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Raman spectroscopy and dynamic light scattering analysis of egg hen binder behaviors

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Abstract

In ancient Egyptian history and the Coptic period, artists used various organic and natural materials to produce specific effects in artifacts as a coating for their paintings. Organic materials play an essential role in the degradation of the archaeological organism, while one of the significant dilemmas is identifying the media themselves. Most of these identification methods are destructive. We used a non-destructive analysis technique, namely infrared Raman spectroscopy, to prevent this drawback. The natural polymeric materials represented by hen egg yolk and white were studied as the widely used material starting from the eighteenth dynasty and in the Coptic period in Egypt regarding the interaction between degraded polymers of hen egg yolk and white and elucidate the mechanisms associated with thermally aging egg white and yolk samples. Raman spectroscopy gave valuable results that well illustrated the behavior of aged samples and the interactions between the media used, in addition to dynamic light scattering analysis, which was used to determine the size of proteins, nucleic acids and compounds, to monitor ligand binding, to understand the size of media particles and to draw the carbon footprint of yolks and whites of hen eggs in the steady state and after obsolescence, as these results helped us to preserve, treat and protect precious artifacts..

Keywords: Artificial-aging, Binders, Egg, Proteins, Hen egg, Raman Spectroscopy, Dynamic light scattering, Artificial intelligence, egg yok, egg white.

1. Introduction

Throughout Egyptian culture and art history, painters tried to use different organic and natural materials as paint binders. Objects created by Coptic artisans frequently have a complex combination of materials. The end product is a complicated, varied, and multi-structured object. Organic chemicals aid the degradation of the object, but to mitigate the challenge we would show media [i]. One of the most complicated media used in ancient Egypt and during the history timeline of Egypt is the hen egg in it [ii],[iii],[iv],[v] and Coptic period [vi],[vii]

Many researchers in the field of archaeology and related sciences used the Raman spectrometer analysis and non-destructive techniques, which included the field of natural dye analysis[viii,ix,x], Amber beads[xi], assessing bioapatite preservation in archaeological bone[xii], Historical primers and paints used for aeronautical protection and coloring during World War II (WWII)[xiii], traces of lost pigments[xiv], bricks[xv], photos[xvi], fibers,[xvii,xviii], biomaterials[xix] and food protein [xx], [xxi].

The majority of the viable methods are damaging in this circumstance. As a result, in order to avoid this, we attempt to employ a micro-destruction technique known as Raman spectroscopy. Inorganic materials typically utilize the later. As a result, it would be fascinating to put this technique to the test in an organic medium investigation[xxii].

Thus, this paper suggests Raman spectroscopy on hen egg yolks and whites. The following questions will evaluate the advantage:

1. The applicability of this method to prevent the interference between egg yolk and egg white in hen eggs, which was used in ancient Egypt as a medium
2. The comprehension of various chemically complicated and deteriorating materials
3. The elucidation of aging mechanisms

The egg yolk's original tertiary structure is preserved throughout pigment production. Protein denatures during evaporation, although amino acid components remain unchanged. Egg-white, which is usually beaten or sieved before being employed as a binding medium, receive some denaturation due to the breaking and stretching of globular proteins (including ovalbumin) that cause the egg to froth. Whipping denatures protein by decreasing lysozyme activity (Fig1). The disordered strands of globular protein components are stretched and homogenized with the other watery sections of the white, creating a less viscous and more homogeneous painting medium. Egg proteins, excluding lysozyme, retain their amino acid makeup and structure[xxiii]. Evaporating solvents from the solution, drying the egg, and solidifying the molten substance are the

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primary steps in the egg protein film-forming process (F.F.M)[xxiv] .

