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**Evaluation of a gelatin-based adhesive for historic paintings that  
incorporates citronella oil as an eco-friendly biocide**

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# **Evaluation of a gelatin-based adhesive for historic paintings that incorporates citronella oil as an eco-friendly biocide**

The presented study focuses on evaluating the efficiency of a gelatin-based product that incorporates a plasticizer (glycerol) and a biocide (citronella oil), proposed as an eco-friendly adhesive for polychrome decoration applied in different parts of the architectural complex of the Longshan Temple in Lukang (18th century, Taiwan). Seven laboratory physico-chemical tests were performed: a) viscosity measurement; b) drying curves; c) moisture content determination; d) water vapor permeability test; e) mechanical test; f) adhesion test; g) susceptibility to fungi colonization test, which provide information on the workability, water content and water barrier properties, as well as mechanical, adhesion, and the biocide properties of the proposed product. The obtained results indicate that the workability, mechanical and adhesive properties of the new adhesive are adequate. Permeability in polychromies is slightly reduced due to the additional barrier effect of the adhesive incorporated into the paint film. The efficiency of citronella oil for preventing the growth of fungus *Aspergillus niger* on paintings consolidated with the adhesive was also probed. In parallel to these laboratory trials, the micro-invasive tests carried out, using nanoindentation combined with atomic force microscopy (NI-AFM), provided direct evidence for the improvement in the mechanical properties induced by applying the new adhesive to the original polychromies.

Keywords: gelatin-based adhesive; glycerol; citronella oil; biocide; plasticizer; consolidant.

## **Introduction**

Adhesion and consolidation of ancient and damaged polychromies are difficult and delicate operations because, if carried out inappropriately, they might cause undesirable effects, due to: first, the heterogeneous distribution of the adhesive between/into different strata; second, the alteration of the substance applied as the adhesive; finally,

unexpected reactions of the adhesive with the original materials. [1,2]

Old altarpieces and other polychromed wooden ornaments often present paint layers that flake and fall from the wood support due to the loss of mechanical properties of both the support and paint layers, which require the application of adhesion/consolidation treatments to return cohesion and loss resistance to the entire object. [3] These treatments have been afforded in diverse ways during different historical periods. Most of the materials used as adhesives for paintings, wood and fabric since ancient times were natural products, such as wheat starch paste [4] or animal glues. [5] A 3300-year-old representation of the gluing process of a piece to a wood plank can be seen in a stone found in Thebas. [5] The use of animal glue as adhesive is also mentioned in the Bible (Ecclesiastes, XXII,7). [5] In the Middle Ages, animal glue was used as a binding medium for grounds and as an adhesive in the junctions of plank panels. It was accurately described by Cennino Cennini in his art treatise. [6] Animal glue was also mixed with flours, molasses or Venice turpentine to confer more plasticity. [7,8] Other substances, such as garlic juice, ox gall or urine, could be added to the adhesive as a disinfectant. [6,8] In 17<sup>th</sup> and 18<sup>th</sup> centuries, the use of mixtures of waxes and colophony resin became popular. [7] Nevertheless, these adhesives sometimes required heating, which notably darkened, and mechanical properties were lost and became highly irreversible. [7] Mixtures of drying oils and resins, the so-called “Puckelin” materials, which date back to the beginning and mid-20<sup>th</sup> century, have been reported for consolidation purposes. [9] In the second half of the 20<sup>th</sup> century, a number of synthetic products, such as acrylic and vinyl polymers, has been abundantly used as adhesives or consolidants of paintings. [10,11] These products were chosen after prevalently considering preservation requirements, such as

effectiveness, compatibility with the object and reversibility. However, other important aspects, such as waste disposal and toxicological risks for the environment, were neglected. [12] In addition, the application of most of these products requires the use of volatile organic solvents, such as white spirit, xylene or toluene, most of which are recognized as being potentially carcinogenic. [13] These toxicological risks for the restorer can be reduced by using solvents with lower toxicity, such as ethanol or acetone. [14] In recent times, a notable improvement has been achieved thanks to the development of synthetic polymers prepared as water emulsions. [15] This water-organic system overcomes some drawbacks of traditional application methods using organic polymers. [16] Nevertheless, other issues related to either the stability of these polymers over time or their restricted applications in polychromed sculptures and paintings due to the sensitivity of these artworks to moisture content are still pending. [12] It is well-known that wood used as the support of polychromies in architectural ornaments is a hygroscopic material whose mechanical properties can notably change with moisture content. [5] Thus fluctuations in environmental relative humidity can concomitantly result in dimensional changes that lead to the appearance of cracks in the more rigid grounds and in applied paint layers. [5] Additionally, moisture content favors the formation of colonies of specific microorganisms. In terrestrial environments, wood is degraded mainly by fungi, whereas alteration is due prevalently to bacteria in aquatic and wet burial environments, where availability of oxygen is low. [17]

In parallel to the development of improved synthetic materials for restoration purposes, an interesting discussion has started in recent years to promote the use of sustainable green alternatives for the cultural heritage field. [12] This new green approach not only includes the used products, but also all the phases that characterize a restoration project; in other words, complete holistic “green” restoration is proposed.

[12] Concerning adhesives, focus has been placed on developing new “green” or “eco-friendly” formulations characterized by their low ecotoxicity and suitable life cycle assessment (LCA), and their lower impact on operator health, which agrees with national legislation and international regulations. [18] Some of these new methodologies are based on polymeric substances synthesized by living beings that are generically termed biopolymers. [19] The use of biopolymers in restoration tasks may also offer advantages in reversibility terms. [12] Among the biopolymers proposed in the last few decades as adhesives of art objects, we can mention the seaweed mucilage *JunFunori* or *Funori* obtained from the alga *Gloiopeltis furcate* [20,21], starch from wheat or rice, widely used in East Asian [21] or animal glues [21,22] prepared by heating the collagen protein present in connective tissues of mammals and fish. [23]

Technical gelatin is the purified active component of collagen glues obtained from skin or bone sources. [23] This biopolymer is a hydrocolloid that consists of a heterogeneous mixture of water-soluble proteins with a high average molecular mass. [24] When the aqueous dispersion is subjected to drying, a three-dimensional network forms with zones of intermolecular microcrystalline junctions. [25] Macroscopically, this microstructure results in the formation of a thermo-reversible film. [26] Nevertheless, the dehydration of this system may excessively increase stiffness and brittleness [27] that, consequently, reduces this material’s ability to adhere and consolidate paintings. This problem can be solved by adding a plasticizer that increases workability, improves flow, extendibility and flexibility to the gelatin-based adhesive, and also reduces shrinkage. [28-30] Polyols (glycerol, propylene glycol, diethylene glycol and ethylene glycol) are effective plasticizers of gelatin films used in pharmaceutical applications, food (confectionery, jellies, ice cream) as rubber

substitutes, paper sizing, adhesives, photographic plates and films, etc. [25,28-39] In particular, glycerol is a hydrophilic plasticizer that acts by promoting softness and flexibility. [28,29] If water is present, glycerol holds it, but if little or no water is present, glycerol acts as plasticizer as a result of its solvent power, low volatility and noncrystallinity. [30] This plasticizer has also been used in industrial materials, such as cellophane, paper products, cork and gasket compounds, glues, textiles and printing supplies. [40] Glycerol occurs naturally in a combined form in glycerides, which are the main components of animal and vegetable fats and oils. [41] Large amounts are produced as by-products while manufacturing soaps, oleochemicals or biodiesel. [41] Glycerol is also characterized by its low toxicity and is, therefore, suitable for use in the food industry as this product is on the FDA's Generally Regarded As Safe (GRAS) list. [42] Thus applications of glycerol as a plasticizer also include a varied type of suitable edible products for human consumption, such as candy, cough drops and cigarette tobacco, as well as other products like cosmetic creams, lotions, capsules and toothpastes. [40]

According to Hueck, biodeterioration is defined as any undesirable change in the properties of a material caused by the vital activities of organisms. [43] Biodeterioration of gelatin-based materials can take place due to microbial activity on paintings as this organic material is a potential source of carbon and energy for microorganisms' growth. Specifically, fungi produce enzymes and organic acids that cause the material to breakdown. In particular, the fungi of *Alternaria*, *Aspergillus*, *Cryptococcus*, *Penicillium*, *Cladosporium*, *Mucor*, *Phoma* and *Trichoderma* genera and the bacteria of the *Bacillus*, *Kocuria*, *Pasteurella*, *Sphingomonas* and *Staphylococcus* have been found to be responsible for the biodeterioration of gelatin used in old photographs. [44-46] The fungi of *Alternaria*, *Aspergillus*, *Aureobasidium*, *Chaetonium*, *Cephalosporium*

(*Acremonium*), *Cladosporium*, *Eurotium*, *Fusarium*, *Mucor*, *Penicillium* and *Spicaria* genera [47,48] and the bacteria of the *Arthrobacter*, *Microbacterium*, *Bacillus*, *Paucisalibacillus*, *Sporosarcina*, *Streptomyces* and *Virgibacillus* genera [49-51] have been found active on oil paints, which has resulted in the detachment of paint layers promoted by the excretion of aggressive metabolic products with acidic properties and the production of extracellular enzymes. [50] Wood-rotting fungi cause massive irreversible damage to the wood supports of wooden artworks. [47] Fungi genera such as *Alternaria*, *Aspergillus*, *Chaetomium*, *Chrysonilia*, *Cladosporium*, *Drechslera*, *Exophiala*, *Eurotium*, *Fusarium*, *Mucor*, *Mycelia*, *Penicillium*, *Rhizopus*, *Rhodotorula*, *Trichoderma* and *Verticillium* occur frequently in wooden objects. [48,52]

