



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Deep eutectic solvent and agar: a new green gel to remove proteinaceous-based varnishes from paintings

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Deep eutectic solvent and agar: a new green gel to remove proteinaceous-based varnishes from paintings / Jia Y.; Sciutto G.; Botteon A.; Conti C.; Focarete M.L.; Gualandi C.; Samori' C.; Prati S.; Mazzeo R.. - In: JOURNAL OF CULTURAL HERITAGE. - ISSN 1296-2074. - ELETTRONICO. - 51:(2021), pp. 138-144. [10.1016/j.culher.2021.08.001]

Availability:

This version is available at: <https://hdl.handle.net/11585/860089> since: 2022-02-17

Published:

DOI: <http://doi.org/10.1016/j.culher.2021.08.001>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Yiming Jia, Giorgia Sciutto, Alessandra Botteon, Claudia Conti, Maria Letizia Focarete, Chiara Gualandi, Chiara Samorì, Silvia Prati, Rocco Mazzeo, 2021. “Deep eutectic solvent and agar: a new green gel to remove proteinaceous-based varnishes from paintings”, Journal of Cultural Heritage, 51, 138-144

The final published version is available online at:
<https://doi.org/10.1016/j.culher.2021.08.001>.

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

Deep eutectic solvent and agar: a new green gel to remove proteinaceous-based varnishes from paintings

Yiming Jia^{1,2}, Giorgia Sciutto¹, Alessandra Botteon^{3,4}, Claudia Conti³, Maria Letizia Focarete^{5,6}, Chiara Gualandi^{5,7}, Chiara Samori⁸, Silvia Prati^{1}, Rocco Mazzeo¹*

¹ Department of Chemistry “G. Ciamician”, Microchemistry and Microscopy Art Diagnostic Laboratory (M2ADL), University of Bologna, Via Guaccimanni 42, 48121 Ravenna, Italy

² Chongqing Cultural Heritage Research Institute, 400013 Chongqing, China

³ Institute of Heritage Science (ISPC), National Research Council (CNR), Via Cozzi 53, 20125, Milano, Italy.

⁴ Department of Chemistry, Materials and Chemical Engineering “G. Natta”, Politecnico di Milano, piazza Leonardo da Vinci 32, 20131 Milano, Italy.

⁵ Department of Chemistry “Giacomo Ciamician” and INSTM UdR of Bologna, University of Bologna, Via Selmi 2, 40126 Bologna, Italy

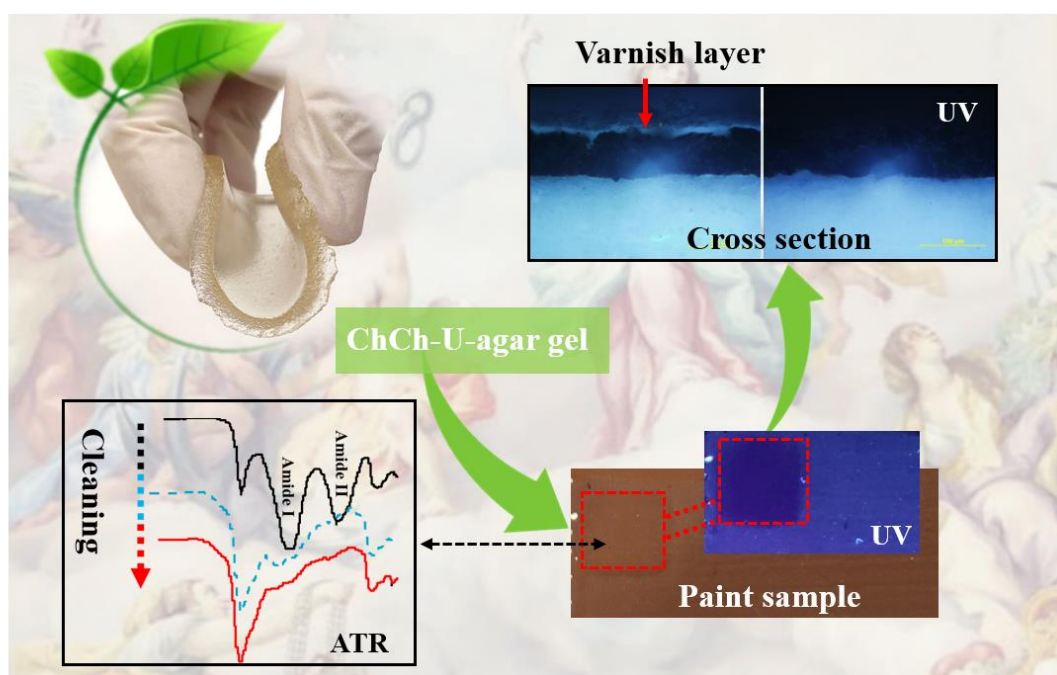
⁶ Health Sciences & Technologies (HST) CIRI, University of Bologna, Via Tolara di Sopra 41/E, 40064 Ozzano Emilia Bologna, Italy

⁷ Interdepartmental Center for Industrial Research on Advanced Applications in Mechanical Engineering and Materials Technology, CIRI-MAM, University of Bologna, Viale Risorgimento, 2, 40136 Bologna, Italy

⁸ Department of Chemistry “G. Ciamician”, University of Bologna, Via Sant’Alberto
163, 48123 Ravenna, Italy

*Corresponding author: Silvia Prati s.prati@unibo.it

Graphical abstract



Highlights

Deep eutectic solvents and agar were used to develop a green cleaning system

ChCh-U-agar gel successfully removes thin proteinaceous coatings

The green cleaning system is effective on both hydrophobic and hydrophilic surfaces

Micro SORS analysis was used to characterize the underneath paint layer

The gel prevents the diffusion of the cleaning agents in the paint layer.

Abstract

The selective removal of thin varnish layers from paintings is still an open challenge in the conservation field. In this paper, a new cleaning system was developed by using a gel composed of fully green components as deep eutectic solvents (DES) and agar. The gel exhibited good rheological properties in terms of gel stiffness, allowing easy handling and removal of the gel. The newly developed cleaning gel was successfully tested for the removal of proteinaceous

layers applied over both hydrophobic and hydrophilic surfaces. Moreover, an innovative non-destructive approach based on micro-Spatially Offset Raman Spectroscopy (micro-SORS) allowed demonstrating how the gel impairs the diffusion of the cleaning agents in the paint layers, highlighting the good solvent retention ability of the gel.

