Treatment of Paper Oxidation and Acidification by Linseed Gel with MgHCO$_3$ and Zinc Oxide (NP)

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Abstract: Collection paper in museums and libraries and stores suffer from acid hydrolysis and oxidations, many factors catalysis the deterioration by acid hydrolysis and oxidations, such as humidity and temperature and light and fungi, paper contains iron ink and colors based on ions heavy metals such as copper and zinc, air pollution, visible and industry light, Residues of manufacturing processes such as bleaching material, additives such as sizing materials, The study proposes a new antioxidants agent by using Line seed gel with magnesium bicarbonate loaded with Zinc oxide (NP), the positive impact of this treatment was evaluated on thermally accelerated ageing on cotton, linen and wooden samples, the samples was verified by FTIR, tensile strength and elongation measurement, Measurement of color change, PH measurement, aromatic hydroxylation assay, (SEM) scanning Electron microscope, Linseed gel with MgHCO$_3$ and Zinc oxide (NP) results to stabilize inks and stop oxidation that occurred and treatment of free radicals, the inks were most stable, The mechanical properties of the samples have been greatly improved, Linseed gel with magnesium bicarbonate loaded with Zinc oxide (NP) consolidation and treatment the samples, the zinc oxide (NP) nano oxide was observed between the fibers which works to protect the cellulose fibers From degradation.

Keywords: Carboxyl Groups, Ageing, Potassium Permanganate, C=O Vibration

1. Introduction

Papers containing iron gall inks (IGI) and Verdigris and Malachite when exposed natural ageing process begin degradation until to occurs holes on Write down and whitening and halos migration of the ink [1]. Atmospheric air oxygen causes the oxidation of cellulose that is introduces side groups, aldehydes, and ketones, which resulting molecules susceptible hydrolyzed, and also the free radicals which resulting by oxidation and light and heat cut the cellulose chain, alcohol groups oxidized to aldehydes groups and later to carboxylic acid which raising the acidy in paper [2-4]. For stability and permanence of paper must be the treatment of de-acidification included antioxidants to lateness oxidative degradation [3]. Chelating compounds use to suppression ions heavy metals and role of preventive antioxidants and non-catalyzed, but many of chelating compounds are not antioxidants [5-7]. Treatment by phytic acid 21, 35, 38-42 give positive effect with iron –gall ink and make stable with iron –gall ink and phytate, and reduce iron not catalyzed oxidation process, but not give with copper. Antioxidants may be given good results in the cases of the sample may not give results in other cases, including the idea of induction of materials other than the previous used [8-10]. Linseed, from the ancient seeds that have been used to get linseed oil, Contains fibers and minerals and vitamins a high proportion linolenic & α-linolenic acid It is a source of Omega 3 fatty. composition of Linseed: the carbohydrate 18-25%, fat 35-46%, protein 23 - 30%, ash & dry matter 3-5%. the fatty acid in linseeds oil contain 23:30% linolenic acid, α-linolenic acid varied from 1.7:50%, nearly 6% palmitic acid, Oleic acid 19%, Steric acid 2.5%. Flax (Linum usitatissimum L.) seeds, contains phenolic compounds which act as antioxidants and antimicrobial in seeds They include mainly phenols, polyphenols, carotenoids, anthocyanins and tocopherols [11-13]. Degradation of historic collection paper is mainly due to the oxidations and acid hydrolysis of cellulose, many of manuscripts and other historical documents exposed and wastage especially contains iron inks and copper colors,
phenolic compounds which act as antioxidants and proposed that leads to antioxidants of paper, linseed is a antimicrobial, linseed is available, not expensive, not toxicity and easy to prepare the gel from linseed..

The aim of the study investigate the role of Linseed gel with magnesium bicarbonate loaded with Zinc oxide (NP) to chelating the free radicals and remove the acidity of paper and save the stability of paper and ink, the positive treatment may be applied for stabilization and permanence of historic collection paper which containing iron gall ink.

2. Materials and Methods

2.1. Samples & Accelerating Ageing

Involved testing the effects of the antioxidants treatment on three holders paper (cotton, linen, wood), cotton paper sheets (17 × 25 cm) composed 100% from cotton fiber (alpha cellulose content ≥ 95), linen paper sheets (17 × 25 cm) composed 100% from linen fiber retting by enzymes (cellulose ≥ 67, Hemicellulose 16.7, lignin 2.5, the rest wax, ashes and other content), wood paper sheets (17 × 25 cm) composed from Kraft pulp fibers [14]. all samples treated with 1% gelatin for one minute to be easy to write the ink.

