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Los Angeles

In situ Synthesis and Characterizations of Bio-ceramic Based Hydroxyapatite in the Remineralization of Calcium-rich Matrices

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Materials Science and Engineering

by

Xiao Ma

2017

ABSTRACT OF THE DISSERTATION

In situ Synthesis and Characterizations of Bio-ceramic Based Hydroxyapatite in the Remineralization of Calcium-rich Matrices

by

Xiao Ma

Doctor of Philosophy in Materials Science and Engineering University of California, Los Angeles, 2017 Professor Ioanna Kakoulli, Chair

The research develops hydroxyapatite (HAP) based, inorganic mineral system with improved properties for the consolidation – providing the binding mechanism to reestablish the loss of cohesion – of powdery porous building materials (calcareous stone, marble, cement) and decorated architectural surfaces (rock-art, wall paintings and mosaics) of archaeological, historic and artistic value. The interdisciplinary nature of this research focused at the interface of materials science, biotechnology and conservation science develops tailored sustainable conservation treatments, which consider fundamental aspects of substrate chemistry and structure to prescribe and metricate treatment protocols. The scientific approach exploits biomimetic principles to induce the formation of HAP crystals by triggering reactions between the Ca-rich substrates of wall paintings and ammonium phosphate precursors such as diammonium hydrogen phosphate (DAP, (NH₄)₂HPO₄),

and ammonium dihydrogen also known as monoammonium dihydrogen phosphate (MAP, NH₄H₂PO₄). Tests were carried out on simulated wall painting panels and on original archaeological substrates in situ. The consolidating effect, influence of the solution and conditions such as concentration and contact time on the extent of hydroxyapatite formation was evaluated through a series of structurally and compositionally sensitive analytics including scanning electron microscopy coupled with energy dispersive X-ray spectroscopy, thermogravimetry, X-ray diffraction, micro-Raman Spectroscopy, mechanical and fluid-transport analyses. Thermodynamic modeling was also performed to evaluate the stable phase equilibria between CaCO₃ and (NH₄)₂HPO₄ / NH₄H₂PO₄ solutions and interpret the HAP formation in aqueous solution. Calculations demonstrated hydroxyapatite precipitation in the systems consisting of CaCO₃/(NH₄)₂HPO₄. From a purely scientific perspective, the research develops new information to relate: (1) the chemical interactions, thermodynamics and kinetics of reactions between the phosphate reactants and CaCO₃, the main component in the stone, plaster or mortar, at various pH levels to the formation of orthophosphates such as HAP and rate/extent of consolidation (2) the impact of HAP formation within the decohesive layers in terms of microstructure-linked, fluidtransport, mechanical, durability and optical properties and (3) the chemical interactions of the reactant precursors with different pigments present in the surface paint layers. Acquired data show the potential of this treatment for the consolidation of powdery calcium carbonate-based surfaces and their protection from weathering and deterioration induced by passage-of-time and environmental action linked effects. These efforts develop quantitative materials science-based structure/property relations to engineer practical, prescriptive consolidation solutions in relation to the reactant chemistry, the substrate properties and the treatment application method relevant to the materials.

The dissertation of Xiao Ma is approved.

Hossein Pirouz Kavehpour

Mark S. Goorsky

Ioanna Kakoulli, Committee Chair

University of California, Los Angeles

2017

To my family

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VITA

2009	Bachelor of Engineering in Materials Science and Engineering
	Huazhong University of Science and Technology, China
2011	Master of Science in Materials Engineering
	Purdue University
2012-2013	Graduate Division Fellowship, UCLA
2015	Ralph C. Altman Award, Fowler Museum
June-August 2015	Graduate Summer Intern, Museum Conservation Institute,
	Smithsonian Institution
September 2015- May 2016	Graduate Intern, Getty Conservation Institute
2016	R. E. Taylor Best Poster Award, Society of Archaeological Science
2016	Martin J. Aitken Best Poster Award, 41 st International Symposium
	of Archaeometry
2016	Dissertation Year Fellowship, UCLA
2012-present	Graduate student researcher, Department of Materials Science and
	Engineering, UCLA

PUBLICATIONS

- Xiao Ma, Yuli Shi, Herant Khanjian, Michael Schilling, Min Li, Hui Fang, Dayong Cui, Ioanna Kakoulli, "Characterization of early imperial lacquerware from Luozhuang Han tomb, China", In Press, Archaeometry.
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- Magdalena Balonis, Xiao Ma, and Ioanna Kakoulli, "Preliminary results on biomimetic methods based on soluble ammonium phosphate precursors for the consolidation of archaeological wall paintings", ACS Symposium Series, Vol. 1147, Archaeological Chemistry VIII (October 2013), Chapter 22, pp 419-447.
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Chapter 1. Introduction

1.1 Overview and Statement of the Problem

Cultural heritage materials and in particular, rock-art, wall paintings and other forms of decorated architectural surfaces were central to culture of ancient people. These are multilayer and multinary systems composed of the paint layer (i.e. pigment and binder) and the substrate (i.e. rock surface or plaster(s)). In ancient and historical times, two different techniques were predominantly employed for painting on walls: fresco (from the Italian meaning 'fresh') and secco (from the Italian meaning 'dry'). In a *fresco* technique pigment powders were mixed with water and applied on a freshly-laid or moist calcium hydroxide/lime (Ca(OH)₂)-rich plaster layer. Upon the setting (hardening) of the lime plaster involving a chemical reaction between the $Ca(OH)_2$ and the carbon dioxide (CO₂) of the atmosphere to form a calcium carbonate lattice, the pigments are fixed/become integral part of the wall. As a result, this process produces durable wall paintings. However, owing to the high alkalinity of the lime and the exothermic reaction during setting, only a small number of pigments are compatible with the *fresco* technique and therefore ancient *fresco* paintings contain limited palette of colors. Secco technique on the other hand, involves no chemical reaction for the fixing of the pigments. It involves the use of dry wall (of any finishing plaster layer), onto which the pigments are 'fixed' by the use of a film-forming organic binding medium such as egg, siccative oil, gum and others [1].

The physical and chemical attributes of the individual components of wall paintings and the environment they are found (catalytic action of moisture, salts, microorganisms, pollution), influence the stability of the system. These are the reasons for which important ancient painted surfaces are often found in poor state of preservation with decohesive matrices and loss of paint. For archaeological wall paintings, the risks of their preservation are even greater as the sudden change in the environmental conditions, mainly temperature, relative humidity and light, once unearthed (excavated) can cause irreversible damages.

Extensive studies have been carried out using a variety of consolidation treatments to improve the condition and establish the loss of cohesion of deteriorated decorated architectural surfaces and wall paintings. These studies critically indicate that choosing a proper consolidating agent for porous materials is challenging. This is specifically related to being able to ensure sufficient action at surface and subsurface level, while also providing mechanical strength and abrasion resistance [2]. The definition of a consolidant provided by Warren [3] can be used as a point of reference:

A consolidant acts at the near-molecular level by fixing or inhibiting the capacity for movement between very small particles, thereby altering the characteristics of the material in terms of its behavior, particularly in the presence of water. It tends to make the material stronger in compression and tension, and may affect inherent characteristics, such as heat and sound transmission and rigidity.

Further, a consolidant should not severely alter the original porosity [3] that could lead to further damage. Ideally, the consolidant should also be photochemically stable to prevent optical changes of the surface of the paint that could affect significantly the saturation of color, gloss and texture. Also, one of the key factors in the consolidation of wall paintings in particular is retreatability, enabling future treatments, if necessary [4].

Polymers, mainly acrylic, vinyl and silicone based, have been widely used as consolidants for cultural heritage artifacts and wall paintings. However, contrary to expectation, in many cases polymers used for the consolidation of wall paintings have often induced further degradation by changing greatly the physicochemical properties of the plasters and paint layers [5].

An alternative method to organic polymers is the in situ formation of more compatible inorganic mineral phases re-establishing the loss of cohesion in disintegrated plaster layers [6-14]. An example is the application of Ca(OH)₂-based consolidants that has been practiced extensively [8, 15-17]. The consolidation mechanism is based on the transformation of Ca(OH)₂ (slaked lime) into calcium carbonate (CaCO₃, carbonated lime) through a carbonation process induced by the CO₂ in the atmosphere through a chemical reaction similar to the setting of the original plaster (Reaction 1).

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O \uparrow$$

Reaction 1. Setting of lime.

However, the low solubility of calcium hydroxide in water (1.73 g/L at 20°C), the low stability of the system and high surface tension of water (as the medium) cause aggregation and phase separation resulting in inefficient penetration, poor consolidation strength and whitening of the surface. Several strategies including "sucrose methods" to increase $Ca(OH)_2$ solubility [9] and the synthesis of $Ca(OH)_2$ nanoparticle-alcohol dispersions to prevent agglomeration and improve penetration have been reported [3]. Yet, in spite of improved working and performance properties, the sensitivity of CaCO₃ to a drop in the pH caused by acid rain and other acid local environments for example, or a phase transformation into gypsum (CaSO₄.2H₂O) in the presence of sulfate ions (known as 'sulfation') poses limitations in the use of Ca(OH)₂-based consolidants. Other inorganic mineralization treatments based on barium or oxalate salts have been questioned due to concerns related to the toxicity of barium (not all nations permit the use of barium in conservation) and the limited penetration of oxalates into a porous medium.

Recent studies [9, 11, 18-20] have demonstrated a considerable potential for developing much improved consolidation methods on limestone and marble matrices by bio-mimicking the growth of hydroxyapatite (HAP, has the formula $Ca_5(PO_4)_3(OH)$, but is usually written as $Ca_{10}(PO_4)_6(OH)_2$, to denote that the crystal unit cell comprises two formula units), the main mineralogical component of teeth and bones [19, 21, 22]. HAP was proved to be effective in binding grain boundaries and improving stone mechanical properties (tensile strength, ultrasonic pulse velocity, resistance to abrasion) [23-25]. However, despite successful results, this method has not been attempted for applications on archaeological wall paintings, nor have any fundamental studies on the thermodynamics of the systems been conducted.

This research, addresses these emerging needs of under-researched areas and creates new information in a two-pronged approach: 1) by exploring similar principles to induce the in situ formation of protective HAP crystals by triggering reactions between the Ca in the calcium carbonate (CaCO₃)-rich plaster layers of wall paintings (as in *fresco* paintings) and ammonium

phosphate precursors, and critically evaluating the reaction chemistries between the ammonium phosphate precursors with selected pigments; 2) through experimental and theoretical thermodynamic modelling of the system.

1.2 Consolidation Principle using Ammonium Phosphate Precursors

HAP is formed in situ by activating reactions between the Ca in the calcium carbonate (CaCO₃)-rich layers and ammonium phosphate precursors. Both diammonium hydrogen phosphate (DAP) and monoammonium dihydrogen phosphate (MAP) precursors were tested. Other soluble phosphate-based salts such as potassium/sodium phosphates could also serve as a source of phosphorous. However, in conservation applications it is not advisable to use Na⁺ and K⁺ cations as upon penetration they may combine with anions present in the system (or deposited from the atmosphere) to form Na and K salts that could be detrimental to the preservation of the wall paintings [19, 20].

The theoretical chemical pathway of the HAP formation using DAP as the precursor is presented below (Reaction 2) [26]. Similar reaction chemistries occur with the MAP precursor as well.

 $10 \text{ CaCO}_3 + 6 (\text{NH}_4)_2 \text{HPO}_4 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6 (\text{OH})_2 + 10 \text{ CO}_2 \uparrow + 12 \text{ NH}_3 \uparrow + 8\text{H}_2\text{O}$ **Reaction 2.** Theoretical pathway of the formation of HAP using diammonium hydrogen phosphate as a precursor.

In reality, the HAP formed through this reaction may be non-stoichiometric; Ca deficient or HAP containing substituted carbonate species may form [21, 27]. The HAP precipitation is expected to be preceded by the formation of intermediate metastable phases, such as amorphous calcium phosphate, monocalcium phosphate monohydrate, dicalcium phosphate dehydrate and/or octacalcium phosphate. These intermediate phases whose formation depends on the reaction conditions (e.g., degree of supersaturation, temperature, pH, presence of foreign ions, etc.), are expected to transform into HAP or its ion-substituted analogues [20, 27, 28]. The resulting hydroxyapatite network is expected to improve cohesion between loose particles at the immediate subsurface of wall painting [29, 30].

The superior qualities of HAP as a consolidating agent for calcium carbonate matrices lies in the fact that it has a much lower solubility ($K_{sp} = 1.6 \times 10^{-117}$ at 25 °C [27]) than calcite (K_{sp} =3.4 x 10⁻⁹ at 25 °C [31]) and it also has a crystal structure similar to calcite – the unit cell being hexagonal for HAP [32] and rhombohedral for calcite, but often described as hexagonal. The lattice parameters of hydroxyapatite and calcite are relatively close: respectively, a = b = 9.43Å and c =6.88Å for HAP [33] and a = b = 9.96 Å and c = 17.07 Å for calcite, considering two molecules per unit cell [34]. This indicates compatibility in the nucleation of the phosphate layer onto the surface of carbonate stones and strong bonding of the newly-formed layer onto the substrate [30]. The other advantages is that it has a dissolution rate about 4-5 orders of magnitude lower than that of calcite: $R_{diss, HAP} = 1 \times 10^{-14}$ moles• cm⁻²•s⁻¹ and $R_{diss, calcite} = 5.4 \times 10^{-9}$ moles• cm⁻²•s⁻¹ at pH = 4 [35, 36]. It is therefore more stable in a range of pH and it is expected to provide additional protection against acid dissolution. It's known that hydroxyapatite is the least soluble and the most stable calcium phosphate phases in aqueous solutions at pH values higher than 4.2 [37, 38].

1.3 Motivation, Significance and Rationale of this Research

While the advantages of HAP as a consolidant mainly for calcium-rich stones such as limestones and marbles have been demonstrated by previous research [23, 24, 39-47], there is clearly a lack of fundamental understanding of the processes of the reaction chemistries (reaction between calcium carbonate substrate and pigments – in the case of wall paintings – and the ammonium phosphate precursors) and also the effect of this treatment on exterior and interior surfaces of varied environments and polychrome artifacts like wall paintings, applied in *fresco*.

In this arena, this research addressed these needs and brings new scholarships with:

- 1. the study of the effects of HAP on wall paintings (lime plasters and pigments);
- the study of the effects of HAP on site (on original surfaces-exterior/interior of varied microclimate;
- the study of the fundamentals based on experimental and theoretical thermodynamic modeling of the chemical reactions between calcium carbonate substrate and the ammonium phosphate precursors.

The significance of this research is twofold and stems from: (1) the fact that previous conservation applications were based on empirical testing and experimentation, and this research is the first to incorporate theoretical modeling with experimental research to understand

biomimetic inorganic mineral systems as alternative – to – polymers consolidating agents for the conservation and protection of porous building materials and decorated surfaces, mainly wall paintings and (2) the interdisciplinary nature of this research at interface between materials engineering, bioengineering, biotechnology and conservation science with expected outcomes to directly and indirectly impact different fields from archaeology and conservation to civil engineering and biomedical research.

The interdisciplinary nature of this research formulates novel, context-sensitive consolidation solutions for decorated surfaces such as rock-art, wall paintings and mosaics of significant archeological, historic and artistic value. The concept and development of the proposed treatment builds on a biomimetic process to control and regulate the deposition of HAP at surface and subsurface levels. The deposition is triggered to occur in a manner wherein it enhances the cohesion and integrity of the material without preventing necessary fluid-exchange with the environment, and/or detrimentally affecting the aesthetic appeal. This research enables faster and more informed translation of new knowledge to the diverse areas of stone and porous building materials rehabilitation as applicable to modern infrastructure, ancient structures and tooth, bone and fossil reconstruction.

Through the targeted application of analytical techniques of high sensitivity and specificity, this research develops quantitative descriptions, which interrelate the substrate properties and precursor reactant chemistry to the extent of cohesion and the pre-to-post consolidation improvements in the material. By carefully considering and evaluating the chemical-impact of the treatment on the overall cohesion and aesthetic properties, this research paves the way for tailormade to-order consolidation treatments for artwork which consider the intrinsic material properties as a critical decision variable to meet desired protection parameters, without damaging effects.

This is an important step forward to alleviate: (1) trial-and-error based empirical routines which have been long used in heritage conservation applications and (2) the application of inappropriate organic or inorganic toxic (e.g., barium-based) consolidating agents. The integration of aspects of archeology, biotechnology and materials science is significant in that, it enables utilization of a broad multidisciplinary skill-set to a social-and-scientific responsibility based conservation need. All in all, by promoting biomimetic treatments based on ecological, biocompatible materials this research advances a new paradigm in forwarding organic solutions to critical heritage conservation applications.

1.4 Dissertation Outline

This research develops and evaluates the effectiveness of using ammonium phosphate as precursors to in situ synthesize hydroxyapatite (as a consolidant) within calcium-rich matrices such wall paintings, complemented by computations and experiments on the thermodynamics of the system. The presentation of the chapters follows an iterative process and focuses on different perspectives of the development, application and evaluation of ammonium phosphate solutions for the formation of HAP.

Chapter 1 provides an introduction and motivation of research. Chapter 2 describes the use of diammonium hydrogen phosphate (DAP) solutions as precursors to react with simulated archaeological wall painting substrate test blocks to in situ synthesize hydroxyapatite. Morphological, microstructural and physicochemical characterization is performed on treated test blocks to study the in situ formation of hydroxyapatite and evaluate its consolidation effect. Chapter 3 elaborates on the chemical reactions between the diammonium hydrogen phosphate solutions and eight common pigments used as colorants in archaeological polychrome surfaces and wall paintings. The phase, microstructural and color changes after treatment with DAP solutions were evaluated. Chapter 4 focuses on the evaluation of hydroxyapatite effects for the consolidation of a Hellenistic-Roman chamber tomb hewn out of marlstone rock at Athienou-Malloura in Cyprus. The phase, composition and microstructural changes of the marlstone after the DAP treatment at different time periods (immediately after and after one year) was assessed. Chapter 5 delves on the fundamental aspect of this research based on theoretical modeling. More specifically, it investigates the thermodynamics of the CaCO₃-(NH₄)₂HPO₄/(NH₄)H₂PO₄-H₂O system. The focus is placed on the morphological, mineralogical and thermodynamic characterization of calcium phosphates precipitating as a result of the reaction between calcium carbonate substrate and different ammonium phosphate solutions. The thermodynamic investigations aimed at providing guidance for the prediction of the formation of calcium orthophosphate phases, such as HAP, during the application of ammonium phosphate solution treatment on the calcium carbonate-rich matrices.

Finally, Chapter 6, summarizes the results of the research and new information developed through this research. Additional experiments based on laboratory simulations for controlled growth of hydroxyapatite, which could further enrich our knowledge on this subject are also suggested in this chapter.