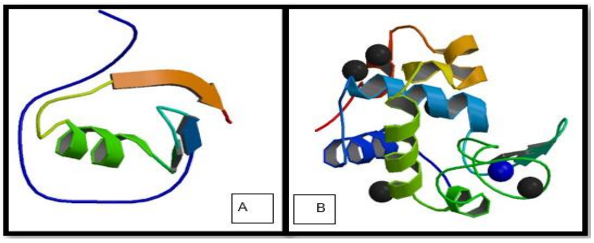


Fig. 1 (A) Ovomucoid, a small protein found in egg white and egg yolk has one alpha-helix and 3 beta sheets. (B) Lysozyme from hen egg white has seven small alpha helices and two beta sheets –here shown bound to sodium and chloride ions (Berman et al., 2000).[xxv]

Protein, nucleic acid, complex sizes, and ligand binding can all be determined with Dynamic light scattering analyses (DLS), which are widely employed in biology labs[xxvi].
Any protein's life cycle in a cell starts with synthesis and continues with co- or post-translational folding[xxvii] and modification, localization to the proper compartment, activity, and, in the end, destruction.[xxviii]
This study aimed to use Raman spectroscopy as a non-destructive procedure to examine the interaction and intervention between the egg white and yolk in samples meant to simulate the effects of aging. Dynamic light scattering was also employed to measure the size of proteins, nucleic acids, and complexes, as well as to track ligand binding and comprehend the particle size of the Media.

2. Material and Methods

2.1. Samples

Pure egg white and egg yolk, frequently used in paint media or varnish, were used to make the samples. Egg white contains the photosensitizer riboflavin, which can efficiently stimulate the oxidation of amino acids, while egg yolk has the same proteinaceous components (mostly ovalbumin and lysozyme). Vitamin A and fatty acid esters are both abundant in egg yolk.

The binding agent in this investigation was concocted using historical formulas found in treatises and artists' diaries. Free-range, corn-fed goose eggs were purchased from the market.[xxix,xxx, xxxi]

To get the most accurate representation of artist supplies in this work, however, corn-fed hen’s eggs were used. A solution of 50% transparent egg white in Nano-pure water was made by beating an egg white until it formed stiff peaks and then let it sit for 24 hours. The foam was then scraped off. The egg's yolk (EY) was removed by puncturing it and allowing the liquid to drain through the film around it; then, the yolk was diluted with nano-pure water to create a 50% emulsion, which could be painted on the surface of the slide substrate with a tiny brush without causing the binder to separate, resulting in a single, utterly opaque layer.

2.3. Artificial Aging and Binding Media

The long-term stability of painting materials is a significant issue for preservationists. As explained above, the processes that occur with age may directly affect the stability and analysis of proteins[xxxii,xxxiii,xxxiv,xxxv] due to their close relationship with the chemical composition of the binding medium.
Protein (amino acid) analysis is significantly affected by aging reactions because producing new products and substantial changes to the molecular structure can make proper identification difficult. The potential for thermally induced alterations in proteinaceous binding medium films has been evaluated using Raman spectroscopy (Figs. 3, 4, and 6)[xxxvi]. A discussion of the artificial aging strategy used in this study follows a brief overview of the techniques often used in laboratories[xxxvii,xxxviii, xxxix] .

Thermal aging
Since the heat significantly impacts the creep, fatigue, and alterations of painted wood, this study aimed to recreate the environmental conditions in Egypt to understand better how they affect media longevity. Therefore, thermal aging must be used to fully comprehend the media's behavior before any mural can be painted. **(Fig.2**)

Fig. 2 Egg protein films (A) Egg yolk (B) Egg white (C) Putting the slides in boxes inside the oven for the thermal aging cycle.

Following is an illustration of the sinusoidal temperature variation used in the employed temperature cycles [xl]:
Cycle (1) at 78°C for 24 hours.
Cycle (2) at 23° C for 24 hours.
Cycle (3) at 78°C for 24 hours.
Cycle (4) 23°C for 120 hours.
~~Cycle (5) at 23° C for 24 hours.~~
Cycle (5) at 78°C for 48 hours.
Cycle (6) at 120° C for 96 hours

2.5 Raman Spectroscopy

The dispersive Raman microscope was used to analyze films made from white egg and egg yolk. During analysis, a diode 785 nm laser with 100 Mw of power. Using a 20 d and 50 d objective, the laser was trained on the material, and a region with a diameter of about 6 m was examined at a spectral resolution of 4 cm^{-1}. The first spectrum was obtained from 3500 cm^{-1} to 250 cm^{-1}, while the second spectrum was recorded from 3200 cm^{-1} to 2700 cm^{-1}. Spectra were cleaned of cosmic noise and spikes, but no adjustments were made.