The ink was prepared according to Potthast & Henniges (2008) Weight of 1.05 gm of ferrous sulphate, then weighed 1.23gm of Gallic acid, 19.75gm of gum Arabic and was dissolving in 250ml deionized water and by filter paper get rid of Impurities, 1.05gm of iron sulfate was add to 1.23gm of Gallic acid and the color change on the compound was began, then 10 ml of the prepared gum was added and one way variability was made and left for an day until the ink was oxidized and ready to write [15]. The ink was written in a handmade method using Inches pen, I wrote a page of an Islamic manuscript on all the samples.

2.2. Application of Treatment

Preparation of gel, 100 g of Linseeds were mixed in 250 ml of deionized water to obtain a substance gel after the seeds were removed, then put the gel in the dryer for an hour for to get rid of water and get the granules. And then weighed about 28 grams of granules and was dissolved in a liter of deionized water until the pH 5–5. 5 It is an appropriate pH to treat oxidation oxycellulose.

Magnesium bicarbonate, de-acidification by aqueous Magnesium bicarbonate Provides good results and maintains paper stability, according to Hey magnesium bicarbonate prepared by dissolved 5 g of magnesium hydroxide to liter water, then carbon dioxide Transferring through the solution for two hours and bubbled in solution becomes clear. The samples treated in aqueous Magnesium bicarbonate for 30 minutes immersion and then dried. [19]. Zinc oxide (NP), Zinc oxide (NP) protect and preservation the collection paper from deterioration of UV light and fungi and bacteria, and the results showed that increased physical and chemical properties [20]. Zinc oxide (NP) (100-70 nm) was used, Zinc oxide (NP) 97% was purchased from Sigma-Aldrich.

Application of treatment, The samples treated in aqueous Magnesium bicarbonate for 30 minutes immersion and then dried, and then the samples treated by poultice of Linseed gel loaded Zinc oxide (NP), and then The samples treated in aqueous Magnesium bicarbonate for 30 minutes immersion due to the acidity of solution does not effect on stability of samples and then dried.

2.3. Measurement of Mechanical Properties

The samples untreated and treated before and after all ageing were measured tensile strength and elongation by H5KT/130-500N NIS MS model 1999 (Shimadzu, Kyoto, Japan), according to TAPPI Standard were measured, the width of samples was 1.5 cm and length was 15cm. The crosshead speed was reduced from 25 to 14 mm/min in order to keep the rate of strength as specified in the TAPPI Standard [21, 22]. Measurements were done in 19 ° C and RH 65%over

Figure 1. Paper from manuscript saved in Al Asfar library in Egypt suffer acidity and oxidation, in the Figure appear discoloration and tideline and holes due to the gall-ink.
24 hours.

2.4. Color Change

UV spectrophotometry used to study the color change of samples and effect of accelerator ageing on samples. The samples untreated and treated before and after all ageing were measured, Color-Eye® Spectrophotometer (OPTIMATCH 3100) was used. The values of the paper color are measured using CIE L*a*b* system [23].

2.5. Aromatic Hydroxylation Assay and pH Measurement

Bathophenanthroline in ethanol (1.6g/L) were prepared to indicator suitable ink, the solution of bathophenanthroline in ethanol were placed on edges of writing inks and far of inks, the color measure and compared development after 5 min by filter paper [24, 25].

PH was measured according to Strlic et al (2004) used by PH-meter Metrohm 691 between pH 4.01 and 7.01, at 20°C RH 50%, drop of deionized water was put on the surface of sample in 5 places and then by PH-meter was measured, and take average of five determinations [26]. PH was measured before and after ageing of untreated samples and treatment and after ageing of treated samples.

2.6. FTIR Spectra & Scanning Electron Microscope

FTIR able to estimate qualitative and quantitative changes of paper caused by acid hydrolysis and oxidation, where carbonyl groups are very active in IR than groups in degraded paper [27, 28]. FTIR model (Nicolet 380 FT-IR) NIS MS was used to analysis, The Nicolet 380 FT-IR spectrometer is available for analysis in the far, mid and near IR regions, spectra was obtained with TGS detector and by using KBr method, Origin lab pro 2019 was used to separate spectra, FTIR solution software in range 400–4000cm⁻¹, and use method Superimpose to comparisons between Spectra [29, 30].