Chapter 2. Application of Diammonium Hydrogen Phosphate (DAP) Precursors for In Situ Consolidation of Calcium Hydroxide Plaster Layers

A significant component of the research builds on laboratory experiments using simulated calcium-rich matrices of poor cohesion to imitate authentic weathered artifacts and conditions at close approximations. Despite the challenges that experimental research may pose due to the difficulties in artificially reproducing exact degradation phenomena, there are considerable advantages associated with simulated laboratory research. First, experiments can be performed under well-defined, controlled conditions. Second, the reproducibility of the experiments allows for statistical analysis. Third, no limitations are imposed in terms of sample availability or on type of instrumentation used for the analysis of samples.

For this reason, test blocks of Ca-rich substrates were prepared using commercial and high purity chemical grade materials to simulate archaeological mortars and plasters, and tested.

2.1 Preparation of Wall Painting Substrate Test Blocks (Mock-ups)

In order to test the hydroxyapatite (HAP) consolidation effect, weak (slightly powdery) test blocks of mortars/plaster layers, with an average total porosity of 20% were prepared by mixing: (1) commercial calcium hydroxide (lime putty or slaked lime) with marble dust (CaCO₃) aggregates (0.2 - 0.6 mm) and deionized water at 1:4:1 volumetric ratio and (2) commercial lime putty with pure quartz (SiO₂) aggregates (0.2 - 0.6 mm) at the same ratio as above. The commercial Ca(OH)₂ was aged between 4-6 years. The mixtures at a consistency of a dense paste (mortar) were prepared using a stirring machine (Kitchen Aid Professional 600 series). Once mixed

adequately, the mix was poured in custom-made stainless-steel molds (5cm x 5cm x 2cm) and left to dry at 25°C and ~60% RH for one week. Consequently, the samples were de-molded and left to set fully for 6 months in order to ensure full conversion of the $Ca(OH)_2$ binder to $CaCO_3$. The calcium hydroxide, marble dust and sand were purchased from Kremer Pigments Inc.

2.2 In situ Formation of HAP using Diammonium Hydrogen Phosphate (DAP) Precursors

DAP solutions were tested as precursors for the formation of HAP. For the application of the reagents cellulose compresses (poultices) were prepared of 1M and 2M DAP. Each compress was approximately 1 cm thick. A permeable thin tissue of compact weave (Tengucho paper) was placed at the interface between the test block and the cellulose poultice to prevent cellulose fibers from sticking on the surface of blocks and to enable easier removal of the poultice (Figure 2-1). 3 to 6 hours of contact time were tested. Polypropylene thin foil was used to cover the cellulose compress in order to slow down the evaporation. During the reaction, a faint smell of ammonia was detected. After completion of the treatment, the cellulose poultice was removed and the test blocks were left to dry in air.

The DAP solutions were made using deionized water and analytical reagent grade purchased from Fisher Scientific. Each cellulose compress was prepared by mixing Arbocel cellulose fibers: BC 200 and BWW 40 at a volumetric ratio of 0.4 (BC 200):0.6 (BWW 40):0.9 (DAP of MAP solution). Tengucho tissue purchased from Hiromi Paper, Inc. was used as the intermediate filter layer.



Figure 2-1. (a) Schematic drawing and (b) photograph illustrating the application of DAP solution on the mock-ups.

The theoretical chemical pathway of the HAP formation using DAP as the precursor is presented in Reaction 2. A schematic diagram of the anticipated consolidation effect with the in situ formation of HAP is illustrated in Figure 2-2. HAP (black dotted surface) forms at the surface and subsurface treated, preferentially in areas occupied by microcrystalline CaCO₃ (plaster binder). The large grey/whitish particles represent aggregates in the plaster layer. The resulting hydroxyapatite network is expected to improve cohesion between loose particles at the immediate subsurface of the wall painting.

 Before consolidation
 After consolidation

Consolidated area. Formation of HAP.

Figure 2-2. Schematic representation of the in situ consolidation of a wall painting plaster. Not in scale.
2.3 Experimental

Phase transformations during setting (hardening) were determined by X-ray diffraction (XRD) on a Bruker D8 Diffractometer using the following measurement parameters: Cu K α radiation λ =1.5405Å, 40kV, 40mA, 10 to 60° 2 θ exploration range with a step size of 0.014° 2 θ . Mineral phases were identified by comparison to the JCPDS (Joint Committee on Powder Diffraction Standards).

The calcium carbonate/calcium hydroxide content was determined using thermal analysis (TG/DTG) performed in a nitrogen environment using a Perkin Elmer STA 6000 thermal analyzer. Temperatures were scanned in the range between 35-980°C and heating rate was set for 10°C/min.

The effectiveness of the treatment in providing consolidation action and the formation of HAP network was evaluated using scanning electron microscopy (SEM) at variable pressure (VP) coupled with energy dispersive x-ray spectroscopy (EDS); X-ray fluorescence spectroscopy (XRF); water sorption test and scotch tape test.

SEM analysis was performed using the FEI Nova[™] NanoSEM 230. Morphological and topographic characteristics of the surface before and after treatment were recorded using the secondary electron detector (SE). The depth of penetration and spatial localization of the newly phosphate-containing forms was assessed using backscattered electron detector (BSE) and energy dispersive x-ray spectroscopy (EDS) on polished sections. Polished sections were prepared by embedding samples in epoxy resin (Buehler EpoxiCure[®]) mixed with EpoxiCure[®] Epoxy

Hardener and placed under vacuum using the Buehler Cast n' Vac 1000. Once the resin had set, the samples were cut using Labcut 1010 low speed diamond saw and ground using Buehler silicon carbide grinding papers from 240 to 1200 grit. Samples were subsequently polished on a Leco[®] GP-25 polishing turntable using water-based diamond suspensions of 6 μm and 1 μm followed by colloidal silica of 0.2 μm spread on Buehler[®] MasterTex polishing cloths.

XRF data provided complementary semi-quantitative results on the phosphorous content using the Thermo Scientific Niton[®] XL3t Series GOLDD[™] technology handheld XRF analyzer with a silver anode and silicon drift detector. As all the consolidation trials were performed on the test blocks positioned vertically, it was anticipated that measured P content would be higher for the slices taken from the bottom of the sample due to the gravity, while slightly lower for the slices taken from the top of the wall painting test blocks. To have the most representative and consistent information sections from the center of the samples were chosen for the preliminary XRF analysis.

The water sorption test was conducted following a modified version of the ASTM standard C1585. For this test both untreated and treated test blocks were kept in the atmospheric air at $20 \pm 3^{\circ}$ C at around 60% RH (relative humidity) for the period of 7 days prior to testing. All sides of each test block (except the surface receiving the treatment) were sealed tightly with duct tape. The surfaces of untreated and treated samples were placed in contact with the water (immersed between 1-3 mm) and the mass of each sample was monitored to the nearest 0.01 g at the intervals specified in the standard.

The absorption, was calculated per the Equation 1.

$$I = \frac{m_t}{a/d}$$

Equation 1. Calculation of water intake.

Where:

I = the absorption;

 m_t = the change in specimen mass in grams, at the time t;

a = the exposed area of the specimen, in mm²;

d = the density of the water in g/mm³.

The scotch tape test (adhesion test) was performed based on a modified version of the ASTM standard D3359. Strips of scotch tape (4 cm long) were pressed onto the surface of each sample using enough fingertip pressure to remove all visible air bubbles and make good contact between the tape (adhesive side) and the surface. The tape was then peeled off in a single smooth motion. Systematic mass analysis of the tape before and after adhesion with the mortar surface provided an indication of the consolidation treatment performance. The results were evaluated visually and quantitatively based on the amount of material separated from the surface before and after the consolidation treatment.

2.4 Results and Discussion using DAP as Precursor: Morphological, Microstructural and Physicochemical Characterization

2.4.1 Simulation of Wall Painting Substrate Test Blocks

The XRD pattern of the mock-ups (Figure 2-3) show that the "marble dust" sample consists of calcite (CaCO₃) and portlandite (Ca(OH)₂) whereas the "quartz" sample consists of calcite, portlandite and SiO₂.



Figure 2-3. XRD patterns of the mock-ups.



Figure 2-4. TG curves of test blocks after 6 months of carbonation in the air: (a) "marble dust" sample and (b) "quartz" sample.

The TG curve of the "marble dust" sample (Figure 2-4(a)) show that the temperature range between 375°C to 480°C corresponded to the dehydration of Ca(OH)₂ while the temperature range between 640°C to 940°C corresponded to the decomposition of CaCO₃. A weight loss of 1.4 wt.% could be observed between 375°C to 480°C, corresponding to a Ca(OH)₂ content of 5.6 wt.% while a weight loss of 41.5 wt.% could be observed between 640°C to 940°C, from which a CaCO₃ fraction could be calculated to be 94.3 wt.%. This indicates that most of the original Ca(OH)₂ binder transformed into calcium carbonate.

Figure 2-4(b) shows the TG curve for the "quartz" sample. From the TG curve above, a 1.6 % weight loss could be observed between 370 °C to 480°C, corresponding to a fraction of a Ca(OH)₂ content of 6.4 wt.% while a 4.1% weight loss could be observed between of 600°C to 787°C, from which a CaCO₃ fraction could be calculated to be 9.3 wt.%. The remaining 84.3 wt.% corresponds to the fraction of SiO₂. Since only Ca(OH)₂ and quartz aggregates (SiO₂) were used for the production of the "quartz" samples, the results indicate that majority of Ca(OH)₂ has transformed into CaCO₃.



Microcrystalline CaCO₃ carbonated from Ca(OH)₂ binder

Figure 2-5. (a-b) Optical photomicrographs of the mock-ups before the DAP treatment; (c-d) Optical photomicrographs of the mock-ups after the 1M DAP treatment. Secondary electron (SE) micrographs of the mock-ups of (e) "marble dust" and (f) "quartz" samples. SE micrographs of mock-ups after treatment in (g) "marble dust" sample and (h) "quartz" sample.

From the SEM images of the mock-ups (Figure 2-5), both samples were very powdery pointing towards a poor binding mechanism between the microcrystalline $CaCO_3$ resulting from the carbonation of the $Ca(OH)_2$ plaster binder and the aggregates.

2.4.2 The Effect of DAP Treatment

Mortar samples (with marble dust aggregates) treated with 1M DAP show the formation of calcium phosphate phases (Figure 2-5(g-h)) as a result of the in situ reaction between the DAP solution and the Ca, mainly from the microcrystalline CaCO₃ binder; though the Ca from the larger marble dust particles in the "marble dust" samples has also contributed in the formation of calcium phosphate phases.

Elemental analysis using EDS of the Ca-P phases combined with morphological characterization of the crystal habit using high resolution secondary electron imaging provided a rough estimation of the Ca-P phases formed. The P content measured by EDS (point analysis) in phosphorous-rich regions (at spatial resolution of $\sim 1 \mu m$) ranged between $\sim 3-6$ wt. % for 1M treatments and ~ 2-5 wt.% for 2M treatments. The newly formed calcium phosphate phase using 1M DAP was identified as hydroxyapatite (HAP), based on its characteristic tabular crystal network. The morphology of the crystals was also very similar to the hydroxyapatite produced by Yang et al. 2011 (Figure 2-6) [19]. Taking into consideration the experimental conditions (25°C and pH~8 which corresponds to 1M DAP), HAP seems also to be thermodynamically the most favored phase to form and it has low solubility ($K_{sp} = 1.6 \times 10^{-117}$ at 25 °C [27]) and crystallographic compatibility with calcite. The theoretical P content of the ideal hydroxyapatite is approximately 18.5 wt.% and the Ca/P molar ratio is close to 1.67. These values significantly differ from what we have observed. However, an ideal hydroxyapatite in nature rarely precipitates from the aqueous solutions and therefore it is more common to form hydroxyapatite, which is nonstoichiometric and substituted by various ions, such as carbonate ions. Also in this research, the reaction between the DAP and the CaCO₃-rich matrices does not involve complete transformations and the overwhelming signal of the unreacted CaCO₃ affects the measured Ca to P ratio.



Figure 2-6. SEM image of the cross-section of test blocks treated by the biomimic method where a porous and interlocked hydroxyapatite structure was produced. Reproduced by Ref. [19] with permission from The Royal Society of Chemistry.

The phosphorous content and profile distribution was also assessed through EDS on polished cross sections at the core of the samples, as shown in Figure 2-7 and Figure 2-8. The P concentration was found to be higher closer to the surface of the sample which was in contact with the DAP saturated compress and in areas surrounded by large pores as more of the DAP solution could enter and fill up nearby pores and react with the surrounding CaCO₃. For the treated "marble dust" sample, the elemental mapping of calcium (Figure 2-7(b)) shows that the Ca is located in areas corresponding to the marble dust particles, as well as, the microcrystalline CaCO₃ binding matrix. The P (Figure 2-7(c)) is located in areas corresponding to the microcrystalline CaCO₃ binding matrix and at the boundaries of the large Ca-rich particles. Since the marble dust is composed of calcite (CaCO₃) it is believed that in the case of the marble dust test blocks, the calcite aggregates also partially react with the DAP solution (Figure 2-7). The extent of this reaction may be kinetically restrained due to the short contact times (between 3-6 hours) of the consolidation treatment tests.



Figure 2-7. SEM-EDS images of a polished section of the "marble dust" sample treated for 6 h with 1M DAP solution; (a) Back-scattered images of the cross-section of the test block; elemental map indicating the spatial distribution of (b) Ca and (c) P.



Figure 2-8. SEM-EDS micrograph of the "quartz" sample treated for 6 h with 1M DAP solution; (a) Back-scattered electron microscopy images of the cross-section of the test block; elemental map indicating the spatial distribution of (b) Ca, (c) P and (d) Si.

For the treated "quartz" sample, Ca is located in areas corresponding to the microcrystalline CaCO₃ binding matrix (Figure 2-8(b)); Si is located in areas corresponding to the quartz particles (Figure 2-8(c)) and P is located in areas corresponding to the microcrystalline CaCO₃ binding matrix (Figure 2-8(d)). As SiO₂ particles are not expected to react with the DAP solution, the formation of calcium phosphate phases for the quartz-based test blocks is considered as the result of the DAP interaction with the microcrystalline CaCO₃ binder surrounding the quartz aggregate.

XRF analysis was performed on both untreated and treated samples. XRF allowed for semiquantitative measurements of the P at relatively large areas of the sample (spot size ~8mm). XRF analysis was carried out on the same cross-sectional areas of the core of the samples analyzed with the EDS. Each of the sections was measured in 3 locations: at the surface, in the center and at the bottom (away from the surface) of each slice (Figure 2-9).

Surface: contact area with DAP compress

Bottom: area away from the DAP compress



For each sample an average (mean) of three XRF readings was calculated. It was observed that the P content decreases with increasing distance from the DAP saturated poultice (Figure 2-10(a)) while the Ca concentration remained constant throughout the samples (Figure 2-10(b)). The results indicated that at the surface (closer to the DAP poultice) the P concentration was the highest. However, even at the bottom of the section (furthest from the surface), phosphorous could still be detected. This is an indication that the phosphorous (induced in the DAP solution) was transported for at least 2 cm distance using this application method.



Figure 2-10. Mean values (n=3) of phosphorus (a) and calcium; (b) contents measured in wt.%, at the surface, in the center and at the bottom of the wall painting test blocks at different consolidation treatments.

The water sorption tests between untreated and treated samples show a final reduction in water sorptivity in the range of ~8-11 wt. %. The results (Figure 2-11), show an exponential trend with noticeably reduced initial sorptivity in the DAP treated samples, compared to the untreated ones (time measured up to ~5 min). The delay in the water intake at the beginning of the test for the treated samples suggests potential change (reduction) in porosity, inhibiting a large quantity of

water from quickly entering the substrate. In particular, the 2M DAP-treated sample exhibited an initial sorption rate markedly lower (between ~20-32 wt. %) than the untreated sample, possibly as a result of the higher pore system modification that occurred on the surface. For the 2M DAP treatments, excessive whitening and formation of a thin 'crust' on the surface was also detected. This indicated the formation of a much denser layer of calcium phosphates at the surface in comparison to the 1M treatments. However, the overall sorptivity trends (t >300 minutes) seemed to be similar regardless to the DAP concentration. This modest reduction in water absorption (when compared to the untreated samples) suggests that water uptake and water vapor exchange between the mortar block and the environment is reduced but not significantly impeded after treatment. Following a tailored application of the DAP precursor with regards to concentration and contact time, it was possible to achieve a controlled hydroxyapatite consolidation effect, almost without changing the overall porosity of the system. Good permeability of the treated CaCO₃plaster substrate allows water to transfer easily from the substrate as water vapor.

Scotch tape (adhesion) tests were also used to evaluate the performance of the consolidation treatment (Figure 2-12). The results indicated that fewer particles were separated from the surface for the samples treated with DAP compared to the untreated samples. The mass change results before and after the consolidation are shown in Table 2-1 and Table 2-2.



Figure 2-11. Water sorptivity measured for wall painting test blocks prepared from lime putty and (a) marble dust aggregates; (b) quartz aggregates, which undergone different consolidation treatments.



Figure 2-12. Photograph showing visually the results from the scotch tape test performed on the "marble dust" sample: untreated sample (left), 1M DAP treated for 6 h (center) and 2M DAP treated for 6 h (right).

Table 2-1. Mass change before and after the consolidation treatment for wall painting test blocks prepared with marble dust aggregates.

Treatment conditions	1M DAP 3h	1M DAP 6h	2M DAP 3h	2M DAP 6h
% of mass change before and after the consolidation	84.73%	88.18%	89.66%	98.52%

Table 2-2. Mass change before and after the consolidation treatment for wall painting test blocks prepared with quartz aggregates.

Treatment conditions	1M DAP 3h	1M DAP 6h	2M DAP 3h	2M DAP 6h
% of mass change before and after the consolidation	68.45%	69.90%	67.48%	83.01%

The higher the percentage is, the better the consolidation effect of the surface. The best consolidation effect was observed for samples treated for 6 hours with 2M diammonium hydrogen phosphate solution (DAP) e.g. for marble dust-based wall painting test blocks, 98% fewer particles detached in comparison with the untreated samples. However, excessive whitening of the surface was observed. Whitening is not desired as it aesthetically affects wall painting's appearance.

2.5 Conclusions

In situ phosphate based consolidation utilizing ammonium phosphate precursors is a promising treatment to re-establish cohesion of powdery Ca-rich matrices such as wall paintings (like *fresco*). Preliminary results indicated the formation of a porous hydroxyapatite network at the surface and subsurface of the wall painting test blocks, as well as reduction of water absorption and insignificant color change. Less 'whitening' of the surface due to the formation of new phases was observed on samples treated with 1M DAP solutions in comparison with 2M DAP solutions. It was found that phosphate solution enters deeply into the wall painting test blocks and penetration depth of the proposed consolidation method is at least up to 2 cm. Consolidation treatment seems effective for wall paintings and calcareous stones but is believed to have also potential for archaeological tooth, bone and fossil reconstruction.