2.6 Mode of Raman

RAMAN AT 785 nm
Each sample was evaluated using a Sentera Bruker FT - Raman spectrometer containing a diode 785 nm[xli] laser with a 6 milli watt (mW) incident laser power. With a 50x

objective, we could concentrate the laser on the material and conduct micro-Raman analysis over an area around 6 m in diameter, with a spectral resolution of 4 cm^{-1}. Since Si, like the tested samples, is transparent to 785 nm light, calibration experiments using this material suggest that the laser's optical penetration depth at this wavelength is no more than 5 m at most. Two distinct spectral bands were utilized during the recording process. First, between 3500 cm^{-1} and 250 cm^{-1} and then between 3200 cm^{-1} and 2700 cm^{-1}. Spectra were manually corrected for detector efficiency and had cosmic noise and spikes removed by hand, but no further adjustments (such as baseline modification by hand) were made.

Raman spectra with a non-zero baseline were likely due to luminescence or scattering caused by the numerous contaminants in the crude egg-based films. As a result of the degradation of luminous chemicals with age, artificially aged samples produce shaper bands with a less noticeable baseline (Table.2) compared to the natural films of **Egg yolk** and **Egg white** (Table.1).

Table 1. Band assignment of Raman spectra obtained from thin films of egg-white and egg-yolk, Bands that have been identified (Phenylalanine, Tyrosine, Tryptophan).

Egg white	Tentative assignment	Ref.	Egg yolk	Tentative assignment	Ref.
2728	V(CH) Aliphatic	Austin et al. 2008; Medhat 2016	2920	ν (CH)	Austin et al. 2008; Medhat 2016
1663	V(C=O)(N) Amide I	Austin et al. 2008; Medhat 2016	2852	ν (CH)	Austin et al. 2008; Medhat 2016
1605	V(CC) Phe / Tyr	Austin et al. 2008; Medhat 2016	2725	ν (CH) Aliphatic	Austin et al. 2008; Medhat 2016
1555	Amide II/Trp	Austin et al. 2008; Medhat 2016	1713	ν (CO)	Austin et al. 2008; Medhat 2016
1455	δ (CH2)	Austin et al. 2008; Medhat 2016	1655	ν (C=O, CN) Amide I	Austin et al. 2008; Medhat 2016
1423	V(CO)	Austin et al. 2008; Medhat 2016	1557	Amide II/ Trp	Austin et al. 2008; Medhat 2016
1390	V(CO)	Austin et al. 2008; Medhat 2016	1439	δ (CH2)	Austin et al. 2008; Medhat 2016
			1263	Amide III	Austin et al. 2008; Medhat 2016
1339	V(CH2)	Austin et al. 2008; Medhat 2016	1121	νas (CH3)/ rocking	Austin et al. 2008; Medhat 2016
1234	Amide II	Austin et al. 2008; Medhat 2016	1008	C-C deformation, Phe	Austin et al. 2008; Medhat 2016
1123	Vas[CH3]/Rocking	Austin et al. 2008; Medhat 2016	876	ν (NH), Trp	Austin et al. 2008; Medhat 2016
1031	ν(CC) aromatic	Austin et al. 2008; Medhat 2016	778	ν (NH), Trp	Austin et al. 2008; Medhat 2016
1002	C-C deformation ,Phe	Austin et al. 2008; Medhat 2016	717	V(CS), Cysteine /Trans and gauche	Austin et al. 2008; Medhat 2016
953	V(CC)Phosphate	Austin et al. 2008; Medhat 2016			

