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THE USE OF POLYSACCHARIDE RIGID GELS IN CLEANING TREATMENTS

Multi analytical approach for the study of their performance, effectiveness and interference with paper artworks

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1. Introduction

It is well known that the conservation of paper artworks depends on different aspects correlated to the intrinsic nature of cellulose (the main component of paper), papermaking and materials added but also the interaction between paper and environment can play an important role. In fact external contaminants, deposition materials, salts, metals, spores, and even organic acids on paper may promote degradation processes of cellulose (i.e.: hydrolysis, oxidation of cellulose) resulting in the mechanical weakening of paper as well as compromising the structural integrity of paper.
Moreover the presence of dust and airborne particles, in combination with specific environmental conditions promote mold growth and pest infestation in paper artworks.
These organisms can release hydrolytic enzymes, *cellulasi*, which can catalyse cellulose hydrolysis and at the same time they can release metabolic products often of acidic nature and pigments. The consequence is an aesthetical, physical-mechanical damage, the formation of specific stains, *foxing*, often of biological origin. For this reason the control of temperature and relative humidity (RH) is fundamental for the conservation of paper artworks [1].

In this context and apart from aesthetical considerations, cleaning treatments become crucial for the stability of paper artifacts and archival materials. The removal of such particles by means of cleaning treatments is fundamental to chemically stabilize and preserve artifacts over time. It is also important to note however the appearance of paper artifacts after cleaning treatments is not always significant to demonstrate and validate the effectiveness of the treatment. Appearance is the direct consequence of paper constituent materials. Moreover, the type of fibre, the presence of lignin and the presence of inert materials, etc. not only determine condition of papers but also governs response of paper to conservation materials and methods. It is known that with the evolution in papermaking at the end of 19^{th} century, the use of wood pulp as raw material instead of cotton, flax or hemp fibres, became commonplace. This posed a number of issues related to paper quality, durability and susceptibility to photochemical and mechanical degradation, due to the presence of encrusting substances such as lignin which can promote cellulose oxidation [2,3]. Also, the introduction of the calendaring machine with rollers and the common use of water containing heavy metals contributed to the decline of the quality of paper. For these reasons, and in comparison to ancient handmade paper, industrial paper dated back to late 19^{th} and early 20^{th} centuries is characterized by shorter and crushed cellulose fibres thus becoming more susceptible to chemical degradation (hydrolysis processes, depolymerization of cellulose, oxidation) [3,4]. All these chemical-physical processes can affect also the mechanical properties of paper (the paper can lose its original mechanical properties such as flexibility, strength

and become more brittle) so the durability of the paper can be compromised over the time.

Paper artifacts are very complex systems. They represent a container of history that can also be read in the paper itself with its materials, its manufacture, its texture and surface morphology [5]. It is for this reason that the type of fibre and the original texture must be preserved as historical evidence of papermaking and therefore cleaning treatments must be respectful with the properties of paper. Nevertheless, cleaning treatments can be challenging and complex especially on delicate surfaces.

Traditionally, cleaning treatments have consisted of dry methods (mostly based on erasers, brushes and any other material that help remove mechanically the dirt from the surface) and wet ones. Some traditional dry cleaning treatments have proved to be too aggressive and could partially damage paper surface morphology typically resulting from manufacturing [3].
On the other hand most of the common wet cleaning treatments have been traditionally based on the use of water because of its polarity thus allowing partially extraction of degradation products and reinforcing the cellulose structure by building hydrogen bonds up.
Water can be absorbed by cellulose in a different entity depending on several factors such as hydrophilic degree of paper, the existence of any sizing, the humidity content in the air, etc. The absorption of water molecules (above all in large amount as treatments of immersion in water) can cause the partial swelling of cellulose fibers and dimensional changes. In fact cellulose undergoes the contraction of the structure to a greater or lesser degree depending on the type of paper and its manufacture in the drying phase (when water is desorbed). Such phenomenon poses concerns in the context of conservation, especially during the assembling phase, when the different pieces can present a different trend to the dimensional variation due to the absorption and consequence desorption processes of water [6].

In these last years research has been focused on the development of wet cleaning treatments consisting on the use of water in a gelled form [7,8,9]. By doing this the release of water in the paper can be reduced and controlled and therefore the risks of dimensional change can be minimized.
For this purpose, water gelling agents with high viscosity such as agar and gellan gum gels can be used. The application of such rigid polysaccharide gels makes the water intake by paper gradual depending on the gel concentration [10]. This aspect can be advantageous from a mechanical and structural point of view but also in case of works of arts characterized by the presence of water sensitive medias.

On the one hand water is fundamental to remove degradation products of hydrophilic nature and to improve the mechanical properties of paper thanks the formation of inter-molecular hydrogen bonds; on the other hand water can have an extractive action on hydrophilic components, not only degradation products but also original ones, such as hemicelluloses and sizing agents.

Few studies have been carried out on the evaluation of the effects of the cleaning treatments on paper mid-to-long stability. In this work, the research is focused on the analytical evaluation of aqueous cleaning treatments and in particular, on the comparison between traditional wet cleaning treatments (immersion of paper in distilled water) and the use of water gelling agents such as agar and gellan gum. The aim of this study is to evaluate the interaction of cleaning treatments with the physical, mechanical and chemical properties of paper by analysing simple papers: Whatman paper (commonly known as filter paper) and original papers of different periods dating back from the 16^{th} to 19^{th} centuries and therefore representative of different composition and papermaking processes (see Chapter 2: aim of the research).

1.1 Paper composition

In the following section the main components of paper are described, since paper properties are strongly dependent from materials and papermaking; moreover cellulose (which represents the principal source for the production of paper) and the possible degradation processes are reported.

1.1.1 Cellulose

1.1.1.1 Cellulose structure

Cellulose is a linear polymer characterized by the presence of D-glucose units[1] linked by the formation of β-(1,4)-glycosidic bonds and it represents the main component of paper.
The primary sources from which the cellulose can be obtained are different: wood, cotton, flax, hemp (*Cannabis sativa*), ramie, jute. The content of cellulose depends on the type of raw material: cotton (*Gossypium)* is very rich in cellulose (cellulose content about 98%), the flax (*Linum usitatissimum)* presents a cellulose content of about 70% [11]. It is possible to obtain cellulose almost pure from cotton, while in the other sources, in particular in wood, cellulose is associated with encrusting substances such as hemicelluloses, lignin, pectin, which may influence cellulose properties and behaviour. For this reason the study of the structure and features of cellulose and encrusting substances is fundamental to understand paper properties.

From a chemical point of view the basic structure of cellulose is cellobiose, obtained from a condensation reaction of two molecules of β-glucose (Figure 1.1).
The repetition of cellobiose units obtained after polycondensation reactions builds the cellulose structure (Figure 1.2).

[1] The smallest chemical unit in cellulose is glucose in the pyranose form (β-D-glucopyranose).

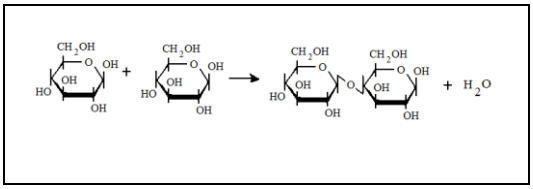


Figure 1.1: The condensation reaction between two β-D-glucopyranose units and the formation of cellobiose.

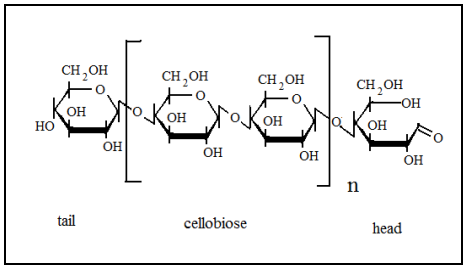


Figure 1.2: Cellulose structure in Haworth projection.

Cellulose is characterized by a linear structure since the type of bond [β-(1,4)-glycosidic bond] allows the elongation of the chain under a linear way, and the structure is stabilized by the chair conformation of pyranose rings where the substituents (OH groups) are placed in plane with the ring[2] [12].

Cellulose $(C_6H_{10}O_5)_n$ can have a different degree of polymerization (DP) (10000 DP for the native fibers and below 1000 for pulped fibers) depending on the fibres sources, on papermaking and on the degradation process it may be subjected. For instance the DP of wood is about 2500, 15000 for cotton and 36000 in the case of flax [2].

Cellulose chains are dominated by the presence of hydrogen bonds (intra and inter-molecular), deriving from OH groups placed in the structure, which play an important role in the stabilization of cellulose structure (Figure 1.3).
The intra-molecular hydrogen bonds engage: the 2-OH of a glucopyranose molecule and the 6-OH of a second unit; the 3-OH of one unit and the oxygen atom of the ring of a second unit. These are responsible for the stabilization of the glycosidic bonds and

[2] In the glucopyranose units there are three hydroxyl groups: two (OH linked to 2 and 3 carbons) lie in plane with the ring and the third one (OH group linked to 6-carbon) is placed out of the ring.

for the axial stiffness of the chain. The inter-molecular hydrogen bonds are set up between the OH (3-OH group of one cellulose chain and 6-OH group of a second cellulose chain) of neighboring cellulose chains [13]. Thanks to this networks of links a β-sheet conformation is obtained and the planes in which the chains lie may interact through the formation of weak bonds (Van Der Waals forces).

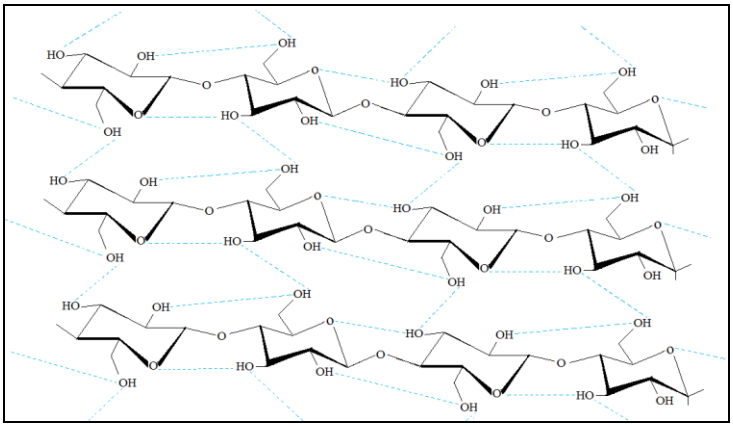


Figure 1.3: Cellulose chains and intra-inter molecular hydrogen bonds.

Cellulose chains aggregate into ordered bundles: *microfibrils* (supramolecular structure), which consist of about 15 elementary fibrils, and they present a diameter of about 0.05 micron. The microfibrils are organized into *fibrils* (diameter about 0.5 micron) and, associated with hemicelluloses, pectin and structural protein form *macrofibrils* (diameter about 5 micron). The whole of the macrofibrils constitutes the *fibre* (diameter about 10 micron, depending on the type of fibre) (Figure 1.4) [2].

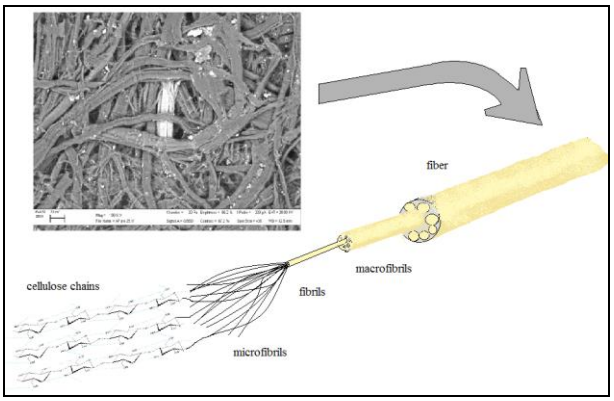


Figure 1.4: Structure of cellulose: cellulose chains, microfibrils, fibrils, macrofibrils and fibre (image of fibres from a 16th century paper, obtained by means of SEM-EDS in backscattered mode).

The microfibril structure of the native cellulose at the solid state is characterized by crystalline phases (the polysaccharide chains are located in an ordered, compact and regular way, in a parallel arrangement to the axis of the microfibril) which are alternated to amorphous phases. In the amorphous phases the chains are characterized by a random, disordered and irregular arrangement and they are not orientated along the axis of microfibril. Polysaccharide chains may participate in the two regions; for this reason the crystalline and amorphous phase are not separated.

Different studies have been carried out on the definition of the structure of the crystalline phase (*cellulose I)* of cellulose by means of X-ray-diffraction analysis. The reticular structure of the crystalline phase is characterized by the repetition of monocline structure elementary cells in which the *b* axe corresponds to the cellobiose structure [11].
The percentage of crystal and amorphous phases, which depends on the raw materials from which the cellulose derives, has an important influence on the mechanical properties of cellulose. In fact, the crystalline phase is responsible of the tensile strength of the polymer and it can be regulated by the flexibility and elongation provided by the amorphous phase. Moreover the degree of crystallinity of cellulose can be influenced also by the state of conservation of fibres and by the external conditions [14].
In addition cellulose, being a hygroscopic polymer (presence of OH groups), may interact with water molecules through the formation of hydrogen bonds. The interaction cellulose-water represents another important aspect to consider in the context of cellulose properties and stability.

1.1.1.2 Degradation processes

a) Hydrolysis processes

Cellulose, as all polymers (in particular polysaccharides), can be subjected to different types of degradation processes depending on its intrinsic nature and the storing conditions. One of the main degradation processes which can affect cellulose is hydrolysis, that consists on the breakage of glycosidic bonds between monosaccharide molecules. This phenomenon can be acid or base-catalysed and it is responsible of the depolymerization of cellulose and the consequent weakening of the structure.

a.1) Acid –catalysed degradation

The hydrolysis of cellulose chains can be acid-catalysed. The mechanism can be summarized in the following way: the first step is the protonation that is the addition of a proton (arising from the dissociation of an acid) to oxygen of glycosidic bond, resulting in the formation of a positive charge on the oxygen; in the second step the water molecule is added to one of carbon atoms involved in glycosidic bond resulting in the breakage of glycosidic bond. From water molecule is removed the hydronium ion thus acting as a catalyst (Figure 1.5) [1]. The acid hydrolysis, unlike the alkaline one, can take place also at room temperature.

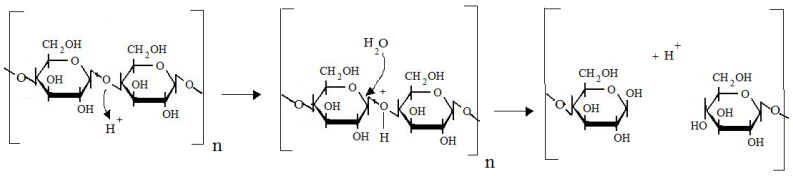


Figure 1.5: Acid-catalysed hydrolysis of cellulose.

In the field of paper conservation, there are different reasons for paper acidity linked to papermaking, the interaction between paper and environmental conditions and the presence of particular inks media.
The sources of paper acidity can be divided into two groups: internal and external causes. Internal causes include all factors which are related to the intrinsic features of paper, this is, to the materials used for papermaking such as the addition of particular sizing agents, the type of papermaking, and the use of acid-nature inks.

External causes are mainly related to the interaction between cellulose and air pollutants such as sulphur dioxide, nitrogen oxides which can promote acid hydrolysis of cellulose.

As described previously, cellulose is characterized by the presence of monosaccharides (glucose units); the primary alcoholic groups (-CH_2-OH) can be oxidized to aldehyde and carboxylic groups (-COOH) and the hydronium ion can be released by the dissociation of carboxylic group.

$$R\text{-}COOH + H_2O \rightleftharpoons R\text{-}COO^- + H_3O^+$$

In other words, the oxidation of cellulose constituents (monosaccharides) can promote acidity and hydrolysis of the chains. Therefore the raw materials and methods used in papermaking may have an important role in the stabilization of cellulose.
For instance paper produced from wood pulp (from the mid-19thcentury) began to be more susceptible to acidity than the typical handmade one, especially with the introduction of alum. Alum (potassium aluminium sulphate - K $Al(SO_4)_2$*12 H_2O)[3] was used since 17th century [15] or even earlier in papermaking, added in gelatine (a surface sizing agent) to prevent gelatine putrefaction, and it was also used as mordant, because it helped the adhesion of the gelatine film to the paper fibers[4]. At the beginning of 1826 [16], gelatine was replaced by rosin (which main constituent is abietic acid $C_{19}H_{29}COOH$) and industrial alum (Al_2 $(SO_4)_3$*18 H_2O) made easer the precipitation and the interaction between rosin and cellulose. Potassium aluminium sulphate and aluminium sulphate are water soluble and they dissociate in the respective cations and anions [17].

$$K\,Al\,(SO_4)_2 \xrightarrow{H_2O} K^+ + Al^{3+} + 2SO_4^{\,2-}$$

$$Al_2(SO_4)_3 \xrightarrow{H_2O} 2Al^{3+} + 3SO_4^{\,2-}$$

[3] Alum can be considered a salt deriving from the neutralization of a weak base $Al(OH)_3$ and a strong acid H_2SO_4 and it presents an acid behaviour (the solution containing alum can obtained a pH=4). *Banik G., Cremonesi P., de La Chapelle A., Montalbano L., Nuove Metodologie nel restauro del material cartaceo. Collana I Talenti, Il Prato, 2003.*

[4] All bivalent ions such as Cu^{2+}, Fe^{2+}, Ca^{2+}, and above all the trivalent Al^{3+}, Fe^{3+} act as cross-linking agent for protein chains such as gelatine molecules.

In water solution Al^{3+} can produce the formation of a hydrated complex, hexaaquaaluminium (III) complex ion, which can hydrolyze and produce acid solution (formation of hydronium ion) [18].

$$[Al(H_2O)_6]^{3+} + H_2O \rightleftarrows [Al(OH)(H_2O)_5]^{2+} + H_3O^+$$

Therefore rosin used in papermaking (with its acidic nature) and the hydrolysis of alum in presence of water can be partially responsible for acidity of cellulose.

In the context of paper conservation, the inks used in different periods can also play a relevant role and ink corrosion of paper may occur. For instance metal-tannic and metal-gall inks [19] commonly used in the past[5], were prepared from vegetable portions (gall nuts of oaks, leaves, bark containing tannin) heated in water and mixed with soluble iron and copper salts ($FeSO_4$, $CuSO_4$). The solution became black after the oxidation of iron (Fe^{2+}) into iron (Fe^{3+}) by air. It was common practice to add an organic binder such as Arabic gum to obtain the final ink [1]. This type of ink presents an acidic nature due also to the presence of H_2SO_4 as by-product reaction [20]. For this reason the presence of acid in trace can catalyze cellulose hydrolysis and as consequence damage occurs. Moreover the presence of metals such as Fe^{3+} may catalyse radical reactions of oxidation [1].

The interaction between cellulose and air pollutants can cause acidity phenomena. The paper, being characterized by a porous structure, can absorb gas contaminants, for instance sulphur dioxide (SO_2), nitrogen oxides (NOx) and ozone (O_3) which can be converted into their respective acids in presence of water and moisture. The consequence may be the progressive breaking of cellulose bonds (acid catalysed hydrolysis) and the weakening of the cellulose fibres.
The possible reactions which may occur are summarized below [21].

$$SO_2\,(g) \rightleftarrows SO_2\,(ads) \rightleftarrows SO_2\,(aq)$$

$$SO_2 + 2H_2O \rightarrow HSO_3^- + H_3O^+ \quad (pKa_1 = 1.81)$$

$$HSO_3^- + H_2O \rightarrow SO_3^{2-} + H_3O^+ \quad (pKa_2 = 6.91)$$

$$HSO_3^- + O_3 \rightarrow HSO_4^- + O_2$$

$$2NO_2 + HSO_3^- + 4H_2O \rightarrow 2NO_2^- + SO_4^{2-} + 3H_3O^+$$

[5] The oldest description of metal-gall inks preparation is reported by Plinio (I century a.d)

As reported in previous studies, a marker for the hydrolysis of cellulose is glucose and oligosaccharide of glucose; a marker for the hydrolysis of hemicelluloses xylan in paper is xylose. It was demonstrated that the content of free glucose and xylose may increase in a considerable way as paper ages [22]. The result of hydrolysis of cellulose and hemicelluloses chains is the decrease in the degree of polymerization with consequences in the structural and mechanical properties.

In this context deacidification treatments[6] are fundamental to neutralize the acidity of paper. Thanks to the formation of an alkaline reserve into the paper after the treatment, the paper may be partially preserved from hydrolysis process. Different studies have been performed on the products and methodologies used for paper deacidification [23] and innovative treatments have been tested [20, 24, 25, 26].

An aspect to consider in case of deacidification treatments of cellulosic materials is that an extreme alkaline environment may compromise cellulose integrity, because base-catalysed hydrolysis processes may occur in case of oxidized cellulose. To prevent this phenomenon, a reductive bleaching treatment can be applied on paper before deacidification. For the reduction of carbonyl groups boron complexes were studied (i.e.: borane-tert-butylammine) [27, 28, 29].

a.2) Base –catalysed degradation

Hydrolysis processes can be also catalysed by bases. Base-catalysed hydrolysis requires an alkaline environment and higher temperatures (>150°C). Oxidized cellulose is more prone to alkaline hydrolysis and β-alkoxy elimination reaction may occur at temperature <100°C even with a diluted alkaline solution.
The hydrogen atom in α position with respect to a carbonyl group can be attached by a base producing water. If in β-position with respect to carbonyl group is present a "good" leaving group (OR^I), the group is eliminated resulting in the formation of a double bond C=C (Figure 1.6).

[6] Deacidification is a treatment mainly based on the use of alkaline substances dissolved in water, alcohol or other organic solvents. Traditionally the treatment may be performed by immersing the paper in an aqueous solution containing the deacidifying agent, by gel form or by mass deacidification systems.

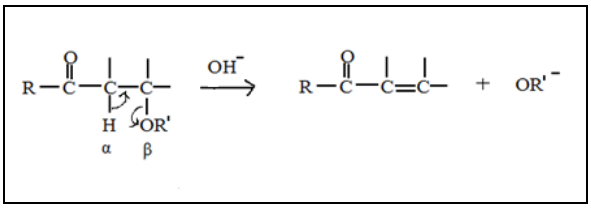


Figure 1.6 : Simplified representation of β-alkoxy-elimination process.

This process may become the main mechanism of degradation of cellulose when oxidized cellulose is subjected to deacidification treatments with pH values higher than those which guarantee the stability of cellulose (pH> 9-9.5).

Another possible form of degradation by bases, which can cause the hydrolysis of cellulose, is *peeling off*. Such process may occur under mild conditions in the case of cellulose not oxidized. The cellulose chains are progressively shortened by a beta-elimination process, which usually involves the reducing end of the cellulose molecule, and this can be interrupted by a "stopping" reaction [30].
In fact the reducing end of cellulose chains (terminal glucose) in an alkaline solution can be subjected to isomerization through the ene-diolic form [31] and the conversion into the corresponding ketose on which a β-alkoxy-elimination, in presence of a base, may occur. If the β-alokoxy-elimination involved the 4-carbon, the remaining part of the chain is released (Figure 1.7) [11].

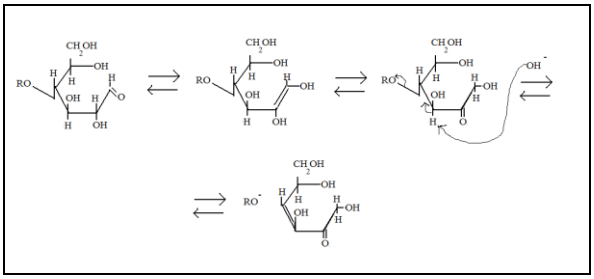


Figure 1.7: Cellulose degradation under basic conditions (*peeling off*).

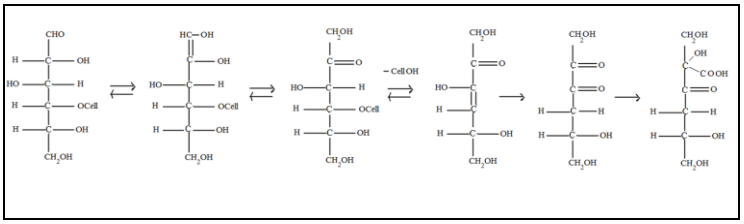


Figure 1.8: Representation of peeling off reaction on cellulose (Cell= cellulose) and formation of isosaccharinic acid.

The ketose residue can become isosaccharinic acid (Figure 1.8). The reaction can be repeated on the new reducing end of the chain; the *peeling off* reaction stops with β-elimination reaction on carbon atoms different from 4-carbon and the rearrangement (benzyl rearrangement) of reducing end oxidized to carboxyl, which determines the formation of metasaccharinic acid and the stabilization of molecules (Figure 1.9). In this way it becomes less susceptible to alkaline degradation.

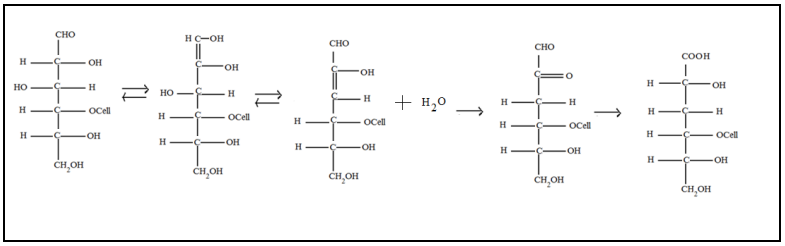


Figure 1.9: Termination of *peeling off* and formation of methasaccharinic acid.

b) Oxidation processes

Cellulose may be also subjected to oxidation processes. In particular primary alcoholic groups (OH linked to 6-carbon) can be oxidized to aldehyde and carboxylic groups; the secondary alcoholic groups (OH linked to 2-carbon and 3-carbon) can be oxidized to ketone (Figure 1.10) [1].

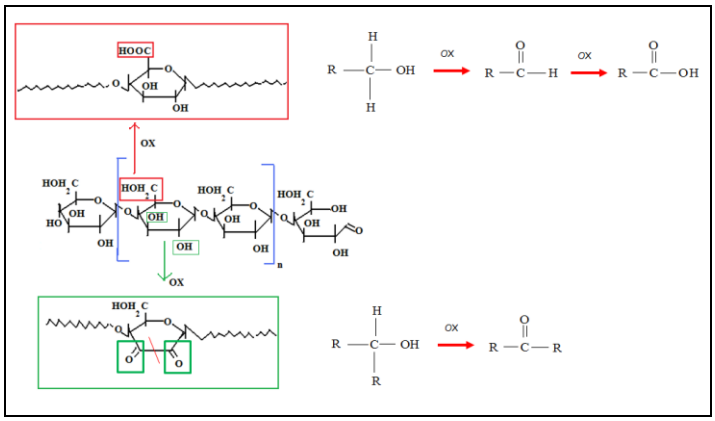


Figure 1.10: Oxidation process of cellulose and formation of ketone and carboxylic groups.