Chapter 3. Effects of Diammonium Hydrogen Phosphate (DAP) Solutions on Pigments

Polychrome architectural surfaces and wall paintings are hierarchical multilayer-structures consisting of a substrate (rock/wall support and or plaster layer) and a paint layer made of a binary system of a pigment (or a mixture of pigments) and a binding medium (film forming). Using a small number of selected pigments, representative of both *fresco* and *secco* applications often encountered on archaeological wall paintings, this chapter investigates as a pressing conservation necessity, the performance of pigments (physical and chemical integrity) and alternation/phase transformation processes (physical and chemical reactions) when in contact with DAP precursors for the formation of hydroxyapatite (HAP) as a potential consolidant for powdery wall paintings.

Research on the effects of diammonium hydrogen phosphate applications for the consolidation – through the formation of hydroxyapatite-HAP – on calcium-carbonate-rich matrices, including limestones, marbles and marlstones has been previously carried out and evaluated [19, 23, 39-41, 44-48]. However, there has no thorough assessment been performed on pigments, often encountered in ancient polychrome artifacts. For the first time, the optical, physical and chemical effects of ammonium phosphate precursor on pigments has been investigated and evaluated. More specifically, research has been conducted on the interactions of DAP solutions with eight pigments commonly found in wall paintings and other polychrome monumental surfaces aiming to answer questions such as:

• Is there any obvious color change after contact with DAP solutions?

- Are there any chemical or morphological changes occurring?
- What are the possible mechanisms leading to color change and/or other forms of physical and chemical phase transformation?

3.1 Materials and Methods

3.1.1 Materials

1M DAP solution was prepared by adding appropriate amount of DAP (Fisher Scientific, purity: 99+%, used as received) into deionized water. In order to study the chemical reaction between the DAP solution and the pigments, eight commercial inorganic pigments were used, including seven pigments commonly used for *fresco* application, such as, cinnabar, lapis lazuli, white chalk, French ochre, green earth, burnt umber, raw sienna and one pigment frequently encountered in *secco* application-red lead. Pigments were purchased from Kremer Pigments Inc. (Table 3-1).

3.1.2 Experimental Design

For the experimental application, 10 g of each pigment were dispersed in 100 ml of 1M DAP solution and in 100 ml of DI water that was used as control, and sealed in a glass bottle. The bottles were kept in dark to avoid any photochemical reactions. The room temperature (T) was

maintained at ~22 °C and the relative humidity (RH) ~50%. The pH values of the solutions were measured using an Oakton EcoTestr[®] pH2 Waterproof pH Tester (standard error: \pm 0.1). Prior to any measurement, the pH meter was calibrated using buffer solutions at pH 7 and pH 10.

pH measurements were taken of the 1M DAP solution and on each pigment dispersion on day 0, a few minutes after pigments were dispersed in the DAP solution, and subsequently on regular intervals: every 24 hours between day 1 and 7, and then day 14, day 21, and day 28. For pigments of cinnabar, lapis lazuli, French ochre, Sienna, burnt umber, green earth, no further phase change was detected by XRD after twenty-eight days of immersion in DAP. So the monitoring of color/phase change of those pigments was focused on the first twenty-eight days. Red lead and chalk however, showed phase and color change even after two months of immersion in the DAP solution. For these two pigments, further monitoring will be required.

Pigment particles were also collected for analysis using different techniques every 24 hours between day 1 and 7, and then on day 14, day 21, day 28 after dispersion into 1M DAP solution. Prior to analysis, the powders were rinsed using DI water and left to dry overnight on filter paper. Samples listed in Table 3-1 were named using the abbreviation of the pigment name and the immersion time. For instance, CIN-raw stands for cinnabar pigment prior to the analysis, whereas CIN-d28 stands for cinnabar precipitate collected twenty-eight days after dispersion in 1M DAP solution. The characterization techniques employed included digital microscopy (DM), X-ray diffraction (XRD), thermo-gravimetric analysis (TGA), scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDS), Fourier transform infrared spectroscopy (FTIR), Raman spectromicroscopy (µ-RS) and fiber optic reflectance spectroscopy

(FORS).

Pigment Name (Color)	Source	Characterization Techniques	Composition	Catalog No.
Cinnabar (deep red)	Hunan District China	DM, SEM-EDS, XRD, μ-RS , FORS	Cinnabar (α-HgS) ; BaSO4	No. 10620
French Ochre (red)	France	DM, SEM-EDS, XRD, FTIR, FORS	Hematite (Fe ₂ O ₃); Kaolinite(Al ₂ Si ₂ O ₅ (OH) ₄) , muscovite (KAl ₂ (AlSi ₃ O ₁₀)(F,OH) ₂) and quartz (SiO ₂)	No. 40090
Chalk (white)	Champagne, France	DM, SEM-EDS, XRD, FTIR, TGA, FORS	Calcite (CaCO3)	No. 58000
Red lead (bright orange)	-	DM, SEM-EDS, XRD, FTIR, FORS	Minium (Pb ₃ O ₄)	No. 42500
Lapis Lazuli (blue)	Chile	DM, SEM-EDS, XRD, FTIR, FORS	Lazurite ((Na,Ca)8(AlSiO4)6(SO4,S,Cl,OH)2); Wollastonite (CaSiO3), Cancrinite (Na6Ca2[(CO3)2Al6Si6O24'2H2O]) and feldspar	No. 10562
Raw Sienna (yellow)	Italy	DM, SEM-EDS, XRD, FTIR, FORS	Goethite (a-FeOOH); Gypsum (CaSO4 2H ₂ O), calcite (CaCO ₃), Montmorillonite ((Na,Ca) _{0.3} (Al,Mg) ₂ Si ₄ O ₁₀ (OH) ₂ ·n(H ₂ O)) and quartz (SiO ₂)	No. 40400
Burnt Umber (brown)	Cyprus	DM, SEM-EDS, XRD, FTIR, FORS	Hematite (Fe ₂ O ₃) and Mn oxides; Calcite (CaCO ₃) and quartz (SiO ₂)	No. 40710
Green Earth (pale green)	Verona Italy	DM, SEM-EDS, XRD, FTIR, FORS	Fe-smectite; Calcite (CaCO ₃), Plagioclase, Hematite (Fe ₂ O ₃) and Chlorites	No. 11000

Table 3-1. Descriptions of the pigments.

3.1.3 Characterization

Using combined optical and analytical techniques, the analysis aimed to provide a qualitative and quantitative assessment of the interactions of DAP with the pigments.

All powders were first examined using a Keyence VHX-1000 Digital Optical Microscope,

between 20x and 200x magnification.

XRD measurements on the pigment powders were performed using a Bruker D8 diffractometer with the following measurement parameters: Cu-K_{α} radiation, λ = 1.5404 Å, voltage

40 kV, beam current 40 mA and a 2-80° 2 θ exploration range with a step size of 0.014° 2 θ . The intensity values of each XRD pattern of the same pigments was normalized to [0, 1] (unity based normalization bringing the values into the range [0, 1]) and offset for comparison purposes.

TGA analysis was performed on selected pigment powders using a Perkin Elmer Pyris Diamond TG/DTA (Thermogravimetric/Differential Thermal Analyzer). Temperatures were scanned in the range between 40 °C to 900 °C, at the heating rate of 20°C/min, in a flowing Ar atmosphere.

Microstructural and elemental analyses of powders were performed on a FEI Nova NanoSEMTM 230 scanning electron microscope at variable pressure (VP) coupled with energy dispersive x-ray spectroscopy (EDS).

FTIR analysis was performed on a JASCO FT/IR-420 Fourier Transform Infrared Spectrometer using the KBr pellet method. Sample powders were ground and dispersed in a KBr matrix with a concentration around 0.5 wt. % and then pressed into a pellet. All spectra were collected at 64 scans with a spectral resolution of 4 cm⁻¹, from 4000 to 400 cm⁻¹. Spectra were matched against the spectral database of the Infrared and Raman Users Group (IRUG) and published literature. The intensity of each FTIR spectra of the same pigments were normalized to to [0, 1] and offset for comparison purposes.

Raman spectra were collected using a Renishaw InVia Raman spectrometer coupled with a Leica DM2500M microscope. A diode near-infrared (NIR) 785nm laser was used for excitation. The spectrometer is equipped with a 1200 lines per millimeter grating and an air-cooled CCD array detector. All measurements were collected at 50x magnification. Wavenumber calibration was performed using the Raman peak of a silicon crystal at 520 cm⁻¹. Each Raman spectrum was recorded for ~10s with 10 accumulations and with a spectral resolution of 2 cm^{-1} . The spectra were acquired using the WiRETM 3.4 software system.

FORS spectra were measured using an Ocean Optics USB 2000+ fiber optical spectrophotometer and the FieldSpec3® Spectroradiometer from Analytical Spectral Devices Inc. (ASD). The spectro-colorimetric measurements allowed quantification of incident and reflected radiation intensities, which roughly equals to human color perception. During the measurement, the white diffuse reference standard was measured every 30 minutes. The intensity values of the FORS spectra of the same pigments were normalized to [0,1] to facilitate comparisons. Color values were recorded in the L^{*}a^{*}b^{*} color space defined in 1976 by CIE (Commission Internationale de l'Eclairage") [49]. The CIE L*a*b* model is a three-dimensional model and the coordinates L^{*} represents the lightness of color (ranging from 0-100, L^{*}=0 represents darkest black and L^{*}=100 indicates brightest white), a^{*} represents red and green (a positive number indicates red, a negative number indicates green) and b^{*} represents yellow and blue (a positive number indicates yellow and a negative number indicates blue). Change in color/color difference (ΔE^*) was calculated with the following formula (Equation 2) by the CIE:

Equation 2. $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

Where ΔL^* , Δa^* and Δb^* are the differences in L^* , a^* and b^* values before and after immersion in DAP solutions. ΔL^* describes the change in luminance, Δa^* the change in red/green components,

and Δb^* the change in yellow/blue components. While generally a $\Delta E^* \leq 2$ is widely acceptable as the value detectable by the human eye [50], a color difference of $\Delta E^* \leq 5$ has been established as the threshold in the field of cultural heritage to evaluate color change after a conservation intervention such as consolidation [20, 51-55].

3.2 Results and Discussions

3.2.1 Cinnabar

Pigment Composition and Physical Characteristics

Cinnabar (α -HgS) is a natural mineral and crystallizes in the hexagonal system, with space group P3₂21. It has a deep red color with angular particles of various sizes up to 100 µm (Figure 3-1(a-d)). XRD and µ-RS analyses on the red pigment particles (raw pigment) identified cinnabar as the main constituent (α -HgS, JCPDS PDF No. 6-256) (Figure 3-1(e)). This was also indicated by µ-Raman (Figure 3-1(f)) with major Raman peaks at 253, 284 and 342 cm⁻¹. Trace impurities of BaSO₄ were also detected in the pigment.

Changes in the Morphology/Structure, Composition and Color

The calculated ΔE^* value for cinnabar pigment particles before and after twenty-eight days of immersion in DAP is 3.536 (Table 3-5). Though this value is above the threshold of color change detected by the human eye [50], it is still below the established value ($\Delta E^* \leq 5$) accepted for cultural heritage consolidation treatments [20, 51-55]. No detectable phase transformation could be observed in the pigment particles (CIN-raw) before immersion in DAP and after twenty-eight days (CIN-d28) in DAP solution, based on XRD analysis, μ -Raman spectroscopy and FORS (Figure 3-1(g)), which showed consistently the characteristic sigmoid-shaped spectrum with an inflection point (maximum at its first derivative, Figure 3-1(h)) ~614 nm corresponding to the bandgap of cinnabar [56].



Figure 3-1. (a) Photomicrograph of the CIN-raw sample; (b) Secondary electron (SE) micrographs of the CIN-raw sample; (c) DM photomicrograph of sample CIN-d28; (d) SE micrographs of sample CIN-d28.



Figure 3-2. (a) XRD pattern of CIN-raw, CIN-d28 and CIN-2m; (b) Raman spectra of CIN-raw and CIN-d28; (c) FORS spectra of cinnabar: CIN-raw, CIN-d1, CIN-d7, CIN-d28; (d) first derivative of the FORS spectra in (c). The intensity values of each XRD pattern, FORS spectra and its first derivative plots were normalized to [0, 1] and offset for comparison purposes.

3.2.2 French Ochre

Pigment Composition and Physical Characteristics

Ochres are natural earth pigments of hydrated ferric oxide and with color varying from dull yellow to red and brown. The darker red ochres are richer in hematite (Fe₂O₃) and yellow ochres contain mainly goethite (α -FeOOH) [57].

Analysis of the French ochre using SEM-EDS, XRD, FTIR and FORS pointed to a hematite (Fe₂O₃)-rich pigment with various amounts of quartz, kaolinite and muscovite (Figure 3-3).

Changes in the Morphology/Structure, Composition and Color

The calculated ΔE^* value for French ochre pigment particles before and after twenty-eight days of immersion in DAP is 3.390 (Table 3-5). Though this value is above the threshold of color change detected by the human eye [50], it is still below the established value ($\Delta E^* \le 5$) accepted for consolidation applications in cultural heritage [20, 51-55]. No detectable phase transformation could be observed in the pigment particles (FRE-raw) before immersion in DAP and after twenty-eight days (FRE-d28) in DAP solution, based on the XRD, FORS and FTIR analysis.

XRD analysis of all samples analyzed (Figure 3-4(a)) identified quartz, muscovite, kaolinite, and hematite. FTIR spectroscopy further corroborated the results from previous analyses (Table 3-2) [58-64]. However, it should be noted that the Fe-O vibration of hematite have vibrational bands at 538 and 469 cm⁻¹, overlapping with the Si-O-Al stretching vibration of kaolinite and the Si-O-Si bending vibration of kaolinite/quartz, respectively. The bands at 1399 cm⁻¹ and 3130 cm⁻¹ are probably due to v₄ the bending vibration and v₃ stretching vibration of surface absorbed NH₄⁺ [65-67].

FORS spectra showed the characteristic inflection point (maximum at its first derivative, Figure 3-4(c)) of hematite at around 580 nm (Figure 3-4(d)). The broad absorption at ~875 nm also characteristic of hematite, could not be seen in this spectrum (cut off at 800 nm). These are attributed to ligand-to-metal charge-transfer transitions in hematite [68].



Figure 3-3. (a) Photomicrograph of the French ochre sample FRE-raw; (b) Micrographs of the FRE-raw. Qtz stands for quartz and Kao stands for kaolinite; (c) Photomicrograph of the sample FRE-d28; (d) Micrographs of the FRE-d28.



Figure 3-4. (a) XRD pattern of the samples FRE-raw and FRE-d28; (b) FTIR spectra of the FRE-raw, FRE-d1 and FRE-d28 samples; (c) FORS spectra of the French ochre samples (FRE-raw, FRE-d1, FRE-d7, FRE-d28); (d) first derivative of the FORS spectra in (c). The intensity values of each XRD pattern, FORS spectra and its first derivative plots were normalized to [0, 1] and offset for comparison purposes.

Compound	Band assignments		
	3696, 3670, 3653 cm ⁻¹ kaolinite's surface hydroxyl groups v(OH)		
	3621 cm ⁻¹ , kaolinite's inner hydroxyl groups v(OH)		
Kaolinite (Al ₂ Si ₂ O ₅ (OH)4)	1030 cm ⁻¹ , Si-O-Si stretching vibration		
	1008 cm ⁻¹ , Si-O-Al stretching vibration		
	938 & 912 cm ⁻¹ , Al-OH deformation vibration		
	693 cm ⁻¹ , Si-O-Si bending vibration (overlaps with quartz)		
	538 cm ⁻¹ , Si-O-Al stretching vibration		
	469 cm ⁻¹ , Si-O-Si bending vibration (overlaps with quartz)		
	1162 cm ⁻¹ , Si-O-Si rocking vibration		
	1096 cm ⁻¹ , Si-O-Si asymmetrical stretching vibration		
Quartz (SiO ₂)	doublets of 777 and 799 cm ⁻¹ , Si-O-Si symmetrical stretching vibration		
	693 cm ⁻¹ , Si-O-Si symmetrical bending vibration (overlaps with kaolinite)		
	469 cm ⁻¹ , Si-O-Si asymmetrical bending vibration (overlaps with kaolinite)		
Surface absorbed free water	3432 cm ⁻¹ , v(OH) O-H stretching		
	$1626 \text{ cm}^{-1}, \delta(\text{OH}) \text{ O-H}$ bending		

Table 3-2. FTIR band assignments for the French ochre pigment

3.2.3 Chalk

Pigment Composition and Physical Characteristics

The chalk pigment analyzed (CHA-raw) is a natural white calcium carbonate (CaCO₃) fine powder (Figure 3-5(a-c)), prepared from the pure microcrystalline chalk with particles size less than 5 μ m.

Changes in the Morphology/Structure, Composition and Color

The calculated ΔE^* value for the chalk pigment particles before and after twenty-eight days of immersion in DAP is 4.893 (Table 3-5). Though this value is above the threshold of color change detected by the human eye [50], it is still below the established value ($\Delta E^* \leq 5$) accepted for consolidation applications in cultural heritage [20, 51-55].

After twenty-eight days in 1M DAP solution, the chalk (CaCO₃) particles showed evident transformation into hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) and octacalcium phosphate (OCP, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$). The habit of the original calcium carbonate crystals had changed into "plate-like" crystals (Figure 3-5(d-f)). EDS mapping of sample CHA-d28 shows that the major elements consist of Ca, O and P corresponding to the newly formed phase(s). This transformation continued even after the period of two months with more calcium carbonate crystals been transformed into calcium phosphate.

XRD analysis (Figure 3-6(a)) showed that the raw chalk pigment solely consisted of calcium carbonate (or calcite) while after 1 day and twenty-eight days, unreacted calcite,

hydroxyapatite and octacalcium phosphate were found to coexist. The consumption of calcite was not complete. FTIR analysis further confirmed the XRD results (Table 3-3) [69]. Bands appearing at 468, 562, 601, 957 and 1033 cm⁻¹ in the analysis of sample CHA-d28 are all vibration modes of the hydroxyapatite phosphate group [70]. The fraction of the calcite was further estimated by TGA, with a weight loss between 600 and 860 °C (linked to the decomposition of calcite). A weight loss of 43.2 wt. %, 16.9 wt. % and 11.55 wt. % was observed for the sample CHA-raw, CHA-d1 and CHA-d28 between 600 to 860 °C (Figure 3-6(c-e)), corresponding a calcite fraction of 98.2 wt.%, 38.4 wt.% and 26.25 wt.%, respectively. Most of the calcite was consumed on the first day of reaction with DAP.

OCP is commonly found to be present as an intermediate phase in the conversion process from the ACP (amorphous calcium phosphates) to HAP (hydroxyapatite). This transition could explain the co-existence of HAP and OCP within the mixtures. While the formation of these phases depends on the reaction conditions such as pH and presence of foreign ions, ultimately, i.e., at thermodynamic equilibrium, they will all transform to non-ideal HAP [71, 72].