940	V(CC)Ploine ,Valine	Austin et al. 2008; Medhat 2016			
927	V(CC)	Austin et al. 2008; Medhat 2016			
852	Ring breathing,Tyr	Austin et al. 2008; Medhat 2016			
829	Ring deformation, Phe Tyr	Austin et al. 2008; Medhat 2016			
757	V(NH), Trp	Austin et al. 2008; Medhat 2016			
670	V(CS) Cysteine /*Trans and gauche*	Austin et al. 2008; Medhat 2016			

Table 2 Band assignment of Raman spectra obtained from thin films of the dark and artificially aged films of EW and EY samples; bands that have not been identified are not assigned but arelisted for completeness

EW (Raman shift, cm^{-1})		Assignment	Ref.	EY ((Raman shift, cm^{-1})		Assignment	Ref.
Natural	Artificiel aged						
2728	2934	V(CH) Aliphatic	Austin et al. 2008; Medhat 2016	2920		ν (CH)	Austin et al. 2008; Medhat 2016
1663	1667	V(C=O)(N) peptide / Amide I	Austin et al. 2008; Medhat 2016	2852	2854	V(CH) Aliphatic	Austin et al. 2008; Medhat 2016
1605	1606	V(CC) Phe / Tyr	Austin et al. 2008; Medhat 2016	2725		ν (CH) Aliphatic	Austin et al. 2008; Medhat 2016
1555	1563	Amide II/Trp	Austin et al. 2008; Medhat 2016	1713	1753	C=O stretching associated with fatty acid esters	Austin et al. 2008; Medhat 2016
1455	1452	δ (CH2)	Austin et al. 2008; Medhat 2016	1655	1652	V(C=O)(N) peptide / Amide I	Austin et al. 2008; Medhat 2016
1423	1408	V(CO)	Austin et al. 2008; Medhat 2016	1557	1561	Amide II/Trp	Austin et al. 2008; Medhat 2016
1390	No BAND	V(CO)	Austin et al. 2008; Medhat 2016	1439	1443	δ (CH2)	Austin et al. 2008; Medhat 2016
				1263	1242	Amide III	Austin et al. 2008; Medhat 2016
1339	1317	V(CH2)	Austin et al. 2008; Medhat 2016	1121	1121	Vas[CH3]/Rocking	Austin et al. 2008; Medhat 2016
1234	1244	Amide II	Austin et al. 2008; Medhat	1008	1005	V(C-C) deformation, Valine	Austin et al. 2008; Medhat

			2016				2016
1123	1161	Vas[CH3]/Rocking	Austin et al. 2008; Medhat 2016	876	873	V(NH),Trp	Austin et al. 2008; Medhat 2016
1031	1029	v(CC) aromatic	Austin et al. 2008; Medhat 2016	778	767	V(NH),Trp	Austin et al. 2008; Medhat 2016
1002	1004	V(C-C) deformation ,Valine	Austin et al. 2008; Medhat 2016	717	718	V(CS) Cysteine /*Trans and gauche*	Austin et al. 2008; Medhat 2016
953	971	V(CC)Phosphate	Austin et al. 2008; Medhat 2016				
940	934	V(CC)Ploine ,Valine	Austin et al. 2008; Medhat 2016				
927	No band	V(CC)	Austin et al. 2008; Medhat 2016				
852	849	Ring breathing, Tyr	Austin et al. 2008; Medhat 2016				
829	828	Ring deformation, Phe, Tyr	Austin et al. 2008; Medhat 2016				
757	no band	V(NH), Trp	Austin et al. 2008; Medhat 2016				
670	698	V(CS) Cysteine /*Trans and gauche*	Austin et al. 2008; Medhat 2016				

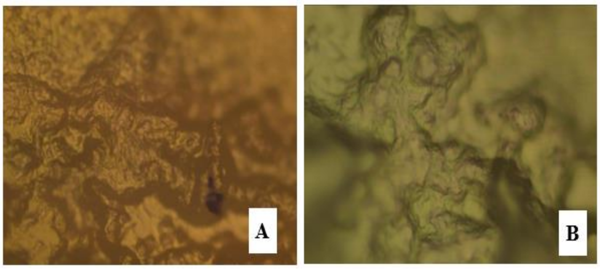
2.7 Dynamic Light Scattering (DLS)

