# Impact of natural deep eutectic solvents-based microalgae extracts on the stability and properties of skincare products

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## Abstract

**Background :** Natural deep eutectic solvents (NaDES) are green ionic liquids prepared by mixing vegetal cellular constituents and metabolites such as sugars, polyols, aminoacids or organic acids. Their specific hydrogen-bond network confers high dissolution power combined with stabilizing ability, which make NaDES perfect media for sustainable valuation of vegetal biomasses. Their composition makes the resulting extracts truly biocompatible and then the solvent removal step in extraction processes becomes superfluous.

**Methods:** Our consortium worked on the extraction properties and formulability of a range of 20 hydrophobic and hydrophilic NaDES, compliant to European and Chinese cosmetic regulation. A part of this work, concerning the formulability of a specific hydrophilic NaDES will be described in this article. Typical situations will be illustrated through examples of an *Arthrospira platensis* microalgae extract. The products modifications in terms of organoleptic properties, stability, rheology and sensory properties will be evaluated.

**Results:** Gly:Glu (1:2, mol/mol) NaDES was considered for formulation. Sensory analysis permitted to attest that this NaDES had appropriate properties for a cosmetic use. This NaDES was included in a basic cosmetic gel at concentrations from 1% to 10% wt/wt. This study indicates that this NaDES could be used to prepare bio-based ingredients that can be introduced

up to 10% in gels. Moreover, the introduction of this specific NaDES-based *A. platensis* extracts has a positive impact on the color and the stability of antioxidant molecules and pigments, notably carotenoids and phycocyanin.
**Conclusion**: The results of this study are in favour of the development of the exploration of NaDES as sustainable cosmetic ingredients.

**Keywords**: Natural deep eutectic solvents, skincare, formulation, rheology, sensory analysis

## Introduction

The cosmetics industry is now well underway in its shift towards product eco-design, including sustainable formulas and processes. The preparation of bio-based ingredients has to follow this movement towards sustainability. In complement to agrosolvents like bio-ethanol or dimethyl carbonate, Natural Deep Eutectic Solvents (NaDES), green ionic liquids based on natural metabolites, seem to be interesting candidates to replace conventional organic solvents in the extraction processes of biomasses.

Emerging in the 2010s, NaDES are composed of a hydrogen bond acceptor, usually choline chloride or betaine, and a hydrogen bonding donor, as sugars or polyols. Their association forms a network of hydrogen bonds resulting in the lowering of the melting point of the solids mixture[1,2,3], making the NaDES liquid at room temperature. NaDES are made of vegetal cellular constituent, which make them renewable and biodegradable[3]. Most commonly used metabolites are choline derivatives, sugars, aminoacids, and organic acids. Moreover, their high dissolution power combined with their stabilizing ability make NaDES perfect media for sustainable valuation of vegetal biomasses[2]. Their composition makes also NaDES truly biocompatible allowing to avoid solvent removal step in extraction processes. NaDES were also described as skin penetration enhancers[4], which add to their attractiveness.

Nevertheless, when it comes to work with NaDES, some difficulty can arise concerning their stability, their extraction capacities, their toxicity or their formulability. Through the example of developments in our labs based on a specific NaDES made of glycerol and glucose, named Gly:Glu in the core of this paper, the critical point of the introduction of a NaDES in a cosmetic product will be highlighted to prepare future users of those new ingredients that will soon be widely present on the market.

NaDES made of Gly:Glu at variable molar ratio is a well-known NaDES described in the literature as a very potent NaDES to extract molecules of interest of biomasses[5,6,7]. Notably, NaDES including glucose and glycerol seem very interesting combination to extract antioxidant molecules from plants, like epigallocatechin-3-gallate from green Tea[5]. Studies performed inside our consortium demonstrated that Gly:Glu (1:2) NaDES was a solvent of choice to extract from microalgae *A. platensis* and stabilize two families of molecules interesting for their cosmetic and colour properties: carotenoids and phycobiliproteins[6,7]. We also demonstrated its absence of toxicity on skin cells in culture[7]. To our knowledge, the compatibility of Gly:Glu (1:2), 20% water with cosmetic products formulas like gel, creams, lotions etc was not published yet.

