Egypt. J. Chem. **Vol. 66**, No. 2 pp. 357 - 366 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Evaluation of the efficiency of sodium alginate for the consolidation of archeological bones

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Abstract

The efficiency of sodium alginate aqueous solutions in different concentrations for the consolidation of bone samples were evaluated in the present work. The bone surface was investigated using a digital light microscope, scanning electron microscope(SEM), and Attenuated total reflection – Fourier transform infrared spectroscopy (ATR-FTIR) in addition to change of color measurement. The results indicated that the total color difference (ΔE) increased by increasing Sodium alginate concentration and was acceptable till 2% polymer concentration. Morphological investigation of the treated samples proved the effect of the aging process on the untreated samples and also the improvement in surface morphology after treatment by sodium alginate at 1% and 2%. ATR/FTIR analysis stated that consolidation materials used led to the chemical stability of the bones in compared with the aged untreated sample.

Keywords: Archeological bones - Ageing - Sodium alginate – Consolidation, analytical techniques.

1. Introduction

Bone consists mainly of organic and inorganic components. The organic component is a complex collagen protein, which usually contains high proportions of the amino acids glycine and hydroxyproline. It is a fundamental property of collagen that it has few large side-chains on the amino acid group. The inorganic component is the mineral element, which is mainly hydroxyapatite[$Ca_{10}(PO_4)_6.(OH)_2$] [1-6].

Archaeological bone artifacts in the burial environment are in direct contact with the soil. The external environment factors in the burial environment such as moisture, temperature, pH of the soil, the interaction with microorganisms, together with the intrinsic physico-chemical properties of bones, such as porosity and crystallinity, can cause deterioration of both collagen and hydroxyapatite [7-9]. It can be added that the improper conditions in the museums and storage (fluctuation in relative humidity and temperature, excessive light, air pollutants, etc.) play also an important role in the deterioration of archaeological bone artifacts [10-17].

Accordingly, archaeological bone artifacts suffer from deterioration, and fragility and weakness are the most common aspects of deterioration. Archaeological bone artifacts need consolidation process, which is considered one of the most crucial treatments in the conservation field. The requirements for the selection of the consolidation material are that it should provide stability, limit water ingress, cohesion and strength, and be easy to use, non-toxic and stable over time. Consolidants used in conservation should also remain unaltered in the presence of soluable salts, which are often found in burial sites, and resistance to the deterioration factors in surrounding environmental conditions [18-20].

Sodium alginate is a linear unbranched, amorphous copolymer usually extracted from various seaweeds and composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G) linked by 1→4 glycosidic bonds [21]. Sodium alginate consolidation had little effect on color change, acidity, and the majority of tensile properties post-aging. Sodium alginate was generally stable to extended periods of light exposure. The application of sodium alginate may reduce the extent of such color change over time when on display [22].

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Receive Date: 23 April 2022, Revise Date: 13 May 2022, Accept Date: 23 May 2022

DOI: 10.21608/EJCHEM.2022.135528.5969

Consolidation with sodium alginate had very little effect on artifact tensile properties pre- and post-accelerated light aging [23].

It should be noted that Sodium alginate was used to consolidate some archaeological materials such as woods, textiles, and leathers. Kronthal et al. [24] used a mixture of sodium alginate and a wheat starch paste with a paper backing material for leather repair. Smith et al. [25] tested the efficiency of sodium alginate for the consolidation of dyed textiles. They found that sodium alginate had no adverse effect on the studied samples and in some cases provided benefits. Walsh-Korb et al. [26] have used sodium alginate to consolidate wood artifacts. They proved that it improved the thermal stability of the lignin component of archaeological wood.

Sodium alginate has become an extremely important family of polysaccharides with a wide range of uses and applications in a variety of fields such as film-forming ability, pH responsiveness, gelling, hydro philicity, biocompatibility, biodegradability, non-toxic, process ability, and ionic crosslinking. It can also be said that its use in the consolidation of archaeological bones is almost not studied yet.

This study aims to evaluate the efficiency of Sodium alginate in the consolidation of archaeological bone artifacts.