Nano Metrix visualized particle size by measuring particle and molecular size in samples of interest. Particle size and size distribution can be calculated from measured Brownian motion diffusion using the Stokes-Einstein relationship. For achieving accurate measurements of particle size by using DLS method, ISO recommendation has to be followed (ISO 22412:2017). The Egyptian Petroleum Research Institute (EPRI) Quanta Chrome Nova 3200 analyzer uses Non-Invasive Back Scatter (NIBS) technology to provide the best sensitivity throughout the broadest size and concentration range possible.[xlii]

3. Results and Discussion

3.1 Raman spectroscopy

The Raman spectra of Egg white (EW) samples (Fig. 4& 5) suggest that artificial aging has little impact on the material, as the ranges of both Egg Yolk (EY) films (Figs. 3&6) exhibit the same bands at the same intensities and positions. Films of artificially aged EW show no evidence of the band 757 cm^{-1} assigned to the N-H stretching tryptophan (Trp)vibrational mode (2). Raman spectral changes (Figs. 3 and 6) reveal a clearer picture of how EY is affected by temperature exposure. After exposure to heat, the Amide I and Amide III bands undergo distinct wave-number shifts (and intensity changes) of 7 cm^{-1} and 21 cm^{-1}, respectively. The Raman spectra of aged EY also reveal a shift in the wave number of the CH_3 by 28 cm^{-1} in the asymmetric stretching/rocking vibrational mode. Notably, the distinctive carbonyl vibration moves from 1713 cm^{-1} to 1741 cm^{-1}, and the bandwidth increases with age in naturally aged EY (1713 cm^{-1} is attributed to the C=O stretching associated with fatty acid esters). The formation of mixed oxidation products, including hydroxides, epoxides, hydroperoxides, and epi dioxides, linked with vibrations in the area from 1605-1660 cm^{-1} is likely responsible for this shift and broadening of the band.



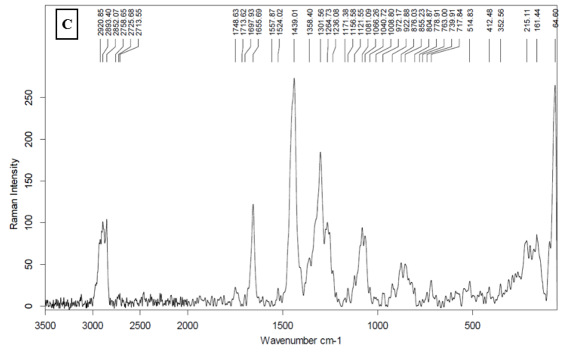
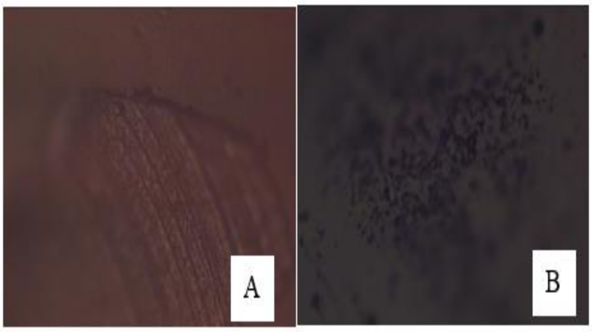


Fig .3 Photomicrographs of the surfaces of egg-yolk recorded with Raman microscope with magnification(A) x20-(B)x50 and(C) Raman spectra of egg-yolk from 3500 to 500 which shows all the functional groups in egg-yolk. No normalization or baseline subtraction has been performed on the spectra.