Scanning electron microscope” SEM”(FEI Model Quanta 200 FEG, with tungsten electron source, at 20KV) unit, Assiut University, Egypt, was used to examined of the surface morphology.

3. Results and Discussion

The experimental setup was made to evaluated the role of linseed gel with MgHCO₃ and Zinc Oxide (NP) to chelating the free radicals and remove the acidity of paper and save the stability of paper and ink, There are a number of methods available for that, these methods are generic chemical methods such as Bathophenanthroline which given indicators suitable of inks and quantitative of Fe ions in the paper due to strips that turn red when exposed to Fe (II) containing solutions. FTIR able to estimate qualitative and quantitative changes of paper throw various ageing and treatment. SEM was used for the investigation of the surface morphology to know behavior the linseed gel with MgHCO₃ and Zinc Oxide (NP), Measurement of Mechanical Properties and PH given indicators stabile of the papers.

3.1. Measurement of Mechanical Properties of Paper & Change of Color

![Figure 2](image.png)

_Figure 2._ Elongation (%) & Tensile strength (N) of samples before and after pre-ageing and heat-moist ageing and treated samples before and after heat-moist.
Figures 2 and 3 shows that the elongation and tensile strength of the samples increased after ageing by potassium permanganate and again after heat-moist ageing cycle, the loss percent ageing by potassium permanganate and heat-moist ageing cycle the loss respectively were 30%, 41% for elongation and tensile strength of cotton paper, 30%, 46% for elongation and tensile strength of linen paper, 53%, 51% for elongation and tensile strength of linen paper. But after treatment by Linseed gel with magnesium bi-carbonate loaded Nano Zinc oxide. The elongation and tensile strength of the samples increased, the respectively were 36%, 52% for elongation and tensile strength of cotton paper, 57%, 44% for elongation and tensile strength of linen paper, 55%, 55% for elongation and tensile strength of linen paper, generally elongation and tensile strength of all treated samples increased. But after thermal aged of treatment samples The tensile strength and elongation of the samples increase.

3.2. Change of Color

It was clear from the data (Table 1), L-value: the samples of cotton and wood paper were near white color but linen paper was silver, after ageing by potassium permanganate and heat-moist ageing the samples became more dark, but after treated with linseed gel with MgHCO₃ and Zinc Oxide (NP) were higher than untreated that’s indicating the lightening of the samples, which are observed values in the naked eyes. a-value: after ageing by potassium permanganate and heat-moist ageing the samples was increase that’s indicating an increase in saturation in red, but after treatment with linseed gel with MgHCO₃ and Zinc Oxide (NP) the samples were less red, the cotton sample was (4.69), the flax sample was (6.13) and the wood sample was (7.12), after the limitation process, the a-value decreased in the three samples the cotton was (3.61), Flax was (4.77) and Wood was (6.24).

b-value: after pre-ageing and heat-moist ageing the samples was increase that’s indicating an increase in saturation in yellow, but after treatment the samples decreased that’s indicating the increase in saturation in blue, the cotton sample was (10.51), the flax sample was (12.97) and the wood sample was (15.77), the value of observation with the naked eye, after the thermal limitation decreased the simple of flax was (11.47) the wood sample was (12.44), the sample was increased slightly cotton was (8.85).

\[ \Delta E \] –value: there was big variation between the samples untreated before and after ageing total color difference was increased for the naked eye in the three sheets after cycle ageing, but after treated with linseed gel with MgHCO₃ and Zinc Oxide (NP) the cotton sample was (5.97) and after aging become (5.19) and the flax sample was (6.57) after aging become (4.23) the wood sample was (5.76) After limitation become (4.99).