The consequences of such structural changes are different: the formation of carboxylic groups, as oxidation product of primary OH, which may contribute to the increase in acidity of the cellulose and catalyze hydrolysis of cellulose chains (consequent decrease in cellulose DP). The formation of ketone groups from oxidation of secondary OH may cause chromatic alterations (i.e.: yellowing and discoloration of paper).
Moreover ketone may create hydrogen bonds weaker than the original OH groups, and for this reason the oxidation phenomenon also determines a negative effect of weakening and the destabilization of the cellulose structure.

Paper can be oxidized when:
- it is exposed to high levels of light, above all to ultraviolet wavelengths (photo-oxidation);
- it is subjected to oxidizing bleaching treatments;
- it is exposed to heat and dramatic humidity conditions (thermo-hygrometric oxidation);
- it presents transition metals such as iron or copper used for instance in iron gall ink or pigments such as copper acetate;
- it is exposed to oxidizing air pollutants.

Several studies have been performed on cellulose oxidation processes [32, 33, 34] but the detailed mechanisms of the oxidation reactions have not been as well established yet. Nevertheless it is known that oxidation processes are based on radical reactions. In fact heat, light and oxygen generate free radicals which can be converted to peroxyl radicals (ROO·) in the presence of oxygen, and to hydroperoxides which are very reactive.

These reactions can be summarized in:

- Initiation (formation of free radicals and peroxyl radicals);
- Propagation (conversion of free radicals to other type of free radicals);
- Termination reactions (combination of two radicals).

The mechanism reported represents one of the processes studied of typical oxidation of organic molecules [35]:

1) Initiation
   a) $RH \rightarrow R\cdot$
   b) $R\cdot + O_2 \rightarrow ROO\cdot$

2) Propagation
   c) $ROO\cdot + R^IH \rightarrow ROOH + R^I\cdot$
   d) $ROOH \rightarrow RO\cdot + \cdot OH$
   e) $RO\cdot \rightarrow$ aldehydes, ketones, aldehydes

3) Termination
   f) $R\cdot + R\cdot \rightarrow RR$

In the initiation process free radicals are formed through the removal of an hydrogen atom. The obtained free radical can absorb oxygen and the formation of an organic peroxyl radical (ROO·) may occur. In the propagation, the peroxyl radical can abstract an hydrogen atom, for instance from another cellulose molecule. The obtained hydroperoxide can split into two radicals (RO· and ·OH) and RO· can transform into aldehyde and ketone groups. The combination of two free radicals determines the termination of the mechanism.
In case of cellulose, if oxidation occurs on 6-carbon, the result is the formation of an aldehyde group (Figure 1.11).

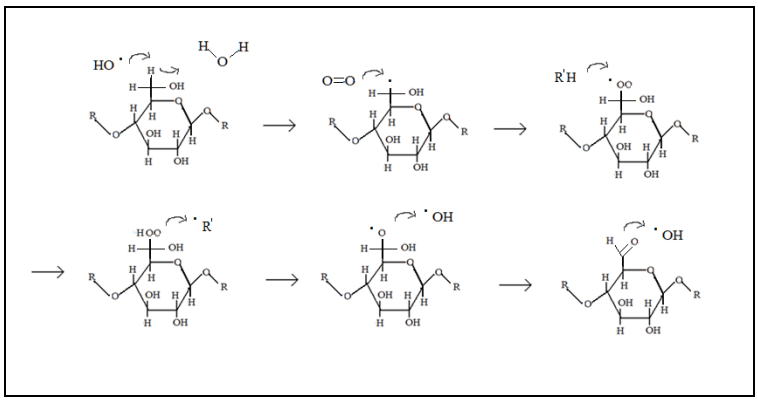


Figure 1.11: Cellulose oxidation with the initial attack at 6-carbon.

The Hydrogen atom at the 6-carbon can be abstracted by a hydroxyl radical and the obtained radical can absorb molecular oxygen by forming a peroxide radical. The peroxide radical can take a hydrogen atom from another organic molecule ($R^{I}H$) by forming an hydroperoxide, which can decompose, especially in the presence of catalysts (iron, copper ions). The obtained hydroxyl radical involved in the mechanism, leads to the formation of an aldehyde group [36].

As described previously, oxidized cellulose is more susceptible to alkaline hydrolysis, for instance when cellulose presents carbonyl groups at 2-carbon (Figure 1.12), and this is a factor which can be taken into account in the case of the deacidification treatments of paper.

At the same time the ketone groups obtained as products of oxidation of OH at 2-carbon and 3-carbon, may take part in a keto-enol equilibrium; the transformation from di-ketone to di-aldheyde (and the possible formation of carboxyl groups), may determine the ring opening with breaking of the 2-carbon and 3-carbon bond. Aldheydic groups can react with OH groups of the ring resulting in emi-acetal form of the ring with 5 or 6 carbon atoms [11].

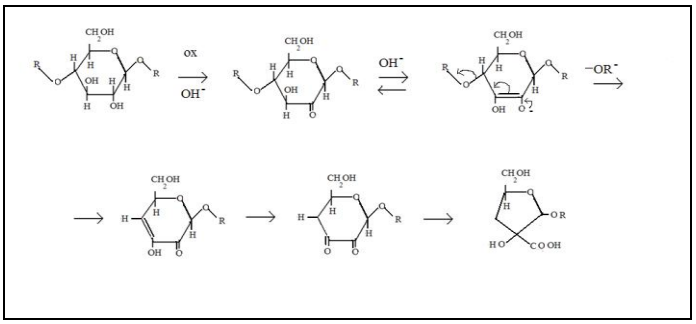


Figure 1.12: Alkaline hydrolysis on oxidized cellulose in 2-carbon.

Either in hydrolysis processes or in oxidation, water plays an important role: thanks to its plasticizing action, it allows the movement of cellulose chains of amorphous regions (which represent the most susceptible part to degradation processes of cellulose) and promote degradation process. Moreover water can be reaction medium because salts, metal dissolved in water can act as catalyst, accelerating degradation processes.

Finally an evident effect of paper oxidation is yellowing, discoloration and darkening phenomenon, caused mainly by the exposure of paper to the light (visible and near ultra-violet radiation, especially the radiation between 360-390 nm), and different studies have been performed on the effects of light on paper [37,38,39]. These phenomena can be a consequence of the formation of organic molecules containing carbonyl groups, double bonds between carbon atoms, the presence of aromatic rings (for instance lignin) in paper. The mechanisms involved are complex, can be several and depend on the type of paper and on its original composition.
Also the rate of moisture to which paper can be exposed and the water content, may have an important role in the discoloration process, in fact high values of relative humidity can accelerate the above degradation process [40,41], but sometimes the presence of very high values of humidity, can promote the formation of localized stains (*foxing*), which can derive from paper impurities (presence of metals) or biological activity (mold) [42].

1.1.2 wood

Wood started to be used in papermaking late in the 19th century by replacing the use of cotton, flax and hemp partially.
The presence of encrusting substances in wood (hemicelluloses and lignin) provides greater structural resistance.
Wood is characterized by the presence of cellulose (40-50%), hemicelluloses (15-35%), lignin (20-35%), proteins (<10%) and inorganic components (<1%). The amount of these components, in particular the encrusting substances, depends on the type of wood (hardwood and softwood).

1.1.2.1 Lignin

Lignin is a complex, amorphous polymer, characterized by the presence of phenylpropanoid monomers (coniferyl, sinapyl and *p*-coumaryl alcohols) which are the main constituents of the network, and acts as binder for the cellulose in wood (Figure 1.13). Lignin presents a high molecular weight (2000-15000 u.m.a), placed between the cellulose fibers and around the cell wall to offer hardness, mechanical strength and protects wood from microorganisms. The percentage distribution of phenylpropanoid monomers depends on the type of wood: in the case of *Gymnosperms* the lignin is composed above all by the polimerization of coniferyl alcohol.

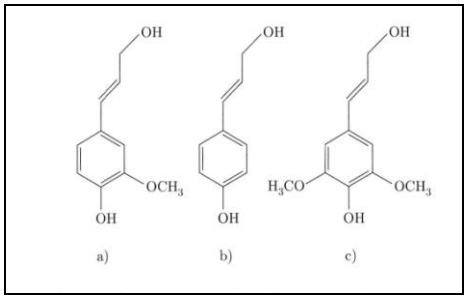


Figure 1.13: Monomers of lignin: a) coniferyl, b) *p*-coumaryl, c) sinapyl alcohols.

Lignin is sensible to UV-VIS light and may induce oxidation processes (photochemical reactions). As far chemical properties are concerned, lignin can be subjected to different reactions, for instance: electrophilic aromatic substitution (i.e.: during the treatment with chlorine for bleaching of paper), oxidation (i.e.: in presence of oxygen and metals with the formation of aromatic aldehydes), hydrolysis processes and salification of

phenol and carboxyl groups in alkaline environment, which make lignin more susceptible to oxidation phenomena [11].
For this reason papermaking also includes treatments for the separation and dissolution of lignin from cellulose (i.e.: by means of *Kraft* and sulfite processes), in order to prevent the formation of such degradation processes in paper [1]. Even if the delignification may prevent oxidation processes due to the presence of lignin, the elimination of lignin may contribute to the variation of the cellulose structure resulting also into change in the mechanical properties.

1.1.2.2 Hemicelluloses

Hemicelluloses are amorphous polysaccharides (DP about 100 units), placed in cell walls of plants and linked to cellulose by hydrogen bonds as well as to lignin by covalent bonds. Hemicelluloses composition and structure depend on the source. Pentose sugars (D-xylose, L-arabinose), hexose sugars (D-mannose, D-glucose, D-galactose) and uronic acids such as D-galacturonic acid and D-glucuronic acid are the main monosaccharides in their structure (Figure 1.14) [11].

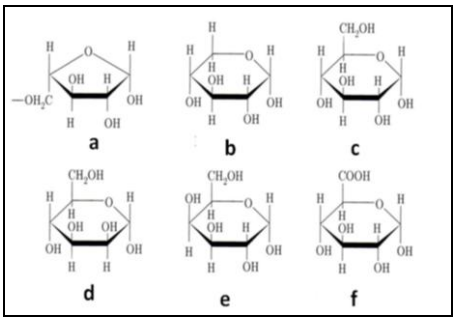


Figure 1.14: Monosaccharides of hemicelluloses: **a) L-arabinose, b)D-xylose, c) D-glucose, d) D-mannose, e) D-galactose, f) D-glucuronic acid.**

Softwood (conifers) consists of hemicelluloses xylan (where the principal component is xylose), araban (with the presence of arabinose units), glucomannan (characterized by the presence of glucose and mannose) and galactan (with galactose) [43, 44]. Hardwood hemicelluloses consist mainly of hemicellulose xylan. The content of pentosan depends

on the type of wood: about 7-10% in case of softwood pulp, and 19-25% in case of harwood pulp.[7]
The chains of hemicelluloses are linear and branched. For this reason they can be swelled by water and solubilised by hot alkali (especially when the lignin has been removed from wood).

The composition of pulps is the result of the type of wood used in papermaking and the pulping process. *Kraft* and sulfite cooking conditions degrade hemicelluloses (with peeling off and hydrolysis reactions) reducing its presence with respect to the original composition of wood [43].

The alteration of the chemical composition of pulps can influence the stability and properties of paper. Studies conducted on papers containing different sugars (xylose, mannose, glucose, arabinose, galactose, glucuronic acid) and subjected to accelerated aging (in dry conditions at 90°C, in moist conditions at 90°C and 50% RH), demonstrated that the presence of hemicelluloses or products of hemicelluloses hydrolysis may contribute to an increment of discoloration phenomenon of paper, depending on the type of sugars present and the type of aging carried out. This phenomenon was more evident in the case of thermal ageing in humid conditions; paper exposed to radiation from "daylight" fluorescent lamp and from near-ultraviolet BLB fluorescent blacklight showed a scarce discoloration in comparison to lignin exposed to UV radiations [45].

[7] Tappi standard T223, 1971

1.1.3 Sizing agents and paper additives

The principal component of paper is cellulose. Paper production began in China (about third century AD) and with the evolution through islamic countries in later centuries, vegetable fibers, cotton, flax, hemp were used as raw materials. Papermaking was based on the use of molds composed of a wooden frame and a wire screen in which a slurry of cellulose swollen fibres formed a uniform thin coating (the sheet) [46]. With the evolution in papermaking at the end of 19th century, wood became the principal raw material and the introduction of new technologies (calendaring machine with rollers) made the production faster. Sizing agents, additives of paper varied widely over the century.

The addition of sizing agents on a paper sheet is fundamental to improve paper properties. The materials added in paper may be different. One of the older sizing agent was animal glue, used in the late 13th and early 14th centuries [47] and continued to be used throughout the 19th century, with the introduction of rosin-alum sizing. Sizing were intended to avoid the penetration of inks into paper, to make possible to write, protecting paper against the settling of dust particles and therefore limiting the access of pollutants. Moreover the introduction of sizing agents can improve the mechanical properties of paper and reduces the negative impact of frequent relative humidity variations on paper [48].

Papermaking also includes the addition of different inorganic fillers (such as calcium carbonate, calcium sulphate dihydrate, titanium dioxide, Kaolinite, etc) in order to improve some features: degree of whiteness and opacity, printability (the inorganic fillers occupy the empty inter-fibre spaces) and degree of smoothing [49].

1.2 The study of the mechanical properties of paper

The study of the mechanical properties of paper is very complex since the mechanical, and morphological properties of paper may be influenced by different factors related to paper composition, papermaking processes, the interaction between paper and environment, paper condition, etc.
Tensile testing helps understand papers behaviour in different conditions, as well as to assess the effects of specific conservation treatments in paper's mid-to-long term stability. Tensile tests (which were carried out for the evaluation of wet cleaning treatments on the mechanical properties of paper, as described in the Chapter 3) can be represented as stress-strain curves (Figure 1.15), where stress (σ) which is the force per cross-sectional area, is plotted as function of strain (ε dimensional change). The initial linear section of the curve represents the elastic region; the slope of the linear section of the curve is the elastic modulus (used for the indication of stiffness (E)). The second region of the curve describes the plastic behaviour (the material experiences an irreversible deformation once the load applied has been removed). The final point of the curve represents the ultimate tensile strength (UTS) and strain at break [50].

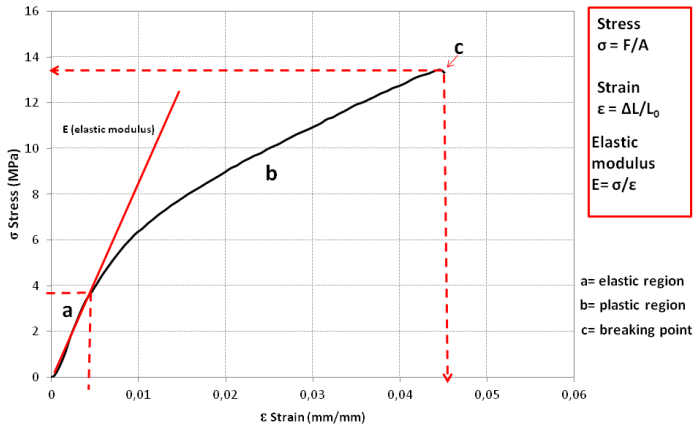


Figure. 1.15: Stress-strain curve of filter paper at 24°C and 50% RH. Stress (σ) is plotted as function of dimensional change (strain, ε). The first region of the curve is the elastic region (a); the second region of the curve is the plastic region (b); the end of the curve represents the breaking point (c) described by an ultimate tensile strength and a strain at break value.

The resulting curve represents the properties of materials. For instance the elastic modulus refers to the stiffness and flexibility of materials. Stress-strain curves can be used to represent the differences between materials but it can also represent changes in the mechanical properties of materials which may occur as consequence of their interaction with external factors.

The mechanical properties of paper depend on different factors correlated to paper composition (presence of cotton, hemp, fibres or wood pulp, and materials added during papermaking), papermaking and to the interaction paper-environmental conditions.
In fact, cellulose (the main constituent of paper) is a polymer characterized by crystal and amorphous regions, in which the crystal phase is responsible for the hardness and the amorphous phase makes cellulose flexible. The final mechanical properties depend on the percentage of crystal and amorphous phases, but also on the condition of cellulose.
Chemical, physical, mechanical properties of paper started to change with the introduction of wood pulp in papermaking (from the late 19^{th} century).
This is correlated also to different papermaking processes and treatments used for the removal of encrusting substances contained in wood (lignin and hemicelluloses) that resulted into the production of paper of shorter fibres, and weakening of paper [22, 51]. Moreover also the total removal of such encrusting substances may be partially responsible for the weakening of paper, because hemicelluloses and lignin also work as binder through the mutual bonding of their hydroxyl groups [52].

Other aspect to consider is the interaction between paper and environment, in particular paper and water. Being constituted by OH groups, cellulose can create inter and intra molecular hydrogen bonds between chains and water molecules and this represents a key aspect in the structural context. On the other hand, the long term effects of storage under certain conditions may affect paper conservation, since the humidity may promote the hydrolysis of cellulose, especially in case of high relative humidity, resulting in loss of strength of paper [53].
Moreover the mechanical properties of paper are influenced by testing conditions. In fact studies such as those conducted by Mecklenburg on cultural materials demonstrated that the relative humidity rate influences the elastic modulus. Paper is more flexible at high relative humidity (RH>60%) [50]. This phenomenon is the result of interaction between water (in humidity form) and cellulose of paper.

The study of the mechanical properties of materials can help monitor the aging of materials [54, 55]. This can be extremely relevant when comparative studies are performed on the same material subjected to different conditions of accelerated aging which can simulate the long term effects of natural aging [53]. This method of

investigation can be extended to the evaluation of the effects of conservative treatments in mechanical properties of historical materials. In the case of paper artworks the investigation of deacidification and cleaning treatments commonly performed for conservation purpose is crucial. In chapter 3 the research carried out for the evaluation of the effects induced by wet cleaning treatments on mechanical properties of paper is reported.

1.3 Aqueous cleaning treatments of paper artworks

Cleaning of paper artworks is a fundamental treatment with conservative purpose. The principal aim of cleaning is the removal of degradation products, contaminants, metals, salts, and any dangerous compounds formed as result of the long term effect aging, which can compromise paper integrity. For instance metals as iron, copper, etc, eventually present in paper, may work as catalyst and contribute to paper degradation, acidity phenomenon and oxidation processes. The products of cellulose hydrolysis can be also subjected to oxidation. Moreover, since paper presents a porous structure strongly dependent from the papermaking and materials added, contaminants may easily penetrate inside paper especially in humid environments, creating the ideal conditions for paper degradation. For this reason the removal of such materials by means of cleaning, can partially contribute to prevent degradation processes.

Even being a treatment with conservative purpose, cleaning represents a delicate treatment that should be performed carefully since paper artworks are complex systems and different aspects should be considered, such as the type of materials and sizing agents present in paper, papermaking, the type of organic binder present in paper, etc.

Nowadays different types of cleaning treatments are available for paper artworks based on the use of aqueous and non-aqueous systems. Cleaning treatments based on the use of water, in particular immersion treatment of paper sheets in distilled water have been widely used throughout history. One may think however how dangerous a treatment by immersion in water might be if water sensitive graphic media are present in paper. Different risks are associated to the use of water in specific conditions. To overcome these problems, a possible alternative can be the use of gel-based aqueous cleaning systems, especially rigid polysaccharide gels. The description of cleaning methodologies is provided in the following section.

1.3.1 Traditional cleaning methods

Traditional cleaning treatments are commonly based on the use of free water. Such treatment is carried out by immersion of paper sheets in distilled water, usually heated at 40°C [56, 57] with the aim to extract contaminants and water-soluble compounds in a homogeneous way [58].

Because of polarity, water can dissolve hydrophilic materials by breaking intermolecular bonds of such compounds and forming hydrogen bonds, dipolar

interactions; on the other hand because of its high dielectric constant, water can also cause the ionization of some molecules, the dissociation of acid/base substances by means of intra-molecular breaking bonds and the formation of new interactions with water.

Water is commonly used in cleaning treatment of paper, but it can be also used in the case of deacidification treatments when a basic substance is added, such as $Ca(OH)_2$, $Ca(HCO_3)_2$, $Mg(HCO_3)_2$ etc.

Nevertheless, some disadvantages may be associated to the immersion treatment:

- The use of heated water at 40°C may promote the extraction not only of degradation products, contaminants, but also sizing agents such as gelatine[9] commonly added in papermaking to improve paper properties as well as other original components of paper. The removal of such materials may cause changes in paper properties, such as the mechanical properties, since paper becomes more susceptible to water (more absorbent);
- The high rate of water absorbed by the paper during immersion in water may be responsible for partial swelling of cellulose, dilatation of fibers (especially the amorphous phase), resulting into general dimensional change of paper [59]. This phenomenon also includes the problem of shrinkage of cellulose and paper during desorption in the drying phase;
- The high amount of water absorbed by paper in an uncontrollable way during immersion treatments may represent a serious risk especially in the case of water sensitive graphic media, given that the solubilisation of such organic binder may be promoted.

The choice of the type of cleaning treatment to be carried out on paper should consider the type of paper and the degree of hydrophilicity. Cleaning treatments of paper may include also the application of organic solvents of different polarity, water-alcohol solution by means of cotton-wool swabs. In this case the application may be restricted to small areas of paper (i.e. the removal of stains of different nature present in paper).

In the last years the use of water gelling agents has been developed with the aim to prevent the problems associated to the use of free water.

[9] The denaturation temperature of collagen contained in gelatine is about 40°C.

1.3.2 Gel-based aqueous cleaning systems

When solvents (i.e.: water) are applied to a surface, part of the solvent can evaporate, another part can penetrate and diffuse inside the material depending on the porosity of materials and the vapour pressure of the solvent. The diffusion of solvent may promote the interaction between the solvent itself and the internal structure of paper, resulting into the solubilisation of compounds as function of polarity.
In the case of paper conservation, sometimes it is not possible to immerse paper sheets in distilled water, for instance, in presence of sensitive organic binder and for others reasons explained previously. In this context a localised treatment could be suitable. The use of water gelling agents can be a possible alternative to traditional cleaning treatments by immersion.

Different types of gelling agents have been tested in order to [60]:

- reduce the penetration of a liquid/solution inside a material;
- slow down the evaporation of solvent;
- improve the wetting power of liquid/solution

In fact, if cleaning solvents are entrapped in a gel matrix the surface tension may be reduced, the viscosity of the cleaning system may increase and the wetting power may be improved. When using solvents in a gelled form, a controlled treatment may be carried out since the diffusion rate of solvent is reduced according to the Washburn equation.

Different gels have been used in cleaning paintings [8] such as soft gels with cellulose ethers (es. Klucel), carboxymethyl cellulose, hydroxypropylcellulose, [61, 62] xanthan gum, polyacrilic acids salified with bases (Carbopol), and rigid polysaccharide gels (agar, agarose gels, gellan gum) [7, 8, 63]. Few studies have been performed on the use of soft gels for the cleaning of paper artifacts. More attention has been given to the use of gellan rigid gel for the cleaning treatment of paper because several advantages have been recognized from a practical point of view [10].

An important aspect to consider in case of the use of gels is the problem of residues. In general soft gels are applied with brush on the surface and then removed with the application of cotton-wool swabs. In this type of application some residues may remain on the surface after treatment. Such risk may be more probable in case of soft gels.
In the case of rigid gels the presence of gels residues may be reduced. In this context some studies carried out on the application of agar gel for the cleaning of gypsum plasters demonstrated the presence of minimal residues left on the gypsum surface after

treatment [64]. The issue of gel residues needs clarification also in the context of paper cleaning.

1.3.2.1 Rigid polysaccharide hydrogels

The use of rigid polysaccharide hydrogels, in particular agar and gellan gum, has been recently experimented for cleaning paper artworks to minimize the impact of free water during aqueous treatments of ancient paper, and to control the release of water molecules [5, 65].

Hydrogels (physical gels) are characterized by a three dimensional porous structure where polymer chains are kept together with the presence of electrostatic interactions. Agar and gellan gum are polysaccharides deriving from red algae and bacterial activity respectively. In the case of agar and gellan gum gels, it is necessary to disperse polysaccharides chains in water, subject the aqueous dispersion to heating (about 100°C) and subsequent cooling. During heating the polysaccharide chains take a random conformation [66]. In the second step, during cooling, the polymer chains start to acquire an ordered conformation with a double helix structure and the free ends of chains disordered. Once the gelation temperature is reached, the sol-gel transition occurs and the double helices interconnect to form bundled of double helices in the final stage [67, 68, 69]. Either agar or gellan gum are thermo-reversible gels.
The result of sol-gel transition is the formation of a porous rigid gels in which properties are strongly dependent from the type of polysaccharide, concentration.

a) Gellan gum

Gellan gum is high molecular mass polysaccharide gum produced by a pure culture fermentation of carbohydrates by *Pseudomonas elodea*, purified by recovery with isopropyl alcohol, dried, and milled.

In 1978 Kaneko and Kang discovered such polymer in *Kelco Division of Merck and Co.* laboratory, California, USA [70]. After toxicity tests in 1998, the product was approved in food industry in Japan. Some specification for gellan gum were performed during *46th Joint Expert Committee on Food Additives (JECFA)* in 1996 [71]. Given its structural, gelling and stabilizing properties, gellan gum started to be employed in pharmaceutical and food industry as well as in microbiological studies as growing

medium as agar. Moreover gellan gum was employed for studies in medicine, in particular for the preparation of films for effective guided bone regeneration [72].

The high molecular mass polysaccharide is principally composed of tetracyclic repeating unit of glucose (β-1,3-D glucose), glucuronic acid (β-1,4-D glucuronic acid) in salified form, glucose (β-1,4-D glucose) and rhamnose (α-1,4-L rhamnose).
In the native form gellan gum is esterified with two acyl groups (L-glycerate and acetate), respectively linked to 2-carbon and 6-carbon of glucose residue (β-1,3-D glucose), adjacent to glucuronic acid unit (Figure 1.16) [66].

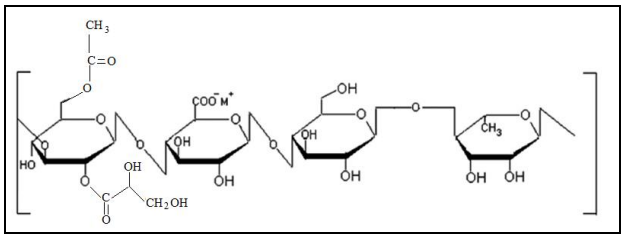


Figure 1.16: Gellan gum molecule in the native form.

Two forms of gellan gum can be considered: *High Acyl* gellan gum (which corresponds to gellan with high content of acyl groups), and *Low Acyl* gellan gum (in which the acetyl groups are removed by alkaline treatment to produce deacetylated gellan gum). The acyl groups are obtained by means of deacetylation treatment in alkaline environment (Figure 1.17).

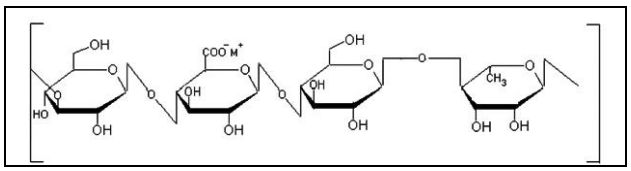


Figure 1.17: *Low Acyl* gellan gum.