## 1. Materials and Methods

*NaDES preparation*: the NaDES used contains glucose as hydrogen bond acceptor and glycerol as a hydrogen bond donor (1:2 molar ratio). The eutectic point was reached by stirring and heating the mixture of raw materials at 80°C until a homogeneous colourless phase was formed. Water was added to reduce the viscosity of the system (20% wt/wt) and obtain a consistency compatible with extraction.

*A. platensis extract preparation and characterization:* Frozen biomass of *A. platensis* was put in contact with NaDES using ultrasonic assisted extraction during 30 min. The resulting mixture was centrifuged and supernatant was recovered. Solid residue was extracted two more times and supernatant were merged. To compare the extract properties with the gels containing 1% of extract, a dilution 1:100 in water was performed. The solution obtained is named SR (for solution of reference) in the rest of the document.

*Topical gels:* Model cosmetic formulations based on COSMOS ingredients were prepared in triplicate as 200g batches with a bench mixer equipped with a dispersing turbine (Turbotest ®, VMI, France). Gels were composed of ultrapure water, antimicrobial agents (benzyl alcohol and dehydroacetic acid, 0.3% wt/wt in the final product) and a thickening agent (sodium carboxymethylcellulose, 3% wt/wt in the final product). The NaDES was introduced at concentrations relevant with their use as an active ingredient in topical formulations, from 1 to 10% wt/wt. A gel without Gly:Glu was prepared as control. This gel is named RF (for reference) in the rest of the document. The extract was included in the gel at 1% wt/wt.

*Rheology study*: A Kinexus pro+ rheometer was used for the measurements (Netzsch, Germany). Analyses were performed at 25°C, with a gap of 0.1 mm and a textured plate-plate mobile of 4 cm of diameter. A study of the viscosity as function of the shear rate was performed between 0.1 and 10000 $s^{-1}$. Gly:Glu NaDES was analysed 72h after preparation while topical gels were observed 72h after preparation and after a stay of 30 days in accelerated aging conditions (40°C, 75% humidity, dark) to evaluate the stability of the systems.

*pH measurements:* pH was measured with a Eutec pHmeter with a an electrode specifically designed for viscous products, at 25°C.

*Colorimetry:* Color measurements were performed with a handheld chromameter C400 (Konika Minolta, Tokyo, Japan). All the measurements were performed at room temperature with the specific accessory provided to measure the color of transparent systems.

*Pigments determination:* Titrations of phycocyanin, carotenoids and chlorophyll were carried on the samples after appropriate dilution by UV-vis spectroscopy using a microplate reader at specific wavelength (Multiskan GO, Thermo Fisher Scientific, SAS)

*Sensory analysis*: Sensory profiles of Glu:Gly and NaDES-loaded gels were performed by a small expert panel (n=4). In case of disagreement within the expert panel, triangle tests were performed on a larger naïve panel (n=20).

## Results and discussion

### 1) Analysis of Gly:Glu NaDES properties

As a first step towards formulation, Gly:Glu sensory characteristics were studied. This NaDES is a translucent, slightly viscous liquid. It shows a slight almond odour which is not unpleasant or unusual in cosmetics. Sensory characteristics of Gly:Glu were further evaluated by an expert panel that build a sensory profile (Figure 1).

This NaDES shows a sticky and thick texture, as well as a low penetration in the skin (forearm), when compared to the other NaDES tested. Those properties force us to limit the concentration of this product in a cosmetic product to ensure a pleasant experience to the consumer. Nevertheless, this is not a real issue here as the NaDES-based ingredients are considered as actives, and will be introduced at low concentration. It was decided to continue the formulability study with concentrations from 1 to 10% wt/wt of Gly:Glu.