2. Materials and methods

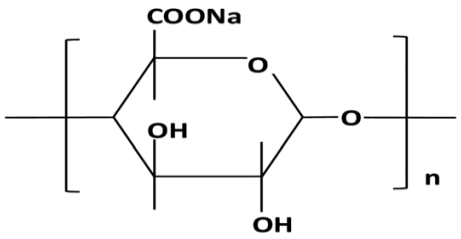
2.1. Materials

2.1.1. Preparation of new bone samples

New bone samples were prepared according to some authors [6, 27]. Bone specimens were prepared from long bones the scapula of sheep. Bone specimens were boiled in water cautiously cleaned from fat or impurities by using scalpels without either scratching the surface or affecting the surface morphology. The marrow materials were removed by using dental tools. Prepared bone samples were then left for one week to dry at room temperature.

2.1.2. Sodium Alginate (SA)

Sodium Alginate (Mw = 100,000 g/mol) was donated by FMC Chemical International AG (Dublin, Ireland) and used as received.



Sodium alginate

Sodium alginate solutions were prepared by dissolving different amounts of sodium alginate powder in water and introducing the solution to an ultrasonic sonifier (350 watts) for 15 min.

2.2. Methods

2.2.1. Application technique for Sodium Alginate

The impregnation technique was used for the application of Sodium Alginate on aged bone samples. The treated bone samples were left to dry naturally at room temperature. The concentrations used with all polymers were: 1%, 2%, and 3%

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2.2.2. Artificial accelerated heat-moist aging used

Artificial accelerated heat-moist aging at 80 ^{o}C and 65% relative humidity for untreated samples according to some authors [28, 29], and at 80 ^{o}C for the treated samples were used for one week. The oven used was a 350 FX-Shel Lab. - Shelldon Manufacturing Inc.

2.3. Investigation techniques

2.3.1. Digital light microscope

The portable USB digital microscope (model PZ01-Shenzhen Super Eyes Co. Ltd., China) was used to examine the surface of the studied samples.

2.3.2. Scanning Electron Microscope (SEM)

A scanning electron microscope (JEOL, JSM 5400 LV EDX link ISIS-Oxford, high vacuum) at the SEM unit, Assiut University, Egypt was used to observe the surface morphology of the samples studied.

2.3.3. Change of color

The effect of heat aging on the studied samples was measured on the chromatic scale CIE 1976 (L, a, b), also called CIELAB, commonly used to compare two samples. The total color difference is found according to the following equation [30-32]:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

2.3.4. Attenuated total reflection – Fourier transform infrared spectroscopy (ATR-FTIR)

The molecular structure of the studied samples was elucidated in the range 4000 to 400cm-1 by attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), Bruker Vertex 70 FTIR spectrometer at the Molecular Modelling and Spectroscopy Unit, National Research Centre, Egypt.

3. Results and discussion

3.1. Investigation of bone surface samples by digital microscope

The digital examination is one of the most important examinations in examining the superficial appearance of archaeological bones. It also gives a simple idea about the change in the bone surface and

the effect of aging on untreated samples, as well as the resistance of treated samples with consolidation materials to heat-moist aging.

- **Investigation of the new and aged untreated bone samples**

The results (Fig. 1A) for the control sample (the new sample before aging) showed that the surface appeared smooth, homogeneous in color, and had Haversian channels (a series of microscopic tubes that are a series of microtubules in the outer region of the bone called the cortical bone. They allow blood vessels and nerves to by passing through it to supply osteoclasts) on all surfaces and they are not distributed in an orderly manner, which distinguishes the bone surface. It can also be said that the surface was flat, which indicates the good preparation of the sample.

The results of the aged untreated sample (Fig. 1B) showed that heat-moist aging affected the surface of the sample, which tended to the noticeable darkening especially around the Haversian canals. The chromatic heterogeneity of each sample was also observed.

- **Investigation of bone samples treated with Sodium Alginate**

The results obtained for the bone sample treated with 1% sodium alginate before aging (Fig. 1C) showed the good distribution of the consolidant on the surface, the surface was smooth, the color homogeneity of the sample, the Haversian channels appeared, and the use of the consolidant at this concentration did not prevent it from its appearing. The aged treated sample (Fig. 1D) showed that the sample was affected by aging, since the color became darker than before aging, and the color heterogeneity appeared. The results showed that aging affected the consolidant in some places on the surface. The results also showed the weakness of the consolidant on the surface compared to other places in the same sample.

