RECENT ADVANCES ON THE USE OF PHOSPHATE TREATMENTS TO PROVIDE HERITAGE STONES WITH ANTI-FOULING ABILITY

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Abstract

Formation of calcium phosphates (CaP) was proposed years ago to consolidate and protect stone substrates from dissolution in rain, thanks to the lower water solubility of CaP (especially hydroxyapatite) compared to calcite, and to enhance the mechanical properties of the stone. CaP can be formed *in situ*, by reacting the calcium-rich substrate with an aqueous solution of a phosphate precursor, typically diammonium hydrogen phosphate (DAP). Given the good protective ability provided by so-formed CaP coatings, the possibility to provide CaP coatings with anti-fouling ability is being investigated within the "SECURE-COATS" project, the first results of which are here presented. Two different strategies have been explored, namely (1) embedding antimicrobial metal nanoparticles (NPs) into CaP coatings, by suspending them in the DAP solution used to form CaP and (2) applying the NPs on already-formed CaP coatings. The results of the present study show that, in the first case, not enough NPs are available on the stone surface to provide effective anti-fouling activity. On the contrary, when the metal NPs were applied onto already-formed CaP coatings, they exerted effective anti-fouling action against two model bacterial strains. After accelerated ageing by dropping a simulated artificial rain onto functionalized stone samples, the anti-fouling ability was retained, thus indicating good durability of the coatings. However, in spite of the initial acceptable colour change right after treatment, the samples exhibited a marked colour change with time, mostly

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due to darkening. Further research is in progress to ascertain the cause of this colour change and to prevent it or at least mitigate it.

Keywords: Calcium phosphate, hydroxyapatite, biodeterioration, antibacterial activity, marble, ageing.

1. Introduction

Coatings made of calcium phosphates (CaP) were proposed 15 years ago to protect stone substrates from dissolution in rain (Naidu *et al.*, 2011), thanks to the lower water solubility of CaP (especially hydroxyapatite, the least soluble CaP at pH>4) compared to calcite, the main mineral constituent of heritage stones, such as marbles and limestones. CaP can be formed *in situ*, by reacting the calcium-rich substrate with an aqueous solution of a phosphate precursor, typically diammonium hydrogen phosphate (DAP, $(NH_4)_2HPO_4$) (Sassoni *et al.*, 2011).

Given the good protective ability provided by so-formed CaP coatings, their ability to provide additional functionalities has been also investigated, namely their consolidating action (by formation of new CaP among loose grains) (Sassoni *et al.*, 2011; Sassoni *et al.*, 2015), self-cleaning action (by combination with photocatalytic nanoparticles) (Sassoni *et al.*, 2018a) and de-sulphating ability (by transformation of gypsum crusts into less soluble CaP) (Sassoni *et al.*, 2018b). Starting from this premise, the possibility to provide CaP coatings with anti-fouling ability is currently being investigated within the "SECURE-COATS" project (https://site.unibo.it/secure-coats/en).

Anti-fouling ability is desired as biodeterioration is a widespread cause of cultural heritage degradation (Figure 1), accounting for some 30% of monument deterioration (Becerra *et al.*, 2018) and expected to be worsened by future climate change (Viles and Cutler, 2012). Notably, already restored monuments can also be subject to biological growth (Cappitelli *et al.*, 2013), as the restoration material can serve as nutriment to the microorganisms.

To provide the stone surfaces with anti-fouling ability, several routes have been explored in the past, especially the use of metal nanoparticles (NPs) with antimicrobial ability. Metal NPs have been either applied directly onto the stone or embedded in organic or inorganic coatings (Pinho and Mosquera, 2011; Colangiuli *et al.*, 2015; Becerra *et al.*, 2018), but compatibility and/or durability issues have frequently been reported.

Therefore, in the present study a new approach is presented, based on the combination of CaP coatings formed *in situ* with antimicrobial metal NPs. Two strategies of combination of CaP coatings and metal NPs were explored, namely: (1) embedding the NPs into the CaP coating, by suspending the NPs in the DAP solution used as precursor of the CaP coating; (2) applying the NPs onto already-formed CaP coatings. The novel coatings were tested on marble, selected as reference substrate. Their anti-fouling ability was assessed right after treatment and then after accelerated ageing, aimed at evaluating the durability of the new coatings.