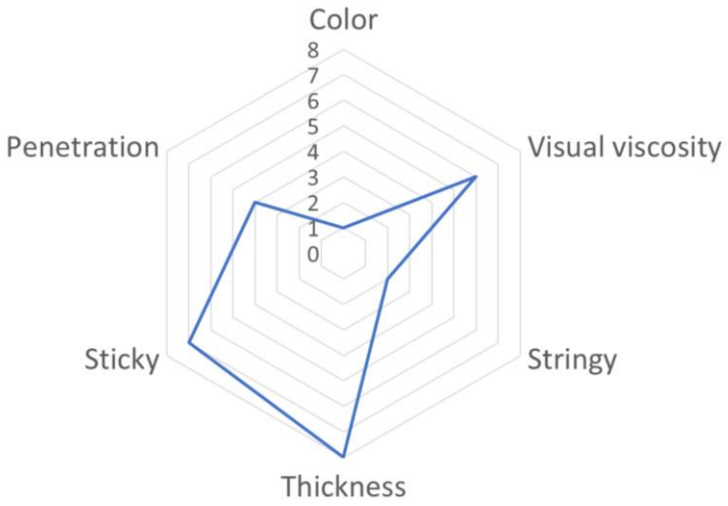


*Figure 1 : Spider profile of Gly :Glu NaDES (the limits of the quantitative scale are determined from the 20 tested NaDES)*

As it contains water, it is possible to measure the pH of this specific NaDES. The obtained values (4.4 ± 0.1) are close to the pH value of the skin (4.5 to 6.5) and do not hinder its introduction in topical formulations. At rest, the product seem slightly viscous, but the rheological measurements show that from low shear-rates, the product loses its viscosity to adopt a newtonian behaviour (Figure 2). As it becomes a liquid with a very low viscosity around 350 mPa.s from a shear rate around 0.2 $s^{-1}$, this NaDES will easily be introduced in a product with the common mixing tools used in laboratories or industry.

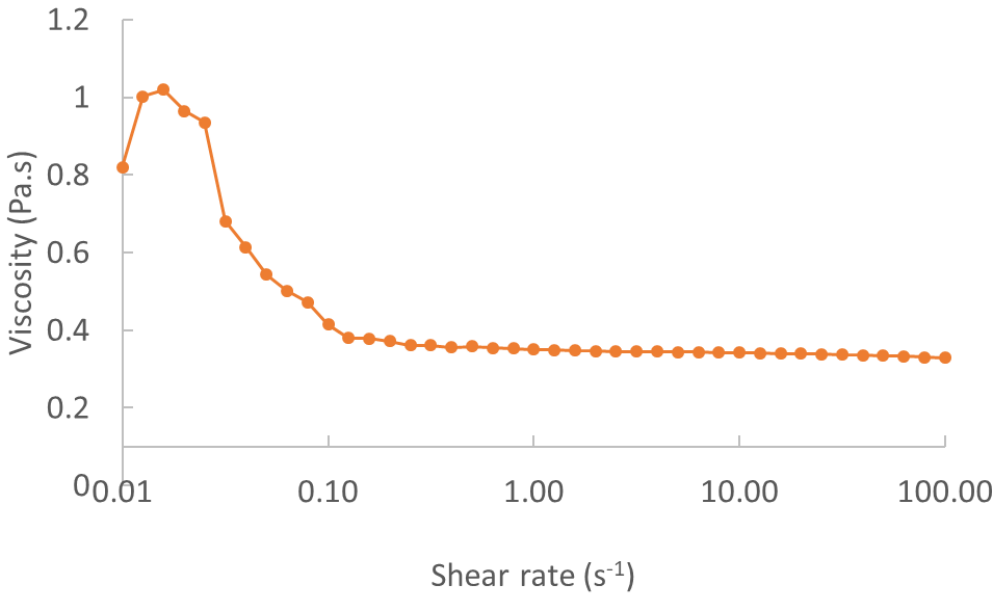


*Figure 2: Rheological behaviour of Gly:Glu (1:2), 20% $H_2O$, at 25°C*

## 2) Analysis of the NaDES-loaded gels

To evaluate the impact of Gly:Glu on the physicochemical characteristics, the stability and the sensoriality of the gels, it was introduced up to 10% wt/wt in the model formulation. The observations will be illustrated by the results obtained with the highest concentration tested, 10% wt/wt of NaDES.

