MODIFICATION OF EAPC-XYL BY PSEUDOMONAS LIPASES BACTERIA TO REMOVE ACRYLIC FROM THE MURAL OIL PAINTINGS

Abeer F. ELHAGGRASSY
Department of Conservation, Faculty of Archaeology, Fayoum University, Egypt

E.mail: afa01@fayoum.edu.eg

ABSTRACT

This paper focuses on the removal of acrylic polymer layers from the surfaces of mural paintings, as one of the serious problems faced by conservators. Nanostructured fluids containing water is a great solution for the immiscible or partially miscible polymer with water. The de-wetting of the polymer layer is usually an essential step to achieve efficient removal. EAPC-XYL system was often used for the removal of paraloid B72 but unfortunately, it has an effect on the noble patina of the pigments. The addition of Pseudomonas stutzeri as a source of lipase enzyme increases the efficiency of the EAPC-XYL in short time which helps in removing of the acrylic polymers without affecting the pigments.

The examination by SEM showed that the applying EAPC-XYL/Pseudomonas stutzeri for only one hour has the effect of removing the polymer’s layers from the mural paintings without affecting the pigments. The FTIR analysis after application showed that the functional groups of the binding medium of the mural paintings is absence of paraloid B72.

KEYWORDS

Nano-emulsions, Acrylic polymer, cleaning, oil wall paintings, conservation, Pseudomonas stutzeri

المالخص

تركز هذه الورقة على إزالة طبقات البوليمر الأكريليك من أسطح اللوحات الجدارية كواحدة من المشاكل الخطيرة التي تواجه الحماية. تعتبر السواكن ذات البنية المائية التي تحتوي على الماء خيارًا رائعا لللوانات غير القابلة للاستحمام أو غير القابلة للأسترمال جزئيًا مع الماء. عادة ما تكون عملية إزالة طبقة البوليمر من طبقة الندى خطوة أساسية لتحقيق الإزالة الفعالة. غالبًا ما كان نظام EAPC-XYL يستخدم لإزالة البالورون B72 ولكن نشوب الحظر، له تأثير على الزنبار Pseudomonas stutzeri

أظهر الفحص الذي أجريه EAPC-XYL / Pseudomonas stutzeri لمدة ساعة واحدة فقط له تأثير على إزالة طبقات البوليمرات من اللوحات الجدارية دون تأثير على الأسماك. أظهر تحليل FTIR بعد التطبيق المجموعات الوظيفية لمسط العلم اللوحات الجدارية في غياب البالورون B72.

الكلمات الدالة

مستحيلات نانوية، بوليمرات الأكريليك – التنظيف، التصوير الجداري الزيتي، الصيانة، الباتيبارو، الباسيلومناس.
INTRODUCTION

Mural paintings are degraded due to the natural ageing of their components. The degradation process occurs at the surface of the pigment layer which leads to the weakness of these mural paintings, and the loss of pigment layers in some cases.\(^1\)

Polymers and Acrylics were used as consolidants for these murals. They are still widespread within the conservation field, although they have produced critical degradation with destructive effects.\(^2\) Their continuous popularity is due to the possibility of applying the same materials at the beginning of the conservation interference and posteriorly reapplied after the conservation process.\(^3\)\(^4\)

When polymers are applied on mural paintings, a surface microfilm is formed even at low concentration. This microfilm certainly changes the mural paintings surface’s porosity and permeability, causing mechanical stresses and photo-degradation of the pigments.\(^5\)\(^6\)

Removing aged polymers from conserved mural paintings must be carried out without affecting the patina of the pigments. This is a critical problem due to the heterogeneous and porosity of the pigment layer itself. Furthermore, as the main reason for polymer degradation is a drastic loss of solubility, it makes their removal by traditional chemical solvent yet more difficult.\(^7\)\(^8\)