Figure 3-5. (a) Photomicrograph of the chalk sample CHA-raw; (b-c) Micrographs of the CHA-raw; (d) Photomicrograph of the sample CHA-d28; (e-f) Micrographs of the CHA-d28 sample; (g-j) SE image and elemental mapping of the sample CHA-d28.



Figure 3-6. (a) XRD pattern of the samples CHA-raw, CHA-d1, CHA-d28 and CHA-2m; (b) FTIR spectra of the CHA-raw, CHA-d1 and CHA-d28 samples; (c-e) TGA of the sample CHA-raw, CHA-d1 and CHA-d28. The intensity of each XRD pattern was normalized to [0, 1] and offset for comparison purposes.

-			
Compound	Band assignments		
Calcite (CaCO ₃)	2513cm ⁻¹ and 1798 cm ⁻¹ , combination bands		
	1420 cm ⁻¹ , v3 asymmetric stretching vibration of CO_3^{2-1}		
	873 cm ⁻¹ , v2 out of plane bending vibration of CO_3^{2-}		
	712 cm ⁻¹ , v4 in-plane bending vibration of CO_3^{2-}		
Hydroxyapatite (Ca ₁₀ (PO ₄) ₆ (OH) ₂)	1033 cm ⁻¹ , triply degenerated asymmetric stretching mode, v3 of P-O		
	bond of the phosphate group		
	957 cm ⁻¹ , non-degenerated symmetric stretching mode, v1 of P-O bond		
	of the phosphate group		
	601 cm ⁻¹ , triply degenerated bending mode, v4 of the O-P-O bonds of		
	the phosphate group		
	562 cm ⁻¹ , triply degenerated bending mode, v4 of the O-P-O bonds of		
	the phosphate group		
	468 cm ⁻¹ , double degenerated bending mode, v2 of the O-P-O bonds of		
	the phosphate group		
Surface absorbed free	3421 cm ⁻¹ , v(OH) O-H stretching		
water	1614 cm ⁻¹ , δ (OH) O-H bending		

Table 3-3. FTIR band assignments for the chalk pigment

3.2.4 Red Lead

Pigment Composition and Physical Characteristics

Read lead, also called minium (Lead (II, IV) oxide, Pb_3O_4), is a bright red to orange pigment. It has a tetragonal crystal structure at room temperature and is composed of octahedrally coordinated Pb^{4+} ions joined together by Pb^{2+} ions which are nestled in a distorted tetrahedron of oxygen ions [73]. It has been widely employed in paintings since antiquity, and identified in a large variety of artworks, including wall paintings, polychrome sculptures, manuscript illuminations and canvas paintings. Both use of pure pigment and the mixing with other pigments have been reported [74]. The red lead analyzed was found to be pure, consisting of minium (Pb_3O_4) with small and irregular particles (Figure 3-7(a-b)) ranging in size from a few microns to 20 μ m.

Changes in the Morphology/Structure, Composition and Color

After dispersing the particle in 1M DAP for twenty-eight days, part of the minium converted into lead hydroxyapatite (also known as hydroxypyromorphite, Pb₁₀(PO₄)₆(OH)₂). The color of the pigment changed from orange red to brownish red after twenty-eight days (Figure 3-7(c)). After two months, the color was further changed to dark brown (Figure 3-7(d)). The calculated ΔE^* value for the red lead pigment particles before and after twenty-eight days of immersion in DAP is 30.643 (Table 3-5). This color change is significant and far beyond the threshold accepted in the field of conservation treatment ($\Delta E^* \leq 5$).

Microscopic observations of the sample RED-d28 showed that most the particles remained the same, while some new elongated crystals could be detected (Figure 3-7(d)). EDS analysis on point 1 (marked with a cross in Figure 3-7(d)) confirmed the presence of Pb (24.65 at. %), P (13.73 at.%) and O (61.62 at.%). The Pb: P: O atomic ratio is close to 5:3:13, indicating the presence of lead hydroxyapatite. After two months, a significant amount of the original pigment particles were transformed into lead hydroxyapatite (Figure 3-7(f)), which accounted for the brown color.

XRD analysis (Figure 3-8) indicated that the raw red lead pigment (sample RED-raw) solely consisted of minium (JCPDS PDF No. 41-1493). The formation of lead hydroxyapatite (JCPDS PDF No. 8-259) was observed to begin only one day after dispersing the pigment in 1MDAP solution (sample RED-d1). After two months, the lead hydroxyapatite became dominant along with the precipitation of platternite (β -PbO₂) and unreacted residual minium (RED-2m in

Figure 3-8). This could be reflected from the analysis using RIR method, which was used to semiquantitatively determine the weight fraction of the phases within a mixture, from which a weight fraction of 78 wt.% minium and 22 wt.% Pb-HAP was obtained for RED-d1, a weight fraction of 74 wt.% minium and 26 wt.% Pb-HAP was obtained for RED-d28; 19.8 wt.% minium, 6.9 wt.% platternite and 73.3 wt.% Pb-HAP was obtained for RED-2m. The change between the first day of reaction and the first twenty-eight days was little while more significant reaction happened over a longer period (two months).

A similar dissolution-precipitation mechanism reported by Guo et al. 2016, and Kang et al. 1988 [73, 75] could be hypothesized for the early stage of the reaction. The dissolution reaction of Pb₃O₄ begins to occur at the surface of Pb₃O₄ (Reaction 3). The Pb₃O₄ first releases Pb²⁺ species from a tetrahedrally-coordinated Pb₃(II,IV)O₄ sites through a ligand substitution leaving unstable octahedral PbO₂ fragments (octahedral arrangement hosting the Pb⁴⁺ ions in the crystalline structure of Pb₃O₄) in the solid. The precipitation of lead hydroxyapatite (Pb₁₀(PO₄)₆(OH)₂) observed after the immersion of the Pb₃O₄ in DAP, suggests a reaction between the supersaturated Pb²⁺ ions released during Pb₃O₄ dissolution and the phosphate (PO₄³⁻) ions.

$$Pb_{3}O_{4} + 2H_{2}O_{(aq)} \rightarrow 2Pb_{(aq)}^{2+} + PbO_{2(s)} + 4OH_{(aq)}^{-}$$

Reaction 3. Dissolution reaction of Pb₃O₄ in aqueous solution.

Following this step, two processes occur simultaneously:

1. The unstable PbO_2 fragments formed from the dissolution of Pb_3O_4 , are reduced to Pb^{2+} as suggested by the reaction 4.

$$PbO_{2(unstable)} + 2e^{-} + 2H_2O_{(aq)} \rightarrow Pb^{2+}_{(aq)} + 40H^{-}_{(aq)}$$

Reaction 4. Reduction of unstable PbO_2 fragment into Pb^{2+} .

2. The nucleation of a newly stable β -PbO₂ from the Pb²⁺ ions as suggested by the reaction 5:

$$Pb_{(aq)}^{2+} + 4OH_{(aq)}^{-} \rightarrow PbO_{2(stable)} + 2e^{-} + 2H_2O_{(aq)}$$

Reaction 5. The oxidation of Pb^{2+} into the formation of stable β -PbO₂ (platternite).

Both Pb_3O_4 and β -PbO₂ are semiconductors and the electrons can transfer between the solid phases. The driving force for the process described, is provided by the decrease in both surface and lattice free energy which results from the dissolution of the octahedral fragment of PbO₂ (labelled as PbO₂(unstable) above) in Pb₃O₄ and the precipitation of β -PbO₂ [75].

During the dissolution reactions (Reaction 3 to Reaction 5) that occur on the surface of Pb_3O_4 , a layer of very fine particles/precipitates of PbO_2 forms during the earliest dissolution stages. Once formed, PbO_2 can remain either as a spectator species or it can be reduced as suggested by Reaction 6 [75, 77, 78], releasing more Pb^{2+} .

$$PbO_{2(s)} + H_2O_{(aq)} \rightarrow Pb^{2+}_{(aq)} + 20H^{-}_{(aq)} + 0.5 O_{2(aq)}$$

Reaction 6. Reductive dissolution of platternite (β -PbO₂) in aqueous solution.

However, the PbO₂ formed on the surface of Pb₃O₄ is likely to passivate the substrate's surface, i.e., inhibiting further dissolution of the Pb₃O₄. However, no such particles/precipitates were detected using XRD during the first month, suggesting that the formation of PbO₂ might have been limited with an amount below the detection limit of XRD. In addition, owing to the very
small porosity of the newly-formed PbO₂ layer on the Pb₃O₄ surface, the (NH₄)₂HPO₄ solution requires longer time to diffuse into the Pb₃O₄ substrate. Based on the XRD analysis, the β -PbO₂ phase only became detectable after two months, which suggests that the dissolution of minium continued along with the continuous formation of Pb-HAP and β -PbO₂. However, the sudden increase in the precipitation of Pb-HAP and the kinetics of its precipitation rate between twenty-eight days and two months still need further investigation. No previous research into the formation mechanism of lead hydroxyapatite, in a more comparable system, has ever been published and therefore future research is pivotal to understand the reaction kinetics of the system.



Figure 3-7. (a) Photomicrograph of the red lead sample RED-raw; (b) Micrographs of the RED-raw; (c) Photomicrograph of the sample RED-d28; (d) Micrographs of the sample RED-d28, the elongated particle was identified as lead hydroxyapatite (Pb-HAP) by the EDS point analysis (e) Photomicrograph of the sample RED-2m; (f) Micrographs of the sample RED-2m.



Figure 3-8. (a) XRD pattern of the samples RED-raw to RED-2m; (b) close view of (a) between 2θ of $20-31^{\circ}$. The intensity values of each XRD pattern was normalized to [0, 1] and offset for comparison purposes.

3.2.5 Lapis Lazuli

Pigment Composition and Physical Characteristics

Lapis lazuli denotes a complex rock mineral, of which the blue color is attributed to the entrapped polysulfide radical anion $(S_n, n=2,3)$ chromophores in one of its component minerals, namely lazurite $[(Na,Ca)_8(Al, Si)_{12}O_{24}(S,Cl,SO_4,OH)_2]$. Lazurite is a feldspathoid mineral of the sodalite group, which also includes sodalite $(Na_8Al_6Si_6O_{24}Cl_2)$, nosean $(Na_8Al_6Si_6O_{24}SO_4)$, and haüyne $[(Ca, Na)_{4-8}Al_6Si_6O_{24}(SO_4,S)_{1-2}]$. Other impurities of lapis lazuli include calcite, pyrite (FeS₂), quartz, diopside (CaMgSi₂O₆), sanidine/albite $[(Na,K)(Si_3Al)O_8]$, wollastonite (CaSiO₃), dolomite and others [79-81].

Some of the most important lapis lazuli mines are located in Afghanistan, Siberia, Chile and North America. In antiquity and during historical times, the stone has been widely used for production of jewelry and small artifacts and as a pigment in paintings and illuminated manuscripts. During the 19th century, due to the industrial production of the artificial pigment ultramarine blue (analogue to the natural rock), the natural pigment declined in popularity.

The lapis lazuli pigment analyzed for this research (sample LAP-raw) was found to consist of different particles (Figure 3-9(a-b)) including lazurite, wollastonite, cancrinite and feldspars with particles size ranging from a few microns to 50 microns.

Changes in the Morphology/Structure, Composition and Color

The calculated ΔE^* value for lapis lazuli pigment particles before and after twenty-eight days of immersion in DAP is 4.163 (Table 3-5). Though clearly above the threshold of color change detected by the human eye [50] it is still below the established $\Delta E^* \leq 5$ accepted in cultural heritage in cultural heritage [20, 51-55].

No detectable phase change could be observed in the pigment particles (LAP-raw) before immersion in DAP and after twenty-eight days (LAP-d28) in DAP solution, based on the XRD and FTIR analysis.

XRD analysis (Figure 3-10(e)) of the raw pigment particles (LAP-raw) and those dispersed in 1M of DAP solution for twenty-eight days (sample LAP-d28) showed no change in the lapis lazuli pigment consisting of lazurite, wollastonite, cancrinite and feldspars. FTIR analysis (Figure 3-10(b)), showed bands at 1100-900 cm⁻¹ region that could be assigned to overlapping of Al, Si-O₄ tetrahedra asymmetric stretching vibration of lazurite and O-Si-O asymmetric stretching vibration of wollastonite as well as, bands at 700-600 cm⁻¹ region, which could be assigned to overlapping of Al, Si-O₄ tetrahedra symmetric stretching vibration of lazurite and O-Si-O symmetric stretching vibration of wollastonite [82]. The band at 568 cm⁻¹ and the band at 452 cm⁻¹ represent the terminal ⁻O-Si-O⁻ bonds bending vibration and (Si)O-Si-O(Si) bending vibration, respectively [83-85].

The visible spectrum of the lapis lazuli is dominated by an absorption band around 600 nm, corresponding to the electronic transitions for S_3^- (see Figure 3-10(c)).



Figure 3-9. (a-b) Micrographs of the lapis lazuli pigment sample LAP-raw; (c-d) Micrographs of the sample LAPd28.



Figure 3-10. (a)XRD pattern of the samples LAP-raw, LAP-d28 and LAP-2m; (b) FTIR spectra of the LAP-raw and LAP-d28 samples; (c) FORS spectra of the samples (LAP-raw, LAP-d1, LAP-d7, LAP-d28). The intensity values of each XRD pattern, FORS spectra and its first derivative plots were normalized to [0, 1] and offset for comparison purposes.

3.2.6 Raw Sienna

Pigment Composition and Physical Characteristics

Sienna is an earth pigment containing mainly hydrated ferric oxide (mineral goethite) with silica and clays. It has a yellowish brown color. It took the name from the city-state of Siena and was extensively used during the renaissance. When heated at elevated temperatures it transforms into reddish brown- a pigment known as burnt sienna [59]. Along with some other natural earthochres, umbers and green earths, sienna has been used as important colorant and ground layer of historical paintings.

The sample of raw sienna analyzed consisted of goethite, montmorillonite/clay, gypsum, calcite and quartz. Microscopic examination of sample SIE-raw (Figure 3-11(a-b)) showed that the pigment consists of different particles of sizes ranging from sub-micron to 50 µm.

Changes in the Morphology/Structure, Composition and Color

The calculated ΔE^* value for raw sienna pigment particles before and after twenty-eight days of immersion in DAP is 2.558 (Table 3-5). This value is only slightly above the threshold of color change detected by the human eye [50], however it is still below the established $\Delta E^* \leq 5$ accepted in cultural heritage [20, 51-55].

After twenty-eight days immersion into DAP solution, phosphates could be identified within the microstructure, by the FTIR and SEM/EDS analysis. XRD analysis of the SIE-raw and SIE-d28 (Figure 3-12(a)) has shown that the raw sienna pigment consists of goethite (α -FeOOH), gypsum (CaSO₄·2H₂O), calcite, quartz, and montmorillonite/clay. In sample SIE-d1, gypsum was absent from the XRD pattern, whereas, calcite could still be detected. This is due to the dissolution of gypsum into DAP solution, while the transformation of calcite into HAP or OCP is not complete. For SIE-d28, however, the calcite peaks were absent, indicating that the amount of calcite was probably below detection limit. FTIR analysis (Figure 3-12(b)) has also shown similar results

(Table 3-4) [86-93]. The appearance of phosphate group and the disappearance of gypsum and calcite in the FTIR spectrum, further indicates that the calcite and gypsum were converted into calcium phosphates such as HAP. Reflectance spectra of the yellow iron hydroxide pigment (goethite) shows the characteristic inflection point (maximum at its first derivative, Figure 3-12(d)) at around 545 nm and absorptions at 640 and ~900 nm (the latter not visible in the spectrum) ((Figure 3-12(c)).



Figure 3-11. (a-b) Micrographs of the raw sienna pigment sample SIE-raw; (c-d) Micrographs of the sample SIE-d28;



Figure 3-12. (a) XRD pattern of the sample SIE-raw, SIE-d1, SIE-d28 and SIE-2m; (b) FTIR spectra of the SIE-raw and SIE-d28 samples; (c) FORS spectra of the samples (SIE-raw, SIE-d1, SIE-d7, SIE-d28); (d) first derivative of the FORS spectra in (c). The intensity values of each XRD pattern, FORS spectra and its first derivative plots were normalized to [0, 1] and offset for comparison purposes.

	Compound	Band assignments					
		3541 cm ⁻¹ , v3 asymmetric stretching vibration of water					
		3399 cm ⁻¹ , v3 asymmetric stretching vibration of water					
	Gypsum (CaSO4 [·] 2H ₂ O)	1685 & 1621 cm ⁻¹ , OH bending vibration					
		1112 cm ⁻¹ , v3 asymmetric vibration of SO ₄ tetrahedron					
		669 cm ⁻¹ , v4 stretching vibration of SO ₄ tetrahedron (overlap with goethite)					
		600 cm ⁻¹ , v4 bending vibration of SO ₄ tetrahedron (overlap with goethite)					
		1096 cm ⁻¹ , Si-O-Si asymmetrical stretching vibration					
	Quartz (SiO ₂)	798 cm ⁻¹ , Si-O-Si symmetrical stretching vibration (overlap with silicate					
		clay and goethite)					
		Broad band centered around 3141 cm ⁻¹ , v2 O-H					
	Goethite (α-FeOOH)	899 cm ⁻¹ , plane deformational mode δO-H					
SIE-raw		798 cm ⁻¹ , γ O-H out of plane deformation mode (overlap with SiO ₂ and					
		silicate clay)					
		669 cm ⁻¹ , combination band (overlap with gypsum)					
		600 cm ⁻¹ , FeO ₆ lattice mode (overlap with gypsum)					
		469 cm ⁻¹ , FeO ₆ lattice mode (overlap with silicate clay)					
	Calcium carbonate (CaCO ₃)	712 cm ⁻¹ , v4 in-plane bending vibration of $CO_3^{2^-}$					
		877 cm^{-1} , v2 out of plane bending vibration of CO_3^{2-1}					
		1425 cm ⁻¹ , v3 asymmetric stretching vibration of CO_3^{2-1}					
		2513cm ⁻¹ and 1797 cm ⁻¹ , combination bands					
	Silicate clay	3621 cm ⁻¹ , OH stretching of structural hydroxyl group					
		1029 cm ⁻¹ , Si-O-Si stretching					
		798 cm ⁻¹ , Si-O-Si symmetrical stretching vibration (overlap with quartz and					
		goethite)					
		469 cm ⁻¹ , Si-O-Si deformation					
	Goethite	Same as day 0					
	(a-FeOOH)						
	Quartz	Same as day 0					
SIE-d28	Silicate clay	Same as day 0					
	Phosphate (PO ₄ ³⁻)	468 cm ⁻¹ , v2 of O-P-O bond of the phosphate group (overlap with goethite					
		and silicate clay)					
		559 cm ⁻¹ , v4 of O-P-O bond of the phosphate group					
		601 cm ⁻¹ , v4 of O-P-O bond of the phosphate group (overlap with goethite)					
		1034 cm ⁻¹ , v3 of O-P-O bond of the phosphate group					

Table 3-4. FTIR band assignments for the sienna pigment

3.2.7 Burnt Umber

Pigment Composition and Physical Characteristics

Umber is a natural brown or reddish-brown earth pigment that contains hydrated iron oxide

(mainly goethite) and manganese dioxide (pyrolucite). Compared to ochre and sienna, it is darker

in color. It's called raw umber in its natural form and when heated, the color becomes more intense.

