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New green solvents for cleaning and conservation of Cultural Heritage: Deep Eutectic Solvents (DESs)

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***Abstract* – Deep Eutectic Solvents (DESs) are a relatively new class of unconventional green solvents. They are obtained by mixing two or more components, a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), which form a liquid at room temperature due to their lower melting point compared to the starting materials. DESs are characterized by their low toxicity, high environmental compatibility, non-flammability, and non-volatility. While DESs have found applications in various fields such as organometallic chemistry and pharmaceutical production, their potential use in restoration of cultural heritage has not been explored until now. This research project aims to synthesize DESs and investigate their effectiveness as green products for restoration practices.**

The results obtained from the first case studies highlight the promising use of DESs as green solvents and biocides, able to effectively removing the products of alteration and degradation of Cultural Heritage.

I. INTRODUCTION

Deep Eutectic Solvents (DESs) have emerged as a new class of green solvents with unique chemical and physical characteristics, offering significant potential for various applications [1]. By combining hydrogen bond donors (HBD) and acceptors (HBA), DESs exhibit a liquid state at room temperature, making them a viable alternative to traditional solvents [2]. DESs possess properties such as low toxicity, high environmental friendliness, and biodegradability [3], which make them suitable for potential use in restoration sector. However, their application in the Cultural Heritage field is still to be discovered, representing an innovative challenge in this research area.

This study aims to fill this knowledge gap by synthesizing DES and investigating their effectiveness as unconventional and sustainable materials for applications in the Cultural Heritage field. In the context of restoration, a critical issue to be addressed is biological degradation, which occurs due to the growth and colonization of macro- and micro-organisms leading to the formation of biofilms and biodeterioration processes of the surfaces of artworks [4]. Traditional approaches involve the use of biocidal products, that usually result to be insufficient and unable to meet the specific requirements of each case [5].

Thus, the objective of this study is to evaluate the biocidal activity of synthesized DESs for the removal of biodeteriorated layers from materials of cultural interest, with the aim of establishing a sustainable and gentle approach for the cleaning and conservation of artworks. This approach allows to avoid the use of unsafe chemicals that could potentially damage these precious artifacts. Furthermore, the use of DESs as biocidal agents in cultural heritage area offers benefits such as reduced environmental impact and increased operator safety. In fact, DESs offer several advantages in this sense, because they are non-toxic, ecological, biodegradable, non-flammable and non-volatile. Furthermore, several DESs reported in the literature have been shown to have antibacterial and antifungal properties [6].

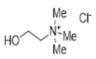
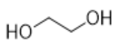
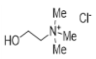
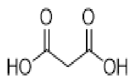
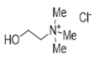
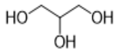
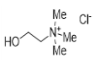
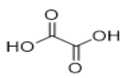
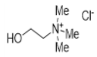
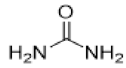
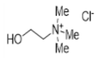
To further advance the goal of using non-toxic solvents in restoration, the research is also focusing on the synthesis of hydrophobic DESs. This is particularly relevant for surfaces exposed to the outside, which require protective treatments to limit not only the proliferation of biodeteriogens, but also the absorption of dirt carried by rainwater and the damages caused by freeze-thaw cycles of the water [7]. Water is the primary vehicle for degradation agents in these scenarios. Hydrophobic DESs can also be employed for the removal of aged non-polar coatings. Traditionally, non-polar materials such as waxes were used as protective agents on artworks, to prevent damages from rain and air pollution, and to improve the surface appearance [8]. However, over time, these wax coatings degrade and accumulate dust and pollutants, causing the texture and appearance of the artifacts to change [9]. Conventional flammable and toxic solvents are commonly used for wax removal, suggesting the exploration of DESs as a potential alternative.

By systematically investigating DESs and their properties, this research project seeks to contribute to the development of sustainable and effective strategies for the cleaning, and protection of Cultural Heritage.

