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The use of ELISA for the identification of proteinaceous binding media from an eighteenth-century Damascene reception room

Julia Schultz*[1], Julie Arslanoglu[2] and Karin Petersen[1]
[1]University of Applied Sciences and Arts of Hildesheim (HAWK), Germany, [2]The Metropolitan Museum of Art, New York

Introduction

The identification of binding media in artworks can provide important information about the objects' composition and history as well as aid in preservation, treatment, display and storage. Natural binding media (e.g. proteins, gums, oils, waxes and resins) have been extensively used throughout history. Accurate protein identification continues to be challenging because traditional analytical approaches (spectroscopic and chromatographic techniques) can be limited by sample mixtures and natural degradation, as well as microbiological contamination.

Immunological or antibody-based techniques present an alternative approach to protein identification. In particular, the Enzyme-linked Immunosorbent Assay (ELISA) is able to distinguish among proteins (casein, ovalbumin, collagen: marker proteins for milk, avian eggs and animal tissue/bone) and can also verify the presence of gums. The major benefit of this technique is its acute specificity and high sensitivity that allows unambiguous identification and differentiation of closely related binding media, even in complex mixtures, on a relatively small sample.

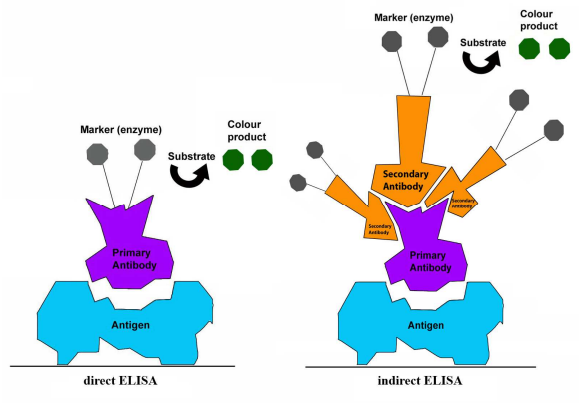
Indirect ELISA was applied to the examination of the binding media used in the polychrome surfaces of the so-called "Nur al-Din" Room, an early 18th century reception room from a house in Damascus in the collection of The Metropolitan Museum of Art, New York. The room's wooden panelling and ceilings are ornamented in the *'ajami* technique using gesso relief with painted, metal-gilded and glazed surfaces in various colours and patterns. FTIR analysis showed, among other components, protein and oil as binding media for most of the samples but no further specification of the proteins was possible. A more detailed description of the analytical study is the subject of several publications.[a b]

Nur al-Din Room: dated 119 A.H./1707 A.D.; Ottoman; Damascus, Syria; The Metropolitan Museum of Art. Gift of The Hagop Kevorkian Fund, 1970, 1970.170

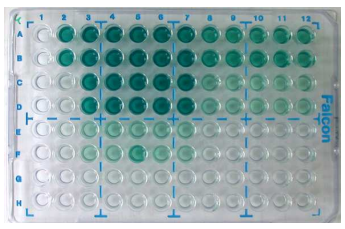
Method

The basis of immunological approaches is the specific reaction of antigens and antibodies. Antibodies are proteins created in mammals to bind foreign molecules such as viruses (or in this case, specific proteins or gums), called "antigens", for identification and neutralization by the immune system. By attaching a detection system (e.g. enzyme) to the antibodies, an antigen-antibody-complex can be visualized and measured by a spectrophotometer. The addition of a secondary antibody (indirect ELISA) increases the sensitivity of the assay. ELISA only gives information on the protein and gum content of the whole sample and does not give information about the location of the materials of interest in multi-layer samples.

In ELISA, in simple terms, an unknown amount of antigen is extracted from a sample; this antigen, immobilized on the surface (such as a plastic microwell plate), is then incubated with a solution of specific antibody, which can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substrate is added such that the enzyme converts to yield a detectable colorimetric signal. If the target protein or gum is present the wells turn coloured, otherwise they remain colourless. Indirect ELISA experiments reported here were carried out with a horseradish peroxidase (HRP) reporting system developed at the HAWK and further optimized at The Metropolitan Museum of Art.[c]



Immunological principle



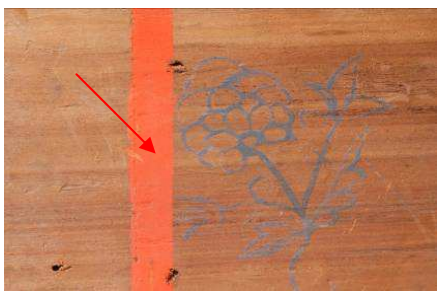
Coloured microwell plate

Results

Eleven paint samples were analyzed with indirect ELISA for ovalbumin, casein, and collagen and the presence of gums. Collagen was identified as the wood size and as the binding media of the ground and *'ajami* relief decoration. Egg was found consistently in the red, yellow, pink, green and blue paints. Neither casein nor gums were detected as paint media. These results indicate that different binders were selected for the wood and ground preparation in contrast to the different paint layers. And they also demonstrate the validity of indirect ELISA as a complement to spectroscopic, chromatographic, mass-spectrometric techniques for the unambiguous detection of different proteins and gums in mixtures.

