [82–84]. In this perspective, the combination of these factors combined with classical wet chemistry methods (illustrated in Figure 5) can lead to a drastic reduction of particle sizes, bringing them to sizes below 5 nm, characterizing them as ultrasmall nanoparticles (USNPs) with unique optical, magnetic, and catalytic properties [85,86].

The first application reported in the literature involving DES in NPs synthesis was proposed by Liao et al. [31]. In this work, DES based on ChCl:U at a molar ratio of 1:2 (*mol:mol*) was used as a stabilizing medium in the synthesis of gold NPs (AuNPs) with controlled shapes at room temperature. The synthesis conducted with DES and L-ascorbic acid (reducer) led to the formation of snowflake-like AuNPs (300 nm), while star- and thorn-like shapes of AuNPs were observed for the addition of water at 5000 ppm and >10000 ppm to the system, respectively. Furthermore, these AuNPs were dispersed on the surface of a glassy carbon electrode (GCE) to evaluate the electrocatalytic potential in reducing hydrogen peroxide (H_2O_2) in a 0.1 mol L^{-1} phosphate buffer solution. The star-type AuNPs exhibited higher electrocatalytic activity than the others due to the high density of stepped atoms on their high-index (331) facets [31]. In practical terms, this work was innovative in two situations: the first controlled synthesis of NPs in DES and applying these NPs in electrochemical sensors.

Since then, other works have been developed using DES in MNP synthesis processes. Chirea et al., used systems based on ChCl:U and ChCl:ethylene glycol (EG), both in a molar ratio of 1:2 (*mol:mol*), to synthesize gold nanowire networks (AuNWNs) *via* reduction by NaBH₄ at 40 °C [87]. AuNWNs presented average sizes of 17.7 and 23.5 nm, respectively. Furthermore, the authors highlight that for both DES, adduct formations between ChCl, AuCl₄⁻, urea and/or ethylene glycol that can stabilize the AuNWNs thought structures [HO-CH₂-CH₂-N⁺(CH₃)₃][AuCl₄⁻•2(NH₂)₂CO and [HO-CH₂-CH₂-N⁺(CH₃)₃][AuCl₄⁻•2(HO-CH₂-CH₂-OH) for ChCl:U and ChCl:EG, respectively.

Gu et al., synthesized tin oxide (SnO₂) NPs by homogeneous precipitation at room temperature in DES based on ChCl:EG at a molar ratio of 1:2 (*mol:mol*) using H₄N₂•H₂O (hydrazine hydrate) as a reductant [88]. SnO₂NPs clusters presented nanocrystallites with average sizes of 4.0 nm, and according to the authors, the average size occurs because the particles can be confined within the DES structures, and crystal growth is suppressed. Additionally, SnO₂NPs were used as an anode for lithium-ion batteries (LIB), which exhibited initial charge and retention capacities of 1045 mAh/g and 58% (after 30 cycles), respectively. Denoting that the material presents a high surface area, making it a promising anode for LIB.

More recently, Baby et al., demonstrated the effect of 5 DES with ChCl-based HBA and HBDs consisting of malonic acid/MA (1:1 molar ratio, *mol:mol*), oxalic acid/OA (1:1 molar ratio, *mol:mol*), urea/U (1:2 molar ratio, *mol:mol*), ethylene glycol/EG (1:2 molar ratio, *mol:mol*) and fructose/F (2:1 molar ratio, *mol:mol*) in the synthesis of magnesium ferrite (MgFe₂O₄) NPs [65]. In the synthesis process, Fe₂O₃ and MgO were added to the DESs, keeping the system at 80 °C (ChCl with U, EG and F) and 25 °C (ChCl with MA and OA) until complete dissolution, and then calcined (500 °C) to obtain MgFe₂O₄NPs. In general, the authors state that there was effective formation of MgFe₂O₄NPs at 500 °C and that the DESs form supramolecular networks so that the electrostatic interactions effectively adjust the physical properties of these solvents, allowing them to act simultaneously as a homogeneous medium for stabilizing metal ions and as a template. Also, the authors further applied these MgFe₂O₄NPs in modifying bare-GCE to simultaneously determine nitrofurantoin (NFT) and 4-nitrophenol (4-NP). Comparatively, MgFe₂O₄NPs synthesized in ChCl:F exhibited better figures of merit with high sensitivity, low detection limits (NFT = 3.3 x 10⁻⁸ mol L⁻¹ and 4-NP = 7.0 x 10⁻⁹

mol L⁻¹), good linear range (0 – 3.4 x 10⁻⁴ mol L⁻¹), good selectivity, and reproducibility using differential pulse voltammetry (DPV) technique.

ChCl:U-based DES has also been used as an electrolyte in the synthesis of palladium NPs (PdNPs) by electrodeposition at 32.5 and 44.5 °C as demonstrated by Hammons et al., the electrodeposited PdNPs are stabilized by adsorbed species larger than choline at both temperatures and form ionic layers above the particle assemblies that depend on the presence of DES [89]. Zhang et al., developed a synthesis of CuInS₂ nanorods structured with chalcopyrites in DES based on ChCl:U (1:2 molar ratio, *mol:mol*) using microwave heating [90]. The dimensions of the obtained CuInS₂ nanorods were 40 nm in diameter and about 400 nm in length. The authors also suggest that DES favors the environment for the formation of nanorods.

As mentioned earlier, the number of studies involving NP synthesis in DES has increased (Figure 3) due to the multiple applications in nanotechnology and because these solvents have unique properties, besides being moldable and meeting the principles of green chemistry. In this sense, the proposition of greener routes in the processes of controlled synthesis of nanomaterials with low energy cost is necessary, especially in the elaboration of materials based on NPs to develop electrochemical sensors, which generally improve the electrocatalytic performance in redox processes, as highlighted in previously mentioned works. Additionally, DES has also been explored as a potential alternative either in developing sensors as binders, conductive films, and modifying agents or as additive electrolyte background modifiers in electrochemical determinations of various analytes.

1.2.2 Electrochemical sensors

DES has been widely used in electrochemical sensor applications in several research areas due to their tunable properties, which can improve the performance of these devices. Figure 6 presents a graph with the results obtained in the WoS database for the search term "*Electrochemical Sensors and Deep Eutectic Solvent*" between the period from 2004 to 2024, indicating that 170 papers were published in journals indexed in this platform. These data show that, in the area of electrochemical sensors, DES has become a possibility for the development of more sustainable alternatives for the applications of these sensors since these solvents have the appeal of being greener.

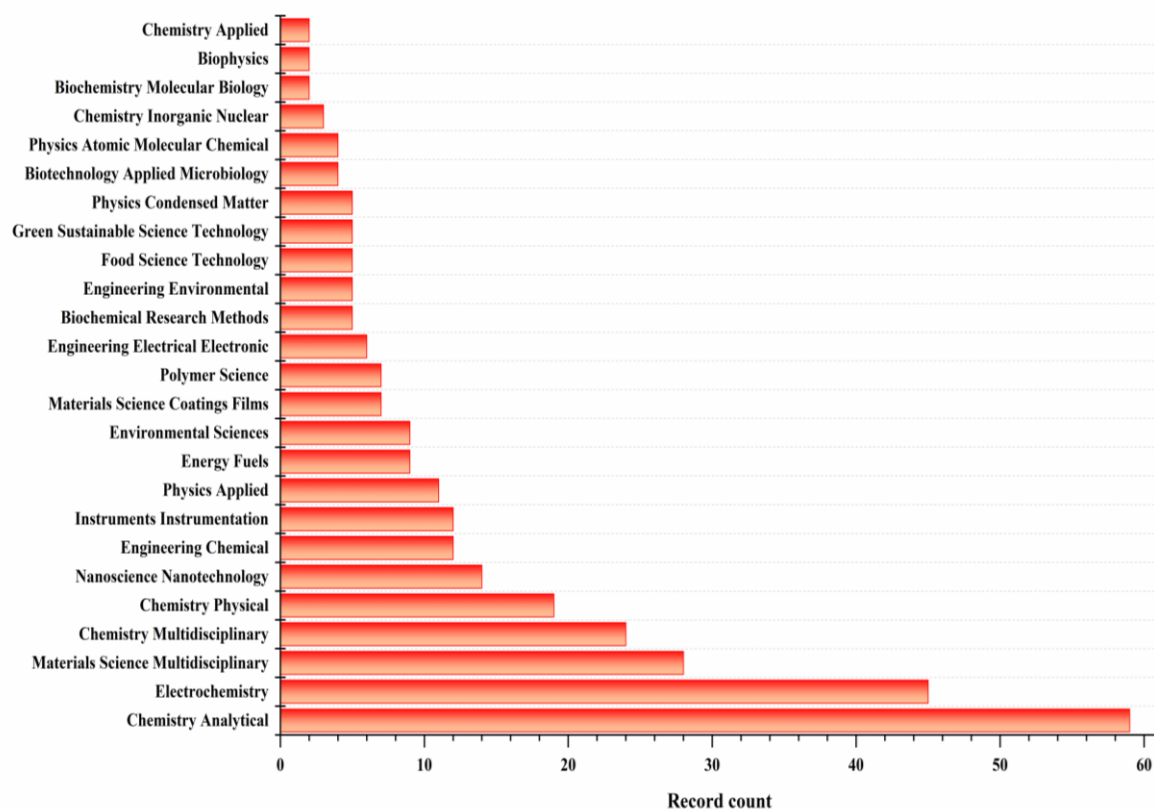


Figure 6. Record of papers on electrochemical sensors and DES in journals in the Web of Science database (period from 2004 to 2024).

The WoS data show that the field of *Electrochemistry* represents 26.5 % of the publications (45 papers), which is expected to increase in the coming decades. Since DES have the potential to be used as a reaction medium for electropolymerizing conductive films [91], electrolyte additives [92], binders [93] and modifying agents [62], they are also promising for the synthesis of NPs that can be applied to electrochemical sensors together with carbonaceous materials.

In recent years, electrochemical sensors have been widely used to detect various chemical substances of interest, such as biomarkers [94], emerging contaminants [95], heavy metals [96] and other pollutants in biological [97], environmental, food [98], and pharmaceutical samples [62]. These devices have gained prominence for offering a faster, cheaper, more accurate and reproducible detection alternative, with low detection and quantification limits, and being more accessible compared to chromatographic techniques [99]. Another advantage is the possibility of miniaturizing electrochemical systems, associated with the widespread use of carbonaceous materials, which stand out for their electrical, optical, thermal, mechanical, chemical and catalytic properties [100].

In the literature, different carbon structures are used to modify electrode surfaces to make them more sensitive. Thus, materials such as carbon nanotubes (CNTs), graphene, carbon black (CB), nanodiamonds (NDs), biochar (BC), and carbon nanohorns (CNH) are some examples used to modify platforms based on screen-printed electrodes (SPCE), 3D electrodes (3D-E), and GCE [99]. Among the mentioned materials, CNT and CB, are widely used in sensor modification. CNT have two main structures, multi-walled carbon nanotubes (MWCNT) and single-walled carbon nanotubes (SWCNT) [100]. MWCNT are basically composed of concentric graphite tubes, with each tube formed by a rolled-up graphite sheet. They have a variety of diameters, generally between 2 – 25 nm, while the distance between the sheets is approximately 0.34 nm, similar to the spacing between graphite layers. SWCNT consists of a single graphite sheet rolled up continuously, creating an individual cylinder with a diameter between 1 – 2 nm. These nanotube groups usually organise themselves into bundles of dozens or even hundreds of nanotubes aligned and in contact [101].

The unique electronic properties of CNTs indicate that they can mediate electron transfer reactions with electroactive species present in solution, making them promising electrode materials. Compared to conventional carbon electrodes, CNTs present superior performance, standing out for their excellent conduction capacity, high chemical stability and, in most cases, improved electron transfer processes [101]. CB is another important structured carbonaceous nanomaterial for use in the fabrication of sensors and biosensors. Morphologically, it is characterized by spherical particles with diameters between 3.0 – 100 nm distributed in large clusters. One of the main attractions of CB is its low cost and large surface area. In addition, it has high thermal stability and electrical conductivity comparable to materials such as graphite, graphene, and CNTs. This is due to the forms of conjugated carbons with sp^2 hybridization in CB and oxygenated groups formed at the edges of graphite-like microcrystallites [102].

The combination of DES with carbonaceous materials, either as film-forming agents or binders for manufacturing carbon paste electrodes (CPE), for example, is still little explored in the literature. Our research group has already developed work with these proposals. Piton et al. [62] used the three classic DES based on ChCl with urea, ethylene glycol and glycerol, all in the molar ratio 1:2 (*mol:mol*) as modifying agents to prepare a composite film with CB and used them in the simultaneous determination of acetaminophen and diclofenac. The results demonstrated that the electrode modified with ChCl:Gly allowed better responses in the electrooxidation of both analytes. The good

performance achieved is credited to the synergistic effect of DES and CB, since the compaction of the film surface caused by DES, which presents oxygenated and nitrogenated groups, allows better conductivity to the film.

In another work proposed by Augusto et al.[93], HDES based on fatty acids (octa, deca, and dodecanoic acids) as HBDs with tetrabutylammonium bromide (TBAB) and 1-octanol as HBAs in a molar ratio of 2:1 (HBD:HBA, *mol:mol*), were used as binders in a CPE to determine hydroquinone in pharmaceutical formulations. The CPE containing 6.0% of the HDES based on DecA:TBAB allowed the best synergistic effect with the graphite particles, and therefore all the kinetic parameters of the target analyte with the proposed electrode were successful for the intended application.

DES improve electrocatalytic performances when combined with carbonaceous materials to construct electrochemical sensors. Likewise, NPs also allow for an increase in surface area, improving the sensitivity of these sensors. Thus, it was highlighted in this first part that DES have an appeal as a green alternative for any application, which makes them a central element of this research, especially in the possibility of using them in NP synthesis processes and combining these particles with carbonaceous materials in the architecture of electrochemical sensors to detect a target analyte. This sequence of applications shows how these solvents can be verticalized with a reduction in steps, fewer hazardous chemicals, and atomic economy, which are some examples that align with the principles of green chemistry as discussed.

1.3 - Doctoral thesis research development

The number of publications involving DES in different research areas in these two decades of consolidating these solvents in the literature is remarkable. Since the first work of the research group of Professor Abbot and collaborators, the diversity of new combinations of precursors to develop another DES, as well as their applications, has been growing throughout this period. In this sense, the research proposal of this doctoral thesis followed stages of elaboration of syntheses of NPs in a DES well established in the literature with general characterizations of both the solvent and the NPs and, subsequently, applying them in the development of electrochemical sensors.

As a first step of the research, studies were developed with DES based on ChCl:U to establish a starting point in the synthesis process of NPs in DES under mild temperature conditions (< 100 °C) based on concepts of the solvothermal approach using conventional reductants, with subsequent application in the development of electrochemical sensors.

All these studies are developed in chapter two, which gave rise to two already published articles.

Once the synthesis process and application were successful, the second step was to develop a new DES that would allow all the characteristics to be combined to eliminate the use of conventional reductants, i.e., to use it as a stabilizing and reducing medium, configuring a “one-pot synthesis” setup, allowing the process to become more environmentally friendly. In this sense, chapter three describes the thermal study *via* DSC of fractions for a system based on ChCl and diethanolamine (DEA) to evaluate the eutectic compositions and determine the eutectic point of this DES and using it for this synthesis proposal, followed by the application of these NPs to an electrochemical sensor.

References

- [1] S.K. Singh, A.W. Savoy, Ionic liquids synthesis and applications: An overview, *J. Mol. Liq.* 297 (2020) 112038. <https://doi.org/10.1016/j.molliq.2019.112038>.
- [2] T. Welton, Ionic liquids: a brief history, *Biophys. Rev.* 10 (2018) 691–706. <https://doi.org/10.1007/s12551-018-0419-2>.
- [3] P.T. Anastas, J.C. Warner, *Green Chemistry: Theory and Practice*, (2000). <https://doi.org/10.1093/oso/9780198506980.001.0001>.
- [4] D.J. Ramón, G. Guillena, *Deep Eutectic Solvents: Synthesis, Properties, and Applications*, Wiley-VCH, Weinheim, Germany, 2020.
- [5] A.P. Abbott, G. Capper, D.L. Davies, H.L. Munro, R.K. Rasheed, Preparation of novel, moisture-stable, Lewis-acidic ionic liquids containing quaternary ammonium salts with functional side chains†, *Chem. Commun.* (2001) 2010–2011. <https://doi.org/10.1039/b106357j>.
- [6] A.P. Abbott, G. Capper, D.L. Davies, R.K. Rasheed, V. Tambyrajah, Novel solvent properties of choline chloride/urea mixtures, *Chem. Commun.* (2003) 70–71. <https://doi.org/10.1039/b210714g>.
- [7] E.L. Smith, A.P. Abbott, K.S. Ryder, Deep Eutectic Solvents (DESs) and Their Applications, *Chem. Rev.* 114 (2014) 11060–11082. <https://doi.org/10.1021/cr300162p>.
- [8] P. Anastas, N. Eghbali, *Green Chemistry : Principles and Practice*, *Chem. Soc. Rev.* (2010) 301–312. <https://doi.org/10.1039/b918763b>.
- [9] Y. Marcus, Deep Eutectic Solvents, 2019. <https://doi.org/10.1007/978-3-030-00608-2>.
- [10] Q. Zhang, K.D.O. Vigier, S. Royer, F. Jérôme, Deep eutectic solvents: syntheses, properties and applications, *Chem. Soc. Rev.* (2012) 7108–7146. <https://doi.org/10.1039/c2cs35178a>.
- [11] Y.H. Choi, J. Van Spronsen, Y. Dai, M. Verberne, F. Hollmann, I.W.C.E. Arends, G. Witkamp, R. Verpoorte, Are Natural Deep Eutectic Solvents the Missing Link in Understanding Cellular Metabolism and Physiology ?, *Plant Physiol.* 156 (2011) 1701–1705. <https://doi.org/10.1104/pp.111.178426>.
- [12] F.J.V. Gomez, M. Espino, M.A. Fernández, M.F. Silva, A Greener Approach to Prepare Natural Deep Eutectic Solvents, *ChemistrySelect.* 3 (2018) 6122–6125. <https://doi.org/10.1002/slct.201800713>.
- [13] S. Rahman, R. Roy, B. Jadhav, N. Hossain, M.A. Halim, D.E. Raynie, Formulation , structure , and applications of therapeutic and amino acid-based deep eutectic solvents : An overview, *J. Mol. Liq.* 321 (2021) 114745. <https://doi.org/10.1016/j.molliq.2020.114745>.
- [14] P.W. Stott, A.C. Williams, B.W. Barry, Transdermal delivery from eutectic systems: Enhanced permeation of a model drug, ibuprofen, *J. Control. Release.* 50 (1998) 297–308. [https://doi.org/10.1016/S0168-3659\(97\)00153-3](https://doi.org/10.1016/S0168-3659(97)00153-3).
- [15] L.I.N. Tomé, V. Baião, W. da Silva, C.M.A. Brett, Deep eutectic solvents for the production and application of new materials, *Appl. Mater. Today.* 10 (2018) 30–50. <https://doi.org/10.1016/j.apmt.2017.11.005>.
- [16] D.J.G.P. van Osch, L.F. Zubeir, A. van den Bruinhorst, M.A.A. Rocha, M.C. Kroon, Hydrophobic Deep Eutectic Solvents: Water-Immiscible Extractants, *Green Chem.* (2015). <https://doi.org/10.1039/C5GC01451D>.
- [17] M.Q. Farooq, N.M. Abbasi, J.L. Anderson, Deep eutectic solvents in separations: Methods of preparation, polarity, and applications in extractions and capillary electrochromatography, *J. Chromatogr. A.* 1633 (2020) 461613. <https://doi.org/10.1016/j.chroma.2020.461613>.
- [18] B. Gurkan, H. Squire, E. Pentzer, Metal-Free Deep Eutectic Solvents: Preparation, Physical Properties, and Significance, *J. Phys. Chem. Lett.* 10 (2019) 7956–7964. <https://doi.org/10.1021/acs.jpclett.9b01980>.
- [19] M.C. Gutiérrez, M.L. Ferrer, L. Yuste, F. Rojo, F. Del Monte, Bacteria incorporation in deep-eutectic solvents through freeze-drying, *Angew. Chemie - Int. Ed.* 49 (2010) 2158–

2162. <https://doi.org/10.1002/anie.200905212>.
- [20] M.C. Gutiérrez, M.L. Ferrer, C.R. Mateo, F. Del Monte, Freeze-drying of aqueous solutions of deep eutectic solvents: A suitable approach to deep eutectic suspensions of self-assembled structures, *Langmuir*. 25 (2009) 5509–5515. <https://doi.org/10.1021/la900552b>.
- [21] Y. Dai, G.J. Witkamp, R. Verpoorte, Y.H. Choi, Tailoring properties of natural deep eutectic solvents with water to facilitate their applications, *Food Chem.* 187 (2015) 14–19. <https://doi.org/10.1016/j.foodchem.2015.03.123>.
- [22] Y. Dai, J. van Spronsen, G.J. Witkamp, R. Verpoorte, Y.H. Choi, Natural deep eutectic solvents as new potential media for green technology, *Anal. Chim. Acta.* 766 (2013) 61–68. <https://doi.org/10.1016/j.aca.2012.12.019>.
- [23] P.L. Pisano, M. Espino, M. de los Á. Fernández, M.F. Silva, A.C. Olivieri, Structural analysis of natural deep eutectic solvents. Theoretical and experimental study, *Microchem. J.* 143 (2018) 252–258. <https://doi.org/10.1016/j.microc.2018.08.016>.
- [24] C. Florindo, F.S. Oliveira, L.P.N. Rebelo, A.M. Fernandes, I.M. Marrucho, Insights into the synthesis and properties of deep eutectic solvents based on cholinium chloride and carboxylic acids, *ACS Sustain. Chem. Eng.* 2 (2014) 2416–2425. <https://doi.org/10.1021/sc500439w>.
- [25] Y. Cui, C. Li, J. Yin, S. Li, Y. Jia, M. Bao, Design, synthesis and properties of acidic deep eutectic solvents based on choline chloride, *J. Mol. Liq.* 236 (2017) 338–343. <https://doi.org/10.1016/j.molliq.2017.04.052>.
- [26] D.E. Crawford, L.A. Wright, S.L. James, A.P. Abbott, Efficient continuous synthesis of high purity deep eutectic solvents by twin screw extrusion, *Chem. Commun.* 52 (2016) 4215–4218. <https://doi.org/10.1039/c5cc09685e>.
- [27] A.P.R. Santana, J.A. Mora-Vargas, T.G.S. Guimarães, C.D.B. Amaral, A. Oliveira, M.H. Gonzalez, Sustainable synthesis of natural deep eutectic solvents (NADES) by different methods, *J. Mol. Liq.* 293 (2019) 17–20. <https://doi.org/10.1016/j.molliq.2019.111452>.
- [28] Y.H. Hsieh, Y. Li, Z. Pan, Z. Chen, J. Lu, J. Yuan, Z. Zhu, J. Zhang, Ultrasonication-assisted synthesis of alcohol-based deep eutectic solvents for extraction of active compounds from ginger, *Ultrason. Sonochem.* 63 (2020) 104915. <https://doi.org/10.1016/j.ultsonch.2019.104915>.
- [29] T. Zhekenov, N. Toksanbayev, Z. Kazakbayeva, D. Shah, F.S. Mjalli, Formation of type III Deep Eutectic Solvents and effect of water on their intermolecular interactions, *Fluid Phase Equilib.* 441 (2017) 43–48. <https://doi.org/10.1016/j.fluid.2017.01.022>.
- [30] A.P. Abbott, D. Boothby, G. Capper, D.L. Davies, R.K. Rasheed, Deep Eutectic Solvents formed between choline chloride and carboxylic acids: Versatile alternatives to ionic liquids, *J. Am. Chem. Soc.* 126 (2004) 9142–9147. <https://doi.org/10.1021/ja048266j>.
- [31] H.G. Liao, Y.X. Jiang, Z.Y. Zhou, S.P. Chen, S.G. Sun, Shape-controlled synthesis of gold nanoparticles in deep eutectic solvents for studies of structure-functionality relationships in electrocatalysis, *Angew. Chemie - Int. Ed.* 47 (2008) 9100–9103. <https://doi.org/10.1002/anie.200803202>.
- [32] Q. Zhang, K. De Oliveira Vigier, S. Royer, F. Jérôme, Deep eutectic solvents: Syntheses, properties and applications, *Chem. Soc. Rev.* 41 (2012) 7108–7146. <https://doi.org/10.1039/c2cs35178a>.
- [33] B. Tang, K.H. Row, Recent developments in deep eutectic solvents in chemical sciences, *Monatshefte Fur Chemie.* 144 (2013) 1427–1454. <https://doi.org/10.1007/s00706-013-1050-3>.
- [34] A.P. Abbott, J.C. Barron, K.S. Ryder, D. Wilson, Eutectic-based ionic liquids with metal-containing anions and cations, *Chem. - A Eur. J.* 13 (2007) 6495–6501. <https://doi.org/10.1002/chem.200601738>.
- [35] A.P. Abbott, R.C. Harris, K.S. Ryder, Application of Hole Theory to Define Ionic Liquids by their Transport Properties †, *J. Phys. Chem. B.* (2007) 4910–4913. <https://doi.org/10.1021/jp0671998>.
- [36] A. Hayyan, F.S. Mjalli, I.M. Alnashef, Y.M. Al-Wahaibi, T. Al-Wahaibi, M.A. Hashim, Glucose-based deep eutectic solvents: Physical properties, *J. Mol. Liq.* 178 (2013) 137–

141. <https://doi.org/10.1016/j.molliq.2012.11.025>.
- [37] T. El Achkar, H. Greige-Gerges, S. Fourmentin, Basics and properties of deep eutectic solvents: a review, *Environ. Chem. Lett.* 19 (2021) 3397–3408. <https://doi.org/10.1007/s10311-021-01225-8>.
- [38] R.J. Nunes, B. Saramago, I.M. Marrucho, Surface Tension of dl -Menthol:Octanoic Acid Eutectic Mixtures, *J. Chem. Eng. Data.* 64 (2019) 4915–4923. <https://doi.org/10.1021/acs.jced.9b00424>.
- [39] T. Li, Y. Song, J. Li, M. Zhang, Y. Shi, J. Fan, New low viscous hydrophobic deep eutectic solvents in vortex-assisted liquid-liquid microextraction for the determination of phthalate esters from food-contacted plastics, *Food Chem.* 309 (2020) 125752. <https://doi.org/10.1016/j.foodchem.2019.125752>.
- [40] M. Jablonsky, V. Majova, K. Ondrigova, J. Sima, Preparation and characterization of physicochemical properties and application of novel ternary deep eutectic solvents, *Cellulose.* 26 (2019) 3031–3045. <https://doi.org/10.1007/s10570-019-02322-2>.
- [41] A.P. Abbott, G. Capper, D.L. Davies, K.J. McKenzie, S.U. Obi, Solubility of metal oxides in deep eutectic solvents based on choline chloride, *J. Chem. Eng. Data.* 51 (2006) 1280–1282. <https://doi.org/10.1021/je060038c>.
- [42] R. Haghighbakhsh, M. Taherzadeh, A.R.C. Duarte, S. Raeissi, A general model for the surface tensions of deep eutectic solvents, *J. Mol. Liq.* 307 (2020) 112972. <https://doi.org/10.1016/j.molliq.2020.112972>.
- [43] C. Reichardt, Solvatochromic dyes as solvent polarity indicators, *Chem. Rev.* 94 (1994) 2319–2358. <https://doi.org/10.1021/cr00032a005>.
- [44] M.J. Kamlet, J.L. Abboud, R.W. Taft, The Solvatochromic Comparison Method. 6. The π^* Scale of Solvent Polarities1, *J. Am. Chem. Soc.* 99 (1977) 6027–6038. <https://doi.org/10.1021/ja00460a031>.
- [45] M.J. Kamlet, The b-scale of solvent hydrogen-bond acceptor (HBA) basicities, *J. Am. Chem. Soc.* 98 (1976) 377–383. <http://pubs.acs.org/doi/pdf/10.1021/ja00418a009>.
- [46] A.K. Dwamena, D.E. Raynie, Solvatochromic Parameters of Deep Eutectic Solvents: Effect of Different Carboxylic Acids as Hydrogen Bond Donor, *J. Chem. Eng. Data.* 65 (2020) 640–646. <https://doi.org/10.1021/acs.jced.9b00872>.
- [47] A.R.R. Teles, E. V. Capela, R.S. Carmo, J.A.P. Coutinho, A.J.D. Silvestre, M.G. Freire, Solvatochromic parameters of deep eutectic solvents formed by ammonium-based salts and carboxylic acids, *Fluid Phase Equilib.* 448 (2017) 15–21. <https://doi.org/10.1016/j.fluid.2017.04.020>.
- [48] R.S. Berry, When the melting and freezing points are not the same, *Sci. Am.* 263 (1990) 68–74. <https://doi.org/10.1038/scientificamerican0890-68>.
- [49] J.D. Dunitz, Are crystal structures predictable?, *Chem. Commun.* 3 (2003) 545–548. <https://doi.org/10.1039/b211531j>.
- [50] S.H. Yalkowsky, Carnelley's rule and the prediction of melting point, *J. Pharm. Sci.* 103 (2014) 2629–2634. <https://doi.org/10.1002/jps.24034>.
- [51] R.J.C. Brown, R.F.C. Brown, Melting Point and Molecular Symmetry, *J. Chem. Educ.* 77 (2000) 724–731. <https://doi.org/10.1021/ed077p724>.
- [52] C. Yuan, X. Zhang, Y. Ren, S. Feng, J. Liu, J. Wang, L. Su, Temperature- and pressure-induced phase transitions of choline chloride-urea deep eutectic solvent, *J. Mol. Liq.* 291 (2019). <https://doi.org/10.1016/j.molliq.2019.111343>.
- [53] H.G. Morrison, C.C. Sun, S. Neervannan, Characterization of thermal behavior of deep eutectic solvents and their potential as drug solubilization vehicles, *Int. J. Pharm.* 378 (2009) 136–139. <https://doi.org/10.1016/j.ijpharm.2009.05.039>.
- [54] P.C. Gomes-Junior, K.K. de Lima Augusto, G.P. Longatto, R. de Oliveira Gonçalves, T.A. Silva, É.T.G. Cavalheiro, O. Fatibello-Filho, Ultrasmall platinum nanoparticles synthesized in reline deep eutectic solvent explored towards the voltammetric sensing of riboflavin in beverages and biological fluids, *Sensors Actuators B Chem.* 395 (2023). <https://doi.org/10.1016/j.snb.2023.134489>.
- [55] A. van den Bruinhorst, M. Costa Gomes, Is there depth to eutectic solvents?, *Curr. Opin. Green Sustain. Chem.* 37 (2022) 100659. <https://doi.org/10.1016/j.cogsc.2022.100659>.

- [56] D.O. Abranches, J.A.P. Coutinho, Everything You Wanted to Know about Deep Eutectic Solvents but Were Afraid to Be Told, *Annu. Rev. Chem. Biomol. Eng.* 14 (2023) 141–163. <https://doi.org/10.1146/annurev-chembioeng-101121-085323>.
- [57] M.A.R. Martins, S.P. Pinho, J.A.P. Coutinho, Insights into the Nature of Eutectic and Deep Eutectic Mixtures, *J. Solution Chem.* 48 (2019) 962–982. <https://doi.org/10.1007/s10953-018-0793-1>.
- [58] E. Alipanahpour Dil, M. Ghaedi, A. Asfaram, L. Tayebi, F. Mehrabi, A ferrofluidic hydrophobic deep eutectic solvent for the extraction of doxycycline from urine, blood plasma and milk samples prior to its determination by high-performance liquid chromatography-ultraviolet, *J. Chromatogr. A.* 1613 (2020). <https://doi.org/10.1016/j.chroma.2019.460695>.
- [59] M. Faraji, M. Mahmoodi-Maymand, F. Dastmalchi, Green, fast and simple dispersive liquid-liquid microextraction method by using hydrophobic deep eutectic solvent for analysis of folic acid in fortified flour samples before liquid chromatography determination, *Food Chem.* 320 (2020). <https://doi.org/10.1016/j.foodchem.2020.126486>.
- [60] E. Shabani, D. Zappi, L. Berisha, D. Dini, M.L. Antonelli, C. Sadun, Deep eutectic solvents (DES) as green extraction media for antioxidants electrochemical quantification in extra-virgin olive oils, *Talanta.* 215 (2020) 1–7. <https://doi.org/10.1016/j.talanta.2020.120880>.
- [61] P. Makoś, A. Przyjazny, G. Boczkaj, Hydrophobic deep eutectic solvents as “green” extraction media for polycyclic aromatic hydrocarbons in aqueous samples, *J. Chromatogr. A.* 1570 (2018) 28–37. <https://doi.org/10.1016/j.chroma.2018.07.070>.
- [62] G.R. Piton, K.K.L. Augusto, A. Wong, F.C. Moraes, O. Fatibello-Filho, A Novel Electrochemical Glassy Carbon Electrode Modified with Carbon Black and Glyceline Deep Eutectic Solvent within a Crosslinked Chitosan Film for Simultaneous Determination of Acetaminophen and Diclofenac, *Electroanalysis.* (2021) 1–11. <https://doi.org/10.1002/elan.202100325>.
- [63] W. Silva, M.E. Ghica, C.M.A. Brett, Novel nanocomposite film modified electrode based on poly (brilliant cresyl blue) -deep eutectic solvent / carbon nanotubes and its biosensing applications, 317 (2019) 766–777.
- [64] F. Chen, S. Xie, J. Zhang, R. Liu, Synthesis of spherical Fe₃O₄ magnetic nanoparticles by co-precipitation in choline chloride/urea deep eutectic solvent, *Mater. Lett.* 112 (2013) 177–179. <https://doi.org/10.1016/j.matlet.2013.09.022>.
- [65] J.N. Baby, B. Sriram, S.F. Wang, M. George, Effect of Various Deep Eutectic Solvents on the Sustainable Synthesis of MgFe₂O₄ Nanoparticles for Simultaneous Electrochemical Determination of Nitrofurantoin and 4-Nitrophenol, *ACS Sustain. Chem. Eng.* 8 (2020) 1479–1486. <https://doi.org/10.1021/acssuschemeng.9b05755>.
- [66] P.G. Jamkhande, N.W. Ghule, A.H. Bamer, M.G. Kalaskar, Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications, *J. Drug Deliv. Sci. Technol.* 53 (2019) 101174. <https://doi.org/10.1016/j.jddst.2019.101174>.
- [67] P.S. Goh, K.C. Wong, A.F. Ismail, Nanocomposite membranes for liquid and gas separations from the perspective of nanostructure dimensions, *Membranes (Basel).* 10 (2020) 1–29. <https://doi.org/10.3390/membranes10100297>.
- [68] K.A. Altammar, A review on nanoparticles: characteristics, synthesis, applications, and challenges, *Front. Microbiol.* 14 (2023) 1–20. <https://doi.org/10.3389/fmicb.2023.1155622>.
- [69] C. Daruich De Souza, B. Ribeiro Nogueira, M.E.C.M. Rostelato, Review of the methodologies used in the synthesis gold nanoparticles by chemical reduction, *J. Alloys Compd.* 798 (2019) 714–740. <https://doi.org/10.1016/j.jallcom.2019.05.153>.
- [70] L.P. Singh, S.K. Bhattacharyya, R. Kumar, G. Mishra, U. Sharma, G. Singh, S. Ahalawat, Sol-Gel processing of silica nanoparticles and their applications, *Adv. Colloid Interface Sci.* 214 (2014) 17–37. <https://doi.org/10.1016/j.cis.2014.10.007>.
- [71] J.A. Darr, J. Zhang, N.M. Makwana, X. Weng, Continuous Hydrothermal Synthesis of Inorganic Nanoparticles: Applications and Future Directions, *Chem. Rev.* 117 (2017)

- 11125–11238. <https://doi.org/10.1021/acs.chemrev.6b00417>.
- [72] J. Lai, W. Niu, R. Luque, G. Xu, Solvothermal synthesis of metal nanocrystals and their applications, *Nano Today*. 10 (2015) 240–267. <https://doi.org/10.1016/j.nantod.2015.03.001>.
- [73] J.H.T. Luong, K.B. Male, J.D. Glennon, Boron-doped diamond electrode: Synthesis, characterization, functionalization and analytical applications, *Analyst*. 134 (2009) 1965–1979. <https://doi.org/10.1039/b910206j>.
- [74] I. Gurrappa, L. Binder, Electrodeposition of nanostructured coatings and their characterization - A review, *Sci. Technol. Adv. Mater.* 9 (2008). <https://doi.org/10.1088/1468-6996/9/4/043001>.
- [75] Y.J. Zhu, F. Chen, Microwave-assisted preparation of inorganic nanostructures in liquid phase, *Chem. Rev.* 114 (2014) 6462–6555. <https://doi.org/10.1021/cr400366s>.
- [76] K.N. Thakkar, S.S. Mhatre, R.Y. Parikh, Biological synthesis of metallic nanoparticles, *Nanomedicine Nanotechnology, Biol. Med.* 6 (2010) 257–262. <https://doi.org/10.1016/j.nano.2009.07.002>.
- [77] T. Prasad Yadav, R. Manohar Yadav, D. Pratap Singh, Mechanical Milling: a Top Down Approach for the Synthesis of Nanomaterials and Nanocomposites, *Nanosci. Nanotechnol.* 2 (2012) 22–48. <https://doi.org/10.5923/j.nn.20120203.01>.
- [78] N.G. Semaltianos, Nanoparticles by laser ablation, *Crit. Rev. Solid State Mater. Sci.* 35 (2010) 105–124. <https://doi.org/10.1080/10408431003788233>.
- [79] H. Wender, P. Migowski, A.F. Feil, S.R. Teixeira, J. Dupont, Sputtering deposition of nanoparticles onto liquid substrates: Recent advances and future trends, *Coord. Chem. Rev.* 257 (2013) 2468–2483. <https://doi.org/10.1016/j.ccr.2013.01.013>.
- [80] A. Asadi, F. Pourfattah, I. Miklós Szilágyi, M. Afrand, G. Żyła, H. Seon Ahn, S. Wongwises, H. Minh Nguyen, A. Arabkoohsar, O. Mahian, Effect of sonication characteristics on stability, thermophysical properties, and heat transfer of nanofluids: A comprehensive review, *Ultrason. Sonochem.* 58 (2019). <https://doi.org/10.1016/j.ultsonch.2019.104701>.
- [81] X. Lu, C. Wang, Y. Wei, One-dimensional composite nanomaterials: Synthesis by electrospinning and their applications, *Small*. 5 (2009) 2349–2370. <https://doi.org/10.1002/sml.200900445>.
- [82] M. Zobel, R.B. Neder, S.A.J. Kimber, Universal solvent restructuring induced by colloidal nanoparticles, *Science* (80-.). 347 (2015) 292–294. <https://doi.org/10.1126/science.1261412>.
- [83] T.M.D. Dang, T.T.T. Le, E. Fribourg-Blanc, M.C. Dang, The influence of solvents and surfactants on the preparation of copper nanoparticles by a chemical reduction method, *Adv. Nat. Sci. Nanosci. Nanotechnol.* 2 (2011). <https://doi.org/10.1088/2043-6262/2/2/025004>.
- [84] M.H. Hussain, N. Fitrah, A. Bakar, A.N. Mustapa, K. Low, N.H. Othman, F. Adam, Synthesis of Various Size Gold Nanoparticles by Chemical Reduction Method with Different Solvent Polarity, *Nanoscale Res. Lett.* 15 (2020) 140–150.
- [85] B.H. Kim, M.J. Hackett, J. Park, T. Hyeon, Synthesis, characterization, and application of ultrasmall nanoparticles, *Chem. Mater.* 26 (2014) 59–71. <https://doi.org/10.1021/cm402225z>.
- [86] O. V. Kharissova, B.I. Kharisov, V.M. Jiménez-Pérez, B. Muñoz Flores, U. Ortiz Méndez, Ultrasmall particles and nanocomposites: State of the art, *RSC Adv.* 3 (2013) 22648–22682. <https://doi.org/10.1039/c3ra43418d>.
- [87] M. Chirea, A. Freitas, B.S. Vasile, C. Ghitulica, C.M. Pereira, F. Silva, Gold nanowire networks: Synthesis, characterization, and catalytic activity, *Langmuir*. 27 (2011) 3906–3913. <https://doi.org/10.1021/la104092b>.
- [88] C.D. Gu, Y.J. Mai, J.P. Zhou, J.P. Tu, SnO₂ nanocrystallite: Novel synthetic route from deep eutectic solvent and lithium storage performance, *Funct. Mater. Lett.* 4 (2011) 377–381. <https://doi.org/10.1142/S1793604711002251>.
- [89] J.A. Hammons, T. Muselle, J. Ustarroz, M. Tzedaki, M. Raes, A. Hubin, H. Terryn, Stability, assembly, and particle/solvent interactions of Pd nanoparticles electrodeposited

- from a deep eutectic solvent, *J. Phys. Chem. C*. 117 (2013) 14381–14389. <https://doi.org/10.1021/jp403739y>.
- [90] J. Zhang, J. Chen, Q. Li, Microwave heating synthesis and formation mechanism of chalcopyrite structured CuInS₂ nanorods in deep eutectic solvent, *Mater. Res. Bull.* 63 (2015) 88–92. <https://doi.org/10.1016/j.materresbull.2014.11.046>.
- [91] K.P. Prathish, R.C. Carvalho, C.M.A. Brett, Electrochemistry Communications Highly sensitive poly (3 , 4-ethylenedioxythiophene) modified electrodes by electropolymerisation in deep eutectic solvents, *Electrochem. Commun.* 44 (2014) 8–11. <https://doi.org/10.1016/j.elecom.2014.03.026>.
- [92] F.J.V. Gomez, M. Espino, M. de los Angeles Fernandez, J. Raba, M.F. Silva, Enhanced electrochemical detection of quercetin by Natural Deep Eutectic Solvents, *Anal. Chim. Acta*. 936 (2016) 91–96. <https://doi.org/10.1016/j.aca.2016.07.022>.
- [93] K.K. de L. Augusto, G.R. Piton, P.C. Gomes-Junior, G.P. Longato, F.C. de Moraes, O. Fatibello-Filho, Enhancing the electrochemical sensitivity of hydroquinone using a hydrophobic deep eutectic solvent-based carbon paste electrode †, *Anal. Methods*. 14 (2022) 2003–2013. <https://doi.org/10.1039/d2ay00473a>.
- [94] P. Cardoso Gomes-Junior, E. Dias Nascimento, K. Kenlderi de Lima Augusto, G. Patelli Longatto, R. Censi Faria, E. Piccin, O. Fatibello-Filho, Voltammetric determination of uric acid using a miniaturized platform based on screen-printed electrodes modified with platinum nanoparticles, *Microchem. J.* 207 (2024) 111931. <https://doi.org/10.1016/j.microc.2024.111931>.
- [95] A. Wong, M. Del, P. Taboada, Biomimetic sensor based on 5,10,15,20-tetrakis(pentafluorophenyl)-21H,23H-porphyrin iron (III) chloride and MWCNT for selective detection of 2,4-D, *Sensors Actuators B. Chem.* 181 (2013) 332–339. <https://doi.org/10.1016/j.snb.2012.12.095>.
- [96] N. Serrano, J.M. Díaz-Cruz, C. Ariño, M. Esteban, Ex situ deposited bismuth film on screen-printed carbon electrode: A disposable device for stripping voltammetry of heavy metal ions, *Electroanalysis*. 22 (2010) 1460–1467. <https://doi.org/10.1002/elan.200900183>.
- [97] A. Wong, A.M. Santos, R. da Fonseca Alves, F.C. Vicentini, O. Fatibello-Filho, M. Del Pilar Taboada Sotomayor, Simultaneous determination of direct yellow 50, tryptophan, carbendazim, and caffeine in environmental and biological fluid samples using graphite pencil electrode modified with palladium nanoparticles, *Talanta*. 222 (2021). <https://doi.org/10.1016/j.talanta.2020.121539>.
- [98] J. Hui, L. Ruiyi, D. Zhenzhong, G. Xiaoyan, X. Junshan, S. Jinsong, L. Zaijun, Platinum nanoparticle-graphene quantum dot nanocage as a promising Schottky heterojunction electrocatalyst for electrochemical detection of vanillin in baby milk powder, *Microchem. J.* 186 (2023) 108320. <https://doi.org/10.1016/j.microc.2022.108320>.
- [99] R.M. Silva, A.D. da Silva, J.R. Camargo, B.S. de Castro, L.M. Meireles, P.S. Silva, B.C. Janegitz, T.A. Silva, Carbon Nanomaterials-Based Screen-Printed Electrodes for Sensing Applications, *Biosensors*. 13 (2023). <https://doi.org/10.3390/bios13040453>.
- [100] Y. Gogotsi, *Carbon nanomaterials*, Taylor & Francis Group, Philadelphia, Pennsylvania, USA, 2006.
- [101] Q. Zhao, Z. Gan, Q. Zhuang, Electrochemical sensors based on carbon nanotubes, *Electroanalysis*. 14 (2002) 1609–1613. <https://doi.org/10.1002/elan.200290000>.
- [102] L.M.C. Ferreira, P.S. Silva, K.K.L. Augusto, P.C. Gomes-Júnior, S.O.D. Farra, T.A. Silva, O. Fatibello-Filho, F.C. Vicentini, Using nanostructured carbon black-based electrochemical (bio)sensors for pharmaceutical and biomedical analyses: A comprehensive review, *J. Pharm. Biomed. Anal.* 221 (2022) 115032. <https://doi.org/10.1016/j.jpba.2022.115032>.