Keywords: Deep eutectic solvent; Agar gel; Proteinaceous; Green chemistry; Micro-spatially offset Raman spectroscopy

1. Introduction

The selective removal of varnish layers from paintings is still an open challenge in the conservation field [1], in particular when the varnish has similar solubility properties with the binder present in the above painting layers. A particularly challenging situation occurs when a proteinaceous varnish or overpainting has to be removed.

Proteinaceous materials, including animal glue, egg, and casein from milk have been traditionally used as binders, coatings and adhesives in paintings thanks to their flexibility, versatility, availability and adhesive properties [3-5]. Casoli et al. studied the effects of cleaning egg tempera paintings with solvents of different polarity [6]: while apolar solvents exerted an effect on the leaching of the lipidic components, water and polar solvents were found capable of extracting the proteinaceous components [6]. The most challenging situation occurs when the proteinaceous layer to be removed is applied on a layer of similar polarity. In this situation, the use of aqueous systems may be extremely detrimental [7-10], causing wrinkles in the hydrophilic layers and leaching of proteinaceous components from not aged films [7,9,10].

Hydrogels have been used for the removal of proteinaceous coatings and repaintings, developing selective bio-based procedures which exploit the use of bacteria and protease enzymes [2,11,12]. However, their application requires the use of complex procedures which strongly depend on application time, pH or temperature.

Agar is a natural polysaccharide produced from several species of marine seaweeds, which can form semi-rigid and thermo-reversible hydrogels by heating and cooling. Agar hydrogels form a rigid network that allows to control of the release of water and to avoid mechanical stresses during cleaning [4-6,10,13-15]. Thanks to these features, agar hydrogels can also be applied on water-sensitive surfaces, however, this application time should be carefully limited [16].

To avoid the challenging use of water, in the present research we developed and tested a new environmentally friendly gel obtained with agar and deep eutectic solvent (DES) for the removal of thin proteinaceous layers from both hydrophobic and hydrophilic surfaces. DESs are solvents composed of eutectic mixtures of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) that have a melting point much lower than those of the individual components [17]. DESs have physico-chemical properties similar to those of common ionic liquids (ILs), but DESs are not entirely composed of ionic species, and can also be obtained from non-ionic species [18]. They present several advantages over traditional ILs, such as low price, easy storage and preparation [19]. DESs have been applied in many areas of chemistry, including catalysis and organic synthesis [20-22], separation technology [23], electrochemistry [24] and pharmaceutical [25] due to their excellent physico-chemical properties, such as low

vapor pressure, non-flammability, good solubility and conductivity, wide electrochemical window and large liquid range [17,18]. DES are also able to swell and solubilize proteins and lipids even in the absence of water [26,27].

To the author's knowledge, up to now DESs have not been tested to produce gels for cleaning purposes. Other ionic liquids composed of imidazolium or pyridinium cations with different alkyl chains and anions were found to be effective in producing soft, viscoelastic and flexible gels with biopolymers comprising agarose [28]. These materials exhibit smart properties such as conductivity and self-healing that can be used in sensors and electrochemical materials [29,30]. The DES here prepared was composed by choline chloride and urea (ChCh-U, 1:2 molar ratio, named as reline). Choline chloride (ChCl) acts as HBA, and is a biodegradable and non-toxic quaternary ammonium salt that can be either extracted from biomass or readily synthesized from fossil reserves, whereas urea acts as HBD [18]. The choice of using non-toxic and biodegradable materials addresses the recent requirements of new safer and sustainable restoration products and is in line with the new Horizon 2021 priorities [2,3,6-10,13, 31-34]. In the present paper, DES can be prepared from natural components such as].

The new green gel (ChCh-U-agar) was tested on mock ups coated with whole egg or rabbit glue applied on both hydrophobic (oil painting layer) and hydrophilic (egg tempera painting layer) surfaces. The cleaning performance was evaluated with optical microscopy and FTIR microscopy. Moreover, to evaluate ChCh-U diffusion inside the paint layer an innovative analytical method based on micro-Spatially Offset Raman Spectroscopy (micro-SORS) was tested. Micro-SORS is a non-destructive technique that allows obtaining information at the micrometer scale on the molecular composition of the inner portions of materials, without the need of resorting to a cross-section [35]. Recently, micro-SORS has been optimized to monitor the diffusion of ChCh-U into a gypsum substrate, permitting to non-invasively obtain an overview of the diffusion trend of this compound into the matrix, opening ways to the evaluation of its transport processes [36].

2. Materials and methods

Choline chloride and urea were purchased from Sigma-Aldrich. Agar was purchased from C.T.S. All the chemical reagents were commercially available and directly used without treatment.

2.1 Synthesis of the gels

ChCh-U-agar gel was produced by mixing choline chloride (ChCh) and urea (U), at the mole ratio 1:2 in a Petri dish. The mixture was stirred at 100 °C for 5 min until a homogeneous colourless liquid was obtained. Then agar was added to the DES (10 wt%) and the mixture was stirred until the gel was formed. The prepared ChCh-U-agar gel was stored in the fridge and used within 1 week.

The EtOH-H₂O/agar gel was synthesized by mixing ethanol and deionized water in a volume ratio of 1:1. Agar was added to the obtained solution in a ratio of 1: 30 g/mL. The mixture was stirred at 70 °C for 4 min then cooled down to form a homogeneous colourless gel.

2.2 Characterisation of the DES-agar gel

Rheology experiments were carried out on an Anton Paar Rheometer MCR 102 using a cone-plate configuration (25 mm diameter, 1°). The experiment was performed at constant temperature of 28 °C controlled by the integrated Peltier system and a Julabo AWC100 cooling system. To keep the sample hydrated a solvent trap was used (H-PTD200). Amplitude and frequency sweep analyses were performed with a fixed gap value of 0.5 mm on the gel samples. Oscillatory amplitude sweep experiments (γ : 0.01-100%) were carried out in order to determine the linear viscoelastic (LVE) range at a fixed frequency of 10 rad s⁻¹. Once the LVE of each hydrogel was established, frequency sweep tests were performed (ω : 0.1-100 rad s⁻¹) at a constant strain within the LVE region of the sample. Rheological measurements were performed on a set of 3 replicates for each type of gel system studied and the trend showed no significant differences.