For the sample treated with a concentration of 2% before aging (Fig. 1E), the distribution of the hardener appeared to be good on the surface, no air bubbles were seen and the surface seemed homogeneous in level and color, but the sample seemed to tend to a very light dark color compared to the sample treated with a concentration of 1% of the same sample. The results (Fig. 1F) showed that the sample treated after aging was homogeneous in color except for the presence of a very light gray color in the places where the Haversian channels are present, and this may due to the effect of aging on the sample.

The results showed that the sample treated with 3% before aging (Fig. 1G) had a semi-homogeneous color, with a good distribution of the polymer on the surface, and the appearance of Haversian channels, which indicates a good distribution of the polymer. It should also be noted here that very slight chromaticity was observed on the surface of the sample compared to the sample treated with a concentration of 1% before aging. For the sample treated after aging (Fig. 1H), the effect of aging on the sample was clearly visible, which appeared in the form of parallel black lines on all the surfaces of the sample, and this indicates the dark color of the consolidant material on the surface.

3.2. Change of color

The measurement of color change is considered one of the most important parameters used for the evaluation of conservation materials, especially for consolidation materials. It is a vital requirement for the selection of polymers, which should not change the color of the treated sample before or after aging.

The following is an explanation of the results obtained from measurements of color change:

Lightness (*L)

Regarding the results obtained for the aged untreated sample, the loss in *L value was 14% compared to the control sample (untreated sample) (Table 1). Abdel-Maksoud [27), Abdel-Maksoud and Khattab [32] reported that the change of color increased with increasing temperature and ageing time.

The results showed that the treated samples before aging with sodium alginate with different concentrations (1%, 2%, 3%) had a decrease in lightness by 0.73%, 1.04%, and 2.47%, respectively. It can be said that the higher the concentration of sodium alginate led to the decrease in lightness. The samples treated after aging with the three concentrations showed that the treated samples were affected by the aging used, and the loss in the lightness with three concentrations were 1.80%, 4.23, and 3.44% respectively.

Red-green value (*a)

The results obtained (Table 1) showed that the a* values of the aged untreated sample increased (68.13%) compared to the control sample.

For samples treated with sodium alginate (see Table 1) at different concentrations (1%, 2%, 3%) before aging, the red color decreased with the increase in the concentration of the polymer compared to the aged untreated sample, but it was higher than the control sample. The loss in a* value reached 83.75%, 73.13%, and 62.50% with the three concentrations respectively. The loss in the red color increased more with the aged treated samples, and their loss reached 53.13%, 37.50%, and 12.50 with the three concentrations used respectively.