| Samples            | Cotton sample | Linen sample | Wooden sample |
|--------------------|---------------|--------------|---------------|
|                    | Color values  | Color values | Color values  |
|                    | L*  A*  B*  AE| L*  A*  B*  AE| L*  A*  B*  AE|
| before ageing      | 89.83 0.035 6.15 0| 82.13 0.5 7.38 0| 93.11 -0.2 4.73 0|
| Primary-Ageing     | 67.84 5.04 13.54 23.67| 61.88 4.06 11.32 20.93| 65.91 4.46 11.61 28.44|
| H-moist ageing     | 53.55 6.13 13.15 16.21| 39.89 7.75 16.5 28.67| 38.71 8.52 18.49 28.44|
| Treated            | 58.8 4.69 10.51 5.97| 45.19 6.13 12.97 6.57| 43.66 7.12 15.77 5.76|
| Aged treated       | 63.17 3.61 8.85 5.19| 43.74 4.77 11.47 4.23| 42.33 6.24 12.44 4.99|
3.3. Aromatic Hydroxylation Assay and PH Measurement

The results showed that in all samples after ageing by potassium permanganate and thermal aging the inks not suitable because getting red color and many of inks remove to strips, but before treatment by Linseed gel with MgHCO₃ and Zinc oxide (NP), strips be white due to heavy metals removal and inks be suitable (Rabee, R et al 2018) after thermal ageing to treatment sample no change has occurred.

Table 2 shows the effect of treatment on the acidity of paper, PH has effect on stability of paper and catalyzed of oxidation of inks and paper (Zervos 2010) treatment with magnesium MgHCO₃ treated different acids group, after treatment the value of PH increase from 4.5 to 6.2 in cotton sample and from 4.3 to 6.9 in linen sample and from 4.1 to 6.5 in wood sample.

3.4. FTIR Spectra & Scanning Electron Microscope

3.4.1. Spectra of Cotton Sample

![Figure 4. (A) of cotton paper samples, before and after pre-ageing and sample before and after heat-moist ageing, and treated and after heat-moist ageing of treated sample.](image)

3.4.2. Spectra of Linen Samples

![Figure 5. (B) of linen paper samples, before and after pre-ageing and sample before and after heat-moist ageing, and treated and after heat-moist ageing of treated sample.](image)
Table 2. Shows the measurement of PH of cotton, linen and wood paper samples, untreated samples before and after ageing and treated before and after heat-moist ageing.

| Sample                        | cotton | linen | wood |
|-------------------------------|--------|-------|------|
| before ageing by KMnO₄        | 5.3    | 5.1   | 4.8  |
| Sample after ageing by KMnO₄ | 4.7    | 4.8   | 4.6  |
| Sample after thermal ageing  | 4.5    | 4.3   | 4.1  |
| Treated sample                | 6.2    | 6.9   | 6.5  |
| Aged Treated sample           | 6.1    | 6.7   | 6.3  |

3.4.3. Spectra of Wooden Samples

- In the Figure 4. Showed that, The Sample of (A) of cotton before ageing showed a hydrogen bonding O-H stretching at 3444 cm⁻¹ band is a strong, after pre-ageing by KMnO₄ appeared at 3441 cm⁻¹ band, and after thermal ageing appeared at 3435 cm⁻¹ band is very small, but after treatment by Line seed gel with magnesium bicarbonate loaded with Zinc oxide (NP) appeared at 3435 cm⁻¹ band with a high frequency shift, and after thermal ageing to treatment sample appeared 3343 cm⁻¹ band, whereas bands before ageing showed 1737, 1721 cm⁻¹ band of C=O vibrations, and after ageing by KMnO₄ appeared a strong band of C=O vibrations, and after thermal ageing appeared strong 1737, 1721 cm⁻¹ band with a high frequency shift, due to more of carbonyl groups were formed as a result of oxidation of C-OH groups cellulose molecules, but after treatment and thermal ageing to treatment the bands of sample don’t appeared C=O vibrations.

The sample before ageing showed a sharp peak at 1428 cm⁻¹ band of -CH vibrations, but after pre-ageing by KMnO₄ appeared at 1426 cm⁻¹ band, and after thermal ageing appeared 1425 cm⁻¹ band very small, and after treatment appeared at 1429 cm⁻¹ band due to increase the crystallization of cellulose, and after thermal ageing to treatment sample no change has occurred.

The sample before ageing showed at 1642 cm⁻¹ band of H-O-H Bending of Adsorbed water is a sharp, and after ageing by KMnO₄ appeared at 1641 cm⁻¹ band, and after thermal ageing appeared 1639 cm⁻¹ band due to drying water, but after treatment appeared 1641 cm⁻¹ band due to increase the moisture content, and after thermal ageing to treatment sample no change has occurred.