In such instances, addition of a biocide to the adhesive can be a suitable strategy to prevent the microbiological contamination of art objects. Prevention of biodeterioration of artworks has been practiced since ancient times. Ancient Egyptians knew the biocidal properties of some natural products, such as cedar tar oil, which was used in their embalming practices. [53] Leonardo da Vinci prepared wood supports by applying arsenic, mercury chloride and basic copper acetate to prevent staining. [54] Since recent times biocides have been frequently included in commercial binders, coatings and adhesives. [55] Despite biocides playing an important role in protection and mitigation strategies for retarding the biodeterioration of artworks, their use is not always completely efficient and stopping the biodeterioration of artworks is not completely achieved. [17] Moreover, they can result in some gloss change in painting. [56]

In parallel to the current use of chemical substances commercially available as biocides for cultural heritage the development of novel biocides of natural sources, which exhibit low or no toxicity, has been considered important and desirable. [12,57]



Given their natural origin and their lower toxic characteristics, essential oils can be considered ideal candidates to replace conventional synthetic biocides. [57,58] Thus studies on the antifungal and antibacterial properties of several essential oils have been carried out to assess the potential application of these natural products as atmospheric preservatives in archives and museums [59] as well as to conserve food [60] and wooden structures in buildings. [57] The essential oil extracted from the genus *Cymbopogon* spp. belonging to the Poaceae family (Gramineae) and including *Cymbopogon nardus* (L.) Rendle (Ceylon type) and *Cymbopogon winterianus* Jowitt (Java type) species, is popularly known by diverse names, which include fragrant grass, lemongrass, citronella grass or citronella. This essential oil is used in the food, cosmetic and pharmaceutical industries.[61,62] In the pharmaceutical industry, citronella oil is included in most mosquito repellent lotions suitable for human use.[63] Recent studies have also demonstrated that citronella oil performs antibacterial, [64] antifungal [59,60] and antioxidant action. [65] In particular, this essential oil has a proven inhibitory effect on the growth of *Aspergillus*, *Eurotium*, *Penicillium* and *Trichoderma* fungi. [66-68] The biocide mechanisms of essential oils are scarcely known and it is considered that their antimicrobial activity is not attributed to a single specific mechanism. [69] Nevertheless,  $\alpha$ -citral and  $\beta$ -citral enhanced by myrcene [70] and limonene, [71] geraniol,  $\alpha$ -terpineol, [71 ],  $\alpha$ -pinene, [ 66,71]  $\beta$ -pinene, citronellal, linalool [66] and citronellol [67], have been identified as the main bioactive compounds present in citronella oil. A former analytical study conducted by the authors on citronella oil of the *Cymbopogon nardus* species has demonstrated that this essential oil is composed of over 50 terpenoids (monoterpenoids mainly and sesquiterpenoids), including citronellal,  $\beta$ -citronellol, geraniol,  $\alpha$ -citral,  $\beta$ -citral, citronellyl acetate, geraniol acetate, dl-

limonene,  $\alpha$ -pinene, linalool, eugenol,  $\beta$ -elemene,  $\alpha$ -muurolene,  $\alpha$ -cadinene,  $\delta$ -cadinene, elemol, germacrene D-4-ol,  $\tau$ -eudesmol,  $\tau$ -cadinol and  $\alpha$ -cadinol, myrcene,  $\alpha$ -thujene,  $\alpha$ -pinene, camphene,  $\beta$ -phellandrene,  $\alpha$ - phellandrene,  $\alpha$ -terpineol and 2- $\beta$ -pinene. [72]

All compounds that are used as biocides have to be approved by the respective authorities to guarantee basic protection for the environment and safe use conditions for operators. The median lethal dose,  $LD_{50}$ , of the natural citronella oil of Ceylon type (*Cymbopogon nardus*) used herein is over 5000 mg.kg<sup>-1</sup> (acute oral and acute inhalation) and over 2000 mg.kg<sup>-1</sup>(dermal). [73] These values enable citronella oil to be considered a category 5 substance according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). [74] The use of citronella oil is currently authorized as a biocide of a herbicide type in the European Union. [75] The Taiwan Food and Drug Administration (FDA) has registered the citronella oil of Ceylon type as a natural species. [76] The citronella oil of Ceylon type is included by the US Department of Health and Human Services in the Generally Regarded As Safe (GRAS) list for human consumption in commonly used quantities. [77]

The presented study focused on evaluating the efficiency of a gelatin-based adhesive plasticized with glycerol, which also includes citronella oil for the consolidation of paintings. [72] The combination of gelatin, glycerol and citronella oil, a natural polymer, a low-toxicity plasticizer and a natural biocide of an essential oil type, respectively, provides the desirable eco-friendly characteristics for a new adhesive. The polychrome decoration applied to different parts of the architectural complex of the Longshan Temple in Lukang (18<sup>th</sup> century, Taiwan) was chosen as the case study.

Seven laboratory tests were done to characterize the adhesive on a series of test specimens that simulated the artistic technique used in the polychromed architectural

ornaments of the Buddhist Temple. The workability of the adhesive was characterized by these tests: a) viscosity measurement and b) drying curves. The barrier and diffusive properties of the adhesive applied to polychromies were characterized by these tests: c) moisture content determination and d) water vapor permeability. The mechanical properties of the adhesive were tested by: e) a mechanical test, while the adhesion properties were evaluated by: f) an adhesion test.

The present research aimed to propose a new adhesive based on a natural product, gelatin, this being a commonly used material by microorganisms as a potential source of carbon and energy. Consequently, this material is very sensitive to colonization by microorganisms. The incorporation of this adhesive to polychromies could, thus, constitute an additional source of infestation by microorganisms. In order to reduce the possible growth of microorganisms, a biocide was included in the adhesive formulation. The effectiveness of the biocide included in the adhesive formulation to prevent growing and spoilage of the fungi of *Aspergillus* and other genera that are highly active on gelatin, as previously described, was evaluated by: g) susceptibility to fungi colonization tests.

After this first series of experiments run on laboratory specimens, a second series of field tests was performed in which the adhesion treatment was carried out *in situ* on detached fragments of the original polychromies in order to perfectly reproduce real conditions. Nanoindentation-atomic force microscopy (NI-AFM) analyses [78] were performed on the microsamples excised from both the treated and untreated original fragments of polychromies. This technique can be considered micro-invasive as the analysis can be performed on microsamples of a few  $\mu\text{m}^3$ . This technique provided valuable data about the enhancement of mechanical properties due to the *in situ* conservation treatments, with minimum damage to monuments.

## Experimental

### *Description of the polychromies of the Buddhist Longshan Temple in Lukang*

Lukang is a city located in the Northwestern part of the island of Taiwan. Taiwan is characterized by a transition subtropical-to-tropical climate, with an annual average temperature of 22°C, temperature fluctuations between 37°C in August and 10°C in January and February, and an average relative humidity of 79%. The island is strongly affected by monsoons, which form in May and June, with high precipitation values (i.e. 539.5 mm in 2017). [79] Another characteristic of Taiwan is its location in a seismic region and, on average, some 16000 earthquakes of different magnitudes are recorded by the Taiwanese Seismic observatories every year. [80] These earthquakes can increase fissures on polychromies. The microclimate of the Buddhist Longshan Temple of Lukang is determined by its location in the center of the city, which encompasses 39.46 km<sup>2</sup> and has a population of 86779 inhabitants. The temple is opened daily and thousands of devotees arriving with offerings that consist of burning incense candles. This practice increases the pollutants in the atmosphere inside the temple. These environmental conditions favor the prevalent growth of the microorganisms of genera *Penicillium* and *Aspergillus* in Taiwanese wooden artworks. [81]

Taiwan's temples combine architectural local style and different styles from the Southern Chinese mainland, which include sumptuous polychrome decoration on different building parts made of wood, such as doors, coffered ceilings or columns. [82] Some botanical wood species have been traditionally used in Taiwanese temples: *Cunninghamia lanceolata*, the so-called China-fir, which belongs to the *cupressaceae* family; *Cinnamomum camphora*, which belongs to the *taxodiaceae* family, has been extensively employed during the Qing dynasty period (1683-1895). [83,84] During the

Japanese colonial period (1895-1945), the Formosan cypress (*Chamaecyparis formosensis*), [83] Formosan michelia (*Michelia compressa* (Maxim.) Sargent), *Machilus thunbergii*, China berry (*Melia azedarach*) and Taiwan zelkova (*Zelkova serrata*) were used. [84]

The Buddhist Longshan Temple in Lukang (Figure 1a), devoted to the Guanyin (Goddess of Clemency), was built in 1786. According to historical sources, a number of successive interventions have been made since the temple was erected until the present-day, the most recent of which was made by the local master, Mr. Kuo Tsingling, between 1956 and 1964.[82] The present study focused on the polychromies applied in four different architectural elements: a) the beams in the Main Palace (*Zhang-dian*), which date back to 1829; b) the coffered ceiling on the Stage (*Zau-jing*) (Figure 1b) and c) the Door (*Wu-men*) (bottom of Figure 1b), both located in the Main Access and date back to 1958; d) the columns in the Main Palace (*Tzén-díen*), which date back to 1829 and 1958. A scheme that illustrates the sampling points is found in Figure 2.

A complete analytical study performed on the temple has provided knowledge about the artistic technique and the materials used in the polychromies (Figure 3). [85,86] The outer paint layer consisted of a pigment (vermilion, ochre yellow, ultramarine blue and, less frequently, chrome oxide green) bound with tung oil. An inner ground was applied underneath, prepared with chalk (polychromies date back to 1956, 1964) or natural earth (polychromies date back to 1829), bound with porcine blood and tung oil, according to the traditional Asian technique. [87] Eventually, an imprimatur made with lithopone and tung oil was found (polychromies date back to 1956, 1964). A technical examination of the polychromies has evidenced that paint flaking and falling from the ground and the flaking and falling of the entire polychromy from the wood support are the most frequent alterations. Flaking usually leads to

polychromies warping, cracking and, finally, dropping (Figure 4). For this reason, the application of an adhesive that provides cohesion and fixes the detached fragments of polychromy strata was considered the most suitable intervention treatment.