High Acyl gellan gum present a molecular weight of 1-2 x 10^6 Da; the molecular weight of *Low Acyl* gellan gum is about 2-3 x 10^5 Da. The structure of gellan gum and the presence of acyl groups may strongly influence the rheological, mechanical, physical properties of gel: the polymer with high content of acyl groups determines the formation of the soft, elastic gel since the presence of glycerate and acetate groups can inhibit the association of polymer chains, making the formation of a compact, closed structure difficult. On the contrary, the deacetylated form makes possible the formation of rigid, compact, viscoelastic gels possible [73].

As described previously, the formation of the gel includes a first step in which polysaccharides are dispersed in an aqueous solution, subjected to heating and subsequently to gradual cooling.
During heating (T> 90°C, 100°C), the gellan gum (in water solution) can acquire a *random coil* structure. In the following step, during cooling, a transition from a disordered to a double helices ordered structure occurs, in which the ends of the chains remain disordered (*coil helix transition*) (Figure 1.18). The double helices start to interact by means of weak interactions (Van Der Waals interaction and hydrogen bonds) where also water molecules are involved and the *sol-gel transition* takes place.

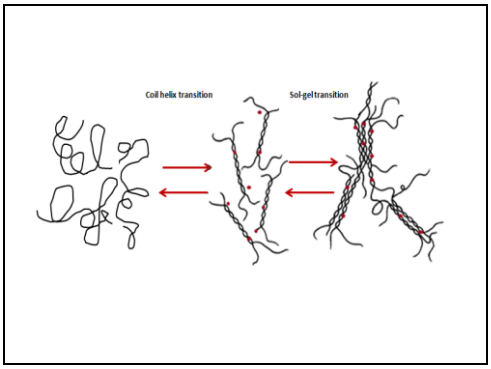
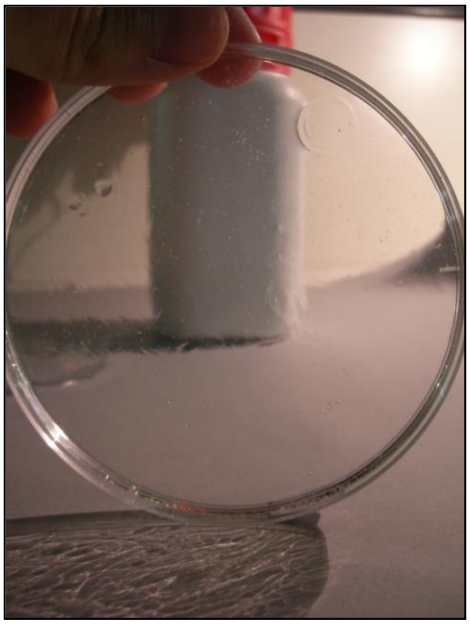


Figure 1.18: Gel formation.

Therefore, in the case of gellan gum, the formation of gel is possible only in presence of cations (monovalent or bivalent) such as calcium, in aqueous solution. These cations neutralize repulsive electrostatic interactions between helices due to the presence of carboxylate groups, and promote the interaction between gellan gum molecules. In this way thermo-reversible gels can be obtained with a compact structure and a degree of porosity in which a certain amount of water can be entrapped.

The gelation temperature can be different (from 50 to 30°C) depending on the gellan concentration in solution, the amount of acyl groups present in the structure and on the amount of cations present in water.

The process of gel formation has been researched. Several theories were developed on the formation of gel network, such as the formation of a branched, fibrous network described after application of light scattering analysis and observation by means of atomic force microscopy (AFM) [74].



Gellan gum gel properties are strongly dependent on several aspects, such as gellan gum structure, concentration, the amount of acyl groups, the amount of cations, pH value of the solution, etc. In the field of conservation the *Low acyl* gellan gum was selected in the study carried out by ICRCAPL[10], because the low amount of acyl present in the structure allowed the formation of rigid, compact, very transparent, viscoelastic gels (Figure 1.19).

Figure 1.19: Gellan gum gel 2% (w/v) with 5 mm of thickness: observation in transmitted VIS light. It is evident the transparency of the gel.

These types of gel may be considered "molecular sponges", in which a certain amount of water molecules can be entrapped inside pores and then released in a gradual, controlled way (*syneresis process*). For this reason gellan gum structure plays a relevant role.

[10] Istituto Centrale per il Restauro e la Conservazione del Patrimonio Archivistico e Librario (ICRCPAL), Via Milano, 76 00184 Roma, www.icpal.beniculturali.it

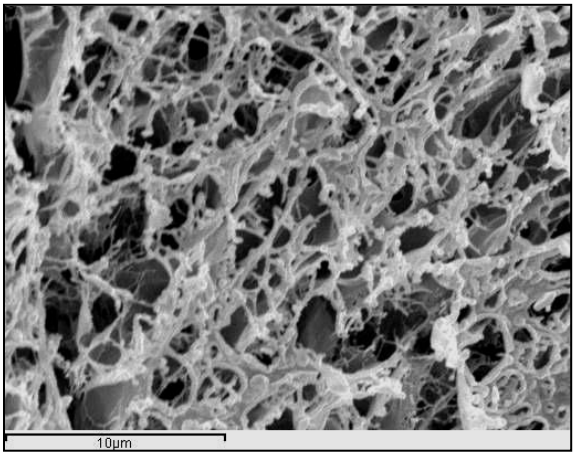


Figure 1.20: Image of gellan gum gel 3% prepared with 2.53 mM of calcium acquired by means of Cryo-SEM in the cross section at 5000x magnification.

In fact, gellan gum gel, as all hydrogels, presents a porous structure, but in this specific case it is characterized by the presence of two microstructures of pores (pores size 0.1-1 µm) which constitute the gel network (Figure 1.20).

The relationship between its microstructure and the property of holding water molecules inside the structure and syneresis was studied by means of Cryo-SEM analysis [75]. Studies showed that the first microstructure, characterized by smaller pores, was responsible for structural stabilization of gel, and pores size did not depend on calcium concentration. Moreover this microstructure regulated the absorption and release process of water molecules. On the contrary, in the second microstructure, (characterized by larger pores), pore size depended on calcium concentration.
The consequence was the formation of flexible and stiff gels respectively as a function of the amount of calcium below and above the critical concentration of calcium (about 240 mg/L for gellan gum 1% w/v), which also influenced the rheological properties of the resulting gels.

As described previously, such porous structure and gel concentration influence the release of water molecules. This aspect is fundamental in the application methodologies of gels. Some studies conducted by ICRCPAL on the use of gellan gum at different concentrations for the cleaning of Whatman and ancient papers of different periods (16^{th}, 18^{th} and 19^{th} centuries) demonstrated that lower concentration of gellan gum may release more water on paper than gels at higher concentrations after 1 hour of treatment [10].

For instance the increase in weight of Whatman paper treated with gellan gum (4% w/v concentration) was 76% due to the absorption of water molecules; on the contrary, when 1% gellan gum gel was used, the absorption of water caused an increase in paper weight of 164,6%. The absorption of water was lower in the case of ancient papers because of the presence of sizing agents and inorganic filler. The behaviour of gellan gum gels at different concentrations was compared to immersion treatment; the increment in the Whatman paper weight due to the absorption of water during immersion was 261% [10]. These preliminary studies underlined the possibility of searching for an alternative that helped control the release of water using gels at different concentration, which would also allow the use of gels in case of water sensitive media.

Cleaning treatment of paper by means of gellan gum is usually performed by applying the gel on paper surface and if necessary, using a Japanese paper sheet between paper and gel to eventually protect water sensitive organic binders present in paper. [76]. The contact between gel and paper surface is fundamental to promote the extraction of degradation products, contaminants, salts, etc, from paper (Figure 1.21).

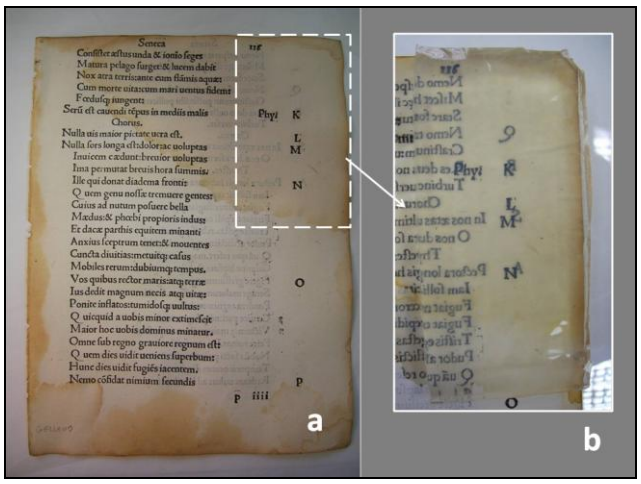


Figure 1.21: Cleaning treatment by means of gellan gum 2% (w/v) of a 16th century paper: a) the paper before the cleaning; b) application of gellan gum on the paper surface. The transparency of gel is evident.

In chapters 5 and 6 the analytical study of the extraction of paper components during cleaning treatment by rigid gels is presented. In fact the aspect of the characterization of compounds extracted by rigid gels is fundamental to understand the effects induced by gels in paper properties during cleaning treatments.
The description of the mechanism of interaction between gel and compounds extracted during cleaning treatment of paper is very complex. In this context the structure of gel could play a relevant role and the cleaning mechanism could be the result of physical interactions between the gel and compounds extracted. After cleaning treatment of degraded paper, a slow diffusion of extracted products inside gel, often appreciated in the section of gel, is evident. This diffusion from the surface in contact with the paper to the internal structure of the gel is often evidenced by the yellowing of gel. (Figure 1.22).

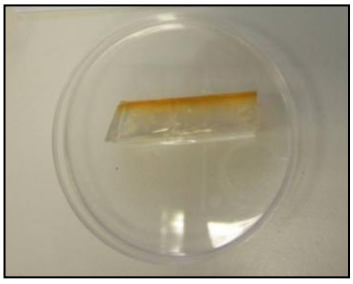


Figure 1.22: Observation of gellan gum gel 2% (w/v) in section after application on a 17th century paper for the cleaning treatment. A yellowing of the gel is observed after the application on paper caused by the migration of degradation products, organic acids from paper to the gel structure.

A possible explication for this phenomenon could be that after the solubilisation of hydrophilic compounds extracted by the gradual release of water by gel, electrostatic interactions and eventually hydrogen bonds between polysaccharide chains of gel and the extracted compounds may be established. These interactions could promote the entrapment of such components inside the porous structure of gel.

b) Agar gel

Agar is a complex polysaccharide extracted from seaweed *(Rhodophyta).* It is employed in food, microbiological industry [64]. From the chemical point of view it is characterized by galactose present in two macromolecules: agarose and agaropectin. Agarose is a linear polimer of galactose. It is formed by agarobiose, disaccharide characterized by the presence of a galactopyranose unit and a anydrogalactopyranose unit linked by α- 1,4- glycosidic bond (Figure 1.23). Agarobiose represents the gelling fraction of agar [60].

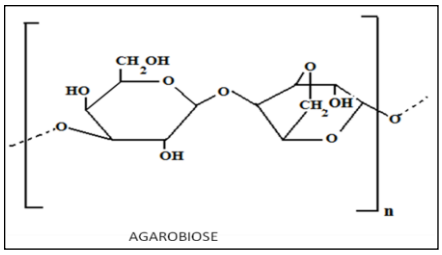


Figure 1.23: Agarobiose structure

Agaropectin is the salified fraction, containing sulphate groups and it is not responsible for the formation of gel.

Agar and agarose are not water soluble at room temperature. For this reason it is necessary to heat water at least 80°C for gel preparation (temperature of agar solubilzation) and then disperse such polymer in water. When such dispersion is cooled, polymer chains acquire an ordered conformation, in particular a helix structure, which make possible the interaction between chains and the formation of a compact network. In this case the gelation temperature is about 40°C (lower temperature than that of gellan gum). Below this temperature, agar dispersed in water becomes a rigid gel. In the case of agar gel is not necessary the introduction of cations in solution for the formation of the gel. Also for agar, gel syneresis process occurs and this phenomenon is responsible of a gradual release of water molecules by gel.

Both agar and gellan gum are thermo-reversible gels. In the case of agar, the final gel obtained results less transparent than the gellan gum one (Figure 1.24).

Figure 1.24: Agar rigid gel 2% after gelation.

Several studies were performed on the use of agar gel in the conservation field. As described previously, agar gel was and is still applied for cleaning treatments of artworks [63, 64, 9, 77]. Thanks to its porous structure and the low gelation temperature, agar gel may be also used as medium for enzymes in the field of conservation which can be very useful when a more specific cleaning action is needed (i.e.: the removal of organic adhesives of starch, animal glue, stains or repainting oil present in artistic objects) [78, 1].

2. Aim of the research

The aim of the research is the analytical evaluation of the effects of aqueous cleaning treatments in the mechanical, physical and chemical properties of paper. Aqueous cleaning treatments considered are immersion in distilled water (which represents the most diffused method used in the context of paper conservation) and the application of rigid polysaccharide gels such as agar and gellan gum gels.
Such treatments are carried out on paper samples of different periods (16^{th}, 17^{th}, 18^{th} and 19^{th} century) as well as on Whatman paper, since they are representative of different manufacturing, and for this reason, they present different features.

This work can be divided in three sections:

- *Introduction*: where the principal conditions of paper degradation are presented and it is described how paper composition and interaction between paper and environmental conditions may affect paper conservation. The introduction includes also the description of the commonest aqueous cleaning treatments usually applied on paper artworks, analyzing also the issue of interaction water-cellulose and the effects in paper properties caused by such interaction;
- *Experimental section:* (chapters 3, 4, 5, 6, 7) in which the effects induced by cleaning treatments on paper are analyzed by considering different aspects (i.e.: the effect of cleaning in paper morphology, mechanical properties, the evaluation of the extraction of original components of paper induced by aqueous cleaning treatments as well as the problem of eventual residues left by rigid gels on paper);
- *Conclusions:* that represents a general summary of the results obtained.

The research considers different aspects. Firstly, the influence of aqueous cleaning treatments in the **mechanical properties** of paper is evaluated by means of tensile tests. In this context such tests are carried out on modern almost pure-cellulose paper (filter paper) with a known pore size, in order to eliminate any variables due to papermaking, condition, etc. The research is extended also to assess the effects of accelerated aging (thermo-hygrometric aging) in the mechanical properties of paper, by verifying if paper treated may be more susceptible to degradation and consequently weaker with respect to the control samples. In this context the results of tensile testing are compared to the microscopic morphological observations of both fracture and fibres after cleaning treatments as well as after accelerated artificial aging.

Secondly, in the field of paper artworks, the original texture of paper represents a fundamental aspect to preserve since it is the result of the different types of

papermaking developed over the centuries. In paper artworks some of the traditional cleaning treatments consist in applying cellulose ethers by brush and then removing them by cotton swabs. These treatments may cause a surface degradation of paper. Considering this phenomenon it may be crucial to understand what is the effect induced by different types of aqueous cleaning treatments (ranging from immersion treatments to less invasive ones such as gelled systems) on the **morphological properties** of paper surface; in other words, to evaluate if aqueous cleaning treatments performed on paper may cause alteration in the morphology of paper surface. This aspect was evaluated by comparing different aqueous cleaning treatments performed on ancient paper and analyzing the effects of such treatments in paper surface by means of SEM-EDS analysis and by the use of 3d profilometer.

A third aspect considered in the work is the extraction ability of aqueous cleaning treatments (immersion washing treatment compared to the application of rigid gels of agar and gellan gum) of components present in paper. As known, the principal purpose of aqueous cleaning treatments is the removal, the extraction of degradation products and contaminants of hydrophilic nature, in order to preserve paper from degradation processes. In this context, few attention has traditionally been given to the problem of the **extraction of original components** of paper caused by water during aqueous treatments and to changes induced in the mechanical properties of paper as consequence of this phenomenon. For this reason in chapters 5 and 6, the extraction of original components such as hemicelluloses and sizing agents (gelatine) from paper induced by the selected cleaning methodologies is also evaluated by means of Gas Chromatography Mass Spectrometry analysis (GC-MS). Such evaluation is performed by qualitative and semi-quantitative analysis of analytes extracted from paper dating from different periods (from 16^{th} to 19^{th} century).

The last aspect evaluated is the **residue issue.** In chapter 7 a qualitative and semi-quantitative study by GC-MS analysis of eventually monosaccharides left by gels is preformed, by comparing among the application of different gels (in different concentrations) on Whatman paper. Such study represents a preliminary evaluation. In the future it might be interesting to develop a similar study on actual paper artworks.

Considering all aspects described briefly in this section, the general aim of the work is to verify what methodology can be less invasive and more respectful with the intrinsic characteristics and behaviour of paper when paper has to be subjected to any sort of aqueous cleaning treatment. For this reason a comparative evaluation of different cleaning treatments was performed.
Such results may help paper conservators to accomplish cleaning treatments in a critical way evaluating both the effectiveness of the removal of degradation products as well as

the problem of the extraction of original components from paper which may influence paper properties and mid-to-long term stability. For this reason cleaning treatments should be designed considering the type and characteristics of paper and its condition. In this context, the scientific characterization of paper by means of chemical and physical analysis may be of great help to understand not only the materials present in the work of art but also the degradation products found in them and, therefore to select the most appropriate methods and materials once conservation decisions have been taken.

3. Evaluation of the influence of wet cleaning treatments in the mechanical properties of paper

The research can be divided into two phases: the first part was intended to evaluate the effects of the previous wet cleaning treatments in the mechanical properties of paper by tensile tests. Having performed a study on the mechanical properties of filter paper, the main aim was to verify if the water intake induced by cleaning treatments can contribute to a significant modification of the mechanical properties of paper, in terms of ultimate tensile strength, deformation at break and flexibility. For this purpose the research correlated different kinds of aqueous cleaning treatments. The selected treatments (immersion in distilled water called also *washing treatment*, and the application of rigid gels of agar and gellan gum) were performed on filter paper of known composition with the aim to reduce the variables to the minimum.

The second part of the research was intended to assess the effects of thermo-hygrometric accelerated artificial aging (70°C and 65% RH[1]) in the mechanical properties of treated and untreated samples subjected to similar artificial aging conditions. The objective was to verify if cleaning treatments could enhance any sort of degradation in the mid-to-long term, in other words, if treated samples might be more prone to degradation after treatment. This was done by correlating the mechanical properties of the different samples selected.

The choice of appropriate conditions of accelerated aging, as a function of the materials to study, and the real correspondence between accelerated and natural aging is still controversial nowadays. For instance, different studies have been performed on the evaluation of the effects of accelerated aging in the mechanical, physical, chemical properties of papers, in which different aging conditions have been compared (dry heated aging and moist heat aging at different temperature, and UV accelerated aging) [22,79,80]. It was demonstrated that the dependence between cellulose degradation and relative humidity was much more relevant than temperature. In moist heat aging, the higher relative humidity, the more significant loss of strength is.

Considering thermo-hygrometric accelerated aging, according the NORMAL 5630-3 an aging timeframe of 144 hours was suggested. In some studies focused on the evaluation of the mechanical properties of paper subjected to thermo-hygrometric accelerated aging different temperatures and relative humidity conditions were compared and many

[1] Conditions similar to the normal UNIISO 5630-3 accelerated aging moist heat treatment.

more hours of aging were needed to appreciate significant variations in paper properties (i.e. loss of strength caused by hydrolytic phenomena of cellulose).[22].
On the other hand, and according to some studies, dry heat ageing was not suggested because in such condition paper seems to experience a different aging with respect to natural aging [81]. For instance, an indicator of paper degradation could be glucose and oligomers of glucose released as consequence of cellulose hydrolysis processes, and xylose which derives from hemicelluloses hydrolysis. The amount of these compounds is higher in the case of acidic and oxidized paper. In the case of dry aging of paper at 90 to 150°C, only small amounts of these water soluble compounds were detected by GC-MS analysis [22]. Moreover, accelerated aging with the use of temperatures higher than 100°C may cause intensive oxidation, dehydration or alkoxy elimination of cellulose chains, and it may alter the water sorption capacity in paper [81]. For these reasons this type of accelerated aging of cellulose seems to be less appropriate. A different accelerated artificial aging could be the use of UV radiation, which can induce photoxidation and hydrolytic phenomena.

Accelerated aging may produce degradation of paper but sometimes it is difficult to ensure that, in specific conditions, it correlates to exactly the same reactions (and degradation) than those that take place during natural aging. The determination of the most convenient accelerated aging conditions should be made according to the material itself and considering also the environment conditions in which material could be stored. On the other hand and considering the most common conditions in which paper might be subjected in uncontrolled environments, it is possible to observe that temperature and humidity play a relevant role in the definition of paper degradation. For this reason specific thermo-hygrometric accelerated aging conditions were chosen for the evaluation of the mechanical properties of paper.

In addition, paper represents a very complex system because its properties are defined by a combination of factors (composition, papermaking processes, additives, the interaction with environment, etc.) which should be taken into account in every single study. For this reason it is important to specify that, the study carried out relates only to the selected paper and in such specific conditions.

In this research the morphological observation of paper was performed by means of Light Microscopy and Scanning Electron Microscopy (SEM) to add fundamental information and explain the mechanical properties of the selected paper samples. Moreover colorimetric analysis was carried out to assess the effects of cleaning treatment and eventual changes in the appearance of paper.

3.1 Materials and samples preparation

3.1.1 Samples

The research was performed on filter paper samples (type: LABOR n.s cod. 05520045, INCOFAR) (Table 3.1).

Table 3.1: Filter paper used

Sample	Grammatura ($g \cdot m^2$)	Thickness (μm)	pH
Filter paper (type: Labor)	67	130	6.78

Paper sheets were subjected to the selected wet cleaning treatments (immersion washing in distilled water, application of agar and gellan gum gels 1-2-3%). After natural drying (25°C and 55% RH) paper strips measuring 5 x140 mm were obtained (Figures 3.1, 3.2).

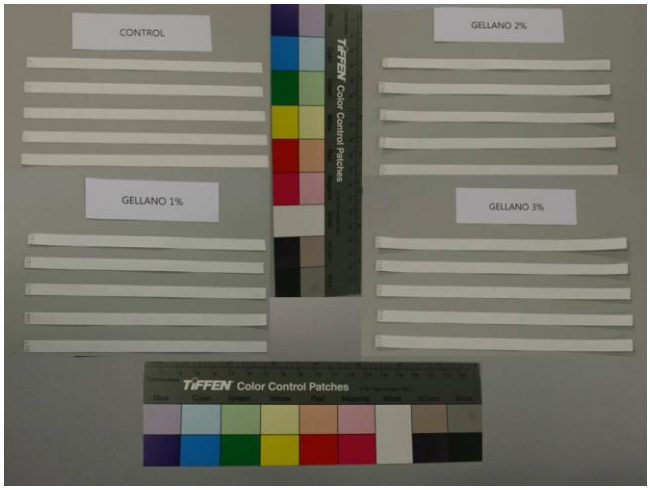


Figure 3.1: Paper samples for tensile tests.

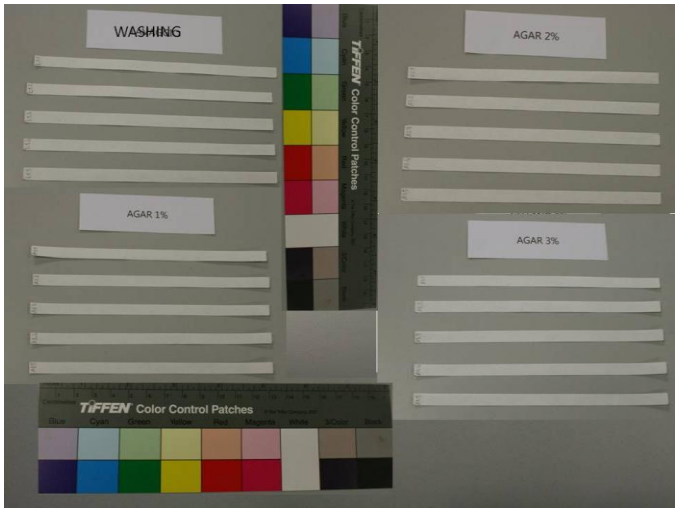


Figure 3.2: Paper samples for tensile tests.

The samples were cut and tested in the cross-machine direction. All tests were carried out in the same conditions and the results were correlated. The tensile tests were performed on unaged and aged samples (control and treated samples in both cases) after different exposure to artificial aging (24, 72 and 144h).

For each set of samples three replicates were tested for the evaluation of mechanical properties (Table 3.2).

Table 3.2: Paper samples after aqueous treatment and subjected to tensile testing

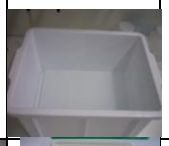
Treatment	**Unaged samples**		**Aged samples**		
			24 h	**72h**	**144h**
Control (untreated samples)	C-0h		C-24h	C-72h	C-144h
Immersion washing in distilled water	W-0h		W-24h	W-72h	W-144h
Agar gel	1%	A1-0h	A1-24h	A1-72h	A1-144h
	2%	A2-0h	A2-24h	A2-72h	A2-144h
	3%	A3-0h	A3-24h	A3-72h	A3-144h
Gellan gum	1%	G1-0h	G1-24h	G1-72h	G1-144h
	2%	G2-0h	G2-24h	G2-72h	G2-144h
	3%	G3-0h	G3-24h	G3-72h	G3-144h

3.1.2 Cleaning treatments

The paper was subjected to different aqueous cleaning treatments, in particular (Table 3.3):

- Immersion in distilled water (duration: 10 minutes; pH of water: 6);
- Application of agar rigid gels at different concentrations (1-2-3% w/v) (duration of the treatment: 15 minutes);
- Application of gellan gum rigid gels at different concentrations (1-2-3% w/v) (duration of the treatment: 15 minutes).

Table 3.3: Steps of cleaning treatments

Treatment	Preparation	Application	Paper sheets after cleaning treatment
Immersion washing in distilled water (10 minutes)			
Application of agar gel 1% (15 minutes)			
Application of agar gel 2% (15 minutes)			
Application of agar gel 3% (15 minutes)			
Application of gellan gum gel 1% (15 minutes)			
Application of gellan gum gel 2% (15 minutes)			
Application of gellan gum gel 3% (15 minutes)			

Immersion treatment was performed by immersing the different paper sheets in a bath of distilled water at room temperature.

Agar gels (1-2-3% w/v) were prepared by adding the quantity of agar in powder (10, 20 and 30 g, respectively) to distilled water (1L) previously heated to 85°C [63]. Resulting dispersions were heated by microwave oven at 900W for 10 minutes and then poured into a plastic container to cool down thus reaching the gelling temperature (38°C).

Gellan gum gels (1-2-3% w/v) were prepared by dissolving calcium acetate (0.4 g) and gellan gum in powder (10, 20, 30 g, respectively) in distilled water (1L) at room temperature [73,10]. Resulting solutions were then heated by microwave oven (900 W for 10 minutes), poured into a plastic container and cooled down thus reaching gelling temperature (40°C) (Figure 3.3).

Figure 3.3: Agar and gellan gum gels (1-2-3% w/v) used for the cleaning treatments.

The different gels were applied on the surface of the paper sheets and removed after 15 minutes (Figures 3.4, 3.5).