The sample before ageing showed at 1033 cm⁻¹ band of C-O-C vibrations, and after pre-ageing by KMnO₄ appeared 1033 cm⁻¹ band, and after thermal ageing appeared 1033 cm⁻¹ band with crease intensity, but after treatment appeared at 1034 cm⁻¹ band due to increase the hydroxyl groups, and after thermal ageing to treatment sample no change has occurred.

The sample before ageing showed at 898 cm⁻¹ band of C-O-O B-glucosidic Bending vibrations, but after ageing by KMnO₄ appeared at 895 cm⁻¹ band, and after thermal ageing appeared 895 cm⁻¹ band with increase the intensity, but after treatment appeared 898 cm⁻¹ band because crease the religion of crystallinity, and after thermal ageing to treatment sample no change has occurred.

- In the Figure 5. Showed that, the sample of (B) of linen before ageing showed at 3441 cm⁻¹ band of O-H stretching is a higher, and after ageing by KMnO₄ appeared at 3438 cm⁻¹ band, after thermal ageing appeared at 3435 cm⁻¹ band is very small, but after treatment by Line seed gel with magnesium
bicarbonate loaded with Zinc oxide (NP) appeared at 3441 cm\(^{-1}\) band with a high frequency shift, and after thermal ageing to treatment sample appeared 3441 cm\(^{-1}\) band, whereas The sample before ageing showed 1736, 1721 cm\(^{-1}\) band of C=O vibrations, and after ageing by KMnO\(_4\) appeared a strong band of C=O vibrations, and after thermal ageing appeared strong 1737, 1721 cm\(^{-1}\) band with a high frequency shift, but after treatment and thermal ageing to treat the band of sample don’t appeared C=O vibrations.

The sample before ageing showed a sharp peak at 1428 cm\(^{-1}\) band of -CH vibrations, but after ageing by KMnO\(_4\) appeared at 1426 cm\(^{-1}\) band, and after thermal ageing appeared at 1425 cm\(^{-1}\) band very small, after treatment appeared at 1428 cm\(^{-1}\) band because increase the crystallization of cellulose, and after thermal ageing to treatment sample no change has occurred.

The sample before ageing showed at 1642 cm\(^{-1}\) band of H-O-H Bending of Adsorbed water, and after ageing by KMnO\(_4\) appeared 1641 cm\(^{-1}\) band, and after thermal ageing appeared 1639 cm\(^{-1}\) band due to drying water, but after treatment appeared 1641 cm\(^{-1}\) band because increase the moisture content, and after thermal ageing to treatment sample no change has occurred.

The sample before ageing showed at 1033 cm\(^{-1}\) band of C-O-C vibrations, but after ageing by KMnO\(_4\) appeared 1033 cm\(^{-1}\) band, and after thermal ageing appeared 1033 cm\(^{-1}\) band with crease intensity, but after treatment appeared 1033 cm\(^{-1}\) band due to increase the hydroxyl groups, and after thermal ageing to treatment sample no change has occurred.

The sample before ageing showed at 897 cm\(^{-1}\) band of C-O-O B-glucosidic Bending vibrations, but after ageing by KMnO\(_4\) appeared 895 cm\(^{-1}\) band, and after thermal ageing appeared 895 cm\(^{-1}\) band, but after treatment appeared 898 cm\(^{-1}\) band because crease the religion of crystallinity, and after thermal ageing to treatment sample no change has occurred.

- in the Figure 6. Showed that, sample (C) of wood before ageing showed hydrogen bonding OH stretching at 3444 cm\(^{-1}\) band of is strong and wide, and after ageing by KMnO\(_4\) appeared at 3441 cm\(^{-1}\) band, after thermal ageing appeared at 3434 cm\(^{-1}\) band is very small, but after treatment by Linseed gel with MgHCO\(_3\) and Zinc oxide (NP) appeared strong and wide at 3434 cm\(^{-1}\) band with a high frequency shift, and after thermal ageing to treatment sample appeared 3434 cm\(^{-1}\) band, whereas The sample before ageing showed 1737, 1721 cm\(^{-1}\) band of C=O vibrations, and after ageing by KMnO\(_4\) appeared a strong band of C=O vibrations, and after thermal ageing appeared strong 1737, 1721 cm\(^{-1}\) band with a high frequency shift, but after treatment and thermal ageing to treatment the band of sample don’t appeared C=O vibrations.