### ***Reagents and materials***

Technical gelatin made of animal bones, probably of bovine origin, was purchased from CTS® (Spain). Citronella oil (*Cymbopogon nardus* Rendal) was supplied by Anshin camphor oil, Ltd. (Hsingchu, Taiwan). Glycerol (food grade,  $\geq 99.5\%$ ) was purchased from Guinama® (Spain). Chalk was supplied by Tǎi yǔ Industrial Co., Ltd. (Taipei, Taiwán). Porcine blood was purchased in the Lukang abattoir, tung oil (*Aleurites foraii*) was supplied by Jin iǒn sèin Paint Co., Ltd. (Changhua, Taiwán). Ultramarine blue, iron oxide yellow, vermilion and lithopone were supplied by Kremer Pigmente (Germany).

### ***Adhesive formulations***

Two different adhesive formulations of gelatin plasticized with glycerol, including citronella essential oil as a biocide agent, were prepared. Formulation A was proposed as the most suitable adhesive to adhere and consolidate paint layer flaking and falling from the ground. Formulation B was proposed as the most suitable adhesive to adhere and consolidate the polychromy fragments (including all the strata) detached from the wood support.

Dispersions of gelatin were prepared in a first step by dispersing gelatin in water with constant stirring at 800 rpm and 80°C for *at ca* 12 h at room temperature to obtain a homogeneous solution so that the concentration of the proteins in the final film-forming solutions would be 10 (w/w)% (dispersion A<sub>0</sub>) and 25 (w/w)% (dispersion B<sub>0</sub>). The pH value of the gelatin aqueous solutions was 7±0.5 at 22°C. Separately citronella oil was emulsified in glycerol at concentrations of 0.5 (w/w)% for dispersion A<sub>0</sub> and 1

(w/w)% for dispersion  $B_0$ . Afterward, the citronella-glycerol emulsion (which we refer to hereafter as plasticizer or modified plasticizer) was added to the gelatin dispersion by stirring at 700 rpm for 15 min to remove air bubbles. Thus the plasticizer:gelatin ratios of 0.3 and 0.5 (w/w) were respectively obtained for the adhesive formulations  $A$  and  $B$ , and each one was prepared from dispersions  $A_0$  and  $B_0$ . Adhesive dispersions  $A$  and  $B$  were applied to a series of reconstructed paint and polychromy specimens by spreading the product using a thin brush.

### ***Reconstructed paint and polychromy specimens***

The reconstructed paint and polychromy specimens were prepared depending on the property to be evaluated.

A first series of specimens (A) (see Figure 5) was prepared to assess the effectiveness of the adhesion between the polychromy and the panel support using adhesives  $A$  and  $B$  (adhesion test). Ground layers were prepared with chalk (60 wt%) and bound with porcine blood (30 wt%) and tung oil (10wt%) without adding siccativ. They were applied on mylar sheets and stored for 1 month at room temperature ( $22\pm 1^\circ\text{C}$ ) and RH ( $55\%\pm 2\%$ ). Next ground layers were removed from the mylar sheets and were adhered to wood panels (*Cunninghamia lanceolata*) using adhesives  $A$  and  $B$ . Specimens were tested after a second 1-month drying period under the above laboratory conditions.

In order to assess the changes in the mechanical, moisture content and water vapor barrier properties of the polychromies after applying the adhesive to the detached paint layers, a series B of reconstructed oil paint films was prepared by binding three selected pigments (vermillion, ochre yellow and ultramarine blue) with tung oil at 30 % wt (see Fig. 5). Paints were then spread onto a sheet of mylar to facilitate film recovery so that rectangular strips measuring 10x30x0.1 mm were formed. Film

thickness was measured by a micrometer with a sensitivity of 0.001 mm. Five to ten thickness measurements were taken per film, and averages were taken as the result. After a 1-month drying period in the above laboratory conditions, the reconstructed oil paint films were brush-coated with both adhesives *A* and *B*, separately. The untreated paint films were reserved for comparison results. The treated films were tested after a 1-month drying period under the above-mentioned conditions.

A third series *C* of specimens was prepared to perform the susceptibility to fungi colonization tests (see Fig. 5). Different specimens were prepared with the lithopone, ultramarine blue, vermilion and iron oxide yellow pigments, mixed with tung oil, which were applied to a ground layer prepared with chalk (60 wt%) bound with porcine blood (30 wt%) and tung oil (10 wt%), according to the traditional Asian technique. A wood panel (*Cunninghamia lanceolata*) was used as a support. After a 1-month drying period under laboratory conditions, specimens were treated with both adhesives *A* and *B* and stored for a second 1-month drying period.

In parallel to this series of reconstructed polychromy specimens, a fourth series *D* of the films of adhesives *A* and *B* were prepared with and without adding citronella oil to assess the biocidal efficiency of citronella oil to prevent the biodeterioration of the adhesive (see Fig. 5).

## ***Experimental techniques***

### *Viscosity measurements*

The dynamic viscosity of the freshly prepared water dispersions was determined by a Visco Star Plus R digital viscometer supplied by (Fungilab, Spain). This instrument is a rotational viscometer of the Brookfield type that can measure the viscosity of liquids in accordance with ISO 2555 standard “Plastics - Resins in the liquid state or as emulsions



or dispersions. Determination of apparent viscosity by the Brookfield Test method”.

[88] In this method a spindle or disc is driven at a constant rotational frequency in the product under study. The resistance exerted by the fluid on the spindle, which depends on the product's viscosity, causes a torque, as indicated by a suitable meter. Spindles with diameters 46.93, 34.69 and 27.3 mm, which provide a suitable shear speed of 200 rpm, were used in adhesives *A* and *B* according to their different viscosities. Triplicate measurements per formulation were taken at different temperatures within the 25-45°C range.

#### *Drying curves*

Graphs showing weight loss *versus* drying time from the thin films of adhesives *A* and *B* spread on glass slides were obtained. Trials were run at room temperature (22°C) and the weight of specimens was recorded on a digital balance (Precisa XT120A, supplied by Precisa, Zürich, Switzerland) at an accuracy of 0.0001 g until the change in weight became constant.

#### *Moisture content determination*

The moisture content (*MC*) of each film was determined by the method proposed by Mei and Zhao. [89] Small reconstructed paint film specimens, both untreated and treated with adhesives *A* and *B*, were cut (approximately (1×1) cm), placed on previously dried aluminum slides, and dried inside a laboratory oven (Raypa, supplied by Espinar SL, Madrid, Spain) that contained desiccants at 105±2°C for 24 h. After drying, specimens were left inside the oven and cooled to room temperature. The weights of the aluminum slides were recorded before and after drying by a digital balance (Precisa XT120A) at an accuracy of 0.0001 g. Moisture content (*MC*) was calculated from:

$$M = \frac{M_i - M_f}{M_i - M_d} 100 \quad (1)$$

where  $M_i$ ,  $M_d$  and  $M_f$  are the weights of an aluminum slide with a film specimen before drying, an empty aluminum slide, and an aluminum slide with a film specimen after drying, respectively. Three replications of each film treatment were used for the *MC* calculations.

#### *Water vapor permeability (WVP)*

The *WVP* of the films was determined gravimetrically in three replicates according to the ASTM E96-01 method. [90] Films were sealed with paraffin and an O-ring rubber on glass cells, which were 2 cm (o.d.), 2 cm (i.d.), 2 cm (depth), and were filled with silica gel. Cells were then placed inside a desiccator that contained saturated  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{HNO}_3$  solution 52 % $\pm$ 2 *RH*, which was maintained at 22°C. The *RH* gradient was 50:0 (*RH* outside: *RH* inside cells). Cells were weighed ( $\pm$ 0.0001 g) at different times until the change in weight became constant.

#### *Mechanical properties*

Ultimate strength and elongation and elastic modulus (*EM*) were measured by a microcomputer-controlled electronic testing machine (Deben-Gatan Microtest). The test specimens were conditioned at 22°C and at 50 $\pm$ 3% *RH* in a desiccator that contained magnesium nitrate saturated solution ( $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{HNO}_3$ ) for at least 2 days prior to the analysis. The measurement was taken immediately after the specimen was removed from desiccators. The initial grip separation was set at 10 mm, and the rectangular strip

specimen was 30 mm long, 10 mm wide and  $0.1 \pm 1\%$  mm thick. The crosshead speed was set at  $0.4 \text{ mm} \cdot \text{min}^{-1}$ . The mechanical parameters of the strips were measured in the static mode. The measurements for each film type were repeated at least 3 times, and the averages were taken as the results.

#### *Adhesion test*

Measurements were performed by using an electronic adhesion tester KN-10 (Neurtek S.A., Eibar, Spain) and by taking into account the UNE normative procedures for paintings and coatings, as well as mortars (UNE-EN ISO 4624:2003, UNE-EN 1015-12). [91] The adhesion test was performed on five replicates for each type of tested adhesive. The test was performed as follows: a holder was adhered to the surface of the specimen to be tested by making a cut in the paint film around it to isolate it. The instrument was placed on the surface and, by turning the wheel, a higher starting force was progressively applied. Upon detachment, the electronic display showed the applied force. Scale: from 0.05 to 10.00 kN. Resolution: 0.01 kN.

#### *Susceptibility to fungi colonization tests*

The susceptibility to fungi colonization tests aimed to evaluate the efficiency of the citronella oil included in the formulation of the adhesive in inhibiting the growth of fungi on the adhesive. Two separate experiments were planned, in which the efficiency of the biocide was tested on the specimens of the pure adhesive formulations and, secondly, on the specimens to which the adhesive was applied to polychromies. The strains of fungi used in this study were selected according to prior studies that had identified the microorganisms responsible for gelatin, paintings and wood biodeterioration described in a previous section. [44-48,52]

The protocol for testing fungal activity on consolidated specimens was as follows:

1) Preparation of fungal spores. Fungi were grown on PDA medium and tubes were incubated at 28°C for 6-8 days. Cultures were resuspended in 4 mL of Tween 80 (0.1% in distilled water) and vigorously stirred. Tube content was filtered under sterile conditions through a funnel using glass wool to retain mycelia. Spores were collected by centrifugation at 13000 x g and were then washed with 1 mL of distilled water (x3) and finally resuspended in 1 mL of ultrapure distilled water.