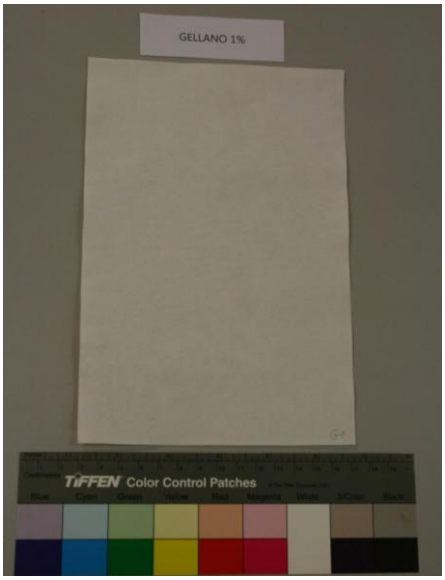


Figure 3.4: Paper sheet after cleaning treatment with gellan gum gel 1%.

Figure 3.5: Paper sheet after cleaning treatment with agar gel 1%.

3.2 Instruments and analytical procedure

3.2.1 Tensile testing of paper samples

EQUIPMENT

Static uniaxial tensile testing was carried out with the equipment donated by the *Smithsonian Museum Conservation Institute* (Washington D.C.). The equipment consists of a rectangular methacrylate box that contains several tensile testers. This box acts as a climatic chamber where relative humidity (RH) and temperature (T) can be controlled. The tensile testers are located in the upper part of the chamber whereas the light bulb and the tray are located on the lower level. A small fan makes the air circulate within the chamber. The fan accelerates the humidity absorption of the silica gel and creates stable and homogenous environmental conditions inside the chamber. A high precision dew point hygrometer is connected to the chamber and a small hygrometer is also located inside it.

MOUNTING AND CONDITIONING OF SAMPLES

Samples were clamped in the testing gauges and the allowed to come into equilibrium in the chamber for 24 hours at 48.0 (±0.5% RH) and 24 (±0.5°C) prior to testing. Increments of strain 0.0625 mm were applied progressively at 30 second intervals. Three tests were run for each type of samples in order to get consistent results.

TESTS' CONDITIONS

The paper specimens were subjected to tensile loading. The tests were performed at a displacement rate of 3.75 mm/min and at 50.0 (± 2%) RH and 23 (±1ºC). Such crosshead speed represents a speed at which induced strains might occur on works of art according to the specialized literature. Data analysis was undertaken using a customized Microsoft Excel spreadsheet. The results obtained from tensile-tests were expressed by means of stress-strain curves. The elastic modulus was calculated considering the slope of the linear section of the curve in the elastic region (data processing by Microsoft excel 2007). A micrometer (Outside Micrometer Mitutoyo, model: M317-25; measuring range: 0-25mm; graduation 0.01 mm) was used to measure thickness of samples. The accelerated aging of samples (in thermo-hygrometric controlled conditions) was performed on a climatic chamber DYCOMETAL MODEL CCK-25/300, and different set of samples was prepared and subjected to different hours of aging (24h,72h, 144h).

3.2.2 Observation of the fracture area after tensile testing by means of Light Microscopy

Paper samples were observed by means of stereo-microscope (LEICA, MZ APO; observation of samples and images acquisition: LEICA MICROSYSTEMS) (magnifications: 8x, 50x). The type of the fracture after tensile-tests was evaluated for unaged and aged samples (144 h).

3.2.3 Scanning Electron Microscopy (SEM) observations

A scanning electron microscope JEOL JSM 6300 equipped with an energy-dispersive X-ray analyzer was used to morphological observations, to evaluate the effects induced by cleaning treatments and the effect of ageing on paper samples. The images were acquired in secondary electron mode with an acceleration voltage of 5 KeV and working distance of 14 mm. The samples were previously coated with a graphite layer (0.1µm of thickness) by sputtering.

3.2.4 Colorimetric analysis of paper samples

Colorimetric analysis were performed on papers before and after cleaning treatments by means of Minolta CM-2600d spectrophotometer (KONICA MINOLTA SENSING, Inc.) interfaced to a PC. Measurements were taken with the excluded and included specular component (SCE and SCI), using illuminant CIE D65 (6500ºK) and 10º standard observer (CIE'64). For each samples the measures were acquired in 6 different points, and they were repeated also after accelerated aging. The average of L*, a*, b* values was considered and the color-difference ΔE was calculated [82].

$$\Delta E^*_{ab}=\sqrt{(L^*_1-L^*_2)^2 + (a^*_1- a^*_2)^2 + (b^*_1- b^*_2)^2}$$

Moreover the colour-difference ΔE_{00} according to CIEDE2000 was also considered, which represents an extension of the CIE 1976 (L*a*b*). In this case the colour-difference was calculated including corrections for change in colour-difference perception, in particular on lightness, chroma, hue an chroma-hue interaction [83, 84,85]. The Reflectance measures were performed in the range 400-700 nm. The colour-difference was calculated and the colour representation was carried out by *Colour & Colorimetry* program[2].

[2] The software *Colour & Colorimetry* is matched to the book: Claudio Oleari, Misurare il Colore, II edizione, Hoepli, 2008, ISBN 978-88-203-4126-8.

3.3 Results and Discussion

3.3.1 Tensile testing of paper samples

In this section the main differences between the mechanical properties of control paper (untreated paper) and paper subjected to wet cleaning treatments (application of agar and gellan gum gel as well as washing by immersion in distilled water) are presented. The same type of comparison was performed on aged samples (control and treated samples) at different aging steps (24h, 72h, 144h).
The measured mechanical properties are mainly focused on the evaluation of the stress-strain curves obtained where samples' maximum deformation at break, UTS can be observed.

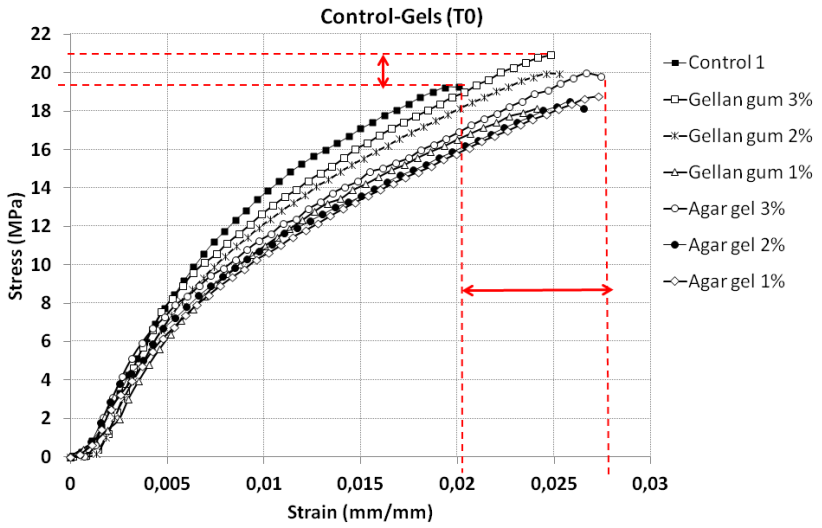


Figure 3.6: Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples (control, treatment with gellan gum and agar gels 3% - 2% - 1%). The force per cross-sectional area (nominal stress) is plotted as a function of dimensional change (strain).

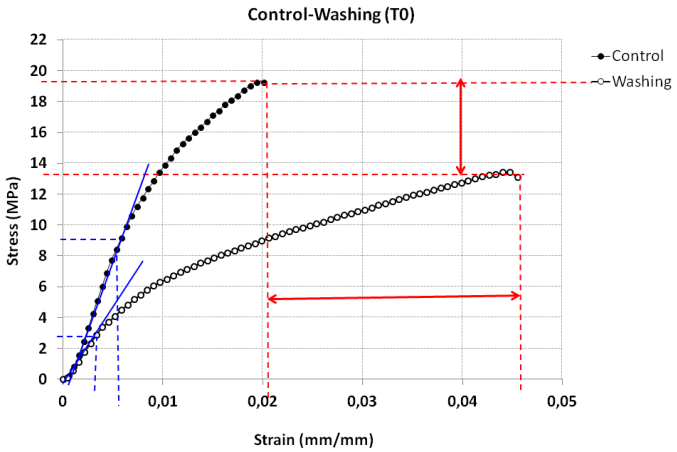


Figure 3.7:Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples (control and immersion washing treatment). The force per cross-sectional area (nominal stress) is plotted as a function of dimensional change (strain).

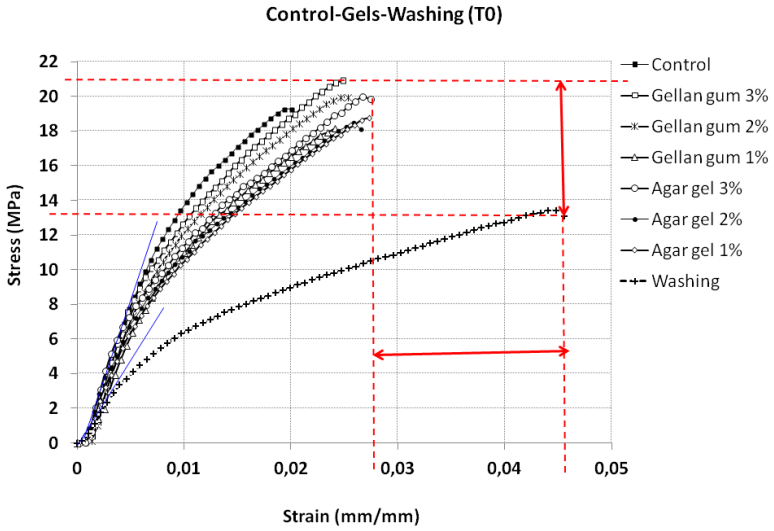


Figure 3.8: Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples (control, immersion washing, treatment with gellan gum and agar gels 3%- 2%- 1%). The force per cross-sectional area (nominal stress) is plotted as a function of dimensional change (strain).

The stress-strain curves of samples paper treated by means of rigid gels compared to the control are shown in Figure 3.6, in which the force per cross-sectional area (nominal stress) was plotted as a function of dimensional change (strain). The initial linear portion defines the elastic region where deformations are reversible; the slope of this linear section of the curve represents the elastic modulus (which is a measure of the stiffness of a material). The second region of the curve is the plastic region where deformations are permanent. The end of the curve represents the breaking point which corresponds to both a value of ultimate tensile strength and breaking strain.

Considering the gels at different concentrations a trend to a slight increase in the strength with increasing gel concentration (specially in the case of gellan gum) was observed. Nevertheless, the differences between different rigid gels as well as different concentrations were not significant enough.

The controlled water intake offered by gels resulted into a moderate increase in strain in comparison to the control and a slight increase in the strength especially in the case of those gels with the highest concentration. In fact the use of gellan gum (3%) produced an increase in the elongation at break (19%), and a moderate increase in the strength to failure (8%) in comparison to the control samples. Agar gel (3%) also caused an increase in the elongation at break (27%), and a slight increase in the ultimate tensile strength (3%) in comparison to the control samples.

Cleaning by immersion in distilled water produced a consistent increase in the strain to failure (56%), a loss of strength (32%) and a slight decrease in the elastic modulus in comparison to the control samples, as shown in Figure 3.7. The water intake due to the treatment seems to make the paper more flexible and extensible compared to the control one. This could be explained by the formation of inter-molecular hydrogen bonds between cellulose chains and the water molecules during immersion treatments.
Such phenomenon can be controlled with the application of water in gelled systems (Figure 3.8). The water intake depends on gel concentration (higher water intake with lower concentrations), and this can also be observed in terms of the mechanical properties of the samples treated with gels at different concentrations in correlation to those of the control paper and to the samples treated by immersion. The difference of breaking strains between samples treated with agar gel (1%) and those subjected to immersion was 40%. Nevertheless a higher strength (37%) between samples treated with gellan gum (3%) and those subjected to immersion was also observed. On the one hand the gradual water intake (as it happens with rigid gels) can promote the partial formation of hydrogen bonds between cellulose chains and the water molecules, resulting in the improvement of the mechanical properties of paper (moderate increment in the strength and strain in comparison to the control samples). On the other hand, the

exaggerated water intake offered by immersion can promote significant alterations in the elastic modulus and the mechanical properties. In fact the high rate of water absorbed by cellulose fibres of paper may cause the formation of hydrogen bonds between water molecules and cellulose, with the partial weakening and destruction of inter-molecular interactions between cellulose chains. Therefore the swelling action of water may determine the separation of cellulose chains and at the same time makes them more flexible thanks to its plasticizer behaviour [86]. The result is a significant increase in flexibility, a decrease in the elastic modulus and a slight weakening of the structure (lower UTS in comparison to the control sample).

Another aspect to consider is paper composition. The type of pulp, the additives, the presence or not of inorganic fillers may influence the mechanical properties of paper in a different way and will induce a different behaviour to paper if subjected to cleaning treatments. Different studies demonstrated that the strength may be influenced also by the cellulose content. In fact for a content of cellulose up to 80% the strength can increase, after which a decrease may occur [87,88,89]. The content of cellulose, especially in the case of industrial paper, depends on the type of papermaking and the treatments applied for the removal of incrusting substances (i.e.: hemicelluloses and lignin) from pulp wood. The rate of hemicelluloses in pulp wood may also influence the mechanical properties of paper partially. In fact, hemicelluloses may have an important role for the internal cohesion of the cell wall; if they are removed a partial weakening of cellulose fibres may occur [52]. For this reason it could be interesting also to consider the extraction of hemicelluloses that could eventually take place during immersion treatments.

Having obtained similar results for gel at different concentrations in the tensile tests carried out for aged samples treated with agar and gellan gum gels, only the 3% concentration was selected and the results are shown in the next figures.

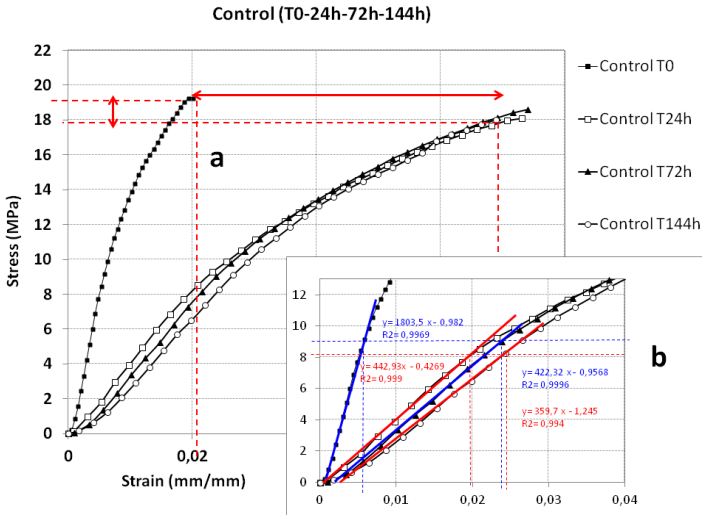


Figure 3.9: Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples: a) unaged control (control T0) and control subjected to different aging exposure (T24h, T72h, T144h); b) magnification of the elastic region.

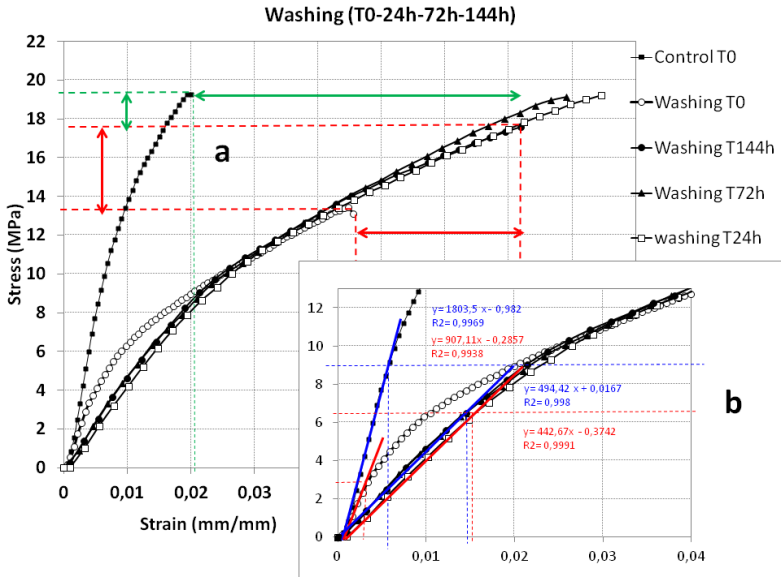


Figure 3.10: Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples: a) unaged control (control T0) and paper treated with immersion washing subjected to different aging exposure (T0, T24h, T72h, T144h); b) magnification of the elastic region.

Similar samples were subjected to accelerated aging (70°C and 65% RH) for different exposure (24,74 and,144 hours) and then tensile tests were carried out to evaluate if any variation in the mechanical properties of papers induced by accelerated aging had taken place as well as to verify if such variations were more significant in the case of the treated samples.

The stress-strain curve of unaged control paper was compared to the curves of control paper subjected to different aging exposures (24, 72 and 144h), as shown in Figure 3.9. The aging conditions seemed to have caused an increase in the strain to failure (70%), lower UTS (6%) as well as a decrease in the elastic modulus (about 60%) with respect to the unaged control samples. Paper flexiblity seemed to have increased slightly with longer exposures. This phenomenon could be related to the humid aging conditions to which paper was subjected, where the increased flexiblity could be the consequence of the swelling action of water from humidity condensation.

Unaged paper samples subjected to immersion in distilled water were compared to the unaged control as well as to similar samples subjected to different aging conditions (70°C and 65% RH for 24, 72 and 144 h) (Figure 3.10). The stress-strain curve obtained evidences how immersion treatments could cause an "acceleration" of the aging phenomenon of paper. A decrease in the ultimate tensile strength (26%) and the strain (37%) were observed for the unaged sample treated by immersion compared to the curve obtained after 144 hours of aging. Even in this case the accelerated aging caused a decrease in the elastic modulus (about 46% in comparison to the same samples unaged) and an increase in the portion of the elastic region in comparison to unaged samples.

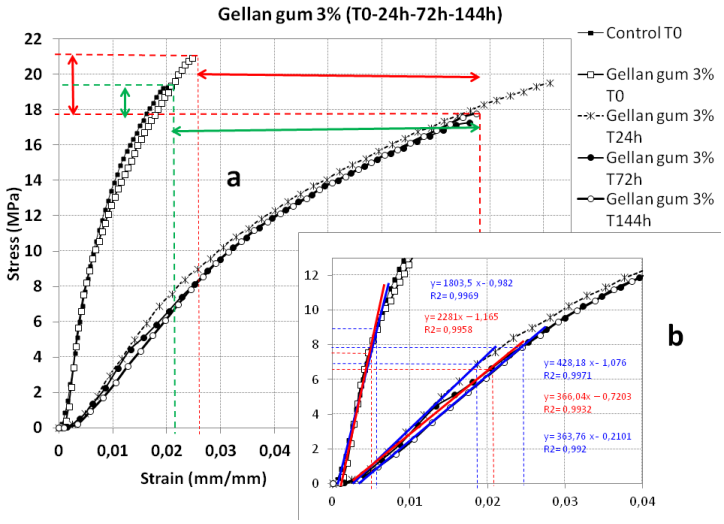


Figure 3.11: Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples: a) unaged control (control T0) and paper treated with gellan gum gel 3% unaged (gellan gum 3% T0) and subjected to different aging exposure (T24h, T72h, T144h); b) magnification of the elastic region.

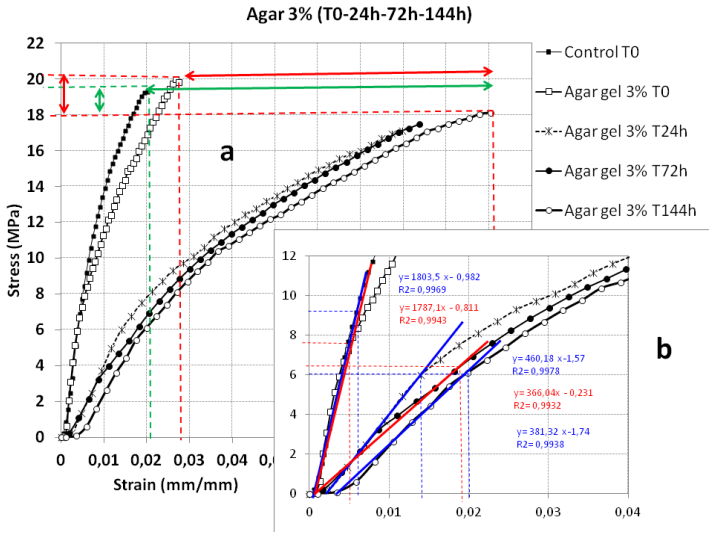


Figure 3.12: Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples: a) unaged control (control T0) and paper treated with agar gel 3% unaged (agar gel 3% T0) and subjected to different aging exposure (T24h, T72h, T144h); b) magnification of the elastic region.

In the case of samples treated with rigid gels (Figures 3.11, 3.12), the accelerated aging caused a loss of ultimate tensile strength (about 15%), an increase in the strain (about 68%) and a decrease in the elastic modulus comparable to the aged control sample.

The sample treated with agar evidenced a loss of breaking strength of about 13%, and an increment in the elongation at break (75%) after aging. The aging effect on samples treated by rigid gels was comparable to that of control samples: in the case of paper samples treated with gellan gum gels, the loss of ultimate tensile strength of about 8% and increase in the strain of about 74% after aging (comparison between the samples treated with gellan gum aged for 144 h and the unaged control), was similar to the effects observed in the control sample (loss of strength of about 6% and increase in the strain of about 71% after aging) (Figure 3.13). The same behaviour was evidenced in the case of samples treated with agar (loss of strength of 6% and increase in the strain of 80%) (Figure 3.14).

In the case of samples immersed in water, the main differences in comparison to the control were already observed between unaged samples; after 144 hours, a lower ultimate tensile strength (9%) and an increment in the strain (72%) in comparison to the unaged control were observed (Figures 3.15, 3.16).

Those samples treated with gellan gum gel (3%) and subjected to aging evidenced a similar behaviour than samples treated with agar gel (3%), but a slight increment in the strain was observed in the case of samples treated with agar gel (3%) subjected to 144 h of aging compared to the samples treated with gellan gum gel (3%) and subjected to 144 h of aging (Figure 3.17).

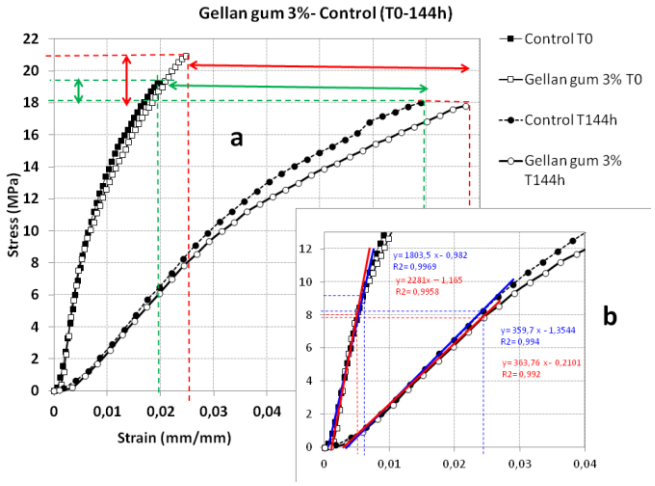


Figure 3.13: Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples: a) unaged control (Control T0), control aged at 144h (Control T144h), unaged paper treated with gellan gum gel 3% (gellan gum T0) and paper treated with gellan gum gel 3% aged at 144 hours (gellan gum 3% T144h); b) magnification of the elastic region.

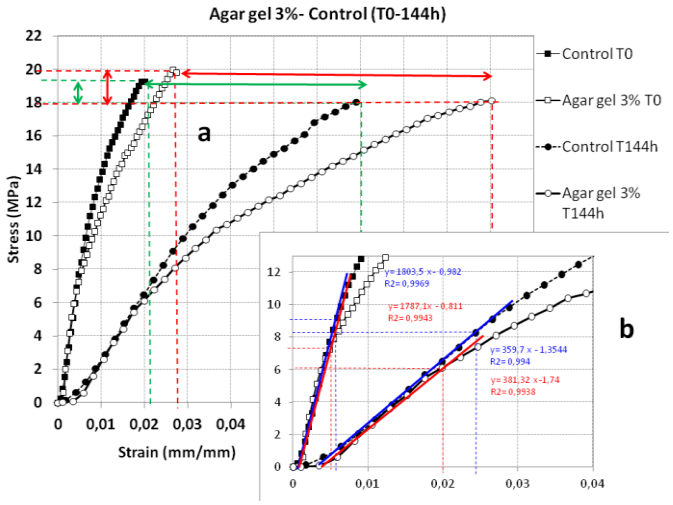


Figure 3.14: Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples: a) unaged control (Control T0), control aged at 144h (Control T144h), unaged paper treated with agar gel 3% (agar gel 3% T0) and paper treated with agar gel 3% aged at 144 hours (agar gel 3% T144h); b) magnification of the elastic region.

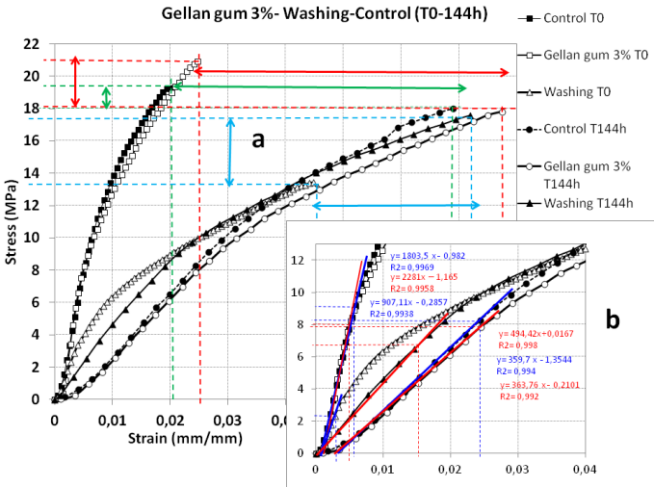


Figure 3.15: Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples: a) unaged control (Control T0), control aged at 144h (Control T144h), paper treated with gellan gum gel 3% unaged (gellan gum T0) and aged at 144 hours (gellan gum 3% T144h), unaged paper treated with immersion washing (Washing T0) and aged at 144 hours (Washing T144h); b) magnification of the elastic region.