Chapter II

Research Articles

Chapter II || Research Articles

2.1. Research Article I

Sensors & Actuators: B. Chemical 395 (2023) 134489 / Impact factor 8.0

Ultrasmall platinum nanoparticles synthesized in reline deep eutectic solvent explored towards the voltammetric sensing of riboflavin in beverages and biological fluids

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Abstract

We report a solvothermal synthesis of ultrasmall platinum nanoparticles (USPtNPs) with an average size of 0.204 (± 0.008) nm using reline deep eutectic solvent (DES) as a stabilizing medium at a temperature of 80 °C. SEM and EDS were used to morphologically characterize the USPtNPs. These revealed approximately spherical shapes with emission lines characteristic of platinum. DES was characterized by FT-IR and NMR, which indicated its formation through hydrogen bonds between the precursors. DSC was also used to characterize the precursors and the formed DES. Furthermore, an electrochemical sensor for determining riboflavin (RB) in commercial energy drinks and biological fluids was developed using the USPtNPs together with multiwalled carbon nanotubes (MWCNT). The USPtNPs-DES/MWCNT/GCE film was characterized by SEM, which demonstrated the characteristic tubular morphology of MWCNT. The RB voltammetric response was studied, and a significant enhanced on the current response was observed, especially due to the USPtNPs. This sensor displayed a linear response for RB in the range of 0.02 to 1.20 $\mu\text{mol L}^{-1}$, with a limit of detection of 1.8 nmol L^{-1} . Besides detectability at nanomolar range, excellent performance was verified for repeatability and anti-interference features. The sensor based on extraordinary USPtNPs synthesized in DES in a simpler and environmentally friendly way was successfully applied to determine RB in different matrices.

Keywords: green synthesis; ultrasmall nanoparticles; reline deep eutectic solvent; voltammetry; carbon nanotubes, vitamin B2; modified electrodes.

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2.1.1 Introduction

Nanomaterials' use in different science areas has grown significantly in recent years. Especially metal nanoparticles, which can have electrical, optical, magnetic, catalytic, and biocompatible properties altered according to their size, making them promising in several applications [1,2]. In this context, the so-called ultrasmall nanoparticles (USNPs), with sizes smaller than 3.0 nanometers, has gained importance due to their unique properties, reflected by the drastically increment of surface area because of size reduction. This class of nanoparticles has been extensively explored for biological applications, particularly in drug delivery and in the development of treatments for cancerous diseases [1,3–5].

More recently, USNPs smaller than 7.0 nm based on copper oxides [6], platinum-nickel nanocomposites [7], and bimetallic particles based on gold-silver [8] have been explored in applications for non-enzymatic sensors to detect hydrogen peroxide and dopamine. These sensors exhibited good biocompatibility, high electrical conductivity, and excellent sensitivities. In addition, the development of electrochemical sensors based on nickel (5.0 nm) and platinum (5.0 nm) USNPs for simultaneous determinations of catechol and hydroquinone [9], and ascorbic acid, dopamine, and uric acid [10], respectively, demonstrated high electrocatalytic activities and sensitivities.

Despite the advances in the applications of USNPs for electrochemical sensing, the improved performance of these devices depends on the size of the USNPs. Usually, similar methods of synthesis for conventional NPs have been used for USNPs. However, it is crucial to ensure that the ultrasmall particles are not in the form of agglomerates. The use of surfactants, for example, can prevent aggregation as they act as dispersing agent in polymer matrices and also allow milder temperature conditions and shorter synthesis time. Furthermore, hydrothermal methods [4], solvothermics [6], microwave radiation [7], thermal decomposition methods [8], among others, were applied in the synthesis of USNPs.

In most of these USNPs synthesis processes, the steps are usually time-consuming, laborious and in some cases require high energy expenditure and sophisticated equipment. In addition, solvents with low or no biodegradability are employed in these methods. Therefore, one of the main highlights of this work is the simpler and greener platinum USNPs synthesis approach using reline deep eutectic solvents (DES). DES were introduced by Abbot et al. in 2001 [11] and, since then, they

proved to be alternatives to non-aqueous solvents and ionic liquids (IL) with various applications, especially because of their low production cost, low toxicity, biodegradability, high thermal stability, among other features [12]. These characteristics have made DESs an alternative to replace conventional solvents in various applications.

Besides the synthesis of USPtNPs based on green DES, this work also presents the application of USPtNPs synthesized in DES to decorate an electrochemical sensor based on multiwalled carbon nanotubes (MWCNT). The sensor was applied it in the voltammetric determination of riboflavin (RB – or vitamin B2) in food (energy drinks) and biological fluids (urine and serum). To the best of our knowledge, no electrochemical sensors based on USPtNPs synthesized in DES have been reported for RB determination. There are only few studies that employ DES as modifiers [13,14] or in certain steps nanomaterials synthesis for further manufacture of sensors [15–18].

2.1.2 Materials and Methods

2.1.2.1 Chemical and Solutions

All chemicals were obtained from Sigma-Aldrich (St. Louis, USA), Synth (Diadema, São Paulo), Neon (São Paulo, Brazil), Panreac (Darmstadt, Germany), or Vetec Ltda. (Rio de Janeiro, Brazil) with the highest purity grade available and used without further purification. Bovine serum albumin (lyophilized powder, crystalized, $\geq 98.0\%$) and MWCNT (O.D. $\times$ L 6 – 9 nm $\times$ 5 μm , $> 95\%$ carbon) powder were acquired from Sigma-Aldrich (St. Louis, USA). All aqueous solutions were prepared with ultrapure water (resistivity $\geq 18.0\text{ M}\Omega\text{ cm}$) obtained from a Millipore Milli-Q system (Billerica, USA). Phosphate and Britton-Robinson buffers were used as supporting electrolyte at 0.2 mol L^{-1} . A $1.0 \times 10^{-2}\text{ mol L}^{-1}$ RB standard stock solution was prepared by dissolution in ultrapure water. Dilution processes were adopted to obtain different concentrations of standard solutions.

2.1.2.2 Instruments

DSC curves were obtained in a Q10 Differential Scanning Calorimetric module controlled by the Thermal Advantage Series® software (v.5.5.24), both from TA Instruments (New Castle, USA). Experiments were conducted using a sample mass of $5.0 \pm 0.1\text{ mg}$ at a heating rate of $10\text{ }^{\circ}\text{C min}^{-1}$, under dynamic N_2 atmosphere flowing at 50 mL min^{-1} and in a closed aluminum sample holder with a pin hole ($\varnothing = 0.7\text{ mm}$) in the center of the cover. Measurements were performed between -70 and $120\text{ }^{\circ}\text{C}$ for DES,

–30 and 160 °C for urea and 30 to 330 °C for choline chloride. The endothermic peak temperatures were used to characterize the melting event.

A Fourier transform infrared spectrophotometer (FT-IR) IRPrestige-21 (Shimadzu Instrument, Kyoto – Japan) (wavenumber range of 4000–500 cm^{-1} , room temperature) was used to verify the presence of functional groups. ^1H NMR spectra were recorded on a Bruker spectrometer (Billerica, USA) (400 MHz for ^1H) to determine possible hydrogen interactions. Residual solvent signal was used as reference for the ^1H NMR spectra, with non-deuterated dimethylsulfoxide ($\text{DMSO-}d_6$): δ ^1H = 2.50 ppm.

Viscosities were measured with an IKA Rotavisc viscometer (model lo-vi) (Campinas, Brazil), electrical conductivities were determined using a CA 150 Marconi conductivity meter (Piracicaba, São Paulo), and water content of the DES was determined by Karl-Fischer method using an 899 KF Coulometer (Metrohm, Utrecht – Netherlands). Morphological characterizations of the composite film and nanoparticles were carried out on a field-emission gun scanning electron microscopy (FEG/SEM, FEI Magellan 400 L) and a transmission electron microscopy (TEM, FEI Tecnai G2 F20 microscope operating at 200 kV).

Electrochemical measurements were performed using a three-electrode single-compartment glass cell containing an $\text{Ag}|\text{AgCl}|\text{KCl}_{(3.0 \text{ mol L}^{-1})}$ reference electrode, a platinum plate as counter electrode, and bare GCE ($\phi = 3.0 \text{ mm}$) or modified-GCE as working electrode. An Autolab PGSTAT-20 (Eco Chemie, Utrecht, the Netherlands) potentiostat/galvanostat was employed in all experiments.

2.1.2.3 Reline DES preparation, USPtNPs synthesis, and comparative PtNPs synthesis methods

Reline DES synthesis was performed similarly to the procedure proposed by Abbott et al. [19] with a molar ratio of 1:2 for choline chloride and urea. The process involved mixing the precursors in a closed glass and constant stirring at 80 °C for 60 min, until a homogeneous liquid was obtained. The DES was stored in a glass flask with molecular sieves. In the USPtNPs synthesis, 10 mL of DES were heated up to 60 °C and then 25 mg of $\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ were added under stirring until the platinum salt dissolution. Then, 40 mg of sodium borohydride were added to the reaction vessel, and the temperature was maintained at 80 °C for 30 minutes. After this, the reaction mixture showed a color change (yellow to gray), which served as a qualitative indicative of the

USPtsNPs formation. Finally, after cooled at room temperature, the USPtsNPs were stored in amber vials and kept under the same conditions as the DES.

The procedure adopted for the comparative citrate-capped platinum nanoparticles (PtNPs) synthesis was similar to that proposed by Moglianetti et al [20] with some changes. A volume of 50 μL of $0.1 \text{ mol L}^{-1} \text{H}_2\text{PtCl}_6 \cdot 6 \text{H}_2\text{O}$ solution was added to a flask containing 10 mL of water at 90°C under constant stirring (same system synthesis used for DES). After 1 min, 400 μL of 35 mmol L^{-1} sodium citrate and 3 mmol L^{-1} citric acid solution were added, and quickly 200 μL of $50 \text{ mmol L}^{-1} \text{NaBH}_4$ solution was transferred to the system. The reaction was held for 15 min at 95°C until the color change (yellow to dark brown) and immediately cooled in an ice bath to retard growth.

2.1.2.4 Preparation of the modified electrode

The GCE surface was polished with 1.0 and 0.5 μm alumina slurry and washed with deionized water. The modifying dispersions containing the nanoparticles (USPtsNPs or PtNPs) and MWCNT were prepared as follows: 1.0 mg of MWCNT was suspended in 100 μL of USPtsNPs or PtNPs, 100 μL of NafionTM in methanol (25 % v/v), and 800 μL of N, N-dimethylformamide (DMF) in water (10 % v/v). The suspensions were taken for 30 min in an ultrasonic bath for complete dispersion of the materials. Then, 8.0 μL of these suspensions was dropped individually on the GCE surface and allowed to dry for 20 minutes under an IR lamp, forming the film.

2.1.2.5 Samples preparation

Commercial energy drink samples from three different brands containing RB were purchased in a local market. The samples were diluted twice with ultrapure water, then aliquots of each sample were added in the electrochemical cell containing supporting electrolyte followed by successive additions of standard RB solution.

Synthetic urine samples were prepared as previously reported by Laube et al. [21], with $0.20 \text{ mmol L}^{-1} \text{KCl}$, $0.18 \text{ mmol L}^{-1} \text{NH}_4\text{Cl}$, $0.10 \text{ mmol L}^{-1} \text{NaCl}$, $0.10 \text{ mmol L}^{-1} \text{CaCl}_2$, $0.15 \text{ mmol L}^{-1} \text{KH}_2\text{PO}_4$, and 0.18 mmol L^{-1} urea in a 25 mL volumetric flask. This sample was spiked at three concentrations of RB (0.05 , 0.35 and $0.73 \mu\text{mol L}^{-1}$, respectively) and analyzed in terms of recovery percentage. The serum (bovine serum) sample was diluted 10-fold with ultrapure water and also evaluated at three known spiking concentrations of RB (0.12 , 0.37 , and $0.84 \mu\text{mol L}^{-1}$, respectively), and analyzed by recovery percentage.

2.1.3 Results and Discussion

2.1.3.1 Physical chemical characterization of DES

The DES was subjected to different characterizations to determine some important properties such as electrical conductivity, viscosity, water content, and thermal behavior (vitreous transitions and melting point). The water content of DES, in general, influences the viscosity, density, conductivity, surface tension, and polarity. Typically, choline-based hydrophilic eutectic solvents presents variable water content [22]. The DES presented a water content of $(4.3 \pm 0.1)\%$, being this result similar to those reported for eutectic choline chloride-based mixtures [22–25].

A low electrical conductivity $(0.39 \pm 0.01) \text{ mS cm}^{-1}$ was found for the DES at room temperature. This value is in accordance with those reported in the literature ($< 2 \text{ mS cm}^{-1}$) at room temperature [14,19,25]. DESs normally present low conductivities as result of the low mobility of the ions that form aggregates of large ionic pairs. However, the conductivity of the DES is significantly altered with temperature [25].

The viscosity of DESs depend on the nature of their precursors, and viscosities greater than $10,000 \text{ mPa s}$ can be achieved [26]. The prepared DES presented viscosity of $2291 \pm 74 \text{ mPa s}$ at room temperature. This value is due to the relative size of the ions, together with their low cohesion and molecular mobility. In addition, in binary eutectic systems, the hydrogen bonds and Van der Waals interactions contribute to increase the viscosity, especially by the proton donor species. Moreover, with temperature increasing, the DES viscosity resembles the viscosity of a pure organic solvent [25,26].

2.1.3.2 Thermal characterization of DES

Fig. 7 shows the DSC curves obtained for DES and its precursors. The choline chloride and urea DSC curves showed endothermic peaks at 588.6 ($315.2 \text{ }^\circ\text{C}$) and 409.9 K ($136.5 \text{ }^\circ\text{C}$), respectively, attributed to their melting temperature (T_m) (Insets in Fig. 7 (i) and (ii)). These values are in agreement with those reported in the literature for choline chloride [27,28] and urea [29,30]. In addition, another endothermic peak at *c.a.* 344.3 K ($71.1 \text{ }^\circ\text{C}$) was observed for choline chloride, which was associated with a crystallographic arrangement phase transition of this precursor [31,32]. On the other hand, in the DSC curve of the eutectic mixture (Fig. 7), no thermal event associated with DES fusion was observed. However, an event corresponding to an exothermic glass transition (T_g) at temperature 279.7 K ($6.58 \text{ }^\circ\text{C}$) with enthalpy of -11.87 J g^{-1} and positive entropy was

observed (Table 2). As a result of these thermodynamic characteristics, the possible degree of disorders in the accommodations of the ionic clusters formed by the precursors of DES may present limited energies to promote a cold-crystallization event (solid $\rightarrow$ solid transition by loss of energy with consequent crystalline ordering of DES), which probably did not cause the fusion of DES. In addition, effects of thermal inertia (T.I) were observed in all DSC curves, and these can be defined as temperature deviations of the sample from a reference [33].

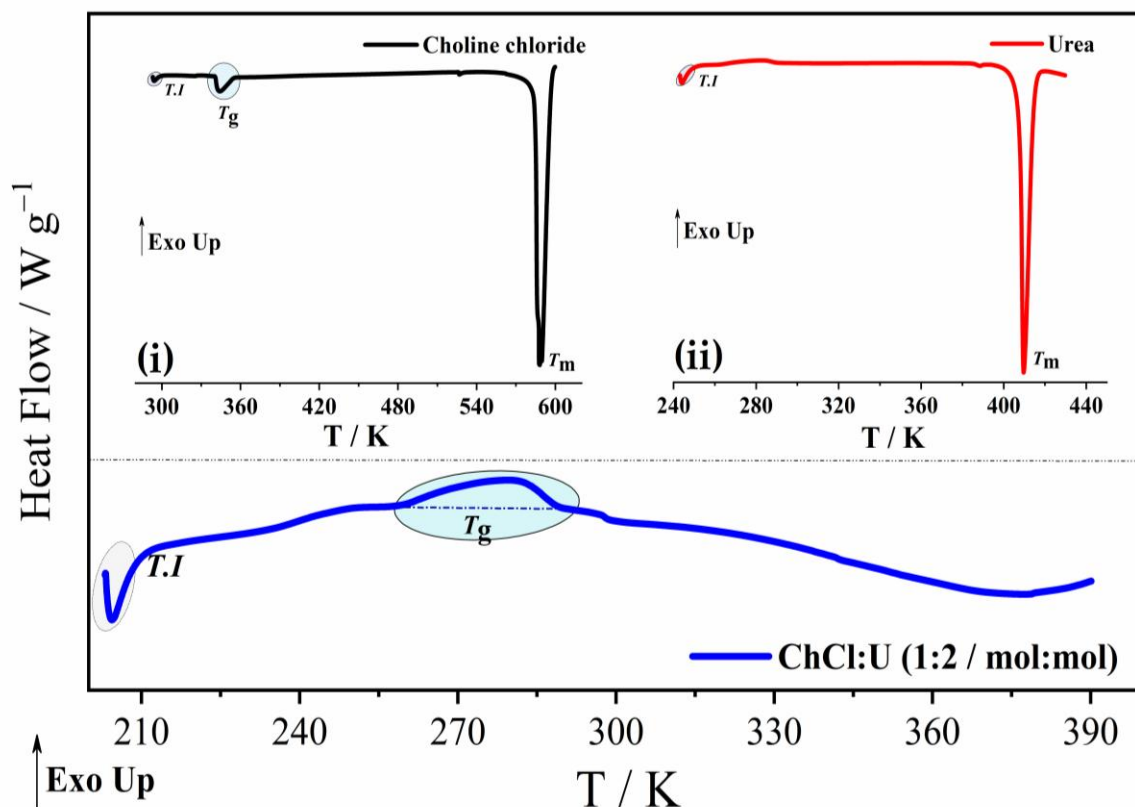


Figure 7. DSC curves were obtained for DES (ChCl:U / 1:2 (mol:mol)) and inset: choline chloride and urea.

Table 2. Thermal events observed in the DSC curves for DES and their precursors

	Temperatures (K)		Enthalpies (J g ⁻¹)		*Entropies (J g ⁻¹ K ⁻¹)	
Choline chloride	^a T _g	344.3	^c Δ _g H	49.2	^e Δ _g S	- 0.143
	^b T _m	588.6	^d Δ _m H	975.7	^f Δ _m S	- 1.658
Urea	T _m	409.9	Δ _m H	235.5	Δ _m S	- 0.574
ChCl:U	T _g	279.7	ΔH _g	- 11.9	Δ _g S	0.042

^a Glass transition; ^b Melting temperature; ^c Glass transition enthalpy; ^d Melting enthalpy; ^e Glass transition entropy; ^f Melting entropy. *ΔS = -ΔH (J g⁻¹) / T (K).

In fact, under the experimental conditions adopted, by cooling with the application of an isotherm, it was expected that DES crystallization would be complete or partial. Thus, a melting event would occur during the heating step in the studied range. However, solvents composed by molecules or complex ions, as is the case of DES, when cooled, can form non-crystalline (or vitreous) structures with irregular arrangements. Usually, these arrangements are conditioned to intermolecular forces between the constituents (Van der Waals, hydrogen bonds, ionic, among others) [34]. As a consequence, they do not present well-defined T_m , only a transit from a more rigid solid state to a flexible at temperature intervals, which are called glass transition temperatures (T_g) [34,35].

In literature, T_m values are reported to determine the eutectic point for the mixture of choline chloride and urea at molar ratio 1:2. Morrison et al. [36] and Meng et al. [27] reported eutectic points of 17 °C and 25 °C, respectively, while Yuan et al. [28] reported only exothermic and endothermal peaks at temperatures of -28 °C and 10 °C, respectively. On the other hand, in the pioneer work on DES, Abbot and collaborators [19] reported the eutectic point from the freezing temperature (T_f) of the mixture at 12 °C.

2.1.3.3 NMR and FTIR analysis of DES

^1H NMR and FT-IR spectroscopies were explored to verify the interactions between the components of the eutectic mixture. The ^1H NMR chemical shifts of the eutectic mixture were compared with the chemical shifts of each precursor. Figs. 8 and 9 show the peak assignments of NMR and IR bands before and after the formation of the eutectic mixture.

In the ^1H NMR spectrum of the eutectic solvent (Fig. 8 (b)), the singlet at δ 3.13 ppm corresponds to methyl groups, and the signals at δ 3.81 and 3.43 ppm belong to methylene groups of choline chloride. The wide singlet at δ 5.63 ppm refers to the OH-NH₂ hydrogens. According to the NMR spectroscopic investigation, it can be observed that the ^1H NMR spectrum of urea (Fig. 8 (a)) presented a peak of 5.51 ppm, attributed to the -NH group. This peak shifted to a lower field (from 5.51 to 5.63 ppm) after mixing with choline chloride. This result revealed the presence of hydrogen bonds between the constituents, weakening the N-H bond. In addition, it is also possible to observe the change of chemical shift of the signal referring to the -OH group of choline chloride to the larger field (Fig. 8 (c)). Due to chemical interactions and the effect of hydrogen bonds, the signals showed different chemical shifts when compared to the precursors' NMR [37].

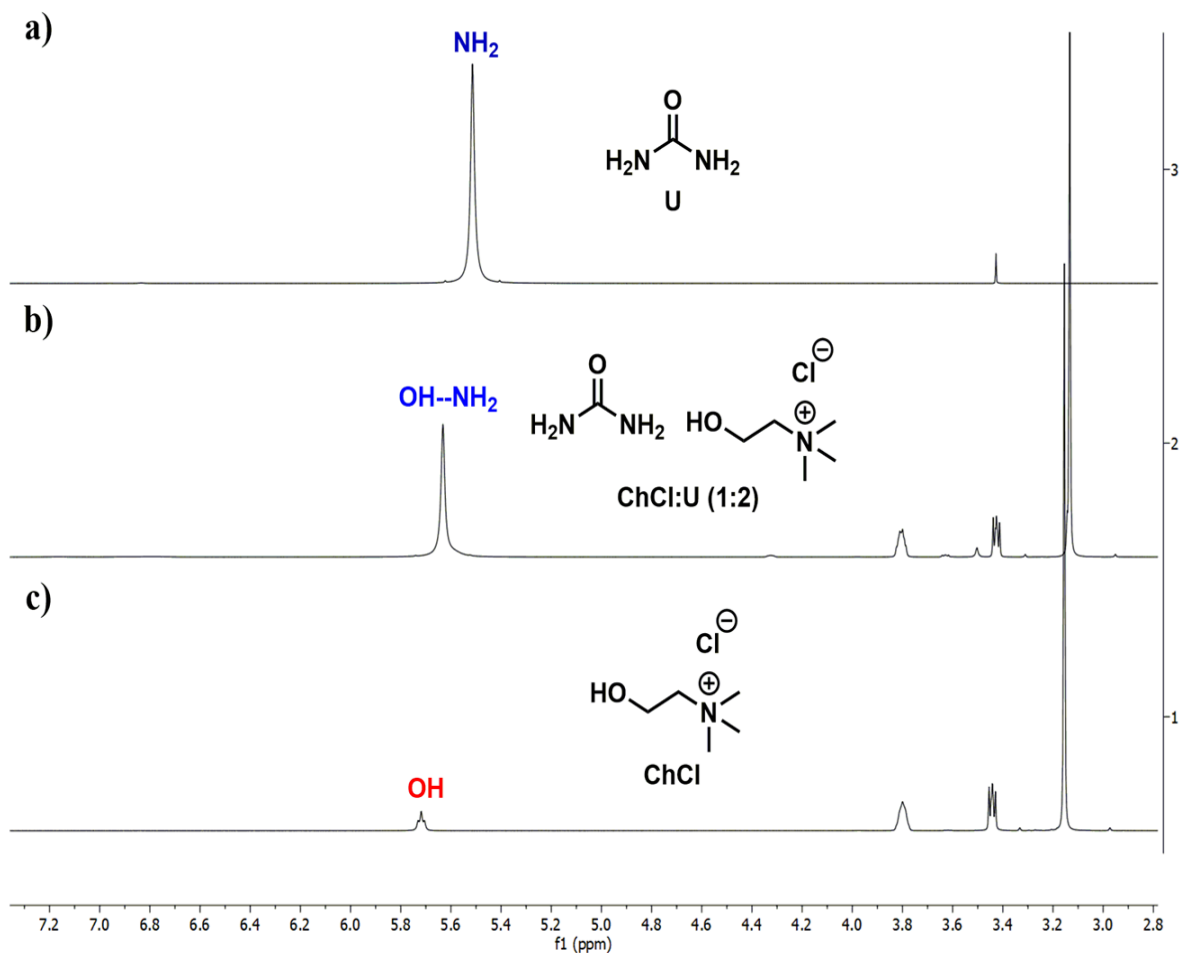


Figure 8. ^1H NMR (400 MHz) analysis for eutectic mixture and its components: **(a)** Urea, **(b)** ChCl:U / 1:2 (*mol:mol*), and **(c)** choline chloride. DMSO-d_6 .

In Fig. 9 (a), the characteristic bands of urea are observed in the FT-IR spectrum, corresponding to the following bonds: N–H at 3444 , 3348 , 1624 and 1153 cm^{-1} , C=O at 1678 cm^{-1} , C–N at 1458 cm^{-1} . In the choline chloride spectrum (Fig. 9 (b)), broad absorption bands were recorded at 3250 cm^{-1} (O–H stretch in the aliphatic structure) and $3000\text{--}2800\text{ cm}^{-1}$ (C–H stretch in methyl and methylene groups). The absorption around 1479 cm^{-1} can be attributed to the C–N stretching. In the spectrum of the synthesized DES (Fig. 9 (c)), a slight change in the absorption band of the C=O stretch at 1668 cm^{-1} was verified, compared with pure urea. Furthermore, an intense broad absorption band at $3431\text{--}3174\text{ cm}^{-1}$ referring to intermolecular NH–OH interactions was obtained.

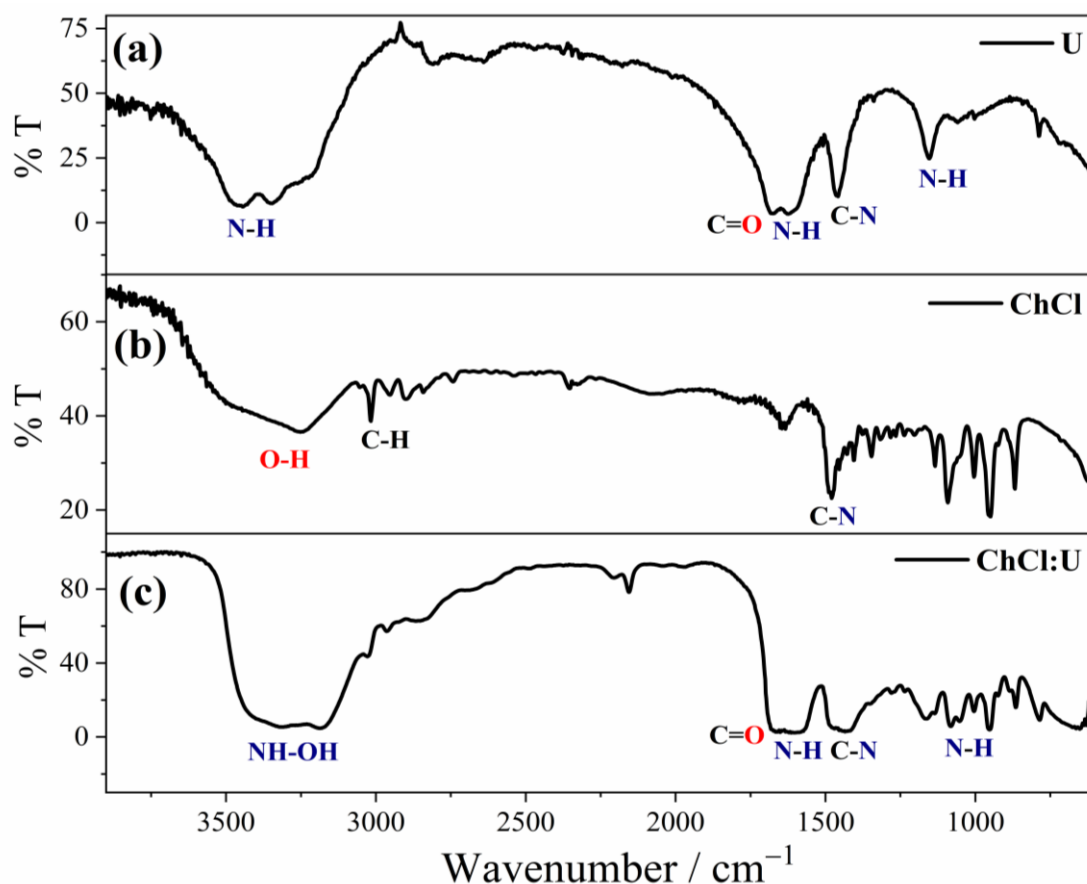


Figure 9. FT-IR spectroscopy analysis for eutectic mixture and their components: (a) urea, (b) choline chloride, and (c) ChCl:U / 1:2 (mol:mol).

2.1.3.4 Characterization of MWCNT and USP_tNPs-DES/MWCNT composite

Initially, the USP_tNPs synthesized in DES and citrate-capped platinum nanoparticles (PtNPs) were morphologically characterized by TEM. As can be seen in Fig. 10 (a), USP_tNPs with uniform size distribution and approximately spherical shape were obtained, with an average size of (0.204 ± 0.008) nm (Fig. 10 (b)). These results consider only particles that did not agglomerate. From Fig. 10 (c), several emission lines from platinum were observed in the EDS spectrum. Similarly, as can be seen in Figs. 10 (d), (e) and (f) the PtNPs showed distinct shapes, with average size of (2.8 ± 0.9) nm and several emissions lines in the EDS spectrum, respectively.

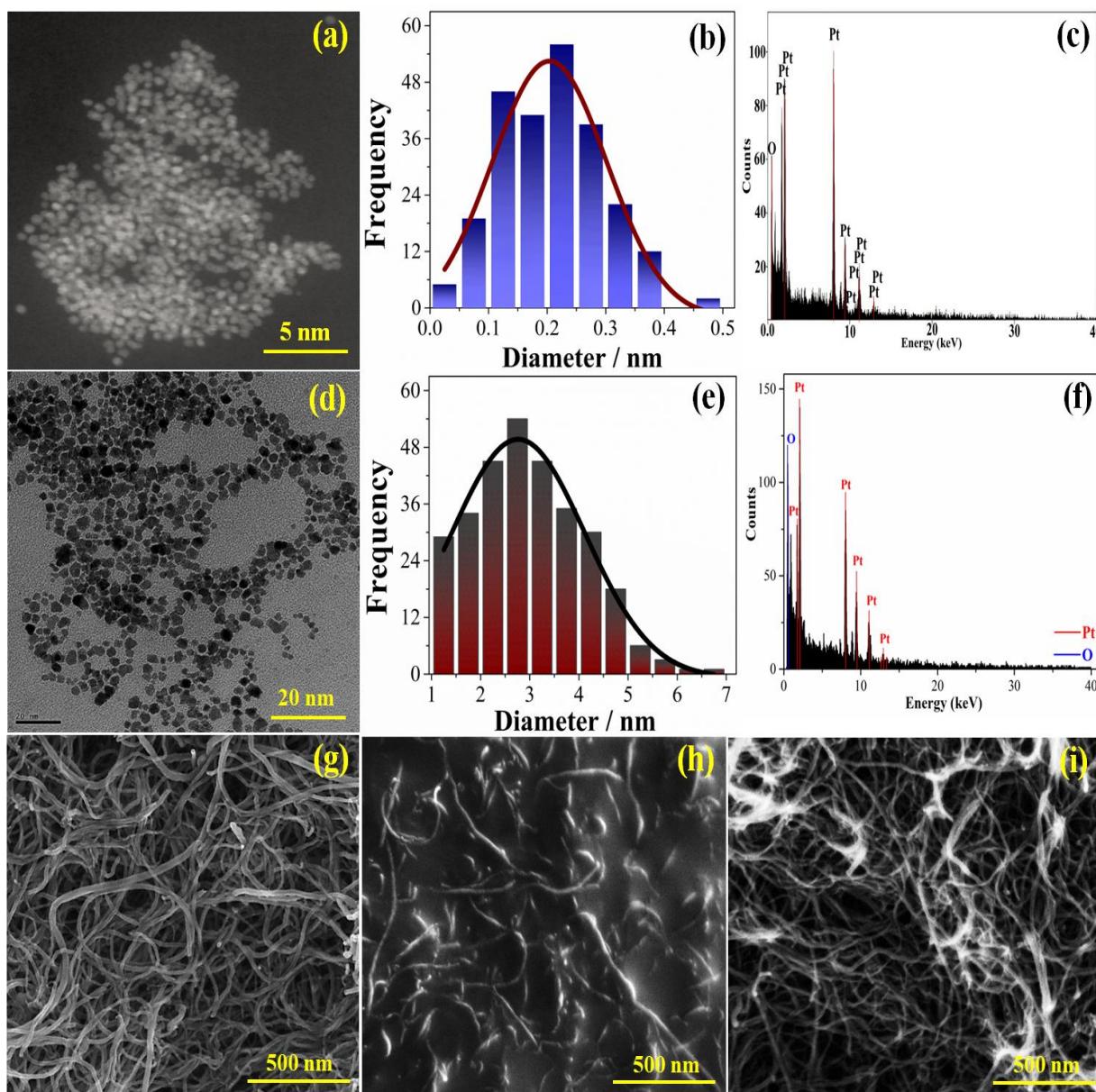


Figure 10. (a) TEM image for USPtNPs. (b) Histogram of diameter distribution obtained for the USPtNPs. (c) EDS spectrum of USPtNPs. (d) TEM image for PtNPs. (e) Histogram of diameter distribution obtained for the PtNPs. (f) EDS spectrum of PtNPs. SEM images obtained for the (g) MWCNT and (h and i) for USPtNPs-DES/MWCNT and PtNPs/MWCNT composite film, respectively.

Thus, when using DES as a stabilizing medium, the average size of the synthesized USPtNPs was considerably lower than that PtNPs obtained in citrate medium (13.8 times larger) and other methods reported in the literature. In the works of Inwati et al. [38] and Ano et al. [39], average sizes ranging from 33 to 48 nm (165 and 240 times larger) and 1.9 nm (9.5 times larger), respectively, were reported for PtNPs synthesized by microwave irradiation. PtNPs synthesized through polyol method with modifications presented by Long et al. [40], yielded PtNPs with average sizes ranging from 5 to 12 nm

(25 to 60 times larger). In addition, electrodeposition methods [41] report PtNPs with average sizes of 13.4 nm (67 times larger).

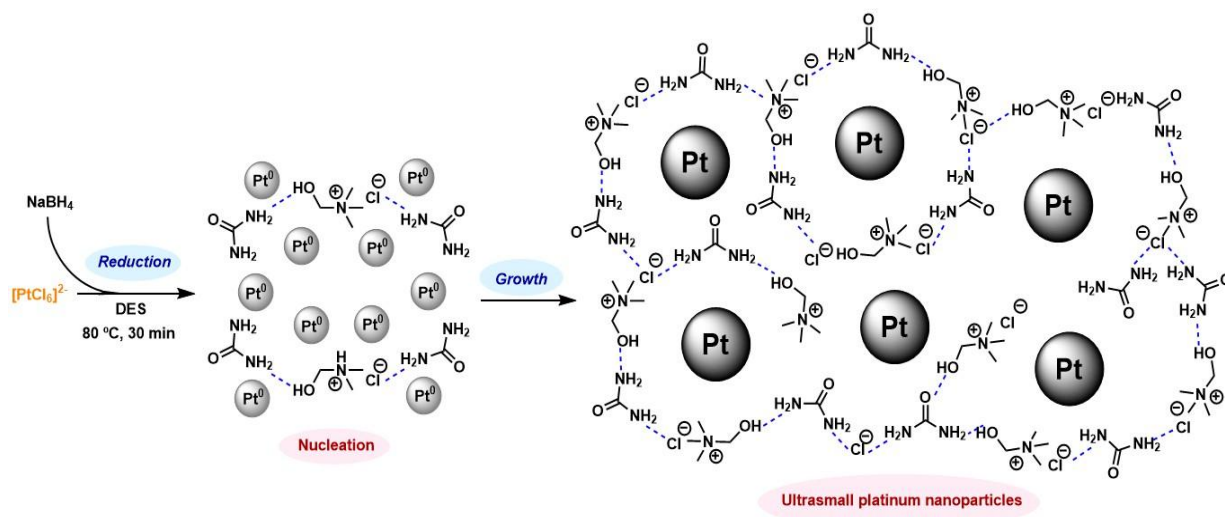
These results place the obtained PtNPs in a more specific classification, of the ultrasmall nanoparticles (USNPs). It is important to emphasize that this is one of the main highlights of this work, when considering the other processes already known in the literature for the synthesis of USNPs using varied solvents. As can be seen in Table 3, the synthesis method reported here is much simpler than those cited, whose require sophisticated equipment or laborious steps that imply in more energy expenditure and waste generation, features that weaken some principles of green chemistry, such as atomic economy, energy efficiency, and safer solvent use [42].

Table 3. Comparative analysis of methods employed in ultrasmall nanoparticles synthesis

Ultrasmall nanoparticles	Type of synthesis	Reductor	Medium	Size / nm	Reference
USPt-nanoclusters	Colloidal suspension	NaBH ₄	Tetrahydrofuran	1.5	[47]
USMoS ₂ -NPs	Sonicated suspension	–	Dimethylformamide	1.5	[48]
USWO _x -NPs	Solvothermal	Trimethylamine N-oxide	Oleyamine (1-amino-9-octadecene)	1.6	[49]
USZnO ₂ -NPs	Microwave-assited	–	Mixture of tetrabutylammonium hydroxide and tertahydrofuran	2.6	[50]
USTPP-PtNPs	Hydrothermal	NaBH ₄	Aqueous	4.8	[4]
USPtNPs	Solvothermal	NaBH ₄	Deep eutectic solvent	0.204	This work

*USPt-nanoclusters: ultrasmall platinum nanoclusters; USMoS₂-NPs: ultrasmall molybdenum sulfide nanoparticles; USWO_x-NPs: ultrasmall tungsten oxide nanoparticles; USZnO₂-NPs: ultrasmall zinc oxide nanoparticles; USTPP-PtNPs: ultrasmall triphenylphosphonium-coated platinum nanoparticles; NaBH₄: sodium borohydride.

In general, it is common to use surfactants as protective molecules that act by preventing aggregation through bonds with nanoparticles [43,44]. Thus, it is possible that the good performance of the proposed synthesis is associated with the ability of DES to keep them physically isolated, especially in the growth step. Therefore, the possible mechanism of USPtNPs synthesis in DES displayed in Scheme 1 was proposed.



Scheme 1. Suggested mechanism for synthesis of USPtNPs in DES.

In Scheme 1, the steps involving nucleation and growth as in any synthesis process based on building nanostructures atom by atom (*bottom-up* approach) are included; the solvent is one of the key points to ensure uniformity in the synthesis, considering that particle growth can be controlled by solvent molecules forming an adsorption layer around them [44]. In this sense, in the growth stage, DES can control and stabilize through interactions formed between its ionic complexes and USPtNPs that can accommodate uniformly in cavities, attenuating the effects of agglomeration, and this can result in more uniform particles.

In addition, DES are reported as promising green solvents, and in this work we applied for the first time a DES in the USNPs synthesis, without its thermal decomposition. In fact, the synthesis was conducted below 100 °C. Thus, the use of DES provided a less energetic and more efficient synthesis route for USNPs. It seems that there is a relationship between the diffusion and mobility of the molecular and/or ionic aggregates formed in DES, which may reduce the extent of thermal gradients in the reaction system [45]. This allow the nucleation and growth reactions of USNPs to proceed smoothly and uniformly.

Figs. 10 (h) and (i) show the SEM images of the USPtNPs-DES/MWCNT and PtNPs/MWCNT films deposited from a DMF and Nafion™ dispersion. As can be seen, the MWCNTs were uniformly dispersed in the films. However, in the USPtNPs-DES/MWCNT film, it can be noticed that there is a coating on MWCNT, indicating the presence of DES residues. In Fig. 10 (g), the SEM image certify the morphology of the used MWCNT. Crossed tubular connections characteristic of this carbonaceous material were noted [46].

2.1.3.5 Electrochemical behavior of RB at the proposed sensor

The electrochemical behavior of RB at the bare and modified GCEs was evaluated by cyclic voltammetry (CV). Figs. 11 (a)–(d) illustrate the voltametric profile of RB on the bare GCE, MWCNT/GCE, PtNPs/MWCNT/GCE, and USPtNPs-DES/MWCNT/GCE, respectively.