During the heating, the hydrated iron oxide (goethite) is partially transformed into hematite.

The raw umber analyzed was found to consist of hematite, manganese dioxide, calcite, quartz, and clay (Figure 3-13(a-b)), with particle sizes ranging from sub-micron to 20 μm.

Changes in the Morphology/Structure, Composition and Color

The calculated ΔE^* value for burnt umber pigment particles before and after twenty-eight days of immersion in DAP is 1.658 (Table 3-5) and therefore the color change of this pigment remained below detection.

After twenty-eight days immersion into DAP solution, phosphates could be identified from the microstructure, by the SEM/EDS analysis. XRD analysis (Figure 3-14(a)) of sample BUR-raw showed that the raw burnt umber pigment consists of hematite, calcite and quartz, whereas, no calcite could be detected after twenty-eight days (sample BUR-d28). FTIR analysis (Figure 3-14(b)) showed bands at 1423, 879 cm⁻¹ corresponding to vibration of CaCO₃ and bands at 1030, 778 & 797 and 463 cm⁻¹ corresponding to the vibration of silicate (possibly clay and SiO₂). The bands at 532 and 463 cm⁻¹ are indicative of the Fe-O vibration of hematite. After twenty-eight days, no calcite could be detected by FTIR.

FORS spectra of burned umber (Figure 3-14(c)) showed the same features as those of the French ochre (Figure 3-4(c-d)) since the main component of both pigments is hematite.



Figure 3-13. (a-b) Micrographs of the burnt umber pigment sample BUR-raw; (c-d) Micrographs of the sample BUR-d28.



Figure 3-14. (a)XRD pattern of the sample BUR-raw, BUR-d1, BUR-d28 and BUR-2m; (b) FTIR spectra of the BUR-raw and BUR-d28 samples; (c) FORS spectra of the samples (BUR-raw, BUR-d1, BUR-d7, BUR-d28); (d) first derivative of the FORS spectra in (c). The intensity values of each XRD pattern, FORS spectra and its first derivative plots were normalized to [0, 1] and offset for comparison purposes.

3.2.8 "Green Earth"

Pigment Composition and Physical Characteristics

Similar to other earth pigments, green earth has been used as pigments since antiquity and it's still in use in the artwork creation. The main component of green earths is the mineral celadonite or glauconite. Other greenish clayed minerals in green earth pigments include smectites, chlorites, serpentines and pyroxenes [94]. The "green earth" pigment analyzed for this study (GRE-raw) was found to consist mainly of Fe-smectite, hematite, calcite, plagioclase and chlorites (Figure 3-15(a-b)) with particle size ranging from sub-micron to up to 30 µm.

Changes in the Morphology/Structure, Composition and Color

The calculated ΔE^* value for burnt umber pigment particles before and after twenty-eight days of immersion in DAP is 3.106 (Table 3-5). Though this value is above the threshold of color change detected by the human eye [50], it is still below the established value ($\Delta E^* \leq 5$), accepted for consolidation treatments in the field of cultural heritage [20, 51-55].

After twenty-eight days immersion into DAP solution (Figure 3-15(c-d)), phosphates could be identified within the microstructure by the FTIR analysis. XRD analysis (Figure 3-16(a)) of sample GRE-raw confirmed the presence of Fe-smectite, hematite, calcite, plagioclase and chlorites. Similar results were also reported in the literature and the coexistence of anatase, goethite and khandite mineral was also reported by using μ RS analysis on individual particles by A. Coccato [95, 96]. FTIR analysis (Figure 3-16(b)) has further corroborated the results with bands at 2513, 1797, 1431, 873, 712 cm⁻¹ corresponding to the vibration modes of CaCO₃. The presence of Fe-rich smectite could be identified with vibration bands at 3559 cm⁻¹ (Al-Fe-OH asymmetric stretching vibration), 3424 cm⁻¹ (Fe-Fe-OH asymmetric stretching vibration), 1016 cm⁻¹ (Si-O asymmetrical stretching vibration), 675 cm⁻¹ (Al-Fe-OH translational vibration) and 457 cm⁻¹ (Si-O bending deformation) [97-100]. After twenty-eight days, no calcite could be detected in the XRD pattern and in FTIR spectra, bands corresponding to the vibrational modes of phosphates could be identified, at 601cm⁻¹ (v4 of O-P-O bond of the phosphate group) and 562 cm⁻¹ (v4 of O-P-O bond of the phosphate group).

FORS spectra (Figure 3-16 (c)) showed a broad band around 650 nm probably due to Fe^{2+} -> Fe^{3+} intervalence charge transfer of the Fe-smectite [101]. Most of the Fe occupy in the octahedral sites of the smectite, which contained enough Fe to make it green. The bands at high energies are unknown but may be due to the exchange-enhanced transitions to the triplet Fe^{2+} ligand field states.



Figure 3-15. (a-b) Micrographs of the "Green Earth" pigment sample GRE-raw; (c-d) Micrographs of the sample GRE-d28.



Figure 3-16. (a)XRD pattern of the sample GRE-raw, GRE-d1, GRE-d28 and GRE-2m; (b) FTIR spectra of the GRE-raw and GRE-d28 samples; (c) FORS spectra of the samples (GRE-raw, GRE-d1, GRE-d7, GRE-d28). The intensity values of each XRD pattern, FTIR spectra and FORS spectra were normalized to [0, 1] and offset for comparison purposes.

3.2.9 Summary of color and phase changes in pigments

The color values of the pigments before immersion into DAP solutions and after twentyeight days reaction with DAP, as well as, the ΔE^* values, are listed in Table 3-5 and Table 3-6. As mentioned above, color change with value $\Delta E^* \leq 2$ is widely accepted as the value detectable by the human eye, while a color difference of $\Delta E^* \leq 5$ has been established as the threshold in the field of cultural heritage to evaluate color change after a conservation intervention such as consolidation. In this research, it was demonstrated that while the color difference ΔE^* of all *fresco* pigments tested, including cinnabar (deep red), French ochre (yellow) and lapis lazuli (blue), chalk (white), raw sienna (yellow), and green earth (pale green) were above the threshold detected by the human eye ($\Delta E^*>2$), with the exception of burnt umber (brown) which showed no detectable color change ($\Delta E^*<2$), they all showed ΔE^* values below the accepted threshold ($\Delta E^* \leq 5$) for cultural heritage studies. Red lead, however, has shown a significant color value change with $\Delta E^*=30.643$, which is well above the accepted values.

Pigment	Raw pigment value			Value after 28 days reaction with DAP			Change in color ΔE^*	
Name	L	a*	b*	L	a*	b*	B 00101 = 2	
Cinnabar	33.493	32.735	18.287	31.734	30.861	15.858	3.536	
French Ochre	34.757	25.404	24.694	33.714	25.005	21.493	3.390	
Chalk	74.286	2.243	4.657	69.862	2.751	2.629	4.893	
Red lead	47.804	46.654	52.714	37.312	28.597	30.289	30.643	
Lapis Lazuli	43.95	4.205	-17.459	41.106	5.451	-14.686	4.163	
Raw Sienna	41.895	14.123	33.497	43.884	12.643	32.868	2.558	

Table 3-5. Color values in pigments after twenty-eight days immersion in DAP solutions.

Burnt Umber	22.098	11.364	13.126	21.233	12.588	13.835	1.658
Green Earth	42.492	1.57	10.854	45.262	0.269	10.324	3.106

Pigments such as chalk and calcite found as impurity in some of the colored pigments, have also undergone evident phase change from calcium carbonate into calcium phosphates such as hydroxyapatite. In this case, however, this phase change could be considered as 'favorable' given that it provides an additional binding mechanism and adds to the consolidation effect. Conversely, the changes occurred in the red lead (Pb₃O₄) pigment can be characterized as 'non-favorable', resulting into significant color change from bright orange to brown (with a ΔE^* =30.643) and phase transformation from lead tetroxide into lead hydroxyapatite possibly via the dissolutionprecipitation mechanism described above, causing irreversible damage.

In this research we were able to demonstrate measureable color difference and phase transformations on pigments, occurring immediately after treatment and after sometime (two months) under controlled environmental conditions and to assess the direct impact/modification to the color and phases of the pigments induced by the DAP precursor. While the stability or any further changes that may occur after treatment in an uncontrolled environment (variable temperature, light, relative humidity (RH), pH, pollutants etc.) would be very interesting in the general understanding of effects of the microenvironment in the preservation of wall paintings after consolidation, this poses a separate research question that was not part of this initial

study on colors. Further, research would be required on the long-term environmental effects of DAP treated pigments and pigmented surfaces.

Pigment	Obvious Color change	Main Component (DAP reaction)	Impurities/DAP Reaction	Safe to apply DAP directly
Cinnabar	Х	Cinnabar (X)	-	\checkmark
French Ochre	Х	Hematite (X)	Х	\checkmark
Chalk	Х	Calcite (√)	-	\checkmark
Red Lead	$\sqrt{(\text{orange to brown})}$	Minium (√)	-	Х
Lapis Lazuli	Х	Lazurite (X)	Х	\checkmark
Raw Sienna	Х	Goethite (X)	\checkmark	\checkmark
Burnt Umber	Х	Hematite and Pyrolusite (X)	\checkmark	\checkmark
Green Earth	X	Fe-smectite (X)	\checkmark	\checkmark

 Table 3-6. Summary of color/phase changes of the pigments

As pigments such as red lead are sensitive to the ammonium phosphate solution treatment based on observed phase and color changes monitored during the first day of DAP infusion, a pretreatment with cyclododecane (CDD, $C_{12}H_{24}$) could be performed prior to the application of the reactant solution. CDD could form a temporary surface protective layer over the pigment or colorant, as it sublimes (leaving no residues behind) at room temperature after sometime, depending on the substrate, the environment and the method of application [102]. CDD can be applied in molten form, in solution or spray thus providing options in the treatment based on the surface condition, areas of application and treatment requirements.

3.2.10 pH Value of the Supernatant Solutions



The change of pH value of the DAP solutions with time is shown in Figure 3-17.

Figure 3-17. The change of pH value of the DAP solutions with time (the number in the X axis means the number of days of measurement) with a standard error of ± 0.1 .

The pH value of the French ochre, lapis lazuli and cinnabar was almost constant (~8.3) during the first twenty-eight days. This is consistent with the fact that no significant color, phase or morphological change could be observed in these pigments.

By comparing the pH value of the solution at day 0 and day 28, an increase in the pH was observed for calcium carbonate containing pigments including chalk, bunt umber, raw sienna, and "green earth". This is due to the chemical reaction of the calcium carbonate with DAP for the formation of phosphate phases that caused the increase in the pH value of the solution. A slight increase was also observed for the pH value of the solution containing red lead after twenty-eight days reaction with DAP. That was due to the reaction of the minium (Pb₃O₄) with ammonium phosphate that leads to the formation of lead hydroxyapatite that caused the increase in the pH value.

3.3 Conclusions

Here, the chemical interactions of DAP with eight pigments have been investigated. The pigments studied include seven pigments commonly used for *fresco* application -cinnabar, French ochre, chalk, lapis lazuli, raw sienna, burnt umber and "green earth" and one pigment commonly used for *secco* application - red lead. For the experiments, 10 g of each pigment were immersed into 100ml of 1M DAP solution and kept at room temperature for up to two months. In order to study the effect of the treatment on the pigments' color, morphology and chemistry, the raw pigments – before treatment – and the reaction products after twenty-eight days have been analyzed and compared using combined analytical techniques including DM, XRD, FTIR, TGA, SEM-EDS, µRS and FORS.

While detectable to the human color changes occurred for most of the pigments analyzed, these were below the threshold accepted for cultural heritage consolidation treatments. Evident phase transformations into HAP was identified in calcium carbonate containing pigments, including chalk, and calcite found as an impurity in the pigments: raw sienna, burnt umber and "green earth".

Significant color and phase change was observed in the red lead pigment. This was mainly caused by the reactivity of the lead-pigment with the DAP precursor. The red lead (lead tetroxide) reacted with the ammonium hydrogen phosphate and turned into lead hydroxyapatite, with a color change from bright orange to brown. DAP treatment on painted surfaces pigmented with red lead could therefore cause serious and irreversible damage to the artwork (both chromatically and chemically). Pre-treatment with cyclododecane would be tested prior to the application of the treatment solution if red lead is detected in the polychromy.

Chapter 4. Evaluation of In Situ Hydroxyapatite Consolidation Effects on a Hellenistic-Roman Rock-cut Chamber Tomb at Athienou-*Malloura* in Cyprus

Following experimental results from our previous research conducted in laboratory controlled conditions [30], diammonium hydrogen phosphate (DAP) was tested as the precursor for the in situ precipitation of HAP for the consolidation of a Hellenistic-Roman marlstone rockcut chamber tomb (Tomb 27) on the NE slope of Magara Tepeși ("Hill of the Tombs") at Athienou-*Malloura* in Cyprus. DAP solutions were applied in three distinctive parts of the tomb (A, B and C). Prior to the treatment trials, the surface was cleaned using a soft brush. Desalination as a pretreatment was also performed to evaluate the impact on the hydroxyapatite consolidating-effect, phase and microstructure of the treated areas. Compositional and microstructural evolutions of the test areas were compared and scientifically assessed immediately following treatment, and after the period of one year. To develop comprehensive understanding of the occurring physical, chemical and microstructural changes before and after treatment and with no desalination or with desalination pretreatment, different analytical techniques including XRD, FTIR, DM and SEM-EDS were employed for the analysis of the samples. Results indicated an immediate formation of hydroxyapatite after the DAP application with no significant variation between the three test areas. In addition, there were no noticeable differences in the phase and microstructure of the treated rock between non-desalinated and desalinated areas.

4.1 Mağara Tepeşi Tomb No. 27 in Athienou-Malloura, Cyprus

Since 1989, the Athienou Archaeological Project (AAP) initiated excavations at the site of Athienou-Malloura situated within the Malloura Valley in central Cyprus (Figure 4-1). The valley is located within the area of chalk hills composed of marine sediments (Paleocene-Oligocene and Miocene in age, ca. 67-5 million years ago). The excavation and survey on the site revealed that a long settlement history began early in the Cypro-Geometric Period (1050-750 BC), and habitation continued through the Early Byzantine times. After the Christian-Arab Conflict and the late Byzantine Period (AD 647-1191), Malloura was resettled at the time of Frankish rule (AD 1191-1489) and flourished during the Venetian period (AD 1489-1571). The settlement declined during the Ottoman rule (AD 1571-1878) and was finally abandoned [103].

A series of tombs at the necropolis of Mağara Tepeşi disclosed that the valley was used for funerary purposes from the Archaic to Roman periods [104]. During the excavation and survey, a cluster of four small looted chamber tombs on top of the hill (Tombs 50-53) and four large rock-cut massive white chalk chamber tombs (Tomb 25-28, Figure 4-1 (b)) was exposed at the base of the northeast slope of Mağara Tepeşi [104]. Among all the tombs, Tomb 27 (Figure 4-1 (c-e)) is the largest and most architecturally elaborated. The tomb dates primarily to Hellenistic and Roman times [105]. It includes a stepped *dromos*, a sunken forecourt with two small *loculi*, a side tunnel and double benches in the burial chamber (7m x 4m) [103]. Nearly 10000 pot sherds and skeletal

material from no less than 31 individuals were found by careful sieving of the fill from the Tomb 27.

In terms of climatic conditions, the Malloura Valley is in one of the hottest and driest parts of Cyprus with a summer temperature average about 32°C and mean winter temperatures between 16-20°C [106]. The post-excavation exposure caused the soft and fissured marlstone to become extremely vulnerable to exfoliation. Thus, in order to slow the deterioration of the marlstone affected from intense Cypriot sun, wind and seasonal rainfall, shelters were built over the tomb (Figure 4-1(b-c)). A staircase was also constructed in the *dromos* of Tomb 27 over the original ancient steps (Figure 4-1(d)), in order to facilitate safe entry and to protect the tomb fabric.



Figure 4-1. (a) Map of Cyprus showing the location of Athienou-*Malloura*; (b) Tomb 25-28 at the base of the northeast slope of Mağara Tepeşi; (c) close view of the Tomb 27; (d) access stair to Tomb 27; (e) stepped sunken forecourt of Tomb 27.

4.2 In-situ Application of 1M DAP Solutions

Three different test areas of Tomb 27 with distinct microenvironments were chosen for the application of DAP treatment to assess the consolidating effects of HAP on the powdery marlstone.

These included: Area A – above the *loculus* on the south wall of the forecourt, Area B – west wall south side of the entrance in the interior of the chamber and Area C – on the east wall of the chamber above the carved benches affected by microbial growth (Figure 4-2). Prior to the treatment with DAP all areas were first gently cleaned using soft brush. Subsequently areas A and B were divided into two sections and one of these sections in each respective area was desalinated. The entire of area C was also desalinated after mechanical removal of a green surface layer caused by microorganisms' colonization. Desalination was performed using cellulose compresses (poultices), prepared with Arbocel BC200 from Kremer Pigmente (~ 0.3 mm cellulose fibers) soaked in deionized (DI) water. The poultices were applied on area A1, B1 and C and left for 24 hours until dry.

For the HAP treatment, 1M DAP solution was applied on the test areas using Arbocel BWW40 cellulose compresses, (of ~200 μ m cellulose fibers) from Kremer Pigmente. A solid to liquid volume ratio of about 3:2 was used. Each poultice was applied on the surface over a Japanese tissue (used as an intermediate layer) at approximate thickness of 1 cm and left to react for 3 hours. Polypropylene thin foil was used to cover the poultice in order to prevent the solution from evaporating too fast. After the completion of treatment, the poultice was carefully removed (Figure 4-2(c), (h) and (m)) and left to dry naturally.



Figure 4-2. On-site treatment of DAP solution using poultice method on (a-f) Area A, (g-k) Area B and (l-o) Area C respectively. Two different locations were chosen within Area A (A1&A2) and Area B (B1&B2) for comparison in order to evaluate the effects of desalination on the hydroxyapatite formation.

4.3 Reaction between Gypsum and 1M DAP Solution

Since gypsum (CaSO₄·2H₂O) salts were found in the untreated samples a controlled laboratory experiment was carried out to evaluate the effect of DAP treatment on gypsum. For this experiment, 10 grams of gypsum (BAKER ANALYZED® Reagent) was mixed with 100ml 1M

DAP solution in a glass container at room temperature (~ 22 °C) and left for twenty-eight days. After this period, the morphology, microstructure and mineral composition of the reactant product was characterized.