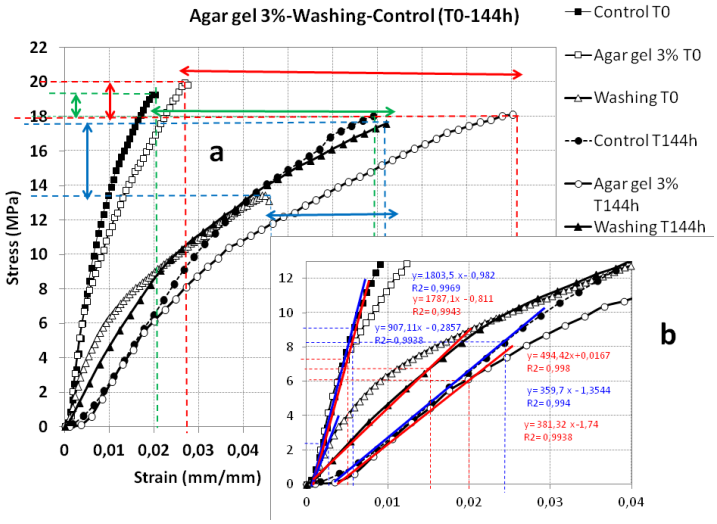


Figure 3.16: Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples: a) unaged control (Control T0), control aged at 144h (Control T144h), paper treated with agar gel 3% unaged (agar gel T0) and aged at 144 hours (agar gel 3% T144h), paper treated with immersion washing unaged (Washing T0) and aged at 144hours (Washing T144h); b) magnification of the elastic region.

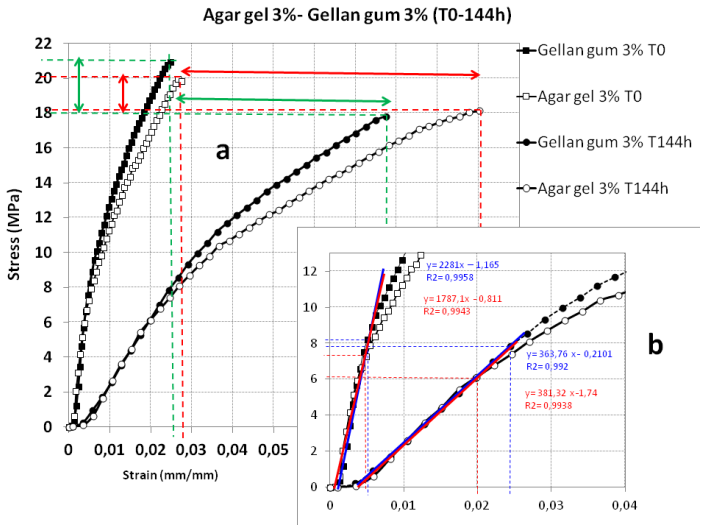


Figure 3.17: Stress-Strain curve at 24°C and 50% RH in the crossmachine direction for paper samples: a) paper treated with gellan gum gel 3% unaged (gellan gum 3% T0) and aged at 144 hours (gellan gum 3% T144h), paper treated with agar gel 3% unaged (agar gel 3% T0) and aged at 144 hours (agar gel 3% T144h); b) magnification of the elastic region.

The UTS average values expressed in MPa, and the breaking strain of three replicates of samples are presented in Tables 3.4, 3.5 with the standard deviation, for the different aging exposures. Results are also represented in figures 3.18, 3.19, 3.20, 3.21 and 3.22.

Table 3.4: Average of ultimate tensile strength, breaking strain of unaged samples

Sample	Ultimate Tensile Strength (MPa)	Breaking Strain (%)
Control	17.5±1.6	1.8±0.2
Washing	13.6±0.7	4.6±0.1
Agar 1%	18.3±2.0	2.7±0.1
Agar 2%	18.4±1.3	2.7±0.3
Agar 3%	19.7±0.1	2.7±0.2
Gellan 1%	18.8±0.9	2.5±0.1
Gellan 2%	19.3±0.5	2.4±0.2
Gellan 3%	20.3±1.0	2.4±0.2

Table 3.5: Average of utlimate tensile strength, breaking strain, elastic modulus of samples at different aging exposures.

Sample	Ultimate Tensile Strength (MPa)	Breaking Strain (%)	Elastic Modulus (MPa)
Control (T0)	17.5±1.6	1.8±0.2	1812±5
Washing (T0)	13.6±0.7	4.6±0.1	921±20
Agar 3% (T0)	19.7±0.1	2.7±0.2	1620±18
Gellan 3% (T0)	20.3±1.0	2.4±0.2	1860±6
Control (T24h)	18.4±0.8	7.3±1.1	419±34
Washing (T24h)	18.8±1.8	8.3±0.2	417±37
Agar 3% (T24h)	18.1±1.6	8.8±0.7	461±1
Gellan 3%(T24h)	19.4±0.2	8.5±0.6	426±4
Control (T72h)	18.8±1.5	7.2±0.6	432±14
Washing (T72h)	19.7±0.5	8.5±0.6	410±8
Agar 3% (T72h)	18.4±1.4	8.4±0.6	420±7
Gellan 3%(T72h)	17.5±1.0	7.1±0.8	361±8
Control (T144h)	18.3±0.4	7.4±0.9	372±16
Washing (T144h)	16.9±2.3	5.9±1.2	414±6
Agar 3% (T144h)	17.9±0.3	8.8±1.1	388±10
Gellan3%(T144h)	16.4±1.2	7.4±0.4	366±2

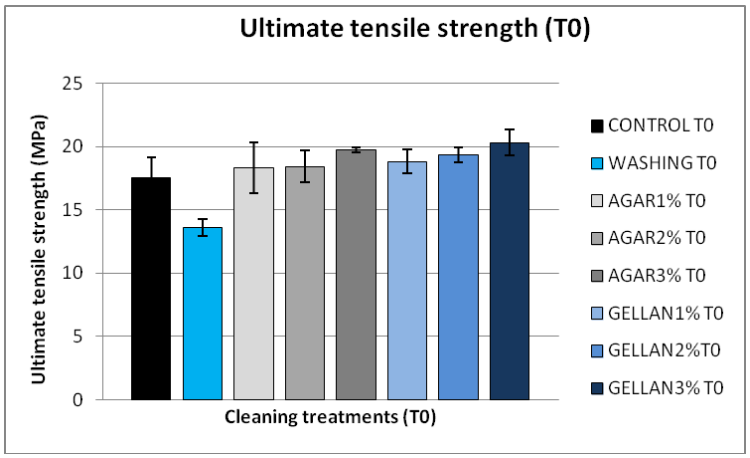


Figure 3.18: Ultimate tensile strength values of unaged paper samples. Bars represent mean ± relative standard deviation (%RSD).

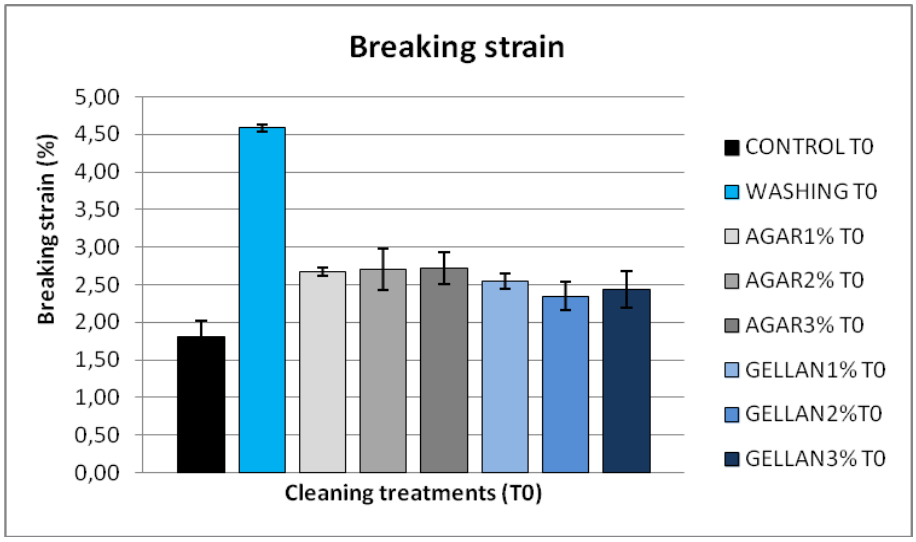


Figure 3.19: Breaking strain (%) of unaged paper. Bars represent mean ± relative standard deviation (%RSD).

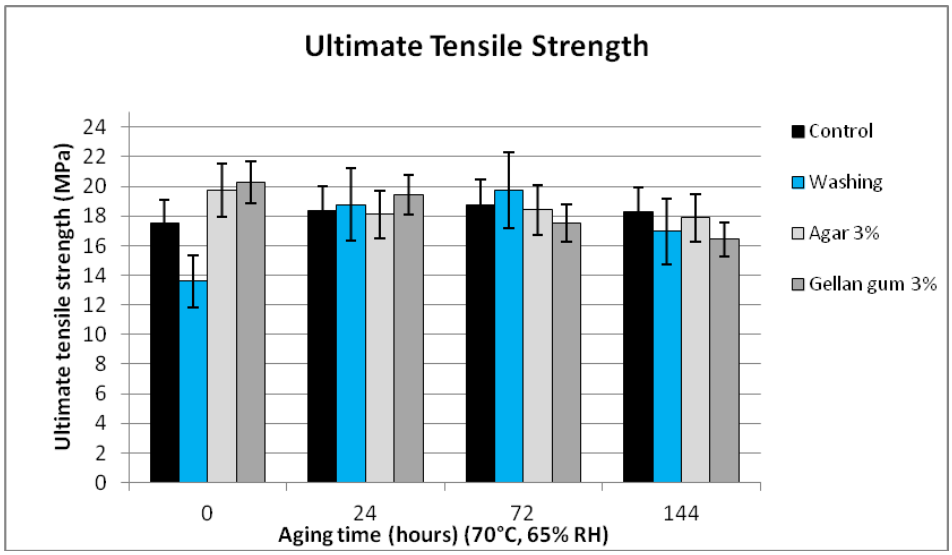


Figure 3.20: Ultimate tensile strength values of samples for different aging exposures. Bars represent mean ± relative standard deviation (%RSD).

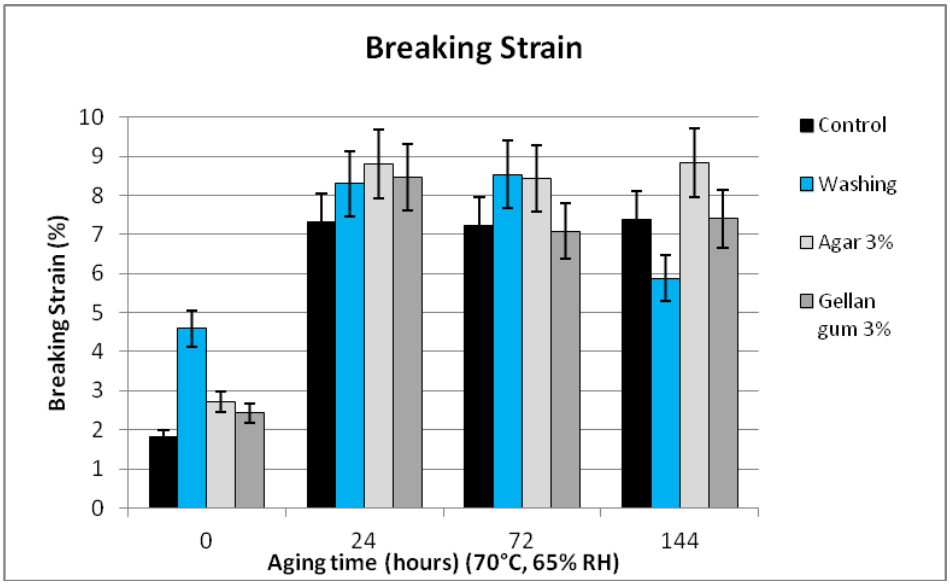


Figure 3.21: Ultimate breaking strain (%) of samples for different aging exposures. Bars represent mean ± relative standard deviation (%RSD).

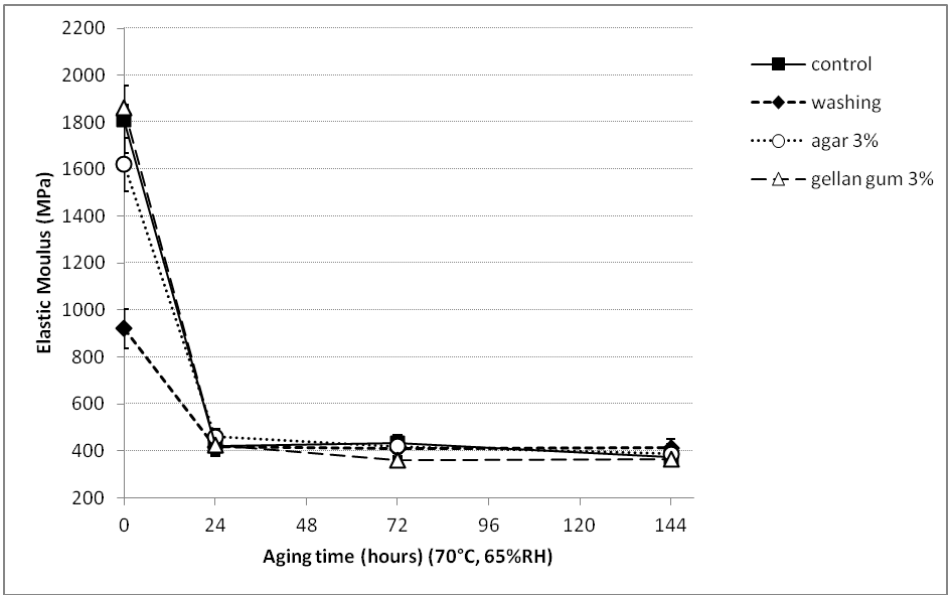


Figure 3.22: Average of elastic modulus values of samples for different aging exposures and relative standard deviation (%RSD).

The results obtained showed that the application of rigid gels on paper, far beyond their cleaning action, contributed to a moderate improvement of the mechanical properties of paper, this is, a slight increment in the ultimate tensile strength and breaking strain compared to the control unaged paper. Immersion treatments caused an evident variation in the mechanical properties of paper and a slight weakening compared to the control sample. The general trend observed for unaged samples were: an increase in flexibility and deformation, an increase in the breaking strain (60%) and a loss of strength of about 22%.

The variation of the mechanical properties of paper induced by immersion treatment is significant and it suggests that specific conservation treatments will require a special attention. For instance the absorption and desorption of a high rate of water may contribute to some dimensional alterations of paper resulting into mechanical stress of the structure. This phenomenon depends on paper composition (type of fibre as well as the eventual presence of additives depending on papermaking processes) and it may occur in a different magnitude depending on the fibre direction. This could be an issue in those cases when conservators treat documents or paper artifacts that need a later assembling since dimensional changes could compromise the perfect adjustment of the different fragments [6].

When assessing the effects of accelerated aging (70°C and 65% RH) in the mechanical properties of treated and untreated paper samples an increase in flexibility with a variation of the elastic modulus due to the swelling action of water as well as a moderate loss of strength were observed. Curves obtained for all samples aged for 144 hours (samples treated with agar and gellan gum gels at 3% as well as samples subjected to immersion) were similar to the aged control samples (144 h). The samples treated with rigid gels don't seem to have altered their properties in a negative way, and even if subjected to accelerated thermo-hygrometric conditions for 144 hours, they presented similar behaviour to the control. These results may suggest that samples treated with gels do not present a higher susceptibility to degradation than the control after having been subjected to thermo-hygrometric accelerated aging.

3.3.2 Observation of the fracture area after tensile testing by means of Light Microscopy

The fracture area was evaluated by means of stereo-microscope observations after tensile testing. The aim was to assess any difference in the failure typology between unaged and aged samples by means of qualitative morphological observations.

Control T0-144h

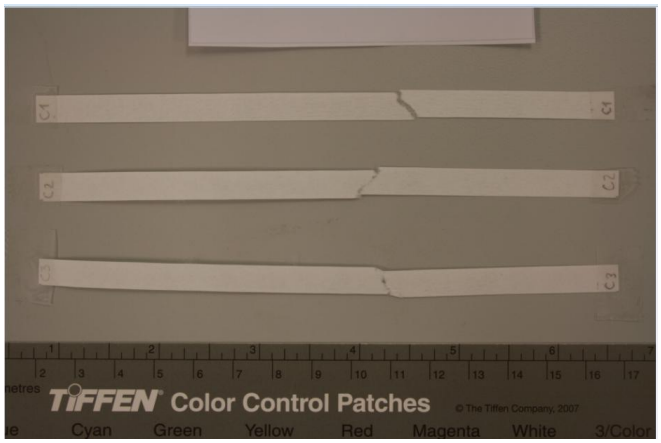


Figure 3.23: Control samples (C-0h) after the tensile tests.

Figure 3.24: Control samples (C-144h) after the tensile tests.

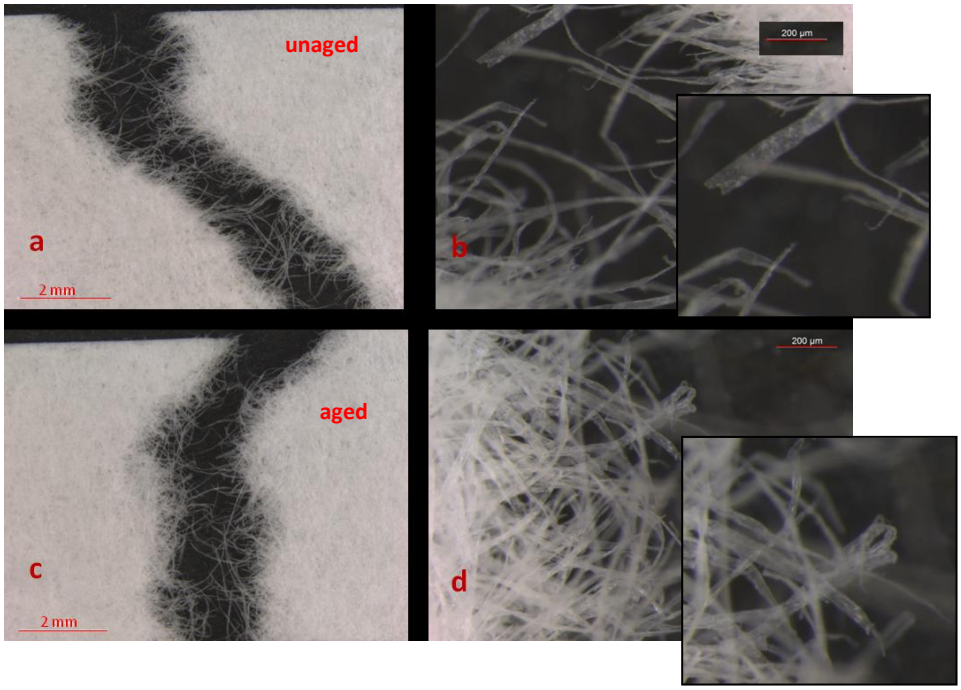


Figure 3.25: Stereo-microscope observation of control fracture after tensile testing: unaged sample (C1-0h) (a) 8x magnification, (b) 50x magnification; aged sample (C1-144h) (c) 8x magnification, (d) 50x magnification. In the case of unaged sample the fracture was defined, sharp as shown in the magnification of figure (b). On the contrary, after aging the fracture involved the fibrillar structure, resulting in a defibration of cellulose structure as shown in the magnification of figure (d).

Observing the fracture area, slight differences are evident between aged and unaged samples, and they could derive in part from the previous treatments and the humid conditions of accelerated aging. A granular break was observed in unaged control samples [90], which occurred more or less perpendicularly across the fibres (Figures 3.23, 3.25 a-b). Under a certain level of tension, the stress could have been transferred along the elements of the fibres making them break. For instance, it is possible to observe this kind of fracture in cellulose fibres (cotton) in which the inter-molecular hydrogen bonds between the chains play a relevant role at the structural point of view.

Samples subjected to thermo-hygometric accelerated aging (70°C and 65% RH) for 144 hours showed some slight changes in the fracture area. In this case, a fibrillar break had been taken place (Figures 3.24,3.25 c-d) [91]. In fact the water absorption by cellulose fibres could have caused the weakening of inter-molecular hydrogen bonds between the fibrils and, as consequence, a slight separation of them and the break had taken place [92].

Immersion washing 0-144h

Figure 3.26: Samples treated by immersion washing (W-0h) after the tensile tests.

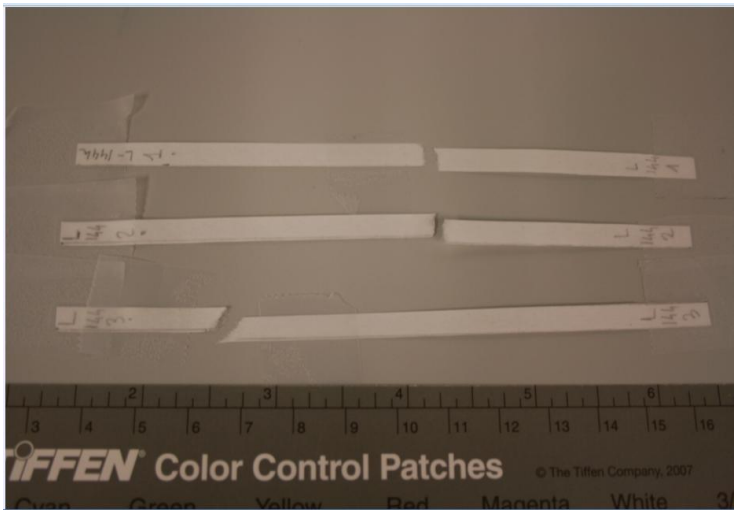


Figure 3.27: Samples treated by immersion washing (W-144h) after the tensile tests.

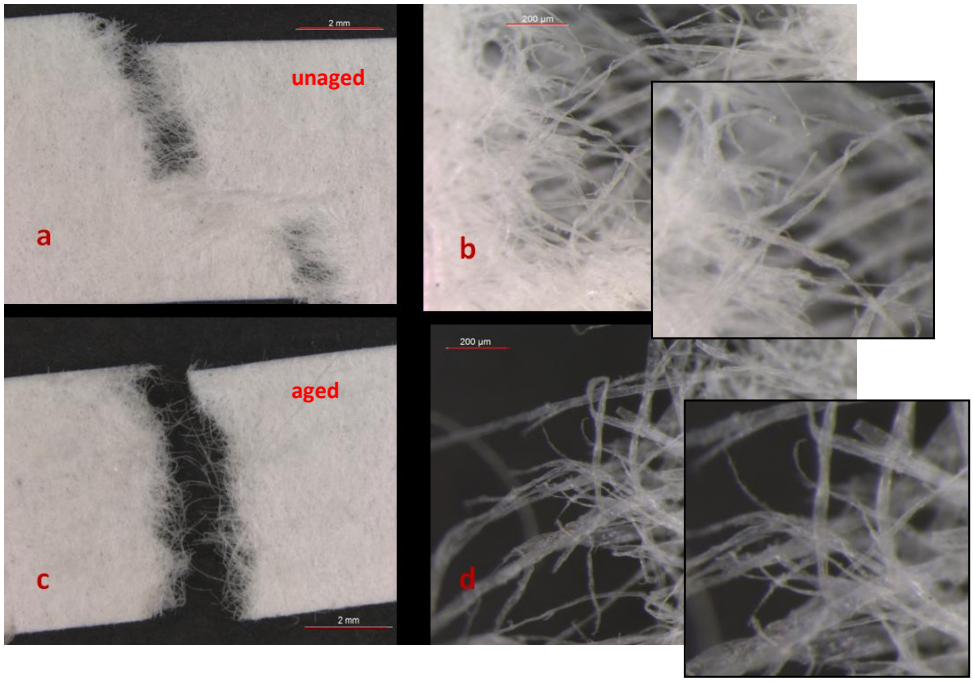


Figure 3.28: Stereo-microscope observation of fracture of sample treated by immersion washing after tensile-test: unaged sample (W1-0h) (a) 8x magnification, (b) 50x magnification; aged sample (W1-144h) (c) 8x magnification, (d) 50x magnification. Both unaged and aged samples evidence a fibrillar fracture as shown in the magnifications of figure (b) and (d). This may be caused by the swelling action of water during immersion treatment and water humidity during accelerated aging.

In the case of samples subjected to immersion in distilled water both the unaged and aged samples (144 hours) seem to present a similar fracture profile from the morphological point of view. In this case, the swelling effect of water absorbed by cellulose during immersion is similar to the swelling caused by humidity during accelerated aging and this explains the fibrillar break observed in both aged and unaged samples (Figures 3.26, 3.27, 3.28 a-b-c-d).

Agar 3% 0-144h

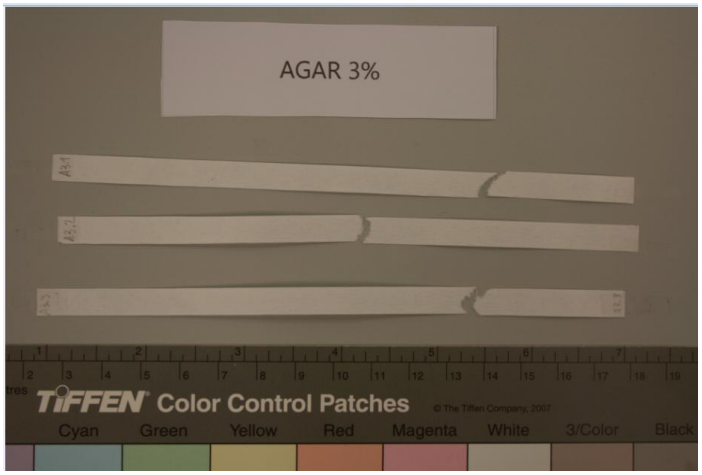


Figure 3.29: Samples treated with agar gel 3% (A3-0h) after the tensile tests.

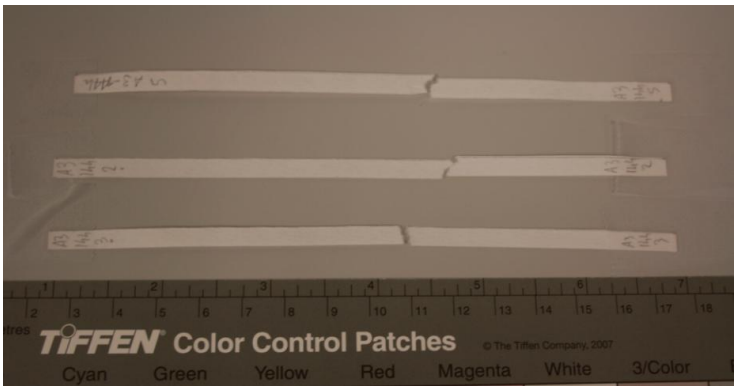


Figure 3.30: Samples treated with agar gel 3% (A3-144h) after the tensile tests.