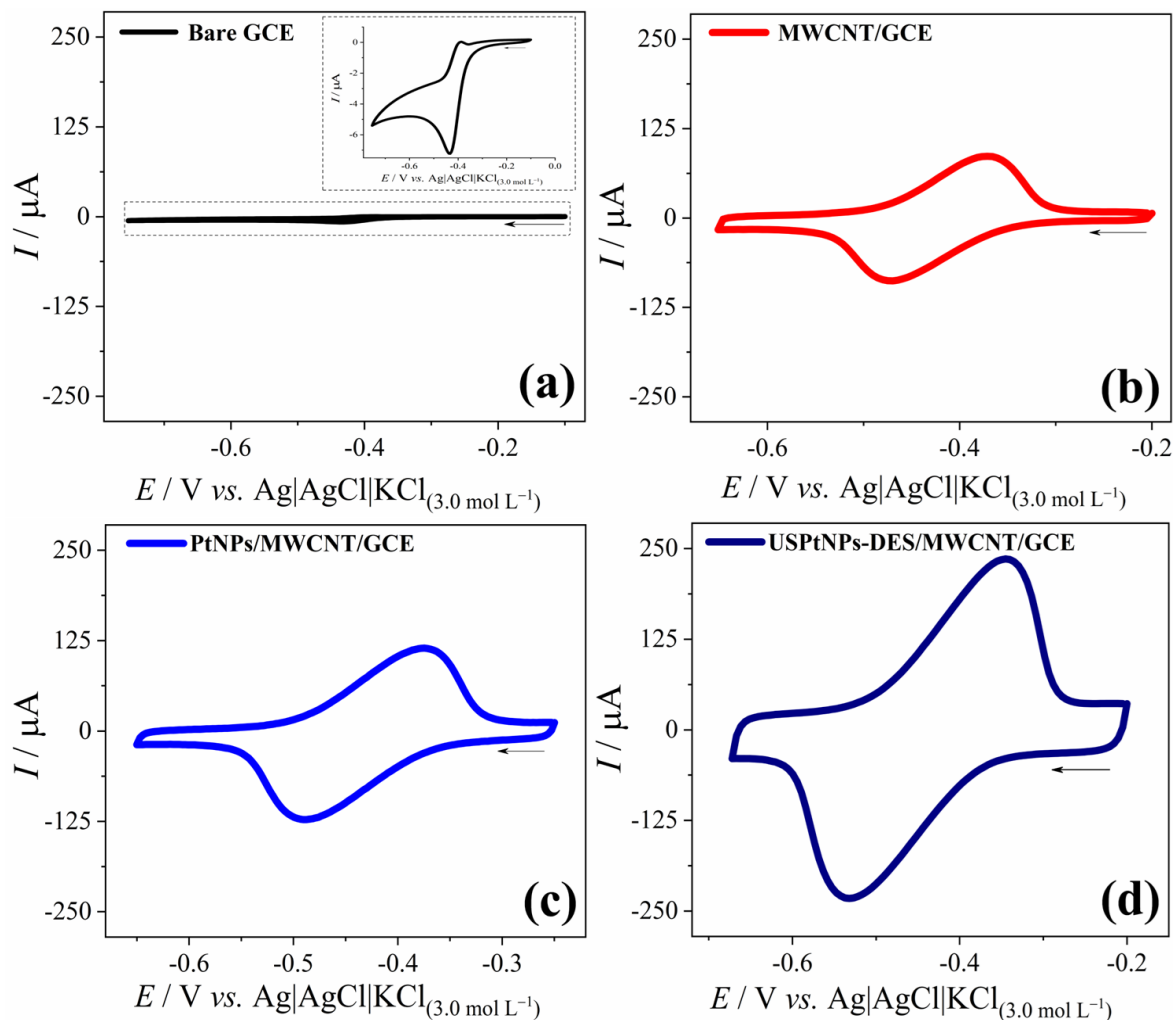


Figure 11. Cyclic voltammograms obtained for $1.0 \times 10^{-4} \text{ mol L}^{-1}$ RB in 0.2 mol L^{-1} phosphate buffer solution (pH 7.0) using different working electrodes: (a) bare CGE, (b) MWCNT/GCE, (c) PtNPs/MWCNT/GCE and (d) USPtNPs-DES/MWCNT/GCE. $v = 50 \text{ mV s}^{-1}$.

As shown, well-defined anodic (I_{ap}) and cathodic (I_{cp}) peaks were registered using all the modified electrodes. However, the electrochemical profile for GCE indicates effects of electron transfer resistance in RB redox process, mainly in oxidation of the species that present I_{ap} 2 times smaller than I_{cp} . In addition, I_{ap}/I_{cp} values suggest a quasi-reversible behavior for the bare GCE and reversible for MWCNT/GCE, PtNPs/MWCNT/GCE and USPtNPs-DES/MWCNT/GCE, as shown in Table 4.

Table 4. Anodic peak current (I_{ap}), cathodic peak current (I_{cp}), current peak ratio (I_{ap}/I_{cp}), anodic peak potential (E_{ap}), cathodic peak potential (E_{cp}), and peak-to-peak potential separation (ΔE_p) obtained for RB using bare GCE and modified electrodes

Electrodes	$I_{ap} / \mu A$	$I_{cp} / \mu A$	I_{ap}/I_{cp}	E_{ap} / V	E_{cp} / V	$\Delta E_p / V$
Bare GCE	+3.23	−7.24	0.45	−0.39	−0.43	0.04
MWCNT/GCE	+86.40	−87.80	0.98	−0.37	−0.47	0.10
PtNPs/MWCNT/GCE	+114.56	−122.47	0.94	−0.37	−0.49	0.12
USPtNPs-DES/MWCNT/GCE	+235.72	−232.36	1.01	−0.35	−0.53	0.18

In quantitative terms, peak currents increased considerably with the incorporation of MWCNT, PtNPs, and USPtNPs synthesized in DES. Indeed, modification with MWCNT increased the electrocatalytic activity for RB, with a current increment of 27 times compared to GCE [46]. When PtNPs and USPtNPs were incorporated with MWCNT, an almost 1.5- and 3-fold increase, respectively, in both currents of RB redox process was observed, comparatively to modification with only MWCNT. This may be attributed to the reduction in electronic transfer resistance and the increase in the surface area of the electrode, owing to the very low average size of the USPtNPs (0.204 nm) synthesized in DES. In general, sensors modified with nanomaterials to determine RB show high faradaic currents [51–55].

2.1.3.5.1 Effect of pH and composition of supporting electrolyte

The influence of pH was investigated by SWV to analyze the magnitude of the current response of RB using the USPtNPs-DES/MWCNT/GCE sensor. Britton-Robinson buffer (BRB) (0.2 mol L^{-1}) with pH ranging from 2.0 to 9.0 was utilized. As shown in Fig. 12 (a), the cathodic peak currents reached similar magnitudes at pH 2.0, 7.0 and 9.0. Considering the composition of the target biological samples (serum and urine), pH 7.0 was chosen as the best condition.

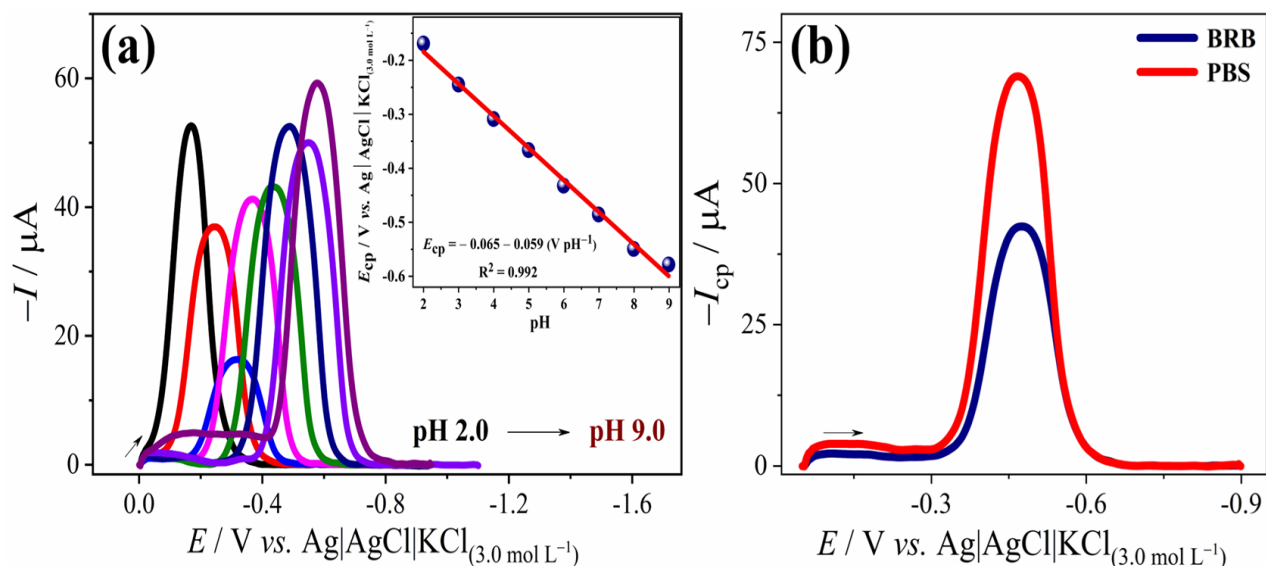


Figure 12. (a) SWV voltammograms obtained for RB at different pH values (inset: Plot of E_{cp} vs. pH, for $n = 3$). (b) SWV voltammograms obtained for $5.0 \times 10^{-7} \text{ mol L}^{-1}$ RB at pH 7.0 for the supporting electrolytes: 0.2 mol L^{-1} phosphate and 0.2 mol L^{-1} Britton-Robinson buffers (SWV parameters: $f = 8 \text{ Hz}$, $a = 100 \text{ mV}$, and $\Delta E_s = 5 \text{ mV}$).

The dependence of the cathodic peak potential (E_{cp}) vs. pH (insert Fig. 12 (a)) was linear, with E_{cp} displacement to more negative values with pH increasing, suggesting the involvement of protons in the RB electroreduction mechanism. Furthermore, the slope of -0.059 V pH^{-1} indicates that the same number of protons and electrons are involved in the reduction process, as described in the literature [56,57]. This finding is in accordance with previous literature, which reports the involvement of two electrons and two protons in the RB reduction [20,55].

The effect of the supporting electrolyte composition was also evaluated by comparing the voltammetric responses of RB at BRB and phosphate buffer solutions (both at pH 7.0) with same ionic strength (0.2 mol L^{-1}). As shown in Fig. 12 (b), in 0.2 mol L^{-1} phosphate buffer solution (pH 7.0) the peak current for the RB electroreduction was higher, therefore, this solution was chosen as the electrolyte.

2.1.3.5.2 potential scan rate study

Cyclic voltammograms recorded in the potential scan rate from 10 mV s^{-1} to 100 mV s^{-1} are presented in Fig. 13 (a). The respective plots of $\log -I_{cp}$ vs. $\log v$ and $-I_{cp}$ vs. $v^{1/2}$ (Figs. 13 (b, c, and d)) were constructed. For $\log -I$ vs. $\log v$ curves, slopes close to 0.5 suggest diffusion control, while slopes close to 1.0 indicate adsorption-controlled processes [58].

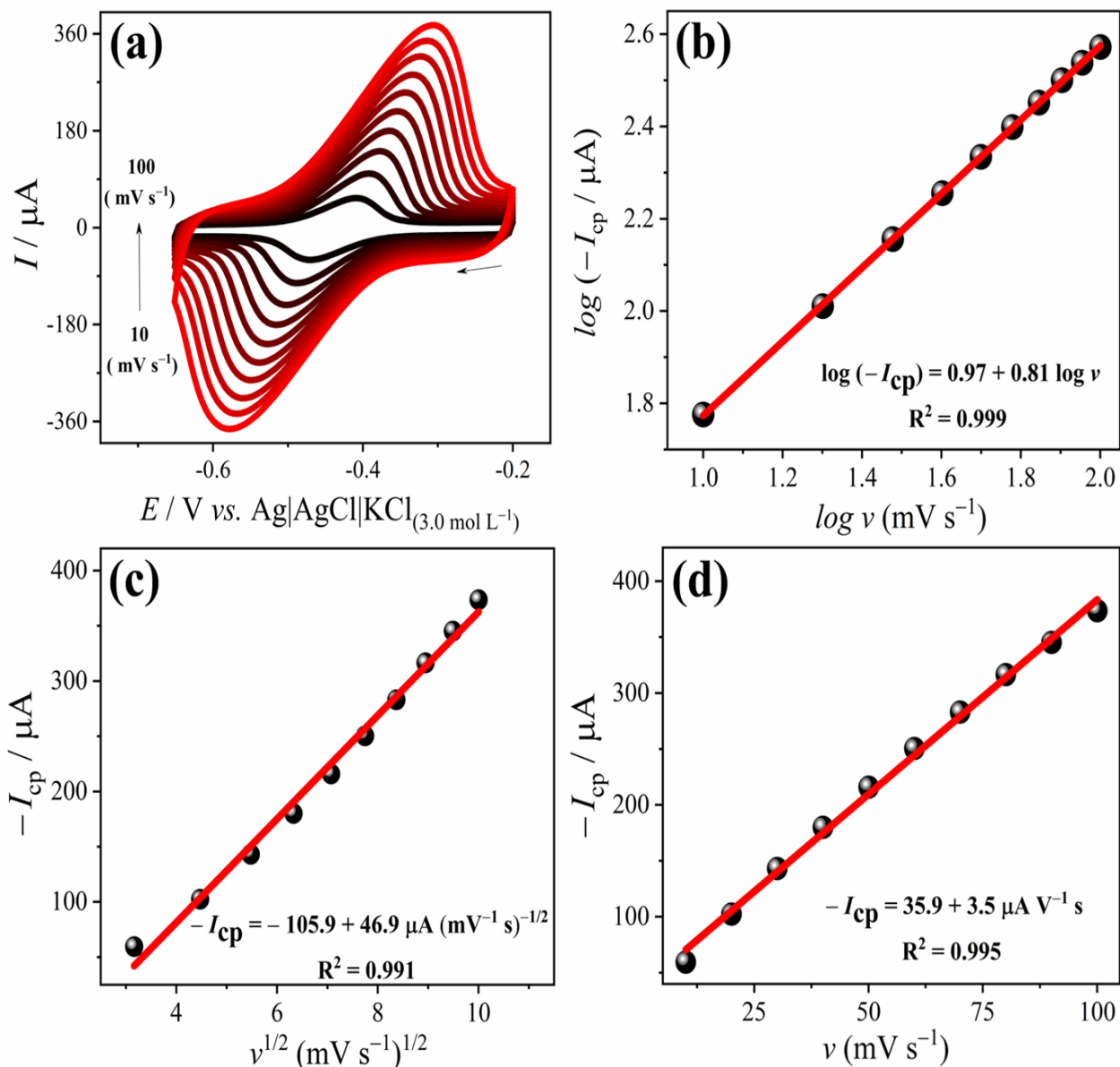


Figure 13. (a) Cyclic voltammograms obtained at different scan rates (10 – 100 mV s⁻¹) for 5.0 × 10⁻⁷ mol L⁻¹ RB using the USPtNPs-DES/MWCNT/GCE. (b), (c), and (d) plots for $\log(-I_{cp})$ vs. $\log v$, $-I_{cp}$ vs. $v^{1/2}$, and $-I_{cp}$ vs. v , respectively.

As can be seen in Fig. 13 (a), an experimental slope of 0.81 (closer to 1.0) was obtained for the $\log -I$ vs. $\log v$, suggesting that the RB reduction process was controlled by adsorption or by both phenomena (adsorption/diffusion). Therefore, to determine the dominant process on the electron transfer of RB reduction, the dependence of $-I$ vs. $v^{1/2}$ and $-I$ vs. v was also examined. Note that plots of Fig. 13 (c and d) presented a linear trend, which indicates that RB electroreduction was a mixed-controlled redox process on the USPtNPs-DES/MWCNT/GCE sensor. In fact, works in the literature have reported the adsorption of RB in modified electrodes [57,59].

2.1.3.6 Analytical performance

Initially, the parameters of SWV were optimized, being this technique chosen as it usually provides better analytical performance towards analytes featuring reversible redox reaction [58]. Thus, from the monitoring of cathodic RB response in terms of resolution and intensity, the best SWV parameters were as shown in Table 5.

Table 5. Optimization of SWV parameters towards RB determination

Parameter	Range	Selected
Pulse frequency (f , Hz)	8 – 100	8
Potential pulse amplitude (a , mV)	10 – 100	100
Potential increment (ΔE_s , mV)	1 – 10	5

Under optimal conditions, an analytical curve was constructed for RB in the concentration range from 2.0×10^{-8} to 1.0×10^{-6} mol L⁻¹ using the USPtNPs-DES/MWCNT/GCE sensor. The SWVs and the respective analytical curve are shown in Fig. 14.

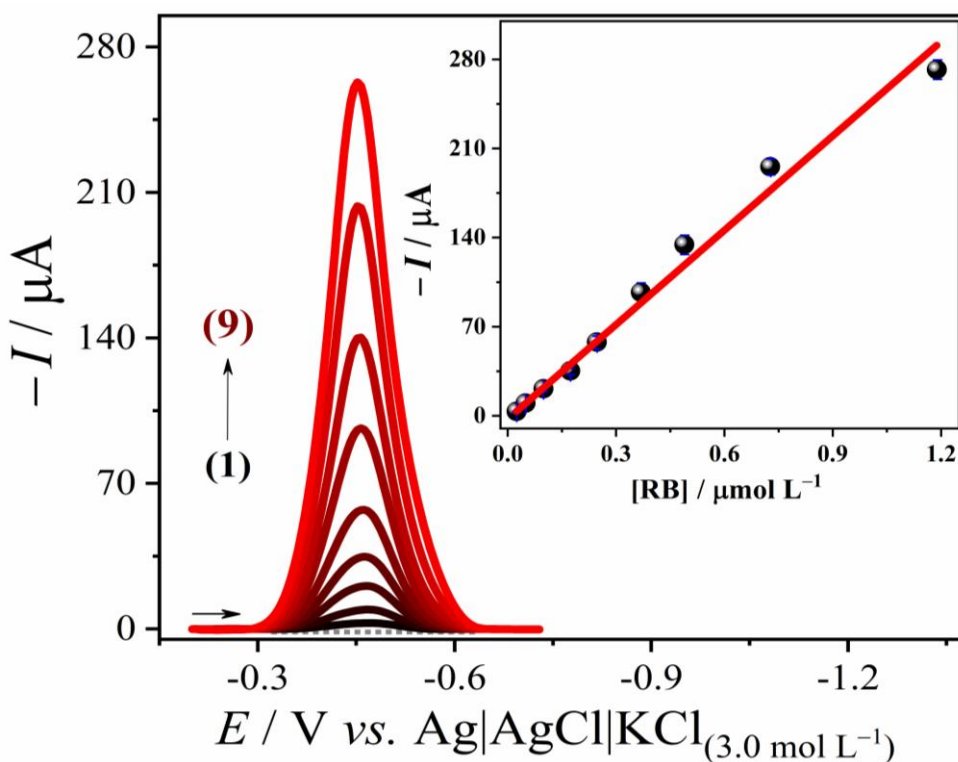


Figure 14. SWV voltammograms obtained at different RB concentrations ranging from 2.0×10^{-8} mol L⁻¹ (1) to 1.0×10^{-6} mol L⁻¹ (9) using the USPtNPs-DES/MWCNT/GCE sensor. Supporting electrolyte: 0.2 mol L⁻¹ phosphate buffer solution (pH 7.0). SWV parameters $f = 8$ Hz, $a = 100$ mV, and $\Delta E_s = 5$ mV. Inset: Analytical curve obtained for RB ($-I$ vs. $[RB]$) and $n = 3$.

The following linear regression equation was obtained for the analytical curve: $-I / \mu\text{A} = -3.2 \mu\text{A} + 247.1 \mu\text{A L } \mu\text{mol}^{-1} [\text{RB}] / \mu\text{mol L}^{-1}$ ($R^2 = 0.991$). The limits of detection (LOD) and quantification (LOQ) were calculated as follows: $\text{LOD} = 3 \times s_b / m$ and, $\text{LOQ} = 10 \times s_b / m$, where s_b is the standard deviation of ten blank measurements ($n = 10$) and m is the analytical sensitivity (slope of the analytical curve). LOD and LOQ of $1.8 \times 10^{-9} \text{ mol L}^{-1}$ (1.8 nmol L^{-1}) and $1.0 \times 10^{-8} \text{ mol L}^{-1}$ were obtained, respectively.

The analytical parameters obtained for RB with the proposed sensor are compared with other sensors present in the literature (Table 6). The proposed sensor showed satisfactory performance in relation to the other reported in the literature. This is a result of the incorporation of USPtNPs synthesized in DES (0.204 nm), providing increased surface area. As a result, the sensitivity was superior when compared to other sensors for the determination of RB using voltammetric methods.

Table 6. Comparison of analytical parameters for the voltammetric determination of RB

Sensors*	RB			Sensitivity ($\text{A L mol}^{-1} \text{ cm}^{-2}$)	Reference
	Linear range ($\mu\text{mol L}^{-1}$)	LOD ($\mu\text{mol L}^{-1}$)	LOQ ($\mu\text{mol L}^{-1}$)		
44-DABP/GCE	0.013 – 7.09	8.5×10^{-3}	0.028	27.54	[60]
$\text{Fe}_3\text{O}_4\text{NPs-ePAD}$	2.0 – 20.0	0.25	2.0	4.87	[55]
BiFE	0.3 – 0.8	0.1	0.3	215.2	[20]
R-CoP/GCN/GCE	0.06 – 3468.8	1.0×10^{-3}	3.6×10^{-3}	1.27	[17]
$\text{TiO}_2\text{NPs/CPE}$	2.4 – 240	1.6	NI**	3.3×10^3	[53]
$-\text{C}_6\text{H}_4\text{COOH/dsDNA/GCE}$	0.08 – 1.0	0.06	0.15	646.5	[61]
SAMs/L-Cys/AuE	5.0×10^{-5} – 5.0	2.5×10^{-5}	NI	NI	[58]
USPtNPs-DES/MWCNT/GCE	0.02 – 1.20	1.8×10^{-3}	0.01	3.5×10^3	This work

*44-DABP/GCE: Glassy carbon electrode modified with 4,4'-diamino benzophenone; $\text{Fe}_3\text{O}_4\text{NPs-ePAD}$: Electrochemical paper-based analytical devices modified with magnetite nanoparticles; BiFE: Bismuth-film electrode; R-CoP/GCN/GCE: Glassy carbon electrode modified with ruthenium doped cobalt phosphide embedded graphitic carbon nitride; $\text{TiO}_2\text{NPs/CPE}$: Carbon paste electrode modified with titanium oxide nanoparticles; $-\text{C}_6\text{H}_4\text{COOH/dsDNA/GCE}$: Glassy carbon electrode modified with the carboxyphenyl layer and DNA (amino probe DNA ($5' \Rightarrow 3'$): $\text{H}_2\text{N} - \text{C}_6 - \text{GAGCGATGCCGA} \text{ACTGCTCG}$ complementary DNA ($5' \Rightarrow 3'$): $\text{CGAGCAGTTCGGCATCGCTC}$) immobilization; SAMs/L-Cys/AuE: Gold electrode modified with a self-assembled monolayer of L-cysteine. **Not informed.

The inter-day and intra-day repeatability studies were performed to evaluate the sensor precision. In the intra-day repeatability, the RB voltammetric response was measured five times in a day ($n = 5$ for each RB concentration). For the inter-day repeatability, SWV measurements were conducted for three days. As shown in Table 7,

the RSD results obtained for the intra- and inter-day precision were below to 7 and 5%, respectively. These values attest that the precision of the SWV method using the proposed USPtNPs-DES/MWCNT/GCE sensor was very satisfactory.

Table 7. Repeatability study for RB using the USPtNPs-DES/MWCNT/GCE sensor

Day	Intra-day			Day	Inter-day		
	[RB] / $\mu\text{mol L}^{-1}$	$-I_{\text{cp}}$ / μA	RSD / %		[RB] / $\mu\text{mol L}^{-1}$	$-I_{\text{cp}}$ / μA	RSD / %
1	0.20	31 ± 2	6.2	1	0.20	32 ± 2	4.5
	0.50	102 ± 5	5.2	2			
	0.98	158 ± 4	2.7	3			
2	0.20	34 ± 1	4.2	1	0.50	103 ± 1	0.9
	0.50	104 ± 4	3.8	2			
	0.98	159 ± 3	1.8	3			
3	0.20	33 ± 2	5.7	1	0.98	160 ± 2	1.4
	0.50	102 ± 1	1.1	2			
	0.98	163 ± 3	1.6	3			

The USPtNPs-DES/MWCNT/GCE sensor was designed to determine RB in commercial energy drinks samples. Thus, the interference of concomitant substances typically found in these samples was evaluated. For this, the RB concentration was fixed at $2.0 \times 10^{-7} \text{ mol L}^{-1}$, and the possible interfering substances were added in three concentration ratios: 1:10, 1:1 and 10:1 ([concomitant]:[RB]). The effect of the following substances was tested: ascorbic acid (vitamin C), vitamins B1, B5, B6, B7, B9, B12, glucose, and caffeine. Table 8 provides the relative error obtained for the comparison of voltammetric responses of RB in the absence and presence of the possible interfering compound. Based on the results, it was evident that the studied substances had no significant effect on the RB voltammetric response, with error below 10%. This suggests that the proposed sensor presented good selectivity over the set of tested species.

Table 8. Riboflavin determination in the presence of possible interfering species

Interferents	Error* / %		
	1:10	1:1	10:1
Ascorbic acid	−3.9	0.9	5.8
Vitamin B1	−7.8	−4.6	−0.4
Vitamin B5	−8.7	−2.3	4.8
Vitamin B6	−9.4	−7.5	0.8
Vitamin B7	−6.3	−0.6	1.6
Vitamin B9	−8.5	−0.7	6.9
Vitamin B12	−9.4	7.4	7.4
Glucose	−5.2	4.8	6.9
Caffeine	−9.0	−2.9	3.1

*Error: $(-I_{\text{cp, presence}} - I_{\text{cp, absence}}) / -I_{\text{cp, absence}} \times 100\%$

2.1.3.7 Determination of RB in food samples and biological fluids

For food samples, three commercial energy drinks were subjected to the voltammetric analysis applying the proposed sensor. Table 9 displays the labelled values of the RB content and those determined by applying the USPtNPs-DES/MWCNTs/GCE sensor. The errors obtained were below 7%. Thus, the results demonstrate that the developed voltammetric approach is applicable to the RB determination in commercial energy drinks.

Table 9. Determination of riboflavin in commercial energy drink samples

Samples*	RB content / mg		
	Labeled	Found	Error ^a / %
A1	1.30	1.22 ± 0.02	−6.5
A2	1.30	1.23 ± 0.02	−5.7
A3	0.65	0.62 ± 0.01	−4.8

*n = 3. ^aError = $[(\text{RB content})_{\text{Found}} - (\text{RB content})_{\text{Labeled}}] / (\text{RB content})_{\text{Found}} \times 100\%$.

The efficiency of the voltammetric sensor was also tested for biological fluid samples spiked with a RB standard at the following concentrations: $5.0 \times 10^{-8} \text{ mol L}^{-1}$, $2.5 \times 10^{-7} \text{ mol L}^{-1}$ and $7.3 \times 10^{-7} \text{ mol L}^{-1}$ for synthetic urine; and $1.2 \times 10^{-7} \text{ mol L}^{-1}$, $3.7 \times 10^{-7} \text{ mol L}^{-1}$ and $8.4 \times 10^{-7} \text{ mol L}^{-1}$ for bovine serum. The recoveries obtained are presented in Table 10. They varied from 93 to 108 %, which denotes no significative matrix effect in the response with the USPtNPs-DES/MWCNT/GCE sensor in media of

complex composition. This suggests the sensor potentiality for the determination of RB in biological samples.

Table 10. Determination of riboflavin in synthetic urine and serum samples

Samples*	[RB] / ($\mu\text{mol L}^{-1}$)		
	Added	Found	Recovery ^a / %
¹ U1	0.05	0.05 ± 0.01	100
U2	0.25	0.25 ± 0.01	100
U3	0.73	0.70 ± 0.04	96
² S1	0.12	0.13 ± 0.01	108
S2	0.37	0.37 ± 0.01	100
S3	0.84	0.78 ± 0.03	93

* $n = 3$; ¹Urine; ²Serum; ^aRecovery percentage = $([\text{RB}]_{\text{Found}}/[\text{RB}]_{\text{Added}}) \times 100\%$.

2.1.4 Conclusions

Here, we demonstrated for the first time a synthesis approach based on solvothermal concepts using a deep eutectic solvent based on choline chloride and urea for ultrasmall platinum nanoparticles. We consider that the reduction of USPtnPs were due to DES, which thermally stabilizes the synthesis reaction and affords uniform nucleation and growth steps of these particles. This may be the possible reason of the drastic reduction in the size of the synthesized USPtnPs. Furthermore, we consider that the proposed solvothermal synthesis using reline DES needs to be further explored. In fact, the excellent performance achieved in this pioneering work opens new perspectives for synthesis of ultrasmall nanoparticles using DES, as these provide greener alternatives. In addition, the synthesized USPtnPs with a mean diameter of 0.204 nm were applied for the first time in the construction of an electrochemical sensor based on multi-walled carbon nanotubes, which was utilized to detect RB in commercial samples of energy drinks and simulated biological fluids. The USPtnPs-DES/MWCNT/GCE sensor exhibited high sensitivity for the determination of RB. USPtnPs contributed significantly to the faradic responses of the RB, considering the excellent LOD and LOQ values, recoveries, repeatabilities, and quantifications.

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References

- [1] J. Xu, M. Song, Z. Fang, L. Zheng, X. Huang, K. Liu, Applications and challenges of ultra-small particle size nanoparticles in tumor therapy, *J. Control. Release.* 353 (2023) 699–712. <https://doi.org/10.1016/j.jconrel.2022.12.028>.
- [2] B.H. Kim, M.J. Hackett, J. Park, T. Hyeon, Synthesis, characterization, and application of ultrasmall nanoparticles, *Chem. Mater.* 26 (2014) 59–71. <https://doi.org/10.1021/cm402225z>.
- [3] S.E. Kim, L. Zhang, K. Ma, M. Riegman, F. Chen, I. Ingold, M. Conrad, M.Z. Turker, M. Gao, X. Jiang, S. Monette, M. Pauliah, M. Gonen, P. Zanzonico, T. Quinn, U. Wiesner, M.S. Bradbury, M. Overholtzer, Ultrasmall nanoparticles induce ferroptosis in nutrient-deprived cancer cells and suppress tumour growth, *Nat. Nanotechnol.* 11 (2016) 977–985. <https://doi.org/10.1038/nnano.2016.164>.
- [4] Z. Ma, Y. Zhang, J. Zhang, W. Zhang, M.F. Foda, X. Dai, H. Han, Ultrasmall Peptide-Coated Platinum Nanoparticles for Precise NIR-II Photothermal Therapy by Mitochondrial Targeting, *ACS Appl. Mater. Interfaces.* 12 (2020) 39434–39443. <https://doi.org/10.1021/acsami.0c11469>.
- [5] M. Fan, Y. Han, S. Gao, H. Yan, L. Cao, Z. Li, X.J. Liang, J. Zhang, Ultrasmall gold nanoparticles in cancer diagnosis and therapy, *Theranostics.* 10 (2020) 494–4957. <https://doi.org/10.7150/thno.42471>.
- [6] X. Qiao, M. Arsalan, X. Ma, Y. Wang, S. Yang, Y. Wang, Q. Sheng, T. Yue, A hybrid of ultrathin metal-organic framework sheet and ultrasmall copper nanoparticles for detection of hydrogen peroxide with enhanced activity, *Anal. Bioanal. Chem.* 413 (2021) 839–851. <https://doi.org/10.1007/s00216-020-03038-0>.
- [7] Y. Yu, J. Peng, M. Pan, Y. Ming, Y. Li, L. Yuan, Q. Liu, R. Han, Y. Hao, Y. Yang, D. Hu, H. Li, Z. Qian, A Nonenzymatic Hydrogen Peroxide Electrochemical Sensing and Application in Cancer Diagnosis, *Small Methods.* 5 (2021) 1–11. <https://doi.org/10.1002/smt.202001212>.
- [8] P. Balasubramanian, S. Bin He, A. Jansirani, H.P. Peng, L.L. Huang, H.H. Deng, W. Chen, Bimetallic AgAu decorated MWCNTs enable robust nonenzyme electrochemical sensors for in-situ quantification of dopamine and H₂O₂ biomarkers expelled from PC-12 cells, *J. Electroanal. Chem.* 878 (2020) 114554. <https://doi.org/10.1016/j.jelechem.2020.114554>.
- [9] M. Arsalan, X. Qiao, A. Awais, Y. Wang, S. Yang, Q. Sheng, T. Yue, Enhanced Sensitive Electrochemical Sensor for Simultaneous Catechol and Hydroquinone Detection by Using Ultrasmall Ternary Pt-based Nanomaterial, *Electroanalysis.* 33 (2021) 1528–1538. <https://doi.org/10.1002/elan.202100026>.
- [10] W. He, Y. Ding, W. Zhang, L. Ji, X. Zhang, F. Yang, A highly sensitive sensor for simultaneous determination of ascorbic acid, dopamine and uric acid based on ultra-small Ni nanoparticles, *J. Electroanal. Chem.* 775 (2016) 205–211. <https://doi.org/10.1016/j.jelechem.2016.06.001>.
- [11] A.P. Abbott, G. Capper, D.L. Davies, H.L. Munro, R.K. Rasheed, Preparation of novel, moisture-stable, Lewis-acidic ionic liquids containing quaternary ammonium salts with functional side chains[†], *Chem. Commun.* (2001) 2010–2011. <https://doi.org/10.1039/b106357j>.
- [12] A. Shishov, A. Bulatov, M. Locatelli, S. Carradori, V. Andrich, Application of deep eutectic solvents in analytical chemistry. A review, *Microchem. J.* 135 (2017) 33–38. <https://doi.org/10.1016/j.microc.2017.07.015>.
- [13] K.K. de L. Augusto, G.R. Piton, P.C. Gomes-Junior, G.P. Longato, F.C. de Moraes, O. Fatibello-Filho, Enhancing the electrochemical sensitivity of hydroquinone using a hydrophobic deep eutectic solvent-based carbon paste electrode [†], *Anal. Methods.* 14 (2022) 2003–2013. <https://doi.org/10.1039/d2ay00473a>.

- [14] G.R. Piton, K.K.L. Augusto, A. Wong, F.C. Moraes, O. Fatibello-Filho, A Novel Electrochemical Glassy Carbon Electrode Modified with Carbon Black and Glyceline Deep Eutectic Solvent within a Crosslinked Chitosan Film for Simultaneous Determination of Acetaminophen and Diclofenac, *Electroanalysis*. (2021) 1–11. <https://doi.org/10.1002/elan.202100325>.
- [15] S. Kumar-Krishnan, M. Guadalupe-Ferreira García, E. Prokhorov, M. Estevez-González, R. Pérez, R. Esparza, M. Meyyappan, Synthesis of gold nanoparticles supported on functionalized nanosilica using deep eutectic solvent for an electrochemical enzymatic glucose biosensor, *J. Mater. Chem. B*. 5 (2017) 7072–7081. <https://doi.org/10.1039/c7tb01346a>.
- [16] J.N. Baby, B. Sriram, S.F. Wang, M. George, Effect of Various Deep Eutectic Solvents on the Sustainable Synthesis of MgFe₂O₄ Nanoparticles for Simultaneous Electrochemical Determination of Nitrofurantoin and 4-Nitrophenol, *ACS Sustain. Chem. Eng.* 8 (2020) 1479–1486. <https://doi.org/10.1021/acssuschemeng.9b05755>.
- [17] X. Li, K.H. Row, Preparation of levofloxacin-imprinted nanoparticles using designed deep eutectic solvents for the selective removal of: Levofloxacin pollutants from environmental waste water, *Analyst*. 145 (2020) 2958–2965. <https://doi.org/10.1039/c9an02556a>.
- [18] B. Dalkiran, C.M.A. Brett, A novel nanostructured poly(thionine)-deep eutectic solvent/CuO nanoparticle film-modified disposable pencil graphite electrode for determination of acetaminophen in the presence of ascorbic acid, *Anal. Bioanal. Chem.* 413 (2021) 1149–1157. <https://doi.org/10.1007/s00216-020-03078-6>.
- [19] A.P. Abbott, G. Capper, D.L. Davies, R.K. Rasheed, V. Tambyrajah, Novel solvent properties of choline chloride/urea mixtures, *Chem. Commun.* (2003) 70–71. <https://doi.org/10.1039/b210714g>.
- [20] M. Moglianetti, J. Solla-Gullón, P. Donati, D. Pedone, D. Debellis, T. Sibillano, R. Brescia, C. Giannini, V. Montiel, J.M. Feliu, P.P. Pompa, Citrate-Coated, Size-Tunable Octahedral Platinum Nanocrystals: A Novel Route for Advanced Electrocatalysts, *ACS Appl. Mater. Interfaces*. 10 (2018) 41608–41617. <https://doi.org/10.1021/acsami.8b11774>.
- [21] N. Laube, B. Mohr, A. Hesse, Laser-probe-based investigation of the evolution of particle size distributions of calcium oxalate particles formed in artificial urines, *J. Cryst. Growth*. 233 (2001) 367–374. [https://doi.org/10.1016/S0022-0248\(01\)01547-0](https://doi.org/10.1016/S0022-0248(01)01547-0).
- [22] M. Vilková, P. Justyna, V. Andruch, The role of water in deep eutectic solvent-base extraction, *J. Mol. Liq.* 304 (2020). <https://doi.org/10.1016/j.molliq.2020.112747>.
- [23] J.T.M. Amphlett, S. Choi, The effect of increasing water content on transition metal speciation in deep eutectic solvents, *J. Mol. Liq.* 332 (2021). <https://doi.org/10.1016/j.molliq.2021.115845>.
- [24] R. Craveiro, I. Aroso, V. Flammia, T. Carvalho, M.T. Viciosa, M. Dionísio, S. Barreiros, R.L. Reis, A.R.C. Duarte, A. Paiva, Properties and thermal behavior of natural deep eutectic solvents, *J. Mol. Liq.* 215 (2016) 534–540. <https://doi.org/10.1016/j.molliq.2016.01.038>.
- [25] M. Jablonsky, V. Majova, K. Ondrigova, J. Sima, Preparation and characterization of physicochemical properties and application of novel ternary deep eutectic solvents, *Cellulose*. 26 (2019) 3031–3045. <https://doi.org/10.1007/s10570-019-02322-2>.
- [26] A.P. Abbott, R.C. Harris, K.S. Ryder, Application of Hole Theory to Define Ionic Liquids by their Transport Properties †, *J. Phys. Chem. B*. (2007) 4910–4913. <https://doi.org/10.1021/jp0671998>.
- [27] X. Meng, K. Ballerat-busserolles, J. Andanson, Impact of water on the melting temperature of urea + choline chloride deep eutectic solvent, *New J. Chem.* (2016) 4492–4499. <https://doi.org/10.1039/c5nj02677f>.
- [28] C. Yuan, X. Zhang, Y. Ren, S. Feng, J. Liu, J. Wang, L. Su, Temperature- and pressure-induced phase transitions of choline chloride-urea deep eutectic solvent, *J. Mol. Liq.* 291 (2019). <https://doi.org/10.1016/j.molliq.2019.111343>.
- [29] T.S.B.A. V Kuznetsov, Phase equilibria in binary subsystems of urea – biuret – water system, *J. Therm. Anal. Calorim.* 101 (2010) 33–40. <https://doi.org/10.1007/s10973-010->

- 0829-2.
- [30] M. Kuhnert-brandstüitter, A. Burger, Phase diagrams of urea inclusion compounds, *J. Therm. Anal.* 50 (1997) 559–567. <https://doi.org/0.1007/BF01979028>.
 - [31] I.M. Aroso, A. Paiva, R.L. Reis, A. Rita, C. Duarte, Natural deep eutectic solvents from choline chloride and betaine – Physicochemical properties, *J. Mol. Liq.* 241 (2017) 654–661. <https://doi.org/10.1016/j.molliq.2017.06.051>.
 - [32] R.L. Collin, Polymorphism and Radiation Decomposition of Choline Chloride, *J. Am. Chem. Soc.* 79 (1957) 6086. <https://doi.org/10.1021/ja01579a064>.
 - [33] V. Kocí, J. Sesták, R. Cerný, Thermal inertia and evaluation of reaction kinetics: A critical review, *Measurement*. 198 (2022). <https://doi.org/10.1016/j.measurement.2022.111354>.
 - [34] P. Gabbott, Principles and Applications of Thermal Analysis, 1 st editi, Blackwell Publishing Ltd, 2008.
 - [35] A. Alhadid, L. Mokrushina, M. Minceva, Formation of glassy phases and polymorphism in deep eutectic solvents, *J. Mol. Liq.* 314 (2020). <https://doi.org/10.1016/j.molliq.2020.113667>.
 - [36] H.G. Morrison, C.C. Sun, S. Neervannan, Characterization of thermal behavior of deep eutectic solvents and their potential as drug solubilization vehicles, *Int. J. Pharm.* 378 (2009) 136–139. <https://doi.org/10.1016/j.ijpharm.2009.05.039>.
 - [37] L.I.N. Tomé, V. Baião, W. da Silva, C.M.A. Brett, Deep eutectic solvents for the production and application of new materials, *Appl. Mater. Today*. 10 (2018) 30–50. <https://doi.org/10.1016/j.apmt.2017.11.005>.
 - [38] G.K. Inwati, Y. Rao, M. Singh, In Situ Free Radical Growth Mechanism of Platinum Nanoparticles by Microwave Irradiation and Electrocatalytic Properties, *Nanoscale Res. Lett.* (2016). <https://doi.org/10.1186/s11671-016-1653-9>.
 - [39] T. Ano, S. Tsubaki, A. Liu, M. Matsuhisa, S. Fujii, W. Chun, Y. Wada, K. Motokura, Probing the tmperature of supported platinum nanoparticles under microwave irradiation by in situ and operando XAFS, *Commun. Chem.* (2020) 2–12. <https://doi.org/10.1038/s42004-020-0333-y>.
 - [40] N.V. Long, N.D. Chien, T. Hayakawa, H. Hirata, G. Lakshminarayana, M. Nogami, The synthesis and characterization of platinum nanoparticles: a method of controlling the size and morphology, *Nanotechnology*. 21 (2010) 1–16. <https://doi.org/10.1088/0957-4484/21/3/035605>.
 - [41] L.C.S. Figueiredo-Filho, E.R. Sartori, O. Fatibello-Filho, Electroanalytical determination of the linuron herbicide using a cathodically pretreated boron- doped diamond electrode: comparison with a boron-doped diamond electrode modified with platinum nanoparticles, *Anal. Methods*. 7 (2015) 643–649. <https://doi.org/10.1039/c4ay02182g>.
 - [42] P. Anastas, N. Eghbali, Green Chemistry : Principles and Practice, *Chem. Soc. Rev.* (2010) 301–312. <https://doi.org/10.1039/b918763b>.
 - [43] T.M.D. Dang, T.T.T. Le, E. Fribourg-Blanc, M.C. Dang, The influence of solvents and surfactants on the preparation of copper nanoparticles by a chemicalreduction method, *Adv. Nat. Sci. Nanosci. Nanotechnol.* 2 (2011). <https://doi.org/10.1088/2043-6262/2/2/025004>.
 - [44] M. Zobel, R.B. Neder, S.A.J. Kimber, Universal solvent restructuring induced by colloidal nanoparticles, *Science* (80-.). 347 (2015) 292–294. <https://doi.org/10.1126/science.1261412>.
 - [45] D.J. Adams, P.J. Dyson, Chemistry In Alternative Reaction Media, John Wiley & Sons Ltd, Chichester, UK, 2004.
 - [46] Y. Gogotsi, Carbon nanomaterials, Taylor & Francis Group, Philadelphia, Pennsylvania, USA, 2006.
 - [47] C. Liu, G. Li, D.R. Kauffman, G. Pang, R. Jin, Synthesis of ultrasmall platinum nanoparticles and structural relaxation, *J. Colloid Interface Sci.* 423 (2014) 123–128. <https://doi.org/10.1016/j.jcis.2014.02.022>.
 - [48] T. Wang, H. Zhu, J. Zhuo, Z. Zhu, P. Papakonstantinou, G. Lubarsky, J. Lin, M. Li, Biosensor based on ultrasmall MoS2 nanoparticles for electrochemical detection of H2O2 released by cells at the nanomolar level, *Anal. Chem.* 85 (2013) 10289–10295.