II. APPLICATIONS AND RESULTS

The preparation of DESs' mixture involves weighing the components, HBA and HBD, at the appropriate molar ratio. These components are mixed and heated between 80°C and 100°C until the formation of homogeneous liquid. Table 1 provides the chemical structures of the components of each synthesized hydrophilic DESs, and their molar ratio. DESs are obtained through a simple and cheap synthetic procedure. Once obtained, the DESs were used in their pure form, without further modifications.

Table 1. Composition a of DESs

DES code	HBA	HBD	Molar ratio
DES 1	 Choline Chloride	 Ethylene glycol	1:2
DES 2	 Choline Chloride	 Malonic Acid	1:1
DES 3	 Choline Chloride	 Glycerol	1:2
DES 4	 Choline Chloride	 Oxalic Acid	1:1
DES 5	 Choline Chloride	 Urea	1:2
DES 6	 Choline Chloride	$Zn(Cl)_2$	1:2

The biocidal activity of the synthesized DES was analysed by antimicrobial susceptibility tests conducted on microbial strains taken in situ directly from the surfaces of an artifact. The results obtained from the biological tests showed that DES 1, 2 and 5 have no antibacterial activity, while DES 3, 4 and 6 have good antibacterial activity.

To evaluate their biocidal action in the Cultural Heritage sector, the mixtures of DESs were applied to specific areas (Area 1 and Area 2) of a biodegraded mosaic located in the Archaeological Park of Ostia Antica [10], reported in Fig. 1.

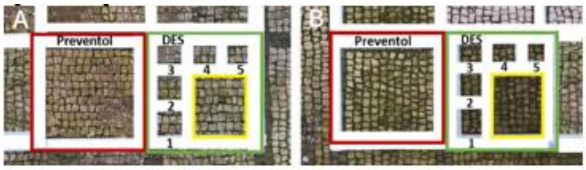


Fig. 1. Application of DESs in two areas of the mosaic in the Archeological Park of Ostia Antica (Rome): Area 1 (A) and Area 2 (B).

To estimate the interaction of DESs with the material, laboratory absorption tests were conducted through electrical conductivity, instead, to evaluate the biocidal activity of DESs, investigative analyses, such as ultraviolet fluorescence, bioluminescence and spectrocolorimetry were performed. From the examination carried out, the best results were obtained with the application of DES 2, DES 4 and DES 5. Acid-based DESs (Choline Chloride/Malonic Acid = DES 2; Choline Chloride/Oxalic Acid = DES 4) have a higher biocidal action than DES 5 (Choline Chloride/Urea) because, due to their residual acidity, they cause the death of microorganisms and prevent their proliferation. Furthermore, DES 6 was also applied in situ at the archaeological park of Ostia Antica. DES 6 was compared with the application of Preventol RI50 and a saturated solution of zinc chloride, see Fig. 2.

Fig. 2. Application of DES 6 (above) and after application (below) in area of the mosaic in the Archeological Park of Ostia Antica (Rome).

The zinc chloride component is known for its biocidal property, but DES containing zinc chloride also show similar biocidal effects. In fact, the application of DES 6 resulted in having an effective cleaning action.

Additionally, DES 6 was also tested during the conservative treatment of the ruins of a wall painting known as "Déesis" of Motta San Giovanni (Reggio Calabria). It was tested on a portion of the pictorial film affected by the presence of a biological patina, as reported in Fig. 3.

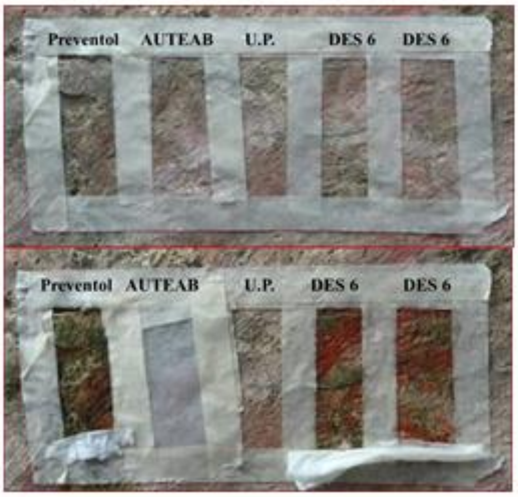


Fig. 3. Before application of DES 6 (above) and after application (below) during conservative treatment of wall painting in Motta San Giovanni (Reggio Calabria).