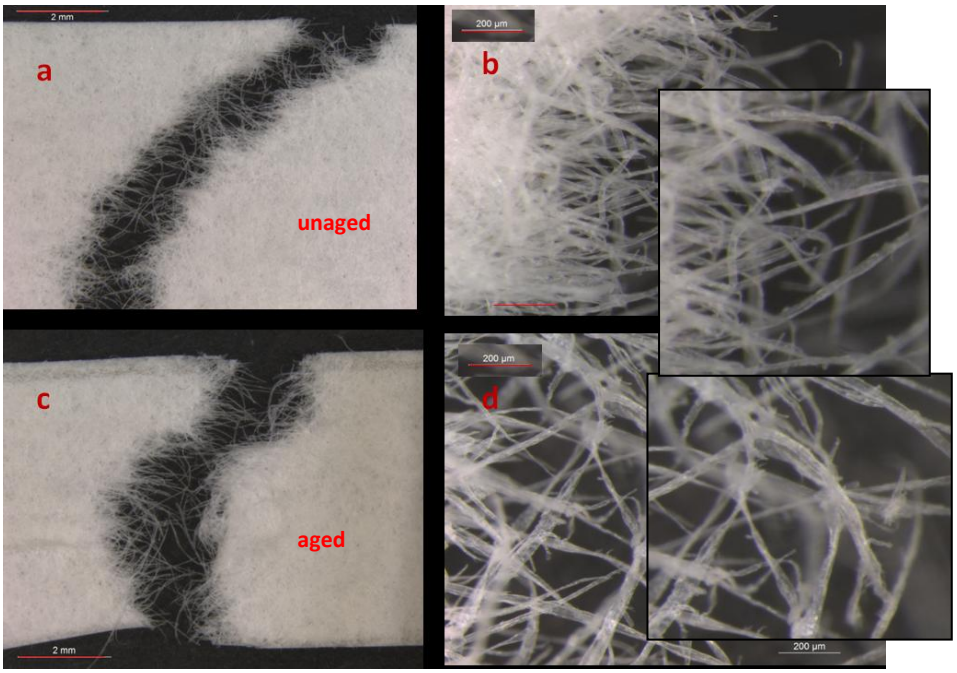


Figure 3.31: Stereo-microscope observation of fracture of sample treated with agar gel 3%: unaged sample (A3-0h) (a) 8x magnification, (b) 50x magnification; aged sample (A3-144h) (c) 8x magnification, (d) 50x magnification. In this case unaged sample presents a granular fracture; on the contrary sample subjected to aging (144 hours) presents a fibrillar fracture.

From a morphological point of view, the samples treated with agar gel (3%) presented the same behaviour than the control; Whereas unaged samples present a granular fracture (Figures 3.29, 3.31 a-b), the aged samples showed a fibrillar break (Figures 3.30, 3.31 c-d). Also for samples treated with gellan gum (3%) the same behaviour was observed: granular fracture in the case of unaged samples (Figures 3.32, 3.34 a-b) and fibrillar break for the aged ones (Figures 3.33, 3.34 c-d).

Other samples treated with gels at different concentrations (before and after accelerated aging) showed a similar behaviour than the control ones and no significant morphological differences were detected in their fracture areas.

Gellan gum 3% 0-144h

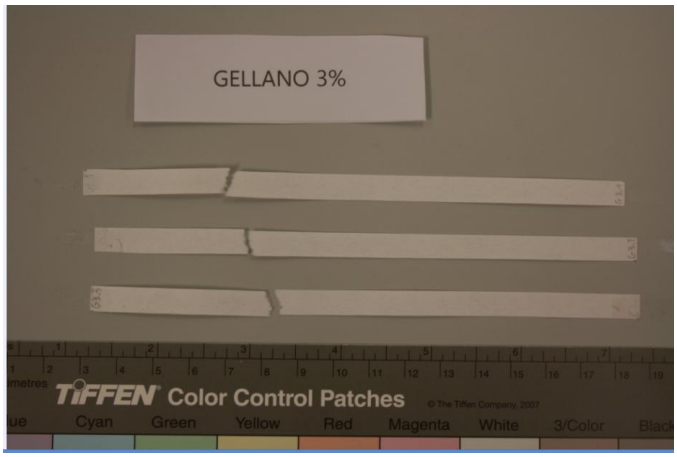


Figure 3.32: Samples treated with gellan gum gel 3% (G3-0h) after the tensile tests.

Figure 3.33: Samples treated with gellan gum gel 3% (G3-144h) after the tensile tests.

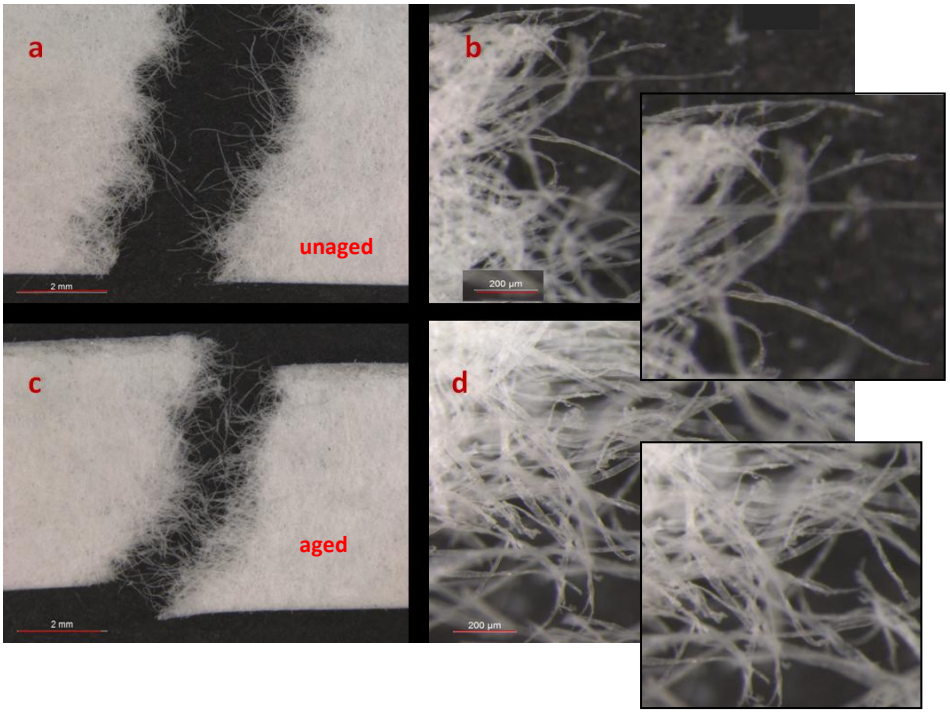


Figure 3.34: Stereo-microscope observation of fracture of sample treated with gellan gum gel 3%: unaged sample (G3-0h) (a) 8x magnification, (b) 50x magnification; aged sample (G3-144h) (c) 8x magnification, (d) 50x magnification. The unaged sample treated with gellan gum presents a granular fracture as in the case of sample treated with agar gel; after the aging (144 hours) the fracture evidences a fibrillar behaviour as shown in the magnification of figure (d).

Agar gel 1%

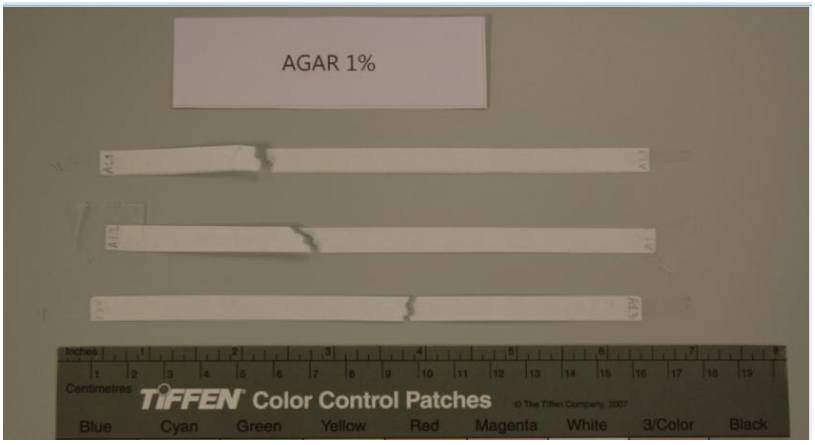


Figure 3.35: Samples treated with agar gel 1% (A1-0h) after the tensile tests.

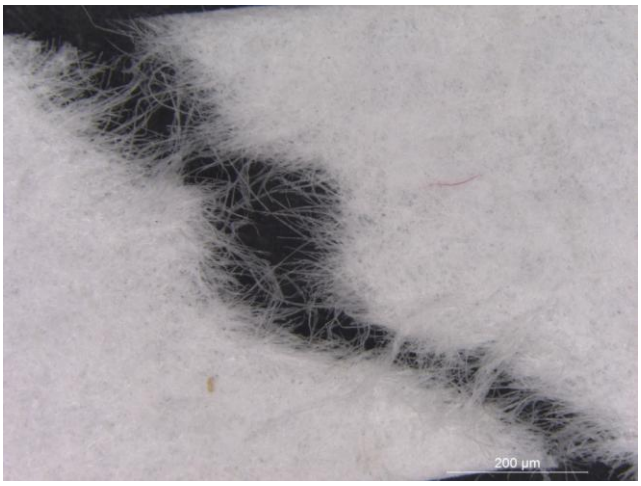


Figure 3.36: Stereo-microscope observation of tensile failure of sample treated with agar gel 1% (A1-0h) (magnification: 8x). Sample presents a granular morphology.

Agar gel 2%

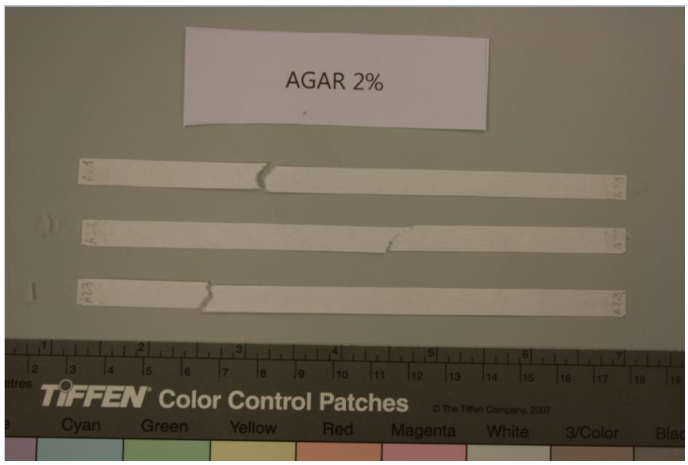


Figure 3.37: Samples treated with agar gel 2% (A2-0h) after the tensile tests.

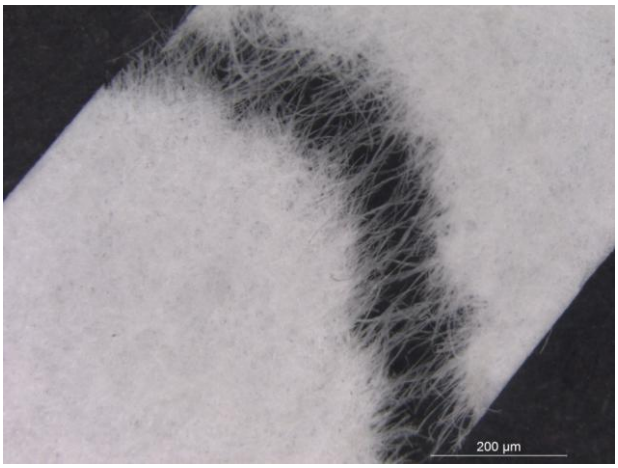


Figure 3.38: Stereo-microscope observation of tensile failure of sample treated with agar gel 2% (A2-0h) (magnification: 8x). Sample presents a granular fracture and no significant differences are observed with the sample treated with agar gel 1% with respect to control sample.

Gellan gum 1%

Figure 3.39: Samples treated with gellan gum gel 1% (G1-0h) after the tensile tests.

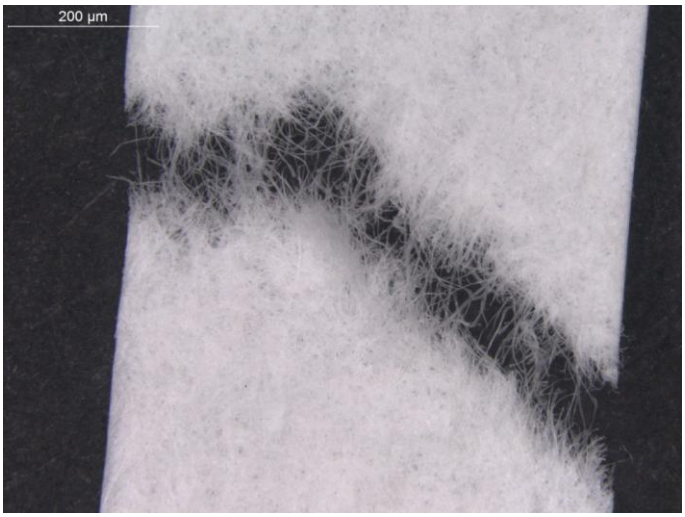


Figure 3.40: Stereo-microscope observation of tensile failure of sample treated with gellan gum gel 1% (G1-0h) (magnification: 8x). Sample presents a granular facture. Slight defibration phenomenon is observed in some regions of the fracture.

Gellan gum 2%

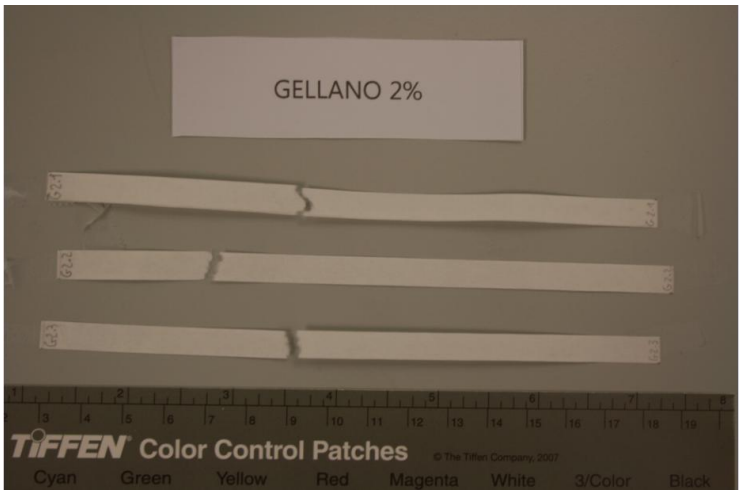


Figure 3.41: Samples treated with gellan gum gel 2% (G2-0h) after the tensile tests.

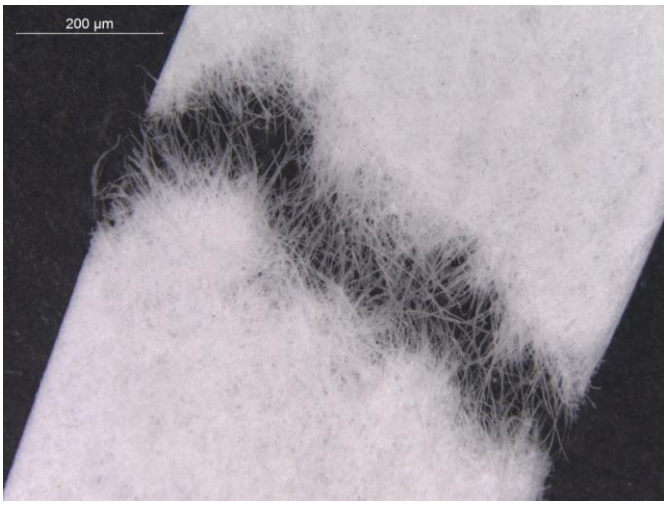


Figure 3.42: Stereo-microscope observation of tensile failure of sample treated with gellan gum gel 2% (G2-0h) (magnification: 8x). Also in this case the fracture is granular even if in some regions slight defibration phenomenon is observed.

3.3.3 Scanning Electron Microscopy (SEM) observations

Scanning electron microscopy analysis was performed for morphological observations of fibres. The aim was to evaluate the effect of different aqueous cleaning methods and materials as well as the effect of different exposures of treated and untreated samples to artificial accelerated aging. For this purpose changes observed in fibres' diameter were correlated among the different samples. Fibres diameter was measured using software INCA Microanalysis suite (from Oxford-Link-Isis EDX), and evaluating the images of samples acquired at 250 x of magnification. For each image 120 measures of diameter in different regions were performed, and the average of the obtained values was considered.

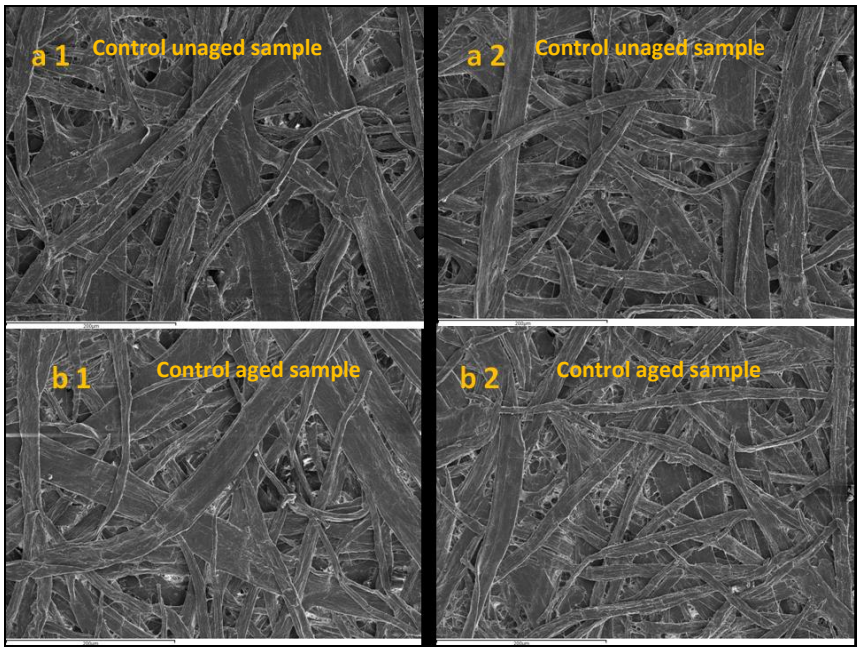


Figure 3.43: Control samples, unaged (C-0h: a1-a2) and aged after 144 hours (C-144h: b1-b2). SEM images were acquired in secondary electrons mode (magnification:250x) in two different regions of samples. Paper did not present inorganic fillers. Control sample after aging showed a slight dimensional change in the diameter of fibre and a reduction of inter-fibres porosity compared to the unaged control.

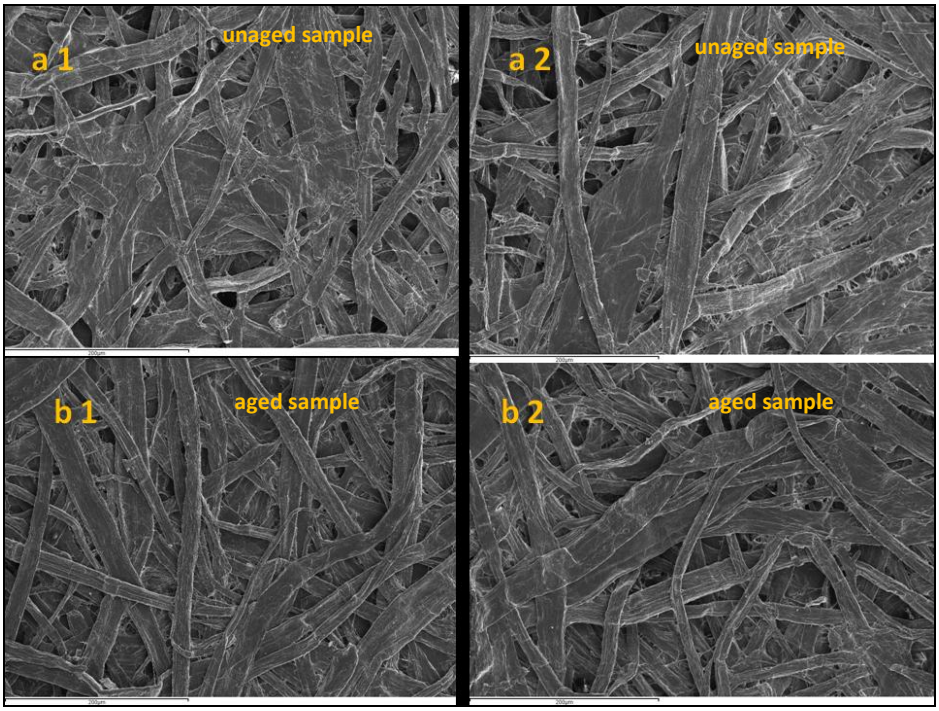


Figure 3.44: Samples treated with agar gel 3%, unaged (A3-0h: a1-a2) and aged after 144 hours (A3-144h: b1-b2). SEM images were acquired in secondary electrons mode (magnification:250x) in two different regions of samples. Sample treated with agar gel 3% after aging showed a slight dimensional change in the diameter of fibre and a reduction of inter-fibres porosity.

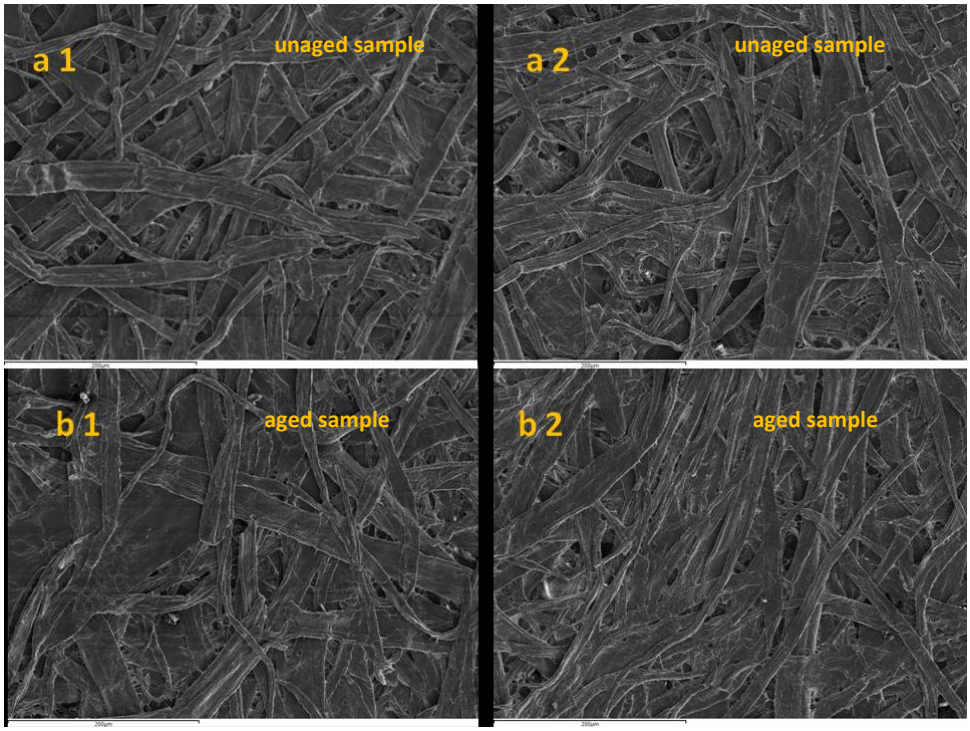


Figure 3.45: Samples treated with gellan gum 3%, unaged (G3-0h: a1-a2) and aged after 144 hours (G3-144h: b1-b2). SEM images were acquired in secondary electrons mode (magnification:250x) in two different regions of samples. Sample treated with gellan gum 3% after aging showed a slight dimensional change in the diameter of fibre and a reduction of inter-fibres porosity.

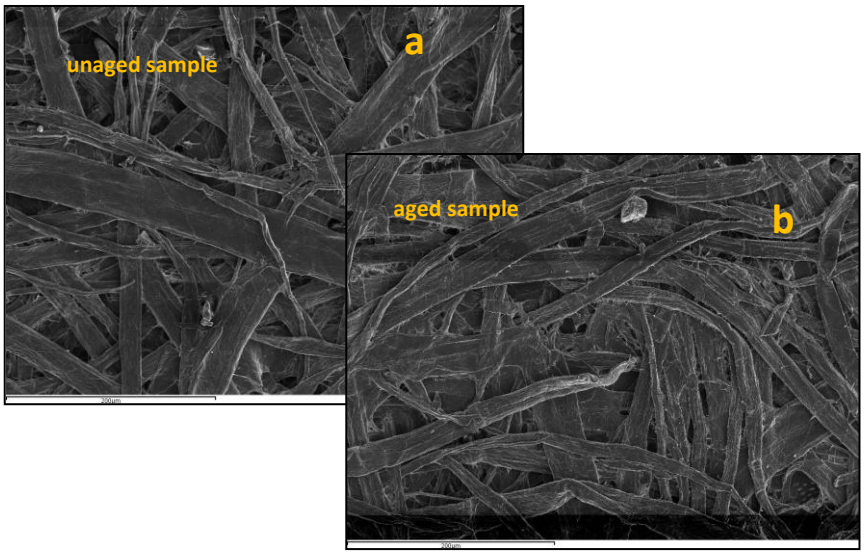


Figure 3.46: Samples treated by immersion in distilled water, unaged (W-0h: a) and aged after 144 hours (W-144h: b). SEM images were acquired in secondary electrons mode (magnification:250x). After immersion sample showed a slight increase in diameter fibres caused by swelling action of water. After thermo-hygrometric aging fibres showed a slight decrease in their diameter compared to the unaged sample.

SEM observation showed very slight morphological differences between unaged and aged samples. In fact, in the case of control aged samples, a slight increase in the diameter of fibres (about 9%) was observed (Figure 3.43). Increase in the diameter of fibres with aging was detected also in samples treated with agar gel (about 6%) and for gellan gum gel (about 6%) (Figures 3.44, 3.45). This phenomenon may be explained by the humid conditions in which paper samples had been aged. In fact the water, gradually absorbed by paper, had caused a moderate swelling of fibres resulting in a slight change in diameter, that can be correlated to changes observed in the mechanical properties of paper as shown in the previous section, thus evidencing that porosity of paper might have changed slightly either in fibres' porosity itself as well as in the inter-fibrillar spaces [93]. In samples subjected to immersion however, the opposite behaviour was detected: a decrease in fibres' diameter (about 5%) seemed to be observed after aging. In this case, the maximum level of cellulose swelling was obtained in those samples subjected to immersion treatments; after aging, paper samples could have partially lost the water previously absorbed, resulting in a slight dimensional changes and a decrease in diameter of fibres.

The magnitude of the dimensional changes observed in paper may be different depending on the type of fibres present and in the case of paper containing inorganic fillers.

Table 3.6: Average fibre diameter (µm).

Sample	Average figer diameter (µm) ± SD
C-0h	23.1± 10.4
C-144h	25.5± 11.4
A3-0h	22.7 ± 8.7
A3-144h	24.0 ± 9.6
G3-0h	22.1 ± 6.8
G3-144h	23.4 ± 11.0
W-0h	24.7 ± 10.3
W-144h	23.6 ± 8.9

At higher magnifications no significant differences were observed between unaged and aged samples (Figure 3.17) and between treated samples.