Figure 1: Examples of biodeterioration (the dark areas are affected by biological growth, while in the pale areas the biological growth is inhibited by leaching of copper ions from letters and symbols.

2. Materials and methods

2.1 Stone

Carrara marble was used for the tests. Samples with 30 x 20 x 3 mm^3 were sawn from a single slab provided by Imbellone Michelangelo s.a.s., Italy.

2.2 Functionalized coatings

The treatment conditions described in the following were considered:

- "UT" = Untreated reference;
- "DAP" = Treatment with a solution containing 0.1 M DAP+0.1 mM $CaCl_2$ (applied by poulticing);
- "DAP with NPs" = Treatment with a dispersion of metal NPs in a 0.1 M DAP+0.1 mM $CaCl_2$ solution (applied by brushing 4 times), so as to embed the NPs in the CaP coating;
- "DAP then NPs" = Treatment with a solution containing 0.1 M DAP+0.1 mM $CaCl_2$ (applied by poulticing), followed by application (by poulticing) of the metal NPs over the already-formed CaP coating.

All the treatments that were carried out by poulticing involved mixing cellulose pulp and the respective solution in a 1:4 w/w ratio. The poultice was left on the samples for 24 hours, preventing evaporation by wrapping with a plastic film, then the samples were rinsed with water and dried at 40 °C. No detailed information can be provided about the composition of the metal NPs and their application method, because of confidentiality reasons.

2.3 Accelerated ageing

Accelerated ageing was performed by exposing samples to simulated rain, by using a solution designed to reproduce the rain composition in current urban environment. The rain was let drop continuously on the samples, disposed with a 45° with respect to the horizontal, for 2 days.

2.4 Characterization

2.4.1 Coating composition

The composition of the CaP coating formed after treatment was determined by X-ray diffractometry (XRD), performed by analysing the treated surface of the samples with a PANalytical X'Pert PRO diffractometer.

2.4.2 Coating morphology

The morphology of the CaP coating and the presence of metal NPs were determined by observation with a scanning electron microscope (SEM, TESCAN MIRA3), after making the samples conductive by coating with graphite. To facilitate the identification of the metal NPs, observations were carried out by using backscattered electrons (BSE).

2.4.3 Colour change

The CIELab colour parameters were determined on untreated and treated samples, by using a NH310 Portable Colorimeter. Three measurements were performed in each sample and then the resulting colour coordinates were averaged. The colour change caused by the treatment was calculated according to the formula $\Delta E = (\Delta L^2+\Delta a^2+\Delta b^2)^{0.5}$.

2.4.4 Anti-fouling ability

The antifouling ability was evaluated against two model bacterial strains, namely Escherichia coli (Gram -) and Staphylococcus aureus (Gram +). The samples were initially inoculated with bacterial cells and then the antibacterial and anti-fouling ability was assessed against non-adherent cells and adherent cells, respectively, after 24 hours of exposure. Bacterial enumeration was assessed by recovering the bacterial cells from the samples and performing serial dilutions followed by transferring an aliquot on LB agar plates and counting colony forming units (CFU). To enumerate the adherent cells, the samples were sonicated before the serial dilution and plate inoculation.

3. Results and discussion

3.1 Effect of the treatments

Compared to the untreated reference, the "DAP" sample exhibited new peaks at about 26° and 32° (Figure 2), which can be attributed to the formation of carbonate hydroxyapatite. Peaks are broad and poorly defined, which suggests that the newly formed carbonate hydroxyapatite is poorly crystalline and nanocrystalline in nature. While in the case the "DAP then NPs" sample the new peaks are clearly visible, in the case of the "DAP with NPs" sample new peaks owing to new phases are hardly detectable. When the functionalized coatings were observed by SEM, abundant presence of metal NPs was distinguishable in the case of the “DAP then NPs” sample, while more isolated NPs were observed on the surface of the “DAP with NPs” sample (Figure 3). In fact, as a result of the NP dispersion in the DAP solution, so that NPs are already present during the formation of the CaP coating, the NPs end up being embedded in the coating, so that fewer particles are present on the sample surface.

In terms of colour change after treatment, the “DAP” sample exhibited minor alterations (Table 1), consistent with results previously reported in the literature (Sassoni, 2018). When NPs were introduced into the DAP solution (“DAP with NPs” sample), a higher colour change was experienced, exceeding the common acceptability threshold of ΔE* = 5 (Table 1). On the contrary, when the NPs were applied over the coating (“DAP then NPs”), a minimal colour change occurred, which results below the visibility limit of ΔE* = 2.3 (Table 1).