Nanoemulsions consist of oil, water and an emulsifier which is necessary for the formation of nano-sized droplets that help to decrease the interfacial tension, the surface energy per unit area between the water phase and the oil one of the emulsions. The addition of an emulsifier is critical for the stabilising of nanoemulsions through repugnant steric hindrance and electrostatic interactions.\(^9\)\(^10\)\(^11\)

Treatment by nanoemulsion using (SDS), 1-pentanol (1-PeOH) as a co-surfactant, addition to a small amount of p-xylene and nitro diluent were applied at three mural paintings: the Loggia del Bigallo in Florence, the Filippo Lippi frescoes in Prato Cathedral, and ‘a secco’ painting in the Villa del Barone in Prato and the results were acceptable.\(^12\)

An oil-in-water nanoemulsion containing SDS/dodecane/n-butanol/ H\(_2\)O was efficient in the removal of the polar materials from mural paintings of the Oratory of San Nicola al

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Nanoemulsions are ordinarily prepared in two processes: first a microemulsion that is then converted to a nanoemulsion.\textsuperscript{16}

Nanoemulsions Fig. 1, have smart properties like nanodroplet size, high surface area per unit volume, tunable rheology, kinetic stability of liquid-in-liquid dispersions, and limpid appearance.\textsuperscript{17}

The formulation of o/w (oil/water) nanoemulsions requires small amounts of solvent which reduces the toxicity and environmental impact. The cleaning process with nanoemulsions provides a controlled cleaning process without affecting the pigments.\textsuperscript{18}

EAPC is a fluid that is composed of five components: water, sodium dodecyl sulfate (SDS), 1-pentanol (PeOH), propylene carbonate (PC), and ethyl acetate (EA). EAPC provides

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{nanoemulsion_application.png}
\caption{Application and properties of nanoemulsion} \label{fig:nanoemulsion}
\end{figure}

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effective results in the removal of methacrylic/acrylic or vinyl/acrylic copolymers but unfortunately it causes some colour change.\(^{20}\)

EAPC system was successfully used before to remove polylsioxane resin applied on mural paintings in Nazareth (Israel) and is considered as one of the most efficient systems for removing polymers for now.\(^{21}\) \(^{22}\) \(^{23}\)

The present study is concerned with using *Pseudomonas stutzeri* as a source of Lipase which can increase the effect of EAPC, and the effect of using *Pseudomonas stutzeri* to remove any other organic matter. We will also point out the effective application time. *Pseudomonas stutzeri* is used to remove animal glue from protected mural paintings.\(^{24}\) \(^{25}\) \(^{26}\)

The mosque of El Gamaly Yosouf is one of the monuments where the mural oil paintings were consolidated on multiple occasions with polymers and acrylic (Fig. 2). Fragments were taken to be examined and analysed, but all the results showed only polymers without the components of the layer.

The aim of this study is to modify the nanoemulsion to make it safe to be used for mural paintings, without affecting the pigment of the oil wall paintings.

![Fig. 2. The mural oil paintings of the mosque present the name of righteous Caliphs and Ahl el-Kahf names.](image)

\(^{21}\) Cappitelli, “Biocleaning of Cultural Heritage Surfaces”, 2016
\(^{24}\) Lustrato et al, “Fast biocleaning of mediaeval frescoes using viable bacterial cells”, 2012
\(^{25}\) Rosado et al, ) “Enzymatic Monitorization of Mural Paintings Biodegradation and Biodeterioration”, 2013
\(^{26}\) ElHagrassy “Bio-restoration of mural paintings using viable cells of Pseudomonas stutzeri and characterization of these murals”, 2019
MATERIALS AND METHODS

Chemicals and bacteria

(A) Chemicals

Sodium dodecyl sulfate (Sigma-Aldrich), 1-pentanol (Sigma-Aldrich), ethyl acetate (PioChem), p-xylene (Sigma-Aldrich), ethyl acetate (Sigma Aldrich) and Distilled Water (PioChem) were used without purification. Furthermore, Broth media was purchased (Z699187- Sigma-Aldrich) and Paraloid B72 was purchased from (PioChem, India).