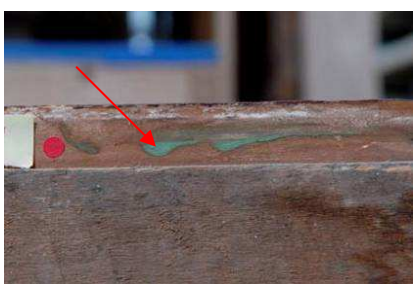
'ajami (#CA5_03)

blue paint (#CA5_01)

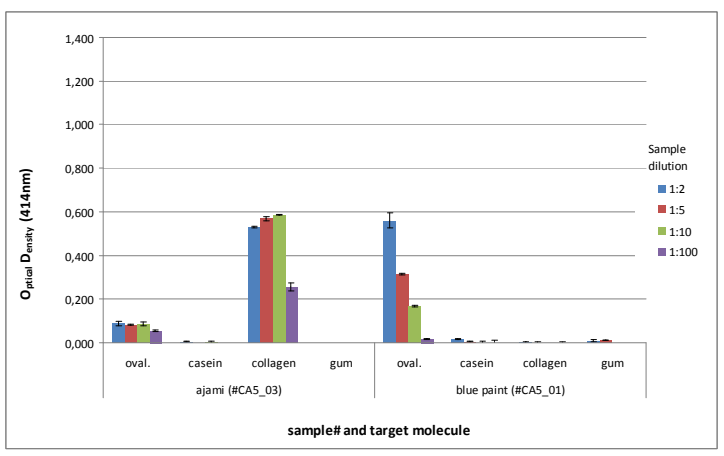


red paint (#NAr_04)

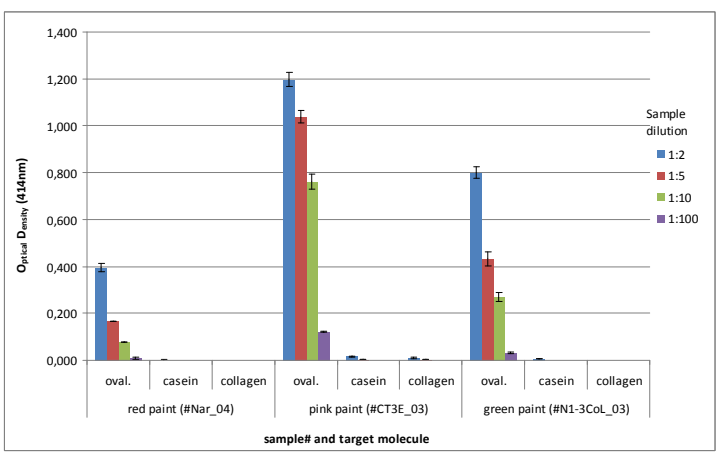
pink paint (#CT3E_03)



green paint (#N1-3CoL_03)



ELISA results for the identification of the binding media of the *'ajami* and blue paint sample. Both collagen and ovalbumin are clearly present in the *'ajami* sample, while only ovalbumin is present in the blue paint. This result suggests that the ovalbumin traces found in the *'ajami* are a result of migration/penetration from layers above. Thus, the binder for the *'ajami* is collagen and the binder for the blue paint is egg.



ELISA results of the binding media analysis show that the binder of the red, pink and green paint samples is egg-based. The identification of ovalbumin (the protein in egg white) in the paint samples can actually indicate that the binder was whole egg, yolk or white alone, due to the difficulty of fully separating white from yolk, and the sensitivity of the ELISA method. Thus, one limitation of this method (with the antibodies used) is that it does not distinguish between different formulations of egg in the binder.[b]

Summary of ELISA results

sample#	description	oval.	casein	collagen	gums
N7.1	blue (smalt) and white (lead white) paint layers	+++	-	-	-
E9a_01	wood size	-	-	++	-
CA5_03	*'ajami* (gypsum) layer	+ (?)	-	++	-
CA5_01	blue (smalt) paint layer, only	++	-	-	-
CA5_02	blue (smalt), pink and white (lead white and red lake) paint layers	++	-	+	-
S7-9CoU_01	blue (smalt) paint layer from a protected backside area	++	-	-	-
CT3S_01	hemp fiber/glue	++	-	+++	-
NAr_04	red (red lead) paint layer	++	-	-	n/a
CT3E_05	ground (gypsum) layer	-	-	++	n/a
CT3E_03	pink (organic red lake/lead white) paint layer	+++	-	-	n/a
N1-3CoL_03	green (verdigris/ lead white) paint layer	++	-	-	n/a
SAr_01	yellow (orpiment) paint layer	++	-	-	-

-	no detection
+ (?)	positive, weak signal, OD (414nm) <0.1, extremely questionable
+	positive detection, weak signal, OD (414nm) <0.3
++	positive detection, medium signal, OD (414nm) >0.3
+++	positive detection, strong signal, OD (414nm) >0.9
n/a	not tested

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References cited:
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*correspondence:
julia.schultz@hawk-hhg.de