2.3 Samples preparation

To evaluate the cleaning efficiency, the gel was tested on mock-ups presenting thin layers of proteinaceous coatings (glue and whole egg) applied both on a hydrophobic surface, prepared using linseed oil as binder, and on a hydrophilic surface in which the paint binder was egg tempera. The painting mock-ups were produced according to traditional painting techniques [37]: the preparation layer by dissolving 1.0 g of animal glue in 5.0 mL of hot distilled water and mixing it with 6.0 g of grinded gypsum and the oil painting layer by mixing 1.0 g of red burnt ochre in 0.5 mL of linseed oil. The egg tempera was prepared by adding 1.0 g of red burnt ochre 101.0 mL of egg binder mixture, composed by egg white, yolk, and distilled water in a 1:1:1 (v/v) ratio. Whole egg coating was prepared by mixing egg white, yolk, and distilled water in a 1:1:1 (v/v) ratio. Glue coating was obtained by dissolving 1.0 g of rabbit glue in 10.0 mL of distilled water.

2.4 Cleaning procedure

All the different mock-ups were treated by applying the gel for 15 minutes on the painting surface, changing the gel every 5 minutes and using cotton swabs between each application. Due to the high viscosity and non-volatility of DES, trace residues can be eliminated to an undetectable level with additional 10 s of EtOH-H₂O/agar gel treatment. Since the use of aqueous system may cause adverse effects on the painting layer, in particular when the layer is hydrophilic, the application time of the EtOH-H₂O/agar gel was optimised in order to prevent the swelling of the painting.

2.5 Evaluation of the cleaning performances

Optical microscopy in visible and ultraviolet light (Olympus Optical Microscope BX51, Tokyo, Japan) was used to observe the cross sections of mock-ups before and after the cleaning treatment.

Total Attenuated Reflectance (ATR) FTIR analysis was performed directly over the surface after the cleaning treatments to assess the cleaning efficiency and the presence of gels residues after the treatment. A Thermo Nicolet (Thermo Fisher Scientific, Waltham, MA, USA), iNTM10MX imaging microscope, equipped with a mercury-cadmium-telluride (MCT) detector cooled by liquid nitrogen, was used. The measurements were performed using a slide-on ATR objective, equipped with a conical germanium crystal, in the range 4000-675 cm⁻¹, with a spectral resolution of 4 cm⁻¹ with 64 scans and an optical aperture of 150×150 μm. The

spectroscopic analysis was performed on 3 different areas treated with the same cleaning procedure and 4 spectra were recorded before and after the treatment.

In the solvent diffusion experiments, a cotton swab soaked with ChCh-U was rolled over an area of about 1x1 cm of a mock up prepared with egg tempera. The surface was treated for 5 min and the excess of solvents was removed with two dried cotton swabs. ChCh-U-agar gel was put in contact with a similar area of the same mock up for 10 min, then the gel was removed and cleaned as previously described with two dried cotton swabs.

Micro-SORS Raman analyses were performed immediately after the treatments to evaluate and compare the solvent diffusion. A Senterra dispersive Raman microscope (Bruker Optik GmbH) equipped with a Peltier cooled charge coupled device detector (1024×256 pixels) and a 785 nm excitation laser was used. Reference spectra of ChCh-U and mock-up were performed using a 20× objective (NA 0.4 and WD 1.3 mm), a laser power ranging from 10 to 50 mW and an acquisition time ranging from 50 s to 100 s. Micro-SORS sequences were acquired with the defocusing micro-SORS modality [36], consisting on the acquisition of measurements on the intact mock-up at imaged and defocused positions moving the 20× objective away from the sample in z direction ($\Delta z = 50, 100, 200, 300, 400, 500, 600, 700, 800 \mu\text{m}$). The theoretical laser spot diameter at imaged position was 2.4 μm . The laser power was 10 mW and the accumulation time of 100 s per spectrum. Micro-SORS spectra are baseline corrected and normalized for comparison. The progressive change of ChCh-U DES signal was evaluated calculating the intensity ratio between two selected Raman bands of ChCh-U DES and red ochre, the pigment used in the mock-up. Calculations and plot construction were carried out using OPUS and ORIGIN software.

3. Results and discussion

3.1 ChCh-U agar gel mechanical properties

Rheological analyses have been performed to measure storage and loss moduli (G' and G'' , respectively) (Figure 1). The gel is characterized by a “solid-like” behaviour, with the storage modulus approximately an order of magnitude higher than the loss modulus and a LVE region of 1 % (Figure 1a), determined according to ISO 6721-10:2015. Furthermore, the frequency sweep analysis (Figure 1b) pointed out that G' and G'' were almost independent of the frequency in the range from 0.1 to 100 rad/s (always with $G' > G''$), confirming the “solid-like” rheological behaviour for the analysed organogel.

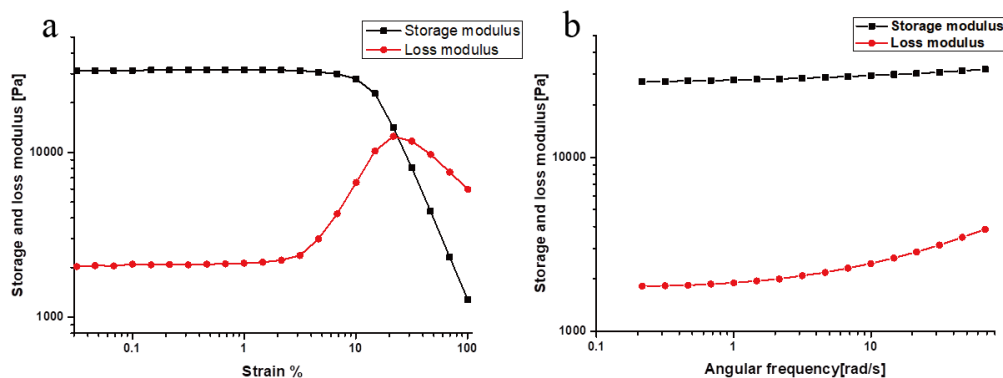


Figure 1. a) Strain dependence and b) frequency dependence of storage modulus (black) and loss modulus (red).