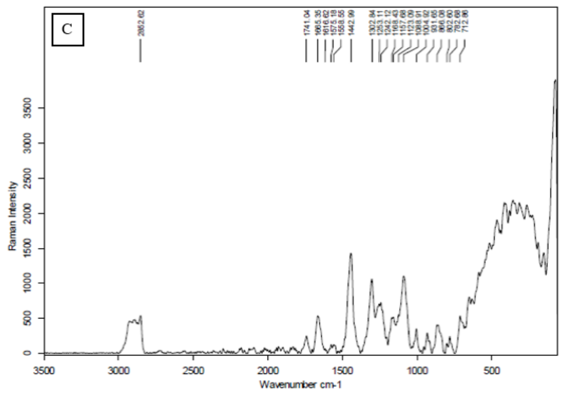
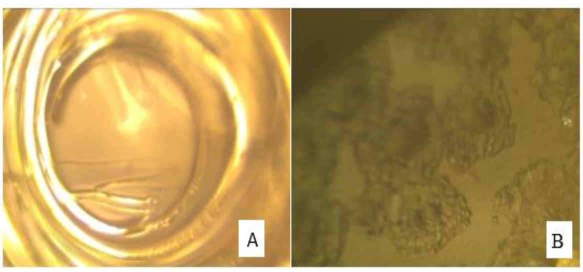


Fig.4 Photomicrographs of the surfaces of egg-yolk after the artificial aging process recorded with Raman microscope with magnification(A) x20-(B) x50 and (C) Raman Spectra of egg-yolk from 3500 to 500 which shows all the functional groups. No normalization or baseline subtraction has been performed on the spectra



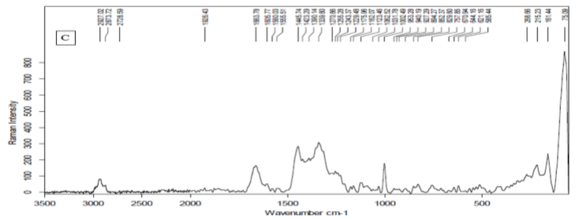
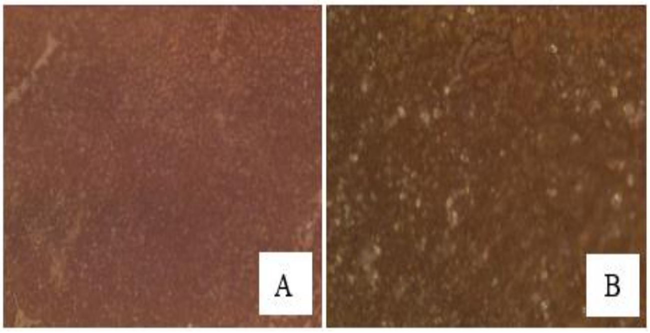


Fig. 5 Photomicrographs of the surfaces of egg-white recorded with Raman microscope with magnification (A) x20- (B)x50 and (C)Raman spectra of egg-white from 3500 to 500 which shows all the functional groups in egg-white. No normalization or baseline subtraction has been performed on the spectra.



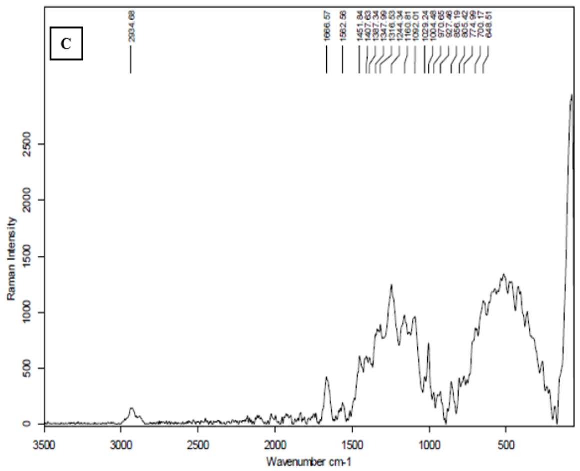


Fig.6 photomicrographs of the surfaces of egg-white after artificial aging process recorded with Raman microscope with magnification (A) x20-(B) x50 and (C) Raman Spectra of egg white from 3500 to 500 which shows all the functional groups. No normalization or baseline subtraction has been performed on the spectra.