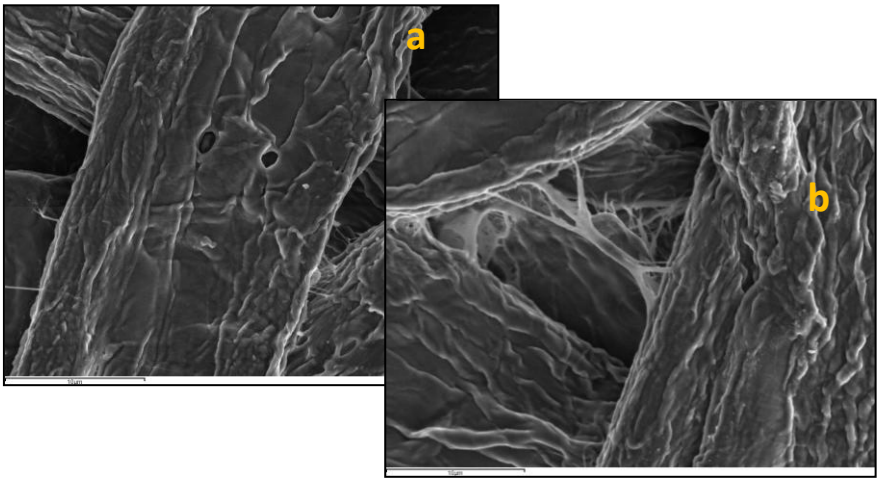


Figure 3.47: Control sample: unaged (a) and aged (b) after 144 hours. SEM images were acquired in secondary electron mode (magnification: 3500x). In figure (a) typical fibres of wood are observed. Comparing unaged and aged control sample, no significant changes in the morphology of fibre wall are observed.

3.3.4 Colorimetric analysis of paper samples

Colorimetric analysis was performed on paper samples before and after the cleaning treatments; the measures were repeated after accelerated artificial aging. The aim was to verify the effects of cleaning treatments in the reflectance of light (400-700 nm) as well as in paper chromatism by measuring colorimetric coordinates (and having the control samples as reference material) and to evaluate if treated samples may be more susceptible to colour variation than untreated ones in time.

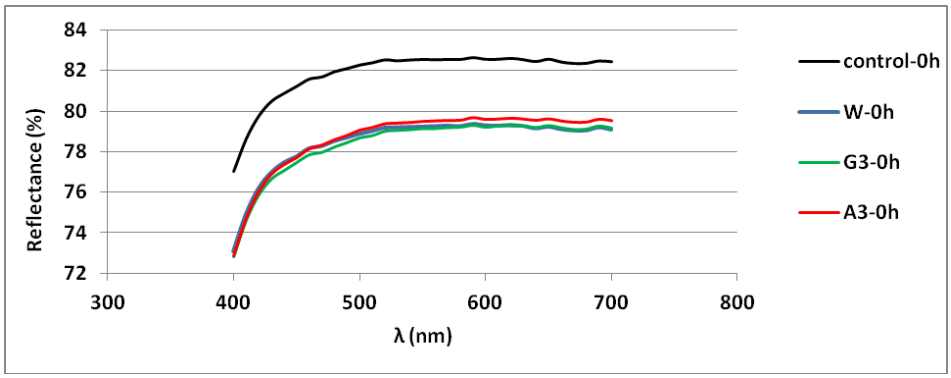


Figure 3.48: Reflectance (%) of unaged control sample (control-0h) and unaged samples treated by immersion in water (W-0h), gellan gum gel 3% (G3-0h) and agar gel 3% (A3-0h).

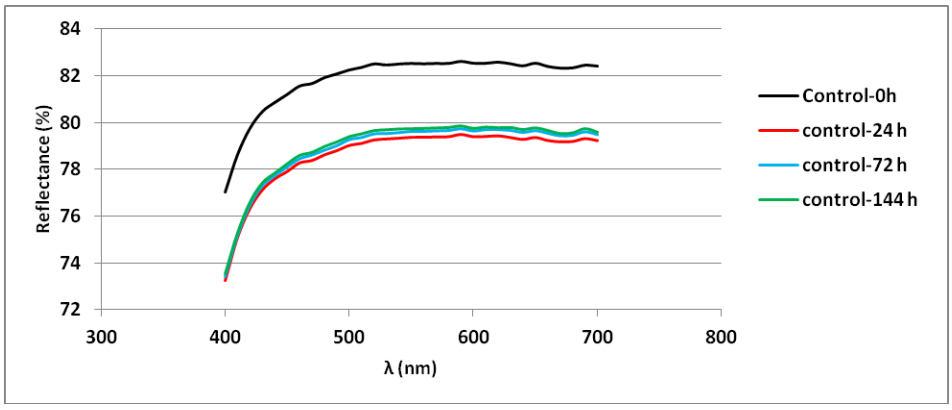


Figure 3.49: Reflectance (%) of control samples subjected to different aging exposures (control-0h, 24h, 72h, 144h).

The preliminary analysis showed a decrease of about 4% in the reflectance in treated samples in comparison to the control due probably to the extraction action caused by cleaning treatments (Figure 3.48). The same behaviour was observed during the accelerated aging of the control samples: the reflectance decrease of about 4% as the aging exposure increased (Figure 3.49).

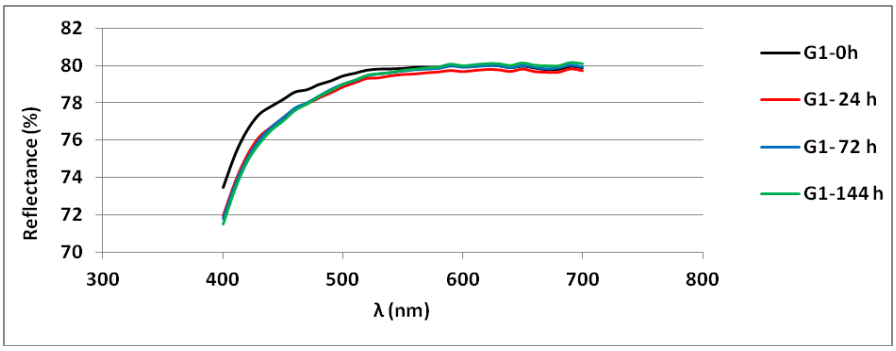


Figure 3.50: Reflectance (%) of paper treated with gellan gum gel 1%, subjected to different aging exposures (G1-0h, 24h, 72h, 144h).

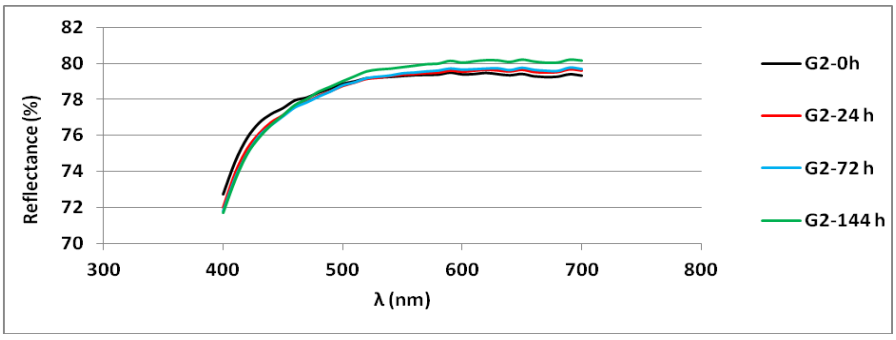


Figure 3.51: Reflectance (%) of paper treated with gellan gum gel 2%, subjected to different exposure of aging (G2-0h, 24h, 72h, 144h).

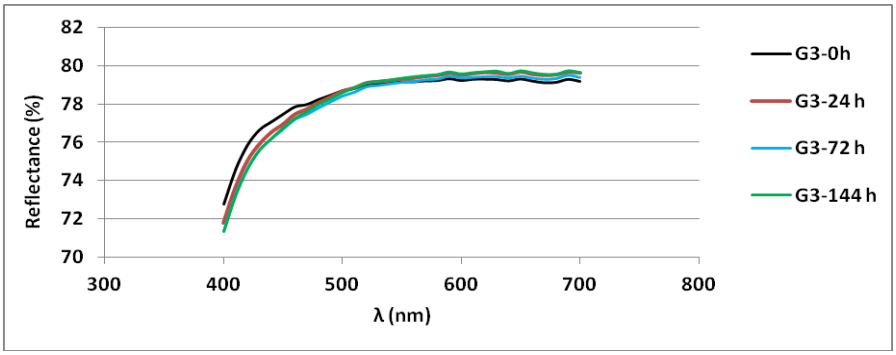


Figure 3.52: Reflectance (%) of paper treated with gellan gum gel 3%, subjected to different aging exposures (G3-0h, 24h, 72h, 144h).

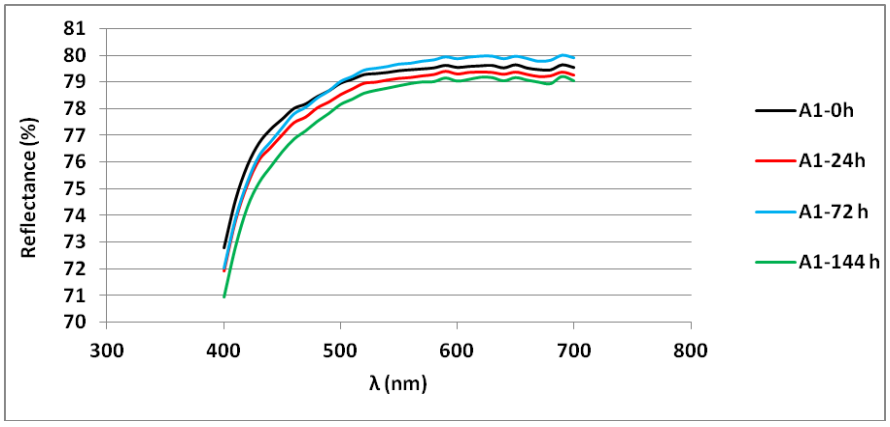


Figure 3.53: Reflectance (%) of paper treated with agar gel 1%, subjected to different aging exposures (A1-0h, 24h, 72h, 144h).

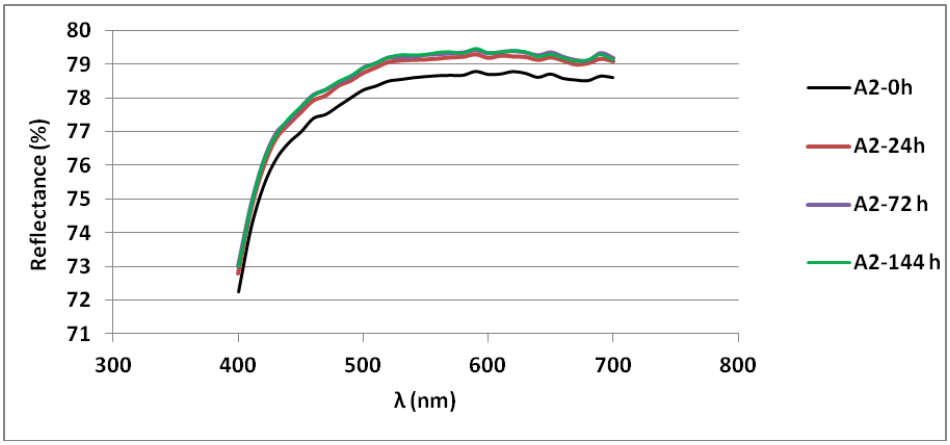


Figure 3.54: Reflectance (%) of paper treated with agar gel 2%, subjected to different aging exposures (A2-0h, 24h, 72h, 144h).

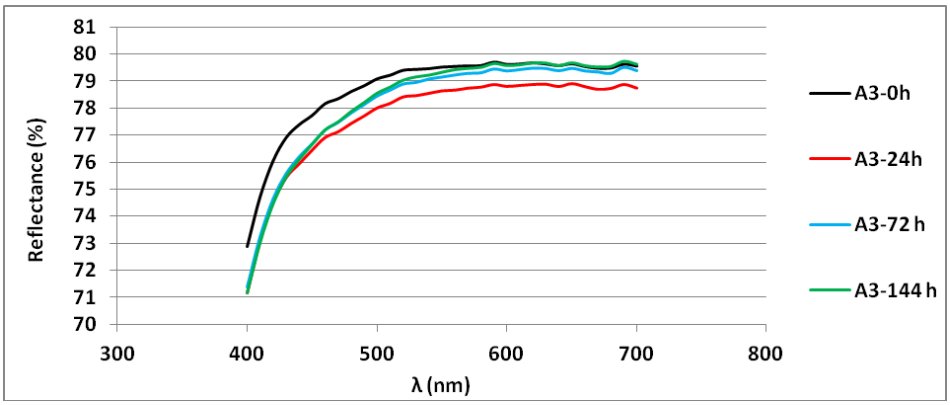


Figure 3.55: Reflectance (%) of paper treated with agar gel 3%, subjected to different aging exposures (A3-0h, 24h, 72h, 144h).

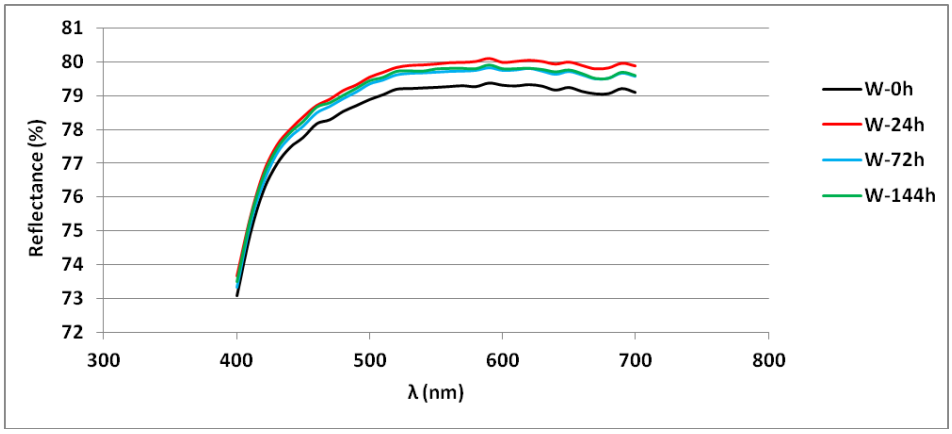


Figure 3.56: Reflectance (%) of paper treated by immersion washing, subjected to different aging exposures (W-0h, 24h, 72h, 144h).

In the case of paper treated with gellan gum gel (1-2-3%) the reflectance remained unchanged after aging and small variations were detected in the case of those samples treated by immersion washing as well as in those treated with agar gel (Figures 3.50-3.56).

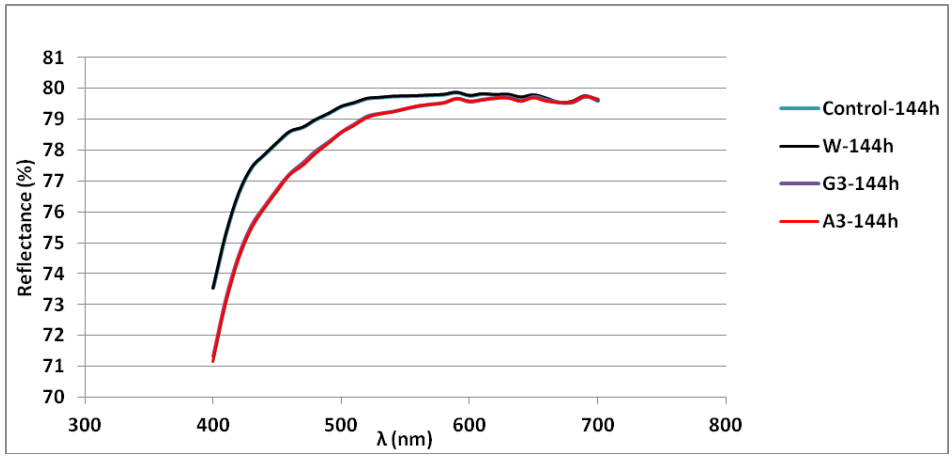


Figure 3.57: Reflectance (%) of paper untreated and treated by immersion, gellan gum gel 3%, agar gel 3% after 144 hours of aging (control-144h, W-144h, G3-144h, A3-144h).

After 144 h of exposure to artificial accelerated aging, the reflectance of the control samples was similar to the paper treated by immersion, and only small variations were detected in the reflectance of short wavelengths in the case of those samples treated with gellan gum gel (3%) and agar gel (3%) (Figure 3.57). The evaluation of changes was carried out by acquiring the colorimetric coordinates of the samples before and after cleaning treatments. Similar measurements were repeated after subjecting the samples to accelerated aging. The evaluation was carried out either by including the specular component (SCI) and by excluding the specular component (SCE) (Tables 3.7, 3.8, 3.9).

Table 3.7: Colorimetric coordinates (L_{10}*, a_{10}*, b_{10}*) according to CIELAB system with CIE 1964 observer

	SCI			SCE		
Sample	**L_{10}***	**a_{10}***	**b_{10}***	**L_{10}***	**a_{10}***	**b_{10}***
C-0h	91.57	-0.39	1.18	91.55	-0.36	1.17
A1-0h	91.36	-0.35	1.40	91.34	-0.32	1.39
A2-0h	91.00	-0.36	1.26	91.00	-0.33	1.25
A3-0h	91.40	-0.37	1.35	91.38	-0.35	1.34
G1-0h	91.56	-0.36	1.28	91.56	-0.34	1.27
G2-0h	91.31	-0.37	1.35	91.29	-0.36	1.35
G3-0h	91.23	-0.34	1.29	91.22	-0.32	1.29
W-0h	91.28	-0.37	1.14	91.26	-0.35	1.13
C-144h	91.51	-0.38	1.18	91.45	-0.36	1.17
A1-144h	91.08	-0.42	1.89	91.05	-0.40	1.88
A2-144h	91.31	-0.37	1.22	91.30	-0.35	1.21
A3-144h	91.29	-0.43	2.03	91.28	-0.41	2.00
G1-144h	91.48	-0.43	2.03	91.46	-0.41	2.01
G2-144h	91.50	-0.43	2.02	91.48	-0.41	2.00
G3-144h	91.30	-0.42	1.97	91.27	-0.40	1.96
W-144h	91.53	-0.39	1.17	91.51	-0.37	1.16

Table 3.8: Variation in colorimetric coordinates (ΔL_{10}^*, Δa_{10}^*, Δb_{10}^*), colour-difference (ΔE^*_{ab}) according to CIELAB system.

	SCI				SCE			
Sample	ΔL_{10}^*	Δa_{10}^*	Δb_{10}^*	ΔE^*_{ab}	ΔL_{10}^*	Δa_{10}^*	Δb_{10}^*	ΔE^*_{ab}
A1%	-0.21	0.04	0.21	**0.37**	-0.21	0.04	0.21	**0.30**
A2%	-0.57	0.03	0.07	**0.58**	-0.55	0.03	0.08	**0.56**
A3%	-0.17	0.02	0.16	**0.36**	-0.17	0.01	0.16	**0.24**
G1%	-0.01	0.03	0.10	**0.23**	0.01	0.02	0.10	**0.10**
G2%	-0.26	0.02	0.17	**0.38**	-0.26	0.00	0.17	**0.31**
G3%	-0.34	0.05	0.11	**0.42**	-0.33	0.04	0.11	**0.36**
W	-0.29	0.02	-0.05	**0.32**	-0.29	0.01	-0.04	**0.29**
C (0-144h)	-0.06	0.01	-0.01	**0.18**	-0.10	0.003	-0.002	**0.10**
A1 (0-144h)	-0.28	-0.07	0.49	**0.60**	-0.28	-0.08	0.49	**0.57**
A2 (0-144h)	0.31	-0.01	-0.04	**0.34**	0.30	-0.02	-0.04	**0.30**
A3 (0-144h)	-0.11	-0.06	0.68	**0.74**	-0.10	-0.06	0.67	**0.68**
G1 (0-144h)	-0.09	-0.07	0.75	**0.81**	-0.10	-0.07	0.74	**0.75**
G2 (0-144h)	0.19	-0.06	0.67	**0.74**	0.19	-0.05	0.65	**0.68**
G3 (0-144h)	0.07	-0.08	0.68	**0.70**	0.06	-0.08	0.67	**0.68**
W (0-144h)	0.24	-0.02	0.04	**0.25**	0.25	-0.02	0.04	**0.25**

Table 3.9: Colour-difference (ΔE_{00}) according to CIEDE2000

	SCI	SCE
Sample	**ΔE_{00}**	**ΔE_{00}**
A1%	0.25	0.25
A2%	0.36	0.35
A3%	0.20	0.19
G1%	0.11	0.10
G2%	0.23	0.23
G3%	0.25	0.24
W	0.19	0.18
C (0-144h)	0.04	0.06
A1 (0-144h)	0.50	0.50
A2 (0-144h)	0.20	0.19
A3 (0-144h)	0.64	0.62
G1 (0-144h)	0.70	0.70
G2 (0-144h)	0.64	0.62
G3 (0-144h)	0.64	0.63
W (0-144h)	0.16	0.16

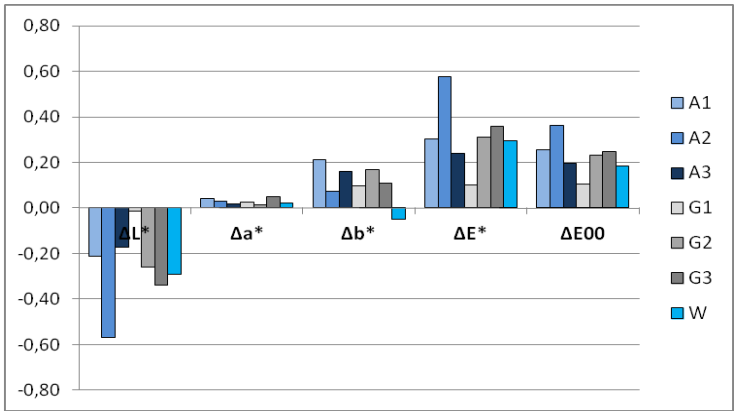


Figure 3.58: Difference in colorimetric coordinates and colour-difference (according CIELAB system and CIEDE2000) by including the specular component (SCI) of paper samples subjected to cleaning treatments: agar gel 1-2-3% (A1, A2, A3), gellan gum gel 1-2-3% (G1, G2, G3), immersion washing (W).

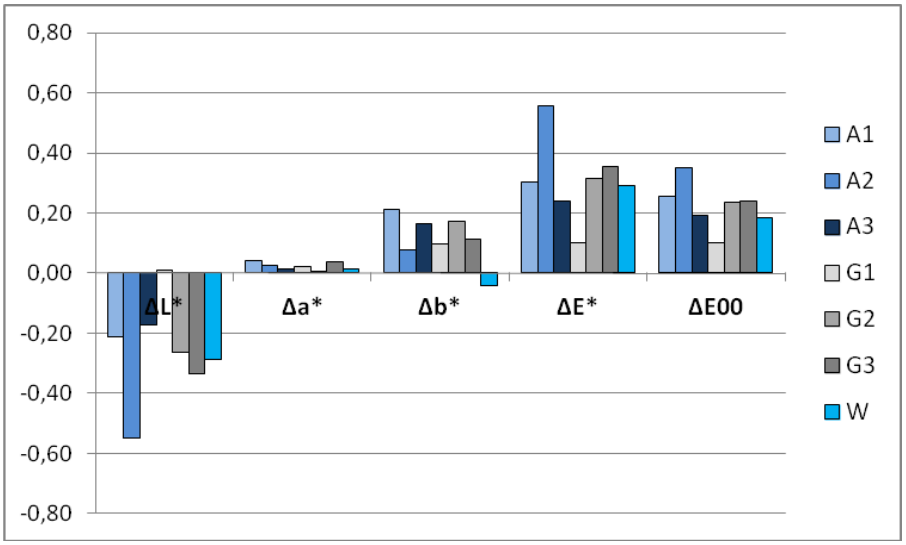


Figure 3.59: Difference in colorimetric coordinates and colour-difference (according CIELAB system and CIEDE2000) by excluding the specular component (SCE) of paper samples subjected to cleaning treatments: agar gel 1-2-3% (A1, A2, A3), gellan gum gel 1-2-3% (G1, G2, G3), immersion washing (W).

The cleaning treatments performed on filter paper samples caused a slight decrease in lightness (L*), a slight increment in component a* and b*, and ΔE below 0.60 (Figure 3.58). The ΔE_{00} according to CIEDE2000 showed a value below 0.40.

The effect of cleaning on paper artworks from different periods was evaluated in previous studies [94]. The application of gellan gum rigid gels as well as other gelling agents (i.e.: Klucel G 2% w/v in water and hydro-alcohol solution) on ancient papers caused different effects: a significant colour-difference higher than 2 was detected, as well as a decrease in the colorimetric coordinate b* of about 2 units (which corresponds to a decrease in the yellowing of paper) and an increment in lightness (L*). These results suggest that the effects of cleaning treatments are strongly influenced by the type of paper, its manufacture and conditions (i.e.: oxidation processes in progress).

Since cleaning treatments can extract degradation products partially responsible of the yellowing of paper, the effects of ΔE is more evident in the case of ancient papers. In the case of filter paper (representative of what an industrial manufactured paper of almost pure cellulose and in good condition would be) the effect was not significant. For this reason the colour-difference was moderate and not perceived. Also in the case

of excluded specular component the colorimetric variation was very slight (Figure 3.59). The similarity of results obtained by including and excluding specular component may be explained by the cleaning action: the cleaning treatments didn't cause any alteration in the surface roughness of papers.

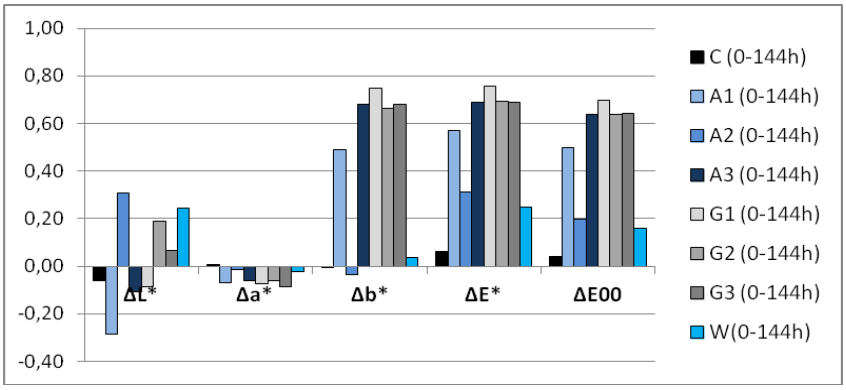


Figure 3.60: Difference in colorimetric coordinates and colour-difference (according CIELAB system and CIEDE2000) by including the specular component (SCI) of paper samples untreated (C) and treated: agar gel 1-2-3% (A1, A2, A3), gellan gum gel 1-2-3% (G1, G2, G3), immersion washing (W), subjected to accelerated aging at 144 hours.

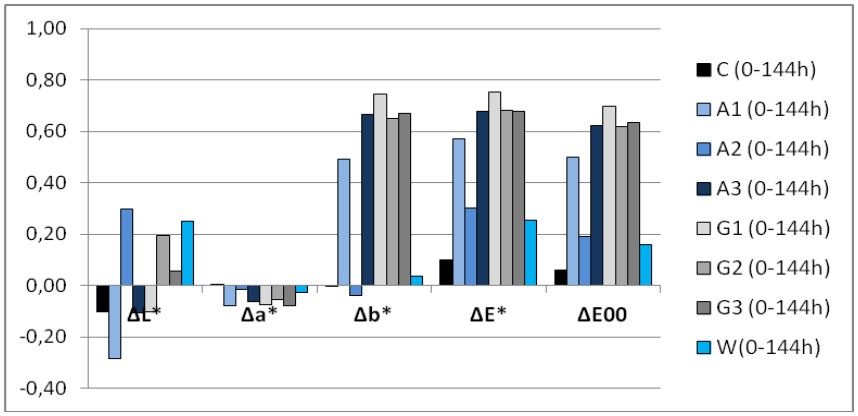


Figure 3.61: Difference in colorimetric coordinates and colour-difference (according CIELAB system and CIEDE2000) by excluding the specular component (SCE) of paper samples untreated (C) and treated: agar gel 1-2-3% (A1, A2, A3), gellan gum gel 1-2-3% (G1, G2, G3), immersion washing (W), subjected to accelerated aging at 144 hours.