3.2 Diffusion behaviour of the ChCh-U solvent

Micro-SORS experiments were carried out to evaluate the diffusion behaviour of the DES solvent into the thickness of the mock-up. Both ChCh-U-agar gel and ChCh-U soaked in cotton swabs were applied on an egg tempera mock-up to simulate the cleaning treatments. Figure 2a reports the average of the Raman spectra acquired on the surface of the mock-up before the treatments. Bands at 409 cm^{-1} , 291 cm^{-1} and 224 cm^{-1} are ascribable to red ochre [38], while egg binder showed several broad bands in the region between 1200 and 1600 cm^{-1} [39]. The presence of carbonate is attributed to calcite (1089 cm^{-1}) and/or dolomite (1094 cm^{-1}) probably added to the red pigment or present as impurity [40]. Figure 2a also reports the reference Raman spectrum of ChCh-U solvent, with bands at 1450 and 999 cm^{-1} which refer to C-N asymmetric and C-N symmetric stretching, respectively, attributed to urea. Besides, the other C-N asymmetric stretching band at 956 cm^{-1} and C-N symmetric stretching band at 716 cm^{-1} are assigned to choline [41].

Micro-SORS spectra were acquired immediately after the treatment with cotton swabs soaked with ChCh-U and after the ChCh-U-agar gel cleaning. In Figure 2b and c representative sequences are reported, normalized to the red ochre signal at 409 cm^{-1} for comparison. The main differences between the cotton swabs and agar gel cleaning treatments concern the amount of ChCh-U at imaged positions and its diffusion within the mock-up: after cotton swabs treatment a relatively higher amount of ChCh-U is present on the mock-up surface, and its diffusion has been detected up to $200\text{ }\mu\text{m}$ of defocusing distance (figure 2b), whereas after agar gel cleaning the amount of ChCh-U is lower, barely visible, and at $200\text{ }\mu\text{m}$ of defocusing distance its signal is no longer detectable (this spectrum is not reported in figure since it is extremely noisy).

To properly monitor the micro-SORS sequence trends, the intensity ratio between the ChCh-U band at 716 cm^{-1} and the red ochre band at 409 cm^{-1} has been calculated (Figure 2d) for both treatments. As expected, this ratio progressively decreases from the imaged position up to $200\text{ }\mu\text{m}$ of defocusing distance; after this distance the micro-SORS spectra show a high degree of noise, thus calculations are not reported. However, also within this small defocusing range the differences between the treatments are unequivocal: agar gel application shows lower overall

ratio values and at 200 μm of defocusing distance ChCh-U signal is not detected, thus the ratio value tends to zero. This finding confirms the retention capability of the ChCh-U agar gel.

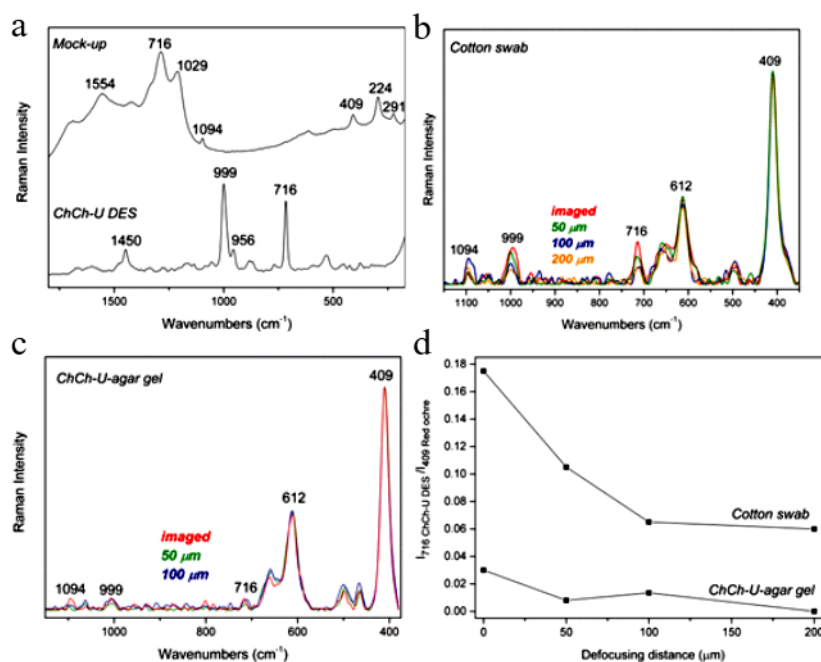


Figure 2. a) Reference Raman spectra of the painted mock-up and ChCh-U, b) Micro-SORS sequence after ChCh-U cotton swab application and c) after ChCh-U-agar gel application, normalized to the red ochre signal at 409 cm^{-1} (the defocusing distances in μm are also reported), d) ChCh-U DES/Red ochre intensity ratios of micro-SORS sequences after ChCh-U cotton swab and agar gel applications.

3.3 Cleaning efficacy and residues

3.3.1 Cleaning efficacy on hydrophobic surfaces

Oil painting mock-ups varnished with either glue or whole egg were used to evaluate the cleaning efficiency of ChCh-U-agar gel. Cross-sections of paint samples were collected from the mock-ups before and after the cleaning treatments and compared with fragments collected from the unvarnished areas. Under optical microscopy, samples collected from the unvarnished area (Figure 3a and 3b) show a superficial thin yellow fluorescent layer, unevenly distributed, which can be ascribed to a surface enrichment of the oil binder, due to local pigment sedimentation. Observing the cross sections of sample taken from the varnished areas, it was possible to identify the thin fluorescent layer of either the glue or the whole egg varnish above the suspended oil. Figure 3 reports the cross sections obtained on the glue varnished sample before and after the cleaning, showing the complete and selective removal of the proteinaceous layer after the treatment with the ChCh-U agar gel. Similar results were obtained on mock-ups varnished with whole egg.