Studies on analogous systems suggest that exposure to light and heat oxidizes double bonds in fatty acid esters, creating various oxygenated triacylglycerides, which may account for the observed 30 cm^{-1} shift and broadening. The variations in Amide I and Amide III are not attributable to conformational change within the proteins detected in the films, and all the bands, as mentioned earlier, likely reflect the changes in fatty acid components of the film. As a result, the C=O band's presence is crucial for the rapid differentiation of egg white EW from egg yolk EY, and the band's position and form are indicators of chemical changes in the material due to exposure to light and heat. In EY, age does not affect the assigned vibrational bands for Trp. It is probable that the unsaturated fatty acid esters primarily quench free radicals or singlet oxygen, hence restricting the photooxidation of Trp.[xliii]

3.2. Particle size analyzer

Dynamic light scattering (DLS) is a standard technique used to measure the size distribution of nanoparticles in suspension. It determines the size of the particles from fluctuations of the scattered light resulting from the Brownian motion of the particles. The Z average values of samples were reported as determined to be 207 nm and 358 nm. Figure 7(A-B) represents particle size distribution (PDS) that contains three parameters such as size (r.nm), Mean Number (%), and Std Dev Number %. The data in Figure 7 displays the DLS spectra of materials to illustrate their textural characteristics. Due to the buildup of egg yolk and white components, the average pore size increased from 207 to 358.[xliv]

The zeta potential values have a significant impact on the behavior of nanoparticles and their ability to reduce the dispersion rate of stable and durable very small particles in aqueous solutions.

As shown in **Fig. 7D,** the zeta potential of sample is -29 mV. According to the result, it can be speculated that egg yolk and egg white in aqueous solution, owing to its negativity of its surface .

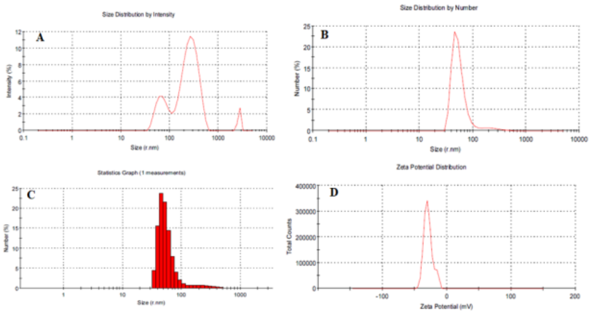


Fig 7 **Dynamic Light Scattering curve and Zeta Potential curve painting materials**

3.3 Prospective future work

Artificial intelligence uses artificial neural networks to examine ancient Egypt's historical use of natural ingredients as paint binders. Thanks to its low overhead, adaptability, generalizability, and user-friendliness, AI has been put to good use in the study of ancient Egyptian history. Throughout history, RNNs, CNNs, DTs, FFBPNNs, and ANFISs (Adaptive Network Based Fuzzy Inference Systems) have been among the most popular forms of artificial intelligence.[xlv]

4. Conclusions

Using Raman spectroscopy, we could differentiate between different chemical changes in the hen tempera and quickly determine their significance temperature-treated egg. The most notable modification is a broadening of the peak and a decrease in the wavenumber of the C-O vibrational band. Changes in the wavenumbers of the Amide I Raman band and the C-C stretching vibrational modes after heating the egg yolk are additional indicators of chemical changes in the material. These shifts have not been attributed to any particular molecular changes but may be related to chain fragmentation due to oxidation[xlvi]. Research is needed to determine how time and pigments have affected the Raman spectra of other regularly used binding mediums, such as Tempera and Fresco. The particles range in size from 207 nm to 358 nm.

The results of this investigation corroborate those of literature which identify egg yolk as the medium of an 18th Century icon.

Credit authorship contribution statement

Abd El Rahman Medhat: Conceptualization; Methodology; writing. E.G. Zaki , Khaled Elnagar and Mona Fouad : analysis; writing

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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