The organoleptic characters of the NaDES-based gel were not different from the reference. The gel was transparent with a few bubbles easily eliminated by a short centrifugation. The pH was around 6, which is compatible with a skin application. The viscosity of the gel was visually higher than the control gel, which seemed to show that a specific hydrogen bond network was created inside the product. In the aim of understanding this phenomenon, a rheological profile of the product was performed and is presented in Figure 3.

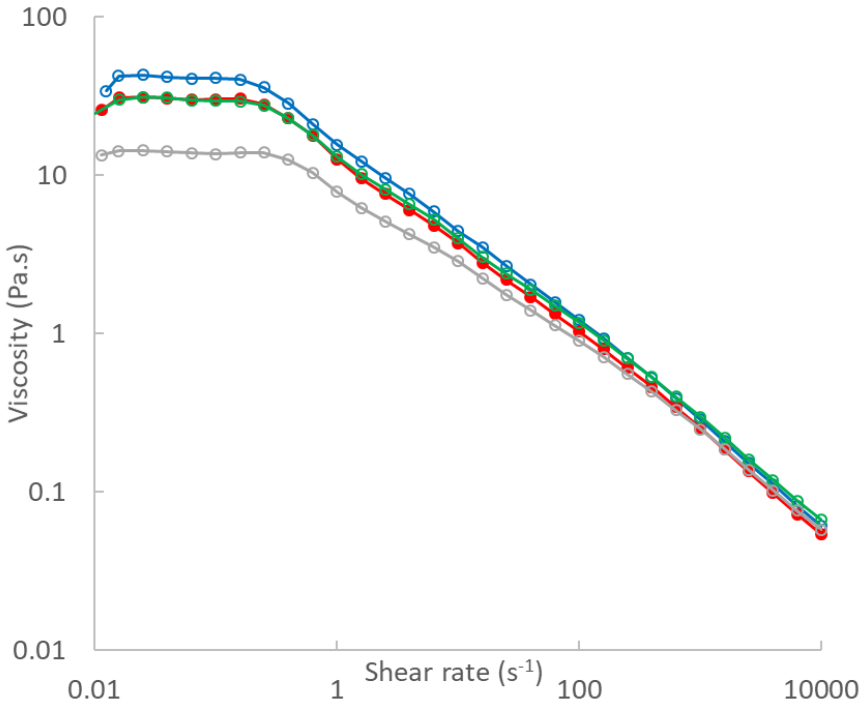


*Figure 3: Rheological behaviour of gels, red: control gel; blue: 10% wt/wt Gly:Glu loaded gel, grey : control gel after 30 days in accelerated aging conditions, green : 10% wt/wt Gly:Glu loaded gel after 30 days in accelerated aging conditions*

The RF shows a shear-thinning behaviour exhibiting a yield point (red curve). The 10% Gly:Glu loaded gel has a similar rheological behaviour (blue curve), but shows a higher yield point (≈ 42 Pa.s and ≈31 Pa.s respectively). This difference in viscosity decreases in the range of the medium shearing rates (from 1 to 1000 $s^{-1}$) to become not significant in the range of the

high shearing rates (from 1000 to 10000 $s^{-1}$). This result sheds light on two important points. Firstly, this rheological behaviour is in good agreement with the existence of a hydrogen bond network that will be broken in the range of the high shearing rate, leading to a drop in viscosity to reach the reference values. Secondly, this results gives us information about the scale-up ability of such formulas. Indeed, the range from medium to high shear rates permits to anticipate the mixing, packaging, spreading *etc*. of a cosmetic product. The shear rates zone around 1000 $s^{-1}$ is particularly interesting as it describes the spreading of the product[8]. On this part of the curve, the difference in viscosity is not significant and close to 30 mPa.s. Therefore, it can be expected that the introduction of 10% of this Gly:Glu NaDES will not have an impact on the spreading that can be detected by the consumer.

After 30 days of storage at 40°C, 75% of humidity in the dark, a decrease of the viscosity of both gels can be observed for the low shear-rates (Figure 3, grey and green curve). This decrease is more marked for the control gel (grey curve) than for the Gly:Glu-loaded gel (green curve). This could be a positive effect of the creation of a hydrogen bond network between the NaDES components and the sodium carboxymethylcellulose.