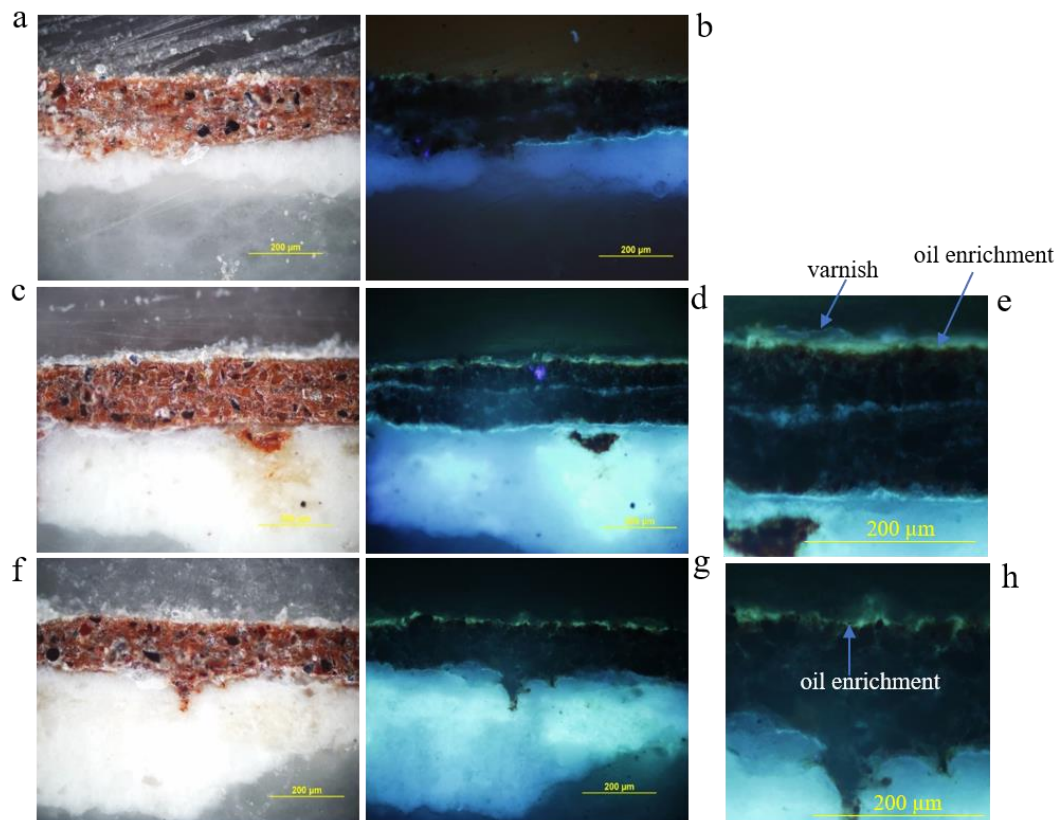


Figure 3. Cross-sections of oil painting reconstructions, a) VIS and b) UV photomicrographs before cleaning, c), VIS, d) and e) UV photomicrographs of the mock-up varnished with glue before cleaning, f) VIS, g) and h) UV photomicrographs of the of the mock up varnished with glue after the cleaning.

To assess the cleaning efficacy, ATR-IR analyses were performed on the treated areas. In Figure 4, spectra acquired on the mock-up varnished with glue are reported.

After the treatment, the acquired spectra presented the same features of spectra acquired on the unvarnished area and the characteristic signals of the proteinaceous varnish (N-H stretching band at 3281 cm^{-1} , amide I and amide II bands at 1640 and 1539 cm^{-1}) completely disappeared (figure 4b and c).

It is worth noting that, after the application of the ChCh-U agar gel, some residues of ChCh-U were observed as the presence of the C=O stretching band at 1659 cm^{-1} and of the N-H scissoring band at 1620 cm^{-1} suggests (Figure 4c). To remove the traces of ChCh-U, a gel composed by agar, EtOH and water was gently applied for 10 sec. Thanks to the high solubility of the DES solvent in water and EtOH, the gel was able to efficiently remove the residues present on the surface with a very short application time, as demonstrated by the absence of the diagnostic bands of ChCh-U in the spectra acquired after this treatment (Figure 4d).

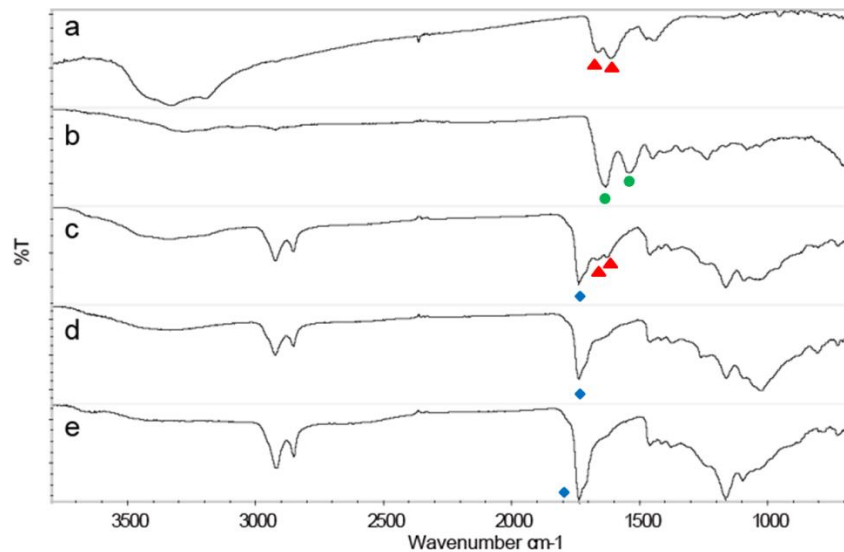


Figure 4. ATR-IR spectra acquired on a) ChCh-U DES reference, b) glue varnish before cleaning, c) after ChCh-U-agar gel cleaning, d) after ChCh-U-agar gel + EtOH-H₂O/agar gel cleaning, e) an unvarnished area. The symbol ▲ indicates ChCh-U DES bands, the symbol ● indicates the glue characteristic bands and the symbol ◆ indicates the characteristic bands of oil used as binder in the painting layer.