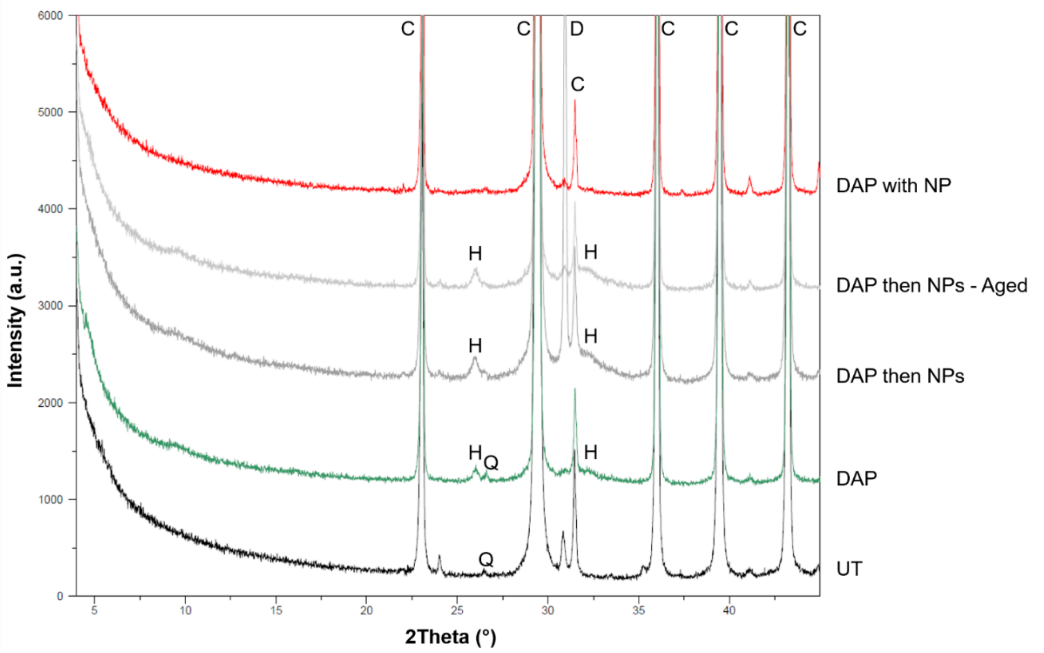

Figure 2: XRD graphs (C = Calcite, D = Dolomite, Q = Quartz, H = Carbonated Hydroxyapatite).

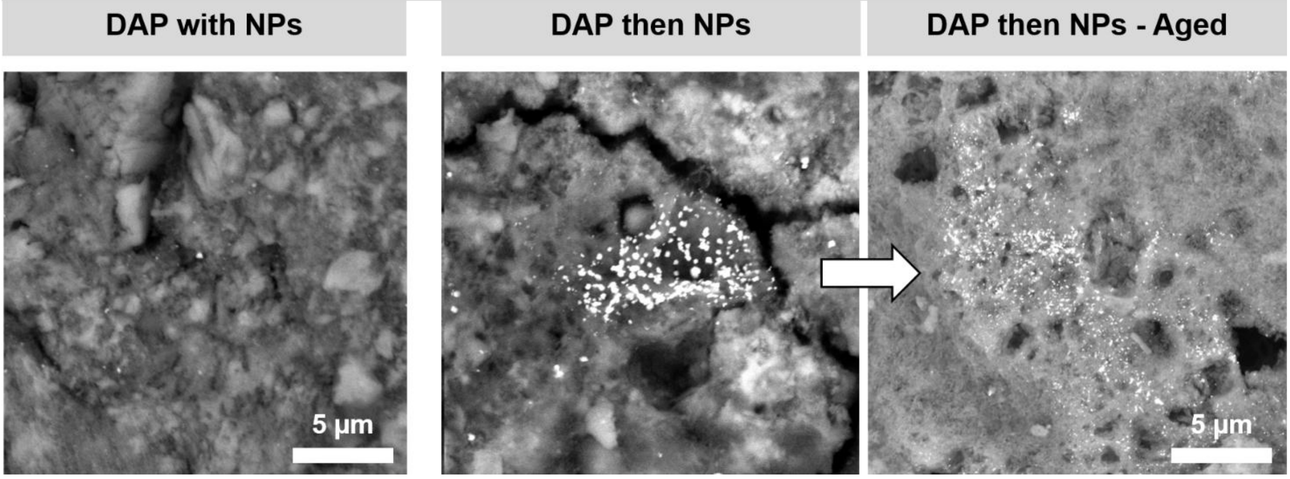

Figure 3: SEM images of the surface of marble samples functionalized by the two strategies.