4.4 Archaeological Sample Preparations

With the aid of a scalpel, small stratigraphic fragments (containing the surface and subsurface of the rock) and powdered samples were removed from the surface of all test areas for analysis. These included samples from the areas A, B, and C: 1) before treatment; 2) forty-eight hours after treatment and 3) one year after treatment.

Stratigraphic samples were prepared as polished cross-sections using a Buehler EpoxiCure® epoxy resin mixed with EpoxiCure® Epoxy Hardener as prescribed. The resin was poured into custom-made rubber cubic molds (1.5x1x1cm) to fill half of their volume. After hardening of the resin, samples were placed in the mold with the top surface facing downwards. More resin was then poured over the sample until it was completely covered and placed under the vacuum using a Buehler Cast n'Vac 1000 vacuum system. Once the resin had set, the samples were cut perpendicular to the surface exposing both the surface and subsurface, and grinded using Buehler silicon carbide grinding papers from 240 to 800 grit and subsequently polished on a Leco® GP-25 polishing turntable using water-based diamond suspensions of 6µm and 1µm and Buehler® MasterTex polishing cloths.

4.5 Characterization Techniques

All samples were first examined using a Keyence VHX-1000 Digital Optical Microscope, utilizing between 20- 200x magnification.

XRD measurements on the samples were performed on a Rigaku R-Axis Spider X-ray Diffractometer, with the following measurement parameters: Cu K α radiation λ =1.5405Å, 50 kV, 40 mA for 10 mins. Mineral phases were identified by using the JCPDS (Joint Committee on Powder Diffraction Standards).

For TGA analysis, marlstone samples, both untreated and treated, were grinded in a mortar to very fine powders. TGA analysis was then performed using a Perkin Elmer Pyris Diamond TG/DTA (Thermogravimetric/Differential Thermal Analysis) instrument. Temperatures were scanned in the range between 40 °C to 1000 °C, at the heating rate of 15 °C/min, in a flowing air atmosphere.

Microstructural and elemental analyses of cross-section samples as well as powders separated from each layer were performed on a FEI Nova NanoSEMTM 230 scanning electron microscope at variable pressure (VP) coupled with energy dispersive x-ray spectroscopy (EDS).

FTIR analysis was performed on a JASCO FT/IR-420 Fourier Transform Infrared Spectrometer using the KBr pellet method. Sample powders were ground and dispersed in a KBr matrix with a concentration around 0.5 wt. % and then pressed into a pellet. All spectra were collected at 64 scans with a spectral resolution of 4 cm⁻¹, from 4000 to 400 cm⁻¹. Spectra were

matched against the spectral database of the Infrared and Raman Users Group (IRUG) and literature data.

DNA assays for detection and identification of microorganisms were conducted. The Kapa LTP library kit was used to make the sequencing library. The workflow consists of fragmentation of whole genome DNA, end repair to generate blunt ends, A-tailing, adaptor ligation and PCR amplification. Different adaptors were used for multiplexing samples in one lane. Sequencing was performed on Illumina HiSeq 3000 for a pair read 150 run. Data quality check was done on Illumina SAV. Demultiplexing was performed with Illumina Bcl2fastq2 v 2.17 program.

4.6 **Results and Discussion**

4.6.1 Untreated Marlstone Substrate

The chalky marlstone sampled from Mağara Tepeşi Tomb 27 was found to consist of calcite (ICSD PDF No: 5-586), quartz (ICSD PDF No: 46-1045) and traces of montmorillonite (ICSD PDF No: 13-0135) and plagioclase, as evidenced from the XRD pattern (Figure 4-3(a)).

The FTIR analysis (Figure 4-3(b)), corroborated the results by XRD. Bands at 711 cm⁻¹ (v4 in-plane bending vibration of CO_3^{2-}), 875 cm⁻¹ (v2 out of plane bending vibration of CO_3^{2-}), 1428 cm⁻¹ (v3 symmetric stretch vibration of CO_3^{2-}), 1798 cm⁻¹, 2515 cm⁻¹ (combination bands) are characteristic of calcite (CaCO₃) [62, 107]. Band at 1037 cm⁻¹ are attributed to the Si-O

vibration from the clay minerals and those at 3419 cm⁻¹, 1624 cm⁻¹ to the OH stretching and bending of the absorbed water. Bands at 467 cm⁻¹ (Si-O-Si rocking vibration), 797 cm⁻¹ (Si-O-Si bending vibration), 1078 cm⁻¹ and 1162 cm⁻¹ (Si-O-Si asymmetrical stretching) correspond to characteristic bands of silica (SiO₂) [108, 109]. Other bands at 2919 cm⁻¹, 2850 cm⁻¹ correspond to the asymmetric stretching and symmetric stretching of C-H band respectively and they possibly derive from organic surface contaminants.

The TGA curve (Figure 4-3(c)) showed a weight loss of 26.1 wt. % within the temperature range of 626 to 770 °C, which corresponds to the decomposition of calcite. This yielded a weight fraction of 59.3 wt. % of calcite within the marlstone, with the remaining amounts of silica and clay/feldspars. As expected, there were no mineralogical or microstructural difference in the substrate of the different areas tested as the tomb was entirely carved out of the chalk hills, rich in coccoliths (calcareous spherical platelets produced by single-cell planktonic algae of the class *Coccolithophorida*) [110] [111]. Elements of O, Na, Mg, Al, Si, Cl, K, Ca, Fe and Ti were detected by the EDS analysis. The elemental mapping of the polished cross-section of the marlstone (Figure 4-3(j-o)) clearly illustrates the heterogeneity of the substrate.

As the test areas are located in different parts of the tombs, there is are small variations in the state of preservation pertaining to the localized microenvironment. Area A was characterized by salt efflorescence and delamination; area B showed surface encrustation and C extensive microbial growth, identified by DNA assays as bacteria (Figure 4-3(d-f)). Salt efflorescence of NaCl was also identified in some of the samples.



Figure 4-3. (a) XRD pattern of the untreated samples A, B, C; (b) FTIR spectrum (normalized) of the untreated marlstone samples A, B, C (c) TGA curve of the untreated sample B; (d-f) Digital micrograph of untreated sample A, B and C respectively; (g-h) SEM micrograph of untreated sample A and untreated sample B; (i) SE micrograph of the bacterial colony on the surface in sample C; (j) Back scattered electron (BSE) micrograph and (k-o) EDS elemental mapping on the polished cross-section of marlstone.

4.7 Treated Marlstone Substrate

4.7.1 Samples Taken Immediately After the DAP Treatment

Samples collected from the areas A, B and C immediately after the DAP treatment showed evidence of reaction chemistries and the formation of calcium phosphate phases. There were no observed differences in the composition and/or microstructure between the various test areas and those that have been desalinated.

X-ray diffraction analysis of the samples following the DAP application (Figure 4-4(a)), showed that the peaks of hydroxyapatite (ICSD PDF No: 9-432) could be observed at 2-theta angle (Bragg diffraction angle) of 25.9° and around 31.8°. None of the more soluble calcium phosphate phases such as brushite, was detected in the XRD pattern. FTIR analysis of the sample taken soon after the DAP treatment (Figure 4-4(b)) also shows the appearance of two bands, one at 603 and another one at 563 cm⁻¹, which correspond to the v4 bending mode of PO₄³⁻ [47, 112, 113]. The two bands of 603 and 563 cm⁻¹ were interpreted as the indication of the HAP formation [23, 41, 44]. Bands at 1039 cm⁻¹ and 467 cm⁻¹ were intensified, probably due to the overlapping of the vibration mode of PO₄³⁻ (467 cm⁻¹, 1039 cm⁻¹, v2 bending mode and v3 anti-symmetric stretching mode of PO₄³⁻ respectively) [114], with the vibration mode of Si-O as described above.

No significant difference could be observed between the marlstone samples taken immediate after the treatment apart from a surface color saturation (Figure 4-4(c-e)). However, high resolution imaging using SEM indicated the formation of a new crystal network (Figure 4-4(f, h)) in the calcite-rich regions of the marlstone that were in contact with the DAP saturated compresses. On the fractured rough surface (silica-rich) and in regions further away from the surface the formation of these new phases was uneven (Figure 4-4(g)). The presence of phosphorus, indicating the presence of new P-rich phases was also confirmed by EDS analysis.



Figure 4-4. (a) XRD pattern of the samples collected immediately after the DAP treatment; (b) FTIR spectrum of the samples soon after DAP treatment (normalized); (c-e) Digital micrographs of the samples soon after treatment (a: A2, b: B1 and e: C); (f-h) Secondary electron (SE) micrographs of the samples showing the newly formed HAP.

After the treatment with DAP, gypsum-rich areas indicated the presence of the gypsum analogue phosphate mineral brushite (CaHPO₄•H₂O). The transformation of gypsum into brushite when exposed to 1M DAP was also confirmed by the laboratory controlled experiment described below.

4.7.2 Sample Taken One Year after DAP Application

One year after DAP treatment, XRD analysis of the samples showed that the peaks belonging to hydroxyapatite became more evident (Figure 4-5(a)), when compared to the XRD pattern of the sample taken immediately after treatment (Figure 4-5(a)). This was also reflected in the FTIR spectrum (Figure 4-5(b)). The bands at 1034 cm⁻¹ were intensified, whereas the bands (absorbance) corresponding to the symmetric stretch vibration of CaCO₃ (1429 cm⁻¹) decreased. SEM micrographs also showed more clearly the formation of HAP crystals in the samples taken after one year (Figure 4-5(h)). EDS mapping of cross-section samples taken one year after the DAP treatment (Figure 4-6) further demonstrated the progressing formation of a Ca, P-rich mineral phases network.


Figure 4-5. (a) XRD patterns of the samples one year after DAP treatment; (b) FTIR spectra of the samples taken one year after DAP treatment; (c-f) Digital micrographs of the samples taken one year after DAP treatment; (g-k) Secondary electron (SE) micrographs.



Figure 4-6. Backscattered electron (BSE) micrograph and EDS mapping of the cross-section of the sample taken one year after the DAP treatment (a) BSE image; (b-e) elemental mapping; (f-h) point analysis (points: 1, 2, 3 are shown in (a)).

One year after the DAP application, the two areas B and C (in the interior of the chamber) showed the formation of random black spots on the surface, indicative of bacterial growth (Figure 4-5). The bioresceptivity of the stone in this area was most likely increased by the synergistic

activity of the mineralogical composition of the stone, its surface properties (surface texture, porosity, permeability) and the surrounding environmental condition (high moisture levels), pH, light and temperature [115]. No microbial growth was observed in region A (south wall of the forecourt) after the one year elapsing from the time of treatment, probably due to the local environmental differences in the three different regions, such as light, temperature and moisture.

4.8 Reaction between Gypsum and 1M DAP Solution

The reaction between 1M DAP and gypsum showed that after twenty-eight days, gypsum (CaSO₄•2H₂O) and anhydrate gypsum (CaSO₄) was converted into brushite (CaHPO₄•2H₂O) (Figure 4-7(a-b)). This chemical transformation was also observed with a change in the morphology of the gypsum particles before (Figure 4-7(c, e)) and after exposure to 1M DAP solution (Figure 4-7(d, f)). Elemental mapping (Figure 4-7(g-j)) also showed that after twenty-eight days, the original gypsum was changed into a calcium phosphate phase with predominant elements: O, Ca and P. Quantitative elemental analysis showed a Ca/P ratio close to 1:1, evidencing brushite as the reaction product. This also explained the co-existence of brushite, hydroxyapatite and calcite on some of the on-site treated areas, where gypsum was most likely present.



Figure 4-7. (a) XRD pattern of the untreated gypsum particles; (b) XRD pattern of the treated gypsum particles; (c) Digital photomicrograph of untreated gypsum particles; (d) Digital photomicrograph of the gypsum particles after twenty-eight days exposure to 1M DAP solution; (e) Secondary electron (SE) micrograph of the untreated gypsum powders; (f) SE micrograph of the treated powders (g-h) Backscattered electron (BSE) micrograph and elemental mapping of the gypsum particles after twenty-eight days exposure to 1M DAP solution.

4.9 Conclusions

Diammonium hydrogen phosphate solutions were tested as precursors for hydroxyapatite (HAP) consolidation treatment of the powdery chalky rock substrate of Tomb 27 of Mağara Tepeşi at Athienou-*Malloura* in Cyprus. This was identified as a marlstone consisted of calcite, SiO₂, montmorillonite and plagioclase. Coccoliths and other fossils were detected within the microstructure indicative of the marine environment origin of the rock. Three different areas were chosen for testing of the DAP treatment. Desalination was performed on selected areas to evaluate its effect on the formation of HAP and to detect possible compositional and microstructural changes. The phases and microstructure of the different areas were analyzed and compared among the samples collected immediately after treatment and after one year. A combination of analytical techniques including XRD, FTIR, DM and SEM-EDS were employed.

Hydroxyapatite was found to form immediately after DAP treatment and no significant difference could be observed among the different test areas. Calcite was transformed into a HAP network that covered the calcite grains of the substrate and provided cohesion to the matrix. By comparing the composition and morphology of the marlstone sample taken one year after the DAP treatment to those taken immediately after the DAP treatment, it was clear that the reaction between the calcium carbonate and DAP further proceeded in the period subsequent to the treatment. This could be due to the fact that some unreacted DAP further reacted with the calcite. No significant differences were observed between the desalinated and non-desalinated areas. Gypsum salts which cannot be removed by desalination seem to have been transformed into brushite. Furthermore, microorganism formation was favored in some of the test areas in the interior of the chamber where the environment is cooler, darker and moist.

While the consolidating effect of this mineral treatment was attested to by the formation of HAP (until the entire DAP was consumed) further research will be conducted aiming to increase the formation of HAP crystal network. Pre-treatment of the surface with Ca(OH)₂ nanoparticle-alcohol dispersions [19, 24, 39] will be tested in an attempt to suppress the dissolution of the calcium ions from the marlstone substrate and increase the hydroxyapatite crystal network density. In addition, a second treatment using saturated limewater solution and/or Ca(OH)₂ nanoparticles-alcohol dispersions could follow the DAP treatment to remove unreacted DAP within the marlstone [41, 44, 45].

Microbial growth observed before and after treatment is a function of the mineral matrix of the stone and the environment that surrounds these areas. The mineral composition of the stone, nutrient availability, pH, salinity, surface texture, moisture content, porosity, permeability, climatic and other microenvironment conditions, play a determinant role on the microbial ability to colonize the surface of monuments, the type and extent of microbial colonization.

Chapter 5. Equilibrium Thermodynamics of Chemical Reactions between Calcium Carbonate and Ammonium Phosphate Precursors

This chapter attempts to provide an insight into the formation of stable phase assemblages in the CaCO₃-(NH₄)₂HPO₄/(NH₄)H₂PO₄-H₂O system. The main focus is placed on the morphological, mineralogical and thermodynamic characterization of calcium phosphates precipitating as a result of reaction between calcium carbonate substrate and different ammonium phosphate solutions. Correlation between formed stable calcium phosphate phases with the type and concentration of the ammonium phosphate precursor is provided. Advantages and limitations of various analytical approaches used in this study including X-Ray diffraction, scanning electron microscopy and thermal analysis are critically evaluated. Additionally, possible impacts of the solution type on the kinetics of phase transformations are discussed.

5.1 Calcium Orthophosphates

Calcium orthophosphates are a family of minerals of significant importance due to their wide applications in medicine, biology, geology, materials sciences, archaeology as also conservation science [28, 32, 102, 116-124]. As per a simplified general classification calcium phosphates can be subdivided into two groups, i.e., (a) phases that are obtained via high temperature reaction processes and (b) phases that precipitate via reactions in aqueous solution [20]. A listing of common phases is noted in Table 5-1 [27, 125].

Table 5-1. Selected calcium orthophosphate minerals and a tabulation of their properties [27, 117]. The Gibbs energy of formation (Δ Gf) is estimated from La Iglesia [27, 117] (Phases noted in bold text are reported to be thermodynamically stable when precipitated from aqueous solutions at 25°C and 1 bar).

Name	Formula	Ca/P ratio	Molar volume [cm ³ /mol]	ΔG_{f} [kJ/mol]	Comment(s)
Hydroxyapatite (HAP)	Ca10(PO4)6(OH)2	1.67	317.9	-12590.0	Ideal composition, stoichiometric phase very rare; at room temperature when precipitate from aqueous solutions substituted by other ions
Ca-deficient hydroxyapatite	Ca _(10-x) (HPO ₄) _x (PO ₄) _{6-x} (OH) _{2-x} 0 <x<1< td=""><td>1.50 - 1.67</td><td>300.16- 317.9</td><td>11848.0- to- 12590.0</td><td>Metastable with regard to hydroxyapatite; unsubstituted formula does not exist in biological systems; 'real' Ca-deficient HAP will have Ca²⁺; OH⁻ and/or HPO₄, PO₄²⁻ groups substituted by other ions</td></x<1<>	1.50 - 1.67	300.16- 317.9	11848.0- to- 12590.0	Metastable with regard to hydroxyapatite; unsubstituted formula does not exist in biological systems; 'real' Ca-deficient HAP will have Ca ²⁺ ; OH ⁻ and/or HPO ₄ , PO ₄ ²⁻ groups substituted by other ions
Ca-deficient (NH4 substituted) AB-type HAP	$Ca_{9.23}(NH_4)_{0.12}[(PO_4)_{5.36}(CO_3)_{0.64}]$ $(OH)_{0.54}(CO_3)_{0.34}$	1.72		-11761.9	Experimental composition given by Lee et al [126]: CO ₃ content ~ 6.05 mass %, NH ₄ content ~ 0.22 mass %

Brushite	CaHPO ₄ · 2H ₂ O	1.00	75.77	-2152.0	Reported to form for pH range ~ 2.0-6.5
Monetite	CaHPO ₄	1.00	46.54	-1674.0	Stable > 100°C
Whitlockite	Ca ₃ (PO ₄) ₂	1.50	95.99	-3864.0	Does not precipitate in aqueous solutions
Monocalcium phosphate	Ca(H ₂ PO ₄) ₂	0.50	91.78	-2834.0	Forms at temperatures in excess of 100°C
Monocalcium phosphate hydrate	Ca(H2PO4)2·H2O	0.50	115.06	-3073.6	Reported to be stable at low pH (< 2.0); high solubility in water ~18 g/L
Octacalcium phosphate (OCP)	Ca8(HPO4)2(PO4)4·5H2O	1.33	376.54	-12268.0	Precipitates at room temperature but metastable with regard to hydroxyapatite
Amorphous calcium phosphates	Ca _x H _y (PO ₄) ₂ ·nH ₂ O n=3.5-4; 15-20% H ₂ O	1.20- 2.20	~	~	Precipitate at room temperature but metastable with regard to hydroxyapatite
Hilgenstockite	Ca4(PO4)2O	2.00	120.08	-4606.0	Forms at temperatures in excess of 1300°C
Ammonium calcium phosphate heptahydrate	Ca(NH4)PO4·7H2O	1	169.23	-3525.82	Reported to be unstable and decompose in room temperature to hydroxyapatite phase

Hydroxyapatite Ca₅(PO₄)₃OH (HAP), often written as Ca₁₀(PO₄)₆(OH)₂ is a naturally occurring member of calcium orthophosphate family. It is a member of the complex apatite group and may have a variable composition [127] wherein: (a) the OH⁻ group is replaced by other anions (A-type substitution)[27], (b) the PO₄³⁻ group undergoes anion substitution (B-type substitution) [27, 128, 129] or (c) both OH⁻ and PO₄³⁻ groups undergo simultaneous substitution (AB- substitution) [130-132]. Obviously, depending upon the imposed conditions (i.e., temperature, pressure, aqueous chemistry etc.) the extent of anionic-replacement can be partial or complete. 'Ideal' stoichiometric HAP crystallizes in the monoclinic space group P21/b, however at temperatures above 250 °C a transition into a hexagonal ordering is noted (space group P63/m) [21, 130, 133].