Bacteria

Viable cells of *Pseudomonas stutzeri* ATCC 17589 strain (American Type Culture Collection, Rockville, MD, USA).

Preparing replica and artificial aging

In order to evaluate the performance of nanoemulsion, a cubic replica size of 5cm of the mural oil paintings was prepared and aged. Ten cycles of thermal aging were carried out for 8 hours in an oven at 60°C followed by 8 hours in room temperature.

FTIR Spectroscopy

Absorption spectra in the IR region were collected using a Nicolet Magna 75 FTIR spectrometer. Thirty-two signal-averaged scans acquired the samples. Few milligrams of each sample were mixed in KBr (IR grade, Merck) pellet performed in accordance with ASTM E334. The acrylic material was extracted by iodoform 95% (BioChem company, India) to avoid the painted pigments elements.

SEM- EDX

SEM images were taken using a Jeol (Tokyo, Japan) JSM 5600 LV equipped with an Oxford Instruments 6587 EDX microanalysis detector. The images were taken under low vacuum conditions where samples did not show any charging effects. EDX microanalysis was carried out to obtain information about the elemental composition of the sample.

Spectrophotometer


Preparation of the nanoemulsion

Two systems were used in this study, the traditional EAPC and EAPC after adding viable *Pseudomonas stutzeri* cells. EAPC system consists of five components which are: “SDS, 3.7%, 1-pentanol, 7%, ethyl acetate, 8%, propylene carbonate, 8% in H2O, 73.3%”. In addition to using the classical XYL system which consists of p-xylene, 2.5%, SDS, 3.9%

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Prada, J.L. et al., “Laboratory Tests Simulating the Weathering Processes of the Stone of Sant Miquel Church in Cardona (Barcelona, Spain), Protection and Conservation of Cultural Heritage of the Mediterranean Cities”, 2002

DOI: 10.36816/shedet.006.10 - 193 -
and 1-pentanol, 6.5% in H₂O, 89.1%.”  

This study uses *Pseudomonas stutzeri* as a source of Lipase catalyst to remove the organic solvent and acrylic polymer. The *Pseudomonas stutzeri* has been added as a final step in preparing the previous nanoemulsion; it has then been dispersed using a stirrer set up at 500 rpm. Finally, it was applied on the replica.

**RESULTS**

**FTIR analyses**

The analyses by FTIR techniques indicated Paraloid B72 as a polymer used for consolidation (Fig. 3). The characteristic bands of Paraloid B72 broad strong band at ≈1452: 1480 cm⁻¹ due to a methyl group of the ethyl methacrylate monomeric unit, C=O stretching band of CO₃²⁻ at ≈ 1725 cm⁻¹ and O-C-O bending band of Carbonate group at ≈ 865 cm⁻¹. Small C-H stretching bands are in the range between 2988 and 2957 cm⁻¹.

![FTIR spectrum](image)

Fig.3. FTIR spectrum for the extracted acrylic material of the mural oil paintings of the mosque

**EDX-SEM**

The examination by SEM showed that the mural oil painting consists of two layers, the ground layer and the painted layer (Fig.4).

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Fig. 4: Scanning electron micrograph (SEM) indicates two layers (1) the ground layer, (2) the painted layer full of polymers.

Even with the naked eye, the upper painted layer and the ground one appears to be consolidated several times with polymers and acrylic (Fig. 5). The EDX analysis shows high level of carbon beak.

Fig. 5. Scanning electron micrograph showing the upper layer of the mural oil paintings.