2) Inoculation and incubation of culture media. The films obtained by drying adhesives A and B, as well as the treated and untreated reconstructed polychromy specimens, were used as supports of cultures. Next 20 µL of the spores suspension, adjusted to a concentration of 10<sup>5</sup>-10<sup>6</sup> spores.mL<sup>-1</sup>, were inoculated in each support. The following strains from the *Colección Española de Cultivos Tipo* [92] were inoculated: *Aspergillus niger* (CECT-2088), *Chaetomium globosum* (CECT-2701), *Fusarium oxysporum* (CECT-2154), *Mucor rouxii* (CECT-2655), *Penicillium chrysogenum* (CECT-2655) and *Trichoderma pseudokoningii*. (CECT-2937). Specimens were incubated at 28°C and 75% RH in the darks for 28 days. Fungal colonization progression was monitored by stereomicroscopic observations at the incubation times of 1, 7, 14 and 28 days. A magnifying-glass Wild Heerbrugg and a Nikon Eclipse TS100 microscope equipped with a Nikon DS-5M digital camera were used.

#### *Nanoindentation-atomic force microscopy*

NI-AFM is an advanced instrumental technique that allows the differentiation of mechanical properties of materials on the nanoscale. To determine the elastic modulus (*EM*) in the microsamples of the original polychromies before and after applying the adhesives developed in this work, a Multimode AFM (Digital Instruments VEECO

Methodology Group, USA) with a NanoScope IIa controller was used, which was equipped with a J-type scanner (max. scan size of (150x150x6) mm). The *EM* of each sample was obtained in the scan asyst peak-force quantitative nanomechanical mode (*QNM*) with a tip Scan Asyst (Bruker) ( $k=3\text{N.m}^{-1}$ ). The *EM* values were calculated by the Bruker Nanoscope 1.40 Analysis software. Figure 6 shows a map of material deformation as the indenter (the cantilever tip) tracks the surface of the sample. From this experimental retract plot, the value of the Reduced Elastic Modulus ( $E_r$ ) was obtained for each pixel by mathematically fitting the retract curve region in Figure 6. This process was carried out using the Derjaguin, Muller, Toropov (*DMT*) model [93], which established the equation:

$$F_{\text{tip}} = \frac{4}{3} E_r \sqrt{Ra^3} + F_{\text{adh}} \quad (2)$$

where  $F_{\text{tip}}$  is the force on the tip,  $F_{\text{adh}}$  is the adhesion force,  $d$  is the tip-sample separation and  $R$  is the radius of the tip end. In NI-AFM, the geometry of the indenter must be well known to obtain accurate results. Thus tip radius  $R$  is calculated by an indirect method using a polystyrene sample with a known *EM* of 2.7 GPa. The indirect method includes the prior determination of deflection by pressing the tip onto a Sapphire disc, and the spring constant of the cantilever by performing a thermal tune. The *EM* of each sample is calculated from reduced modulus  $E_r$ , given by the expression:

$$E_r = \left[ \frac{1-\nu_t^2}{E_t} + \frac{1-\nu_s^2}{E_s} \right]^{-1} \quad (3)$$

where  $\nu_t$  and  $E_t$  are the Poisson's ratio and *EM* of the tip and  $\nu_s$  and  $E_s$  are the Poisson's ratio and *EM* of the sample. The applied method assumed that the tip modulus

$E_t$  was much larger than sample modulus  $E_s$  so that the former came close to infinite.

Thus Equation (3) was reduced to:

$$E_t = \left[ \frac{1 - \nu_s^2}{E_s} \right]^{-1} \quad (4)$$

Sample elastic modulus  $E_s$  can be calculated using the sample Poisson's ratio. This last parameter generally ranges between about 0.2 and 0.5 (perfectly incompressible). A value of 0.3 was used in the present study. This value is recommended for samples with an  $EM$  that falls within the  $1 \text{ GPa} < E_s < 10 \text{ GPa}$  range. In this way,  $EM$  value ( $E_s$ ) was collected automatically by the instrument for each pixel of the image. Images with scan sizes of  $(1 \times 1) \mu\text{m}$  and  $(5 \times 5) \mu\text{m}$  that consisted of 256 lines by pixels, and taken at a scan rate of 0.4-0.5 Hz, were created for each sample during one same scan, which allowed the morphological data and  $EM$  to be collected.  $EM$  was calculated as the average value of the individual  $EM$  measured in each pixel of an image. At least three images were acquired in each sample. Repeatability was at ca. 5%.

Both adhesives  $A$  and  $B$  were applied in an *in situ* treatment on the areas of the Longshan Temple polychromies, where the blue paint layer was partially detached from the ground. The blue areas of the original polychromies were selected to perform this series of *in situ* trials as they were notably damaged in the temple (see Fig. 4). A second series of trials was carried out in the areas of the polychromies where the paint film had been completely loss and the ground layer was partially detached from the wood support.

### *Field emission scanning electron microscopy (FESEM)*

Secondary electron images of the reconstructed paint films were obtained using a Zeiss model ULTRA 55. The 0.8 kV accelerating voltage was applied in the electron source. Images were acquired with a secondary electron detector Everhart-Thornley model. Samples were directly observed to avoid interference with the particulate structure of the coating element used to eliminate charging effects. The other operating conditions were: working distance of the detector 2.9-4.0 mm, pressure in the sample chamber,  $6.4 \cdot 10^{-5}$  Pa, gun pressure,  $9.2 \cdot 10^{-8}$  Pa.

## **Results and Discussion**

### *Workability properties*

#### *Viscosity of the adhesive dispersions*

Figure 7 shows the values of viscosity measured within the temperature range of 25-45°C in freshly prepared adhesive dispersions *A* and *B*. Both studied adhesive dispersions exhibited an exponential dependence of viscosity on temperature. As we can also see, adhesive dispersion *A*, prepared at lower gelatin concentration and with a lower plasticizer:gelatin ratio, obtained lower viscosity values (59-42 mPa.s) than adhesive dispersion *B*, with a high gelatin concentration and plasticizer:gelatin ratio (700-100 mPa.s). Prior studies have demonstrated that the plasticization of a biopolymer results in lowering the glass-rubber transition temperature of the polymer concomitantly with the reduction in viscosity. [30] The increase in the polymer concentration resulted in an increased dispersion viscosity. With the two studied adhesives, the higher gelatin concentration was the predominant effect that was contributed to the higher viscosity value obtained in adhesive *B*.

Despite adhesive dispersion *B* exhibiting a notably higher viscosity value, the viscosity values of both adhesives were notably lower than those found for the adhesives commonly used in the consolidation of paintings based on synthetic polymers. Thus the viscosity reported for acrylic polymer Paraloid B72 fell within the 200-980 mPas.s range (sol. at 40% in acetone, xylene, toluene at 25°C), and the viscosity of acrylic and PVAc emulsions was higher than 1000 mPas.s (20°C) and 2000 mPas.s (20°C), respectively.[94]

#### *Drying curve*

Figure 8 shows the drying curves obtained for adhesives *A* and *B*. A shorter drying time was found in adhesive *A*, prepared with the more diluted gelatin dispersion and at a lower plastizicer:gelatin ratio, which underwent 78% water loss after 30 min and reached a constant weight after 39 min and retained 0.13% of water. Adhesive *B* underwent 28% water loss after 30 min and a constant weight was reached after 124 min by retaining 0.79% of water. The latter has been associated with a major glycerol content, which enables more water to be absorbed in the dry film. [95-98]

The drying times reported for both adhesives *A* and *B* fell within the workability range of 30-300 min, as recommended for conservation treatments on paintings and polychromies. [10,99,100]

#### ***Water absorption and barrier properties***

##### *Moisture content determination*

Table 1 shows the *MC* values for both the treated and untreated paint films. It is noteworthy that the deviation of the results due to the possible loss of the more volatile



compounds of the citronella oil in the adhesive specimens can be neglected as the total amount of biocide present in the film was 10 orders below the calculated standard deviation for the experimentally obtained *MC* values.

The values obtained for untreated films, which fell within the 3.69-5.38% range, were low due to the hydrophobic behavior of the polymerized tung oil used as a binding medium, which prevents water vapor absorption. The found differences were due mainly to the microstructure of the formed paint films and the intrinsic hygroscopic nature of pigments. Figure 9 shows the secondary electron images of the cross-sections of the films prepared with the vermilion, ultramarine blue and iron oxide yellow pigments obtained by FESEM. The film prepared with vermilion pigment appears to have an amorphous texture due to the abundant binding medium (Fig. 9-a). In contrast, the films prepared with ultramarine blue and iron oxide yellow exhibited a higher pigment volume concentration (*PVC*) (Fig. 9-b,c). The iron oxide yellow pigment presented a high *PVC* with a wide distribution of particle sizes that ranged from a few nm to a diameter of 30  $\mu\text{m}$  (Fig. 9-c), whereas ultramarine blue presented a narrower particle size distribution with grains less than 5  $\mu\text{m}$  (Fig. 9-b). These two films were underbound, which implied air gaps around the pigment particles (see the arrows in Figs. 10-a,b). The presence of air gaps surrounding the pigment particles affects a number of the paint film's physical properties, such as reflectance due to variations in the refractive index among the air, binding medium and pigment particles. [101] Porosity is also affected and the paints with a high *PVC* are more porous due to the presence of air in the paint film. [101] The higher porosity of the yellow and blue films justified the higher *MC* values over 5% found in these films. Mechanical properties were also affected (*vide infra*). In particular, the extendibility and strength of the paint film can decrease and result in lack of cohesion within the paint film, which is prone to

cracking.