Table 1: Colour change ΔE after treatment.*

	ΔL*	**Δa***	**Δb***	**ΔE***
DAP	-2.4	-0.2	-0.4	2.5
DAP with NPs	4.9	1.3	6.4	8.1
DAP then NPs	0.9	0.2	0.5	1.0
DAP then NPs - Aged	-11.0	2.4	2.4	11.6

Consistent with the number of NPs detected on the sample surface by SEM, the anti-fouling ability was found to be significantly higher for the “DAP then NPs” samples than for the “DAP with NPs” ones, as reported in Table 2. In particular, while in the latter sample there was basically no improvement compared to the non-functionalized “DAP” samples, the “DAP then NPs” condition exhibited strong antibacterial and anti-fouling effect against Escherichia coli (100% bacterial reduction) (Table 2). The “DAP then NPs” formulation was also active against Staphylococcus aureus although at a lower level (60% bacterial reduction considering the anti-fouling effect). This is in line with previous studies demonstrating different antibacterial efficacy of the same metal NPs depending on the bacterial strain under analysis (Ghezzi *et al.,* 2023).

Table 2: Bacterial reduction (%) compared to the DAP sample (control).

	Escherichia coli		***Staphylococcus aureus***	
	Non-adherent cells	Adherent cells	Non-adherent cells	Adherent cells
DAP with NPs	Negligible	Negligible	Negligible	Negligible
DAP then NPs	100%	100%	100%	60%
DAP then NPs - Aged	100%	100%	100%	60%

3.2 Effect of accelerated ageing

The analysis of the effect of accelerated ageing was limited to the “DAP then NPs” samples, as these showed suitable anti-fouling ability before ageing, while the “DAP with NPs” ones provided insufficient efficacy.

In terms of stability of CaP coating, XRD analysis confirmed that the coating was still present after accelerated ageing (Figure 2), with no visible difference compared to the condition before ageing. Similarly, also the presence of the metal NPs was confirmed after accelerated ageing, as pointed out by SEM observation (Figure 3). Consistent with the permanence of the metal NPs in the functionalized coating, the anti-fouling ability was persevered after accelerated ageing (Table 2). However, after accelerated ageing a marked colour change was registered (Table 1), mostly due to a decrease in the L* parameter, which indicates substantial darkening. Such a colour change was experienced also by samples that were not subjected to dripping of the simulated rain, but that were simply exposed to air. As a consequence, the darkening of the samples is thought to be due to the NPs undergoing some reaction that is currently being investigated.

4. Conclusions

The results of the present study, aimed at evaluating the effectiveness, compatibility and durability of anti-fouling coatings produced by combination of calcium phosphate (CaP) coatings formed in situ with antimicrobial metal nanoparticles (NPs), allow to derive the following conclusions:

- When the metal NP were suspended in the DAP solution (“DAP with NPs” condition), the NPs end up being embedded in the CaP coating, thus not being available to provide effective anti-fouling ability;

- When the metal NPs were applied onto the already-formed CaP coating ("DAP then NPs" condition), they were clearly visible by SEM on the sample surface and, consistently, they exerted effective anti-fouling action against both the model bacterial strains under analysis;
- When the "DAP then NPs" samples were subjected to accelerated ageing by dropping a simulated artificial rain, the anti-fouling ability was retained. However, the samples exhibited marked colour change with time, mostly due to darkening. Further research is in progress to ascertain the cause of the colour change and to prevent it or at least mitigate it.

Acknowledgements

This research received funding from the Italian Ministry for University and Research (MUR) through the project "SECURE COATS" (Safe, eco-friendly, and durable coatings to prevent deterioration of heritage stones, MUR code: "202288LY27"), funded by EU – Next Generation EU, Mission 4, Component 1, CUP: J53D23003410006.

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