Before applying the products, the surface was photographed using a portable digital microscope. The products were applied with a brush and left on the surface for two days. After this time, the products were washed with demineralized water.

The areas treated with the selected products showed no microbial growth in the months following the application, while the untreated portion soon started to show the first step of biological colonization.

Further, all the synthesized DESs were applied to a magmatic effusive rock exposed at the University of Calabria, which has a homogeneous biological patina. The magmatic effusive rock originates from Lipari, Aeolian Islands (Messina), and it is composed of andesitic lava flows containing cordierite. It is located outdoor, and DESs application was carried out during the winter season. After application, their biocidal action was monitored over time by measuring the colorimetric variation and photographic documentation with a digital microscope, carried out once a month for four months. After carrying out the analyzes and comparing it, it was possible to note that no biological growth was observed on the areas treated with DES 1, 2, 4, 5 (Fig. 4).

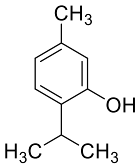
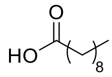
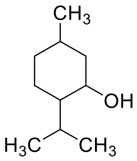
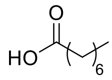
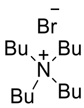
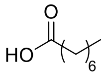
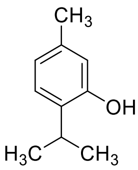
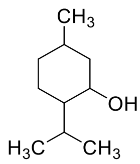
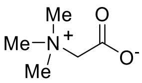
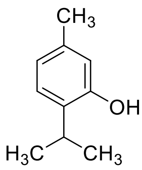
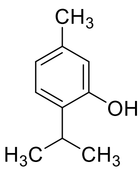
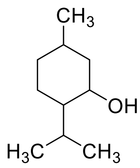
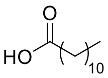
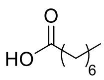
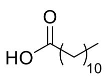
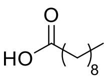
Fig. 4. After DESs application (above) and four months after application (below) on an effusive magmatic rock at the University of Calabria.

The search for new environmentally friendly solvents has extended to the field of Hydrophobic Deep Eutectic Solvents (HDESs). The composition of each hydrophobic DES, including the chemical structure of its components and their respective molar ratios, has been detailed in Table 2.

Substances of a nonpolar nature typically consist of long alkyl chains, making them solid at room temperature due to their higher melting points. However, the synthesis of DESs show a remarkable ability to significantly lower their melting points, offering the possibility of converting nonpolar materials from solid to liquid states that can be used as solvents.

Table 2. Composition of hydrophobic DESs

DES code	HBA	HBD	Molar ratio

HDES 1	 Thymol	 Decanoic acid	1:1
HDES 2	 L-(-) Menthol	 Octanoic acid	1:1
HDES 3	 Tetrabutylammonium bromide	 Octanoic acid	1:1
HDES 4	 Thymol	 L-(-)-Menthol	1:2
HDES 5	 Betaine	 Thymol	1:3
HDES 6	 Thymol	 L-(-)-Menthol	1:1
HDES 7	 Dodecanoic acid	 Octanoic Acid	1:3
HDES 8	 Dodecanoic acid	 Decanoic cid	1:2

Based on these considerations, the ability of HDESs has been exploited for the removal of non-polar coatings, such as waxes, from art surfaces. The following HDESs were tested in laboratory using slide samples having a non-polar coating [11]. They were used to test the removal properties on beeswax and two microcrystalline waxes (R21 and Renaissance), commonly used in the past on stone and metal artifacts. The interaction between each DES and wax was evaluated in laboratory by solubility tests, contact angle measurements and cleaning tests.