A higher *MC* value than that exhibited by the vermilion paint film was found in the vermilion films treated with adhesives *A* and *B*. The increased *MC* value was associated with the water absorbed by the adhesive due to the hydrophilic nature of the gelatin and the ability of glycerol to absorb water. The slightly higher *MC* value of 4.72% reported in the film treated with adhesive *A* was ascribed to the film's better ability to absorb water due to the open polymer network of the dried adhesive prepared with a diluted gelatin dispersion. [72]

The opposite behavior to the treated vermilion film was observed with the treated iron oxide and ultramarine blue films, which had lower *MC* values than their untreated counterparts. These results suggested that the adhesives exerted an effective consolidation action on these paint films by filling gaps and reducing the film's microporosity. This effect was more intense in the iron oxide yellow film ( $\Delta MC$  40% and 41%, respectively, for adhesives *A* and *B*), which was associated with a major content of air gaps accessible to the adhesive in this paint film. The *MC* values found for both the films treated with adhesive *B* were slightly lower than those obtained in the films treated with adhesive *A*. This result, as was the case of the vermilion film, was associated with the *A* adhesive-treated film's better ability to absorb water due to the open gelatin network given the lower plasticizer content in this formulation.

#### *Water vapor permeability (WVP)*

The *WVP* found in the reconstructed oil paint films untreated and consolidated with adhesives *A* and *B* are shown in Table 2. The *WVP* values obtained for the paint specimens were notably low (0.095-0.063 g.mm.m<sup>-2</sup>.kPa.day), which was expected for the films consisting in a particulate solid pigment bound with a hydrophobic substance,

such as polymerized tung oil. The differences found between the *WVP* values obtained with the untreated paint films prepared with each pigment, as already described for the *MC* data, were associated with the diverse particle size distribution and the morphology of the pigments that determined a more or less compact packaging of pigment particles in the film on the micro- and nanoscale and, consequently, influenced the diffusivity of gases through the film. Figure 9-a shows a compact vermilion film with a low *PVC* and the almost complete absence of air gaps. The hydrophobic nature of polymerized tung oil prevented water vapor diffusion throughout the film and had an effective barrier effect, as evidenced by the lowest *WVP* value reported for this film. In contrast, the ultramarine blue film shown in Fig. 9-b and 10-a presented a structure with a dense packaging of pigment grains surrounded by thin air gaps. This structure favored water vapor diffusion, as evidenced by the high *WVP* reported in this film. The iron oxide yellow paint film shown in Fig. 9-c and 10-b was composed of goethite aggregates with densely packed varied morphologies (rounded or angular). Similarly to the ultramarine paint film, air gaps surrounded the goethite aggregates, which resulted in a slightly lower *WVP* value than that reported for the ultramarine blue film.

As also expected, the application of adhesives *A* and *B* led the *WVP* value of the paint films to lower as result of the decreased porosity caused by the consolidating action of the adhesives that filled air gaps and fissures. The comparison made between the *WVP* values obtained in the paint films consolidated with adhesives *A* and *B* ( $\Delta WVP$  within the 0.006-0.028 g.mm.m<sup>-2</sup>.kPa.day range for adhesive *A* and the 0.014-0.029 g.mm.m<sup>-2</sup>.kPa.day range for adhesive *B*) indicated that the adhesive *B* showed a slightly more reduced *WVP*, which was ascribed to the more effective filling of air gaps as result of the consolidation treatment. As with *MC*, the major glycerol content in this adhesive, which opens the polymeric network of protein chains, contributes to minimize the

reduction of *WVP* in this adhesive formed from a more concentrated gelatin dispersion. The comparison between the values obtained in the three different paint films indicated that the iron oxide yellow and ultramarine blue paint films exhibited more marked reductions in *WVP* after treatment (30% and 12%, respectively, for adhesive A, and 31% and 24%, respectively, for adhesive B), whereas the drop in the *WVP* value for the vermilion paint film was not so remarkable (10% for adhesive A and 22% for adhesive B). That was associated with the efficiency of the adhesives for filling air gaps in the iron oxide yellow and ultramarine blue paint films.

It should be noted that the reduction in the *WVP* values reported herein for the reconstructed paint specimens after the consolidation treatment could have a beneficial effect. First, filling air gaps helped improve film cohesion by gaining mechanical strength. [101] Second, water vapor diffusion through the paint film was reduced, and thus avoided alterations associated with the presence of water in paint film, such as hydrolysis of binding media (both the tung oil present in the paint layer and the blood protein molecules mixed with tung oil present in the ground).[102] Other negative effects that are frequently reported in aged oil and tempera paintings, such as growth of microorganisms and solubilization and migration of water soluble products, such as released short chain fatty acids, metal soaps and salts formed from pigments, could be restricted given the reduction in the diffusion of water throughout the paint film. [102]

### ***Mechanical properties: stress-strain testing***

Oil paint films can be considered a kind of particle-reinforced polymer in which pigment particles (the dispersed phase) are surrounded by a continuous phase, the matrix, constituted by the binding medium in a similar structure to that of artificially made large-particle composites or multiphase materials whose constituent phases are chemically dissimilar and separated by an interface. Therefore, the mechanical

properties and dimensional response of oil paint films are governed by the polymerized oil medium and are modulated by the interactions that take place between the pigment and the medium. [102] Previous research had shown that, in general, the stiffness and strength of oil paint films progressively increase during aging, while strain to failure decreases. [102] Figure 11 a-c represents the different stress-strain curves of the studied reconstructed oil paint specimens, together with those of different paint specimens treated with adhesives *A* and *B*. These graphs provide interesting insight into the role played by pigments in the mechanical properties of paint films, and also into their behavior after the consolidation treatment. The comparison of the stress-strain curves of the untreated oil paint films (see Figures 11 a-c) shows that the ultramarine blue and iron oxide yellow films exhibited typical linear elastic deformation behavior with *EM* values of 4.50 and 3.74 MPa, respectively (see Table 3). [102] This finding is characteristic of stiff oil paint films, whereas the vermilion paint film exhibited a less stiff behavior, as suggests by its lower *EM* value (1.39 MPa). [102] Concomitantly, the two former paint films displayed more ultimate strength values of 6.8 and 4.82 MPa, respectively, which contrasts with the lower value of 2.17 MPa reported for the vermilion film (see Table 3). The values of the ultimate elongations reported for the three films within the 1.54-1.59% range indicated that the distensibility of the untreated films was low.

This different mechanical behavior reported for the three studied oil paint films corresponded with their microstructure. As shown in Figure 9, the ultramarine blue and iron oxide yellow films had higher *PVC* values than the vermilion paint film. Hence this mechanical test evidenced that the higher pigment content, the stiffer the paint film becomes. The shape and size of pigment also contribute to film rigidity. The homogeneity in particle size of the ultramarine blue, unlike the larger particle size

distribution observed in iron oxide yellow and vermilion, contributed to this pigment being better packed in the film that exhibited the stiffest behavior.

The stress-strain graphs for the films after applying adhesives *A* and *B* are also shown in Figure 11 a-c. Addition of adhesive generally results in the increased ductility of the films characterized by a lower *EM*, with a reduction in the linear elastic region, followed by a continuous rise of load with an increase in strain until the film breaks. Adhesive *B*, prepared from a dispersion with a higher gelatin concentration and a higher plasticizer:gelatin ratio, gave a lower *EM*, longer ultimate elongations and maximum tensile stresses.

Figure 11-a illustrates that the iron oxide yellow films treated with adhesives *A* and *B* exhibited a tougher behavior than the untreated film, which is characterized by the stress-strain curves that exhibited a smaller linear elastic region followed by a large nonlinear elastic deformation region. The progressive reduction in their *EM* values to 1.87 and 1.09 MPa (50% and 71% of reduction in the *EM* value) for the films treated with adhesives *A* and *B*, respectively, corresponded to the higher plasticizer content in adhesive *B*. Higher ultimate strength and elongation were also achieved with adhesive *B*. Figure 11-b shows that the application of adhesive *A* to the vermilion film did not significantly modify the *EM* value, but resulted in enhanced film toughness that exhibited a nonlinear elastic deformation region, which expanded the curve and thus resulted in a greater ultimate elongation and strength. The effect of the higher plasticizer content in adhesive *B*, combined with the low *PVC* of the vermilion paint film, was evidenced in the film treated with this adhesive, in which *EM* notably reduced to 0.44 MPa. Concurrently, the stress-strain curves showed a short linear elastic deformation region, followed by a large nonlinear elastic deformation region. Greater ultimate strength and elongation were also reached with this treatment. Finally, Figure 11-c

showed the stress-strain curves reported for the ultramarine blue film treated with adhesives *A* and *B*. As seen, the application of adhesives in this film led to *EM* values of 3.43 and 3.16 MPa, which were lower than those reported for the untreated film. In both cases, the stress-strain curve presented a larger linear deformation region with a slight decrease in the slope in the final part, which resulted in greater ultimate strength and elongation, as for the iron oxide yellow and vermilion films.

The comparison of the results obtained for the three paint film types suggested that adhesives performed a more effective consolidation action on the iron oxide yellow film, for which the major decrease in the *EM* value was found. The stress-strain curves of this film treated with the adhesives had characteristic profiles of hard and tough materials. This result was associated with the more effective filling of gaps achieved in this film after treatment with the adhesive, as already evidenced with the notable decrease in *MC* and *WVP* achieved in this film. The lowest *PVC* of the vermilion film justified the softer and tough behaviors of this film after treatment with the adhesives. Finally, an improvement in mechanical properties is also reported for the stiffest ultramarine blue film treated with adhesives *A* and *B* with a reduction in the *EM* and ultimate elongation values.