Naturally occurring HAP (e.g. in bones or dentine) is non-stoichiometric wherein the PO₄³⁻ and/or OH⁻ groups are replaced by other species such as CO₃²⁻. In addition to anionic constituents, Ca^{2+} ions in HAP can also be (partially) substituted by species such as Sr^{2+} or Na^+ [134]. It should be noted, through the course of all iso/alio-valent ionic substitutions, charge neutrality is maintained via variations in composition. Changes in the composition result in changes in the crystal structure and solubility [135-138]. Various ionic substitutions and calcium deficiency result in the hexagonal lattice. The precipitation of HAP in aqueous solution is preceded by intermediate step precursors, including: monocalcium phosphate monohydrate (MCPM), dicalcium phosphate dihydrate (DCPD, brushite), octacalcium phosphate (OCP), amorphous calcium phosphate (ACP) and/or calcium deficient HAP (CDHA). While the formation of these phases depends on the reaction conditions (e.g., pH, foreign ions), eventually (i.e., at thermodynamic equilibrium) they will all transform to non-ideal HAP [139]. Apart from HAP, brushite (CaHPO₄·2H₂O, monoclinic crystal system) a phosphate analog of gypsum can achieve stability in aqueous calcium and phosphate rich environments: and persists as the dominant phase for pH levels ranging between 2.0 to 6.5 [20, 27]. Monocalcium phosphate monohydrate (MCPM) is noted to be stable at highly

acidic conditions (pH < 2) and exhibit high solubility in water (~18 g/L) [27]. Finally, ammonium calcium phosphate heptahydrate phase which is an analogue of struvite have been reported $Ca(NH_4)PO_4$? H_2O : but noted to only display metastability until final conversion to HAP [140].

5.2 Thermodynamic Calculations

Thermodynamic modeling is a powerful technique, widely applied to describe phase relations of metallic/inorganic substances. While a substantial body of work has described phase relations and stability in the system CaO-P₂O₅-H₂O, less information is available to describe how the presence of carbonate(s) may alter phase equilibria [140-143]. Thus and as a crucial objective, this work via a combined experimental and computational strategy describes phase relations in "aqueous systems" comprising calcium carbonate (CaCO₃) and various (i.e., mono-, di-, tri-) ammonium phosphates. Special focus is placed on describing relevant phase equilibria, phase coexistence and boundaries in selected calcium orthophosphate systems that precipitate from aqueous solution and at room temperature and pressure.

Phase equilibria in the system CaCO₃-(NH₄)₂HPO₄/(NH₄)H₂PO₄/(NH₄)₃PO₄-H₂O were computed at 25°C and 1 bar using the GEMS-PSI software [144]. GEMS is a broad-purpose geochemical modeling code which applies a Gibbs free energy minimization criterion to compute equilibrium phase assemblages and ionic speciations in a complex chemical system from its total bulk elemental composition. Chemical interactions involving solids, solid solutions, and aqueous electrolytes are considered simultaneously. Aqueous ion activities and speciation were calculated using the GEMS database, by applying the Truesdell-Jones modification of the extended Debye-Hückel equation to account for electrolytic non-ideality [145]. The Gibbs free energy of formation $(\Delta G_f, kJ/mole)$ of all relevant phosphate-bearing phases (Table 5-1) which were added to the standard built in GEMS database of minerals, was estimated based on the thermodynamic properties of their constituent oxides, as provided by La Iglesia [126, 146].

HAP which precipitates from aqueous solution in carbonate rich environments often is reported to display B-type substitutions wherein CO_3^{2-} replace PO_4^{3-} in the crystalline lattice [129]. Some studies show that mixed AB-type of substitutions may also occur where OH^- is partially replaced by CO_3^{2-} [147]. In any event, the level of CO_3^{2-} substitution in HAP is broadly ranges between 4-8 mass % [129, 130, 132, 148]. To account for dissimilar valence, the Ca/P (calcium: phosphate atomic ratio) ratio elevates beyond 1.67 recorded for 'ideal' HAP [130, 132]. Since at this time there is a lack of critical thermodynamic data for CO_3^{2-} substituted, Ca^{2+} deficient HAP: properties for experimentally derived composition corresponding to as proposed by Lee et al. [130] $Ca_{9,23}(NH_4)_{0.12}[(PO_4)_{5.36}(CO_3)_{0.64}](OH)_{0.54}(CO_3)_{0.34}$ was used for calculations. This composition represents the AB-type substitutions (see Table 5-1) noted to form under aqueous conditions as a result of reaction between calcium carbonate and diammonium hydrogen phosphate solution, and corresponds to a "carbonate-substitution level" of around 6 mass %.

In addition to anionic substitution, HAP often displays cation substitution (i.e., calcium deficiency) wherein Ca^{2+} ions may be replaced Na^+ or K^+ species to account for CO_3^{2-} substitution induced charge imbalance. In the context of this study, NH_4^+ sourced from the ammonium

precursors is anticipated to replace Ca^{2+} . Composition provided by Lee et al. [130] accounts for this substitution on the order of 0.22 mass % [130]. This low level of substitution level is believed to be induced due to the larger size of the NH₄⁺ ion [149], which results in misfits at atomic occupation sites. Due to the lack of experimental data no consideration was made of the potential of protonation of anionic species [32, 150, 151] or solid solution formation, though this is likely an oversimplification [32]. Obviously, in line with the "thermodynamic equilibrium" condition, no account is made for any kinetic influences in the calculations.

5.3 Experimental

Analytical reagent grade CaCO₃ (particle size $< 30 \ \mu$ m) was equilibrated with 100ml (NH₄)₂HPO₄ or (NH₄)H₂PO₄ solution at 0.2, 0.5, 1 and 2 molar levels (prepared using high purity water) in HDPE-bottles at 25 °C for a period of 6 months. The ammonium phosphate powders were purchased from Fisher Chemical and used as received. 10 grams of the CaCO₃ powders were used during each test. The solutions after equilibrating with the calcium carbonate powders for 6 months were centrifuged (Eppendorf Centrifuge 5430). Solids present after the reaction were dried in the desiccator for 1 week prior to further analysis.

XRD measurements on each layers were performed on a Bruker D8 Diffractometer with the following measurement parameters: Cu K α radiation λ =1.5405Å, 40kV, 40mA, 2 to 50° 2 θ exploration range with a step size of 0.014° 2 θ . Microstructural and elemental analyses of the powder samples were performed using FEI Nova NanoSEM 230 SEM. A Mettler Toledo TGA/SDTA 851e with STARe software, v. 8.10, was used for simultaneous TG and DTA measurements. After purging the furnace at 30 °C for 3 minutes, samples were heated in open 70 µl alumina crucibles to 1000 °C with a heating rate of 20°C/min. Nitrogen was used as purge gas and its flow was adjusted to 50ml/min.

5.4 Results

5.4.1 Experimental Results

Figure 5-1 shows the X-ray diffraction patterns (XRD) of reaction products between calcite (CaCO₃) powders and ammonium phosphate solutions: (NH₄)₂HPO₄ (DAP) and (NH₄)H₂PO₄ (MAP) of different molarities. Diffraction pattern of raw (untreated) calcite powder was also provided for comparison. Reaction between calcite powders and the diammonium phosphate (DAP, (NH₄)₂HPO₄) solutions ranging from 0.2M to 2M resulted in the precipitation of the hydroxyapatite phase (Figure 5-1(a)). Calcite was prevalent in all the samples however based on the XRD peak relative intensities it was observed the amount of residual unreacted CaCO₃ has decreased as a function of increasing molarity of diammonium hydrogen phosphate (DAP) solution. Additionally, octacalcium phosphate phase (OCP) was found in case of calcite immersed in 0.5M DAP (S-3), 1M DAP (S-5) and 2M DAP (S-7), with OCP peak intensities escalating as

the molarity of DAP was raised. No apparent peaks that belonged to octacalcium phosphate were identified for the sample treated with 0.2M DAP (S-1).

Similar phases were obtained when calcite interacted with monoammonium dihydrogen phosphate (MAP, (NH₄)H₂PO₄) solutions (Figure 5-1(b)). Hydroxyapatite was produced in samples treated with 0.2M MAP (S-2), 0.5M MAP (S-4) and 1M MAP (S-6). Though unreacted calcite was found in samples subjected to lower concentrations of MAP such as 0.2M (S-2) and 0.5M (S-4) it was noted to be fully consumed in the solutions containing 1M (S-6) and 2M (S-8) MAP.

Octacalcium phosphate (OCP) was noted in calcite immersed in 0.5M (S-4), 1M (S-6) and 2M (S-8) MAP solutions. No apparent peaks that belonged to octacalcium phosphate could be found in the sample treated with 0.2M MAP (S-2). Coexistence of hydroxyapatite, octacalcium phosphate and calcite was observed in sample S-4 (0.5M MAP). Octacalcium phosphate, and hydroxyapatite appeared concurrently in 1M MAP (S-6) sample. Brushite precipitated as a reaction product only in 2M MAP (S-8) treated calcite sample. No detectable amount of hydroxyapatite was found for 2M MAP sample (S-8) though the occurrence of octacalcium phosphate was detected.



Figure 5-1. X-ray diffraction patterns of the reaction products between CaCO₃ powders and (NH₄)₂HPO₄ (DAP) or (NH₄)H₂PO₄ (MAP) solutions: a) 0.2M DAP (S-1), 0.5M DAP (S-3), 1M DAP (S-5), 2M DAP (S-7); (b) 0.2M MAP (S-2), 0.5M MAP (S-4), 1M MAP (S-6); 2M MAP (S-8).



Figure 5-2. TG-DTG curves of calcite samples treated with $(NH_4)_2HPO_4$ (DAP) or $(NH_4)H_2PO_4$ (MAP) solutions: (a) 0.2M DAP (S-1); (b) 0.2M MAP (S-2); (c) 0.5M DAP (S-3); (d) 0.5M MAP (S-4); (e) 1M DAP (S-5); (f) 1M MAP (S-6); (g) 2M DAP (S-7); (h) 2M MAP (S-8).

Sample No.	Sample Description	Composition quantification		
S-1 (0.2M DAP)	Reaction products between 100ml 0.2M DAP solution and 10 g CaCO ₃ powders	Calcite (76.9 wt.%) Hydroxyapatite (23.1 wt.%)		
S-2 (0.2M MAP)	Reaction products between 100ml 0.2M MAP solution and 10 g CaCO ₃ powders	Calcite (67.5 wt.%) Hydroxyapatite (32.5 wt.%)		
S-3 (0.5M DAP)	Reaction products between 100ml 0.5M DAP solution and 10 g CaCO ₃ powders	Calcite (61.9 wt.%) Octacalcium phosphate + Hydroxyapatite (38.1 wt.%)		
S-4 (0.5M MAP)	Reaction products between 100ml 0.5M MAP solution and 10 g CaCO ₃ powders	Calcite (28.2 wt.%) Octacalcium phosphate + Hydroxyapatite (71.8 wt.%)		
S-5 (1M DAP)	Reaction products between 100ml 1M DAP solution and 10 g CaCO ₃ powders	Calcite (32.9 wt.%) Octacalcium phosphate + Hydroxyapatite (67.1 wt.%)		
S-6 (1M MAP)	Reaction products between 100ml 1M MAP solution and 10 g CaCO ₃ powders	Calcite (0 wt.%) Octacalcium phosphate + Hydroxyapatite (100 wt.%)		
S-7 (2M DAP)	Reaction products between 100ml 2M DAP solution and 10 g CaCO ₃ powders	Calcite (23.2 wt.%) Octacalcium phosphate +Hydroxyapatite (76.8 wt.%)		
S-8 (2M MAP)	Reaction products between 100ml 2M MAP solution and 10 g CaCO ₃ powders	Calcite (0 wt.%) Octacalcium phosphate (34.1 wt.%) Brushite (65.9 wt.%)		

Table 5-2. Phase quantification determined based on the thermal (TG/DTG) analysis

In order to obtain thermal decomposition curves and determine the quantity of reaction products between calcite and ammonium phosphate solutions, thermal-gravimetric analysis was performed on all the samples (Figure 5-2). The corresponding phase quantities calculated from the weight loss values were listed in Table 5-2. Absorbed free water was lost in a temperature range from room temperature up to 200°C. Between 600 to 860 °C, associated weight loss was linked to the decomposition of calcite (samples S1, S2, S3, S4, S5 and S7) and its quantity was determined

and listed in Table 5-2. No CaCO₃ decomposition could be observed for samples treated with 1M MAP (S-6) and 2M MAP (S-8), indicating that all calcite was consumed. In all the samples where phases of octacalcium phosphate or hydroxyapatite were identified, associated progressive weight loss was recorded in a temperature range between 200°C and 600° C, above which sharp peak calcite decomposition was noted (samples S1, S2, S3, S4, S5 and S7). A higher quantity of hydroxyapatite or octacalcium phosphate resulted in a more prominent weight loss in that temperature range e.g. when comparing samples S1 and S2 to S4 or S5 and subsequently lower calcite content (Table 5-2) indicating calcium phosphate-rich phases forming at the expense of calcite consumption. In the sample which only consisted of octacalcium phosphate and hydroxyapatite (S6, 1M MAP) continuous weight loss were recorded between 200°C and 1000°C. Additionally another apparent weight loss region could be observed within 40-180 °C and this peak was proportional to the quantity of octacalcium phosphate found (samples S-3, S-4, S-5, S6, S-7, S-8). In specimen where brushite (S8, 2M MAP) was identified three distinguishable mass loss steps were detected at approximately 150 °C and 200 °C and 430 °C.

It is important to highlight that the hydroxyapatite dehydration occurs over a wide temperature range, which was mainly dependent on the partial H₂O pressure. The gradual dehydration of hydroxyapatite leads to the release of OH⁻ ions and transform into oxyhydroxyapatite (OHAP): Ca₁₀(PO₄)₆(OH)_{2-x}O_{x \square x} where \square stands for a vacancy[27, 152, 153]. Beginning from sample S-3, apparent weight loss region could be observed within 40-180 °C which corresponded to the decomposition of octacalcium phosphate. Upon heating up to 180 °C, three water molecule per OCP molecule was removed and the OCP $(Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O)$ was converted into a poorly crystalline apatitic phase $(Ca_8H_4(PO_4)_6(OH)_2)$ as shown in Reaction 7[154].

 $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O \rightarrow H_2O + Ca_8H_4(PO_4)_6 \cdot 4H_2O \rightarrow 2H_2O + Ca_8H_4(PO_4)_6(OH)_2$ Reaction 7. The thermal decomposition of OCP

In case of sample S-8 (2M MAP), as evidenced from the X-ray diffraction, brushite and OCP were found to coexist. A complete dehydration of brushite happened over the temperature range between 110-230°C and decomposition from brushite (CaHPO₄· 2H₂O) into monetite (CaHPO₄) following Reaction 8 is expected [155-157]. Literature reports that the dehydration process of the brushite crystal, occurring at different temperatures is related to the particle size differences [158]. Therefore, here, the peak at 153°C (Figure 5-2(h)) could be possibly related to the partial dehydration of brushite while the peak at around 201°C could correspond to the total dehydration and monetite formations.

 $CaHPO_4 \cdot 2H_2O \rightarrow CaHPO_4 \cdot H_2O + H_2O$

$$CaHPO_4 \cdot H_2O \rightarrow CaHPO_4 + H_2O$$

Reaction 8. Thermal decomposition of brushite.

Estimating quantity of brushite from its decomposition signatures up to 200° C is challenging due the overlapping decomposition with the OCP occurring within the same temperature range. Meanwhile, a further decrease in the weight in the temperature of 360 °C to 500 °C corresponds to possible reaction in which two molecules of CaHPO₄ combine, which leads

to the elimination of the water molecule, and subsequent formation of calcium pyrophosphate (Ca₂P₂O₇), as shown in Reaction 9.

$$2\text{CaHPO}_4 \rightarrow \text{Ca}_2\text{P}_2\text{O}_7 + \text{H}_2\text{O}_7$$

Reaction 9. Thermal decomposition of calcium monohydrogen phosphate

The theoretical mass loss for this step is 5.23 %, thus, thus based on this information a weight fraction of 65.9 % for brushite (Table 5-2) could be estimated. In general by comparing the reaction products for the same ammonium phosphate solutions from 0.2M to 2M, it was noted that the unreacted calcite fraction decreased with the increasing concentration of ammonium phosphate while the 'calcium apatite phases' (such as OCP and HAP) fraction increased.

Figure 5-3 shows SEM images of calcite powders immersed in DAP and MAP solutions. Untreated (raw) calcium carbonate crystals were found to be micrometer-sized rhombohedral crystals with sharp edges (Figure 5-3a). Only calcite was found and no other polymorphs of CaCO₃ could be observed either from the X-ray diffraction pattern (Figure 5-1) or scanning electron microscopy images (Figure 5-3(a)). Calcite powders treated (in the order of increasing concentrations) with DAP solutions are shown on Figure 5-3(b), (d), (f) and (h) and the ones treated with MAP solutions are presented on Figure 5-3(c), (e), (g) and (i). As shown in Figure 5-3(b), in case of DAP solution at lower concentrations the shape of the calcite crystal was maintained while the formation of HAP could be observed on the surface.



Figure 5-3. Scanning electron microscope (SEM) images of : (a) raw calcite rhombohedral crystals with sharp edges; (b) Sample S-1 (0.2M DAP); (c) Sample S-2 (0.2M MAP); (d) Sample S-3 (0.5 DAP); (e) Sample S-4 (0.5M MAP); (f) Sample S-5 (1M DAP); (g) Sample S-6 (1M MAP); (h) Sample S-7 (2M DAP) and (i) Sample S-8 (2M MAP).