A sensory analysis was performed to confirm those observations. It also allows to evaluate the modification in the texture that could be induced by the incorporation of this NaDES in the gel. As the expert panel could not find a sensory difference between the two gels, a triangular test was organized with a naive panel of 20 people. This test lead to the same conclusion: even if Gly:Glu shows sticky properties compared to the other 19 NaDES when tested alone, the inclusion of 10% of this NaDES in a gel did not modify the sensory properties of the gel and the panel could not differentiate the RF from the NaDES-loaded gel.

These results encouraged the consortium to observe the behaviour of Gly:Glu –based extracts in cosmetic formulas.

### 3) Analysis of the *A. platensis* extract-loaded gels

The extraction of the *A. platensis* biomass provided a blue-green slightly viscous extract. This extract is rich in phycocyanin, a blue protein, and in chlorophyll (green) and carotenoids (yellow-red) which are co-extracted and present interesting antioxidant properties. This extract is included at 1% wt/wt in the model cosmetic gel. That is why a 1% wt/wt solution of the extract in water was studied as a reference (SR). After 30 days of storage in accelerated aging conditions, the reference solution appear to be unstable (Figure 4) whereas the extract-loaded

gel was still blue-green. Nevertheless, the colour of the gel seemed slightly different than the initial colour of the extract (Figure 4).

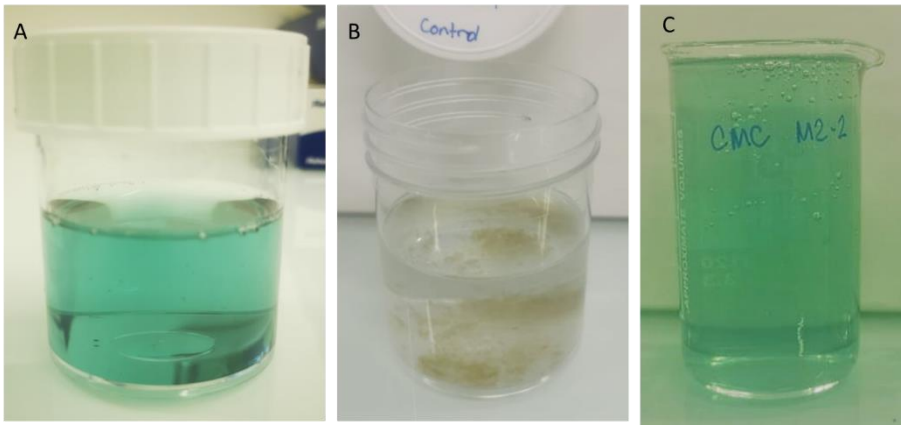


*Figure 4 : A) Initial reference solution of the extract SR, B) SR after 30 days in accelerated aging conditions C) Extract-loaded gel after 30 days in accelerated aging conditions*

A colorimetry study confirmed this observation. The L*a*b values for those three samples are mentioned in Table 1.

*Table 1 : Colorimetry measurements in the CIELAB color space*

| Sample | L | a | b |
|---|---|---|---|
| Reference gel RF, initial | 25.13± 0.01 | 2.78± 0.03 | 0.34± 0.01 |
| Extract Loaded gel, D30<br>*40°C, 75% humidity, dark* | 28.10 ± 0.01 | -0.09 ± 0.03 | -0.33 ± 0.01 |
| Difference (absolute value) | **2.97 , clearer** | **2.97, greener** | **0.67, bluer** |

The parameter L, for lightness, is slightly higher for the gel that SR. It can be linked to the better stabilization of some components in the gel than in water. The parameters a (green to red) and b (blue to yellow) are significantly decreased in the gel, showing that some green and yellow pigments in the wavelengths were degraded in time. It is to notice that the control gel gets yellowish in time (data not shown), which participate to the b value measured a day 30 (D30).