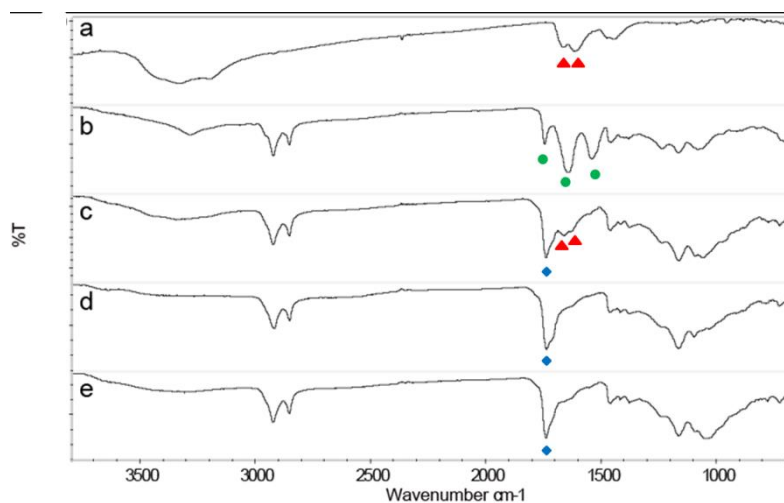


Figure 5. ATR-IR spectra acquired on a) ChCh-U reference, b) whole egg varnish before cleaning, c) mock-up varnished with whole egg after ChCh-U-agar gel cleaning, d) an unvarnished area, e) after ChCh-U-agar gel + EtOH-H₂O/agar gel cleaning. The symbol ▲ indicates ChCh-U bands, the symbol ● indicates the whole egg characteristic bands and the symbol ◆ indicates the characteristic bands of linseed oil used as binder in the painting layer.

The same procedure was applied on a red ochre linseed oil mock-up varnished with whole egg. After the application of the ChCh-U gel, the proteinaceous characteristic bands (amide I at 1640 cm⁻¹ and amide II at 1539 cm⁻¹) disappeared (figure 5b and 5c). Some traces of ChCh-U were detected (figure 5c) and successfully removed after the application of the EtOH-H₂O/agar gel

just for 10 sec (Figure 5d).

3.3.1 Cleaning efficacy on hydrophilic surfaces

The same cleaning procedure, based on the use of ChCh-U agar gel and the subsequent application of the EtOH-H₂O/agar gel just for 10 seconds, was performed on water sensitive egg tempera mock-up which was varnished with a layer of either glue or whole egg. Figure 6 showed that the thin varnishes were efficiently removed after the cleaning treatment.

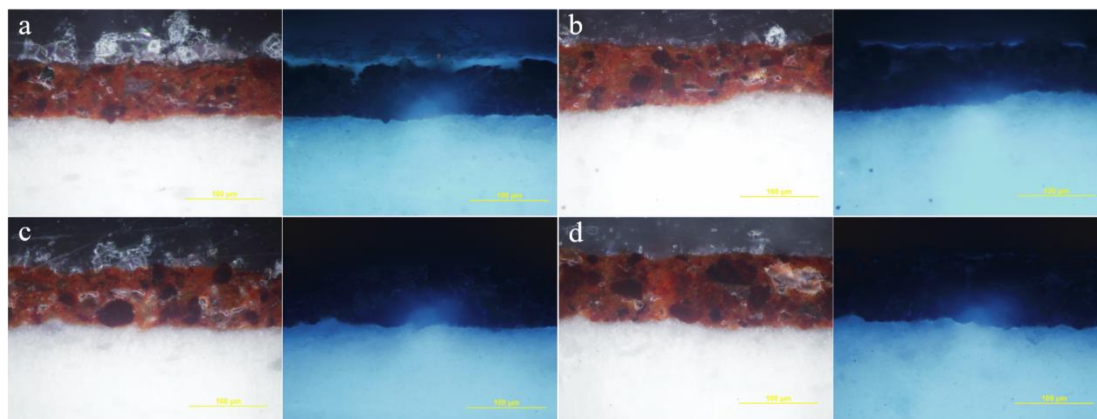


Figure 6. Cross-section microphotographs of oil painting reconstructions varnished with whole egg and glue before and after cleaning, image under visible light (left) and image under UV illumination (right); a) whole egg varnish before cleaning with average thickness of 10.3 μm , b) glue varnish before cleaning with average thickness of 2.8 μm , c) whole egg varnish after cleaning d) glue varnish after cleaning.

Particular attention was devoted to the evaluation of the effects of the EtOH-H₂O agar gel on the water sensitive painting layer. Thanks to the short application time, the EtOH-H₂O agar gel application appeared to be harmless toward the painting layer. Indeed, from a morphological point of view no evidence of strong effects of the treatment on the paint layer was observed and, after its removal, the gel surface did not contain any red particles coming from the painting layer.

Due to the presence of proteinaceous materials both in the paint layer and in the varnish layer, it is challenging to determine whether the varnish was completely removed or not with micro FTIR. However, the change of characteristic bands intensity before and after the treatment allows making some hypothesis. As shown in Figure 7, in the egg varnished mock-up the intensity of the bands attributed to the proteinaceous materials (N-H stretching at 3282 cm^{-1} , amide I at 1635 cm^{-1} and amide II at 1538 cm^{-1}) decreased and the silicate band at 1013 cm^{-1} , ascribable to the ochre pigment, appeared. In the glue varnished mock-ups, the band of silicate was already detectable in the spectra acquire before the cleaning, probably due to limited thickness of the varnish, but after the treatment the relative intensity of the proteinaceous bands decreased with respect to the 1013 cm^{-1} band.

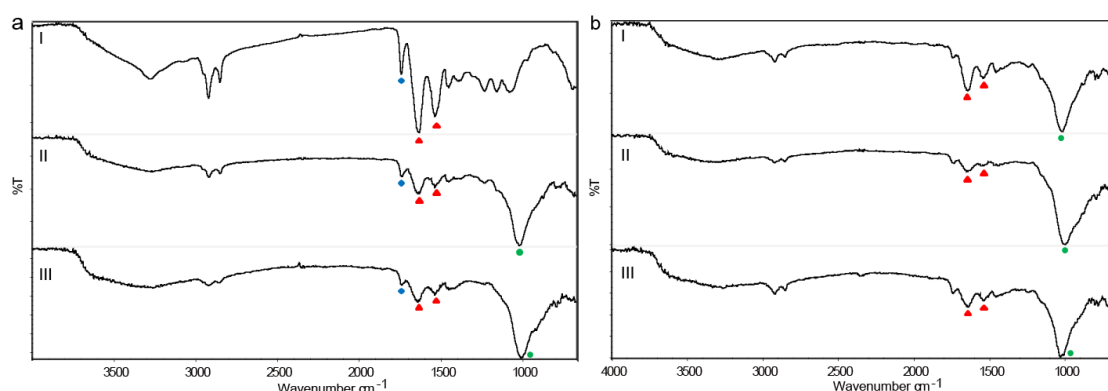


Figure 7. ATR-IR spectra acquired on a) whole egg varnish, b) glue varnish; I, before cleaning, II, after ChCh-U-agar gel + EtOH-H₂O/agar gel cleaning, III, an unvarnished area. The symbol ▲ indicates amide bands, the symbol ● indicates the silicate bands from the pigment and the symbol ◆ indicates the ester bands from egg varnish or binder.