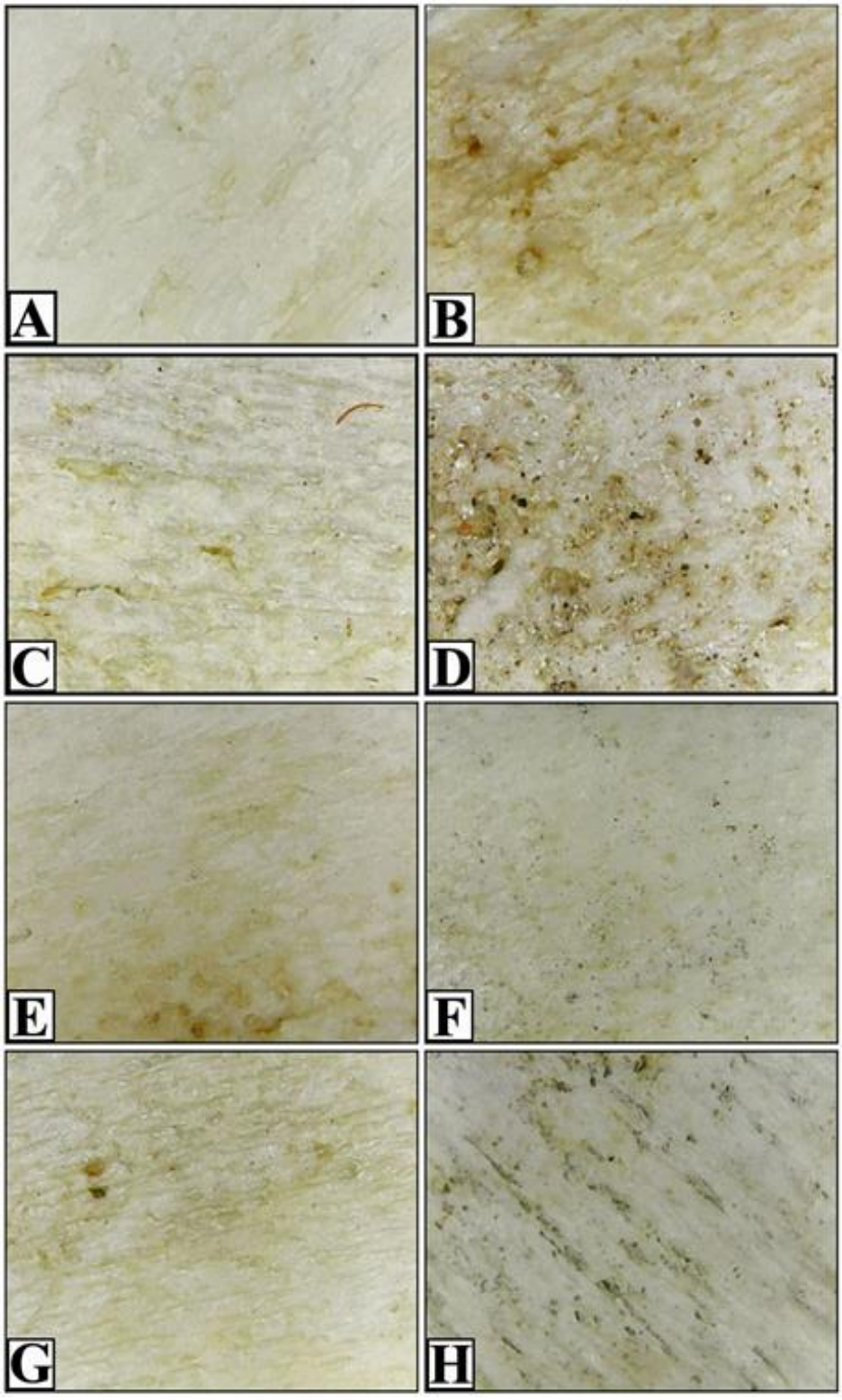

Fig. 1 Digital microscope investigation of bone samples treated with sodium alginate at different concentrations before and after accelerated aging: (A) Control, (B) Aged untreated sample, (C) Treated sample 1%, (D) Aged treated sample 1%, (E) Treated sample 2%, (F) Aged treated sample 2%, (G) Treated sample 3%, (H) Aged treated sample 3%.

Yellow – Blue value (b*)

The obtained results (Table 1) showed that each value of b* was in yellow. The results showed that the aged untreated sample had an increase in yellow color (29.86%) compared to the control sample.

The results showed that samples treated with sodium alginate (Table 1) at the concentrations used before aging had a lower yellow color value compared to the aged untreated sample. The loss was 35.78%, 27.59%, and 4.66% with the concentrations used respectively. The loss in the yellow color of the aged treated sample decreased as the concentration of sodium alginate increased. The aged treated samples showed that the value of the yellow color decreased compared to the aged untreated sample, except for the treated and aged treated samples at 3%, which increased by 2.98%, while the loss in the other samples was 20.02% and 5.17% at 1% and 2% respectively.

Total color differences (ΔE)

The results of the change in the total color (Table 1) of the aged untreated sample was 12.79, and all treated samples showed a bigger change than the aged untreated sample except for the sample treated with the first concentration with sodium alginate before aging. The results also showed that the change in the total color increases more after the application of accelerated aging on the treated samples. The results also showed that the lowest change in the total color was obtained from the first and second concentrations of sodium alginate before and after aging. Also, the concentration of 3% gave a high change compared to the first and second concentrations.