- <https://doi.org/10.1021/ac402114c>.
- [49] N. Soultanidis, W. Zhou, C.J. Kiely, M.S. Wong, Solvothermal synthesis of ultrasmall tungsten oxide nanoparticles, *Langmuir*. 28 (2012) 17771–17777. <https://doi.org/10.1021/la3029462>.
- [50] P.E.J. Saloga, A.F. Thünemann, Microwave-Assisted Synthesis of Ultrasmall Zinc Oxide Nanoparticles, *Langmuir*. 35 (2019) 12469–12482. <https://doi.org/10.1021/acs.langmuir.9b01921>.
- [51] T. Nie, K. Zhang, J. Xu, L. Lu, L. Bai, A facile one-pot strategy for the electrochemical synthesis of poly(3,4-ethylenedioxythiophene)/Zirconia nanocomposite as an effective sensing platform for vitamins B2, B6 and C, *J. Electroanal. Chem.* 717–718 (2014) 1–9. <https://doi.org/10.1016/j.jelechem.2014.01.006>.
- [52] Ebrahim Zarei, M.R. Jamali, J. Bagheri, Application of TiO₂ Nanoparticles Modified Carbon Paste Electrode for the Determination of Vitamin B₂, *J. Anal. Chem.* 74 (2019) 1213–1222. <https://doi.org/10.1134/S1061934819120049>.
- [53] Z. Zhang, J. Xu, Y. Wen, T. Wang, A highly-sensitive VB₂ electrochemical sensor based on one-step co-electrodeposited molecularly imprinted WS₂-PEDOT film supported on graphene oxide-SWCNTs nanocomposite, *Mater. Sci. Eng. C*. 92 (2018) 77–87. <https://doi.org/10.1016/j.msec.2018.06.029>.
- [54] D.F. Pereira, E.R. Santana, A. Spinelli, Electrochemical paper-based analytical devices containing magnetite nanoparticles for the determination of vitamins B₂ and B₆, *Microchem. J.* 179 (2022) 107588. <https://doi.org/10.1016/j.microc.2022.107588>.
- [55] P.K. Sonkar, V. Ganesan, S.K. Sen Gupta, D.K. Yadav, R. Gupta, M. Yadav, Highly dispersed multiwalled carbon nanotubes coupled manganese salen nanostructure for simultaneous electrochemical sensing of vitamin B₂ and B₆, *J. Electroanal. Chem.* 807 (2017) 235–243. <https://doi.org/10.1016/j.jelechem.2017.11.050>.
- [56] É.S. Sá, P.S. Da Silva, C.L. Jost, A. Spinelli, Electrochemical sensor based on bismuth-film electrode for voltammetric studies on vitamin B₂ (riboflavin), *Sensors Actuators, B Chem.* 209 (2015) 423–430. <https://doi.org/10.1016/j.snb.2014.11.136>.
- [57] W. Qijin, Y. Nianjun, Z. Haili, Z. Xinpin, X. Bin, Voltammetric behavior of vitamin B₂ on the gold electrode modified with a self-assembled monolayer of l-cysteine and its application for the determination of vitamin B₂ using linear sweep stripping voltammetry, *Talanta*. 56 (2002) 1167. [https://doi.org/10.1016/S0039-9140\(02\)00015-2](https://doi.org/10.1016/S0039-9140(02)00015-2).
- [58] A.J. Bard, L.R. Faulkner, H.S. White, *Electrochemical Methods: Fundamental and Applications*, 3th editio, John Wiley & Sons, Ltd, New York, 2022.
- [59] T. Tabanlıgil Calam, A novel, efficient and sensitive method for the simultaneous determination of riboflavin (vitamin B₂) and pyridoxine hydrochloride (vitamin B₆) in food and pharmacological samples using an electrochemical sensor based on 4,4'-diamino benzophenone, *Microchem. J.* 169 (2021). <https://doi.org/10.1016/j.microc.2021.106557>.
- [60] R. Shanmugam, C. Koventhan, S.M. Chen, W. Hung, A portable Ru-decorated cobalt phosphide on graphitic carbon nitride sensor: An effective electrochemical evaluation method for vitamin B₂ in the environment and biological samples, *Chem. Eng. J.* 446 (2022) 136909. <https://doi.org/10.1016/j.cej.2022.136909>.
- [61] A. Kowalczyk, M. Sadowska, B. Krasnodebska-ostrega, A.M. Nowicka, Selective and sensitive electrochemical device for direct VB₂ determination in real products, *Talanta*. 163 (2017) 72–77. <https://doi.org/10.1016/j.talanta.2016.10.087>.

2.2. Research Article II

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Synthesis of ultrasmall cerium oxide nanoparticles in deep eutectic solvent and their application in an electrochemical sensor to detect dopamine in biological fluids

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Abstract

We report a solvothermal synthesis of ultrasmall cerium oxide nanoparticles (USCeOxNPs) with an average size of (0.73 ± 0.07) nm using deep eutectic solvent (DES) as a stabilizing medium at a temperature of 90 °C. Transmission electronic microscopy (TEM) and energy dispersive spectroscopy (EDS) were used to morphologically characterize the USCeOxNPs, and selected area electron diffraction pattern (SAED) was used to determine structure and crystallinity. These revealed approximately spherical shapes with emission lines characteristic of cerium and the presence of cubic structures of crystalline cerium oxide nanoparticles (CeO₂NPs). The USCeOxNPs-DES/CB film was characterized by scanning electronic microscopy (SEM), which demonstrated the spherical characteristic of CB with layers slightly covered by DES residues. DES was characterized by Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR), indicating its formation through hydrogen bonds between the precursors. An electrochemical sensor for dopamine (DA) determination in biological fluids was developed using the USCeOxNPs together with carbon black (CB). An enhanced current response was observed on DA voltammetric determination, and this can be attributed to the USCeOxNPs. This sensor displayed linear responses for DA in the range of 5.0×10^{-7} mol L⁻¹ to 3.2×10^{-4} mol L⁻¹, with a limit of detection of 80 nmol L⁻¹. Besides detectability, excellent performances were verified for repeatability and anti-interference. The sensor based on USCeOxNPs synthesized in DES in a simpler and environmentally friendly way was successfully applied to determine DA in biological matrix.

Keywords: Deep eutectic solvent; Solvothermal synthesis; Ultrasmall cerium oxide nanoparticles; Dopamine.

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2.2.1 Introduction

The increasing demand for more sustainable and efficient processes that aim to reduce the use of potentially toxic substances and the generation of waste from chemical reactions has gained importance in recent decades, mostly through guidelines proposed by Anastas and Eghbali that aim for more sustainable and green practices [1]. Thus, nanomaterial synthesis deserves special attention, given that their manufacturing process may require some steps with considerable waste production.

In this context, solvents that combine biodegradable characteristics and thermal stability are essential in nanomaterial synthesis. Several techniques are reported in the literature (i.e., sol-gel, microemulsion, hydrothermal, polyol, chemical vapor deposition, electrodeposition, solvothermal methods, or reducing agents) using various reaction media (i.e., surfactants, organic solvents, and dispersants, among others) in conventional nanoparticle synthesis [2]. However, most of these methodologies use or generate toxic substances and/or are not efficient.

Nanosyntheses are governed by nucleation and growth steps. Controlling these stages allows for the control of morphology and size of particles, which can reach ultrasmall scales (< 5.0 nm), giving them multifunctional properties [3]. In this sense, the roles of solvents, protective agents and reducing agents are fundamental in the process, particularly in *bottom-up* approach syntheses, such as solvothermal and hydrothermal techniques that use reducing agents [4]. Recently, in the work proposed by Gomes-Junior et al., deep eutectic solvents (DES) were explored to synthesize platinum nanoparticles, which showed promising results regarding particle size [5].

DES, in general, presents itself as an environmentally friendly alternative to classical organic solvents and, equally important, its thermal properties are excellent for these applications [6,7]. However, DES has been few explored in the syntheses of metallic nanoparticles (MNPs) under solvothermal conditions and, at the same time, several metals, especially noble metals (e.g., gold and platinum), have been extensively used as precursors in NPs synthesis due to their varied applications [8].

Cerium is also well-explored in nanomaterials production. This rare earth metal belonging to the lanthanide series has two common oxidation states (+3 and +4) and, normally, their nanoxides are the main materials generated in these syntheses [9,10]. In the literature, several works report the use of these materials, ranging from biomedical [10–12] to electrochemical sensors [13–15] applications.

Regarding applications in electrochemical sensors, CeOxNPs have been usually combined with other materials in order to enhance the electrocatalytic performance [13–17]. Ensafi et al. [14], for example, presented a composite sensor based on cerium oxide nanoparticles and reduced graphene oxide to detect the pesticide fenitrothion in water samples. The synergistic effect of the composite materials yielded excellent figures of merit for the sensor, reaching a detection limit of 3.0 nmol L^{-1} . In another work, proposed by Fekry et al. [15], cerium nanoparticles were electrodeposited on a carbon paste electrode, which was used to determine nicotine in cigarette samples. Sensitivity for analyte detection increased when concentrations of sodium dodecyl sulfate were added to the deposition medium. The sensor presented a detection limit of $4.43 \times 10^{-8} \text{ mol L}^{-1}$ for nicotine. Lavanya et al. [13], on the other hand, developed a sensor based on cerium nanoparticles doped with copper to determine purines (guanine and adenosine) and pyrimidines (cytosine and thymine) in a denatured DNA sample, without any enzyme or mediator. The sensor exhibited good performance in terms of repeatability, reproducibility, and stability, with adequate detection limits for the proposed application.

Biological compounds from living systems have a wide spectrum of activities, and determining these compounds can be very important in diagnostic applications. In this sense, dopamine (DA) is a catecholamine neurotransmitter present in the mammalian central nervous system. Abnormal DA concentrations have been associated with neurological disorders such as schizophrenia, Parkinson's disease, and Huntington's disease [18]. Therefore, determining DA levels is essential, especially quickly and accurately. Owing to the non-invasive collection and stability under ambient conditions, urine is commonly used to assay dopamine. The reference value of DA in urine ranges from 60 to $440 \text{ } \mu\text{d day}^{-1}$. This corresponds to $0.2 \text{ to } 1.4 \text{ } \mu\text{mol L}^{-1}$, considering that approximately 2 L of urine is produced per day [19].

In this work, we present the use of DES based on choline chloride and urea as a stabilizing medium in the synthesis of ultrasmall cerium oxide nanoparticles (USCeOxNPs). After morphological characterization, the USCeOxNPs were used together with carbon black (CB) to construct an electrochemical sensor. The USCeOxNPs-DES/CB film was characterized by scanning electron microscopy (SEM) and the sensor was applied for determining dopamine in synthetic urine. This simple and environmentally friendly synthesis of cerium-based nanomaterial in eutectic medium has not been reported in the literature.

2.2.2 Experimental Section

2.2.2.1 Chemicals and solutions

The chemicals were purchased from Sigma-Aldrich (St. Louis, USA), Synth (Diadema, São Paulo), Neon (São Paulo, Brasil), Panreac (Darmstadt, Germany), or Vetec Ltda. (Rio de Janeiro, Brasil) with the highest purity grade available and used without further purification. Carbon black (VXC72R) powder was acquired from Cabot Corporation (São Paulo, Brasil). All aqueous solutions were prepared with ultrapure water (electric resistivity $\geq 18.0 \text{ M}\Omega \text{ cm}$) obtained from a Millipore Milli-Q system (Billerica, USA). Acetate and Britton-Robinson buffers were used as supporting electrolyte at a concentration of 0.2 mol L^{-1} . A $1.0 \times 10^{-2} \text{ mol L}^{-1}$ DA standard stock solution was prepared by dissolution in ultrapure water. Dilution processes were adopted to obtain different concentrations of standard solutions.

2.2.2.2 Apparatus

A Fourier transform infrared spectrophotometer (FT-IR) model IRPrestige-21 (Shimadzu Instrument, Kyoto – Japan) (wavenumber range of $4000\text{--}500 \text{ cm}^{-1}$) was used to study the presence of functional groups on DES. ^1H NMR spectra were recorded on a Bruker spectrometer (Billerica, USA) (400 MHz for ^1H) to determine possible hydrogen interactions between the DES precursors. Residual solvent signal was used as reference for the ^1H NMR spectra, with deuterated dimethylsulfoxide ($\text{DMSO-}d_6$): $\delta \text{ } ^1\text{H} = 2.50 \text{ ppm}$.

Viscosities were measured with an IKA Rotavisc viscometer model lo-vi (Staufen, Germany) and electrical conductivities were determined using a CA 150 Marconi conductivity meter (Piracicaba, São Paulo). The DES water content was determined by the Karl-Fischer method with an 899 KF Coulometer (Metrohm, Utrecht – Netherlands). Morphological characterizations of composite films and nanoparticles

were carried out on a field-emission gun scanning electron microscope (FEG/SEM, FEI Magellan 400 L) and on a transmission electron microscope (TEM, FEI Tecnai G2 F20 microscope operating at 200 kV). For these analyses, the SEM samples (CB film and USCeOxNPs-DES/CB film) were dispersed by drop-casting onto carbon-coated silicon grids. For TEM samples, the carbon-coated copper grids were prepared by drop-casting the particles suspended in an ethanol medium. The Selected Area Electron Diffraction (SAED) pattern analysis was carried out on the same grids.

Electrochemical measurements were performed using a three-electrode single-compartment glass cell containing an Ag|AgCl|KCl(3.0 mol L⁻¹) reference electrode, a platinum plate as counter electrode, and bare GCE ($\phi = 3.0$ mm) or modified-GCE as working electrode. An Autolab PGSTAT-20 (Eco Chemie, Utrecht, the Netherlands) potentiostat/galvanostat was utilized in experiments of cyclic voltammetry (CV), square wave voltammetry (SWV), and chronoamperometry. Electrochemical impedance spectroscopy (EIS) experiments were performed using a Sensit Smart Smartphone potentiostat (PalmSens B.V, Utrecht, Netherlands). EIS measurements were performed at the half-peak potential ($E_{p/2}$) of the DA, with a frequency range of 65 kHz to 0.1 Hz and an amplitude of 6 mV, and 10 points per decade.

2.2.2.3 DES preparation

The DES synthesis was performed similarly to the procedure proposed by Abbott et al. [6]. Choline chloride was the hydrogen bond acceptor (HBA) and urea was the hydrogen bond donor (HBD), with a molar ratio (HBA:HBD) of 1:2. The process involved mixing the precursors together in a closed glass with constant stirring at 80 °C during 30 min. A homogeneous solvent was obtained, which remained a stable liquid even after cooled to room temperature. The DES was then stored in a glass flask with molecular sieves to avoid the incorporation of water.

2.2.2.4 USCeOxNPs synthesis

For the USCeOxNPs synthesis, 5 mL of DES were heated up to 60 °C and then 9.6 mg of Ce(SO₄)₂ were added under stirring until complete dissolution. Then, 12.5 mg of hydrazine sulfate ([N₂H₅⁺] [HSO₄⁻]) was added to the reaction vessel under stirring, and the temperature was controlled at 90 °C for 5 min. After this, the reaction mixture presented a color change (yellow to colorless), which was a qualitative indication of the USCeOxNPs formation. Finally, after cooling at room temperature, the mixture

containing the USCeOxNPs was stored in amber vials under the same conditions as the DES.

2.2.2.5 Preparation of the modified electrode

The GCE surface was polished with 1.0 and 0.5 μm alumina slurry and washed with deionized water. The modifying dispersion containing the USCeOxNPs synthesized at DES medium and CB was prepared as follows: 1.0 mg of CB was suspended in a mixture containing 100 μL of USCeOxNPs suspension, 100 μL of NafionTM in methanol (25 % v/v), and 800 μL of N, N-dimethylformamide. The suspension was then submitted to an ultrasonic bath for 30 min aiming at complete dispersion of the materials. Then, for the formation of a film, 8.0 μL of this suspension was dispersed in the GCE surface and allowed to dry for 20 min under an IR lamp.

2.2.2.5 Samples preparation

Synthetic urine was prepared as previously reported by Laube et al. [21], with 0.20 mmol L⁻¹ KCl, 0.18 mmol L⁻¹ NH₄Cl, 0.10 mmol L⁻¹ NaCl, 0.10 mmol L⁻¹ CaCl₂, 0.15 mmol L⁻¹ KH₂PO₄, and 0.18 mmol L⁻¹ urea in a 25 mL volumetric flask. Then, the pH of the synthetic urine was measured in triplicate, and 5.34 ± 0.04 ($n = 3$). This sample was spiked at three different concentrations of DA (0.75, 2.25, and 15.0 $\mu\text{mol L}^{-1}$, respectively) and analyzed in terms of recovery percentage.

2.2.3 Results and Discussion

2.2.3.1 Physical and chemical characterizations of DES

The DES based on choline chloride and urea (1:2 mol/mol) was firstly characterized aiming at determining some important properties such as water content, viscosity, and electrical conductivity. DES presented a water content of $(4.2 \pm 0.1)\%$ and this result was similar to those reported for eutectic mixtures based on choline chloride [5,22]. It is important to emphasize that the hydrophilic nature of this DES is variable with the water content [23]. Thus, the storage of these solvents in bottles containing molecular sieves is recommended. Moreover, considering the proposed synthesis of USCeOxNPs, it is desirable for the water content to be as low as possible, as water generally interferes directly with the morphology and size of the nanoparticles [24].

The electrical conductivity and viscosity at room temperature were $0.38 \pm 0.1 \text{ mS cm}^{-1}$ and $2325 \pm 50 \text{ mPa s}$, respectively. These values are in accordance with the

literature, as conductivities below 2 mS cm^{-1} are typically reported at room temperature for these solvents. This condition arises from the low mobility of the ions that form agglomerates in the medium [25]. Meanwhile, the DES viscosity can be directly linked to the relative ion sizes, low cohesion, and reduced molecular mobility. These characteristics strongly depend on the nature of the precursors [26,27].

2.2.3.2 NMR and FTIR analysis of DES

Intermolecular interactions between urea and choline chloride in eutectic medium were evaluated by NMR of ^1H , $^{13}\text{C}\{^1\text{H}\}$, and Infrared. The presence of hydrogen bonds was evidenced by comparing the precursors' spectra with those obtained for the DES medium. Analyzing the IR spectra (Fig. 15 (a – c)), choline chloride presented a strong stretch νOH at the range of $3600 - 3000 \text{ cm}^{-1}$ and a νCN at around 1477 cm^{-1} .

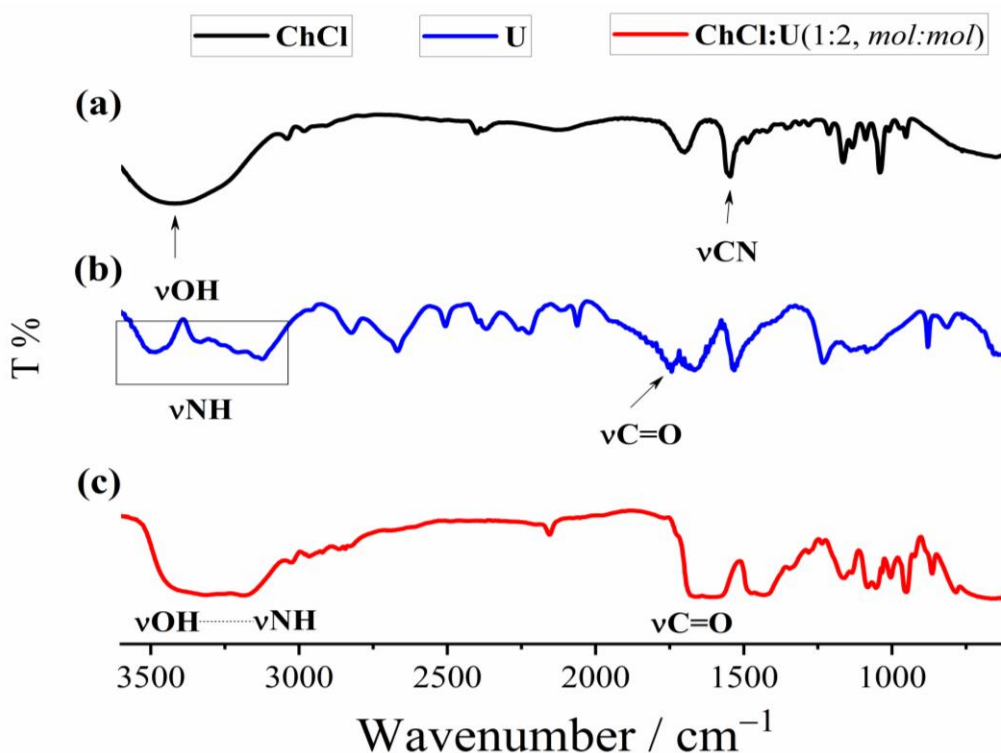


Figure 15. FT-IR spectroscopy analysis for the eutectic mixture and its components: **(a)** choline chloride, **(b)** urea and **(c)** ChCl:U.

The amide group from urea presents characteristics of C=O (carbonyl) stretching bands at about 1687 cm^{-1} , while νNH stretching is observed in less energetic regions (3000 cm^{-1}). When these spectra are compared with the spectrum recorded from the eutectic medium (ChCl:U), broader bands are observed, which is characteristic of the presence of hydrogen bonds. These arise from the interactions between the acidic hydrogens of the N–H (urea) with the O–H group from ChCl [5,28]. Owing to these

widening and overlapping bands, NMR was utilized as it is more sensitive and allows for more conclusive results.

The ^1H and $^{13}\text{C}\{^1\text{H}\}$ spectra of the precursors and DES are shown in the Figures 16 and 17. Note that the proton signals underwent considerable shifts when comparing the precursors' spectra and the DES. For example, the hydrogens susceptible to hydrogen bonds, 5.74 and 5.54 ppm was observed for choline chloride and urea, respectively. For the DES, these signals are overlapped with a frontier shift at 5.60 ppm (Fig. 16), thus showing the possibility of interactions between the species in the DES through hydrogen bonds of precursors.

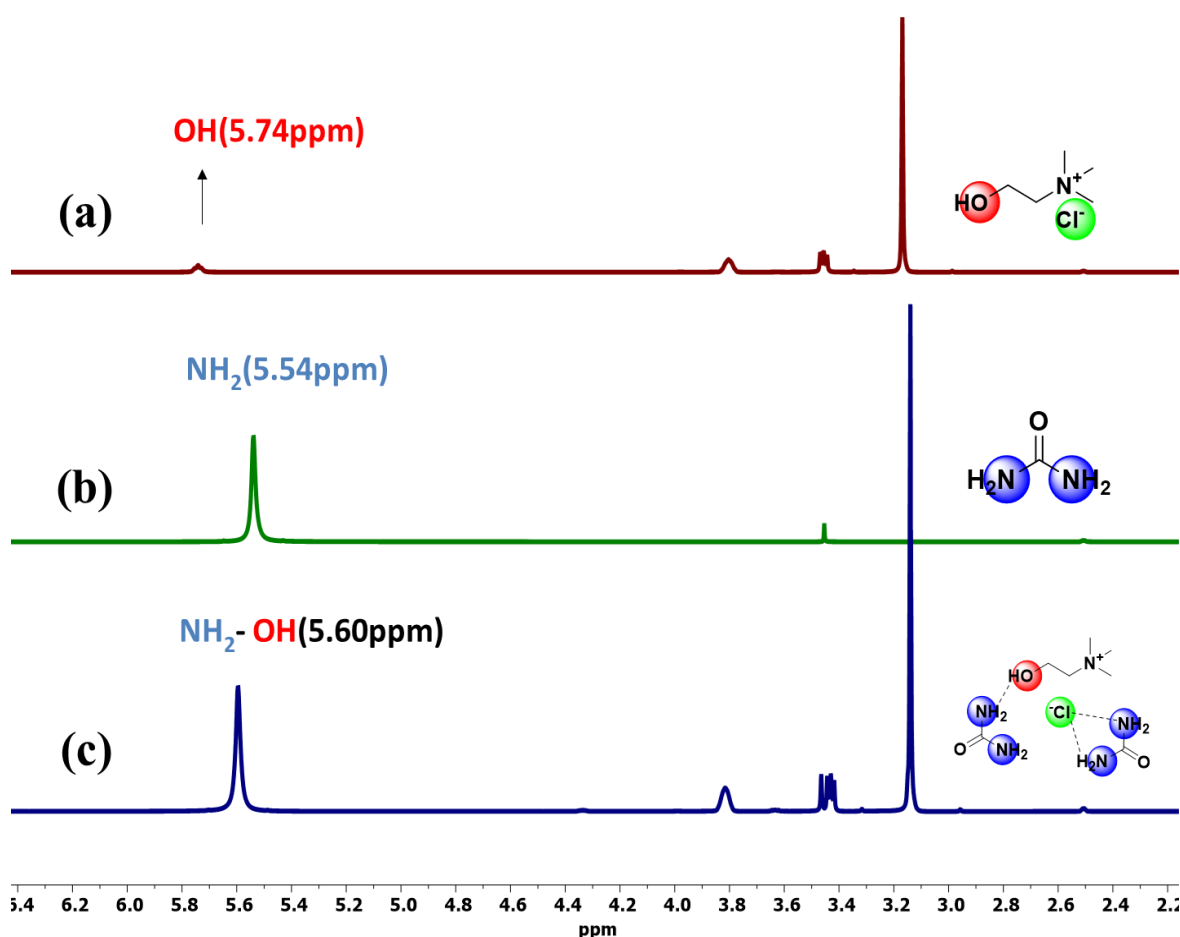


Figure 16. ^1H NMR spectra of (a) choline chloride; (b) Urea and (c) DES (ChCl:U, 1:2 (mol:mol)), obtained in DMSO- d_6 .

Comparative $^{13}\text{C}\{^1\text{H}\}$ analyses provide the expected number of carbons in the formation of the eutectic mixture, with three carbons referring to choline chloride and one in more unshielded regions (160.42 ppm) referring to urea (C=O). Finally, the carbons in the DES did not experiment shifts when compared to the precursors since they do not participate directly in the interactions (Fig. 17).

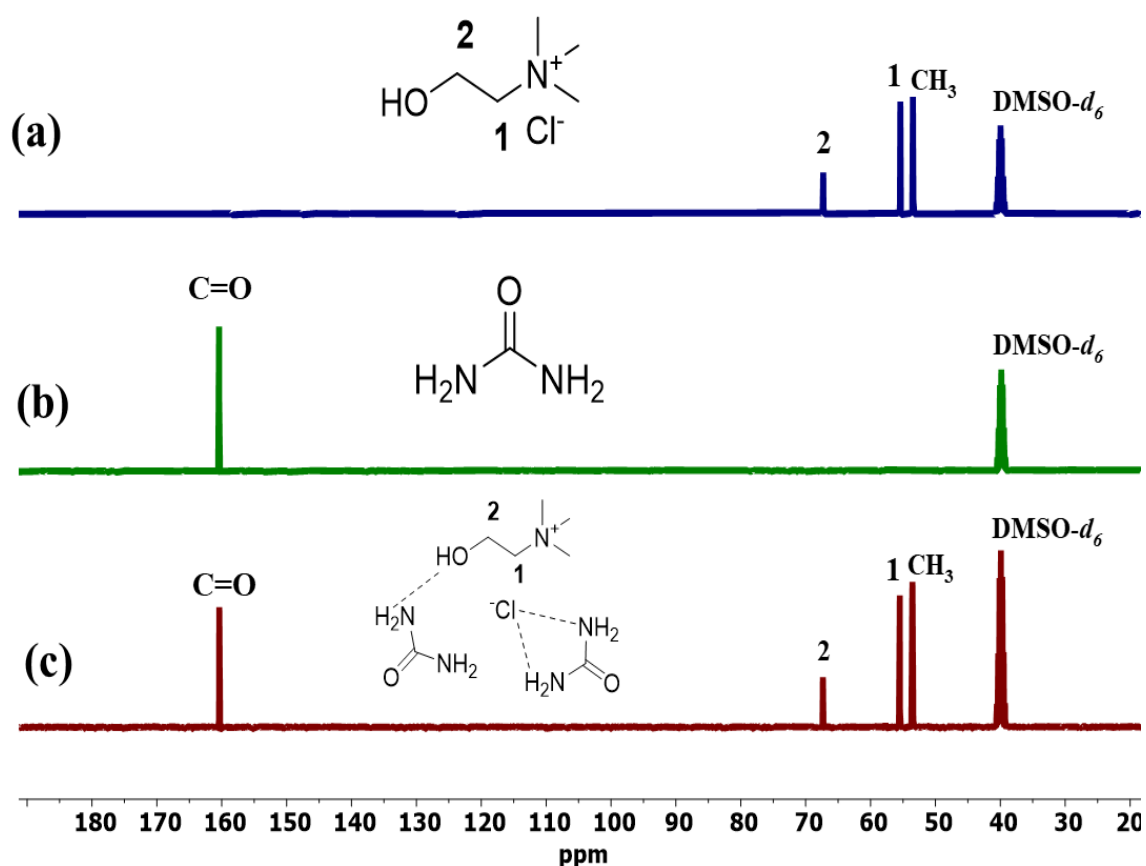


Figure 17. $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of (a) choline chloride; (b) Urea, and (c) DES (ChCl:U, 1:2 (mol:mol)), obtained in DMSO-*d*₆.

2.2.3.3 Characterization of CB and USCeOxNPs-DES/CB composite

Initially, the USCeOxNPs synthesized in DES were morphologically characterized by TEM. As can be seen in Figure 18 (a), USCeOxNPs with uniform size distribution and approximately spherical shape were obtained, with an average size of 0.73 ± 0.07 nm (Fig. 18 (b)). From Figure 18 (c), several emission lines from cerium were observed in the EDS spectrum. The average size of the USCeOxNPs synthesized using DES based on chloride choline and urea (stabilizing medium) was considerably lower than those reported in the literature, where other synthesis processes were employed. These results demonstrate that this solvent can be promising in obtaining nanoparticles with smaller average sizes when compared to conventional reaction media (Table 11).

Table 11. Comparative analysis of methods already utilized for cerium-based nanoparticle synthesis

Type of synthesis	Reductor	Medium	Shape	Size / nm	Reference
Microwave	⁽¹⁾ NI	⁽²⁾ Ionic liquid	NI	7	[29]
Sol-gel	Ammonia	Gelatin solution	Spherical	10.0	[30]
Microwave-hydrothermal	⁽³⁾ Additives	Water	Spherical	1.6 – 20	[31]
Hydrothermal-calcination	Plant extract	Extract of <i>Litchi chinensis</i>	Spherical	10	[36]
Microwave solvothermal	NI	1,4-butanediol	NI	8.0 – 10	[37]
Solvothermal	Hydrazine	Deep eutectic solvent	Spherical	0.73	This work

⁽¹⁾Not informed; ⁽²⁾Ionic liquid based on 1-ethyl-3-methyl-imidazolium bis (trifluoro- methylsulfonyl) imide ([C2mim][NTf2]), 1-butyl-3-methyl-imidazolium bis (trifluoromethylsulfonyl) imide ([C4mim][NTf2]), and 1-hexyl-3-methyl-imidazolium bis (trifluoromethylsulfonyl) imide ([C6mim][NTf2]); ⁽³⁾Sodium hydroxide, ammonia, urea, ethylenediamine, and formamide.

In the work proposed by Goharshadi et al.[29], for example, CeO₂ nanoparticles synthesized by a microwave approach and ionic liquids as stabilizing medium yielded nanoparticles with an average size of 7.0 nm. The ionic liquid had an important role in size reduction owing to the inhibition of the particle aggregation. In the synthesis approach proposed by Ioannou et al.[30], the reaction medium was composed of an aqueous gelatin solution, which enabled the formation of CeO₂ nanoparticles with an average size of 10.0 nm using the sol-gel method. A microwave-assisted polyol method was developed by Soren et al. CeO₂ for nanoparticles synthesizes [30]. The particle sizes ranged from 8.0 to 10.0 nm. Furthermore, the combination of microwave and hydrothermal methods in aqueous media [31] was proper to synthesize CeO₂ nanoparticles with average sizes of 1.6 to 20 nm.

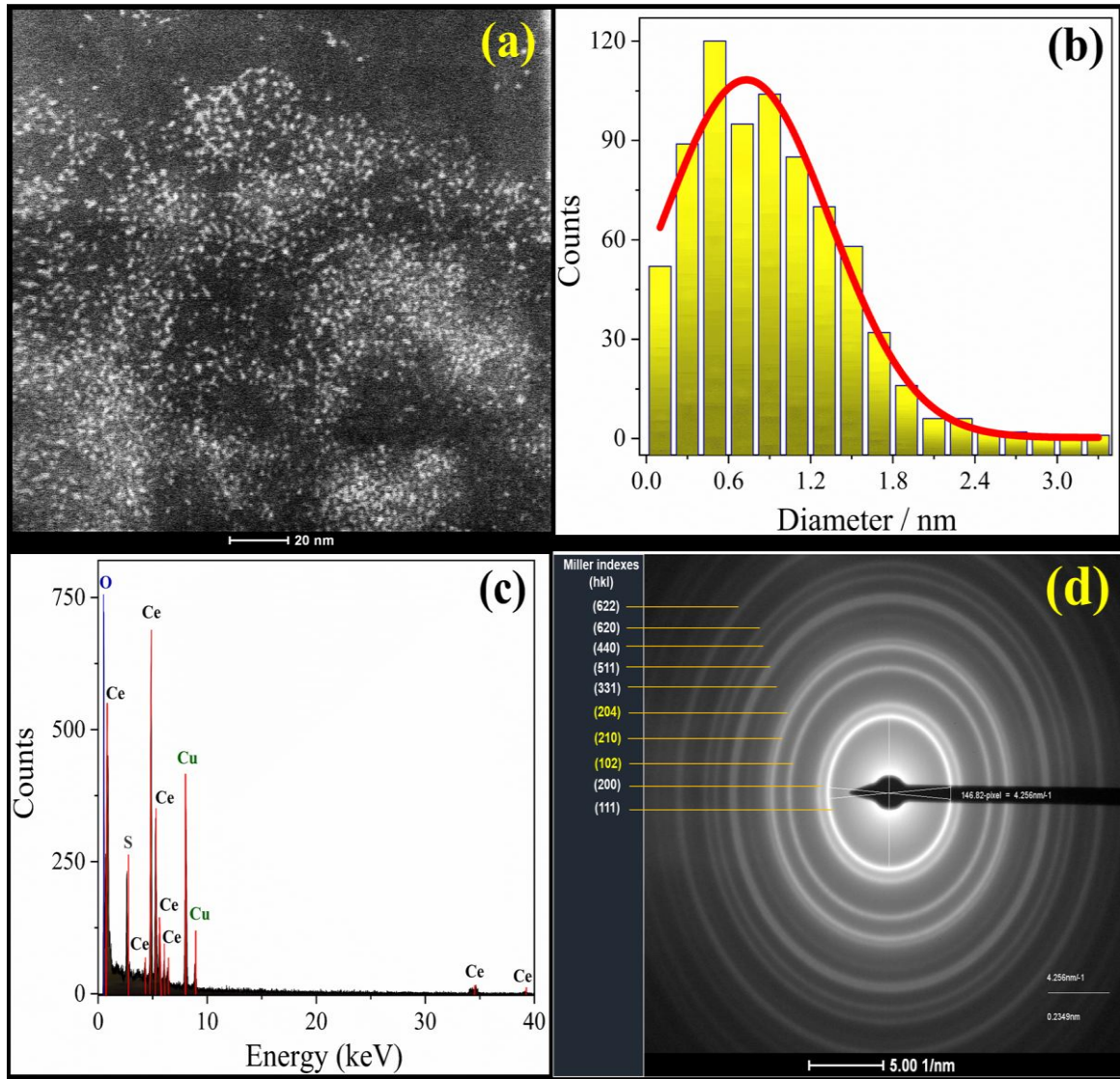


Figure 18. (a) TEM image. (b) Histogram of diameter distribution for the ultrasmall cerium oxide nanoparticles. (c) EDS spectrum of ultrasmall cerium oxide nanoparticles. (d) SAED pattern analysis of ultrasmall cerium oxide nanoparticles.

Additionally, selected area electron diffraction pattern (SAED) was used to analyze the phase structure and crystallinity of USCeOxNPs. Figure 18 (d) shows the diffraction ring patterns and their respective Miller indexes. The patterns of the diffraction rings are associated with crystalline nature. Table 12 presents the interplanar spacing (d) data obtained from some selected areas of the diffraction rings shown in Figure 18 (d), together with the respective Miller index reference value. The SAED standard of the USCeOxNPs reveals the crystalline nature with Miller indices 111, 200, and 331 attesting to the presence of cubic structure CeO₂NPs (JCPDS card no. 53-61386) [32] as reported in the literature for cerium-based nanoparticles [33–35]. However, it is also possible to observe that Figure 18 (d) includes indices (102, 210, and 204) that denote hexagonal

secondary structures, similar to reported by Parimi et al. in the synthesis of CeO₂NPs using hexamethylenetetramine [33].

Table 12. Measured interplanar spacing (d) from selected area diffraction pattern in Figure 17 (d) compared with reference values (Miller indices)

Ring	$1/2r(1/nm)$	$1/r(1/nm)$	r/nm	d -spacing (Å)	Miller indices (hkl)
1	6.46	3.23	0.31	3.09	111
2	7.65	3.82	0.26	2.62	200
3	8.18	4.09	0.24	2.44	210
4	9.76	4.88	0.20	2.05	102
5	13.45	6.72	0.15	1.49	204
6	16.05	8.02	0.12	1.25	331
7	18.89	9.45	0.11	1.06	511
8	21.00	10.50	0.10	0.95	440
9	23.25	11.62	0.09	0.86	620
10	24.55	12.27	0.08	0.81	622

$1/2r$ (1/nm): diameter of rings in the reciprocal space; **$1/r$ (1/nm):** radius of the rings in the reciprocal space (obtained by radius formula – $r = D/2$, where $D = 1/2r$ values); **r/nm :** real radius (1/values of $1/r = r$), these radius value represents the d -spacing of diffraction planes. The d -spacing values are usually given in the unit of angstrom (Å). Therefore, radius values are converted from nm to Å; **d -spacing:** distance between the parallel planes of atoms (minimum distance between two planes).

The results obtained with the USCeOxNPs synthesis in a eutectic medium attest to the approach as very promising from the perspective of greener and simpler, when compared to those previously mentioned. These, when considering the need for sophisticated equipment or laborious steps that involve energy expenditure and waste generation, features that follow principles of green chemistry, such as atomic economy, energy efficiency, and safer use of solvents [1].

The synthesis approach used in this work uses the same class of solvents first proposed by Gomes-Junior et al.[5] to obtain ultrasmall platinum nanoparticles (~0.204 nm). It was observed that the particle sizes may be associated with the DES ability to keep them physically isolated, mainly during the growth stage, by stabilizing and minimizing the agglomeration effects. These thus yield ultrasmall particles at synthesis temperatures below 100 °C. Thus, one of the highlights of the present study is obtaining ultrasmall cerium oxide nanoparticles in a mild (< 100 °C) and simpler way.

We consider that the coexistence of two oxidation states of cerium in the nanoparticles follows two steps: first, the reducing agent $N_2H_5^+$ converts Ce^{4+} into Ce^{3+} . Then, the eutectic system formed between choline chloride and urea can likely source electrons that oxidize Ce^{3+} to Ce^{4+} , as illustrated below.

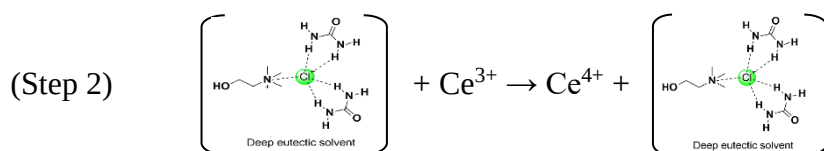
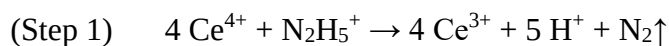


Figure 19 (a) shows the SEM image of a CB film composed by a dispersion of DMF and Nafion™. It can be seen the CB sizes greater than 100 nm with predominantly spherical shapes. This morphological feature is well known for CB, which has sizes ranging from 3.0 to 100 nm. For this reason, CB is also commonly referred as carbon nanoparticles [38]. On the other hand, a SEM image of the USCeOxNPs-DES/CB film, also deposited from a DMF and Nafion™ dispersion, is shown in Figure 19 (b). It presents layers of CB slightly covered, possibly with DES residues, forming a film with a more compact morphology.

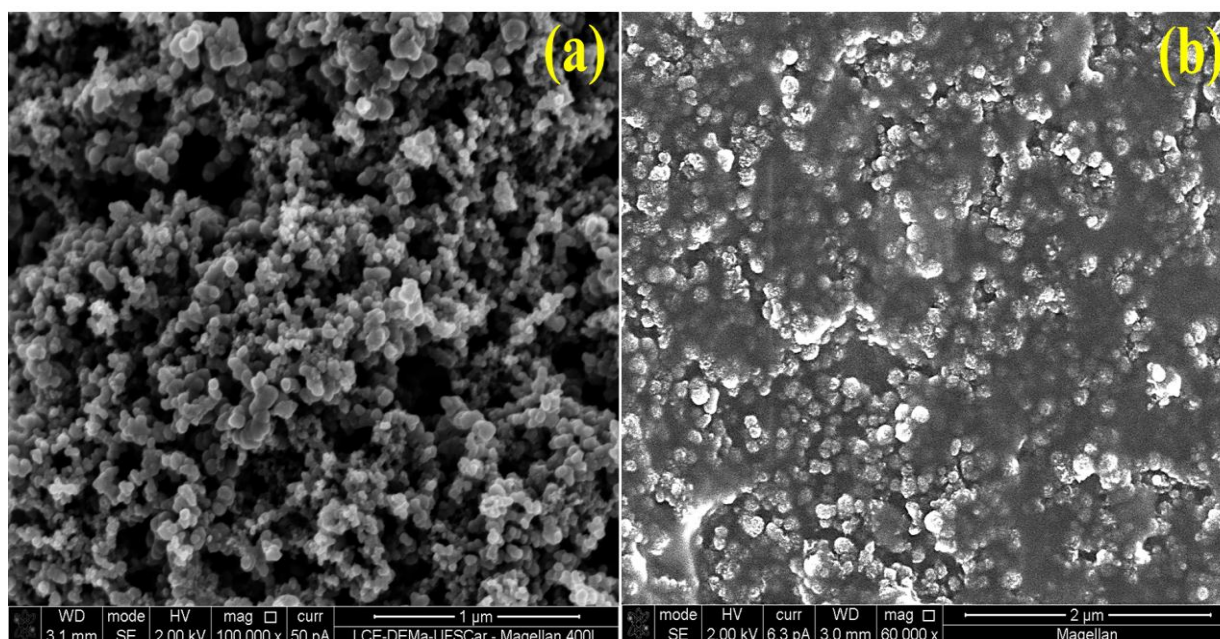


Figure 19. (a) SEM images obtained for the Carbon black; and (b) USCeOxNPs-DES/CB composite film.

2.2.3.4 Electrochemical behaviour of DA at the proposed sensor

Initially, the electrochemical behaviors of DA at the bare and modified GCEs were evaluated by cyclic voltammetry (CV). Figure 20 illustrates the voltametric profile of DA on the bare GCE (Fig. 20 (a)), CB/GCE (Fig. 20 (b)), and USCeO_xNPs-DES/CB/GCE (Fig. 20 (c)). As observed, well-defined anodic (I_{ap}) and cathodic (I_{cp}) peaks were registered using all the electrodes. The I_{ap}/I_{cp} values indicate an almost reversible behavior for all sensors. However, the ΔE_p values in the DA redox process reveal that the

USCeOxNPs-DES/CB/GCE sensor exhibits electrocatalytic activity, as shown in Table 13.