**Viable cell counts**

*Pseudomonas stutzeri* growth rate at 35°C, pH=7.5 on broth medium showed the highest cell density (1.3 O.D.₅₅₀ and >10.1 log CFU ml⁻¹) in the broth.²⁹

**Application on the replica**

The application on the replica was performed under laboratory conditions on the mural oil paintings models. The models were consolidated by paraloid B72 5% in Ethyl alcohol and aged (see 2.3). After ageing, all pigments were measured using a colourimeter before applying the nanoemulsion. The total colour change (ΔE) between the two perimeter colours is ³⁰ ³¹

²⁹ ElHagrassy “Bio-restoration of mural paintings using viable cells of Pseudomonas stutzeri and characterization of these murals”, 2019.
\[ \Delta E^* = \{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\}^{1/2} \]

The cleaning process started by covering the replica with some Japanese paper 0.9mg. Poultices made of cellulose pulp and immersed in the two different fluids were applied for several periods (1h, 2hrs, 3hrs) at room temperature (about 25°C).

After that, the poultices were carefully removed and the pigments were measured again after application (Table 1, 2). The pigments were examined using SEM before and after the application (Fig. 6-7). The FTIR analysis showed that the functional groups before and after treatment were completely different (Fig. 8).

**Table 1. The \( \Delta E \) of the EAPC-XYL system on oil pigment before and after application**

<table>
<thead>
<tr>
<th>Pigments</th>
<th>before</th>
<th>After 1h</th>
<th>After 2hrs</th>
<th>After 3hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>40.22</td>
<td>40.25</td>
<td>40.36</td>
<td>40.52</td>
</tr>
<tr>
<td>Green</td>
<td>46.82</td>
<td>46.89</td>
<td>46.92</td>
<td>47.16</td>
</tr>
<tr>
<td>Blue</td>
<td>49.63</td>
<td>49.71</td>
<td>49.83</td>
<td>49.93</td>
</tr>
<tr>
<td>Red</td>
<td>45.04</td>
<td>45.10</td>
<td>45.15</td>
<td>45.26</td>
</tr>
<tr>
<td>Green-Blue mix</td>
<td>51.87</td>
<td>51.93</td>
<td>51.99</td>
<td>52.18</td>
</tr>
<tr>
<td>Black</td>
<td>45.82</td>
<td>45.86</td>
<td>45.94</td>
<td>46.08</td>
</tr>
</tbody>
</table>

**Table 2. The \( \Delta E \) of the EAPC-XYL / *Pseudomonas stutzeri* cells on oil pigment before and after application**

<table>
<thead>
<tr>
<th>Pigments</th>
<th>before</th>
<th>After 1h</th>
<th>After 2hrs</th>
<th>After 3hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>40.22</td>
<td>40.22</td>
<td>40.25</td>
<td>40.26</td>
</tr>
<tr>
<td>Green</td>
<td>46.82</td>
<td>46.85</td>
<td>46.85</td>
<td>46.88</td>
</tr>
<tr>
<td>Blue</td>
<td>49.63</td>
<td>49.68</td>
<td>49.70</td>
<td>49.72</td>
</tr>
<tr>
<td>Red</td>
<td>45.04</td>
<td>45.06</td>
<td>45.10</td>
<td>45.09</td>
</tr>
<tr>
<td>Green-Blue mix</td>
<td>51.87</td>
<td>51.89</td>
<td>51.88</td>
<td>51.91</td>
</tr>
<tr>
<td>Black</td>
<td>45.82</td>
<td>45.85</td>
<td>45.86</td>
<td>45.86</td>
</tr>
</tbody>
</table>

31 Atodiresei et al., Chromatic Characterization in Cielab System for Natural Dyed Materials, Prior Activation in Atmospheric Plasma Type DBD”, 2013
Fig 6. SEM micrograph presents (a) sample before application, (b) after 1h of applied EAPC-XYL, (c) after 3h of application.

Fig 7. SEM micrograph presents (a) before application of EAPC-XYL / Pseudomonas stutzeri system, and (b) after 1h of application.
Fig 8. FTIR spectrum showing the upper curve represents the mural oil paintings before removing the acrylic, while the lower curve represents the oil binding media of the mural paintings.