3.3. Investigation of the surface morphology using scanning electron microscope (SEM)

The investigation of the surface morphology using SEM became vital in the conservation field. This may be due to the bone surface needing big magnifications to follow the distribution of the polymers used on the surface, and any changes that occurs before and after accelerated aging used.

- **Investigation of the surface morphology of control and aged untreated samples**

The results obtained for the control sample (Fig. 2A) showed the appearance of Haversian channels on the surface with an irregular random distribution of these channels and that the surface appeared flat. The aged untreated sample (Fig. 4B) showed that the surface appeared rough, and the Haversian channels were affected by aging which appeared in the impact on the surface of the sample in some parts.

- **Investigation of the surface morphology of the treated samples with Sodium Alginate**

The results obtained of the treated sample at 1% (Figure 2C) showed the appearance of sodium Alginate on the surface, which covered it completely, and the Haversian channels did not appear. The aged treated sample (Fig. 2D) showed that the polymer that covered the surface was affected by the aging process which led to the shrinkage of the polymer with the presence of the polymer on the surface of the sample.

The sample treated with 2% (Fig. 2E) showed that the polymer covered the surface with a thick layer which led to the absence of any details of the bone surface in addition to the presence of some air bubbles in some places. The sample aged treated sample (Fig. 2F) showed complete coverage of the surface with the polymer and the occurrence of its shrinkage.

The treated sample at 3% (Fig. 2G) showed that the polymer completely covered the surface more densely than the 2% concentration and the polymer hid any details of the bone material. The aged treated sample (Fig. 2H) showed that the full coverage of the polymer material was lost for any details of the bone material with shrinkage in some places on the surface of the sample.

Table 1 Change of color of treated bone samples with sodium alginate at different concentrations before and after accelerated ageing.

samples	Color values			Total color differences (ΔE)
	L*	a*	b*	
Control	80.17	1.02	12.33	0.00
Aged sample	68.71	3.20	17.58	12.79
Sodium alginate (1%)				
Treated sample	68.21	0.52	11.29	12.02
Aged treated sample	66.92	1.50	14.06	13.37
Sodium alginate (2%)				
Treated sample	67.67	-0.86	12.73	12.65
Aged treated sample	65.80	2.00	16.67	15.04
Sodium alginate (3%)				
Treated sample	67.01	-1.20	16.76	14.06
Aged treated sample	66.34	2.80	18.12	15.10

3.4. Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR/FTIR) analysis

The use of ATR/FTIR analysis for the treated bone samples before and after accelerated heat aging is a very important process. The ATR/FTIR analysis was explained accoding to the outlines of Abdel-Maksoud and El-Sayed [2, 3], Abdel-Maksoud et al. [6], and Abdel-Maksoud [27]. The analysis reveals the chemical stability of organic or inorganic phases of bones. It should be noted that the functional groups of organic and inorganic components of the treated bone samples before and after accelerated aging will be explained.

- **ATR/FTIR analysis of the control and aged untreated samples**

The results obtained (Fig. 3) for the control sample (the modern sample before aging) showed the appearance of collagen bands (the main component of organic part in the bones) at wavenumber 3284.51 cm^{-1} which indicated the presence of amide A. This band increased very slightly in the peak position in the aged untreated sample and appeared at wavenumber 3286.57 cm^{-1}. This band also showed a very slightly increased in the absorption intensity. This band represents a wide band exhibiting OH Hydroxyl stretching group which is due to the presence of a hydrogen bond to the hydroxyl group. This range also includes a set of ranges due to the presence of many N-H groups.

The results also showed the appearance of Amide I representing collagen in the control sample at wavenumber 11639.64 cm^{-1} with an absorption intensity of 0.058. For the aged untreated sample this band appeared at a wavenumber of 1637.67 cm^{-1} with an absorption intensity of 0.057. A very small decrease in the intensity of absorption in the aged untreated sample indicated a slight effect on collagen. This band referred to C=O stretching.

The results also showed the appearance of Amide II at wavenumber 1520.11 cm^{-1} with an absorption intensity of 0.051 while this band appeared in the aged untreated sample at wavenumber 1522.74 cm^{-1} with an absorption intensity of 0.048. The decrease in the intensity of absorption compared to the control sample indicated the effect of accelerated aging on the sample. This band is expressed on.NH, CN stretching. It can be said that amide III appeared at the wavenumber 1237.48 cm^{-1} for the aged untreated sample with an absorption intensity of 0.011, and this band did not appear in the control sample.