This study demonstrates the remarkable effectiveness of HDESs in cleaning several varieties of waxes commonly used in the field of cultural heritage conservation. The ability of these HDESs in terms of cleaning comes from their chemical structures that are similar to the specific compositions of waxes. From the results obtained, it was found that HDES 2 and 4 are effective in removing R21 microcrystalline wax, while HDES 2 and 7 are capable of removing Renaissance microcrystalline wax. In addition, HDES 8 and 2 show effective cleaning action in the context of beeswax removal.

HDESs have found application as in situ protective biocidal agents on a portion of the pictorial film of the "Déesis" located in Motta San Giovanni (Reggio Calabria) exposed outdoors, as shown in Fig. 5. This application is possible due to the well-documented antibacterial and antifungal properties inherent in the components of each HDES.

Fig. 5 Application of HDESs during conservative treatment of "Déesis" in Motta San Giovanni (Reggio Calabria).

They have been applied on an area having a homogeneous biological patina during sprint season. Prior to application, colorimetric measurements and photographic documentation were performed on each portion. Subsequently the products were applied with a brush. After one week, each product was removed from the surface by washing with water and TWEEN20, followed by demineralised water and finally pure ethanol. This trial is currently under study.

III. CONCLUSIONS

From the synthesis conducted, it was confirmed that the procedure for the preparation of DESs is simple and nonexpensive. Subsequently, the synthesized DESs were tested both in the laboratory and in situ to evaluate their applicability as biocide solvents for materials of cultural interest.

The first case studies involved several choline chloride-based DESs tested in pure form as biocidal agents. From the results obtained, it was observed that DES 2 (ChCl/MA), DES 4 (ChCl/OA) and DES 5 (ChCl/U) showed the best results, although their biocidal efficacy is lower than Preventol RI50. DES 2 and DES 4, with an acidic base, demonstrated superior biocidal action compared to DES 5, which showed good efficacy at a more basic pH. Acid-based DESs have greater biocidal action because acidic pH causes denaturation of proteins constituting the cell membrane of microorganisms and does not allow bacterial growth. It is important to underline that the use of DESs, compared to Preventol RI50, offers significant advantages in terms of stability, non-volatility, eco-compatibility and safety for operators and the environment. Furthermore, DESs do not require solvents for dilution as they are naturally liquid and low viscosity at room temperature.

The results deriving from these experimental studies indicate that DESs may represent a promising solution as biocidal agents in the cultural heritage sector, encouraging further research in this area. In particular, subsequent research has focused on the study of green water-repellent solvents. Through bibliographic research, hydrophobic DESs with the necessary properties to be employed in the field of restoration have been identified.

Hydrophobic DESs have been tested in the laboratory to evaluate their effectiveness in removing aged non-polar coatings. HDES Thymol/(-)Menthol for microcrystalline wax R21 removal, HDES (-)Menthol/Octanoic acid and HDES Dodecanoic acid/Octanoic acid for microcrystalline wax Renaissance removal, and HDES Dodecanoic acid/ Decanoic acid and HDES (-)Menthol/Octanoic acid for beeswax removal. The cleaning effectiveness of DESs depends on the structural affinity between a given DESs and a particular type of wax. The R21 microcrystalline wax is primarily composed of naphthenic hydrocarbons, which explains the heightened solubility of this wax type when in contact with DESs containing menthol. This phenomenon is especially pronounced in the case of the thymol/menthol DES with a molar ratio of 1:2. While Renaissance microcrystalline wax contains only low-molecular-weight poiliethylene chains and, due to these structural features, could better interfere with octanoic acid-based DESs. Finally, DESs derived from fatty acids with longer alkyl chains show higher affinity for beeswax due to the common presence of -COOH functional groups and long alkyl chains.

DESs are an excellent alternative to toxic and flammable organic solvents commonly used in the cultural heritage sector due to their low toxicity and low volatility. The low volatility allows to use less solvent, as DES stays on the surface longer without evaporating, allowing to effectively remove coatings. The transparent properties, the liquid consistency at room temperature and the effective biocidal and cleaning action on the materials are suitable for their use in the conservation of Cultural Heritage.

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