DISCUSSION

The traditional methodologies for removing polymers and acrylics from mural oil paintings by using organic solvents or physical gels are considered unsuitable. The organic solvents are usually efficient in dissolving the polymer during the initial consolidation process. However, after polymerization, it becomes inefficient to use the same solvent to remove polymer or acrylic.32

In addition, using high concentrations of organic solvents may cause a discolouration of the pigments of the mural paintings. Moreover, the toxicity and volatility of most solvents cannot be neglected, because conservators usually work in poorly ventilated environments.33 34

32 Singh and Arbad, “Ancient Indian Painting Recipes and Mural Art Technique at Ajanta” 2014
34 Giamblanco, et al, “Probing the cleaning of polymeric coatings by nanostructured fluids: A QCM-D study”, 2017
Removing polymers and acrylics by nano-fluids is safe and applicable compared to the use of organic solvents. The re-deposition of the removed polymer from the grains of the mural paintings is avoided due to the aqueous nature of the nano-fluid.\textsuperscript{35} \textsuperscript{36} 

In this study, EAPC-XYL and EAPC-XYL/ \textit{Pseudomonas stutzeri} viable cell were applied on the replica for various duration of time. The results showed that using EAPC-XYL/ \textit{Pseudomonas stutzeri} for only one hour has the same effect as using EAPC-XYL for three hours, due to the lipase enzymes that duplicate the effect of the nano-fluid. However, using EAPC-XYL/ \textit{Pseudomonas stutzeri} has a low effect on the patina of the pigments. The spectrophotometer investigation showed that the ΔE was lower when using EAPC-XYL/ \textit{Pseudomonas stutzeri} than when using the EAPC-XYL.

The SEM of the replica treated with EAPC-XYL/ \textit{Pseudomonas stutzeri} for one hour showed the grains of the pigments without the polymeric layers. The FTIR analysis confirmed that the cleaning process had succeeded, as the FTIR for the mural painting before the removal of acrylic showed the paraloid B72 that was used for consolidation. After the application of the EAPC-XYL/ \textit{Pseudomonas stutzeri} for one hour and the removal of the cellulose poultices, the FTIR spectrum showed the functional groups of the oil binding media without the presence of paraloid B72.

EAPC-XYL/ \textit{Pseudomonas stutzeri} system is more efficient and versatile than the EAPC-XYL nanoemulsion system used in several previous studies.\textsuperscript{37} \textsuperscript{38} \textsuperscript{39} Interactions between EAPC-XYL/\textit{Pseudomonas stutzeri} and the polymer coating the layers were promoted in this study. It was hypothesised that EAPC-XYL/ \textit{Pseudomonas stutzeri} system complies with a mechanism where many processes are taking place in the same time:

i) the aqueous phase (PC and EA) containing solvent interacts with the polymer,\textsuperscript{40} \textsuperscript{41} 

ii) Migration of solvents arises from the nano-droplets to the aqueous phase,

iii) Migration arises from nano-droplets to the polymer.\textsuperscript{42} 

iii) the lipase enzyme lets all the previous processes happen in a short time. As a final result, the polymer layer was swollen and was detached by the cellulose poultices which can be easily removed.

CONCLUSIONS

The removal of aged Paraloid B72 from the consolidated mural oil paintings without affecting the surface is possible by using EAPC-XYL/ \textit{Pseudomonas stutzeri}. Observations

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\bibitem{Cappitelli2016} Cappitelli, “Biocleaning of Cultural Heritage Surfaces” 2016
\bibitem{Giorgi2010} Giorgi, et al, “New methodologies for the conservation of cultural heritage: micellar solutions, microemulsions, and hydroxide nanoparticles”, 2010
\bibitem{Arroyo2012} Arroyo, “Development of innovative nanostructured systems for the cleaning of works of art”, 2012
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by the SEM microscope indicated that the removal of acrylic was successful after only one hour. After the cleaning process, FTIR spectra revealed that there was no dispersed Paraloid within the mural painting layers. Moreover, the spectrophotometer investigation showed that the removal process of the acrylic was effective without altering the noble patina of the pigments. The proposed methodology is applicable, risk-free associated with organic solvents, as well as harmless for both the conservators and the mural oil paintings.
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