Amide III also appeared at wavenumber 1013.12 cm^{-1} with an absorption intensity of 0.11 while in the aged untreated sample it appeared at wavenumber 1016.44 cm^{-1} with an absorption intensity of 0.060. This also indicated that collagen was affected by the accelerated aging process. Amide III refers to C-N stretching and N-H bending.

Concerning the inorganic part the results showed (Fig. 3) the appearance of the band of the carbonate group (-CO3 type A) in the control sample at wavenumber 1455.99 cm^{-1} with an absorption intensity of 0.03 and in the aged untreated sample with an absorption intensity of 0.034. The carbonate group (COO -CO3 type B) appeared at wavenumber 1418.39 cm^{-1} with an absorption intensity of 0.026 for the control sample, and 0.009 for the aged untreated sample.

It was also found the carbonate group (-CO3) in control and aged untreated samples at wavenumber 871.66 $cm^{-1}$with absorption intensity of 0.04 and 0.01 respectively. The phosphate group also appeared at wavenumbers 1028.84 cm^{-1} and 603.61 cm^{-1} with an absorption intensity of 0.10 and 0.06 for the control sample while for the aged untreated sample the absorption intensity was 0.06 and 0.03, respectively.

- **ATR/FTIR analysis of the treated samples with sodium alginate before and after accelerated aging**

The appearance of amide A at wavenumbers 3289.90 cm^{-1} and 3279.08 cm^{-1} at 1% before and after aging with absorption intensity of 0.03 0.02 respectively. This band did not appear in the sample treated at 2% before aging but it appeared in the sample treated after aging at wavenumber 3283.93 cm^{-1} with an absorption intensity of 0.01. This band disappeared in the treated sample before aging and it appeared in the sample treated after aging at wavenumber 3286.83 cm^{-1} with 0.02 absorbance intensity. It was clear from this band that the treatment with this polymer with the first concentration before aging gave higher absorption intensity than the control sample or the aged untreated sample. The treated sample at 1% gave lower absorption intensity than the treated sample and this was also observed for the treated and aged treated samples at 2% and 3% concentrations.

Amide B appeared at wavenumber 2315.68 cm^{-1} , 2316.68 $cm^{-1}$with an absorption intensity of 0.02 for a concentration of 1% before and after aging. It appeared at wavenumber 2316.18 cm^{-1}, 2317.33 cm^{-1} with an absorption intensity of 0.02 for the second concentration before and after aging. It appeared at wavenumber 2317.19 cm^{-1} with strong absorption of 0.02 for 3% concentration before aging and disappeared after aging. The wavenumber and intensity of absorption indicated the chemical stability of the treated samples before and after aging.

. Amide I appeared at wavelengths between 1631-1639.38 cm^{-1} for the three concentrations used before and after aging and the intensity of absorption ranged between 0.01 and 0.06 for the samples treated before and after aging.

But it can be said that the absorption intensity of the treated samples after aging was less compared to the treated sample before aging.