#### ***Adhesion properties: adhesion measurements***

The adhesion test enabled the efficiency of the two adhesives to be evaluated when they were used for fixing the detached layers of polychromy to the wood support. The obtained results are shown in Table 4, where failure type (adhesive or cohesive) is also indicated. As seen, a higher plasticizer content (adhesive *B*) gave higher adhesion values (1.4 MPa). All the replicates exhibited adhesive breaking with over 85% of the area of the polychromy separated from the wood support after applying traction force, whereas only a 15-10% of the area of the polychromy layers displayed cohesive failure.

The best average value of the percentage area of the polychromy separated from the support exhibited by adhesive *B* was associated with the better ability of this formulation to not only adhere the polychromy to the support, but to also consolidate the ground layer, and to thus confer lost cohesive strength and avoid its cohesive breaking. From the painting conservation reversibility perspective, which consists in the ability of an adhesive to be removed from the substrate without damaging the latter, it is considered a basic property. Concerning adhesion junctions, better reversibility is achieved when junction breaking takes place as an adhesive failure because no original materials are lost in this way. [103]

#### ***Susceptibility to fungi colonization tests***

*A. niger* strain growth was observed on the film of the adhesives prepared with no addition of citronella oil as a control. In this case, the characteristic spots formed by the mycelia of these fungi were observed on the surface of the adhesive films (Figure 12). In contrast, no germination of spores was observed on either film formed with adhesives *A* and *B*. This result evidences the biocide efficiency of citronella oil for preventing the growth of fungi in the adhesive.

No growth of fungi was observed in either the treated reconstructed polychromy specimens or in the untreated ones. This result was attributed to the inhibitory properties exhibited by tung oil, as demonstrated in a recent study about the efficacy of drying oil-treated wood against wood-decay fungi. [104]

#### ***Nanoindentation-atomic force microscopy***

Table 5 summarizes the *EM* values obtained in the samples extracted before and after the *in situ* adhesion/consolidation trials. The *EM* values obtained by NI-AFM in the samples from the polychromies were in the same order as those for the 19<sup>th</sup>-century oil



paintings reported in other studies that focused on changes in nanomechanical properties while aging oil paintings. [105] These high values were associated with increased stiffness due to the natural aging of polychromies. Interestingly, the data in Table 5 suggest that the pictorial layers prepared with tung oil are stiffer than those (grounds) prepared with blood as a major component of the binder. The comparison of the  $EM$  values obtained in the samples from the temple door and the column, both before and after the consolidation treatment, with adhesives  $A$  and  $B$  indicated that application of the adhesive, irrespectively of its composition, led to a notable drop in the  $EM$  value of the pictorial layer. Interestingly, adhesive  $A$  is slightly more effective for improving the mechanical properties on the oil paint layer ( $\Delta EM = 1.23$  and  $1.25$  GPa), whereas adhesive  $B$ , with a higher plastizicer content, is more effective on the proteinaceous ground ( $\Delta EM = 0.58$  and  $0.77$  GPa). The latter is probably due to the greater effect of the plasticizer, which improves the flexibility of this protein-rich layer. The similarity between the blood used as a binder and gelatin, which favors the incorporation of the adhesive, could also contribute to better behavior of adhesive  $B$  when applied to the ground.

## **Conclusions**

The study carried out demonstrates that the developed gelatin-based adhesives, including glycerol and citronella oil, satisfactorily accomplish the basic requisites needed for the consolidation of paintings and polychromies:

- The workability properties of the proposed adhesives  $A$  and  $B$  are suitable, as indicated by the notably lower viscosity than those found for the adhesives commonly used in the consolidation of paintings and drying times within the recommended range.

- The water absorption and barrier properties evaluated by the *MC* and *WVP* tests suggest that the adhesive treatment, which fills the accessible fissures and voids present in paints on the microscale, can be beneficial to prevent alterations associated with the presence of water in polychromies.
- The incorporation of citronella oil, a natural product considered practically non-toxic according to international regulations, has demonstrated its effectiveness for the prevention of *Aspergillus niger* growing in proteinaceous adhesives.
- The adhesion properties evaluated by the adhesion test have proved the suitability of adhesives *B* and *A* for adhesion and consolidation purposes by exhibiting suitable reversibility properties.
- The improvement of the mechanical properties, by the enhanced flexibility and mechanical resistance of paints after adhesion treatments, has been confirmed by a mechanical test on laboratory specimens and by NI-AFM measurements on the real samples from the *in situ* treatments.

It can be concluded that, according to the set of tests carried out, adhesive *B* can be proposed as an alternative eco-friendly adhesive to adhere the complete polychromy of the Lukang temple flaking and falling from the wood support, whereas adhesive *A* can be proposed as an alternative eco-friendly adhesive for the paint layer flaking fragments from the ground.

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## References

- [1] Chelazzi D, Chevalier A, Pizzorusso G, Giorgi R, Menu M, Baglioni P. Characterization and degradation of poly(vinyl acetate)-based adhesives for canvas paintings. *Polym. Degrad. Stab.* 2014;107:314-320.
- [2] Carretti E, Dei L. Physicochemical characterization of acrylic polymeric resins coating porous materials of artistic interest. *Prog. Org. Coat.* 2004;49:282-289.
- [3] Berger G, Russell W. The new stress test on canvas paintings and some of their implications on the preservation of paintings. In: Committee for Conservation, 7<sup>th</sup> Triennial Meeting. Copenhagen: ICOM; 1984. pp.7-9.
- [4] Hayakawa N, Kigawa R, Nishimoto T, Sakamoto K, Fukuda S, Kimishima T, Oka Y, Kawanobe W. Characterization of furunori (aged paste) and preparation of a polysaccharide similar to furunori. *Stud. Conserv.* 2007;52:221-232.
- [5] Gettens RJ, Stout GL. *Painting materials. A short Encyclopaedia.* New York: Dover; 1966.
- [6] Cennini C. *Il libro dell' arte [The Book of Art].* Vicenza: Akal; 1982.
- [7] Mills JS, White R. *The Organic Chemistry of Museum Objects.* London: Butterworths; 1987. pp. 150.
- [8] Gómez ML. *La restauración: examen científico aplicado a la conservación de obras de arte.* Madrid: Cátedra; 1988.
- [9] Schönemann A, Eisbein M, Unger A, Dell'Mour M, Frenzel W, Kenndler E. Historic consolidants for wooden works of art in Saxony: an investigation by GC-MS and FTIR analysis. *Stud. Conserv.* 2008;53:118-130.
- [10] Horie, V. *Materials for conservation: Organic consolidants, adhesives and coatings.* Oxford: Elsevier; 2010.
- [11] Mehra VR. *Foderatura a freddo: I testi fondamentale per la metodologia e la pratica.* Padova: Nardini Editore; 2002.
- [12] Balliana E, Ricci G, Pesce C, Zendri E. Assessing the value of green conservation for cultural heritage: positive and critical aspects of already available methodologies. *Int. J. Conserv. Sci.* 2016;7:185-202.
- [13] Directive 2000/39/EC - indicative occupational exposure limit values. Available: <https://osha.europa.eu/en/legislation/directives/directive-2000-39-ec-indicative-occupational-exposure-limit-values>. [Accessed on 21/03/2018].

- [14] Silence P. How are US conservators being green? Results of polling AIC members. *Stud. Conserv.* 2010;55:159-163.
- [15] Learner TJS. *Analysis of Modern Paints*. Los Angeles: The Getty Conservation Institute; 2004.
- [16] Phillips MW. Alkali-soluble acrylic consolidants for plaster: a preliminary investigation. *Stud. Conserv.* 1987;32:145-152.
- [17] Ciferri O. The role of microorganisms in the degradation of cultural heritage. *Rev. Conserv.* 2002;3:35-45.
- [18] International Organization for Standardization. ISO 14040 and ISO 14044. Available: [www.iso.org](http://www.iso.org). [Accessed on 31/03/2018].
- [19] Van de Velde K, Kiekens P. Biopolymers: overview of several properties and consequences on their applications. *Polymer Testing*. 2002;21:433-442.
- [20] Geiger T, Michel F. Studies on the polysaccharide JunFunori used to consolidate matt paint. *Stud. Conserv.* 2005;50:193-204.
- [21] Winter J. Natural adhesives in East Asian paintings. *Stud. Conserv.* 1984;29:117–120.
- [22] Vizárová K, Reháková M, Kirschnerová S, Peller A, Simoň P, Mikulášik R. Stability studies of materials applied in the restoration of a baroque oil painting. *J. Cult. Herit.* 2011;12:190-195.
- [23] Schellmann NC. Animal glues: a review of their key properties relevant to conservation. *Rev. Conserv.* 2007;8:55-66.
- [24] Díaz-Calderón P, Caballero L, Melo F, Enrione J. Molecular configuration of gelatin water suspensions at low concentration. *Food Hydrocolloids*. 2014;39:171-179.
- [25] Cao N, Yang X, Fu Y. Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films. *Food Hydrocolloids*. 2009;23:729-735.
- [26] Achet D, He XW. Determination of the renaturation level in gelatin films. *Polymer*. 1995;36:787-791.
- [27] Vanin FM, Sobral PJA, Menegalli FC, Carvalho RA, Habitante AMQB. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. *Food Hydrocolloids*. 2005;19:899-907.
- [28] Rosen SL. *Fundamental principles of polymeric materials*. New York: Wiley; 1993.