Pigments determinations by UV-Vis spectrometry were performed to understand this colour evolution in time. The reference solution SR showed 24h after its preparation a concentration of 253 ± 20 µg/g of blue phycocyanin, of 6 ± 1 µg/g of yellow-red carotenoids

and of 35 ± 3 µg/g of green chlorophyll, in accordance with the blue-green initial colour of the SR. When the extract is included in the gel, after 30 days in accelerated aging conditions, phycocyanin is well preserved with a concentration of 207 ± 10 µg/g (≈20% of degradation), whereas carotenoids concentration decreases to 3± 1 mg/g (≈50% of degradation) and chlorophyll concentration decreases to 25 ± 5 µg/g (≈29% of degradation). These values are in accordance with a visual impression of a bluer gel. Indeed, the phycocyanin is the more stable coloured molecule in the system. The hypothesis is that carotenoids may have played the role of antioxidants and protected phycocyanin from degradation. This hypothesis is supported by the drop of the antioxidant power of the system, approached by the DPPH assay. The value drops from 25 ± 1 mg/g in the fresh SR to 20 ± 1 mg/g in the gel after 30 days of storage in accelerated aging conditions (20% decrease). This action is reinforced by the presence of the gel components as the SR is not stable at all in time.

These results are particularly promising, as the formula seems to preserve the extract but particularly the natural blue pigment, however this colour is particularly difficult to obtain and stabilize from natural ingredients[7].

## Conclusion

The present article brings together a few results from a larger study concerning the formulability of ingredients based on natural deep eutectic solvents that will be presented in September 2022 at the IFSCC Congress. After we demonstrated that the NaDES Gly:Glu was interesting to extract molecules of interest from spirulina[7], we studied its formulability by introducing it in a model product up to 10% wt/wt. At this high concentration for an active ingredient, it did not modify in a significant manner the consumer's experience with the product. Moreover, it appeared that the composition of this gel favours the stability of interesting blue natural dye phycocyanin. This has to be further studied but could be linked to the hydrogen bond network created in the product between the NaDES ingredients and the thickening agent chosen, sodium carboxymethylcellulose. Those results are in favour of the use of NaDES as new tools for eco-conception of cosmetic ingredients.

**Conflicts of interest**: biomass used for this study was provided by Aqua eco Culture (Lamballe, France).

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**References**

1) Abbott, A.P., Boothby, D., Capper, G., Davies, D.L., Rasheed, R.K., (2004). Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. J. Am. Chem. Soc. 126, 9142–9147.
2) Fernández MLÁ, Boiteux J, Espino M, Gomez FJV, Silva MF (2018) Natural deep eutectic solvents-mediated extractions: The way forward for sustainable analytical developments Anal Chim Acta. 1038:1-10.
3) Liu Y, Friensen JB, McAlpine JB, Lankin DC, Chen SN, Pauli GF (2018) Natural deep eutectic solvents: properties, applications and perspectives, 81(3):679-690.
4) Fernández MLÁ, Boiteux J, Espino M, Gomez FJV, Silva MF (2018) Natural deep eutectic solvents-mediated extractions: The way forward for sustainable analytical developments Anal Chim Acta. 1038:1-10.
5) Jeong K.M., Ko J, Zhao J., Jin Y., Yoo D.E., Young Han S., Lee J. (2017) Multi-functioning deep eutectic solvents as extraction and storage media for bioactive natural products that are readily applicable to cosmetic products, Journal of Cleaner Production, 151, 87-95,
6) Wils L, Leman-Loubière C, Bellin N, Clément-Larosière B, Pinault M, Chevalier S, Enguehard-Gueiffier C, Bodet C, Boudesocque-Delaye L. (2021) Natural deep eutectic solvent formulations for spirulina: preparation, intensification and skin impact. Algal Res 56: 102317.
7) Hilali S., Wils L., Chevalley A., Clément-Larosière B., Boudesocque-Delaye L (2022). Glycerol-based NaDES as green solvents for ultrasound-assisted extraction of phycocyanin from Arthrospira platensis-RSM optimization and ANN modeling. Biomass Conversion and Biorefinery, 12, 157-170.
8) Savary G., Grisel M., and Picard C. (2013) Impact of emollients on the spreading properties of cosmetic products: A combined sensory and instrumental characterization, Colloids and Surfaces B: Biointerfaces, 102, 371-378.