CONCLUSION

This study reports the first attempt to apply a deep eutectic solvent made by choline chloride and urea for cleaning purposes in the cultural heritage field. A new green nonaqueous gel cleaning system based on ChCh-U and agar was developed.

The gel displayed good mechanical properties which allow easy handling during cleaning, especially in the laying and peel off procedures, reducing the presence of residual debris on the paints.

Micro-SORS was successfully applied to characterize the diffusion behavior of the cleaning agent in the paint layer, showing that the gel has better retention capacity and can control the solvent release.

As regards the gel performance for the removal of proteinaceous varnishes, thin whole egg and glue varnished oil and egg tempera painting mock-ups were selected for the test. ATR-IR and optical microscope results showed that the ChCh-U agar gel can efficiently remove the proteinaceous varnishes on both hydrophobic and hydrophilic surfaces. Trace of ChCh-U residues can be easily removed by applying just for few seconds an EtOH-H₂O agar gel. This formulation has a reduced amount of water and may also be used on tempera substrates.

REFERENCES

- [1] D. Chelazzi, R. Giorgi, P. Baglioni, Microemulsions, micelles, and functional gels: How colloids and soft matter preserve works of art. *Angew. Chem. Int. Ed.* 57 (2018) 7296-7303
- [2] R. White, A. Roy, GC-MS and SEM studies on the effects of solvent cleaning on old master paintings from the National Gallery, London. *Stud. Conserv.* 43 (1998) 159-176
- [2] P. Cremonesi, Rigid gels and enzyme cleaning, in: M.F. Mecklenburg (Ed.), *New Insights into the Cleaning of Paintings*, Smithsonian Contribution to Museum Conservation, 2013, pp. 179-184

- [3] G. Leo, L. Cartechini, P. Pucci, A. Sgamellotti, G. Marino, L. Birolo, Proteomic strategies for the identification of proteinaceous binders in paintings. *Anal. Bioanal. Chem.* 395 (2009) 2269-2280
- [4] A. Nevin, I. Osticioli, D. Anglos, A. Burnstock, S. Cather, E. Castellucci, Raman spectra of proteinaceous materials used in paintings: A multivariate analytical approach for classification and identification. *Anal. Chem.* 79 (2007) 6143-6151
- [5] B.H. Tai. Stradivari's Varnish: A review of scientific findings-part II. *J. Violin Soc. Am.* 22 (2009) 1-31
- [6] A. Casoli, M. Berzioli, P. Cremonesi, The chemistry of egg binding medium and its interactions with organic solvents and water, in: M.F. Mecklenburg(Ed.), *New Insights Into the Cleaning of Paintings*, Smithsonian Contributions to Museum Conservation, 2013, pp 39-44
- [7] J.A.L. Domingues, N. Bonelli, R. Giorgi, E. Fratini, F. Gorel, P. Baglioni, Innovative hydrogels based on semi-interpenetrating p(HEMA)/PVP networks for the cleaning of water-sensitive cultural heritage artifacts. *Langmuir* 29 (2013) 2746-2755
- [8] M.F. Pacheco, A.I. Pereira, L.C. Branco, A.J. Parola, Varnish removal from paintings using ionic liquids. *J. Mater. Chem. A* 1 (2013) 7016-7018
- [9] A. Casoli, P. Cremonesi, C. Isca, R. Groppetti, S. Pini, N. Senin, Evaluation of the effect of cleaning on the morphological properties of ancient paper surface. *Cellulose* 20 (2013) 2027-2043
- [10] S. Prati, F. Volpi, R. Fontana, P. Galletti, L. Giorgini, R. Mazzeo, L. Mazzocchetti, C. Samorì, G. Sciutto, E. Tagliavini, Sustainability in art conservation: A novel bio-based organogel for the cleaning of water sensitive works of art. *Pure Appl. Chem.* 90 (2018) 239-251
- [11] C. Pereira, T. Busani, L.C. Branco, I. Joosten, I.C.A. Sandu, Nondestructive characterization and enzyme cleaning of painted surfaces: assessment from the macro to nano level. *Microsc. Microanal.* 19 (2013) 1632
- [12] G. Ranalli, E. Zanardini, L. Rampazzi, C. Corti, A. Andreotti, M.P. Colombini, P. Bosch-Roig, G. Lustrato, C. Giantomassi, D. Zari, P. Virilli, Onsite advanced biocleaning system for historical wall paintings using new agar - gauze bacteria gel. *J. Appl. Microbiol.* 126 (2019) 1785-1796.
- [13] Y. Jia, G. Sciutto, S. Prati, E. Catelli, M. Galeotti, S. Porcinai, L. Mazzocchetti, C. Samorì, P. Galletti, L. Giorgini, E. Tagliavini, R. Mazzeo. A new bio-based organogel for the removal of wax coating from indoor bronze surfaces. *Herit. Sci.* 7 (2019) 34
- [14] M. Anzani, M. Berzioli, M. Cagna, E. Campani, A. Casoli, P. Cremonesi, M. Fratelli, A. Rabbolini, D. Raggiardi, *Gel rigidi di agar per il trattamento di pulitura di manufatti in gesso*, Q. 6, Cesmar7, Il Prato, Padova, 2008
- [15] A. Sansonetti, M. Bertasa, C. Canevali, A. Rabbolini, M. Anzani, D. Scalarone, A review in using agar gels for cleaning art surfaces. *J. Cult. Herit.* 44 (2020) 285-296
- [16] A. Volk, K.J. van den Berg, Agar-a new tool for the surface cleaning of water sensitive oil

paint? in: A. Burnstock, M. de Keijzer, J. Krueger, T. Learner, A. de Tagle, G. Heydenreich, K.J. van den Berg (Eds.), *Issues Contemp, Oil Paint*, Springer, 2014, pp. 389-406