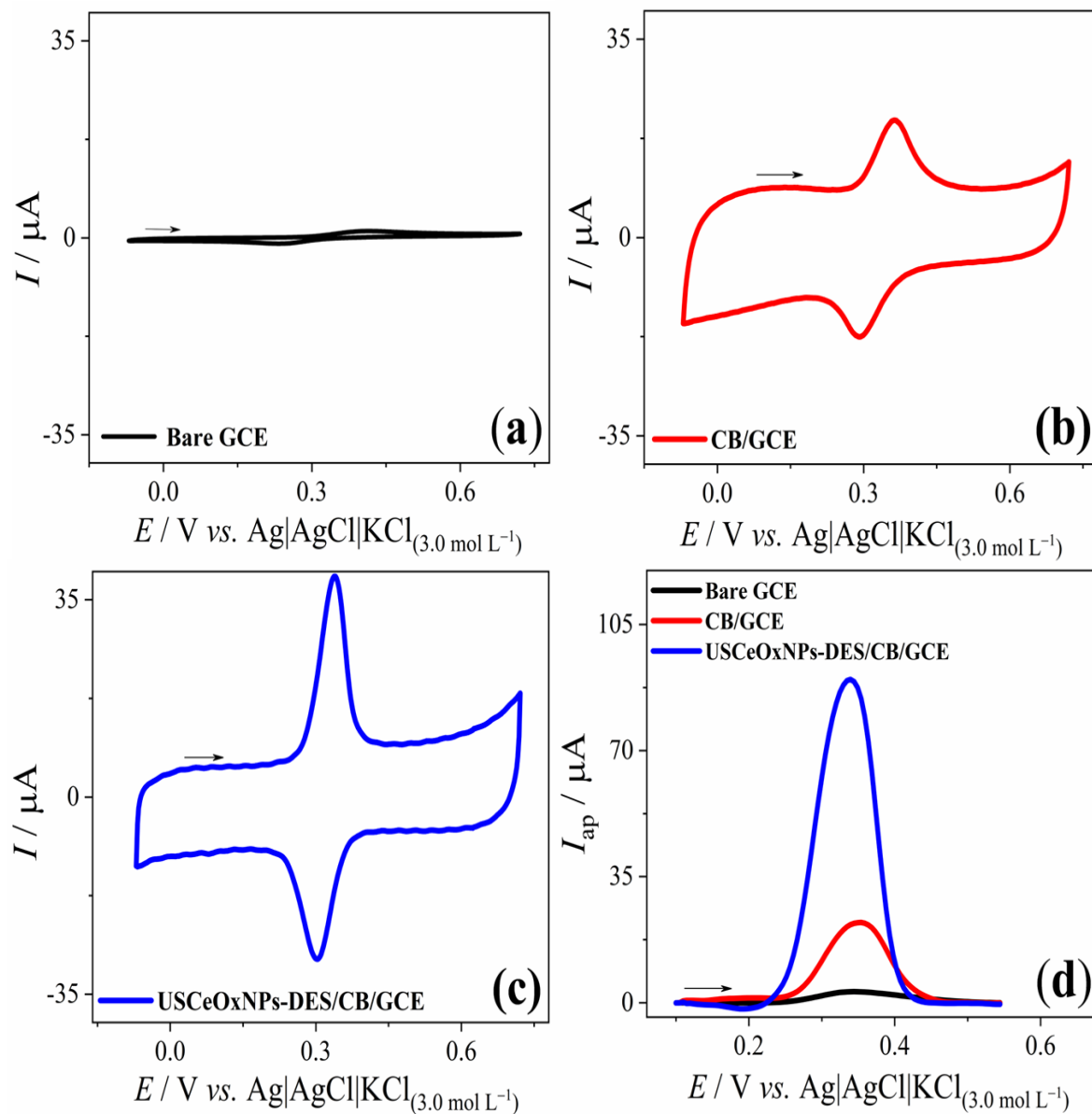


Figure 20. Cyclic voltammograms obtained for $5.0 \times 10^{-5} \text{ mol L}^{-1}$ DA in 0.2 mol L^{-1} BRB solution (pH 5.0) using different working electrodes: bare CGE **(a)**, CB/GCE **(b)**, USCeOxNPs-DES/CB/GCE **(c)** ($\nu = 50 \text{ mV s}^{-1}$). **(d)** SWV voltammograms obtained for $5.0 \times 10^{-5} \text{ mol L}^{-1}$ DA in 0.2 mol L^{-1} BRB solution (pH 5.0). SWV parameters: $f = 25 \text{ Hz}$, $a = 50 \text{ mV}$, and $\Delta E_s = 5 \text{ mV}$.

Table 13. Anodic peak current (I_{ap}), cathodic peak current (I_{cp}), ratio of the peak currents (I_{ap} / I_{cp}), anodic peak potential (E_{ap}), cathodic peak potential (E_{cp}), peak-to-peak potential separation (ΔE_p), electroactive area (A), resistance of the solution (R_s), charge transfer resistance (R_{ct}), constant phase element (CPE), surface roughness (α), and apparent heterogeneous electron-transfer rate constant (k_{app}) obtained for DA using bare GCE and modified electrodes

Electrodes	$I_{ap} / \mu A$	$I_{cp} / \mu A$	I_{ap} / I_{cp}	E_{ap} / V	E_{cp} / V	$\Delta E_p / V$	A / cm^2	R_s / Ω	R_{ct} / Ω	α^*	$CPE / \mu F$	k_{app}
Bare-GCE	+1.22	−0.99	1.24	0.40	0.24	0.16	0.06	131.7	115120	0.79	1.95	4.63×10^{-5}
CB/GCE	+20.7	−17.5	1.18	0.36	0.29	0.07	1.17	274.0	309	0.69	10.0	1.73×10^{-2}
USCeOxNPs-DES/CB/GCE	+39.2	−28.7	1.36	0.34	0.30	0.04	1.59	215.1	241	0.53	10.0	2.21×10^{-2}

* $\alpha = 1$ represents a non-rough surface.

The stability of the DA voltammetric response was also evaluated by CV. After 50 consecutive cycles, the sensor showed a stable response with a signal reduction of less than 7 % in the redox process of DA and a slightly higher response for the electrooxidation (Table 14 and Fig. 21 (a)).

Table 14. Data obtained in the stability study of the USCeO_xNPs-DES/CB/GCE sensor for 5.0×10^{-5} mol L⁻¹ DA in 0.2 mol L⁻¹ acetate buffer solution (pH 4.7)

Cycle	$I_{pa} / \mu A$	E_{pa} / V	$I_{pc} / \mu A$	E_{pc} / V
1	+34	0.35	-31	0.30
5	+33	0.35	-30	0.30
10	+31	0.34	-28	0.30
15	+30	0.34	-27	0.31
20	+29	0.34	-27	0.30
25	+29	0.34	-26	0.30
30	+28	0.34	-26	0.30
35	+28	0.34	-26	0.30
40	+28	0.34	-26	0.30
45	+28	0.34	-25	0.30
50	+27	0.34	-25	0.31
Mean	$+30 \pm 2$	0.34 ± 0.01	-27 ± 2	0.30 ± 0.01
RSD / %	6.9	0.7	6.6	0.6

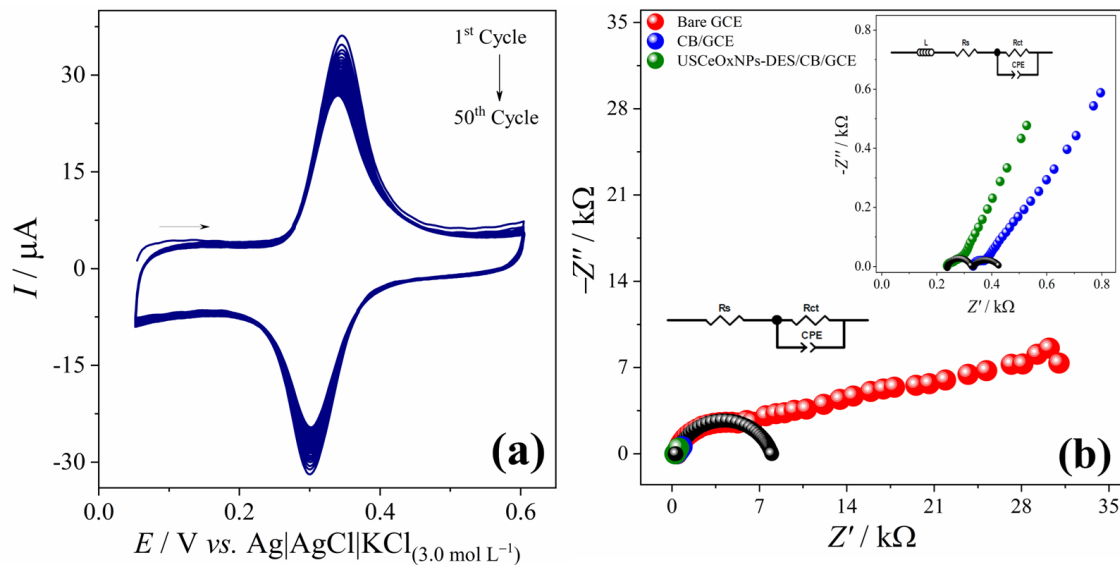


Figure 21. (a) Cyclic voltammograms obtained for 5.0×10^{-5} mol L⁻¹ DA in the sensor stability study ($v = 50$ mV s⁻¹ and supporting electrolyte: 0.2 mol L⁻¹ acetate buffer solution (pH 4.7)), using the USCeO_xNPs-DES/CB/GCE sensor. (b) Nyquist plots for (—) bare GCE, (—) CB/GCE, (—) USCeO_xNPs-DES/CB/GCE electrodes, and (—) fit to simulate the magnitude of the semi-circle (charge transfer resistance) in the Nyquist plot. Inset: the proposed equivalent circuit.

In addition, the current responses for DA electrooxidation were measured using the square-wave voltammetry (SWV) technique. As expected, lower capacitive current effect than CV [39]. The current increments for the sensor based on USCeOxNPs-DES/CB/GCE (89.8 μA) were 28.8 and 4-fold higher than bare GCE (3.12 μA) and CB/GCE (22.3 μA), respectively (Fig. 20 (d)).

From a quantitative point of view, peak currents increased considerably with the incorporation of CB and USCeOxNPs. Moreover, the DA voltammetric profiles presented more reversible, with lower values peak-to-peak potential separation when compared to bare GCE (Table 12). Indeed, modifications with CB and USCeOxNPs increased the catalytic activities for DA in both anodic and cathodic currents compared to bare GCE. Typically, the modification of electrochemical sensors with nanomaterials combined with carbonaceous materials enhances faradaic currents [5,40,41].

Electrochemical impedance spectroscopy (EIS) characterization was carried out to investigate the electrochemical behavior of the proposed analyte, dopamine (DA), in the GCE, CB/GCE, and USCeOxNPs-DES/CB/GCE electrodes. Figure 20 (b) shows the Nyquist diagrams and the proposed equivalent circuits for this electrochemical system. The spectra were adjusted based on the Randles equivalent circuit, consisting of a resistance (assigned to the resistance of the supporting electrolyte solution, R_s) in series with a parallel combination of a constant phase element (CPE), which represents the formation of the electrical double layer as a non-ideal capacitor, and a second resistance (assigned to charge transfer resistance, R_{ct}). Additionally, an inductor (L) was added to correct interference-related problems due to external components for the CB/GCE and USCeOxNPs-DES/CB/GCE electrodes.

The modifications suggested for the GCE electrode resulted in improved kinetic parameters, as evidenced by the CV results. The electrode's R_{ct} values were lower, while the apparent heterogeneous electron-transfer rate constant (k_{app} , Eq. 1) was increased (Table 13).

$$k_{app} = \frac{RT}{F^2 R_{ct} C} \quad (1)$$

From Eq. (1), R represents the molar gas constant (8.314 J mol⁻¹ K⁻¹), T is the thermodynamic temperature (298.15 K), F is the Faraday constant (96485 C mol⁻¹), R_{ct} (Ω) is the charge transfer resistance, and C is the DA concentration (5.0 $\times$ 10⁻⁸ mol cm⁻³).

These improvements may be due to the combined effect of modifying the GCE with CB and USCeOxNPs. This modification enhanced the electrode's conductivity, thereby facilitating reactions on its surface. Specifically, the k_{app} value increased by approximately 1.3, while the R_{ct} value was 1.3 folds lower when compared to the CB/GCE electrode (see Table 13).

2.2.3.5 Effect of pH and supporting electrolyte

The influence of pH was investigated by CV in order to study the magnitude of the current response for DA using the USCeOxNPs-DES/CB/GCE sensor. Britton-Robinson buffer (BRB) (0.2 mol L⁻¹) with pH ranging from 2.0 to 10.0 was utilized. As shown in Figure 22 (a), the anodic peak current was maximum at pH 2.0. However, considering the composition of biological samples (urine), pH values between 4.0 and 5.0 are more appropriate. As the current magnitudes were very similar (Fig. 22 (a, i)), this pH range was chosen as the best condition.

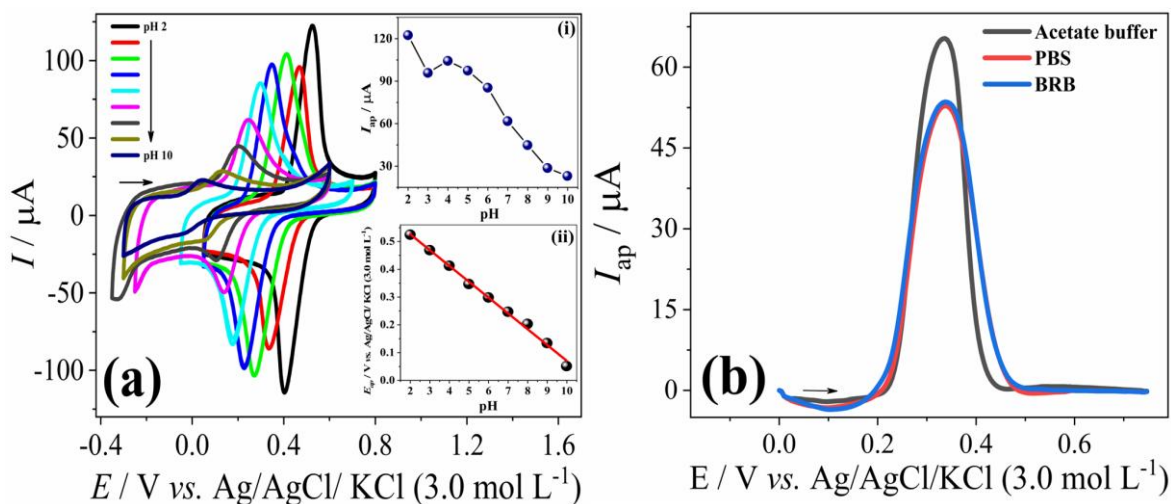
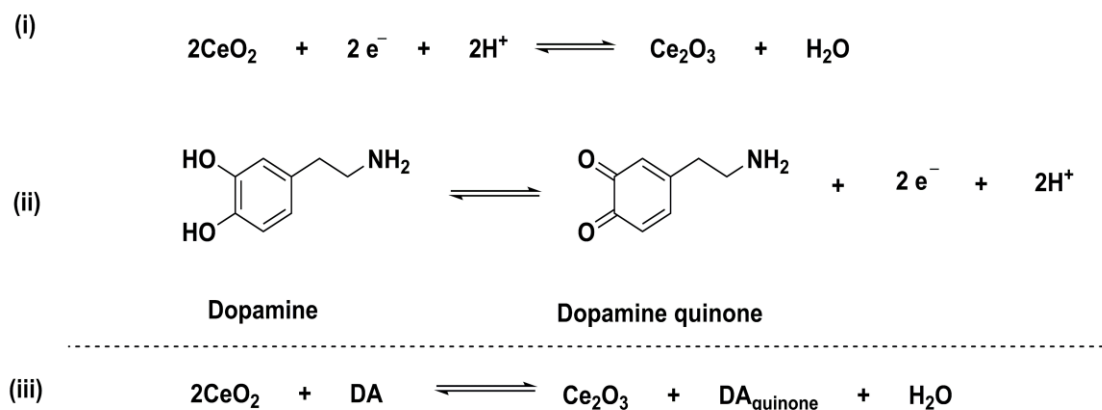


Figure 22. (a) CV voltammograms obtained for 1.0×10^{-3} mol L⁻¹ DA using USCeOxNPs-DES/CB/GCE sensor in 0.2 mol L⁻¹ BRB solutions at different pH values. $v = 50$ mV s⁻¹. (inset: (i) I_{ap} vs. pH and (ii) plot of E_{ap} vs. pH). In (b) SWV voltammograms obtained for 9.9×10^{-6} mol L⁻¹ DA at adjusted pH 4.7 with supporting electrolytes: (—) acetate buffer; (—) phosphate buffer, and (—) Britton-Robinson buffer, at 0.2 mol L⁻¹ (SWV parameters: $f = 25$ Hz, $a = 50$ mV, and $\Delta E_s = 5$ mV).

The dependence of the anodic peak potential (E_{ap}) vs. pH (inset in Fig. 22 (a, ii)) was linear, with E_{ap} shifts to more negative values with pH increasing, suggesting the involvement of protons in the DA electrooxidation mechanism. Additionally, the slope of -0.057 V pH⁻¹ indicates that the same number of protons and electrons are involved in

the oxidation process. This finding is in accordance with previous literature, which reports the involvement of two electrons and two protons in the DA oxidation [42–45]. Considering the catalytic activity of the sensor, Scheme 1 presents the mechanism for DA oxidation. In (i), Ce (IV) is reduced to Ce (III), denoting the catalytic effect of the material based on USCeOxNPs. In (ii), DA is oxidized to dopamine quinone, and (iii) represents the global equation of the DA electrooxidation.



Scheme 1. Electrochemical mechanism for DA electrooxidation in the USCeOxNPs-DES/CB/GCE sensor.

The effect of the supporting electrolyte composition was evaluated within the previously mentioned pH range (between 4.0 and 5.0). DA voltammetric responses were evaluated at BRB, PBS, and acetate buffer solutions adjusted at pH 4.7 with the same ionic strength (0.2 mol L^{-1}). As shown in Figure 22 (b), in 0.2 mol L^{-1} acetate buffer the peak current for the DA electrooxidation was higher. Therefore, this solution was chosen as the electrolyte.

2.2.3.6 Effect of scan rate and sensor stability study

Cyclic voltammograms were recorded in the potential scan rate from 10 mV s^{-1} to 100 mV s^{-1} for a $5.0 \times 10^{-5} \text{ mol L}^{-1}$ DA in acetate buffer 0.2 mol L^{-1} (pH 4.7) at the bare GCE and at the proposed sensor. Analyzing Figure 23 (a, b, and c), along with the respective plots, CB/GCE and USCeOxNPs-DES/CB/GCE sensors presented slopes close to 1.0 for $\log I_{ap}$ vs. $\log v$, indicating adsorption-controlled processes or both adsorption/diffusion processes [39] for DA electrooxidation. Hence, to determine the dominant process on the electron transfer of DA oxidation, the relations I_{ap} vs. v and I_{ap} vs. $v^{1/2}$ were also examined (Table 15). Note that plots of Figure 23 (a, b, and c) presented linear trends, which indicates that DA electrooxidation was a mixed-controlled redox

process on the CB/GCE and USCeOxNPs-DES/CB/GCE sensors. In contrast, in bare GCE the slope value of 0.49 suggests that DA oxidation is diffusional controlled ($\log I_{ap}$ vs. $\log v$). The I_{ap} vs. v and I_{ap} vs. $v^{1/2}$ plots displayed linear trends, which confirm the process' predominance.

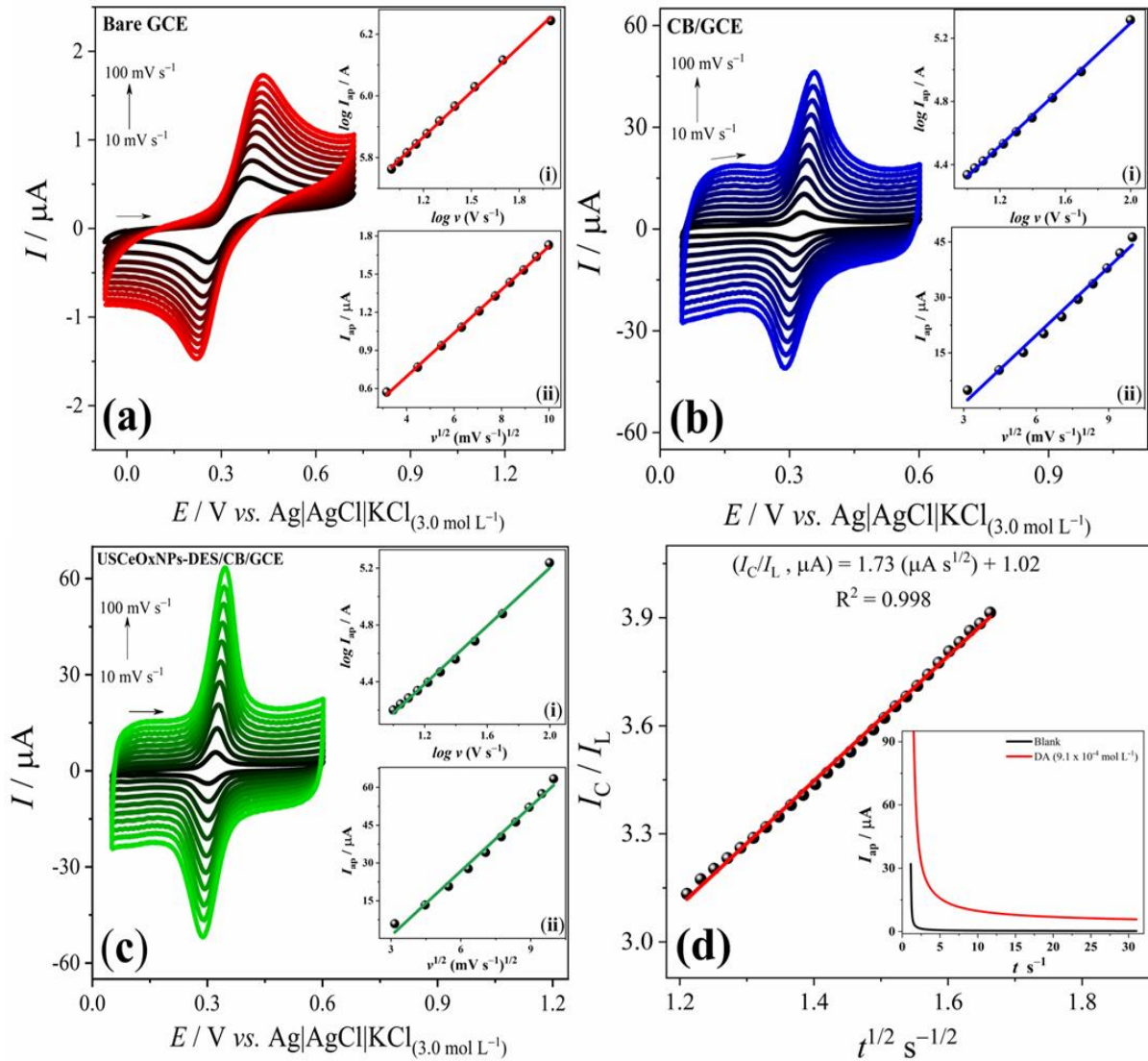


Figure 23. Cyclic voltammograms obtained at different scan rates (10 – 100 mV s⁻¹) for 5.0 × 10⁻⁵ mol L⁻¹ DA in acetate buffer 0.2 mol L⁻¹ (pH 4.7) using (a) bare GCE, (b) CB/GCE, and (c) USCeOxNPs-DES/CB/GCE sensors (plots inset: (i) $\log I_{ap}$ vs. $\log v$, and (ii) I_{ap} vs. $v^{1/2}$). In (d) dependence of I_C/I_L on the $t^{1/2}$ obtained of the chronoamperometry data. Inset: the chronoamperograms obtained in 0.2 mol L⁻¹ acetate buffer (pH 4.7) in the absence (blank) and in 9.0 × 10⁻⁴ mol L⁻¹ DA using the USCeOxNPs-DES/CB/GCE sensor.

Table 15. Merit figures for plots of scan rate study obtained for DA using bare GCE and the modified electrodes

Plots	Equation	Electrodes		
	$y = a + bx$	Bare GCE	CB/GCE	USCeOxNPs-DES/CB/GCE
$\log I_{ap}$ vs. $\log v$	Slope ($\mu\text{A mV}^{-1} \text{s}$)	0.49	0.97	1.03
	Intercept	5.28	3.35	3.15
	R ²	0.998	0.998	0.995
I_{ap} vs. v	Slope ($\mu\text{A mV}^{-1} \text{s}$)	0.01	0.45	0.64
	Intercept	0.53	1.29	1.18
	R ²	0.991	0.997	0.996
I_{ap} vs. $v^{1/2}$	Slope ($\mu\text{A mV}^{1/2} \text{s}^{-1/2}$)	0.009	6.15	8.56
	Intercept	0.171	-17.24	-24.69
	R ²	0.999	0.992	0.991

2.2.3.7 Electroactive area and catalytic rate constant

The electroactive areas for bare GCE, CB/GCE, and USCeOxNPs-DES/CB/GCE sensors were evaluated by CV with $5.0 \times 10^{-5} \text{ mol L}^{-1}$ DA in 0.2 mol L^{-1} acetate buffer solution (pH 4.7) as supporting electrolyte. The scan rate ranged from 10 to 100 mV s^{-1} (Fig. 23 (a, b, and c)). The Randles–Ševčík Eq. (2) [39] is related to a quasi-reversible redox process,

$$I_{p,f}^{\text{quasi}} = (2.69 \times 10^5) n^{3/2} C D^{1/2} A v^{1/2} \quad (2)$$

where $I_{p,f}^{\text{quasi}}$ is the anodic or cathodic peak current (A), n is the number of electrons in the system (for DA, the process involves 2 electrons) [43,44], D is the diffusion coefficient of DA ($6.0 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}$) [46], A is the electroactive area (cm^2), C is the concentration of DA ($5.0 \times 10^{-8} \text{ mol cm}^{-3}$), and v is the potential scan rate (V s^{-1}). The linear equations and the corresponding plots obtained for all sensors are presented in Figure 23 (a, b, and c) and Table 13. The electroactive area obtained for bare GCE, CB/GCE, and USCeOxNPs-DES/CB/GCE were 0.06, 1.17, and 1.59 cm^2 , respectively. This means that the USCeOxNPs-based modification yielded a 26.5- and 1.35-fold increase in electroactive area compared to the bare GCE and CB/GCE, respectively (see Table 13). Based on these results, ultrasmall cerium oxide nanoparticles synthesized in choline chloride-based eutectic solvent, when combined with CB, enhance the sensor's performance to detect DA, given its electrocatalytic properties.

The catalytic rate constant (k_{cat}) on the surface of the USCeOxNPs-DES/CB/GCE sensor was determined using the chronoamperometry technique (Fig. 23 (d)) and applying the following Eq. (3) [39, 47]:

$$I_C / I_L = \pi^{1/2} (k_{\text{cat}} c t)^{1/2} \quad (3)$$

where I_C is the catalytic current (A) of DA, I_L is the diffusion-limited current (A) in the absence of DA, c is the concentration of DA ($9.0 \times 10^{-4} \text{ mol L}^{-1}$), t is time (s), and π is constant. From the slope of I_C / I_L vs. $t^{1/2}$ plot, k_{cat} can be calculated for a given concentration of DA (Fig. 23 (d)). The found value was equal $1.0 \times 10^4 \text{ mol}^{-1} \text{ L s}^{-1}$, which is in accordance with reported in the literature for DA [48], but also supports the results observed in EIS and CV regarding the catalytic performance of the sensor to electrooxidize DA.

2.2.3.8 Analytical performance

SWV technique was chosen as it usually provides better analytical performance towards analytes featuring reversible redox reaction [39]. To monitor the anodic DA response, the optimized parameters in terms of resolution and intensity are shown in Table 16.

Table 16. Optimization of SWV parameters towards DA determination

Parameter	Range	Selected
Pulse frequency (f , Hz)	8 – 100	8
Potential pulse amplitude (a , mV)	10 – 100	80
Potential increment (ΔE_s , mV)	1 – 10	7

Under these optimal experimental conditions, the analytical curve was constructed for DA in the concentration range from 2.0×10^{-8} to $1.0 \times 10^{-6} \text{ mol L}^{-1}$ using the USCeOxNPs-DES/CB/GCE sensor. The SWVs and analytical curves for four linear regions are showed in Figure 24.

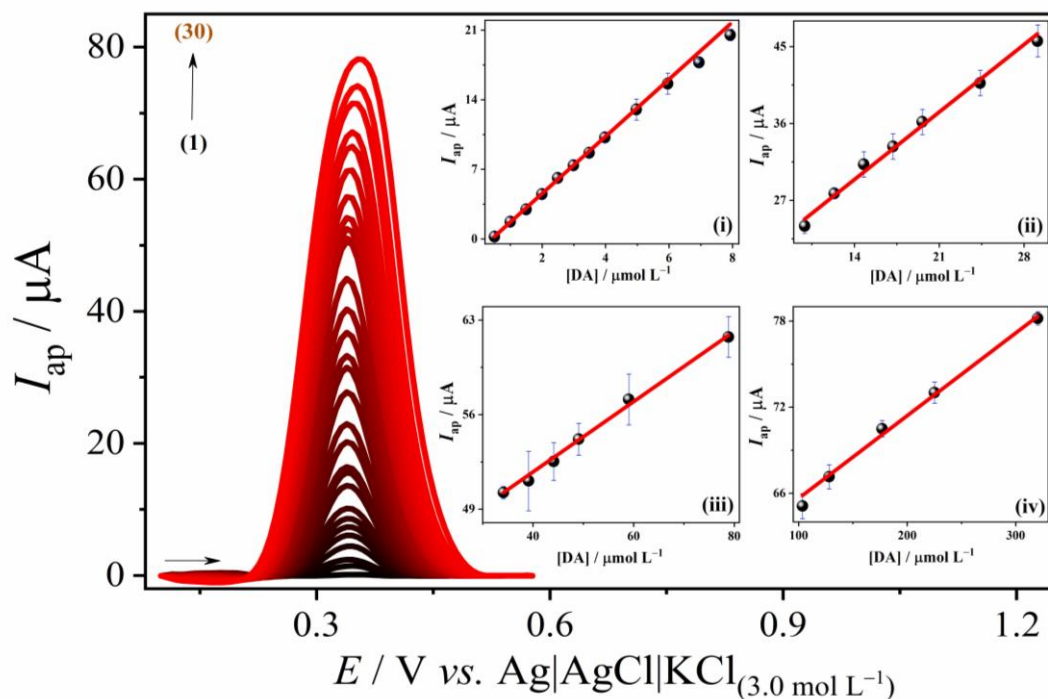


Figure 24. SWV voltammograms obtained at different DA concentrations ranging from $5.0 \times 10^{-7} \text{ mol L}^{-1}$ **(1)** to $3.2 \times 10^{-4} \text{ mol L}^{-1}$ **(30)**, using the USCeO_xNPs-DES/CB/GCE sensor. Supporting electrolyte: 0.2 mol L^{-1} acetate buffer solution (pH 4.7). SWV parameters $f = 8 \text{ Hz}$, $a = 80 \text{ mV}$, and $\Delta E_s = 7 \text{ mV}$. Inset: Analytical curves obtained in four regions for DA (I vs. $[DA]$) – **(i)** from $5.0 \times 10^{-7} \text{ mol L}^{-1}$ to $7.9 \times 10^{-6} \text{ mol L}^{-1}$, **(ii)** $9.9 \times 10^{-6} \text{ mol L}^{-1}$ to $2.9 \times 10^{-5} \text{ mol L}^{-1}$, **(iii)** $3.4 \times 10^{-5} \text{ mol L}^{-1}$ to $7.9 \times 10^{-5} \text{ mol L}^{-1}$, and **(iv)** $1.0 \times 10^{-4} \text{ mol L}^{-1}$ to $3.2 \times 10^{-4} \text{ mol L}^{-1}$.

The following linear regression equations were obtained for the analytical curves: (I) $I / \mu\text{A} = -1.24 \mu\text{A} + 2.87 \mu\text{A L } \mu\text{mol}^{-1} [\text{DA}] / \mu\text{mol L}^{-1}$ ($R^2 = 0.999$), (II) $I / \mu\text{A} = 13.6 \mu\text{A} + 1.13 \mu\text{A L } \mu\text{mol}^{-1} [\text{DA}] / \mu\text{mol L}^{-1}$ ($R^2 = 0.995$), (III) $I / \mu\text{A} = 41.3 \mu\text{A} + 0.25 \mu\text{A L } \mu\text{mol}^{-1} [\text{DA}] / \mu\text{mol L}^{-1}$ ($R^2 = 0.997$), and (IV) $I / \mu\text{A} = 58.8 \mu\text{A} + 0.06 \mu\text{A L } \mu\text{mol}^{-1} [\text{DA}] / \mu\text{mol L}^{-1}$ ($R^2 = 0.994$). The limits of detection (LOD) and quantification (LOQ) were calculated as $\text{LOD} = 3 \times s_b / m$ and $\text{LOQ} = 10 \times s_b / m$, where s_b is the standard deviation of ten blank measurements ($n = 10$) and m is the analytical sensitivity (slope of the analytical curve). LOD and LOQ of $8.0 \times 10^{-8} \text{ mol L}^{-1}$ (80 nmol L^{-1}) and $2.7 \times 10^{-7} \text{ mol L}^{-1}$ were obtained, respectively. Table 17 compares the analytical parameters of the proposed sensor with other sensors present in the literature for DA analysis.

Table 17. Comparison of analytical parameters for the voltammetric determination of DA

Sensors*	Technique	DA			Sensitivity (A L mol ⁻¹ cm ⁻²)	References
		Linear range (μmol L ⁻¹)	LOD (mol L ⁻¹)	R ²		
NiONP-MWCNT-DHP/GCE	DPV	$7.0 \times 10^{-8} - 4.8 \times 10^{-6}$	5.0×10^{-8}	0.991	26.06	[41]
RGO-CB-CTS/GCE	SWV	$3.2 \times 10^{-6} - 3.2 \times 10^{-5}$	2.0×10^{-7}	0.996	0.267	[44]
MWCNT/CCE	DPV	$5.0 \times 10^{-7} - 1.0 \times 10^{-4}$	3.1×10^{-7}	0.998	12.54	[45]
PSFM/GCE	DPV	$5.0 \times 10^{-8} - 4.7 \times 10^{-4}$	3.0×10^{-8}	0.994	NI	[48]
APT/a-CN _x electrode	SWV	$8.0 \times 10^{-8} - 8.3 \times 10^{-6}$	6.6×10^{-8}	0.997	0.886	[49]
Cu/Cu ₂ O-AO/MWCNTs/GCE	DPV	$2.0 \times 10^{-8} - 1.6 \times 10^{-5}$	3.3×10^{-9}	0.989	326.7	[50]
GNP/LC/GCE	DPV	$6.0 \times 10^{-8} - 8.5 \times 10^{-5}$	2.0×10^{-8}	0.997	316.2	[51]
Ti ₃ C ₂ @UIO-66-NH ₂ /GCE	DPV	$1.0 \times 10^{-16} - 2.5 \times 10^{-13}$	8.1×10^{-16}	0.996	14.72×10^9	[52]
OCMCS/GCE	DPV	$6.0 \times 10^{-8} - 7.0 \times 10^{-6}$	1.5×10^{-9}	0.998	10.55	[53]
GNs/HDT+OT/Au electrode	DPV	$2.0 \times 10^{-4} - 1.2 \times 10^{-3}$	9.0×10^{-5}	0.994	NI	[54]
USCeOxNPs-DES/CB/GCE	SWV	$*5.0 \times 10^{-7} - 3.2 \times 10^{-4}$	8.0×10^{-8}	0.999	41.0	This work

NiONP-MWCNT-DHP/GCE – glassy carbon electrode modified with nickel oxide nanoparticles and multiwalled carbon nanotubes within a dihexadecylphosphate film; RGO-CB-CTS/GCE – glassy carbon electrode modified within reduced graphene oxide and carbon black in a chitosan film; MWCNT/CCE – Multi walled carbon nanotube modified carbon-ceramic electrode; PSFM/GCE – poly(sulfonazo III) film-modified glassy carbon electrode; APT/a-CN_x electrode – anodically pretreated electrodes based on amorphous carbon nitride (a-CN_x) film deposited on a stainless steel foil; Cu/Cu₂O-AO/MWCNTs/GCE – glassy carbon electrode modified with copper/Copper oxide (I)2 nanoparticles obtained from oleic acid, and multiwalled carbon nanotubes; GNP/LC/GCE – gold nanoparticles self-assembled glassy carbon electrode pretreated with L-cysteine; Ti₃C₂@UIO-66-NH₂/GCE – glassy carbon electrode modified with titanium carbide membrane doped with metal-organic framework; OCMCS/GCE – glassy carbon electrode modified with O-carboxymethylchitosan; GNs/HDT+OT/Au electrode – gold electrode modified with self-assembly of gold nanoparticles at the surface of mixed film of 1,6-hexanedithiol and 1-octanethiol.

*linear range divided into four regions: (i) from 5.0×10^{-7} mol L⁻¹ to 7.9×10^{-6} mol L⁻¹, (ii) 9.9×10^{-6} mol L⁻¹ to 2.9×10^{-5} mol L⁻¹, (iii) 3.4×10^{-5} mol L⁻¹ to 7.9×10^{-5} mol L⁻¹, and (iv) 1.0×10^{-4} mol L⁻¹ to 3.2×10^{-4} mol L⁻¹. LOD, LOQ, and sensitivity were calculated from the data of the first linear region (5.0×10^{-7} mol L⁻¹ to 7.9×10^{-6} mol L⁻¹).

As can be seen, the detection limit of the proposed sensor is slightly above those demonstrated for the other sensors. However, it presents adequate LOD and linear range for the determination of dopamine in urine samples. The result from incorporating USCeOxNPs synthesized in DES enhanced the current response and sensitivity for determining DA when compared with bare GCE and CB/GCE electrodes. Moreover, it is important to highlight that the proposed sensor architecture is simple, and the nanoparticles were synthesized in a greener way, which represents a reduction in steps in the synthesis process, generating atomic and energy economies. Such characteristics align with the principles of green chemistry and the improvement in the sustainable development of nanomaterials for electrochemical sensor applications.

Inter-day and intra-day repeatability studies were performed to evaluate the sensor precision. In the intra-day repeatability, the DA voltammetric response (SWV) was measured five times in a day ($n = 5$ for each DA concentration). For the inter-day repeatability, SWV measurements were conducted for three consecutive days. As shown in Table 18, the RSD results obtained for the intra- and inter-day precision were below to 5 % in both cases. These values attest that the precision of the SWV method using the USCeOx-DES/CB/GCE sensor was very satisfactory.

Table 18. Repeatability study in the detection of DA using USCeOxNPs-DES/CB/GCE sensor

Day	Intra-day			Day	Inter-day		
	[DA] / $\mu\text{mol L}^{-1}$	I_{ap} / μA	RSD / %		[DA] / $\mu\text{mol L}^{-1}$	I_{ap} / μA	RSD / %
1	1.0	1.7 ± 0.1	3.5	1	1.0	1.6 ± 0.1	3.0
	4.9	19.6 ± 0.2	1.2	2			
	9.8	35.9 ± 0.9	4.0	3			
2	1.0	1.6 ± 0.1	2.3	1	4.9	18.5 ± 0.9	4.9
	4.9	17.3 ± 0.7	4.1	2			
	9.8	34.8 ± 0.1	0.3	3			
3	1.0	1.6 ± 0.1	3.5	1	9.8	35.4 ± 0.4	1.3
	4.9	18.3 ± 0.4	2.4	2			
	9.8	35.6 ± 0.9	3.0	3			

The USCeOx-DES/CB/GCE sensor was designed to determine DA in biological fluids. Thus, the interference of concomitant substances typically found in these samples was evaluated. For this, the DA concentration was fixed at $2.0 \times 10^{-6} \text{ mol L}^{-1}$, and the

possible interfering substances were added in three concentration ratios: 1:10, 1:1, and 10:1 ([concomitant]:[DA]). The effect of the following substances was tested: urea, ammonia, glucose, sulfate ions, chloride ions, sodium ions, potassium ions, oxalate ions, creatinine, uric acid, ascorbic acid, and caffeine. Table 19 provides the relative error obtained for the comparison of voltammetric responses of DA in the absence and presence of the possible interfering compound (Figure 25).

Table 19. Dopamine determination in the presence of possible interfering species

Interferents	Error* / %		
	1:10	1:1	10:1
Urea	2.2	1.7	-3.1
Ammonia	3.4	4.9	2.0
Glucose	-1.5	3.0	1.6
Sulfate (SO ₄ ²⁻)	3.4	4.9	2.0
Chloride (Cl ⁻)	1.0	-3.9	3.0
Sodium ion (Na ⁺)	1.0	-3.9	3.0
Potassium ion (K ⁺)	-3.3	-2.7	-2.7
Oxalate ion (C ₂ O ₄ ²⁻)	-3.3	-2.7	-2.7
Uric acid	4.5	3.3	-4.4
Creatinine	-4.4	-2.1	-1.1
Ascorbic acid	-3.4	-2.7	-2.7
Caffeine	-4.1	-0.2	-0.7

*Error: $(I_{ap, \text{ presence}} - I_{ap, \text{ absence}}) / I_{ap, \text{ absence}} \times 100\%$.

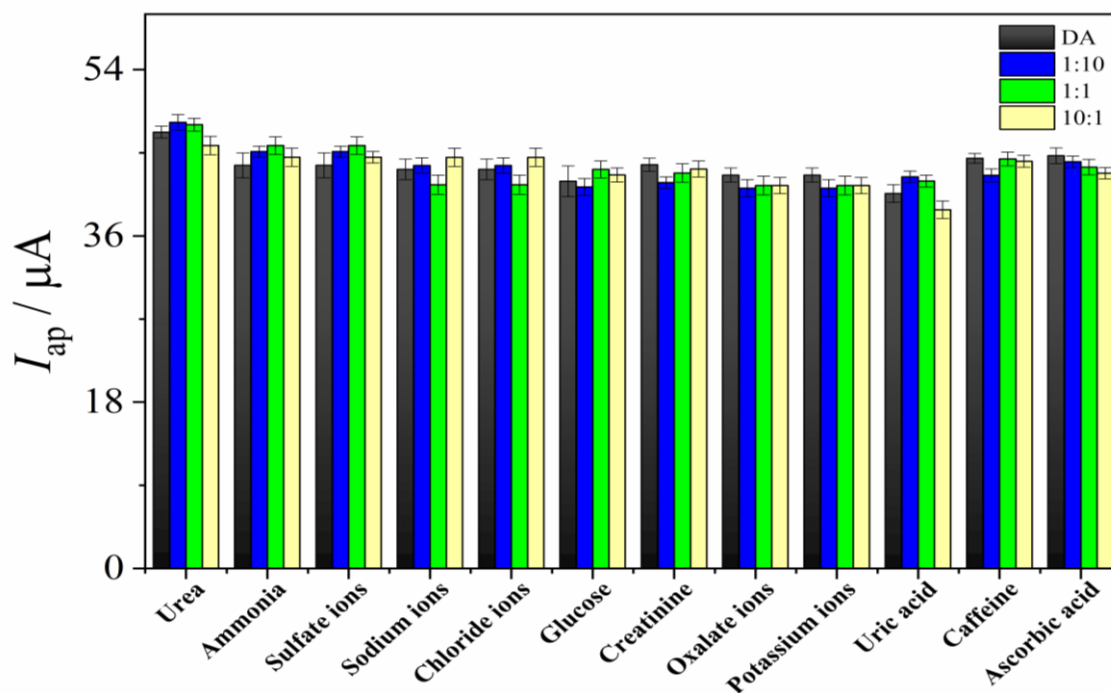


Figure 25. Voltammetric responses obtained of concomitant substances measured in the presence of $2.0 \times 10^{-6} \text{ mol L}^{-1}$ DA using USCeOxNPs-DES/CB/GCE sensor in acetate buffer solution, at 0.2 mol L^{-1} (SWV parameters: $f = 25 \text{ Hz}$, $a = 50 \text{ mV}$, and $\Delta E_s = 5 \text{ mV}$).

Based on the results, it was evident that the studied substances had no significant effect on the DA voltammetric response, with errors below 5 %. This suggests that the proposed sensor presented good selectivity over the set of tested species.