- [29] Gurgel Adeodato Vieira M, Altenhofen da Silva M, Oliveira dos Santos L, Masumi Beppu M. Natural-based plasticizers and biopolymer films: A review. *Eur. Polym. J.* 2011;47:254-263.
- [30] Byun Y, Zhang Y, Geng X. Innovation in food packaging. Chapter 5. Plasticization and Polymer Morphology. New York: Academic press, Elsevier; 2014. pp. 87-108.
- [31] Vanin FM, Sobral PJA, Menegalli FC, Carvalho RA, Habitante AMQB. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. *Food Hydrocolloids.* 2005;19:899-907.
- [32] Jongjareonrak A, Benjakul S, Visessanguan W, Tanaka M. Effects of plasticizers on the properties of edible films from skin gelatin of bigeye snapper and brownstripe red snapper. *Eur. Food Res. Technol.* 2006;222:229-235.
- [33] Carvalho RA, Grosso CRF, Sobral PJA. Effect of Chemical Treatment on the Mechanical Properties, Water Vapour Permeability and Sorption Isotherms of Gelatin-based Films. *Packag. Technol. Sci.* 2008;21:165–169.
- [34] Lim L-T, Mine Y, Tung MA. Barrier and Tensile Properties of Transglutaminase Cross-linked Gelatin Films as Affected by Relative Humidity, Temperature, and Glycerol Content. *J. Food Sci.* 1999;64:616-622.
- [35] Thomazine M, Carvalho RA, Sobral PJA. Physical properties of gelatin films plasticized by blends of glycerol and sorbitol. *J. Food Sci.* 2005;70:172-176.
- [36] Jongjareonrak A, Benjakul S, Visessanguan W, Prodpran T, Tanaka M. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. *Food Hydrocolloids.* 2006;20:492-501.
- [37] Sobral PJA, Menegalli FC, Hubinger MD, Roques MA. Mechanical, water vapor barrier and thermal properties of gelatin based edible films. *Food Hydrocolloids.* 2001;15:423-432.
- [38] Cuq B, Gontard N, Cuq J, Guilbert S. Selected functional properties of fish myofibrillar protein-based films as affected by hydrophilic plasticizers. *J. Agr. Food Chem.* 1997;45:622-626.
- [39] Díaz-Calderón P, MacNaughtan B, Hill S, Mitchell J, Enrione J. Reduction of enthalpy relaxation in gelatine films by addition of polyols. *Int. J. Biol. Macromol.* 2018;109:634–638.
- [40] Morrison LR. Glycerol. In: Kirk-Othmer Encyclopedia of Chemical Technology. 4<sup>th</sup>. New York: Wiley; 2000.

- [41] Ciriminna R, Della C Pina, M Rossi M, Pagliaro M. Understanding the glycerol market. *Eur. J. Lipid Sci. Tech.* 2014;116:1432-1439.
- [42] Rahman M, Brazel CS. The plasticizer market: an assessment of traditional plasticizers and research trends to meet new challenges. *Prog. Polym. Sci.* 2004;29:1223-1248.
- [43] Hueck HJ. The biodeterioration of materials- an appraisal. In: *Biodeterioration of materials*. Walters AH Elphick JS (Eds). London: Elsevier; 1968. pp. 6-12.
- [44] Lourenço MJL, Sampaio JP. Microbial deterioration of gelatin emulsion photographs: Differences of susceptibility between black and white and colour materials. *Int. Biodeter. Biodegrad.* 2009;63:496-502.
- [45] Abrusci C, Marquina D, Del Amo A, Catalina F. Biodegradation of cinematographic gelatin emulsion by bacteria and filamentous fungi using indirect impedance technique. *Int. Biodeter. Biodegrad.* 2007;60:137-143.
- [46] Abrusci C, Martín-González A, Del Amo A, Catalina F, Collado J, Platas G. Isolation and identification of bacteria and fungi from cinematographic films. *Int. Biodeter. Biodegrad.* 2005;56:58-68.
- [47] Strzelczyk AB. Observations on aesthetic and structural changes induced in Polish historic objects by microorganisms. *Int. Biodeter. Biodegrad.* 2004;53:151-156.
- [48] Sterflinger K. Fungi: Their role in deterioration of cultural heritage. *Fungal Biol. Rev.* 2010;24:47-55.
- [49] Ortiz-Miranda IAS, Doménech-Carbó A, Doménech-Carbó MT, Osete-Cortina L, Bolívar-Galiano F, Martín-Sánchez I. Analyzing chemical changes in verdigris pictorial specimens upon bacteria and fungi biodeterioration using voltammetry of microparticles. *Herit. Sci.* 2017;5:8.
- [50] López-Miras M, Piñar G, Romero-Noguera J, Bolívar-Galiano FC, Ethenauer J, Sterflinger K, Martín-Sánchez I, Microbial communities adhering to the obverse and reverse sides of an oil painting on canvas: identification and evaluation of their biodegradative potential. *Aerobiologia.* 2013;29:301-314.
- [51] Doménech-Carbó MT, Osete-Cortina L, de la Cruz-Cañizares J, Bolívar-Galiano F, Romero-Noguera J, Martín-Sánchez I, Fernández-Vivas MA. Study on the microbiodegradation of terpenoid resins-based varnishes from easel painting using pyrolysis-gas chromatography-mass spectrometry and gas chromatography-mass spectrometry. *Anal. Bioanal. Chem.* 2006;385:1265-1280.

- [52] Sabatini L, Sisti M, Campana R. Evaluation of fungal community involved in the biodeterioration process of wooden artworks and canvases in Montefeltro area (Marche, Italy). *Microbiol. Res.* 2018;207:203–210.
- [53] Koller J, Baumer U, Kaup Y, Weser U. Herodotus' and Pliny's embalming materials identified on ancient Egyptian mummies. *Archaeometry.* 2005;47:609-628.
- [54] González García A (Ed). Leonardo da Vinci. *Tratado de Pintura [Painting's Treatise]*. 3<sup>rd</sup> Ed. Madrid: Akal; 1995. pp.430.
- [55] Edge M, Allen NS, Turner D, Robinson J, Seal K. The enhanced performance of biocidal additives in paints and coatings. *Prog. Org. Coat.* 2001;43:10-17.
- [56] Koestler RJ, Parreira E, Santoro ED, Noble P. Visual effects of selected biocides on easel painting materials. *Stud. Conserv.* 1993;38:265-273.
- [57] Yang VW, Clausen CA. Antifungal effect of essential oils on southern yellow pine. *Int. Biodeter. Biodegrad.* 2007;59:302-306.
- [58] Jeong SH, Lee HJ, Kim DW, Chung YJ. New biocide for eco-friendly biofilm removal on outdoor stone Monuments. *Int. Biodeter. Biodegrad.* 2017; in press. <http://dx.doi.org/10.1016/j.ibiod.2017.03.004>.
- [59] Delespaul Q, de Billerbeck VG, Roques CG, Michel G, Marquier-Viñuales C, Bessière JM. The antifungal activity of essential oils as determined by different screening methods. *J. Essent. Oil Res.* 2000;12:256-266.
- [60] Youssef, MM, Pham Q, Achar PN, Sreenivasa MY. Antifungal activity of essential oils on *Aspergillus parasiticus* isolated from peanuts. *J. Plant Prot. Res.* 2016;56:139-142.
- [61] Nerio LE, Olivero-Verbe J, Stashenko E. Repellent activity of essential oils: A review. *Bioresour. Technol.* 2010;101:372–378.
- [62] Abdulazeez MA, Abdullahi AS, James BD. Lemongrass (*Cymbopogon* spp.) Oils. In: Preedy V (Ed.). *Essential Oils in Food Preservation, Flavor and Safety*. Elsevier; 2016. pp. 509-516.
- [63] Tavares M, Mattos da Silva MR, Betzler de Oliveira de Siqueira L, Schuenck Rodrigues RA, Bodjolle-d'Almeida L, Pereira dos Santos E, Ricci-Júnior. Trends in insect repellent formulations: A review. *Int. J. Pharm.* 2018;539:190–209.
- [64] Pattnaik S, Subramanyam VR, Kole C. Antibacterial and antifungal activity of ten essential oils in vitro. *Microbios.* 2006;86:237-246.

- [65] El Asbahani A, Miladi K, Badri W, Sala M, Aït Addi EH, Casabianca H, El Mousadik A, Hartmann D, Jilale A, Renaud FNR, Elaissari A. Essential oils: From extraction to encapsulation. *Int. J. Pharm.* 2015;483:220–243.
- [66] Nakahara K, Alzoreky NS, Yoshihashi T, Nguyen HTT, Trakoontivakorn G. Chemical Composition and Antifungal Activity of Essential Oil from *Cymbopogon nardus* (Citronella Grass). *Japan International Research Center for Agricultural Sciences (JIRCAS) JARQ.* 2003;37:249-252. Available on: <http://www.jircas.affrc.go.jp>. [Accessed on 20/04/2018].
- [67] de Billerbeck V., Roques CG, Bessi re JM, Fonvieille JL, Dargent R. Effects of *Cymbopogon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger*. *Can. J. Microbiol.* 2001;47:9-17.
- [68] Ma-in K, H-Kittikun A. Application of plant essential oils in prevention of fungal growth on Para rubber wood. *Eur. J. Wood Prod.* 2014;72:413-416.
- [69] Millezi AF, Cardoso MG, Alves E, Hilsdorf Piccoli R. Reduction of *Aeromonas hydrophyla* biofilm on stainless steel surface by essential oils. *Braz. J. Microbiol.* 2013;44:73-80.
- [70] Oliveira MMM, Brugnera DF, Cardoso MG, Alves E, Piccoli RH. Disinfectant action of *Cymbopogon* sp. essential oils in different phases of biofilm formation by *Listeria monocytogenes* on stainless steel surface. *Food Control.* 2010;21:549-553.
- [71] Akinkunmi EO, Oladeleb A, Eshoa O, Odusegun I. Effects of storage time on the antimicrobial activities and composition of lemon grass oil. *J. Appl. Res. Med. Aromatic Plant.* 2016;3:105-111.
- [72] Dom nech-Carb  MT, Lee Y, Osete-Cortina L, Mart n-Rey S. Influence of plasticizer and biocide on the functional properties of gelatin-based adhesives used in painting consolidation. *J. Adhes. Sci. Technol.* 2015;291:774-1795.
- [73] United States Environmental Protection Agency. R.E.D. FACTS. Prevention, Pesticides And Toxic Substances (7508W). EPA-738-F-97-002. February 1997. Available: <https://www3.epa.gov/pesticides>. [Accessed on 10/04/2018].
- [74] Globally Harmonized System of Classification and Labeling of Chemicals (GHS). (7th Ed). New York and Geneva: United Nations; 2017. pp. 116-122. Available: United Nations Economic Commission for Europe (UNECE). <https://www.unece.org>. [Accessed on 10/04/2018].