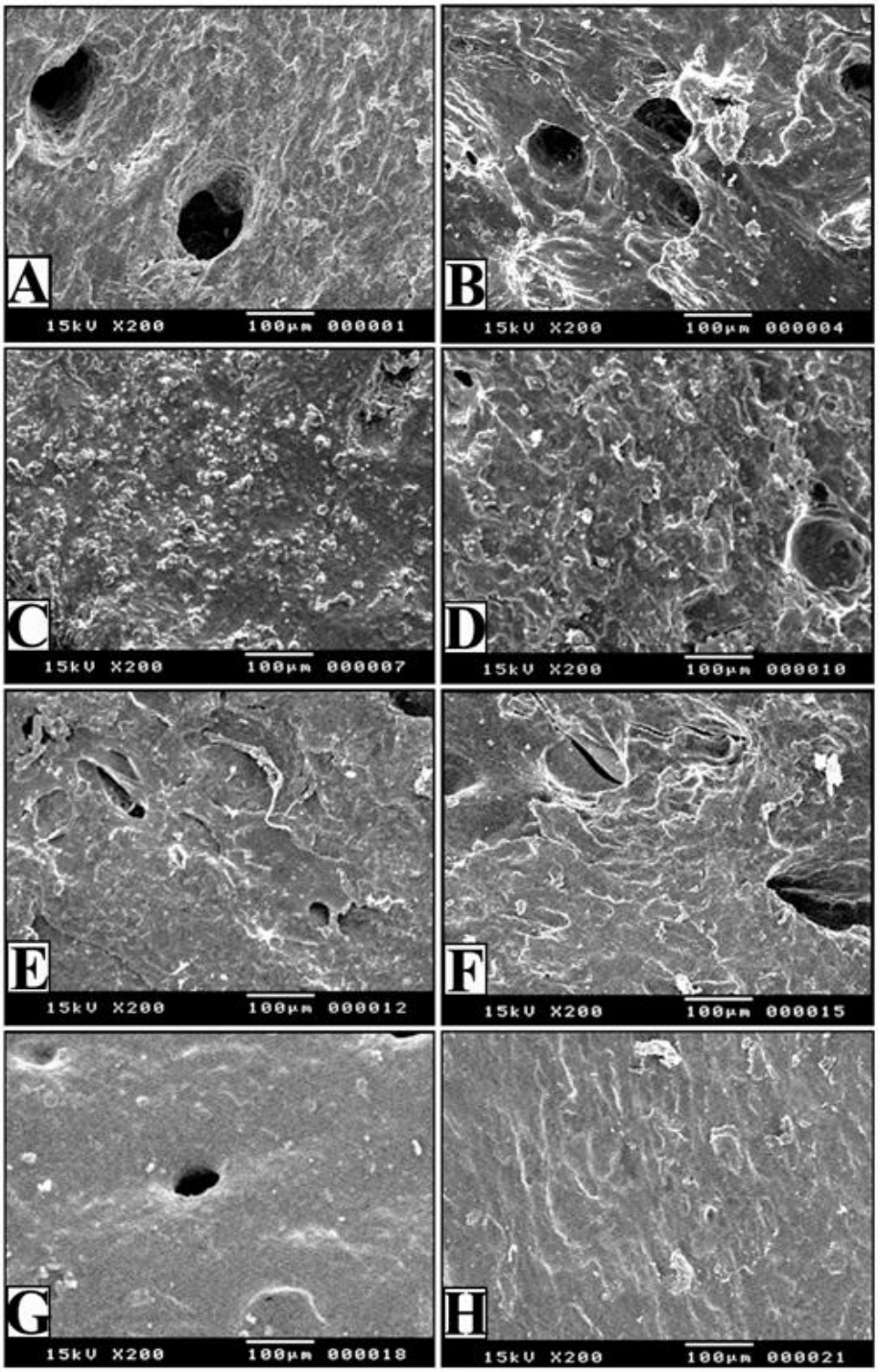

Fig. 2 SEM investigation of bone samples treated with sodium alginate at different concentrations before and after accelerated aging: (A) Control, (B) Aged untreated sample, (C) Treated sample 1%, (D) Aged treated sample 1%, (E) Treated sample 2%, (F) Aged treated sample 2%, (G) Treated sample 3%, (H) Aged treated sample 3%.