2.2.3.9 Determination of DA in biological fluids

The efficiency of the USCeOxNPs-DES/CB/GCE sensor was tested in biological fluid (synthetic urine) samples spiked with a DA at the following concentrations: $7.5 \times 10^{-7} \text{ mol L}^{-1}$, $2.5 \times 10^{-6} \text{ mol L}^{-1}$ and $1.5 \times 10^{-5} \text{ mol L}^{-1}$. The recoveries are presented in Table 20. These varied from 94.6 to 108 %, denoting no significant matrix effect in the response. These results indicate the sensor's potential as an alternative for possible applications in the voltammetric determination of DA in biological samples.

Table 20. Determination of dopamine in synthetic urine

Samples*	[DA] / ($\mu\text{mol L}^{-1}$)		
	Added	Found	Recovery ^a / %
<i>Synthetic urine</i>			
1	0.75	0.71 ± 0.01	94.6
2	2.25	2.43 ± 0.01	108.0
3	14.9	14.4 ± 0.01	96.6

*n = 3; ^aRecovery percentage = $([\text{DA}]_{\text{Found}}/[\text{DA}]_{\text{Added}}) \times 100\%$.

2.2.4 Conclusions

We demonstrate for the first time a synthesis approach for ultrasmall cerium oxide nanoparticles based on solvothermal concepts using a deep eutectic solvent based on choline chloride and urea. We consider that the thermal stability effect of the DES, especially in the nucleation and growth steps of these particles, is the possible reason for the drastic reduction in the size of USCeOxNPs. The excellent performance achieved in this work with cerium-based particles opens new perspectives for the synthesis of other ultrasmall metallic nanoparticles using DES, with the advantage of being a greener alternative. Furthermore, the synthesized USCeOxNPs with an average diameter of 0.73 nm were applied for the first time in the construction of a carbon black-based electrochemical sensor to detect dopamine in simulated biological fluids samples. The proposed USCeOxNPs-DES/CB/GCE sensor showed enhanced faradaic responses, good sensitivity, excellent LOD and LOQ, recoveries, repeatability, and quantifications for DA in urine.

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References

- [1] P. Anastas, N. Eghbali, *Green Chemistry : Principles and Practice*, Chem. Soc. Rev. (2010) 301–312. <https://doi.org/10.1039/b918763b>.
- [2] C. Dhand, N. Dwivedi, X.J. Loh, A.N. Jie Ying, N.K. Verma, R.W. Beuerman, R. Lakshminarayanan, S. Ramakrishna, *Methods and strategies for the synthesis of diverse nanoparticles and their applications: A comprehensive overview*, RSC Adv. 5 (2015) 105003–105037. <https://doi.org/10.1039/c5ra19388e>.
- [3] B.H. Kim, M.J. Hackett, J. Park, T. Hyeon, *Synthesis, characterization, and application of ultrasmall nanoparticles*, Chem. Mater. 26 (2014) 59–71. <https://doi.org/10.1021/cm402225z>.
- [4] H. Duan, D. Wang, Y. Li, *Green chemistry for nanoparticle synthesis*, Chem. Soc. Rev. 44 (2015) 5778–5792. <https://doi.org/10.1039/c4cs00363b>.
- [5] P.C. Gomes-Junior, K.K. de Lima Augusto, G.P. Longatto, R. de Oliveira Gonçalves, T.A. Silva, É.T.G. Cavalheiro, O. Fatibello-Filho, *Ultrasmall platinum nanoparticles synthesized in reline deep eutectic solvent explored towards the voltammetric sensing of riboflavin in beverages and biological fluids*, Sensors Actuators B Chem. 395 (2023) 1–10. <https://doi.org/10.1016/j.snb.2023.134489>.
- [6] L.I.N. Tomé, V. Baião, W. da Silva, C.M.A. Brett, *Deep eutectic solvents for the production and application of new materials*, Appl. Mater. Today. 10 (2018) 30–50. <https://doi.org/10.1016/j.apmt.2017.11.005>.
- [7] C.M.A. Brett, *Deep eutectic solvents and applications in electrochemical sensing*, Curr. Opin. Electrochem. 10 (2018) 143–148. <https://doi.org/10.1016/j.coelec.2018.05.016>.
- [8] P.G. Jamkhande, N.W. Ghule, A.H. Bamer, M.G. Kalaskar, *Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications*, J. Drug Deliv. Sci. Technol. 53 (2019) 101174. <https://doi.org/10.1016/j.jddst.2019.101174>.
- [9] Y. Gao, K. Chen, J.L. Ma, F. Gao, *Cerium oxide nanoparticles in cancer*, Onco. Targets. Ther. 7 (2014) 835–840. <https://doi.org/10.2147/ott.s62057>.
- [10] I. Celardo, E. Traversa, L. Ghibelli, *Cerium oxide nanoparticles: A promise for applications in therapy*, J. Exp. Ther. Oncol. 9 (2011) 47–51.
- [11] S.M. Hirst, A.S. Karakoti, R.D. Tyler, N. Sriranganathan, S. Seal, C.M. Reilly, *Anti-inflammatory properties of cerium oxide nanoparticles*, Small. 5 (2009) 2848–2856. <https://doi.org/10.1002/sml.200901048>.
- [12] A. Rocca, S. Moscato, F. Ronca, S. Nitti, V. Mattoli, M. Giorgi, G. Ciofani, *Pilot in vivo investigation of cerium oxide nanoparticles as a novel anti-obesity pharmaceutical formulation*, Nanomedicine Nanotechnology, Biol. Med. 11 (2015) 1725–1734. <https://doi.org/10.1016/j.nano.2015.05.001>.
- [13] N. Lavanya, J. Nizeyimana Claude, C. Sekar, *Electrochemical determination of purine and pyrimidine bases using copper doped cerium oxide nanoparticles*, J. Colloid Interface Sci. 530 (2018) 202–211. <https://doi.org/10.1016/j.jcis.2018.06.046>.
- [14] A.A. Ensafi, R. Noroozi, N. Zandi—Atashbar, B. Rezaei, *Cerium(IV) oxide decorated on reduced graphene oxide, a selective and sensitive electrochemical sensor for fenitrothion determination*, Sensors Actuators, B Chem. 245 (2017) 980–987. <https://doi.org/10.1016/j.snb.2017.01.186>.
- [15] A.M. Fekry, S.M. Azab, M. Shehata, M.A. Ameer, *A novel electrochemical nicotine*

- sensor based on cerium nanoparticles with anionic surfactant, *RSC Adv.* 5 (2015) 51662–51671. <https://doi.org/10.1039/c5ra06024a>.
- [16] N. Manjula, S. Pulikkutty, T.W. Chen, S.M. Chen, C.H. Fan, M.A. Ali, F.M. Al-Hemaid, Electrochemical sensor based on cerium niobium oxide nanoparticles modified electrode for sensing of environmental toxicity in water samples, *Colloids Surfaces A Physicochem. Eng. Asp.* 637 (2022) 128277. <https://doi.org/10.1016/j.colsurfa.2022.128277>.
- [17] G. Ziyatdinova, L. Gimadutdinova, Cerium(IV) and iron(III) oxides nanoparticles based voltammetric sensor for the sensitive and selective determination of lipoic acid, *Sensors*. 21 (2021). <https://doi.org/10.3390/s21227639>.
- [18] F. Contreras, C. Fouilloux, N. Simonovis, R. Herna, M. Velasco, Dopamine, hypertension and obesity, *J. Hum. Hypertens.* 16 (2002) 13–18. <https://doi.org/10.1038/sj/jhh/1001334>.
- [19] T.G. Beatto, W.E. Gomes, A. Etchegaray, R. Gupta, R.K. Mendes, Dopamine levels determined in synthetic urine using an electrochemical tyrosinase biosensor based on ZnO@Au core-shell, *RSC Adv.* 13 (2023) 33424–33429. <https://doi.org/10.1039/d3ra06277e>.
- [20] A.P. Abbott, G. Capper, D.L. Davies, R.K. Rasheed, V. Tambyrajah, Novel solvent properties of choline chloride/urea mixtures, *Chem. Commun.* (2003) 70–71. <https://doi.org/10.1039/b210714g>.
- [21] N. Laube, B. Mohr, A. Hesse, Laser-probe-based investigation of the evolution of particle size distributions of calcium oxalate particles formed in artificial urines, *J. Cryst. Growth*. 233 (2001) 367–374. [https://doi.org/10.1016/S0022-0248\(01\)01547-0](https://doi.org/10.1016/S0022-0248(01)01547-0).
- [22] G.R. Piton, K.K.L. Augusto, A. Wong, F.C. Moraes, O. Fatibello-Filho, A Novel Electrochemical Glassy Carbon Electrode Modified with Carbon Black and Glyceline Deep Eutectic Solvent within a Crosslinked Chitosan Film for Simultaneous Determination of Acetaminophen and Diclofenac, *Electroanalysis*. (2021) 1–11. <https://doi.org/10.1002/elan.202100325>.
- [23] A. Shishov, A. Bulatov, M. Locatelli, S. Carradori, V. Andrich, Application of deep eutectic solvents in analytical chemistry. A review, *Microchem. J.* 135 (2017) 33–38. <https://doi.org/10.1016/j.microc.2017.07.015>.
- [24] A. Abo-hamad, M. Hayyan, M. Abdulhakim, M. Ali, Potential applications of deep eutectic solvents in nanotechnology, *Chem. Eng. J.* 273 (2015) 551–567. <https://doi.org/10.1016/j.cej.2015.03.091>.
- [25] T. Juric, D. Uka, B.B. Holló, B. Jovic, B. Kordic, B.M. Popovic, Comprehensive physicochemical evaluation of choline chloride-based natural deep eutectic solvents, *J. Mol. Liq.* 343 (2021). <https://doi.org/10.1016/j.molliq.2021.116968>.
- [26] A.P. Abbott, R.C. Harris, K.S. Ryder, Application of Hole Theory to Define Ionic Liquids by their Transport Properties †, *J. Phys. Chem. B.* (2007) 4910–4913. <https://doi.org/10.1021/jp0671998>.
- [27] D.O. Abranches, L.P. Silva, M.A.R. Martins, J.A.P. Coutinho, Differences on the impact of water on the deep eutectic solvents betaine/urea and choline/urea, *J. Chem. Phys.* 155 (2021). <https://doi.org/10.1063/5.0052303>.
- [28] S. Sivrikaya, A novel vortex-assisted liquid phase microextraction method for parabens in cosmetic oil products using deep eutectic solvent, *Int. J. Environ. Anal. Chem.* 99 (2019) 1575–1585. <https://doi.org/10.1080/03067319.2019.1625345>.
- [29] E.K. Goharshadi, S. Samiee, P. Nancarrow, Fabrication of cerium oxide nanoparticles: Characterization and optical properties, *J. Colloid Interface Sci.* 356 (2011) 473–480. <https://doi.org/10.1016/j.jcis.2011.01.063>.
- [30] M.E. Ioannou, G.K. Pouroutzidou, I. Chatzimentor, I. Tsamesidis, N. Florini, I. Tsiaoussis, E. Lymperaki, P. Komninou, E. Kontonasaki, Synthesis and Characterization of Cerium Oxide Nanoparticles: Effect of Cerium Precursor to Gelatin Ratio, *Appl. Sci.* 13 (2023). <https://doi.org/10.3390/app13042676>.
- [31] F. Gao, Q. Lu, S. Komarneni, Fast synthesis of cerium oxide nanoparticles and nanorods, *J. Nanosci. Nanotechnol.* 6 (2006) 3812–3819. <https://doi.org/10.1166/jnn.2006.609>.
- [32] M.C. Morris, H.F. McMurdie, E.H. Evans, B. Paretzkin, H.S. Parker, N.P. Pyrras,

- Standard X-Ray Diffraction Powder Patterns: Section 20 – Data for 71 Substances, Card no. 5, National Institute of Standards and Technology, Swarthmore, PA, 1984. <https://doi.org/10.6028/NBS.MONO.25-20>.
- [33] D. Parimi, V. Sundararajan, O. Sadak, S. Gunasekaran, S.S. Mohideen, A. Sundaramurthy, Synthesis of Positively and Negatively Charged CeO₂ Nanoparticles: Investigation of the Role of Surface Charge on Growth and Development of *Drosophila melanogaster*, *ACS Omega*. 4 (2019) 104–113. <https://doi.org/10.1021/acsomega.8b02747>.
- [34] S. Sathiyamurthy, K.J. Leonard, R.T. Dabestani, Reverse micellar synthesis of cerium oxide nanoparticles, *Nanotechnology*. 16 (2019) 1960–1964. <https://doi.org/10.1088/0957-4484/16/9/089>.
- [35] S.A. Khan, A. Ahmad, Fungus mediated synthesis of biomedically important cerium oxide nanoparticles, *Mater. Res. Bull.* 48 (2013) 4134–4138. <https://doi.org/10.1016/j.materresbull.2013.06.038>.
- [36] K. Kashyap, D.K. Verma, S.K. Pattanayak, F. Khan, Green Synthesized Cerium Oxide Nanoparticles as Efficient Adsorbent for Removal of Fluoride Ion from Aqueous Solution, *Water. Air. Soil Pollut.* 234 (2023). <https://doi.org/10.1007/s11270-023-06191-1>.
- [37] S. Soren, M. Bessoi, P. Parhi, A rapid microwave initiated polyol synthesis of cerium oxide nanoparticle using different cerium precursors, *Ceram. Int.* 41 (2015) 8114–8118. <https://doi.org/10.1016/j.ceramint.2015.03.013>.
- [38] L.M.C. Ferreira, P.S. Silva, K.K.L. Augusto, P.C. Gomes-Júnior, S.O.D. Farra, T.A. Silva, O. Fatibello-Filho, F.C. Vicentini, Using nanostructured carbon black-based electrochemical (bio)sensors for pharmaceutical and biomedical analyses: A comprehensive review, *J. Pharm. Biomed. Anal.* 221 (2022) 115032. <https://doi.org/10.1016/j.jpba.2022.115032>.
- [39] A.J. Bard, L.R. Faulkner, H.S. White, *Electrochemical Methods: Fundamental and Applications*, 3th editio, John Wiley & Sons, Ltd, New York, 2022.
- [40] J.W. Zhang, X. Zhang, Electrode material fabricated by loading cerium oxide nanoparticles on reduced graphene oxide and its application in electrochemical sensor for tryptophan, *J. Alloys Compd.* 842 (2020). <https://doi.org/10.1016/j.jallcom.2020.155934>.
- [41] L.C.S. Figueiredo-Filho, T.A. Silva, F.C. Vicentini, O. Fatibello-Filho, Simultaneous voltammetric determination of dopamine and epinephrine in human body fluid samples using a glassy carbon electrode modified with nickel oxide nanoparticles and carbon nanotubes within a dihexadecylphosphate film, *Analyst*. 139 (2014) 2842–2849. <https://doi.org/10.1039/c4an00229f>.
- [42] A.A. Abdelwahab, A.H. Naggar, M. Abdelmotaleb, M.Y. Emran, Ruthenium Nanoparticles Uniformly-designed Chemically Treated Graphene Oxide Nanosheets for Simultaneous Voltammetric Determination of Dopamine and Acetaminophen, *Electroanalysis*. 32 (2020) 2156–2165. <https://doi.org/10.1002/elan.202060126>.
- [43] E. Haghshenas, T. Madrakian, A. Afkhami, Electrochemically oxidized multiwalled carbon nanotube/glassy carbon electrode as a probe for simultaneous determination of dopamine and doxorubicin in biological samples, *Anal. Bioanal. Chem.* 408 (2016) 2577–2586. <https://doi.org/10.1007/s00216-016-9361-y>.
- [44] M. Baccarin, F.A. Santos, F.C. Vicentini, V. Zucolotto, B.C. Janegitz, O. Fatibello-Filho, Electrochemical sensor based on reduced graphene oxide/carbon black/chitosan composite for the simultaneous determination of dopamine and paracetamol concentrations in urine samples, *J. Electroanal. Chem.* 799 (2017) 436–443. <https://doi.org/10.1016/j.jelechem.2017.06.052>.
- [45] B. Habibi, M.H. Pournaghi-Azar, Simultaneous determination of ascorbic acid, dopamine and uric acid by use of a MWCNT modified carbon-ceramic electrode and differential pulse voltammetry, *Electrochim. Acta*. 55 (2010) 5492–5498. <https://doi.org/10.1016/j.electacta.2010.04.052>.
- [46] G. Gerhardt, R.N. Adams, Determination of Diffusion Coefficients by Flow Injection Analysis, *Anal. Chem.* 54 (1982) 2618–2620. <https://doi.org/10.1021/ac00251a054>.
- [47] X. Wang, L. Hao, Z. Zhang, J. Yan, S. Zhang, Y. Zhang, Design three-dimensional CoFe₂O₄ nanocage via ion exchange route for ultra-sensitive electrochemical sensing of

- nitrobenzene, *Ceram. Int.* 49 (2023) 18699–18707. <https://doi.org/10.1016/j.ceramint.2023.02.247>.
- [48] A.A. Ensafi, M. Taei, T. Khayamian, A. Arabzadeh, Highly selective determination of ascorbic acid, dopamine, and uric acid by differential pulse voltammetry using poly(sulfonazo III) modified glassy carbon electrode, *Sensors Actuators, B Chem.* 147 (2010) 213–221. <https://doi.org/10.1016/j.snb.2010.02.048>.
- [49] R.A. Medeiros, R. Matos, A. Benchikh, B. Saidani, C. Debiemme-Chouvy, C. Deslouis, R.C. Rocha-Filho, O. Fatibello-Filho, Amorphous carbon nitride as an alternative electrode material in electroanalysis: Simultaneous determination of dopamine and ascorbic acid, *Anal. Chim. Acta.* 797 (2013) 30–39. <https://doi.org/10.1016/j.aca.2013.08.018>.
- [50] M. Devaraj, R. Saravanan, R. Deivasigamani, V.K. Gupta, F. Gracia, S. Jayadevan, Fabrication of novel shape Cu and Cu/Cu₂O nanoparticles modified electrode for the determination of dopamine and paracetamol, *J. Mol. Liq.* 221 (2016) 930–941. <https://doi.org/10.1016/j.molliq.2016.06.028>.
- [51] G. Hu, Y. Guo, S. Shao, Simultaneous determination of dopamine and ascorbic acid using the nano-gold self-assembled glassy carbon electrode, *Electroanalysis.* 21 (2009) 1200–1206. <https://doi.org/10.1002/elan.200804510>.
- [52] M. Wen, Y. Xing, G. Liu, S. Hou, S. Hou, Electrochemical sensor based on Ti₃C₂ membrane doped with UiO-66-NH₂ for dopamine, *Microchim. Acta.* 189 (2022) 1–8. <https://doi.org/10.1007/s00604-022-05222-8>.
- [53] C.Y. Wang, Z.X. Wang, A.P. Zhu, X.Y. Hu, Voltammetric determination of dopamine in human serum with amphiphilic chitosan modified glassy carbon electrode, *Sensors.* 6 (2006) 1523–1536. <https://doi.org/10.3390/s6111523>.
- [54] J.B. Raoof, A. Kiani, R. Ojani, Simultaneous voltammetric determination of ascorbic acid and dopamine at the surface of electrodes modified with self-assembled gold nanoparticle films, *J. Solid State Electrochem.* 14 (2010) 1171–1176. <https://doi.org/10.1007/s10008-009-0917-z>.

Chapter III

Development of a deep eutectic solvent based on choline chloride and diethanolamine

Chapter III || Development of a deep eutectic solvent based on choline chloride and diethanolamine

Synthesis and characterization of eutectic solvents based on choline chloride and diethanolamine and its application in the green synthesis of silver nanoparticles for electrochemical sensors

ABSTRACT

Deep eutectic solvents (DES) are emerging as eco-friendly alternatives to hazardous organic solvents due to their biodegradability, low volatility, and thermal stability. This study focuses on a hydrophilic DES composed of choline chloride (ChCl) and diethanolamine (DEA). The eutectic point was determined using differential scanning calorimetry (DSC) at a 1:3 molar ratio with a melting temperature of 285.8 K. Detailed characterizations, including FTIR and NMR spectroscopy for ^1H , provided insights into precursor interactions, while physicochemical properties such as water content, conductivity, and viscosity were also assessed. The DES was utilized to synthesize silver nanoparticles (AgNPs) without a conventional reducing agent and below 100 °C. Various characterization techniques such as transmission electron microscopy (TEM), energy dispersive spectroscopy (EDS), selected area electron diffraction (SAED), and X-ray diffraction (XRD) confirmed the nanoparticles' varied shapes and polycrystalline nature with cubic structures. These AgNPs were then used to create a composite sensor with multi-walled carbon nanotubes (MWCNT) for detecting hydroquinone (H_2Q). Scanning electron microscopy (SEM) showed the MWCNT's tubular morphology. The electrochemical sensor exhibited enhanced performance, including increased faradaic capacitance and reduced charge transfer resistance. Key metrics such as the diffusion coefficient ($(4.1 \pm 0.5) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$) and catalytic constant ($(2.8 \pm 0.2) \times 10^6 \text{ mol}^{-1} \text{ L s}^{-1}$) improved significantly. This work demonstrated the importance of thorough DES characterization and demonstrates its successful application in the green synthesis of nanomaterials for electrochemical sensors.

Keywords: Green synthesis silver nanoparticles; Electrochemical sensors; Deep eutectic solvents; Solid-liquid equilibrium; Phases diagram.

3.1 Introduction

Since deep eutectic solvents (DES) were introduced in the literature [1], several applications involving them as greener alternatives to conventional solvents have been presented. In fact, green technologies require new solvents to gradually replace conventional organic media that exhibit high toxicity and volatility [2]. In this sense, DES have been consolidated due to their easy synthesis, reduced toxicity, higher biodegradability compared to ionic liquids (IL), high thermal stability, and almost endless combinations of precursor (hydrogen bond donor and acceptor (HBD and HBA) species) [3].

DES based on the hydrogen acceptor choline chloride (ChCl) with hydrogen donors such as urea, glycerol, and ethylene glycol are classic binary systems that have been extensively studied regarding their physical and chemical properties, except their thermal characteristics, which are eventually explored [4,5]. Although the “eutectic” and “deep eutectic” concepts for DES still require more precise definitions in the literature [6], theoretical studies based on computational simulations predict the nature of the interactions between precursors and estimate regions of eutectic composition with good precision [7–9]. Furthermore, experimental investigations using differential scanning calorimetry (DSC), although poorly explored, is a powerful technique to access the thermal properties of DES through the elaboration of a solid-liquid equilibrium (SLE) phase diagram [10].

The advantage of constructing an SLE phase diagram is the possibility of predicting the melting temperature of any precursor’s composition. However, elaborating SLE phase diagrams for DES is laborious due to the properties of the precursors, which may be hygroscopic and have high viscosity, in addition to the possible occurrence of chemical reactions after a long time of storage [11].

The preparation of DES with alkanolamines as HBDs has been widely reported because they are widely applied in chemical absorption processes of CO₂ in gas processing units and ammonia production. For these applications, aqueous solutions of DES are usually utilized [12]. Moreover, this class of DES have also been used in the pretreatment of cellulosic biomass [13] and keratin extraction.[14]

Recently, DES has been used to synthesise metal nanoparticles (MNPs) as a stabilizing medium under mild synthesis temperature conditions [15,16]. The results are promising in terms of a synthesis process that meets the principles of green chemistry,

such as atomic economy, generation of less hazardous chemicals, safe solvents, and more biodegradable design [17]. For these reasons, DES has gained prominence in nanomaterial synthesis applications using approaches based on solvothermal [16], electrodeposition [18,19], microwave [20,21], one-pot synthesis [22], and thermal decomposition [23], among others.

The growth of environmentally friendly approaches for the synthesis of metallic nanomaterials is an important topic in nanotechnology [24], especially the "noble" metals (silver, gold, and platinum) with potential biological applications[25]. The literature has also been reporting works applying DES in the synthesis of silver nanoparticles (AgNPs) *via* microwave-assisted synthesis. The DES utilized was based on halide-free choline with glycerol [20]. Liu et al. [28] combined green tea extracts with urea-based DES with glycerol and betaine to synthesize AgNPs, which were tested for anticancer activity *in vitro* [26].

Another application of DES involves the field of electrochemistry aiming to improve the detection of electroactive species in sensors. These mainly involve using DES as a medium for electrodeposition [27,28], binders in carbon paste [29], background electrolytic additive modifiers [30], solvent for anodizing alloys [31], additive solvent for the solution of polymerization [32] and medium for the synthesis of metallic nanoparticles [15,16].

Considering the above, it is clear that characterization of DES is a very important topic to investigate. The construction of the SLE phase diagram of new and previously proposed DESs is unquestionable to attest their eutectic identity as well as their thermal behavior. DES has been diffused in the literature as a potential solvent to replace conventional ones in various applications, such as, for example, in syntheses of more sustainable and functional materials. Particularly, this work presents a detailed characterization of mixtures based on ChCl and DEA in different molar proportions, firstly establishing their thermal properties and identifying the eutectic point. Then, a study on its molecular properties using Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) was carried out. After characterization, the DES was used to develop a greener synthesis of AgNPs, which were applied in the construction of a sensor based on multiwalled carbon nanotubes (MWCNT). The sensor was finally used to evaluate the electrocatalytic properties of AgNPs in the detection of hydroquinone.

3.2 Experimental Section

3.2.1 Chemicals and solutions

The chemicals were obtained from Sigma-Aldrich (St. Louis, USA), Synth (Diadema, São Paulo), Neon (São Paulo, Brasil), Panreac (Darmstadt, Germany), or Vetec (Rio de Janeiro, Brasil), with the highest purity grade available and used without further purification. Only choline chloride (ChCl) was recrystallized in ethanol and dried overnight in an oven at 80 °C. Multiwalled carbon nanotubes (MWCNT; O.D. x L 6 – 9 nm x 5 µm, > 95 % carbon) powder were acquired from Sigma-Aldrich (St. Louis, USA). All aqueous solutions were prepared with ultrapure water (resistivity $\geq 18.0 \text{ M}\Omega \text{ cm}$) obtained from a Millipore Milli-Q system (Billerica, USA).

3.2.2 Apparatus and measurements

DSC curves were obtained in a Q10 Differential Scanning Calorimetric module, controlled by the Thermal Advantage Series® software (v.5.5.24), both from TA Instruments (New Castle, USA). All analyses were carried out using sample mass of $5.0 \pm 0.1 \text{ mg}$ and a heating rate of $10 \text{ }^{\circ}\text{C min}^{-1}$ and under a dynamic N_2 atmosphere flowing at 50 mL min^{-1} . Closed aluminum sample holders with a pin hole ($\varnothing = 0.7 \text{ mm}$) in the center of the lid were used in all experiments. The measurements were performed in the temperature interval of -60 to $120 \text{ }^{\circ}\text{C}$ for mixtures based on ChCl:DEA and for diethanolamine (DEA), and of 30 to $330 \text{ }^{\circ}\text{C}$ for ChCl. The endothermic peak temperatures were used to characterize the melting event. The melting enthalpies of mixtures were estimated based on the 1st derivative of the DSC curve, which was used to determine the initial temperature of the melting event, owing to its overlapping with the exothermic transition.

Viscosity, electrical conductivity, and water content assays studied the physicochemical properties of the proposed hydrophilic eutectic solvents. Viscosity measurements were performed using IKA Rotavisc viscometer model lo-vi (Staufen, Germany). Electrical conductivities were determined using a CA 150 Marconi conductivity meter (Piracicaba, São Paulo). The conductivity cell was calibrated with standard KCl aqueous solutions. The degree of uncertainty of these measurements was 2 %. The water content was determined by the Karl-Fischer method, which was performed using an 899 KF Coulometer (Metrohm, Utrecht, Netherlands) with a generator electrode in the absence of diaphragm. A pyridine-free solvent was used in all measurements

(Hydranal, Coulometer AG, Honeywell, Brazil). All measurements were carried out in triplicate at 298.15 K for water content. For viscosity and conductivity measurements the working temperature were 60 and 80 °C, respectively.

¹H NMR measurements were performed on a Bruker ULTRASHIELD (Billerica, USA) operating at 400 MHz at the hydrogen frequency. The sample temperature was set at 298.15 K. Chemical shifts were referenced to the DMSO-*d*₆ solvent signal. ¹H NMR spectra were recorded with a spectral width of 16 ppm and with 16 scans.

A Fourier transform infrared spectrometer model IRPrestige-21 (Shimadzu Instrument, Kyoto, Japan) was used to characterize the eutectic mixtures. The FTIR spectra were obtained in the range from 400 to 4000 cm⁻¹. An ATR accessory was coupled to the instrument to measure liquid samples and KBr pellets were used for solids samples.

Morphological characterizations of the composite film and nanoparticles were carried out on a field-emission gun scanning electron microscope (FEG/SEM, FEI Magellan 400 L) and a transmission electron microscope (TEM, FEI Tecnai G2 F20 microscope operating at 200 kV). For these analyses, the SEM samples (MWCNT film and AgNPs-DES/MWCNT film) were dispersed by drop-casting onto carbon-coated silicon grids. For TEM samples, the carbon-coated copper grids were prepared by drop-casting the particles suspended in an ethanol medium. The Selected Area Electron Diffraction (SAED) pattern analysis was carried out on the same grids.

X-ray Diffraction (XRD) analyses were carried out on a Bruker diffractometer (Model D8, Advance Eco) operating at room temperature under constant conditions: K α Cu radiation at 40 kV, 25 mA, range of 0.02°, and scans from 5 at 90° for all samples. Lattice interplanar spacing of the crystal (*d*) was calculated using Bragg's Law (Equation 1):

$$n\lambda = 2d \sin\theta \quad \text{or} \quad d = \frac{n\lambda}{2 \sin \theta} \quad (\text{Eqn. 1})$$

where θ is the angle of diffraction (in Radians), λ is the wavelength (1.5406 Å), and *n* is the order of diffraction (*n* = 1). The average crystallite size of silver, *D*, was calculated using the Scherrer equation (Equation 2):

$$D = \frac{k\lambda}{\beta \cos \theta} \quad (\text{Eqn. 2})$$

where the constant *k* is given a value (*k* = 1) and β is the full width at half maximum of peak (FWHM).

Electrochemical measurements were performed using a three-electrode single-compartment glass cell containing an Ag|AgCl|KCl(3.0 mol L^{-1}) reference electrode, a platinum plate as a counter electrode, and bare GCE ($\phi = 3.0 \text{ mm}$) or modified-GCE as the working electrode. Cyclic voltammetry (CV), chronoamperometry, and electrochemical impedance spectroscopy (EIS) were performed using an Autolab PGSTAT-20 (Eco Chemie, Utrecht, the Netherlands) potentiostat/galvanostat with an impedance FRA module. EIS measurements were performed at the half-peak potential ($E_{P/2}$) of hydroquinone (H_2Q), with a frequency range from 65 kHz to 0.1 Hz, amplitude of 10 mV, and 10 points per decade.

3.2.3 hydrophilic mixtures preparation

Hydrophilic mixtures with different molar ratios of ChCl and DEA were prepared (Table 21). For this, the DEA was heated up to 313.15 K (40 °C) before adding ChCl, according to their physical-chemical properties (Table 21). The syntheses were conducted in closed glass by controlling the temperature at 353.15 K (80 °C) and stirring at 500 rpm until a homogeneous liquid was formed. The melting point obtained for each mixture was used to plot a phase diagram aiming at finding the eutectic compositions and the eutectic point for ChCl:DEA.

3.2.4 Silver nanoparticles (AgNPs) synthesis in eutectic system

AgNPs synthesis was carried out in DES at a eutectic composition of ChCl:DEA 1:3 (*mol:mol*). In detail, 25 mg of AgNO_3 were transferred to a flask containing 6.9 g of diethanolamine at 60 °C under constant stirring. After dissolution, 500 μL of a solution containing 35 mmol L^{-1} sodium citrate and 3 mmol L^{-1} citric acid were added and allowed to react for 3 min. Then, 3.1 g of choline chloride were added and allowed to react for 30 min at 80 °C until a color change (colorless to light yellow). Finally, after cooling to room temperature, the AgNPs were stored in an amber vial containing molecular sieves.

3.2.5 Preparation of the modified electrode

The GCE surface was polished with 1.0 and 0.5 μm alumina slurry and washed with deionized water. The modifying dispersion containing the AgNPs and MWCNT was prepared as follows: 1.0 mg of MWCNT was suspended in 800 μL of N, N-dimethylformamide, together with 100 μL of AgNPs, and 100 μL of Nafion™ in methanol (25 % v/v). The suspension was sonicated during 30 min in an ultrasonic bath

for complete dispersion of the materials. Finally, 10 μL of this suspension was dispersed in the GCE surface and allowed to dry for 20 minutes under an IR lamp, forming the film.

3.3 Results and Discussion

3.3.1 Physicochemical characterizations

Fractions of the mixtures based on ChCl and DEA were prepared in different molar ratios as presented in Table 21. As can be seen only the fraction 1:9 (ChCl:DEA), in the evaluated set, remains liquid and stable at 298.15 K, while the others present physical aspects of liquid-solid and solid mixtures with the increase of ChCl in the system. However, at the temperatures of 333.15 and 353.15 K, it is observed that among the evaluated compositions, there is a predominance of the liquid aspect in the mixtures. Fractions of 0.67 (1:2) and 0.50 (1:1) were not completely liquid mixtures even at these temperatures. These results attest that the DES do not form stable liquids at room temperature, especially in molar ratios commonly used in the literature (1:2 / *mol:mol*) for analytical applications.

Table 21. Molar ratio of the prepared hydrophilic mixtures based on choline chloride (ChCl) and diethanolamine (DEA) and the respective physical-chemical properties

Molar ratio (ChCl:DEA)	Components		Physical state		
	ChCl	DEA	298.15 K	333.15 K	353.15 K
0:1	0.00	1.00	L	L	L
1:9	0.10	0.90	L	L	L
1:4	0.20	0.80	L + S	L	L
1:3	0.25	0.75	L + S	L	L
1:2.7	X	0.73	L + S	L	L
1:2.3		0.70	L + S	L	L
1:2	0.33	0.67	S	L + S	L
1:1	0.50	0.50	S	L + S	L + S
1:0	1.00	0.00	S	S	S

X: molar fraction; L: liquid; S: solid.

The water content in a DES can differ according to the precursors used in the preparation, especially in the hydrophilic DES, such as those based on choline chloride, that is hygroscopic. All fractions based on ChCl and DEA (Table 22) were studied regarding the water content and the results ranged from $(0.39 \pm 0.07)\%$ to $(0.58 \pm 0.06)\%$, which are in agreement with the results commonly reported in the literature for choline chloride-based DES [33].

Table 22. Determination of water content of each hydrophilic mixture based on choline chloride (ChCl) and diethanolamine (DEA)

Molar ratio	ChCl:DEA					
	1:9	1:4	1:3	1:2.67	1:2.33	1:2
Water content / %	0.38	0.39	0.32	0.42	0.60	0.54
	0.51	0.41	0.57	0.42	0.66	0.39
	0.29	0.38	0.38	0.37	0.60	0.38
	0.41	0.36	0.59	0.45	0.49	0.43
	0.40	0.39	0.61	0.39	0.58	0.35
	0.38	0.39	0.52	0.36	0.58	0.38
Mean ($\pm$ SD)	0.40 ($\pm$ 0.07)	0.39 ($\pm$ 0.02)	0.50 ($\pm$ 0.12)	0.40 ($\pm$ 0.03)	0.58 ($\pm$ 0.06)	0.41 ($\pm$ 0.07)

It is important to mention that the incorporation of water into the system influences, for example, the viscosity, density, conductivity, and polarity of the DES [18]. However, in some applications, the water content is added intentionally, such as in the case of extraction of bioactive compounds [34].

The conductivity and viscosity of the mixtures were measured at the temperatures of 333.15 and 353.15 K. This is because only the 0.90 fraction is fully liquid at room temperature (see Table 1). Electrical conductivity values ranged from (1.4 ± 0.1) to (2.4 ± 0.1) mS cm⁻¹ at 333.15 K and from (2.1 ± 0.1) to (2.9 ± 0.1) mS cm⁻¹ at 353.15 K. These low values of electrical conductivity were as expected as result of the low mobility of ions that form aggregates of large ion pairs [35]. Although the conductivities were measured at different and higher temperatures when compared to the room temperature, their values were not significantly different from each other (Table 23).

Table 23. Determination of viscosity and conductivity at different temperatures for each hydrophilic mixture based on choline chloride (ChCl) and diethanolamine (DEA)

Molar ratio (ChCl:DEA)	Viscosity / mPa s		Conductivity / mS cm ⁻¹	
	Temperature / °C		Temperature / °C	
	60	80	60	80
1:9	108 ($\pm$ 2)	54 ($\pm$ 3)	1.4 ($\pm$ 0.1)	2.1 ($\pm$ 0.1)
1:4	110 ($\pm$ 2)	56 ($\pm$ 1)	2.2 ($\pm$ 0.2)	2.3 ($\pm$ 0.1)
1:3	112 ($\pm$ 3)	64 ($\pm$ 1)	2.3 ($\pm$ 0.1)	2.3 ($\pm$ 0.1)
1:2.67	115 ($\pm$ 3)	72 ($\pm$ 1)	2.3 ($\pm$ 0.1)	2.7 ($\pm$ 0.2)
1:2.33	117 ($\pm$ 2)	76 ($\pm$ 2)	2.3 ($\pm$ 0.1)	2.8 ($\pm$ 0.2)
1:2	118 ($\pm$ 1)	79 ($\pm$ 1)	2.4 ($\pm$ 0.1)	2.9 ($\pm$ 0.1)

The viscosities of the mixtures were measured under the same temperature conditions mentioned above. At 333.15 K, the values ranged from (108 ± 2) to (188 ± 1) mPa s and at 353.15 K from (54 ± 3) to (78 ± 1) mPa s. As shown in Table 23, at both temperatures, the viscosity increased with the increment of choline chloride which can affect the relative sizes of the ions by reducing the molecular mobility of the system caused by the increase in intermolecular forces such as van der Waals, electrostatics, and hydrogen bonds [1,5].

3.3.2 Thermal characterization of choline chloride and diethanolamine mixture

Differential scanning calorimetry curves were used to measure the melting temperature of mixtures based on ChCl:DEA (Table 21). Peak temperatures were acquired to construct a phase diagram and establish the eutectic point. Figure 25 shows the DSC curves for the precursors and the ChCl and DEA 1:3 (*mol:mol*) mixture, which presented the lowest melting temperature. Figure 26 presents all DSC curves of the other mixtures, and Table 24 summarizes the main thermal events and the thermodynamic data (ΔH_m and ΔS_m) for the compositions studied in this work.

Table 24. Thermodynamic data acquired from the experiments by differential scanning calorimetry

X_{DEA}	T_{trans} / K	T^* / K	T_m / K	$\Delta_m H (J g^{-1})$	$\Delta_m S (J g^{-1} K^{-1})$	γ_{DEA}
1.00	↑253.1	—	↓296.1	163.0	0.55	—
0.90	↑260.4	—	↓290.4	106.1	0.37	0.90
0.80	↑270.7	↑318.0	↓290.7	41.1	0.14	1.00
0.75	↑267.9	↑319.9	↓285.8 ^a	41.4	0.15	0.90
0.73	↑268.1	↑323.0	↓289.9	14.1	0.05	1.00
0.70	↑267.8 / ↓287.7	↑323.6	^c	—	—	—
0.67	↑261.1	323.0	↓293.3	96.3	0.33	1.35
0.50	↓287.9 / ↓345.5	—	^c	—	—	^b
X_{ChCl}	T_{trans} / K	T^* / K	T_m / K	$\Delta_m H (J g^{-1})$	$\Delta_m S (J g^{-1} K^{-1})$	
1.00	↓344.2	—	↓588.6	973.7	1.65	—

^aEutectic point; ^bIt does not represent a eutectic system; ^cNo melting event was detected; T_m : melting temperature; T_{trans} : temperature of phase transition; T^* : temperature of the free fraction of the precursor (ChCl); ΔH_m : melting enthalpy; ΔS_m : melting entropy; γ : activity coefficient of component i (DEA). ↓ Endothermic event; ↑ Exothermic event.

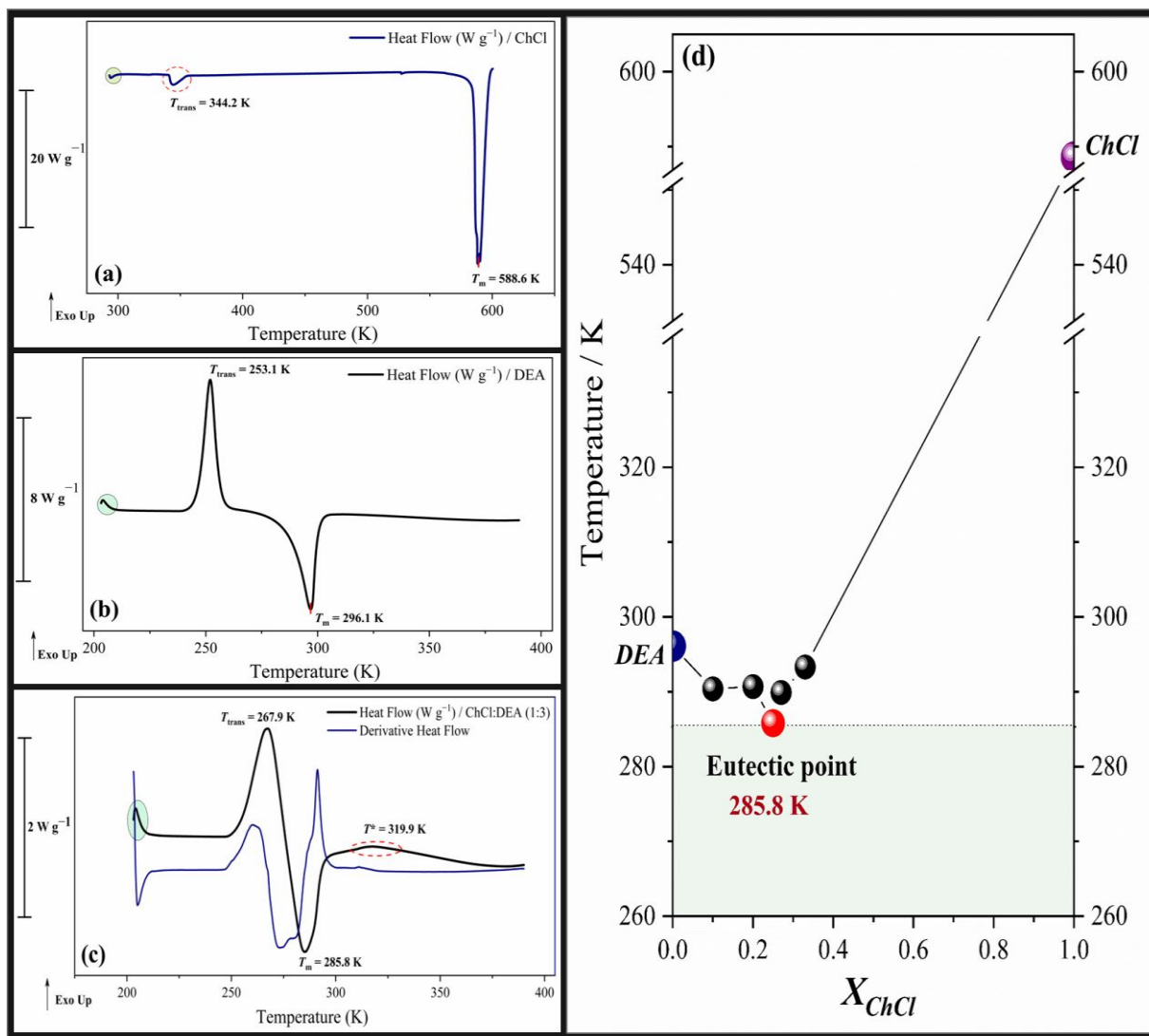


Figure 26. DSC curves with the thermal events of the pure components **(a)** ChCl, **(b)** DEA, mixtures at the eutectic point **(c)** ChCl:DEA in molar ratio 1:3 (*mol:mol*) (overlap: derived from the heat flow), and **(d)** phase diagram obtained for the choline chloride and diethanolamine system.