[17] E.L. Smith, A.P. Abbott, K.S. Ryder, Deep eutectic solvents (DESs) and their applications. *Chem. Rev.* 114 (2014) 11060-11082

[18] Q. Zhang, K.D.O. Vigier, S. Royer, F. Jérôme, Deep eutectic solvents: syntheses, properties and applications. *Chem. Soc. Rev.* 41 (2012) 7108-7146

[19] C. Ruß, B. König, Low melting mixtures in organic synthesis-an alternative to ionic liquids? *Green Chem.* 14 (2012) 2969-2982

[20] S.B. Phadtare, G.S. Shankarling, Halogenation reactions in biodegradable solvent: Efficient bromination of substituted 1-aminoanthra-9, 10-quinone in deep eutectic solvent (choline chloride: urea). *Green Chem.* 12 (2010) 458-462

[21] P.M. Pawar, K.J. Jarag, G.S. Shankarling. Environmentally benign and energy efficient methodology for condensation: an interesting facet to the classical Perkin reaction. *Green Chem.* 13 (2011) 2130-2134

[22] S. Gore, B. Koenig. Efficient synthesis of 3, 4-dihydropyrimidin-2-ones in low melting tartaric acid-urea mixtures. *Green Chem.* 13 (2011) 1009-1013

[23] Q. Zeng, Y. Wang, Y. Huang, X. Ding, J. Chen, K. Xu, Deep eutectic solvents as novel extraction media for protein partitioning. *Analyst* 139 (2014) 2565-2573

[24] E. Gómez, P. Cojocar, L. Magagnin, E. Valles, Electrodeposition of Co, Sm and SmCo from a deep eutectic solvent. *J. Electroanal. Chem.* 658 (2011) 18-24

[25] M.C. Serrano, M.C. Gutiérrez, R. Jiménez, M.L. Ferrer, F.d. Monte, Synthesis of novel lidocaine-releasing poly-(diol-co-citrate) elastomers by using deep eutectic solvents. *Chem Commun.* 48 (2012) 579-581

[26] A. Sanchez-Fernandez, K.J. Edler, T. Arnold, D.A. Venero, A.J. Jackson, Protein conformation in pure and hydrated deep eutectic solvents. *Phys. Chem. Chem. Phys.* 19 (2017) 8667-8670

[27] S.J. Bryant, R. Atkin, G.G. Warr, Spontaneous vesicle formation in a deep eutectic solvent. *Soft Matter* 12 (2016) 1645-1648

[28] T. Singh, T.J. Trivedi, A. Kumar. Dissolution, regeneration and ion-gel formation of agarose in room-temperature ionic liquids. *Green Chem.* 12 (2010) 1029-1035

[29] K. Prasad, D. Mondal, M. Sharma, C. Mukesh, J. Bhatt, Stimuli responsive ion gels based on polysaccharides and other polymers prepared using ionic liquids and deep eutectic solvents. *Carbohydr. Polym.* 180 (2018) 328-336

[30] K. Prasad, M. Sharma, Green solvents for the dissolution and processing of biopolymers, *Curr. Opin. Green Sustain. Chem.* 18 (2019) 72-78

[31] N. Barbabietola, F. Tasso, C. Alisi, P. Marconi, B. Perito, G. Pasquariello, A.S. Sprocati, A safe microbe-based procedure for a gentle removal of aged animal glues from ancient paper. *Int. Biodeter. Biodegr.* 109 (2016) 53-60

- [32] A. Macchia, L. Rivaroli, G. Gianfreda. The GREEN RESCUE: a 'green' experimentation to clean old varnishes on oil paintings. *Nat. Prod. Res.* (2019) 1-11
- [33] C. Samorì, P. Galletti, L. Giorgini, R. Mazzeo, L. Mazzocchetti, S. Prati, G. Sciutto, F. Volpi, E. Tagliavini. The green attitude in art conservation: polyhydroxybutyrate-based gels for the cleaning of oil paintings. *ChemistrySelect* 1 (2016) 4502-4508
- [34] Y. Jia, G. Sciutto, R. Mazzeo, C. Samorì, M.L. Focarete, S. Prati, C. Gualandi, Organogel coupled with microstructured electrospun polymeric nonwovens for the effective cleaning of sensitive surfaces. *ACS Appl. Mater. Interfaces* 12 (2020) 39620-39629
- [35] C. Conti, A. Botteon, C. Colombo, D. Pinna, M. Realini, P. Matousek, Advances in Raman spectroscopy for the non-destructive subsurface analysis of artworks: Micro-SORS. *J. Cult. Herit.* 43 (2020) 319-328.
- [36] A. Botteon, Y. Jia, S. Prati, G. Sciutto, M. Realini, C. Colombo, C. Castiglioni, P. Matousek, C. Conti, Non-invasive characterisation of molecular diffusion of agent into turbid matrix using micro-SORS. *Talanta* 218 (2020) 121078
- [37] C. Cennini, *Il libro dell'arte*, in: F. Brunello (Ed.), Vicenza, 1993
- [38] F. Froment, A. Tournie, P. Colomban. Raman identification of natural red to yellow pigments: ochre and iron-containing ores. *J. Raman Spectrosc.* 39 (2008) 560-568
- [39] I. Osticioli, A. Nevin, D. Anglos, A. Burnstock, S. Cather, M. Becucci, C. Fotakis, E. Castellucci, Micro-Raman and Fluorescence Spectroscopy for the assessment of the effects of the exposure to light on films of egg white and egg yolk. *J. Raman Spectrosc.* 39 (2008) 307-313
- [40] J. Sun, Z. Wu, H. Cheng, Z. Zhang, R.L. Frost, A Raman spectroscopic comparison of calcite and dolomite. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 117 (2014) 158-162
- [41] B. Bozzini, B. Busson, C. Humbert, C. Mele, A. Tadjeddine, Electrochemical fabrication of nanoporous gold decorated with manganese oxide nanowires from eutectic urea/choline chloride ionic liquid. Part III-Electrodeposition of Au-Mn: a study based on in situ Sum-Frequency Generation and Raman spectroscopies, *Electrochim. Acta* 218 (2016) 208-215