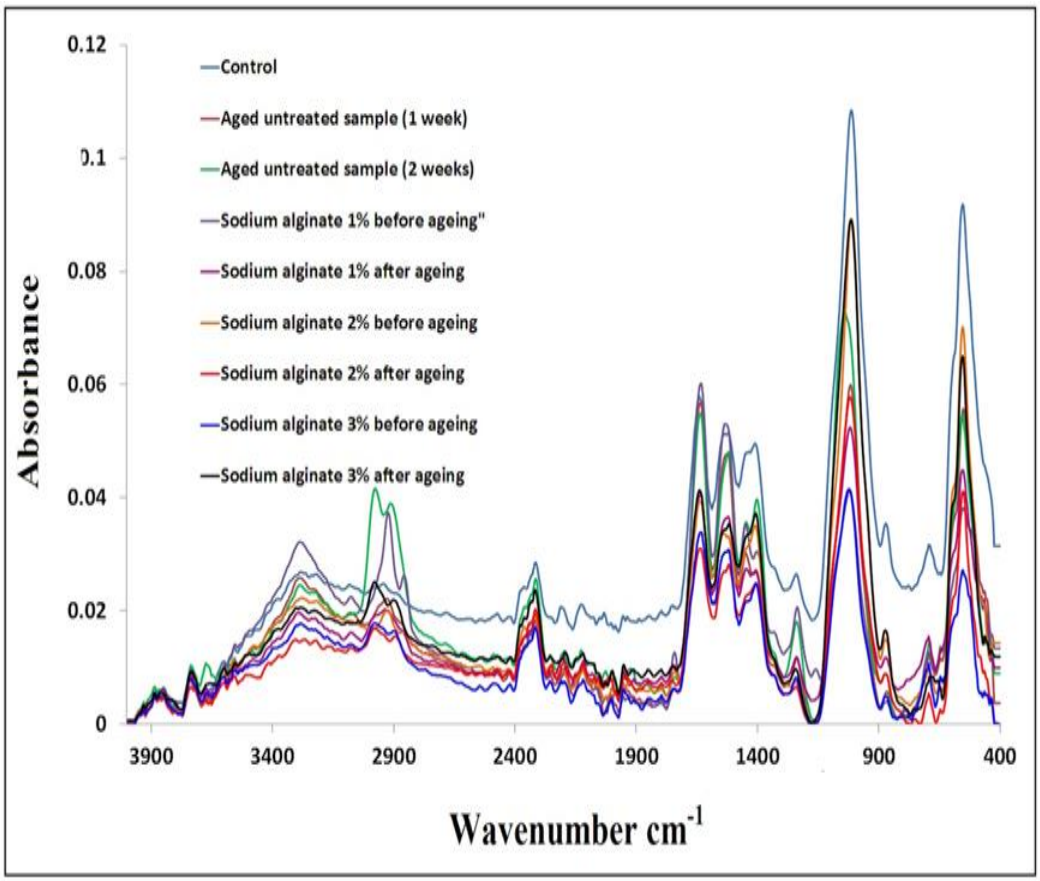

Fig. 3 ATR/FTIR analysis of the treated samples with sodium alginate at different concentrations before and after accelerated aging

Amide II appeared at wavenumber between 1517.64 cm^{-1} and 1535.99 cm^{-1} with intensity ranging between 0.03 and 0.05 for the three concentrations used before and after aging. It can also be said that the samples treated after aging were also less absorbent than the samples treated before aging.

Amide III appeared at wavenumber from 1013.04 cm^{-1} to 1023.39 cm^{-1} and intensities ranging from 0.04 to 0.9 for samples treated with different concentrations before and after aging. There was little change in the intensity of absorption between the treated samples before and after aging.

For the inorganic components in the bones treated with sodium alginate with different concentrations before and after aging (Fig. 3) the carbonate group appeared at wavenumber 1455.99 cm^{-1}, 1418.38 cm^{-1}, 871.77 cm^{-1}. The phosphate group appeared at wavenumber 1028.83 cm^{-1}, .603.61 cm^{-1}. The results also showed that the difference in the absorption intensity was very small which indicated that the polymer was characterized by chemical stability and there was no clear change in the functional groups in both organic and inorganic components.

4. Conclusion

Using of sodium alginate biopolymer in the consolidation of bone samples has been investigated using different aqueous solutions with different polymer concentrations ranging from 1 to 3%. The conclusions of this study can be stated that the positive effect of sodium alginate biopolymer on the consolidation of bone samples depends on the polymer concentration. Surface morphological properties obtained from digital and scanning electron microscopes and chemical stability of the archeological bone resulting from ATR/FTIR analysis were enhanced until 2% polymer concentration and the results are getting worse with 3% polymer concentration. Also the results obtained from color change measurement indicated that the treated and aged treated samples recorded acceptable good values in the total color difference except in the case of using 3% polymer concentration. According to the results of this studySodium alginate at 1% and 2% are good and can be used for the consolidation of archaeological bones. A concentration of 3% of sodium alginate should be excluded from application to archaeological bones.

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