In Fig. 26 (a), the DSC curve for ChCl revealed two endothermic peaks. The first, at 344.2 K, can be attributed to the readjustment of the crystal resulting from the phase transition [37,38]. The second peak, in 588.6 K, is relative to its melting point, which is in agreement with the values reported in the literature for choline chloride [15]. Likewise, DSC curve of diethanolamine (Fig. 26 (b)) presented two well-defined sharp peaks at 253.1 and 296.1 K, which coincide with an exothermic crystallization event and subsequent melting of the compound, respectively. The value found for melting is close to that reported in the literature [39].

Finally, the DSC curve for deep eutectic solvent based on ChCl:DEA in molar ratio 1:3 (*mol:mol*) (Fig. 26 (c)) showed the lowest melting temperature among the

studied fractions with melting below 296.1 K (see Figs. 27 (a – c); and also Table 24). Furthermore, three events are highlighted; the first refers to exothermic crystallization, this event is also present in the other fractions (see Figs. 27 (a – e); and Table 24). The second represents the temperature at the eutectic point, which was 285.8 K. This value is close to that reported by Murshid et al with systems based on ChCl:DEA in the composition of 1:4 (*mol:mol*) with a temperature of 281.03 K [40]. The third event (319.9 K) can probably be related to the excess of ChCl compared to DEA, resulting in a thermal transition profile close to the melting temperature, an event also observed in the other fractions (see Figs. 27 (b – e); and Table 24). In Fig. 27 (f) ($X_{0.50}$) the increase in ChCl content can intensify the interactions (hydrogen bonds) and probably cause the glass transition event at 287.9 K. While the event observed in this fraction at 345.5K denotes the presence of crystallographic readjustment of pure ChCl (344.2 K, see Fig.26 (a)).

The blue curve in Fig. 26 (c) is the 1st derivative of DSC curve that was used to estimate the inflexion point between the exothermic transition at 267.9 K and the melting at 285.8 K, once both are successive overlapping events. The temperature used to define the inflexion was 278.9 K, which was used to estimate the inflection and to evaluate the enthalpy.

Additionally, it is worth highlighting that the first peak of the DSC curves, 294 K for ChChl (Fig. 26 (a)) and 204 K for DEA (Fig. 26 (b)) and the other fractions (Fig. 26 (c)); see Figs. 27 (a – f), is related to the effect of thermal inertia, which can be attributed to a temperature gradient created between the end of the process cooling and the beginning of heating of the fractions and pure compounds [41].

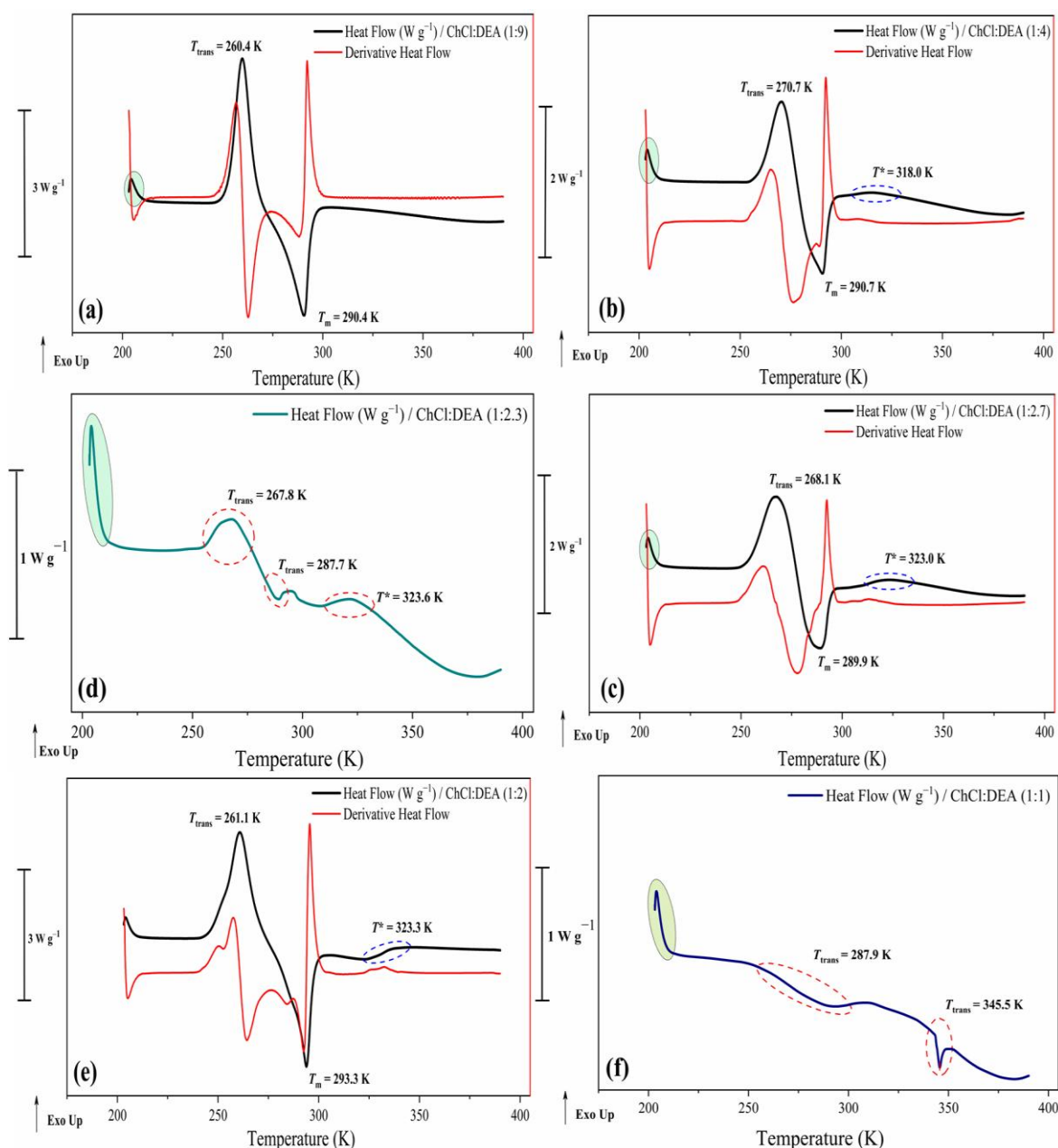


Figure 27. DSC curves for different mixtures of ChCl and DEA (overlap: derived from the heat flow in (a), (b), (c), and (e)).

3.3.4 Determination of solid-liquid equilibria phase behavior of hydrophilic mixtures

Recent studies have shown that the term ‘deep’ for eutectic solvents can be determined from experimental thermodynamic data based on the melting enthalpy (H_m) and temperature (T_m), as well as the behavior of the components in the liquid phase (*i.e.*, activity coefficient, γ) [10,11]. Thus, it is evaluated whether the elaborated eutectic system presents deviations from ideality. Since ‘eutectic’ depends on these deviations of ideality, it is assumed that the presence of negative values will characterize the system as

deep eutectic. When positive, then the system is only eutectic [42]. This behavior can be measured in terms of activity coefficient (γ) according to Equation (3) [11], where $\gamma = 1$ indicates an ideal system and $\gamma < 1$ a deep eutectic system.

$$\ln (x_i\gamma_i) = \frac{\Delta_m H_i}{R} \left(\frac{1}{T_{m,i}} - \frac{1}{T} \right) \quad \text{Eqn. (3)}$$

Following equation (3), x_i , γ_i , $\Delta_m H_i$ (J mol⁻¹), and $T_{m,i}$ (K) are, respectively, the molar ratio, activity coefficient, melting enthalpy, and melting temperature of component i . R (8.314 J mol⁻¹ K⁻¹) and T (K) are the ideal gas constant and the thermodynamic temperature of the system, respectively.

The results obtained in the thermal analysis study (see section 3.2) applied in Eqn (3) enable the elaboration of a phase diagram, which aims to evaluate the thermodynamic behavior of the studied mixtures. The γ values for the ChCl:DEA mixtures were calculated based on the diethanolamine enthalpy and melting temperature data applied to Eqn (1). Table 24 shows the γ results for all studied mixtures, and Figure 26 (d) represents the phase diagram for the mixture based on ChCl:DEA as a function of the molar ratio of chloride choline.

For the ChCl:DEA system, fractions $X_{0.70}$ and $X_{0.50}$ (Table 24) did not present thermal melting events, only phase transitions (Figs. 27 (d) and 27 (f)). However, the fraction $X_{0.67}$ had a lower melting temperature than DEA, indicating that this composition is eutectic (Fig. 27 (e)). The activity coefficient for this mixture showed a value of $\gamma > 1$, confirming that in this ratio there is only a eutectic mixture. Additionally, a variation of γ is observed with the increase in the choline chloride fraction, with fractions $X_{0.80}$ and $X_{0.73}$ approaching a pure composition ($\gamma \rightarrow 1$), around the composition of the eutectic point. Furthermore, values of $\gamma < 1$ suggest that the ChCl:DEA system presents a negative deviation from ideality, classifying it as a deep eutectic solvent. Notably, activity coefficient values lower than 1 were obtained in the fraction 0.90 and 0.75, which represents the eutectic composition of this system.

3.3.5 NMR and FT-IR characterizations

¹H NMR and FTIR of the eutectic mixture usually provide valuable information regarding interactions between precursors. In the ¹H NMR spectrum, the chemical shifts of protons are relative to the chemical environment of each proton. Therefore, the different functional groups, molecular conformations, and intermolecular interactions influence the changes observed in the ¹H NMR spectra [43].

Regarding the formation of the eutectic mixtures, hydrogen bonds or dipole-dipole interactions are common considering the precursors nature. The interactions between them may result in changes in the chemical shifts of the protons. On the other hand, in the infrared spectrum, it is possible to identify molecular interactions through changes in absorption bands and intensities. Thus, these changes provide information of how the molecular structure of the eutectic mixture differs from that of its precursors [43,44]. The ^1H NMR chemical shifts for the eutectic mixture based on choline chloride and diethanolamine (molar ratio of 1:3) and its precursors are presented in Figure 28 (a – c).

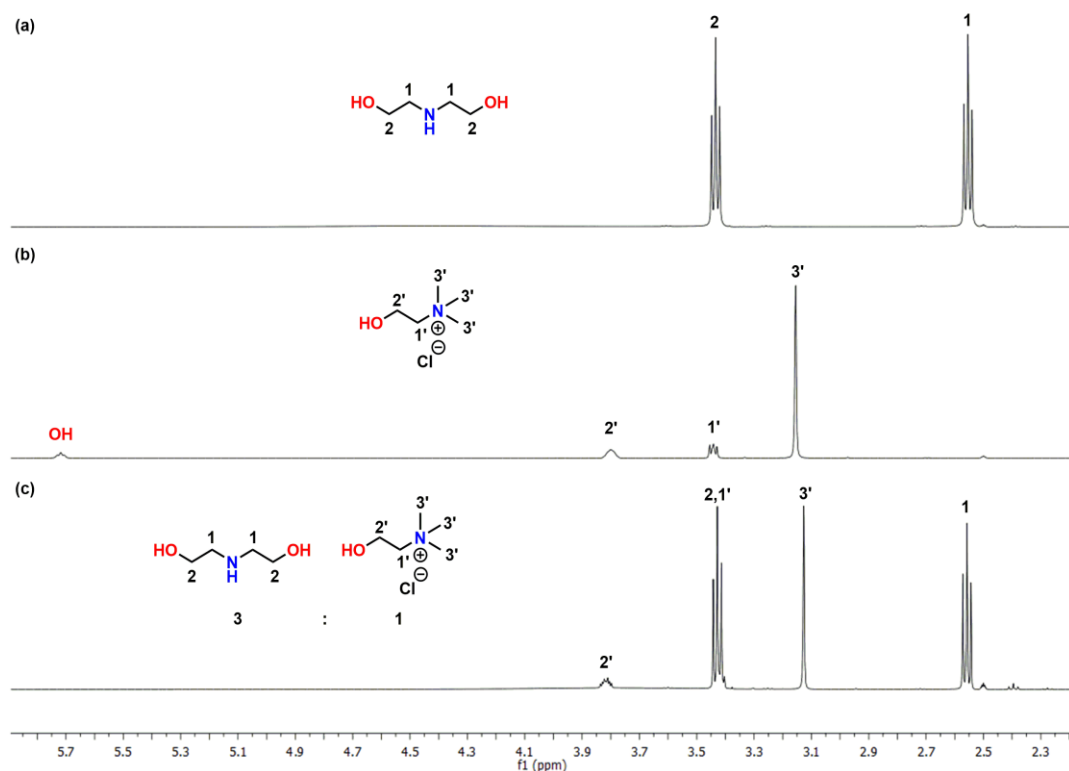


Figure 28. ^1H NMR spectra obtained for the eutectic mixture and its components: (a) diethanolamine (b) choline chloride, and (c) ChCl:DEA in molar ratio 1:3 (*mol:mol*).

As can be seen in Figure 28 (c), proton signals of groups methylene 1' and methylene 3' shift to lower fields of 3.41 and 3.13 ppm, respectively, compared to their individual precursors. The hydroxyl and secondary amine signals could not be detected by ^1H NMR. This is evident in the expanded spectra shown in Figs. 28 (a – c). This may be due to a rapid exchange of protons with the solvent or interference from other more intense signals. The changes observed from chemical shifts suggest possible interactions in the molecular structure of the eutectic mixture compared to the individual precursors.

In the infrared spectrum of choline chloride (Fig. 29 (a)), absorption bands are observed at 3250 cm^{-1} , which correspond to the stretching vibration (ν) of the O—H bonds in the aliphatic structure; absorptions in the range of $3000 - 2800\text{ cm}^{-1}$, which are attributed to stretching vibrations of the C—H bonds in the methyl and methylene groups; and absorption around 1479 cm^{-1} , due to stretching vibrations (δ) of the C—N bonds. The infrared spectrum of diethanolamine (Fig. 29 (b)) presents absorption bands around 3280 cm^{-1} , corresponding to vibrations $\nu\text{O—H}$ and $\nu\text{N—H}$; bands in the range of $3000 - 2800\text{ cm}^{-1}$ ($\nu\text{C—H}$); and absorption at 1454 cm^{-1} ($\delta\text{C—N}$). In the spectrum of the eutectic mixture with molar composition of 1:3 (ChCl:DEA), it is possible to observe the presence of a broad, intense absorption band between $3394 - 3100\text{ cm}^{-1}$ (Fig. 29 (c)), suggesting the presence of intermolecular interactions, particularly NH—OH and OH—OH interactions. Furthermore, the intensity and amplitude of the absorption band in this range indicate the strength and extent of these intermolecular interactions within the eutectic mixture.

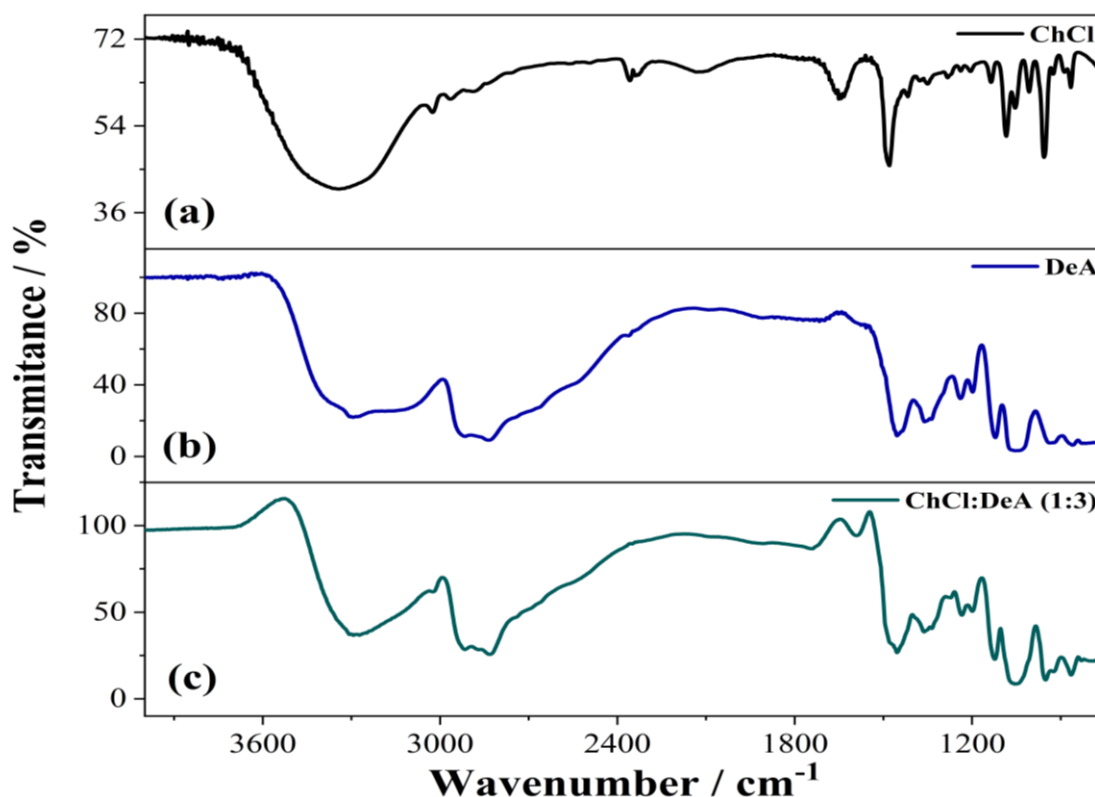


Figure 29. FT-IR spectroscopy analysis for eutectic mixture and its components: (a) choline chloride, (b) diethanolamine, and (c) ChCl:DEA.

3.3.6 Characterization of AgNPs-DES/MWCNT composite

AgNPs synthesized in the DES were morphologically characterized by TEM. As can be seen in Figure 30 (a), it was obtained AgNPs with size distribution ranging from 15.8 to 81.3 nm, an average size of 51.7 nm (inset of Fig. 30 (a)), and distinct shapes (Fig. 30 (a)). From Figure 30 (b), several emission lines for silver were observed in the EDX spectrum. The inset of Figure 30 (b) highlights the selected area of the nanoparticle.

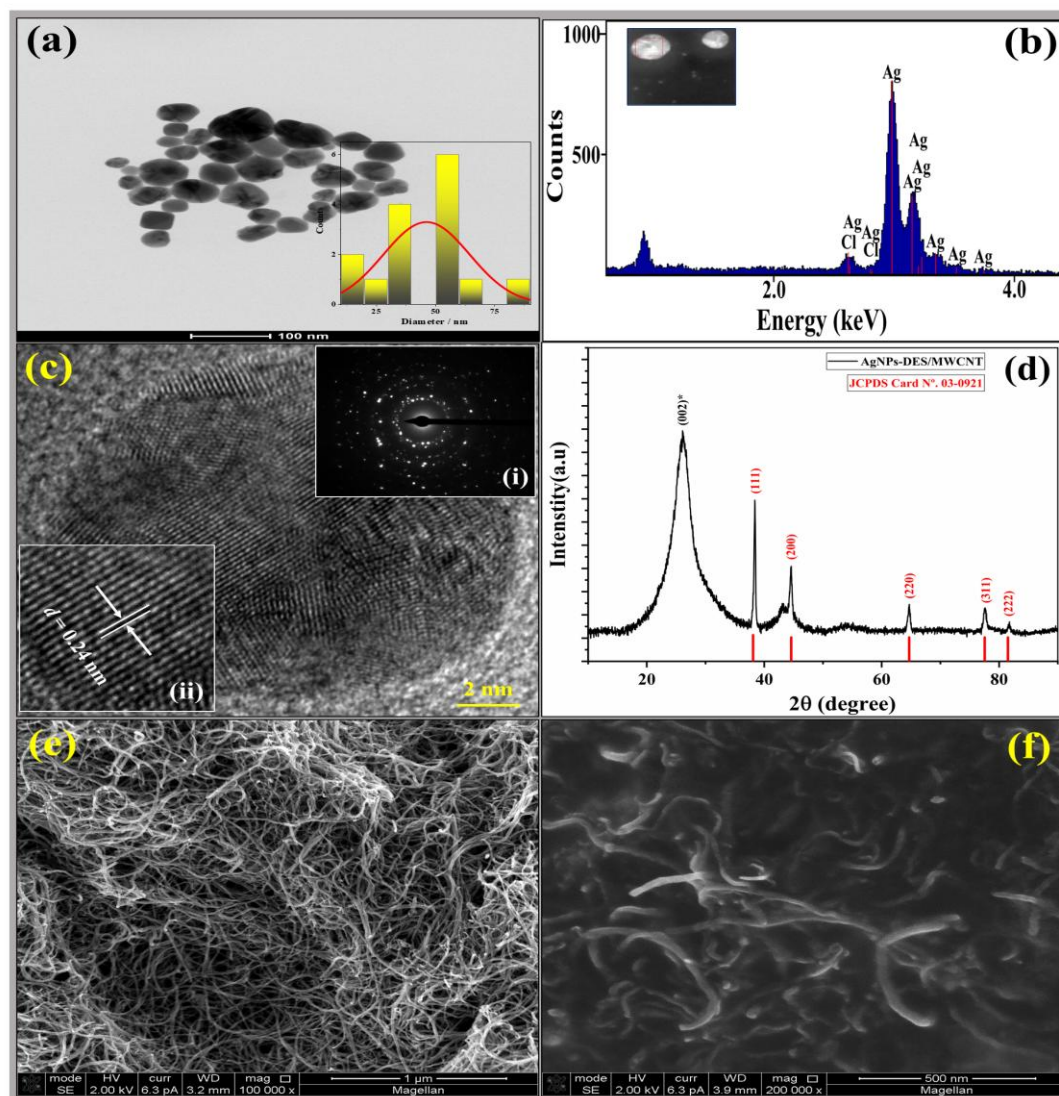


Figure 30. (a) TEM image (inset: histogram of size distribution for the AgNPs-DES). (b) EDX spectrum of silver nanoparticles (inset: collected area). (c) HRTEM image (inset: (i) SAED pattern of AgNPs-DES; and (ii) d-spacing). (d) X-ray diffractogram for AgNPs-DES/MWCNT composite. (e) SEM images obtained for the MWCNT; and (f) AgNPs-DES/MWCNT composite film.

HRTEM image (Fig. 30 (c)) confirmed that the AgNPs synthesized in DES present a polycrystalline nature. The SAED patterns are presented in inset (i) of Fig. 30 (c). The interplanar distance of 0.24 nm (inset (ii) of Fig. 30 (c), and Fig. 31 (a)) indicates that the silver crystals have a cubic crystallographic plane structure [45].

XDR experiments were performed in order to confirm the crystallographic planes. The XRD patterns obtained for AgNPs showed characteristic diffraction peaks at 38.1° , 44.3° , 64.3° , 77.3° , and 81.7° , corresponding to (111), (200), (220), (311), and (222) planes, which corroborate cubic structure for the Ag crystals. (Fig. 30 (d)). Additionally, using Equation (1) (see section 3.1.2.2), d -spacings were calculated for all diffraction angles of AgNPs. From this, 2.4 Angstrom ($\text{\AA}$) (or 0.24 nm) corresponds to (111), ratifying the value observed in the SAED pattern (see Table 25 and inset (ii) of Fig. 31 (c)). Moreover, the diffraction peak at 25.7° corresponds to the (002)* plane referring to the hexagonal highly ordered graphite of MWCNT [46] (see Fig. 31 (b)), which is a component of the AgNPs-DES/MWCNT composite.

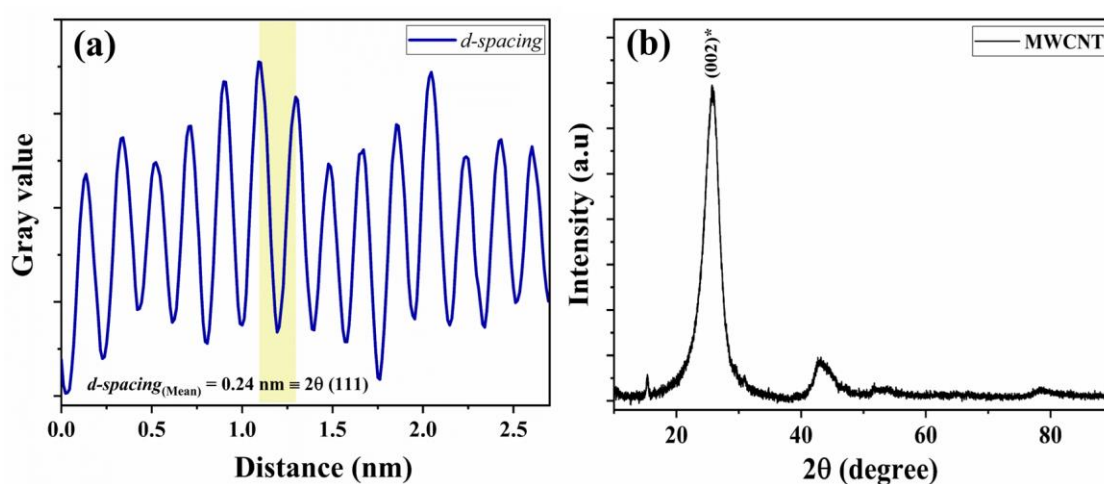


Figure 31. Plot obtained from the interplanar distances of the HRTEM image (a). X-ray diffractogram for MWCNT (b).

Table 25. Data obtained for d -spacing using the Bragg equation

Plane	2θ	θ	d -spacing ($\text{\AA}$)
(002) ^a	26.1	13.1	3.4
(111)	38.5	19.3	2.4
(200)	44.6	22.3	2.0
(220)	64.7	32.4	1.4
(311)	77.7	38.9	1.2
(222)	81.8	40.9	1.2

^aplane referring to the hexagonal highly ordered graphite of MWCNT.

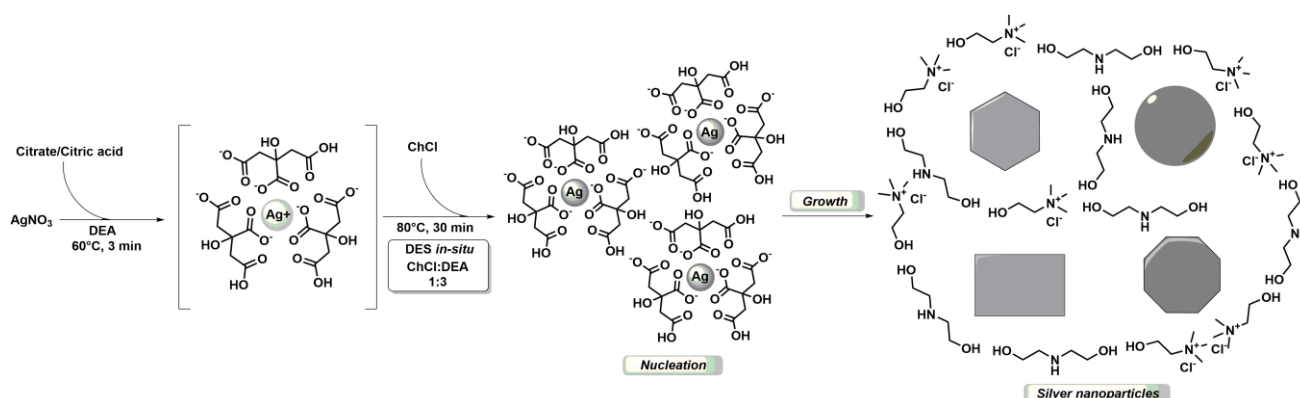
XRD data were used to estimate the crystallinity of AgNPs by following relation: Crystallinity = [Area of crystallite peaks / Area of all peaks (crystalline + amorphous)] x 100%. Thus, 75% of crystallinity was estimated for the AgNPs synthesized in the

elaborated DES. Additionally, Scherrer's equation (see section 3.1.2.2) was used to estimate average crystallite size (Table 26). The results revealed an average crystallite size of (22.5 ± 2.1) nm for AgNPs.

Table 26. Data obtained for crystallite sizes of AgNPs-DES

2θ / degree	FWHM (β) / degree	Crystallite size, D (nm)	D, Average
38.5	0.34	24.5	22.5 (± 2.1)
44.6	0.35	24.4	
64.7	0.46	20.3	
77.7	0.50	20.3	
81.8	0.45	23.2	

Additionally, the possible mechanism of green synthesis of AgNPs in the eutectic composition of the prepared DES was proposed, and the steps are shown in Scheme 1.



Scheme 1. Suggested mechanism for green synthesis of AgNPs in DES based on ChCl:DEA (molar ratio, 1:3 / *mol:mol*).

The first step corresponds to the protective effect of silver ions in DEA medium using a mixture of citric acid and citrate solutions that can minimize the precipitation of silver in the form of chloride in the ChCl addition step, in addition to being able to leave it free to form DES *in-situ* with DEA and also increase the reducing capacity of the medium, since these protective agents have this characteristic. In the step involving nucleation, the synergistic effect promoted by DES with the protective agents favors the complete reduction of the ions to nuclei that subsequently grow to form AgNPs with distinct sizes and shapes (Scheme 1). In this sense, DES is one of the key points for the stability of AgNPs, since the agglomeration effects of the particles are attenuated by the physical isolation of the particles that settle in the cavities formed from the interactions between the DES precursors as previously reported in the literature [15,16].

Finally, Figures 30 (e) and (f) show the SEM images of the MWCNT and AgNPs-films deposited from a DMF and Nafion™ dispersion, respectively. As can be seen, the SEM image certifies the morphology of the used MWCNT. Crossed tubular connections characteristic of this carbonaceous material were evident. Nevertheless, in the AgNPs-DES/MWCNT film, it can be seen that there is a coating on MWCNT, indicating the presence of DES residues, which is in accordance with the literature [15,16].

3.3.7 Electrochemical behaviour of hydroquinone and stability study at the proposed sensor

Electrochemical studies using H₂Q were carried out under conditions similar to those proposed by Augusto et al. [29] regarding the supporting electrolyte and pH conditions. Initially, the electrochemical behaviors of H₂Q at the bare and modified GCEs were evaluated by cyclic voltammetry (CV) (Figure 32).

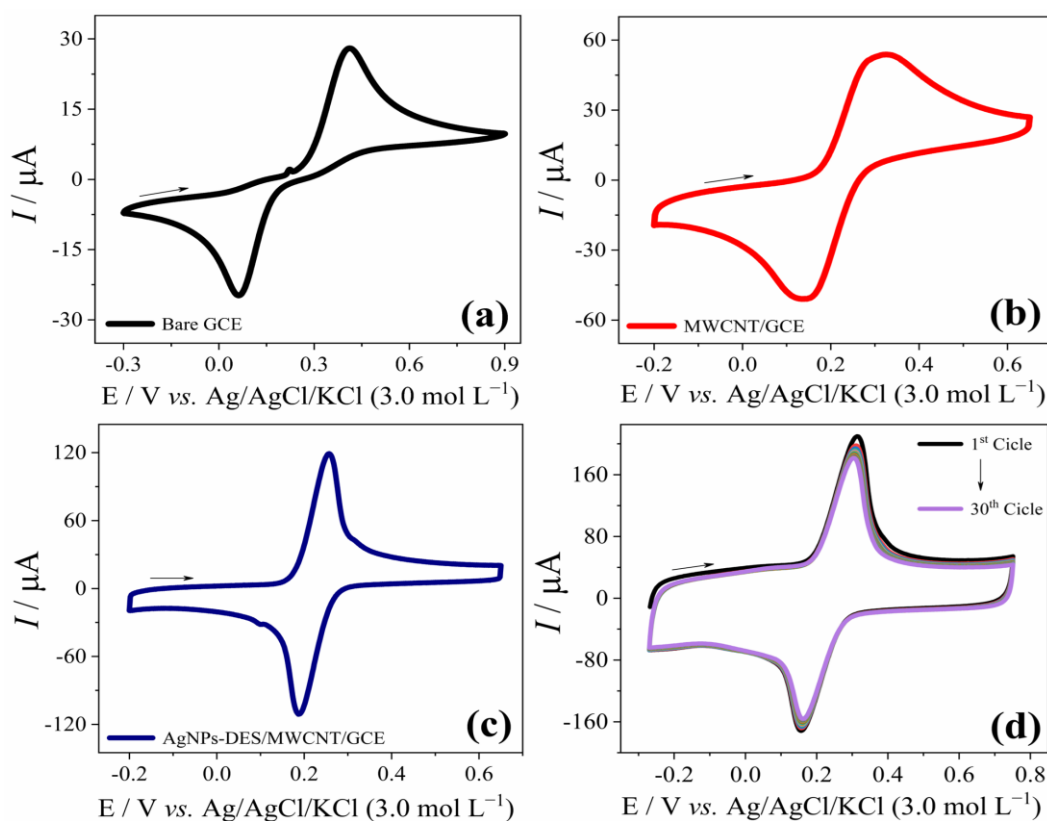


Figure 32. Cyclic voltammograms obtained for 5.0×10^{-3} mol L⁻¹ H₂Q in 0.1 mol L⁻¹ acetate buffer solution (pH 4.7) using different working electrodes: **(a)** bare CGE, **(b)** MWCNT/GCE, and **(c)** AgNPs-DES/MWCNT/GCE, with $\nu = 50$ mV s⁻¹. **(d)** Cyclic voltammograms obtained for 1.0×10^{-3} mol L⁻¹ H₂Q in the sensor stability study using the AgNPs-DES/MWCNT/GCE sensor. Other conditions: $\nu = 50$ mV s⁻¹ and supporting electrolyte: 0.1 mol L⁻¹ acetate buffer solution pH 4.7.

Figure 32 illustrates the voltammetric profile of H₂Q on the bare GCE (Fig. 32 (a)), MWCNT/GCE (Fig. 32 (b)), and AgNPs-DES/MWCNT/GCE (Fig. 32 (c)). As observed, well-defined anodic (I_{ap}) and cathodic (I_{cp}) peaks were registered using all the electrodes. The I_{ap}/I_{cp} values indicate an almost reversible behavior for MWCNT/GCE and AgNPs-DES/MWCNT/GCE sensors (Table 26).

Table 27. Anodic peak current (I_{ap}), cathodic peak current (I_{cp}), ratio of the peak currents (I_{ap} / I_{cp}), anodic peak potential (E_{ap}), cathodic peak potential (E_{cp}), peak potential separation (ΔE_p), resistance of the solution (R_s), charge transfer resistance (R_{ct}), and apparent heterogeneous electron-transfer rate constant (k_{app}) obtained from the application GCE and modified electrodes using $1.0 \times 10^{-3} \text{ mol L}^{-1}$ H₂Q in 0.1 mol L^{-1} acetate buffer (pH 4.7)

Electrodes	$I_{ap} / \mu\text{A}$	$I_{cp} / \mu\text{A}$	I_{ap} / I_{cp}	E_{ap} / V	E_{cp} / V	$\Delta E_p / \text{V}$	R_s / Ω	R_{ct} / Ω	k_{app}
Bare GCE	28.0	-24.8	1.13	0.41	0.06	0.35	138	2890	4.6×10^{-5}
MWCNT/GCE	53.8	-50.9	1.06	0.33	0.14	0.19	102	45.4	2.9×10^{-3}
AgNPs-DES/MWCNT/GCE	119.2	-111.1	1.07	0.26	0.19	0.07	181	3.86	3.4×10^{-2}

However, the peak potential separation (ΔE_p) in the H₂Q redox process reveals that the AgNPs-DES/MWCNT/GCE sensor exhibits better electrocatalytic activity, as shown in Table 26. Furthermore, the stability of the H₂Q voltammetric response was also evaluated by CV. After thirty consecutive cycles, the sensor showed a stable response with a signal reduction less than 5 % in the redox process of H₂Q (Table 27). A slightly higher response for the electrooxidation, with 17 % greater than electroreduction, was observed (see Table 27 and Fig. 32 (d)).

Based on the comparative CV studies, it clearly appears that the peak currents increased significantly with the incorporation of the AgNPs synthesized in the DES. This attests to the excellent electrocatalytic activity of the sensor, as the H₂Q voltammetric profiles were more reversible, with lower peak-to-peak potential separation, when compared to bare GCE and MWCNT/GCE sensors (Table 26). Modifications of sensors based on carbonaceous materials together with nanoparticles synthesized in DES have been reported in the literature with significant enhancements in the figures of merit associated with catalytic activities [15,16].

Table 28. Data obtained in the stability study of the AgNPs-DES/MWCNT/GCE sensor for $1.0 \times 10^{-3} \text{ mol L}^{-1} \text{ H}_2\text{Q}$ in 0.1 mol L^{-1} acetate buffer solution (pH 4.7)

Cycle	$I_{\text{ap}} / \mu\text{A}$	$I_{\text{cp}} / \mu\text{A}$	E_{ap} / V	E_{cp} / V
1	210	-172	0.31	0.16
3	194	-166	0.31	0.16
6	189	-163	0.31	0.16
9	186	-160	0.31	0.16
12	184	-158	0.31	0.16
15	183	-157	0.31	0.16
18	182	-157	0.30	0.16
21	182	-156	0.31	0.16
24	182	-156	0.30	0.16
27	182	-156	0.30	0.16
30	182	-156	0.31	0.16
Mean ($\pm$ SD)	187 ($\pm$ 9)	-160 ($\pm$ 5)	0.31 ($\pm$ 0.01)	0.16 ($\pm$ 0.01)
RSD / %	4.6	3.2	1.0	1.4

Electrochemical impedance spectroscopy (EIS) characterization was carried out to investigate the electrochemical behavior of the H_2Q in the bare GCE, MWCNT/GCE, and AgNPs-DES/MWCNT/GCE electrodes. Figure 33 (c) shows the Nyquist diagrams and the proposed equivalent circuits for this electrochemical system. The spectra were adjusted based on the Randles equivalent circuit, consisting of a resistance (assigned to the resistance of the supporting electrolyte solution, R_s) in series with a parallel combination of a constant phase element (CPE), which represents the formation of the electrical double layer as a non-ideal capacitor, and a second resistance (assigned to charge transfer resistance, R_{ct}).

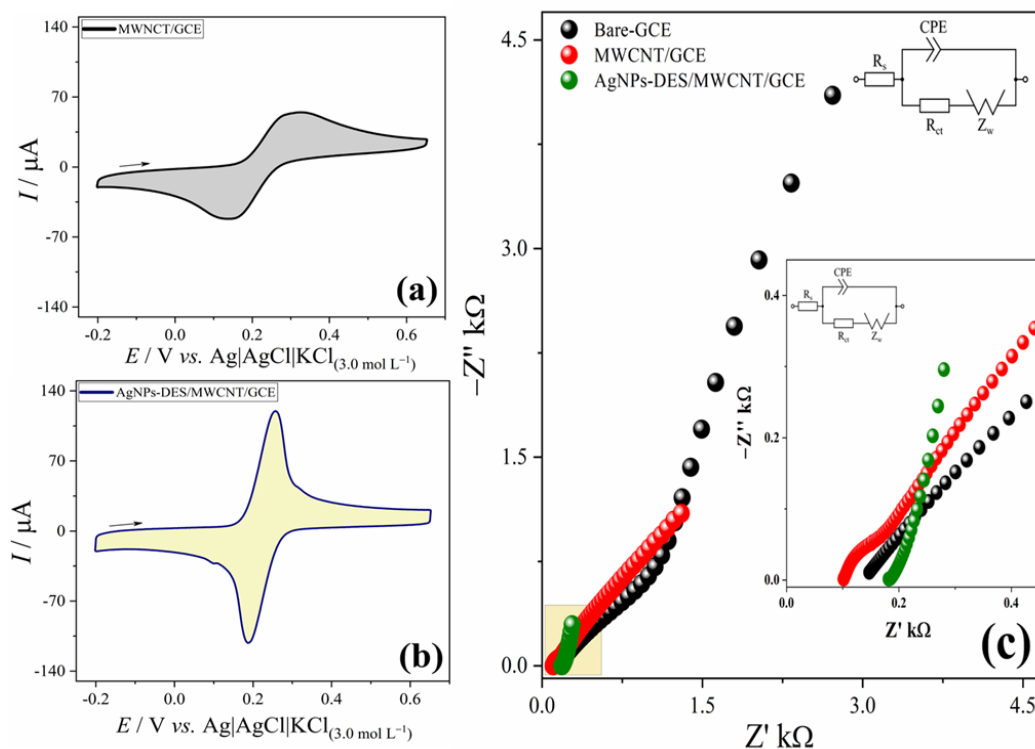


Figure 33. Integrated areas of the cyclic voltammograms obtained for 5.0×10^{-3} mol L⁻¹ H₂Q in 0.1 mol L⁻¹ acetate buffer solution (pH 4.7) using working electrodes: **(a)** MWCNT/GCE (value of area inside the CV, $A = 2.5 \times 10^{-5}$ A V; mass of active material, $m = 12 \pm 0.1$ mg), and **(b)** AgNPs-DES/MWCNT/GCE (value of area inside the CV, $A = 3.3 \times 10^{-5}$ A V; mass of active material, $m = 10 \pm 0.1$ mg), with $v = 50$ mV s⁻¹. **(c)** Nyquist plots for (—) bare-GCE, (—) MWCNT/GCE, and (—) AgNPs-DES/MWCNT/GCE electrodes (Inset: the proposed equivalent circuit). 1.0×10^{-3} mol L⁻¹ H₂Q in 0.1 mol L⁻¹ acetate buffer solution (pH 4.7).

The suggested modifications of the GCE electrode resulted in improved kinetic parameters, as evidenced by the CV results. The sensor's R_{ct} and the apparent heterogeneous electron-transfer rate constant (k_{app} , Equation 4) confirm the performance improvement, of which lower R_{ct} values and higher k_{app} are highlighted, especially with the incorporation of AgNPs (Table 26).

$$k_{app} = \frac{RT}{F^2 R_{ct} C} \quad \text{Eqn. (4)}$$

From equation (5), R represents the molar gas constant (8.314 J mol⁻¹ K⁻¹), T is the thermodynamic temperature (298.15 K), F is the Faraday constant (96485 C mol⁻¹), R_{ct} (Ω) is the charge transfer resistance, and C is the H₂Q concentration (5.0×10^{-8} mol cm⁻³).

The improvements in terms of current magnitude are mainly due to the combined effects of modifying GCE with MWCNT and AgNPs synthesized in DES. The sensor surface also contains eutectic solvent residues (see Fig. 30 (f)) that may also contribute

to the performance. The modification certainly enhanced the conductivity of the electrode, thus facilitating reactions on its surface. Specifically, the k_{app} value increased by approximately 262 times, while the R_{ct} value was 264 times lower when compared to the MWCNT/GCE electrode (see Table 26).

Additionally, the specific faradaic capacitance (C_s) of the AgNPs-DES-based sensor through the H₂Q redox process was determined by CV to attest to its excellent catalytic properties, using equation 5 which results from the proposed model applying CV analysis [47]

$$C_s = \frac{A}{2\Delta V m v} \quad \text{Eqn. (5)}$$

where C_s is the specific faradaic capacitance (F g⁻¹), A is the area inside the cyclic voltammogram (A V), m is the mass of active material (g), v is the scan rate of CV (V s⁻¹), and ΔV is the potential window of CV (total voltage range). The area inside values obtained for the H₂Q CVs are shown in Figure 33 (a and b). The C_s values were 0.2 and 0.4 F g⁻¹ for the MWCNT/GCE and AgNPs-DES/MWCNT sensors, respectively. These values suggest that when AgNPs are incorporated into the sensor, the faradaic responses for H₂Q increase twofold, which denotes electrocatalytic enhancement of the material.