- [75] Commission implementing regulation (EU) no 540/2011 of 25 may 2011 implementing regulation (EC) no 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. Official Journal of the European Union. Date 11.6.2011, pp. L 153/106.
- [76] Taiwan Food and Drug Administration (FDA). Available: <http://www.fda.gov.tw/EN>. [Accessed on 29/03/2018].
- [77] US Department of Health & Human Services. CFR –Code of Federal Regulations Title 21. Title 21 Foods and Drugs. Chapter I Food and drug administration. Subchapter B Food for Human consumption. Part 182 Substances generally recognized as safe. Subpart A General Provisions. Section 182.20 Essential oils. Oleoresins (solvent free) and natural extractives (including distillates) that are generally recognized as safe for their intended use within the meaning of section 409 of the Act. Revised as of April 1 2017 from the U.S. Government. Available: <http://www.accessdata.fda.gov>. [Accessed on 31/03/2018].
- [78] Ferencz R, Sanchez J, Blümich B, Herrmann W. AFM nanoindentation to determine Young's modulus for different EPDM Elastomers. *Polym. Testing*. 2012; 31: 425-432.
- [79] Taiwan Central Weather Bureau. Available: <http://www.cwb.gov.tw/eng/index.htm>. [Accessed 2/04/2018].
- [80] Seismological Center. Taiwan Central Weather Bureau. Available: <http://scweb.cwb.gov.tw/>. [Accessed on 2/04/2018].
- [81] Chang, Ch-P. Studies on Dimensional Stability and Anti-mildewing Ability of Oil-Treated Bamboo. 2012. Available: <http://nchuir.lib.nchu.edu.tw/handle/309270000/110909>. [Accessed on 31-03-2018].
- [82] Cheng S, Lukang, Longshan Temple. *Lukang: Lushue Study of Culture and History*; 2004.
- [83] Lee, Ch-L. *Introduction to Traditional Architecture in Taiwan*. Taipei: Council for Cultural Affairs, Executive Yuan; 1999.
- [84] Cho, Ch-L, Chang K-L. Investigation of Wood Species Used for Timber Construction Historical Buildings in Taiwan. *Journal of Ilan University*. 2003:1.
- [85] Lee Y, Doménech-Carbó MT, Osete-Cortina L, Yusà-Marco DJ. Caracterización química-analítica de los materiales aplicados en la techumbre artesonado del templo Longshan (Lukang, Taiwan). In: Doménech-Carbó, M.T. (Ed.), *Proceedings of the*

Jornadas de Investigación Emergente en Conservación y Restauración de Patrimonio. Valencia: Servicio Publicaciones Universitat Politècnica de València; 2015. pp. 799-806.

[86] Doménech-Carbó A, Doménech-Carbó MT, Lee Y, Osete-Cortina L. Potential application of voltammetry of microparticles for dating porcine blood-based binding media used in Taiwanese architectural polychromies. *Asian J Chem.* 2012;7:2268-2273.

[87] Mazzeo R, Cam D, Chiavari G, Fabbri D, Ling H, Prati S. Analytical study of traditional decorative materials and techniques used in Ming Dynasty wooden architecture. The case of the Drum Tower in Xi'an, P.R. of China. *J. Cult. Herit.* 2004;5:273-283.

[88] International Organization for Standardization. ISO 2555. Available: [www.iso.org](http://www.iso.org). [Accessed on 31/03/2018].

[89] Mei Y, Zhao Y. Barrier and Mechanical Properties of Milk Protein-Based Edible Films Containing Nutraceuticals. *J. Agric. Food Chem.* 2003;51:1914-1918.

[90] ASTM, Standard test method for water vapor transmission of materials. Annual books of ASTM Standards. Designation E 96-01. Philadelphia: ASTM, American Society for Testing Materials; 2001.

[91] Asociación Española de Normalización y Certificación (AENOR). UNE-EN ISO 4624:2003, UNE-EN 1015-12. Available: <http://www.aenor.es>. [Accessed on 31/03/2018].

[92] Colección Española de Cultivos Tipo. Available: <https://www.uv.es/cect>. [Accessed on 31 March 2018].

[93] Derjaguin BV, Muller VM, Toropov Yu P. Effect of contact deformations on the adhesion of particles. *J. Colloid. Interface Sci.* 1975; 53: 314-326.

[94] CTS Conservation Products. Available <http://www.ctseurope.com>. [Accessed on 31 March 2018].

[95] Karbowiak T, Hervet H, Léger L, Champion D, Debeaufort F, Voilley A, Effect of Plasticizers (Water and Glycerol) on the Diffusion of a Small Molecule in Iota-Carrageenan Biopolymer Films for Edible Coating Application. *Biomacromolecules.* 2006;7:2011-2019.

[96] Gennadios A, Weller CL. Moisture adsorption by grain protein films. *Trans. ASAE.* 1994;37:535-539.

- [97] Coupland JN, Shaw NB, Monahan FJ, O’Riordan ED, O’Sullivan M. Modeling the effect of glycerol on the moisture sorption behavior of whey protein edible films. *J. Food Eng.* 2000;43:25-30.
- [98] Kim S-J and Z. Ustunol Z, Solubility and Moisture Sorption Isotherms of Whey-Protein-Based Edible Films as Influenced by Lipid and Plasticizer Incorporation. *J. Agric. Food Chem.* 2001;49:4388-4391.
- [99] Parker J. Testing adhesive emulsions for use in the conservation of ethnographic artefacts. *Conservation News.* 2005;96:24-27.
- [100] Withmore PM, Moran D, Bailie. Shrinkage stresses in art and conservation coatings based on synthetic polymers. *J. Am. Institute Conserv.* 1999;38:429-441.
- [101] Ebert B, Singer B, Grimaldi N. Aquazol as a consolidant for matte paint on Vietnamese paintings. *J. Institute Conserv.* 2012;35:62-76.
- [102] Erhardt D, Tumosa CS, Mecklenburg MF. Long-Term Chemical and Physical Processes in Oil Paint Films. *Stud. Conserv.* 2005;50:143-150.
- [103] Ashley-Smith J, Wilks H (Eds.). *Adhesives and Coatings.* London: The Conservation Unit of Museums & Galleries Commission; 1987. pp. 16-18.
- [104] Humar M, Lesar B. Efficacy of linseed- and tung-oil-treated wood against wood-decay fungus and water uptake. *Int. Biodeter. Biodegrad.* 2013;85:223-227.
- [105] Salvant J, Barthel E, Menu M. Nanoindentation and the micromechanics of Van Gogh oil paints, hal-00593798, 2011. Available: <http://hal.archives-ouvertes.fr>. [Accessed: 14/05/2013].

### **Figure captions**

**Figure 1.-** a) The Longshan Temple of Lukang (Taiwan); b) the Stage and Doors.

**Figure 2.-** Scheme of the sampling points in the Longshan Temple of Lukang.

**Figure 3.-** Scheme of the stratigraphic distribution of the original polychromies of the Temple of Lukang.

**Figure 4.-** Detail of polychromies. Detachment and partial loss of the paint layer can be observed.

**Figure 5.-** Scheme showing the different types of laboratory test specimens prepared with indications about their strata distribution and composition, and the test carried out in each one.

**Figure 6.-** Map of the material deformation obtained with NI-AFM.

**Figure 7.-** Variation of the viscosity of adhesives *A* and *B* in temperature.

**Figure 8.-** The drying curves obtained for adhesives *A* and *B*.

**Figure 9.-** The secondary electron images obtained at 0.8 kV in the cross-section of the paint films: a) vermilion paint film, width image 234  $\mu\text{m}$ ; b) ultramarine blue paint film, width image 730  $\mu\text{m}$ ; c) iron oxide yellow paint film, width image 234  $\mu\text{m}$ .

**Figure 10.-** The secondary electron images obtained at 0.8 kV in the cross-section of: a) ultramarine blue, width image 12  $\mu\text{m}$ ; b) the iron oxide yellow paint film, width image 92  $\mu\text{m}$ . Detail of the air gaps that surround the pigment grains, pointed out with arrows.

**Figure 11.-** The stress-strain curves for the untreated paint films and for those treated with adhesives *A* and *B*: a) iron oxide yellow; b) vermilion; c) ultramarine blue paint films.

**Figure 12.-** Aspect of the surface of the control film prepared with the gelatin-based adhesive plastized with glycerol with no addition of citronella oil after a 28-days incubation (x25). Spots formed by the growth of *A. niger* colonies can be seen on this specimen in which citronella was not included in the formulation of the adhesive.

## **Table captions**

**Table 1.-** Effect of adhesives on the moisture content (%) of the paint films after conditioning at 50% *RH*. The values shown in the table are the means±standard deviation ( $n=3$ ).

**Table 2.-** The mean value and standard deviation of the water vapor permeability (*WVP*) of the reconstructed oil paint specimens.

**Table 3.-** The mean value and standard deviation of the elastic modulus, ultimate strength and ultimate elongation for the paint films untreated and treated with adhesives *A* and *B*.

**Table 4.-** The results obtained in the adhesions tests for the system ground layer-wood support after ground layer consolidation.

**Table 5.-** The mean value of the elastic modulus obtained by NI-AFM in the microsamples excised from the original polychromies before and after the *in situ* consolidation trials performed with adhesives *A* and *B*.