Figure 5-3(d) suggests that the DAP solution induces the formation of a hydroxyapatite 'shell' around calcite grains. White arrow indicates a portion of exposed CaCO₃ which was initially hidden under the HAP type coating. As the concentration of DAP is rising, the alteration of calcite grains seems to be more abundant (Figure 5-3(f) and (h)) and the morphology of 'coating' also slightly changes from the network of small 'plates-like' phase (Figure 5-3(b)) typical for hydroxyapatite [159] to larger 'layer-like' crystals (Figure 5-3(h)). In case of MAP treated samples

for those treated with lower concentrations up to 0.5M MAP (Figure 5-3(e)) HAP phase of a small plates like morphology was found while as concentration of MAP increased to 1M MAP (Figure 5-3(g)) it resembled more a 'layer-like' surface. The EDS measurement on the Ca/P ratio of sample S-8 yielded a value of ~ 1, indicating a complete disintegration of calcite crystals followed by the formation of brushite (Figure 5-3(i)).

5.4.2 Thermodynamics Modelling

To ensure compatibility between experiments and modeling, thermodynamic simulations were performed for the conditions similar to those reflecting experimental setup. Figure 5-4 to Figure 5-9 show solid phases anticipated to coexist in thermodynamic equilibrium for the calcite $(CaCO_3)$ immersed in a different ammonium phosphate precursors at variety of solution concentrations (molality, m). Calculated compositions of aqueous solutions were projected simultaneously to provide an insight on the relationship between solids and corresponding liquids coexisting in equilibrium. It was found (Figure 5-4) that the increasing molality of (NH₄)₂HPO₄ (DAP) resulted in the consumption of calcite (CaCO₃) and the precipitation of carbonate substituted hydroxyapatite (HAP) type of phase. HAP formation was maximized at the point where calcite has been completely exhausted (~0.75 m (NH₄)₂HPO₄). Looking at the aqueous solution, initial consumption of calcite up to ~ 0.2 m DAP caused drop of the pH (Figure 5-5) from 8 to 6. This pH decrease trend was followed by the increased calcium (Ca) ion concentrations coming from the dissolution of calcite. Both pH and Ca started to elevate past ~ 0.2 m DAP and by ~ 0.75 m DAP reached stable values corresponding the equilibrium concentration that would be expected in the saturated HAP solutions. Past this point upon increasing DAP concentrations the rise in the phosphorus aqueous content is expected since all the calcite is depleted and no more calcium is available to form hydroxyapatite phase.

Acidic (NH₄) H₂PO₄ (MAP) solutions (Figure 5-6) were expected to result in the formation of the brushite phase which is reported to be stable in a lower pH ranges in the calcium phosphate systems [32]. However up to the ~ 1.5m MAP it was predicted that hydroxyapatite phase will be a dominant reaction product. As the MAP molarity increased, the brushite formation increased, at the expense of decreasing quantity of HAP. At very high MAP concentrations (above 3m), brushite is expected to completely replace hydroxyapatite phase. Regarding aqueous solutions (Figure 5-7) of MAP, due to its acidic nature, it is projected to cause decrease of pH from 8 to 5.5 once brushite starts to precipitate. (NH₄)₃PO₄ (TAP) demonstrated (Figure 5-8) trends very similar to the DAP except slightly higher pH of 9 (Figure 5-9) instead of 8 as it was in case of DAP when it equilibrates with respect to hydroxyapatite phase. This is related to the higher alkalinity of (NH₄)₃PO₄ as compared to the (NH₄)₂HPO₄.



Figure 5-4: Calculated total volume of solid phases of a hydrated model mixture initially consisting of 10g CaCO₃, 100 ml H₂O and 7 g of air showing phase development and its dependence on changing $(NH_4)_2$ HPO₄ concentrations at 25 °C.



Figure 5-5: Calculated aqueous composition of hydrated mixture initially consisting of $10g CaCO_3$, $100 ml H_2O$ and 7 g of air showing changes in the aqueous composition and dependence on changing $(NH_4)_2HPO_4$ concentrations at 25 °C.



Figure 5-6: Calculated total volume of solid phases of a hydrated model mixture initially consisting of 10g CaCO₃, 100 ml H₂O and 7 g of air showing phase development and its dependence on changing (NH₄)H₂PO₄ concentrations at 25 $^{\circ}$ C.



Figure 5-7: Calculated aqueous composition of hydrated mixture initially consisting of 10 g CaCO₃, 100 ml H₂O and 7 g of air showing changes in the aqueous composition and dependence on changing $(NH_4)H_2PO_4$ concentrations at 25 °C.



concertation of (NH₄)₃ PO₄ solution [m]

Figure 5-8: Calculated total volume of solid phases of a hydrated model mixture initially consisting of 10g CaCO₃, 100 ml H₂O and 7 g of air showing phase development and its dependence on changing $(NH_4)_3PO_4$ concentrations at 25 °C.



Figure 5-9: Calculated aqueous composition of hydrated mixture initially consisting of 10g CaCO₃, 100 ml H₂O and 7 g of air showing changes in the aqueous composition and dependence on changing $(NH_4)_3PO_4$ concentrations at 25 °C.

5.5 Discussion

Predicting the phase equilibria even in the simple systems of CaCO₃-(NH₄)₂HPO₃/(NH₄)H₂PO₄/(NH₄)₃PO₄-H₂O as the ones presented in this study was found to be highly complex. Estimating the extent of hydroxyapatite formation in particular is complicated. It is known that 'hydroxyapatite' is not just a one compound of fixed composition and rather exists over a compositional range [160] which is highly influenced by experimental conditions. Various ions can substitute for each other in various sites [32] depending on the factors such as initial reactant compositions, solution concentration, temperature or pH [149]. For the experiments similar to the ones conducted in this study reaction in aqueous solution and at room temperature carbonate ions are reported to mostly substitute in a B position (phosphate sites) [161]; however, there are the findings showing that in fact it can also substitute for both A (hydroxide) and B sites (phosphate) [147]. The extent of this substitution, the limits for the potential solid solution formation, dependence on the solution concentrations and type of phosphate precursor are currently unknown and have not been determined in this study. Additionally, apart from the carbonate ions other ions also need to be considered e.g. no empirical reports exist on the extent of protonation of phosphate group in calcium deficient hydroxyapatite obtained at different experimental conditions. These factors can contribute to oversimplification of the thermodynamic database and resulting potential discrepancies between experimental and modeling results.

Furthermore, HAP precipitation is reported to be preceded by the formation of several intermediate metastable phases, such as monocalcium phosphate monohydrate and anhydrous (MCPM and MCPA, respectively), dicalcium phosphate dihydrate (brushite) and anhydrous (DCPD and DCPA, respectively), octacalcium phosphate (OCP), amorphous calcium phosphate (ACP) and/or calcium-deficient HAP (CDHA) [20]. Such precursor phases, whose formation depends on reaction conditions (e.g., degree of supersaturation, temperature, pH, presence of foreign ions, etc.), are expected to transform eventually into HAP by dissolution and reprecipitation processes [162-165].

For this reason, therefore octacalcium phosphate (OCP) as well as other metastable phases were excluded from thermodynamic considerations. The only exception was made with regard to brushite (dicalcium phosphate dehydrate) which was found to be thermodynamically stable under certain (acidic) conditions [32]. As mentioned above OCP is commonly found to be present as an intermediate phase in the conversion process from the ACP (amorphous calcium phosphates) to HAP (hydroxyapatite) [166]. This transition could explain the co-existence of HAP and OCP within the mixtures of most of the reaction products (Figure 5-1).

The kinetics of these phase transformations seems strongly affected by the solution concentration as indicated by XRD data (Figure 5-1). After 6 months of sample equilibration of solutions with molarity above 0.5 M (MAP or DAP) an octacalcium phosphate (OCP) seems to persist though thermodynamic calculations; literature data indicate that octacalcium phosphate should fully transform to hydroxyapatite.

This persistence is more prevalent as the concentration of the solution increases, suggesting that it affects the kinetics of the OCP transformation into HAP variant phase. This could explain the relative intensity of OCP was stronger for 2M solutions versus 1M solutions and why OCP was not detected in the case of 0.2M solutions (Figure 5-1). Kinetics of the reaction is also highly influenced by the type of the phosphate precursor. When placed in a more acidic solution of monoammonium dihydrogen phosphate (MAP), calcite undergoes quick dissolution followed by the formation of brushite (Figure 5-1 (b), Figure 5-2(h), Figure 5-3(i)). For the concentrations of 1M MAP and higher calcite is fully consumed.

In the case of diammonium hydrogen phosphate (DAP) reactions seem to be much slower as compared to the monoammonium dihydrogen phosphate (MAP). SEM images (Figure 5-3 (b,d,f)) disclosed formation of hydroxyapatite or octacalcium phosphate variant phase at the surface of the grains but XRD and thermal analysis data (Figure 5-1(a) and Table 5-1) had shown calcite grains underneath to be largely unreacted. It seems that the degree of the reaction of calcite is linked to the molarity of the solution; the higher the concentration, more calcite is consumed (Table 5-1) and calcium phosphate phases such as hydroxyapatite or octacalcium phosphate may form. The reaction of the DAP treatment with the substrate occurs through a slow and controlled dissolution-precipitation reaction of the external parts of calcite grains [167-169] as the surface of the calcite is exposed to the phosphate solution.

In the case of DAP, a shell of hydroxyapatite phases (or their precursors such as octacalcium phosphate) forming around of calcite grains were noted (Figure 5-1(b,d)). Calcium

ions are not anticipated to re-precipitate as calcium carbonate due to the fact that the solubility product of HAP is much lower than the one of calcite [168]. As the OCP/HAP shell thickens, the reaction may also slow down due to the limited access of phosphate ions into the core of the calcite grain. Noticeably, the bigger the calcite grain is, the longer it would take for the reaction to take place. According to the thermodynamic simulations at 2m DAP, all calcite should be consumed (Figure 5-4). However, experimental data indicated that for relatively small calcite particles (< 30 µm), even after 6 months of equilibration, ~ 23% of calcite has still prevailed (Figure 5-3, Table 5-1). These findings indicate limitations of thermodynamic modeling which cannot account for kinetic factors, as well as, intermediate/metastable phase transformations before the final product-in this case HAP, is stabilized.

Literature reports that brushite can be formed in the initial stage during the reaction between the 2M monoammonium phosphate solution with calcium carbonate [170], though this brushite will eventually transform into a more basic calcium phosphate phase of OCP followed by the subsequent formation of HAP. On the other hand some data show that if the pH is kept below 6.5 brushite is noted as a stable phase [32]. Thermodynamic simulations presented herein suggest that hydroxyapatite and brushite may be both thermodynamically stable and coexist under certain experimental conditions (Figure 5-6). There is a level of uncertainty regarding the self-consistency, an accuracy of current thermodynamic data or the exact composition/solid solution formations of hydroxyapatite phases, which may add to the discrepancy between what is theoretically predicted and what is observed experimentally. However, taking these factors into consideration thermodynamic modeling can still serve as a useful generic guideline for understanding long term phase equilibria in these systems.

The amount of air or partial pressure of carbon dioxide which is in contact with calcite and ammonium phosphate solutions is also anticipated to influence phase equilibria. The amount of CO₂ dissolved in the aqueous phase would be dictated by the Henry's law and easily could alter pH as well as carbonate phases for these systems.

5.6 Conclusions

As set of experiments coupled with thermodynamic simulations have attempted to define phase relations between calcite and various ammonium phosphate environments. Results have demonstrated that CaCO₃ undergoes reactions when placed in the ammonium phosphate solutions however kinetics of phase transformations is highly dependent on the concentration and type of solution. When calcite is immersed in diammonium hydrogen phosphate (DAP) a development of the shell of hydroxyapatite (HAP) type phase is noted on the surface. This hydroxyapatite structure is preceded by the formation of metastable octacalcium phosphate (OCP) which is expected to transform to HAP.

This finding is in agreement with current literature reports. As the molarity of DAP increases above 1M the kinetics of this transformation seems to slow down and in some samples OCP was found to prevail even after 6 months of reaction. Also the formation of a layer of HAP/OCP phase around the calcite particles seems to hinder further penetration of phosphate into

the inside of grains as some unreacted calcite was detected even though samples were continuously stirred; DAP solution concentration was high (2M) and CaCO₃ powder was fine (particle size < 30 μ m). From this set of experiments, supported by thermodynamic calculations, a full consumption of calcite and complete formation of HAP was anticipated.

In the case of monoammonium dihydrogen phosphate (MAP) solutions, due to the acidity of MAP, dissolution of CaCO₃ was more rapid and the formation of brushite (DCPD) was noted. Brushite is also reported as an intermediate phase preceding HAP formation in some systems so it would eventually be expected to disappear potentially over the period of time longer that this study. However, this may not be the case as for the certain concentration ranges of MAP thermodynamic calculations predicted coexistence of hydroxyapatite and brushite as two stable phases.

Chapter 6. Conclusions

This research focusing on the synthesis, optimization of application and fundamental research on hydroxyapatite (HAP) consolidation of calcium-rich matrices including pigmented surfaces (i.e. wall paintings, polychrome architecture, rock-art), has provided new insight into the reaction chemistries involved when diammonium hydrogen phosphate (DAP) is used as the precursor. Systematic and comprehensive investigations both experimental and theoretical focused on four fundamental aspects, crucial for a holistic approach:

- application and evaluation of DAP solutions on laboratory simulated wall painting lime (Ca(OH)₂)-based substrates (mainly plaster) test blocks;
- 2. direct effects of DAP solutions on pigments;
- 3. on-site testing and assessment of DAP precursors on archaeological CaCO₃-rich substrates;
- 4. thermodynamic modeling of calcium carbonate and ammonium phosphate precursors.

Results from 1M DAP treatment on CaCO₃-rich plaster test blocks, indicated the formation of a porous hydroxyapatite network at the surface and subsurface of the blocks, as well as, slight reduction of water uptake. A light saturation of the surface could be observed with detectable color change. 2M DAP solutions however, caused evident whitening of the surface due to a high concentration of newly-formed phases of HAP at the surface.

Investigations into the optical, physical and chemical interactions of pigments with DAP solutions focused on the study of eight pigments (coloring compounds) commonly encountered in ancient art. The effects of the treatment on the color, morphology and chemical composition of the pigments were thoroughly investigated. Acceptable color changes with undetectable phase

changes were determined for cinnabar, lapis lazuli and French ochre. In the case of chalk and calcite, the latter found as in impurity in some of the pigments, though it was also used as pigment itself in ancient artwork, both color and phase change were identified. However the ΔE^* value was below the accepted threshold for consolidation treatments on cultural heritage and the phase transformation favored the consolidation mechanisms by the growth of HAP network. Significant phase transformation, accompanied also by a strong color change was identified in the samples containing red lead (minium). This change was attributed to the formation of lead hydroxyapatite through a possible multistep dissolution-precipitation reaction between the DAP precursor and the red lead (minium) pigment [75, 77, 78]. These results indicated that while DAP could potentially be employed on painted surfaces to re-establish loss of cohesion both within the paint layer and/or the substrate, precautions should be taken against the possible reactivity of certain pigments with DAP and color changes that may occur. For instance, DAP treatment on red lead pigmented surfaces could cause serious and irreversible damage to the artwork and should be avoided.

Based on the experimental data verifying the potential (while delineating the risks of possible phase and color change) of DAP (1M) as a consolidant for CaCO₃-rich plaster test blocks and pigmented surfaces, the materials and methods developed in this research, could be safely tested on-site for the consolidation of *fresco* wall paintings.

On-site testing for in situ HAP consolidation through the application of 1M DAP solution was performed on the marlstone (calcite-rich with clays and silt) at Tomb 27 at the Mağara Tepeşi necropolis in Athienou-*Malloura*, Cyprus. A hydroxyapatite network was formed immediately after DAP treatment mainly on the CaCO₃-rich regions of the marlstone. One year after the DAP treatment, the hydroxyapatite formation was found to be further promoted, due to the humid and warm environment of the tomb where further reaction between unreacted DAP and calcite grains proceeded.

For a more fundamental understanding of HAP formation, phase relations between calcium carbonate and ammonium phosphate solutions have been studied both theoretically and experimentally. Results have demonstrated that CaCO₃ undergoes reactions when placed in the ammonium phosphate solutions, however, kinetics of phase transformations is highly dependent on the concentration and type of solution. When calcite is immersed in DAP solutions, development of a shell of HAP type phase is noted on the surface. This hydroxyapatite structure is preceded by the formation of metastable octacalcium phosphate (OCP) which it is expected to transform into HAP. In the case of monoammonium dihydrogen phosphate (MAP) solutions due to the acidity of MAP, dissolution of CaCO₃ is more rapid and the formation of brushite (DCPD) is favored.
6.1 Future Research

6.1.1 HAP Effects on Pigments, Organic Colorants and Organic Binders

While much on CaCO₃-rich substrates and pigments has been covered in this research, multilayered and multinary systems of painted surfaces that include the binary system of the paint layer (containing both the pigment and the binding medium) and the substrate have not been addressed. Binding media in ancient art included natural polysaccharides, proteinaceous materials and to a lesser extend drying oils. Their reactivity to the DAP solutions would need to be further investigated alone as well as within the multinary system they belong.

Pigmented test-blocks to imitate these ancient systems will need to be prepared and tested at laboratory controlled conditions. Similar procedures as described in Chapter 2 and Chapter 3 could be followed. The results will need to be evaluated on multiple aspects including microstructure, morphology, physicochemical properties, as well as, color changes.

6.1.2 Control of HAP growth

Application of nano Ca(OH)₂ stable dispersions in alcohol

To further optimize the treatment and increase the concentration of Ca available for the transformation into HAP especially for stone or plaster substrates rich in SiO_2 (i.e. sandstones or plasters using sand as aggregates), nanoparticles of calcium hydroxide dispersed in alcohol will be synthesized and applied as pre-treatment. These Ca(OH)₂ stable dispersions in alcohol could be

introduced into the test panels by spraying. After complete carbonation (transformation to calcite), ammonium phosphate precursors will be applied. The newly formed CaCO₃ nanoparticles will be in-situ transformed into HAP. These particles will not only help fill large gaps in the macro-porosity but will also enhance the binding mechanism with the formation of the HAP network. After the consolidation treatment, the nanolime could also be applied to remove the unreacted DAP from inside the pores of the calcium carbonate matrices.

Application of peptides

In order to achieve controlled growth of HAP and/or its transitional phases, peptides that are short chains of amino monomer and linked by amide bonds will be tested as pre-treatment. Peptides consisting of octuplet repeats of aspartate-serine-serine (8DSS), may serve as nucleation agents for HAP crystals formation and promote bio-mineralization of calcium-rich matrices. These peptides and the phosphate solutions will be combined to apply to the substrate surface of the test panels [19, 171-173].

Evaluations will then be undertaken to estimate the effects of peptides in encouraging HAP formation and consolidation of the aged test panels. Further phase characterizations will be performed using primarily X-ray diffraction (XRD) and micro-Raman spectroscopy (μ RS). The microstructures of the aged test panels will be carried out using optical microscopy (OM), variable pressure scanning electron microscopy (VPSEM) and transmission electron microscopy (TEM). On the same time, consolidation effects measured in engineering properties such as water

sorptivity, fluid permeability, color value changes, dynamic elastic modulus change, and water absorption, will be performed on the untreated and treated aged test panels.

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