3.3.8 chronoamperometric measurements

The apparent diffusion coefficient (D_{app}) for H₂Q in 0.1 mol L⁻¹ acetate buffer solution (pH 4.7) was determined through chronoamperometry for all sensors. The current–time profiles for electrooxidation were recorded in response to potential steps of 0.35, 0.25, and 0.51 V, respectively, for bare GCE, MWCNT/GCE, and AgNPs-DES/MWCNT/GCE sensors. Different concentrations of analyte (2.5 x 10⁻³ mol L⁻¹ to 1.5 x 10⁻³ mol L⁻¹) were studied (Fig. 34). The faradaic current for the oxidation is related to time^{-1/2}, as predicted by the Cottrell equation (Equation 6)

$$(I \pi^{-1/2} t^{-1/2}) = n F A D^{1/2} C \quad \text{Eqn. (6)}$$

where n is the number of transferred electrons (for H₂Q, the process involves 2 electrons) [29], F is the Faraday constant (96485 C mol⁻¹), A is the electrode area (cm²), and C is the analyte concentration (mol cm⁻³). The plots of I (background corrected current) vs. $t^{-1/2}$ obtained for different H₂Q concentrations are presented in Fig. 34 (a, b, and c). The slopes of the I vs. $t^{-1/2}$ curves were plotted as a function of the H₂Q concentration (mol cm⁻³) and are shown in Fig. 34 (a, b, and c). From the slopes and the Cottrell equation

(Eqn. 6), the values of the apparent diffusion coefficient (D_{app}) for H_2Q were calculated. The experiments were carried out in triplicate and the average values found were $(8.6 \pm 1.0) \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$, $(4.3 \pm 0.2) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, and $(4.1 \pm 0.5) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ for GCE, MWCNT/GCE, and AgNPs-DES/MWCNT/GCE, respectively. These values are in close agreement with those reported for H_2Q in the literature, with values of $2.7 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ in 0.1 mol L^{-1} tetra-*n*-butylammonium perchlorate (TBAP) [48] and $3.5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ in $0.1 \text{ mol L}^{-1} \text{ HClO}_4$ solution [49] for GCE; $(5.2 \pm 0.2) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ in $0.5 \text{ mol L}^{-1} \text{ HClO}_4$ solution [50] for platinum disk electrode modified with poly(3,4-ethylenedioxythiophene) (PEDOT) films.

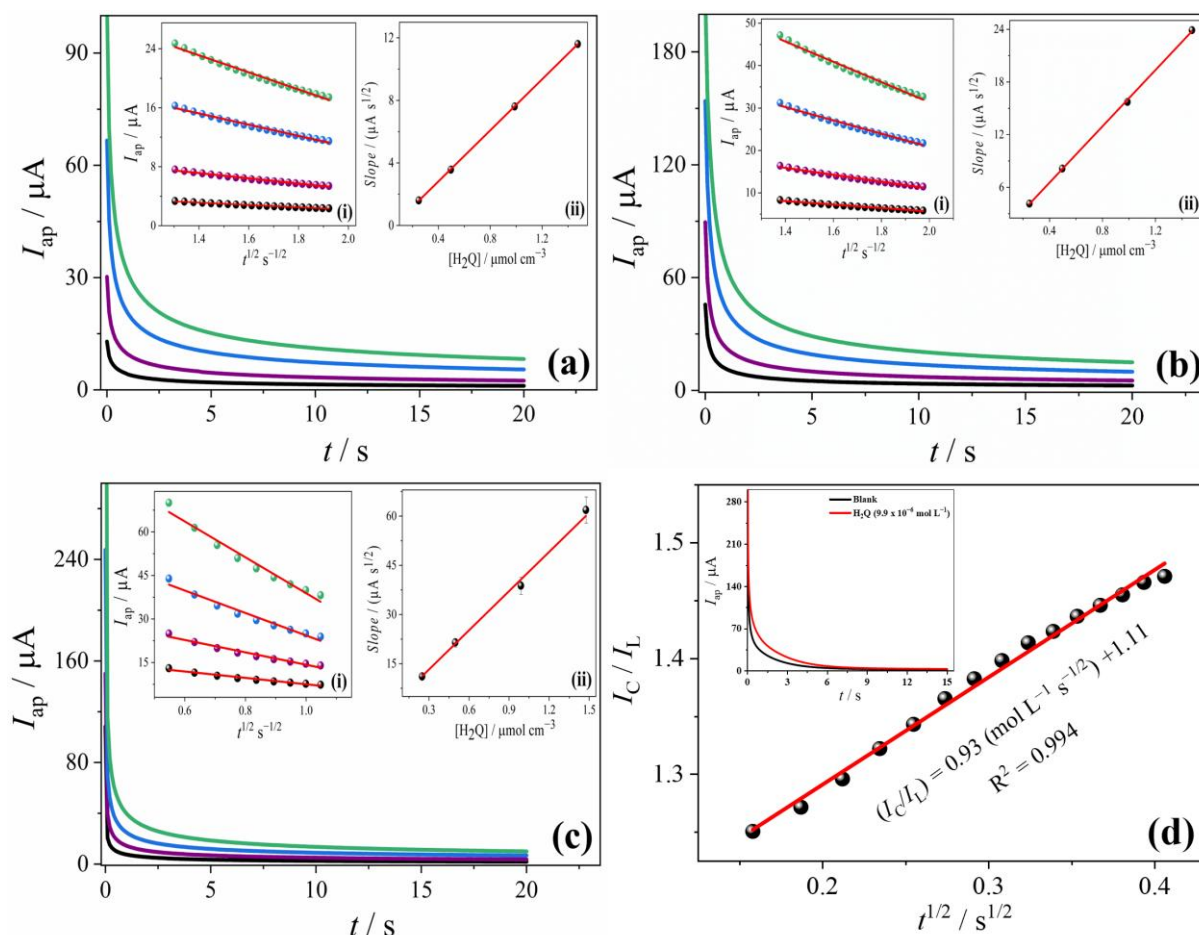


Figure 34. Chronoamperometric i - t profiles of H_2Q at different concentrations obtained at the bare GCE (a), MWCNT/GCE (b), and AgNPs-DES/GCE (c) sensors in 0.1 mol L^{-1} acetate buffer (pH 4.7). The H_2Q concentrations were: (—) 2.5×10^{-4} , (—) 5.0×10^{-4} , (—) 9.9×10^{-4} , and (—) $1.5 \times 10^{-3} \text{ mol L}^{-1}$. Step potential: 0.55 V vs. $\text{Ag}|\text{AgCl}|\text{KCl}$ (3.0 mol L^{-1}). (i) Plots of the background-corrected oxidation current (I_{ap}) vs $t^{-1/2}$. (ii) Plot of the I vs $t^{-1/2}$ slopes vs. H_2Q concentrations for all sensors. (d) Dependence of I_C/I_L on the $t^{1/2}$ obtained of the chronoamperometry data. Inset: the chronoamperograms obtained in 0.1 mol L^{-1} acetate buffer (pH 4.7) in the absence (blank) and $9.9 \times 10^{-5} \text{ mol L}^{-1} \text{ H}_2\text{Q}$ using the AgNPs-DES/MWCNT/GCE.

Additionally, the rate constant for the chemical reaction between hydroquinone and redox sites in the AgNPs-DES/MWCNT film on the GCE surface, k_{cat} could be evaluated by chronoamperometry applied to the Equation (7) [51]:

$$i_{\text{Cat}} / I_{\text{L}} = (\pi k_{\text{cat}} c t)^{1/2} \quad \text{Eqn. (7)}$$

where i_{Cat} is the catalytic current of AgNPs-DES/MWCNT in the presence of H_2Q , I_{L} is the limited current in the absence of H_2Q , c is the bulk concentration of H_2Q ($9.9 \times 10^{-5} \text{ mol L}^{-1}$), k_{cat} and t are the catalytic rate constant ($\text{L mol}^{-1} \text{ s}^{-1}$) and time elapsed (s), respectively. Employing equation (7), k_{cat} was calculated using the slope of the plot $i_{\text{Cat}} / I_{\text{L}}$ vs. $t^{1/2}$ (Fig. 33 (d)), which was $(2.8 \pm 0.2) \times 10^6 \text{ mol}^{-1} \text{ L s}^{-1}$.

3.4 Conclusions

This chapter presented the characterization of a hydrophilic solvent based on choline chloride and diethanolamine through DSC, as well as the proposal of a greener AgNPs synthesis route using the DES developed for potential applications in electrochemical sensors. Based on the $\Delta_m H$ data, the activity coefficient was calculated to demonstrate the “deep” nature of the hydrophilic DES, as well as its eutectic compositions. These results strengthen the importance of fully characterizing all proposed eutectic solvents, as information from the SLE diagram can provide insights on the physical-chemical properties, important to define their applications. The characterization of the DES in its eutectic composition also involved NMR and FT-IR spectroscopy, which highlighted interactions between the precursors through hydrogen interactions. Finally, we demonstrated for the first time a simpler and greener synthesis of AgNPs using ChCl:DEA-based DES as both stabilizing and reducing media, allowing to eliminate the use of conventional reducing agents (i.e, borohydride or hydrazine) and making the approach more environmentally friendly. The AgNPs had an average size of 51.7 nm with cubic crystallographic planes, 75 % of crystallinity, and crystallite sizes 22.5 nm. The synthesized AgNPs were also tested in the development of a MWCNT-based composite sensor to detect H_2Q . The figures of merit demonstrated that the AgNPs synthesized in DES considerably improved the electrocatalytic activities.

References

- [1] A.P. Abbott, G. Capper, D.L. Davies, R.K. Rasheed, V. Tambyrajah, Novel solvent properties of choline chloride/urea mixtures, *Chem. Commun.* (2003) 70–71. <https://doi.org/10.1039/b210714g>.
- [2] E.L. Smith, A.P. Abbott, K.S. Ryder, Deep Eutectic Solvents (DESs) and Their Applications, *Chem. Rev.* 114 (2014) 11060–11082. <https://doi.org/10.1021/cr300162p>.
- [3] L.I.N. Tomé, V. Baião, W. da Silva, C.M.A. Brett, Deep eutectic solvents for the production and application of new materials, *Appl. Mater. Today*. 10 (2018) 30–50. <https://doi.org/10.1016/j.apmt.2017.11.005>.
- [4] A. Mero, S. Koutsoumpou, P. Giannios, I. Stavrakas, K. Moutzouris, A. Mezzetta, L. Guazzelli, Comparison of physicochemical and thermal properties of choline chloride and betaine-based deep eutectic solvents: The influence of hydrogen bond acceptor and hydrogen bond donor nature and their molar ratios, *J. Mol. Liq.* 377 (2023) 121563. <https://doi.org/10.1016/j.molliq.2023.121563>.
- [5] T. Jurić, D. Uka, B.B. Holló, B. Jović, B. Kordić, B.M. Popović, Comprehensive physicochemical evaluation of choline chloride-based natural deep eutectic solvents, *J. Mol. Liq.* 343 (2021). <https://doi.org/10.1016/j.molliq.2021.116968>.
- [6] A. van den Bruinhorst, M. Costa Gomes, Is there depth to eutectic solvents?, *Curr. Opin. Green Sustain. Chem.* 37 (2022) 100659. <https://doi.org/10.1016/j.cogsc.2022.100659>.
- [7] S.L. Perkins, P. Painter, C.M. Colina, Experimental and computational studies of choline chloride-based deep eutectic solvents, *J. Chem. Eng. Data*. 59 (2014) 3652–3662. <https://doi.org/10.1021/je500520h>.
- [8] E.S.C. Ferreira, I. V. Voroshylova, N.M. Figueiredo, C.M. Pereira, M.N.D.S. Cordeiro, Computational and experimental study of propeline: A choline chloride based deep eutectic solvent, *J. Mol. Liq.* 298 (2020) 111978. <https://doi.org/10.1016/j.molliq.2019.111978>.
- [9] P. Dave, A. Lohar, R. Kakkar, Exploration of Choline Chloride Based Type II and Type III Deep Eutectic Solvents: A Computational and Experimental Approach, *ChemistrySelect*. 9 (2024) 1–9. <https://doi.org/10.1002/slct.202305215>.
- [10] A. Alhadid, L. Mokrushina, M. Minceva, Design of deep eutectic systems: A simple approach for preselecting eutectic mixture constituents, *Molecules*. 25 (2020) 1–11. <https://doi.org/10.3390/molecules25051077>.
- [11] M.A.R. Martins, S.P. Pinho, J.A.P. Coutinho, Insights into the Nature of Eutectic and Deep Eutectic Mixtures, *J. Solution Chem.* 48 (2019) 962–982. <https://doi.org/10.1007/s10953-018-0793-1>.
- [12] H. Deguchi, Y. Kubota, Y. Yagi, I. Mitani, Y. Imai, M. Tatsumi, N. Watari, T. Hirata, Y. Kameda, Structure of monoethanolamine and diethanolamine carbamates in aqueous solutions determined by high-energy X-ray scattering, *Ind. Eng. Chem. Res.* 49 (2010) 6–13. <https://doi.org/10.1021/ie9009556>.
- [13] Z. Zhao, X. Chen, M.F. Ali, A.A. Abdeltawab, S.M. Yakout, G. Yu, Pretreatment of wheat straw using basic ethanolamine-based deep eutectic solvents for improving enzymatic hydrolysis, *Bioresour. Technol.* 263 (2018) 325–333. <https://doi.org/10.1016/j.biortech.2018.05.016>.
- [14] J. Wang, Z. Zhao, H. Yuan, H. Gao, Y. Nie, Effective Extraction of Keratin from Human Hair under Mild Conditions Using DES Based on Choline Chloride and Ethanolamine, *ACS Sustain. Chem. Eng.* 12 (2024) 534–542. <https://doi.org/10.1021/acssuschemeng.3c06622>.

- [15] P.C. Gomes-Junior, K.K. de Lima Augusto, G.P. Longatto, R. de Oliveira Gonçalves, T.A. Silva, É.T.G. Cavalheiro, O. Fatibello-Filho, Ultrasmall platinum nanoparticles synthesized in reline deep eutectic solvent explored towards the voltammetric sensing of riboflavin in beverages and biological fluids, *Sensors Actuators B Chem.* 395 (2023). <https://doi.org/10.1016/j.snb.2023.134489>.
- [16] P.C. Gomes-Junior, G.P. Longatto, K.K. de Lima Augusto, J. da Silveira Rocha, E. Piccin, O. Fatibello-Filho, Synthesis of ultrasmall cerium oxide nanoparticles in deep eutectic solvent and their application in an electrochemical sensor to detect dopamine in biological fluid, *Microchim. Acta.* 191 (2024). <https://doi.org/10.1007/s00604-024-06480-4>.
- [17] P. Anastas, N. Eghbali, *Green Chemistry : Principles and Practice*, Chem. Soc. Rev. (2010) 301–312. <https://doi.org/10.1039/b918763b>.
- [18] G. Saravanan, S. Mohan, Structure, composition and corrosion resistance studies of Co-Cr alloy electrodeposited from deep eutectic solvent (DES), *J. Alloys Compd.* 522 (2012) 162–166. <https://doi.org/10.1016/j.jallcom.2012.01.140>.
- [19] P. Cojocar, L. Magagnin, E. Gomez, E. Vallés, Using Deep Eutectic Solvents to electrodeposit CoSm films and nanowires, *Mater. Lett.* 65 (2011) 3597–3600. <https://doi.org/10.1016/j.matlet.2011.08.003>.
- [20] L. Adhikari, N.E. Larm, N. Bhawawet, G.A. Baker, Rapid Microwave-Assisted Synthesis of Silver Nanoparticles in a Halide-Free Deep Eutectic Solvent, *ACS Sustain. Chem. Eng.* 6 (2018) 5725–5731. <https://doi.org/10.1021/acssuschemeng.8b00050>.
- [21] J. Zhang, J. Chen, Q. Li, Microwave heating synthesis and formation mechanism of chalcopyrite structured CuInS₂ nanorods in deep eutectic solvent, *Mater. Res. Bull.* 63 (2015) 88–92. <https://doi.org/10.1016/j.materresbull.2014.11.046>.
- [22] S.D. Çalhan, M.Ö. Alaş, M. Aşık, F.N.D. Kaya, R. Genç, One-pot synthesis of hydrophilic and hydrophobic fluorescent carbon dots using deep eutectic solvents as designer reaction media, *J. Mater. Sci.* 53 (2018) 15362–15375. <https://doi.org/10.1007/s10853-018-2723-4>.
- [23] J.N. Baby, B. Sriram, S.F. Wang, M. George, Effect of Various Deep Eutectic Solvents on the Sustainable Synthesis of MgFe₂O₄ Nanoparticles for Simultaneous Electrochemical Determination of Nitrofurantoin and 4-Nitrophenol, *ACS Sustain. Chem. Eng.* 8 (2020) 1479–1486. <https://doi.org/10.1021/acssuschemeng.9b05755>.
- [24] A. Abo-hamad, M. Hayyan, M. Abdulhakim, M. Ali, Potential applications of deep eutectic solvents in nanotechnology, *Chem. Eng. J.* 273 (2015) 551–567. <https://doi.org/10.1016/j.cej.2015.03.091>.
- [25] O. Pryshchepa, P. Pomastowski, B. Buszewski, Silver nanoparticles: Synthesis, investigation techniques, and properties, *Adv. Colloid Interface Sci.* 284 (2020) 87–100. <https://doi.org/10.1016/j.cis.2020.102246>.
- [26] Y. Liu, S. Kang, K. Li, J. Chen, B. Bae, I. Hwang, E.Y. Ahn, Y. Park, K.H. Chun, J. Lee, Ecofriendly and enhanced biogenic synthesis of silver nanoparticles using deep eutectic solvent-based green tea extracts, *J. Clean. Prod.* 379 (2022). <https://doi.org/10.1016/j.jclepro.2022.134655>.
- [27] F.J.V. Gomez, M. Espino, M. de los Angeles Fernandez, J. Raba, M.F. Silva, Enhanced electrochemical detection of quercetin by Natural Deep Eutectic Solvents, *Anal. Chim. Acta.* 936 (2016) 91–96. <https://doi.org/10.1016/j.aca.2016.07.022>.
- [28] Y.S. Lu, W.Y. Pan, T.C. Hung, Y.T. Hsieh, Electrodeposition of silver in a ternary deep eutectic solvent and the electrochemical sensing ability of the Ag-modified electrode for nitrofurazone, *Langmuir.* 36 (2020) 11358–11365. <https://doi.org/10.1021/acs.langmuir.0c02213>.

- [29] K.K. de L. Augusto, G.R. Piton, P.C. Gomes-Junior, G.P. Longato, F.C. de Moraes, O. Fatibello-Filho, Enhancing the electrochemical sensitivity of hydroquinone using a hydrophobic deep eutectic solvent-based carbon paste electrode †, *Anal. Methods*. 14 (2022) 2003–2013. <https://doi.org/10.1039/d2ay00473a>.
- [30] R. Svigelj, F. Zanette, R. Toniolo, Electrochemical Evaluation of Tyrosinase Enzymatic Activity in Deep Eutectic Solvent and Aqueous Deep Eutectic Solvent, *Sensors*. 23 (2023). <https://doi.org/10.3390/s23083915>.
- [31] R. Dehdari Vais, H. Yadegari, N. Sattarahmady, H. Heli, An anodized nanostructure of Ni/Cu alloy synthesized in ethaline for electrocatalytic oxidation and amperometric determination of L-carnitine, *J. Electroanal. Chem.* 815 (2018) 134–142. <https://doi.org/10.1016/j.jelechem.2018.03.017>.
- [32] X. Liang, Y. Zhou, J.M.S. Almeida, C.M.A. Brett, A novel electrochemical acetaminophen sensor based on multiwalled carbon nanotube and poly(neutral red) modified electrodes with electropolymerization in ternary deep eutectic solvents, *J. Electroanal. Chem.* 936 (2023) 117366. <https://doi.org/10.1016/j.jelechem.2023.117366>.
- [33] R. Craveiro, I. Aroso, V. Flammia, T. Carvalho, M.T. Viciosa, M. Dionísio, S. Barreiros, R.L. Reis, A.R.C. Duarte, A. Paiva, Properties and thermal behavior of natural deep eutectic solvents, *J. Mol. Liq.* 215 (2016) 534–540. <https://doi.org/10.1016/j.molliq.2016.01.038>.
- [34] M.H. Zainal-Abidin, M. Hayyan, A. Hayyan, N.S. Jayakumar, New horizons in the extraction of bioactive compounds using deep eutectic solvents: A review, *Anal. Chim. Acta*. 979 (2017) 1–23. <https://doi.org/10.1016/j.aca.2017.05.012>.
- [35] M. Jablonsky, V. Majova, K. Ondrigova, J. Sima, Preparation and characterization of physicochemical properties and application of novel ternary deep eutectic solvents, *Cellulose*. 26 (2019) 3031–3045. <https://doi.org/10.1007/s10570-019-02322-2>.
- [36] K.A. Omar, R. Sadeghi, Physicochemical properties of deep eutectic solvents: A review, *J. Mol. Liq.* 360 (2022). <https://doi.org/10.1016/j.molliq.2022.119524>.
- [37] I.M. Aroso, A. Paiva, R.L. Reis, A. Rita, C. Duarte, Natural deep eutectic solvents from choline chloride and betaine – Physicochemical properties, *J. Mol. Liq.* 241 (2017) 654–661. <https://doi.org/10.1016/j.molliq.2017.06.051>.
- [38] R.L. Collin, Polymorphism and Radiation Decomposition of Choline Chloride, *J. Am. Chem. Soc.* 79 (1957) 6086. <https://doi.org/10.1021/ja01579a064>.
- [39] I. Adeyemi, M.R.M. Abu-Zahra, I.M. AlNashef, Physicochemical properties of alkanolamine-choline chloride deep eutectic solvents: Measurements, group contribution and artificial intelligence prediction techniques, *J. Mol. Liq.* 256 (2018) 581–590. <https://doi.org/10.1016/j.molliq.2018.02.085>.
- [40] G. Murshid, F.S. Mjalli, J. Naser, S. Al-Zakwani, A. Hayyan, Novel diethanolamine based deep eutectic mixtures for carbon dioxide (CO₂) capture: synthesis and characterisation, *Phys. Chem. Liq.* 57 (2019) 473–490. <https://doi.org/10.1080/00319104.2018.1491043>.
- [41] V. Kocí, J. Sesták, R. Cerný, Thermal inertia and evaluation of reaction kinetics: A critical review, *Measurement*. 198 (2022). <https://doi.org/10.1016/j.measurement.2022.111354>.
- [42] D.O. Abranches, J.A.P. Coutinho, Everything You Wanted to Know about Deep Eutectic Solvents but Were Afraid to Be Told, *Annu. Rev. Chem. Biomol. Eng.* 14 (2023) 141–163. <https://doi.org/10.1146/annurev-chembioeng-101121-085323>.
- [43] M. Dračinský, The Chemical Bond: The Perspective of NMR Spectroscopy, in: *Annu. Reports NMR Spectrosc.*, 2017: pp. 1–40. <https://doi.org/10.1016/bs.arnmr.2016.07.001>.
- [44] P. Aiswarya, T. Jayavarthanam, S. Periandy, S. Suresh, S. Soundharya, Molecular structural analysis, conformers and spectral (FT-IR, FT-Raman, NMR and UV-Visible),

- Importance of solvent role in molecular, ADME and molecular docking investigation on alpha-cyano-4-hydroxycinnamic acid, *Chem. Phys. Impact.* 7 (2023) 100353. <https://doi.org/10.1016/j.chphi.2023.100353>.
- [45] S. Mukherji, S. Bharti, G. Shukla, S. Mukherji, Synthesis and characterization of size-And shape-controlled silver nanoparticles, *Phys. Sci. Rev.* 4 (2019) 1–73. <https://doi.org/10.1515/psr-2017-0082>.
- [46] S. Gómez, N.M. Rendtorff, E.F. Aglietti, Y. Sakka, G. Suarez, Intensity of sulfonitric treatment on multiwall carbon nanotubes, *Chem. Phys. Lett.* 689 (2017) 135–141. <https://doi.org/10.1016/j.cplett.2017.10.020>.
- [47] A.W. Anwar, A. Majeed, N. Iqbal, W. Ullah, A. Shuaib, U. Ilyas, F. Bibi, H.M. Rafique, Specific Capacitance and Cyclic Stability of Graphene Based Metal/Metal Oxide Nanocomposites: A Review, *J. Mater. Sci. Technol.* 31 (2015) 699–707. <https://doi.org/10.1016/j.jmst.2014.12.012>.
- [48] X. Ji, C.E. Banks, D.S. Silvester, A.J. Wain, R.G. Compton, Electrode kinetic studies of the hydroquinone-benzoquinone system and the reaction between hydroquinone and ammonia in propylene carbonate: Application to the indirect electroanalytical sensing of ammonia, *J. Phys. Chem. C.* 111 (2007) 1496–1504. <https://doi.org/10.1021/jp066704y>.
- [49] J. Zhang, A. Zhou, Q. Xie, W. Wei, A long path length spectroelectrochemical behavior analysis of the coabsorption system, *Talanta.* 52 (2000) 903–908. [https://doi.org/10.1016/S0039-9140\(00\)00471-9](https://doi.org/10.1016/S0039-9140(00)00471-9).
- [50] I.C. Monge-Romero, M.F. Suárez-Herrera, Electrocatalysis of the hydroquinone/benzoquinone redox couple at platinum electrodes covered by a thin film of poly(3,4-ethylenedioxythiophene), *Synth. Met.* 175 (2013) 36–41. <https://doi.org/10.1016/j.synthmet.2013.04.027>.
- [51] Z. Galus, Fundamentals of electrochemical analysis, Ellis Horwood ; Halsted Press, a division of Wiley, Chichester, New York, 520 p., 1976. <https://doi.org/LK> - <https://worldcat.org/title/2074368>.

Chapter IV

Conclusions and Perspectives

Chapter IV || Conclusions and Perspectives

This doctoral thesis presented the development of studies on metallic NPs synthesis in deep eutectic solvents and subsequent applications of these particles to electrochemical sensors for voltammetric determinations of some analytes in simulated biological samples and energy drinks.

In summary, chapter one presented an overview of the trends and potential of DES applications. In particular, some strategies for developing NP syntheses using these solvents and how to use DES in electroanalysis, whether as electrolyte additives, binders or modifying agents. It was emphasized that there is a need to study the properties in more depth, aiming at the applications of these DES in general, especially the thermal characterizations to attest their eutectic nature using the differential scanning calorimetry technique. This can be quite useful for creating bases on the “depth” of these solvents from thermodynamic data.

Considering these aspects, the other physical and chemical properties were also highlighted in the first part of the thesis, which provided evidence that the system can be useful for certain applications. The water content, for example, impacts all other properties, since the hydrogen bonds established between the precursors of these solvents are strongly affected by water. Furthermore, it is already established in the literature that solvents are key points in the synthesis of NPs. In the special case of using a DES as a reaction medium and/or stabilizer, the water content can be a factor that alters the morphology of the NPs. Furthermore, these characteristics and the classical descriptions of DES, such as preparation methods, were briefly addressed.

In the second chapter, the syntheses of ultrasmall platinum and cerium oxide nanoparticles were performed for the first time using DES as a stabilizing medium. This yielded a simple, greener and cheaper procedure compared to those traditionally reported in the literature, that use the bottom-up approach and based on the mild solvothermal method ($< 100\text{ }^{\circ}\text{C}$). The generated USNPs presented sizes of 0.204 nm (2.4 Å) and 0.73 nm (7.3 Å) for platinum and cerium, respectively. In both works, the DES based on ChCl:U in the molar ratio of 1:2 (*mol:mol*) acted as a protective agent, preventing the aggregation effect, especially in the nucleation and growth stages. The DES promoted an electrical insulation of these particles in their cavities that led them to extraordinary small sizes that were confirmed in the characterizations by transmission electron microscopy. Moreover, these USNPs were applied for the first time to develop electrochemical sensors

for the voltammetric determination of riboflavin and dopamine, using the USPtNPs and USCeOxNPs, respectively.

In both applications, the sensors presented significantly improved the faradaic responses. For riboflavin, for example, the observed current magnitudes were higher when the USPtNPs were incorporated with MWCNT, corresponding to a 3-fold increase in relation to the sensor modified only with MWCNT. The increase was even higher when the responses were compared with the sensor based on PtNPs synthesized via hydrothermal (conventional) with MWCNT. These results showed that these USPtNPs allowed better electron transfer processes. And this was clear with the other figures of merit related to the determination of riboflavin, as the sensor exhibited a linear response range of 2×10^{-8} to 1.2×10^{-6} mol L⁻¹, with a detection limit of 1.8 nmol L⁻¹, excellent results for repeatability, anti-interference, recoveries from 93 to 108% in synthetic urine and serum samples, and determinations in energy drink samples with errors below 7%. These USPtNPs synthesized pioneeringly within the proposal of this thesis represented the beginning of possible strategies for greener synthesis of USNPs using DES and their use in applications involving sensors.

The work involving the synthesis of USCeOxNPs confirmed the efficiency of this eutectic system in manufacturing USNPs and their application to sensors. The morphological characterizations of USCeOxNPs revealed approximately spherical shapes of the order of 0.73 nm. Dispersive energy emission lines characteristic of cerium and electron diffraction allowed the identification of the presence of cubic crystalline structures, demonstrating that the proposed synthesis was successful. Regarding the sensor developed with USCeOxNPs, it was demonstrated that the modification with these particles together with CB improved the faradaic response of the target analyte. Dopamine was used in this case, which presented linear responses ranging from 5.0×10^{-7} mol L⁻¹ to 3.2×10^{-4} mol L⁻¹, with a detection limit of 80 nmol L⁻¹, good repeatability and anti-interference results, and recovery in synthetic urine samples between 93 and 105%.

Finally, the third chapter presented a complete study involving the development and characterization of a new DES based on ChCl:DEA, for subsequent application of the composition at the eutectic point in the synthesis of silver NPs. These were used for developing a sensor to detect hydroquinone. Regarding the thermal characterization, not all of the molar fractions of the precursors studied presented a thermal event associated with melting. Only the molar fractions $X_{0.90}$, $X_{0.80}$, $X_{0.75}$, $X_{0.73}$, and $X_{0.67}$ presented melting events and, in fact, are eutectic mixtures considering that the T_m were below 296.1 K (T_m

for DEA), while only glass transition events were observed in $X_{0.70}$ and $X_{0.50}$. For the eutectic compositions, the eutectic point was determined in the fraction of $X_{0.75}$, which represents the 1:3 molar ratio of ChCl:DEA. The results obtained for the activity coefficient (γ) calculations suggest that only fractions $X_{0.90}$ and $X_{0.75}$ are deep eutectic systems ($\gamma < 1$), $X_{0.80}$ and $X_{0.73}$ are eutectic systems that tend to ideality ($\gamma \rightarrow 1$) and $X_{0.67}$ (1:2, ChCl:DEA) is just a simple eutectic mixture.

Furthermore, basic characterizations such as water content, conductivity and viscosity were performed. The results for the system are similar to those normally reported for ChCl-based DES that present high viscosities, low conductivities and water content $< 1\%$. Additionally, molecular NMR and FT-IR analyses for the eutectic point composition allowed the identification of possible hydrogen bonds between the precursors, since the proton signals of the methylene 1' and methylene 3' groups shifted to lower fields of 3.41 and 3.13 ppm in the NMR spectra. The absorption bands were observed between $3394 - 3100 \text{ cm}^{-1}$, suggesting the presence of intermolecular interactions of the NH—OH and OH—OH type established between the precursors.

The DES prepared in the eutectic point composition was applied in the green synthesis of AgNPs under mild conditions ($< 100^\circ\text{C}$) and without the addition of conventional reductant (borohydride and hydrazine). DES enhanced the reducing effect together with the mixture of citric acid and citrate (protective agents), as well as stabilized and isolated the AgNPs in their cavities formed between the precursors bonds. The morphological characterizations of the AgNPs confirmed the success of this synthesis. The particles presented distinct shapes with average sizes of 22.5 nm, diffraction peaks characteristic of the planes for cubic structure and a degree of crystallinity of 75%. Regarding the sensor, the incorporation of AgNPs improved the voltammetric profile of the target analyte (hydroquinone) compared to the sensor modified only with MWCNT, the k_{app} value increased by 11.7 times, R_{ct} reduced by 11.8 times, faradaic capacitance was 2 times higher with AgNPs (0.4 F g^{-1}) and the catalytic constant was in the order of $(2.8 \pm 0.2) \times 10^6 \text{ mol}^{-1} \text{ L s}^{-1}$. All these characteristics demonstrated that AgNPs synthesized in DES improved the electrocatalytic properties of the sensor.

Based on the conclusions presented in this doctoral research, it is evident that DES can be used to synthesize NPs through more environmentally friendly routes, as established in the principles of green chemistry. These solvents, by themselves, already meet some principles (atom economy and safe and biodegradable solvent) and can be tuneable for applications such as those presented in this thesis. It is important to

emphasize that some variables need to be investigated/tested in future work, namely: new precursors with the potential to reduce metals, the effect of water content in the synthesis, especially in hydrophilic systems, post-synthesis stability of NPs, solvent volume ratio (DES): metal mass: reducer, and the effect of temperature. This can help expand the knowledge of the potential of this solvent in the synthesis of NPs for future applications.

Curriculum Summary || Súmula Curricular

During the Ph.D. in Chemistry (March 2021 to February 2025), it was studied the use of deep eutectic solvents in the synthesis of metallic nanoparticles and their applications to composite sensors for analyte detection in biological and food samples. Thus, some innovations were discovered and then presented at national or international meetings, as well as publications of first-authored articles and collaborations that derived from this process, all of them in well qualified periodicals. Some of these works are highlighted below. It is important to emphasize that the research was carried out with a scholarship granted by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) under process 88887.597443/2021-00, valid from 01/2021 to 12/31/2024. I am sincerely grateful to this funding institution for its support.

Works presented at meetings

I. National meetings

1º Encontro do INCT Nanovida – Curitiba/PR, Brasil. Título do trabalho: *“Elaboração de sensor eletroquímico com nanopartículas de ouro sintetizadas em solvente eutético profundo”*. Novembro de 2024. Apresentação de Pôster.

Autores: Karen Kenlderi de Lima Augusto; Paulo Cardoso Gomes-Júnior, Gustavo Patelli Longatto e Orlando Fatibello-Filho

1º Encontro do INCT Nanovida – Curitiba/PR, Brasil. Título do trabalho: *“Solvente eutético profundo hidrofóbico: caracterização do ponto eutético e modificação de eletrodo de pasta de carbono para determinação de diuron”*. Novembro de 2024. Apresentação de Pôster.

Autores: Gustavo Patelli Longatto; Paulo Cardoso Gomes Júnior; Karen Kenlderi de Lima Augusto; Charbel Nagib Mouchrek; Evandro Piccin; Orlando Fatibello-Filho

21º ENQA (Encontro Nacional de Química Analítica) | 9º CIAQA (Congresso Ibero-Americano de Química Analítica) – Belém/PA (Brasil). Work title: *“Electrochemical sensor based on gold nanosheets (AuNSs) synthesized in a green ternary mixture for simultaneous determination of acetaminophen and dopamine”* as a poster presentation. (September 2024). Poster presentation.

Authors: Paulo Cardoso Gomes Junior; Karen Kenlderi de Lima Augusto; Gustavo Patelli Longatto; Evandro Piccin; Éder Tadeu Gomes Cavalheiro; Orlando Fatibello Filho

46ª Reunião Anual da Sociedade Brasileira de Química (46ª RASBQ) – Águas de Lindóia – SP (Brasil). Work title: ***Synthesis of platinum nanoparticles in deep eutectic solvent for application in electrochemical sensors***. May 2023. Poster presentation.

Autores: Paulo Cardoso Gomes Junior, Karen Kenlderi de Lima Augusto, Renan de Oliveira Gonçalves, Gustavo Patelli Longatto, Julio César de Oliveira Almeida, Éder Tadeu Gomes Cavalheiro e Orlando-Fatibello.

46ª Reunião Anual da Sociedade Brasileira de Química (46ª RASBQ) – Águas de Lindóia/SP (Brasil). Work title: ***“Hydrophobic deep eutectic solvent based on decanoic acid and tetrabutylammonium bromide: characterization and evaluation towards electrode modification”***. May 2023. Poster presentation.

Autores: Karen Kenlderi de Lima Augusto, Paulo Cardoso Gomes Junior, Renan de Oliveira Gonçalves, Julio César de Oliveira Almeida, Gustavo Patelli Longatto, Éder Tadeu Gomes Cavalheiro e Orlando Fatibello-Filho.

46ª Reunião Anual da Sociedade Brasileira de Química (46ª RASBQ) – Águas de Lindóia – SP (Brasil). Work title: ***A novel electrochemical sensor based on hydrophobic deep eutectic solvent for 17 β -estradiol detection***. May 2023. Poster presentation.

Autores: Julio César de Oliveira Almeida, Karen Kenlderi de Lima Augusto, Paulo Cardoso Gomes Junior, Renan de Oliveira Gonçalves, Gustavo Patelli Longatto, Éder Tadeu Gomes Cavalheiro e Orlando Fatibello-Filho.

46ª Reunião Anual da Sociedade Brasileira de Química (46ª RASBQ) – Águas de Lindóia – SP (Brasil). Work title: ***Development of electrochemical sensors with metallic nanoparticles synthesized in deep eutectic solvents***. May 2023. Poster presentation.

Autores: Gustavo Patelli Longatto, Paulo Cardoso Gomes Junior, Karen Kenlderi de Lima Augusto, Julio César de Oliveira Almeida, Renan de Oliveira Gonçalves, Orlando Fatibello Filho e Éder Tadeu Gomes Cavalheiro.

II. International meetings

XXVI SIBAE: Sociedade Iberoamericana de Eletroquímica, Lisboa – Portugal. Título: “Eletrodo de carbono vítreo modificado com solvente eutético profundo hidrofóbico para a determinação de serotonina”. Maio de 2024. Comunicação oral.

Autores: Karen K. de Lima Augusto, **Paulo C. Gomes-Júnior**, Gustavo P. Longatto, Elena Bernalte, Craig Banks, Orlando Fatibello-Filho

74th – ISE: Annual Meeting of the International Society of Electrochemistry in Lyon, France. ***Platinum nanoparticles synthesized in hydrophilic deep eutectic solvent for application in electrochemical sensor.*** (September 2023). Poster presentation.

Authors: Orlando Fatibello-Filho, **Paulo Gomes-Júnior**, Karen Augusto, Renan Gonçalves, Júlio Almeida, Gustavo Longatto, Eder Cavalheiro .

74th – ISE: Annual Meeting of the International Society of Electrochemistry in Lyon, France. ***Hydrophobic Deep Eutectic Solvent Based on Decanoic Acid and Tetrabutylammonium Bromide: Characterization and Evaluation Towards Electrode Modification.*** (September 2023). Poster presentation.

Authors: Orlando Fatibello-Filho, Karen Augusto, **Paulo Gomes-Júnior**, Renan Gonçalves, Júlio Almeida, Gustavo Longatto, Eder Cavalheiro .

III. Awards

Award received in the panel session of the 46th RASBQ, Division of Electrochemistry and Electroanalytical, as one of the authors of the work entitled ***Synthesis of platinum nanoparticles in deep eutectic solvent for application in electrochemical sensors.*** (May 2023).

Autores: **Paulo Cardoso Gomes Junior**, Karen Kenlder de Lima Augusto, Renan de Oliveira Gonçalves, Gustavo Patelli Longatto, Julio César de Oliveira Almeida, Éder Tadeu Gomes Cavalheiro e Orlando-Fatibello.

Published scientific articles

ROCHA, JOSIAS S.; PEREIRA, GEORGE B.S.; PALMEIRA-MELLO, MARCOS V.; OLIVEIRA, WILLYAN F.; DUTRA, JOCELY L.; TEIXEIRA, TAMARA; DA SILVA, NÁDIJA N.P.; OLIVEIRA, GABRIELA P.; **GOMES-JUNIOR, PAULO C.**; PASSAES, ANNA C.S.J.; ARAUJO-NETO, JOÃO H.; BATISTA, ALZIR A.; ELLENA, JAVIER; DUTRA, JOSÉ D.L.; FARIAS, RENAN L.; ROCHA, FILLIPE V.

Synthesis, characterization of europium(III) and terbium(III) complexes containing lawsone ligand and their interaction with DNA, HSA, and topoisomerases. JOURNAL OF MOLECULAR STRUCTURE, v. 1333, p. 141790, 2025. <https://doi.org/10.1016/j.molstruc.2025.141790>.

AUGUSTO, KAREN KENLDERI DE LIMA; **GOMES-JUNIOR, PAULO CARDOSO**; LONGATTO, GUSTAVO PATELLI ; PICCIN, EVANDRO ; CAVALHEIRO, ÉDER TADEU GOMES; BERNALTE, ELENA; BANKS, CRAIG E. ; FATIBELLO-FILHO, ORLANDO. Electrochemical sensor based on carbon nanohorns and hydrophobic deep eutectic solvent for the determination of serotonin in biological samples. ELECTROCHIMICA ACTA, v. 520, p. 145836, 2025. <https://doi.org/10.1016/j.electacta.2025.145836>.

GOMES-JUNIOR, PAULO CARDOSO; LONGATTO, GUSTAVO PATELLI; DE LIMA AUGUSTO, KAREN KENLDERI; DA SILVEIRA ROCHA, JOSIAS; PICCIN, EVANDRO; FATIBELLO-FILHO, ORLANDO . Synthesis of ultrasmall cerium oxide nanoparticles in deep eutectic solvent and their application in an electrochemical sensor to detect dopamine in biological fluid. MICROCHIMICA ACTA, v. 191, p. 1, 2024. <https://doi.org/10.1007/s00604-024-06480-4>.

CARDOSO GOMES-JUNIOR, PAULO; DIAS NASCIMENTO, EVAIR; KENLDERI DE LIMA AUGUSTO, KAREN; PATELLI LONGATTO, GUSTAVO; CENSI FARIA, RONALDO; PICCIN, EVANDRO; FATIBELLO-FILHO, ORLANDO. Voltammetric determination of uric acid using a miniaturized platform based on screen-printed electrodes modified with platinum nanoparticles. MICROCHEMICAL JOURNAL, v. 207, p. 111931, 2024. <https://doi.org/10.1016/j.microc.2024.111931>.

GOMES-JUNIOR, PAULO CARDOSO; DE LIMA AUGUSTO, KAREN KENLDERI; LONGATTO, GUSTAVO PATELLI; DE OLIVEIRA GONÇALVES, RENAN; SILVA, TIAGO ALMEIDA; CAVALHEIRO, ÉDER TADEU GOMES; FATIBELLO-FILHO, ORLANDO. Ultrasmall platinum nanoparticles synthesized in reline deep eutectic solvent explored towards the voltammetric sensing of riboflavin in beverages and biological fluids. SENSORS AND ACTUATORS B-CHEMICAL, v. 395, p. 134489, 2023. <https://doi.org/10.1016/j.snb.2023.134489>.

FERREIRA, LUÍS M.C.; SILVA, PATRÍCIA S.; AUGUSTO, KAREN K.L.; **GOMES-JÚNIOR, PAULO C.**; FARRA, SINARA O.D.; SILVA, TIAGO A.; FATIBELLO-FILHO, ORLANDO; VICENTINI, FERNANDO C. Using nanostructured carbon black-based electrochemical (bio)sensors for pharmaceutical and biomedical analyses: A comprehensive review. JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS, v. 221, p. 115032, 2022. <https://doi.org/10.1016/j.jpba.2022.115032>.

AUGUSTO, KAREN KENLDERI DE LIMA; PITON, GABRIELA RIZZO; **GOMES-JÚNIOR, PAULO CARDOSO**; LONGATTO, GUSTAVO PATELLI; DE MORAES, FERNANDO CRUZ; FATIBELLO-FILHO, ORLANDO. Enhancing the electrochemical sensitivity of hydroquinone using a hydrophobic deep eutectic solvent-based carbon paste electrode. Analytical Methods, v. 14, p. 10.1039/d2ay004, 2022. <https://doi.org/10.1039/d2ay00473a>.

Links

ORCID: <https://orcid.org/0000-0001-5551-9887>.

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Scopus: <https://www.scopus.com/authid/detail.uri?authorId=57362663600>.

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Ultrasmall platinum nanoparticles synthesized in reline deep eutectic solvent explored towards the voltammetric sensing of riboflavin in beverages and biological fluids

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