The sample before ageing showed a sharp peak at 1428 cm\(^{-1}\) band of -CH vibrations, but after ageing by KMnO\(_4\) appeared at 1425 cm\(^{-1}\) band, and after thermal ageing appeared at 1424 cm\(^{-1}\) band, but after treatment appeared sharp at 1429 cm\(^{-1}\) band because increase the crystallization of cellulose, and after thermal ageing to treatment sample no change has occurred.

The sample before ageing showed a sharp at 1643 cm\(^{-1}\) band of H-O-H Bending of Adsorbed water, after ageing by KMnO\(_4\) appeared at 1641 cm\(^{-1}\) band, and after thermal ageing appeared 1640 cm\(^{-1}\) band due to drying water, after treatment appeared 1641 cm\(^{-1}\) band because increase the moisture content, and after thermal ageing to treatment sample no change has occurred.

The sample before ageing showed at 1033 cm\(^{-1}\) band of C-O-C vibrations, but after ageing by KMnO\(_4\) appeared 1033 cm\(^{-1}\) band, and after thermal ageing appeared 1033 cm\(^{-1}\) band with crease intensity, but after treatment appeared 1035 cm\(^{-1}\) band due to increase the hydroxyl groups, and after thermal ageing to treatment sample no change has occurred. The sample before ageing showed a 897 cm\(^{-1}\) band of C-O-O B-glucosidic Bending vibrations, but after ageing by KMnO\(_4\) appeared 895 cm\(^{-1}\) band, and after thermal ageing appeared 894 cm\(^{-1}\) band with increase the intensity, but after treatment appeared 898 cm\(^{-1}\) band because crease the religion of crystallinity, and after thermal ageing to treatment sample no change has occurred, the results showed that in all samples after ageing by potassium permanganate and thermal aging the inks not suitable because getting dark color and many of inks remove to filter paper, but before treatment by Linseed gel with magnesium bicarbonate treatment and coating Nano Zinc oxide filter paper be white because heavy metals removal ant inks be suitable, and after thermal ageing to treatment sample no change has occurred.

![Figure 7](image-url). Scanned images of the samples of (A) cotton sample, (B) linen sample and (C) wood sample before cycle ageing, showing the fibers seemed clear and strong, and gelatin appeared between the fibers (Magn. 500).
Figure 8. Scanned images of the samples of (A) cotton sample, (B) linen sample and (C) wood sample after cycle ageing, there was cracks shredding and some destruction in the fibers due to ageing (Magn. 500).

Figure 9. Scanned images of samples (A) cotton sample and (B) linen sample, (C) wood sample after treatment by linseed gel with MgHCO$_3$ and zinc oxide (NP), the fibers seem strong and very clear, the Nano oxide was observed between the fibers which works to protect the cellulose fibers From degradation (Magn. 500).

Figure 10. Scanned images of samples of (A) cotton sample, (B) linen sample and (C) wood sample after heat-moist of treated, the fibers seemed clear, and the Nano oxide appeared between fibers (Magn. 500).

4. Conclusion

Using potassium permanganate to ageing paper occur degradation and discoloration impossible to completely remove it.

The choice of treatment materials depends on the ability and effectiveness of the substance stopping acid hydrolysis and chelating of free radicals in inks and paper, and removal of transitional elements in paper that’s cause deterioration by stimulating the oxidation reactions.

Linseed gel with MgHCO$_3$ Zinc oxide (NP) results to stabilize inks and stop oxidation that occurred and treatment of free radicals that evaluate by aromatic hydroxylation assay where the color of filter paper not change after treatment process and circle ageing, FTIR results increased of hydroxyl groups and decreased of carbonyl groups and increased of crystalline religions and crystallinity and water content as described above.

After ageing by potassium permanganate and heat-moist ageing the samples became more dark but after treatment Linseed gel with MgHCO$_3$ and Zinc oxide (NP) indicating the lightening of the samples, measuring color change and calculating different color change is difficult due to the presence of spots and tideline due to oxidative inks and paper and acid degradation, but general after treatment the samples indicating the lightening. The mechanical properties of the samples have been greatly improved, Linseed gel consolidation and treatment the samples.

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