After accelerated aging a slight change in the b* component and in the colour-difference were detected (ΔE^*_{ab}<0.80; ΔE_{00}<70) (Figure 3.60, 3.61). Even in the case of accelerated aging no significant variations in the surface roughness of papers was detected (the results - including and excluding specular component- were similar). Moreover, comparing the colour of paper samples treated by immersion and rigid gels with the control sample, no significant variation in the colour perception was detected, for any of the different backgrounds used (black, grey and white) (Figure 3.62).

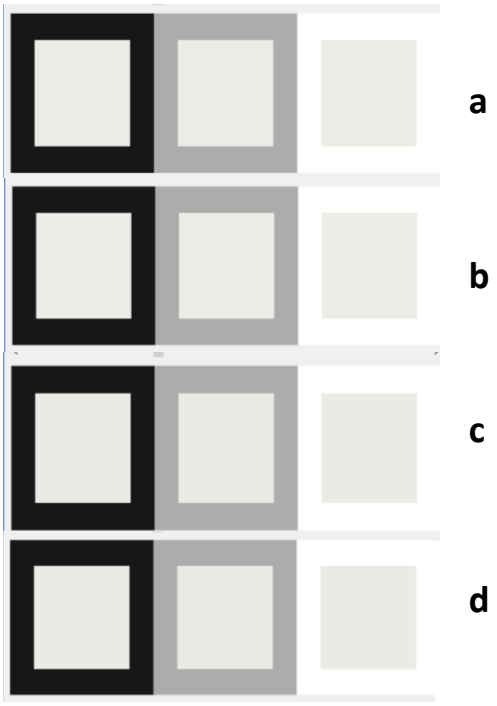


Figure 3.62: The colour detected in paper: untreated (a), treated with agar gel 3% (b), treated with gellan gum gel 3% (c), treated by immersion washing (d).

Also in the case of papers subjected to accelerated aging either treated by immersion or untreated, the perceived colour remained similar (Figure 3.63, 3.64).

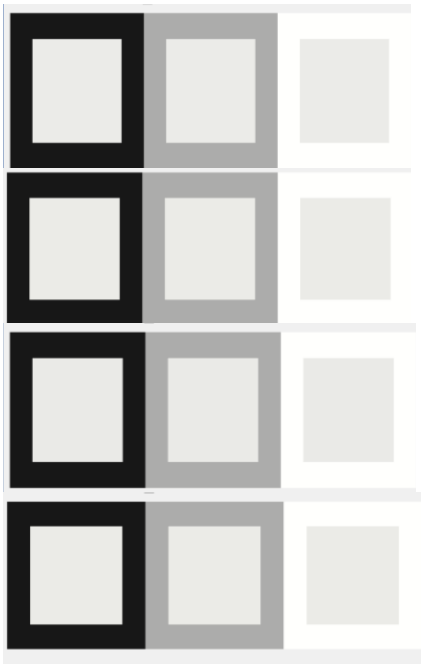


Figure 3.63: Colour detected in paper: untreated (a1), untreated paper subjected to 144 hours of accelerated aging (a2), paper treated by immersion washing (d1) and subjected to accelerated aging (d2).

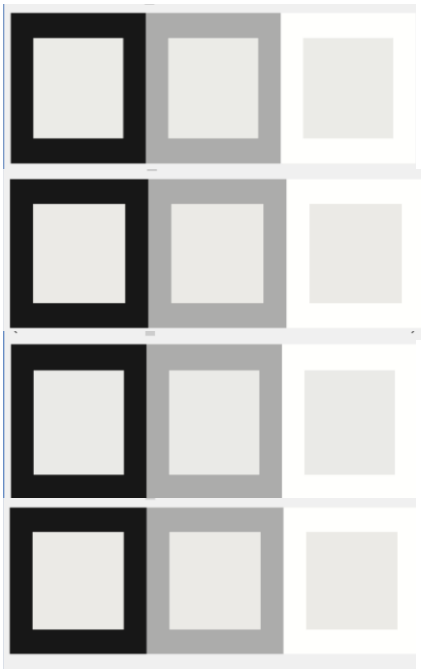


Figure 3.64: Colour detected in paper: treated with agar gel 3% (b1), treated with agar gel 3% and subjected to 144 hours of accelerated aging (b2), treated with gellan gum gel 3% (c1) and subjected to 144 hours of accelerated aging (c2).

3.4 Conclusions

In this research the evaluation of the influence of wet cleaning treatments in the mechanical properties of paper was carried out. For this purpose samples of filter paper were subjected to different cleaning treatments (immersion in distilled water as well as the application of agar and gellan gum rigid gels at different concentrations) and a comparative evaluation was done.

Tensile tests were carried out to evaluate the mechanical properties of paper before and after cleaning treatments to assess if the use of water (either free or in gelled form) may induce any significant change in the mechanical properties of paper in terms of ultimate tensile strength, deformation at break and flexibility. The same evaluation was carried out on treated paper samples subjected to artificial accelerated thermo-hygrometric aging in an attempt to verify if samples subjected to any form of aqueous cleaning may be more prone to degradation than the untreated ones.

Results showed that immersion treatments in distilled water caused a significant change in the mechanical properties of paper (decrease in the ultimate tensile strength, increase in the strain to failure and significant changes in the elastic modulus). For this reason paper became weaker. On the contrary, in the case of the application of rigid gels a slight improvement of the mechanical properties of paper was observed (slight increase in the ultimate tensile strength and strain to failure).

After aging, the treated paper samples presented the same behaviour than the aged control (slight decrease in the ultimate tensile strength and increase in the breaking strain). In other words samples treated with rigid gels and subjected to artificial aging were not weaker than the untreated ones.

Finally and in what refers to the observation of the fracture area, some changes in the type of fracture were observed in those samples subjected to immersion treatments as well as when subjected to accelerated aging. In such cases a transition from a granular to a fibrillar break was evident.

From a microscopic point of view, the water absorption of paper samples after cleaning treatments caused slight dimensional changes in the diameter of fibres. In particular in the case of samples subjected to immersion treatments the diameter of paper fibres increased because of the swelling action of water molecules. These results suggested that higher amount of water released during some cleaning treatments (as it happens

during cleaning by immersion) could cause significant changes both in the mechanical-dimensional and morphological properties of paper. For this reason cleaning treatments by immersion in water should be carefully evaluated.

In the last part of the research colorimetric analysis were carried out in order to assess any colour change induced by the different cleaning treatments evaluated. The comparison between control and treated samples confirmed the existence of slight colour variation ($\Delta E<1$) in the case of treated samples. Such colour changes however, were not evident with the naked eye even after accelerated aging.

4. Evaluation of the effects of cleaning on the superficial morphology of paper

In the conservation field of paper artworks it's well known the importance of cleaning treatments for the removal of degradation products, external contaminants, salts and deposition materials which may compromise paper integrity. In fact, the presence of such products may promote degradation processes (hydrolysis, oxidation of cellulose, etc.), a chemical destabilization that results in the structural weakening of paper.
In paper conservation cleaning treatments range from the use of free water (immersion at 40°C) [56], aqueous gelled systems (such as cellulose ethers, carboxymethyl cellulose, hydroxypropyl cellulose in aqueous solution) [61,62] and polyacrylic acid salified with strong bases, for the partial solubilization of harmful polar, hydrophilic products. In the last years however, hydrogels in the rigid form (agar and gellan gum) had been tested [7, 8, 9, 63, 5]. Such polysaccharide gels exhibit different properties thanks to their porous structure deriving from electrostatic interactions among the polymer chains [67, 68, 69]. By varying the gel concentration, the properties of releasing water molecules change, as well as the absorption capacity of degradation products from paper. The possibility of preparing gels with different concentration and the adjustment of water supply represents an advantage for the application, especially in case of water sensitive media on paper. Although cleaning represents a fundamental treatment for paper conservation, if it is not done carefully it can be responsible of the alteration of paper surface.
Few attention has been given to the evaluation of the effects of cleaning treatments in the morphological properties of ancient paper surfaces although the original morphology of paper surface, the texture, represent an historical evidence of the papermaking.
For this reason the preliminary research described in this section was based on the evaluation of the effects of different cleaning treatments on the surface micromorphology of paper [3]. Cleaning treatments compared were: cleaning by immersion in distilled water (immersion washing), cleaning with water in gelled form (with agar and gellan gum), cleaning with water gelled with cellulose ethers, dry cleaning by Wishab ® sponge (white Wishab Akapad), to verify if eventually changes in surface profile may be attributed to the type of cleaning treatment.
For this purpose the study was carried out on a late 19^{th} century paper (newspaper) since it represented an example of industrial, low quality paper, containing encrusting substances, affected by cellulose oxidation and acidity which was therefore particularly in need of an efficient cleaning treatment. In addition, its extreme fragility and weakness would easily the consequences of any slight erosion on the surface.

Since the morphological properties of paper are strongly influenced by materials used in papermaking, the first part of the work consisted on the preliminary characterisation of paper (fibre composition and eventual additives) by means of chemical tests, Fourier Transform Infrared Spectroscopy analysis (FT-IR), stereo-microscopy observations, Scanning Electron Microscopy observation (SEM-EDS) [3].

The evaluation of the effects of cleaning on the surface roughness of paper was performed by means of stereo-microscopy observations, SEM-EDS analysis and surface microtopography analysis of samples by means of 3d profilometer, in order to identify the most respectful treatment with the morphological properties of paper [3]. The analysis were carried out before and after the cleaning treatments. This technique has proven to be useful in different application in the mechanical, biomedical, textile and forensic domains [96, 97, 98, 99, 100], in archaeological objects [101] and the conservation field [102].

The obtained roughness values were subjected to statistical analysis by means of ANOVA and Tukey HSD post hoc test [3]. This work therefore represents a preliminary study that has the potential to be applied to different types of paper (handmade paper, coated paper, glossy paper, handmade oriental paper) in the future.

4.1 Materials and samples preparation

4.1.1 Samples

Paper samples (2x2 cm) were cut from a 19th century oxidized and acid newspaper page and subjected to selected cleaning treatments.

4.1.2 Cleaning treatments

The cleaning treatments selected were [3]:
1. Cleaning with a solution of Klucel G ® 1–2 % (w/v) (hydroxypropyl cellulose in water) applied by brush and followed by removal with cotton swab and a water-alcohol solution (1:1 v/v);
2. Application of agar rigid gel for 30 min;
3. Application of gellan gum rigid gel for 30 min;
4. Immersion in distilled water;
5. Dry cleaning with Wishab ® sponge.

Preparation of Klucel G ® 1-2 % solution (w/v) in water:
1 g of Klucel G (2 g in the case of Klucel 2%) was added in 100 ml of distilled water at room temperature. The obtained solution was applied with brush on the paper surface. The gel was left on the paper for 5 min and then removed using a water-alcohol solution (1:1 v/v) with a cotton swab.

Preparation of agar gel 2 % (w/v): 2 g of agar was added to distilled water (100 ml) at 85°C [63]. The resulting dispersion was heated, stirred in a water bath for 20 min, then poured into a Pyrex glass container and left until gelation (38 °C). For the cleaning treatment, the gel obtained was applied on the surface of the paper for 30 min and then removed.

Preparation of gellan gel 2 % (w/v): calcium acetate (0.04 g) and 2 g of gellan gum [73, 103] were dissolved in 100 ml of distilled water at room temperature. The resulting solution was then heated in a microwave oven (900 W for 5 min), poured into a Pyrex glass container and cooled down to gelation temperature (40°C). The paper sample was treated by applying the rigid gel on its surface and then removing it after 30 min.

Immersion: Sample was immersed for 10 min in distilled water at room temperature. Although commonly used [56], higher temperature (40–50°C) were avoided in this study because such condition could have promoted the removal of sizing.

Dry cleaning with Wishab ® sponge: the treatment was performed by exerting a slight pressure with an extra soft Wishab[1] sponge for 5 min on paper surface.

4.2 Instruments and analytical procedure

4.2.1 Characterization of cellulose fibres by means of spot tests with Herzberg reagent

The preliminary characterization of samples fibres was performed by Herzberg (chlorine hydride, zinc) spot test.

Preparation of Hertzberg reagent: 40 g of zinc chloride were dissolved in 20 ml of distilled water and cooled; a second solution was prepared by dissolving 4.2 g of potassium iodide and 0.2 g of iodine 10 ml of distilled water; the two solutions were mixed and kept at rest for 1 day.

[1] AKAPADWhite: AKAPAD Dry Cleaning Sponge –white.

A small paper sample (about 0.5 mm^2) was placed on a glass slide and stained by deposing a drop of the reagent solution to the surface. After 18 min, the slides were observed by an optical microscope (OPTIKA N-400 FL) and the paper composition was identified [104].

4.2.2 Fourier Transform Infrared spectroscopy analysis

Paper samples were preliminary analysed by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) to verify the presence of inorganic fillers.
A spectrometer (NICOLET Smart Orbit Nexus TM), equipped with a silicon carbide IR source and a Mercury Cadmium Telluride (MCT) detector, was used to collect spectra for the samples examined in the study. ATR-FTIR spectra were collected in the range of 4,000–400 cm^{-1} (mid-infrared region), with automatic setting of the gain parameter, at a resolution of 4 cm^{-1}, by recording 120 scans using a diamond cell (30,000 ÷ 200 cm^{-1}).

4.2.3 Stereo-microscopy observations of the surface of paper samples

The surface of paper samples was observed before and after the cleaning treatments by means of OPTIKA SZM stereo-microscope (10x magnification) to evaluate any possible surface morphology modification.

4.2.4 Scanning Electron Microscopy analysis (SEM-EDS)

A scanning electron microscope, JEOL JSM-6400 equipped with an energy-dispersive X-ray analyser was used to observe the paper samples and to evaluate the effects of the cleaning treatments. Secondary electron mode was used to acquire the images. The elemental composition of samples was evaluated using an acceleration voltage of 20 keV, lifetime >10 s and working distance equal to 15 mm. The samples were previously coated with a graphite layer (0.1 μm of thickness) by sputtering (JEOL JEE-4X).

4.2.5 Analysis of three-dimensional surface microtopography of the paper samples

Three-dimensional surface microtopography data was acquired from the selected samples by means of a 3D stylus-based profilometer (SM RT-150) [3].
Acquisition system: SB60 stylus, conical tip at 90° with spherical end of 4 μm; maximum vertical resolution <0.1 μm; vertical field of measurement 130 μm.

For each treatment the acquisition was performed 24 times for a total of 168 samples covering an overall projected area of 0.7875 x 0.7875 mm with 64 x 64 scanning points in X and Y and uniform spacing dw=dy= 12.5 μm.
The resulting dataset consisted of a height map (z coordinates arranged over a regular x,y grid).

The analysis of each height map was performed in the following procedure [3]:

- The height map was levelled by subtraction of the least-squares mean plane;
- A visual analysis was performed on a 3D virtual model of the surface reconstructed from the height map. Artificial colours were used to express different levels: blue, green, yellow and red;
- The principal, 3D field parameters for surface finish assessment were evaluated, as defined in ISO/FDIS 25178-2, in order to investigate the main surface finish differences among paper treatments (Table 4.1);
- Special attention was reserved to the Sk field parameter (core height: distance between the highest and lowest level of the scale-limited surface excluding core-protruding hills and dales). Sk is a measure of dispersion of height values similar to Sa (arithmetic mean height) and it can be used to quantify surface roughness, however it is less influenced by localized singularities than the latter (e.g. localized high peaks or deep pits) and thus it is more reliable for running comparisons in this specific application;
- The hypothesis of a statistically significant modification in the surface microtopography of paper induced by the different cleaning treatments under examination was tested in this research by means of one-way analysis of variance (ANOVA) and Tukey post hoc test performed on the distributions of the Sk parameter evaluated for the different samples.

Table 4.1: Definition of the surface parameters employed for the morphological analysis of the paper surface (ISO/FDIS 25178-2).

Symbol	Parameter	Interpretation
Sa	Arithmetic mean height	Sa measures the dispersion of heights with respect to the mean surface height. The higher Sa, the rougher the surface
Ssk	Skewness of the scale-limited surface	Ssk measures the symmetry of the surface height distribution curve with respect to its mean value. It is 0 for a symmetric height distribution (e.g. Gaussian) and ≠0 for an asymmetric distribution. It is>0 if most heights are below the mean, but are counterbalanced by a few regions high above it (hills/peaks); it is<0 if most heights are above the mean, but are counterbalanced by a few regions well below it (dales/pits)
Sku	Kurtosis of the scale-limited surface	Sku measures the spikiness of the surface height distribution curve. It is 3 for a roughly Gaussian distribution;>3 for spikier distributions,<3 for flatter ones
Sk	Core height	Sk represents the core (or kernel) roughness of the surface over which a load may be distributed during most of the functional life of the surface. It is a measure of the nominal roughness and may be used to replace Ra and similar parameters. It is more robust of Ra especially when localized singularities (e.g. very high peaks or deep pits) may affect the outcome of the more traditional parameters
Spk	Reduced peak height	Spk measures peak height above the kernel zone
Svk	Reduced dale heigh	Svk measures valley depth below the kernel zone
Sr1	Material ratio	Sr1 measures the upper bearing area of the surface
Sr2	Material ratio	Sr2 measures the lower bearing area of the surface

4.3 Results and Discussion

4.3.1 Characterization of cellulose fibres by means of spot tests with Herzberg reagent

The paper samples were characterized and both cellulose pulp and wood pulp were found; in fact the preliminary observation showed the presence of cotton with the typical ribbon shaped morphology [4] and the presence of vascular structure typical of wood.
The red tint of sample acquired after the application of Herzberg reactive (typical of cotton or linen) and the formation of a violet-blue colour in the wood fibres confirmed the mixed composition of the paper: wood and cotton (Figure 4.1) [3].

Figure 4.1: Fibres of cotton a) and wood b) of paper sample after the treatment with Herzberg reagent (x100 magnification; x4 zoom camera).

4.3.2 Fourier Transform Infrared spectroscopy analysis

Paper samples were characterized by the presence of calcium sulphate dehydrate (3,534–3,402 cm^{-1} antisymmetric and symmetric OH stretching bands; 1,653–1,620 cm^{-1} stretching bands of intermolecular water; 1,110 cm^{-1} asymmetric SO_4^{2-} stretching band and bending at 670 cm^{-1}), calcium carbonate (1,425 cm^{-1} CO_3^{2-} stretching band; 873 cm^{-1} O–C–O bending band) and silicates (1,029 cm^{-1} asymmetric Si–O–Si stretching bands) used as inorganic fillers [105] as showed by the FT-IR results (Figure 4.2) [3]. These absorptions were detected besides the characteristic peaks of cellulose (absorption at 3000 cm^{-1} corresponding to OH stretching; asymmetric and symmetric stretching of CH_2 at 2900-2800 cm^{-1}; CH_2 symmetric bending at 1375 cm^{-1}; CH bending vibrations at 1335 cm^{-1}; OH in plane bending at 1100 cm^{-1}; ring asymmetric stretching at 1110 cm^{-1}; CO stretching at 1060-1030 cm^{-1} and OH of absorbed water at 1650 cm^{-1}) [106].

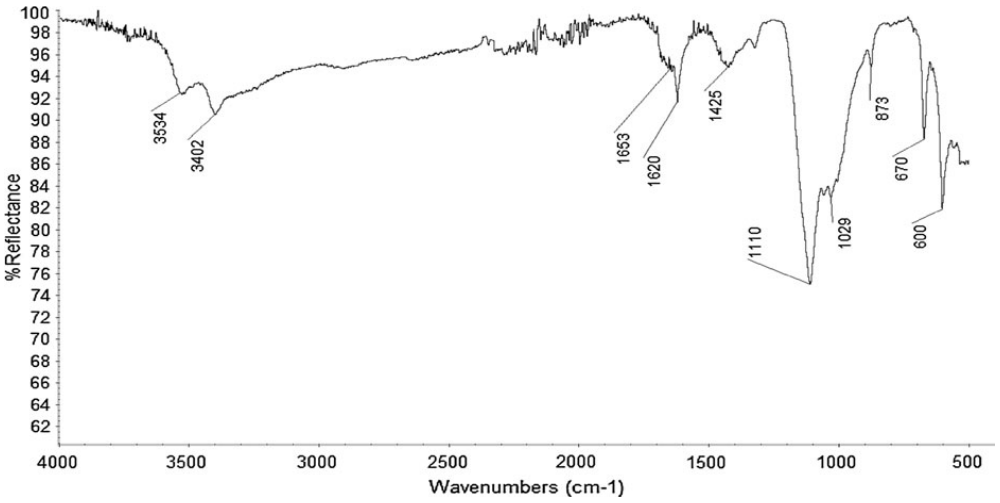


Figure 4.2: FTIR-ATR spectrum of nineteenth century paper sample.

4.3.3 Stereo-microscopy observations of the surface of paper samples

Samples paper were observed by means of stereo-microscopy before and after the cleaning treatments in order to assess the effects of cleaning on surface morphology (Figure 4.3) [3].

Figure 4.3: Stereo-microscopy observation of paper samples: gellan gum 2% treatment (a1= before; a2=after the treatment); agar gel 2% treatment (b1=before; b2= after the treatment); washing by immersion in distilled water treatment (c1= before; c2= after the cleaning treatment); Klucel G ® 2% application (d1= before; d2= after the treatment); cleaning treatment with Wishab ® sponge (e1=before; e2= after the treatment) (x10 magnification; x4 zoom camera).

In the case of gellan gum and agar gels as well as immersion treatment, the surface of paper remained unchanged from a morphological point of view. On the contrary, after the application of Klucel G ® 2% (and hydro-alcohol solution), the morphology of the surface showed a distinctive variation, probably due to the erosion produced by the use of brush and cotton swabs. In the case of Wishab ® sponge, no change in the surface morphology was observed, even if some residues remained on the surface (this could be appreciate only by stereo-microscope observations). Wishab ® residues could be removed by brushing. Different studies have demonstrated that the presence of Wishab

® residues on paper surface can be responsible for an increase in fluorescence when subjected to UV accelerated aging [107].

4.3.4 Scanning Electron Microscopy analysis (SEM-EDS)

The samples were analysed by SEM-EDS in order to evaluate the presence of inorganic fillers and assess the effects of cleaning in the fibres. SEM images showed a regular distribution of cellulosic fibres with consistent presence of inorganic particles. In fact, the microanalysis EDS revealed S, Ca, Mg, Si, Al, K, confirming the presence of gypsum ($CaSO_4$*$2H_2O$), kaolinite ($Al_2Si_2O_5(OH)_4$) (Figure 4.4) and, in some cases, talc ($Mg_3Si_4O_{10}(OH)_2$) (Figure 4.5) [3]. This presence is more evident in the case of untreated paper. Salts of potassium and sodium chloride were detected as well in some of the analysed regions, in particular in the untreated sample. The presence of Fe was also detected, which is known to play an important role in degradation phenomenon of cellulose. In the case of the samples subjected to cleaning the fibres profile was more defined (Figures 4.6 b, 4.6 c) and a lower presence of inorganic particles was observed with respect to the untreated sample (Figure 4.6 a) [3].

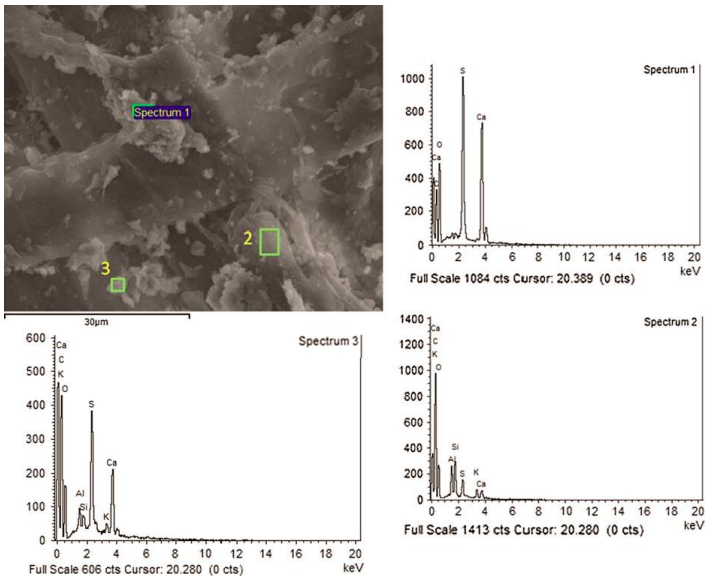


Figure 4.4: SEM–EDS analysis of untreated paper in secondary electron mode with x 2,000 magnification.

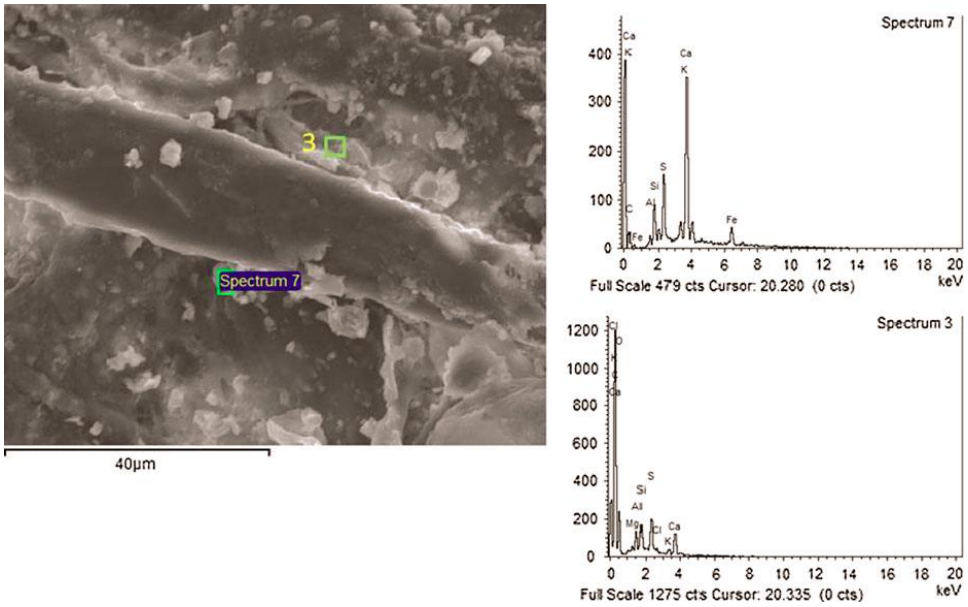


Figure 4.5 : SEM–EDS analysis of untreated paper in secondary electron mode with x 1,500 magnification.

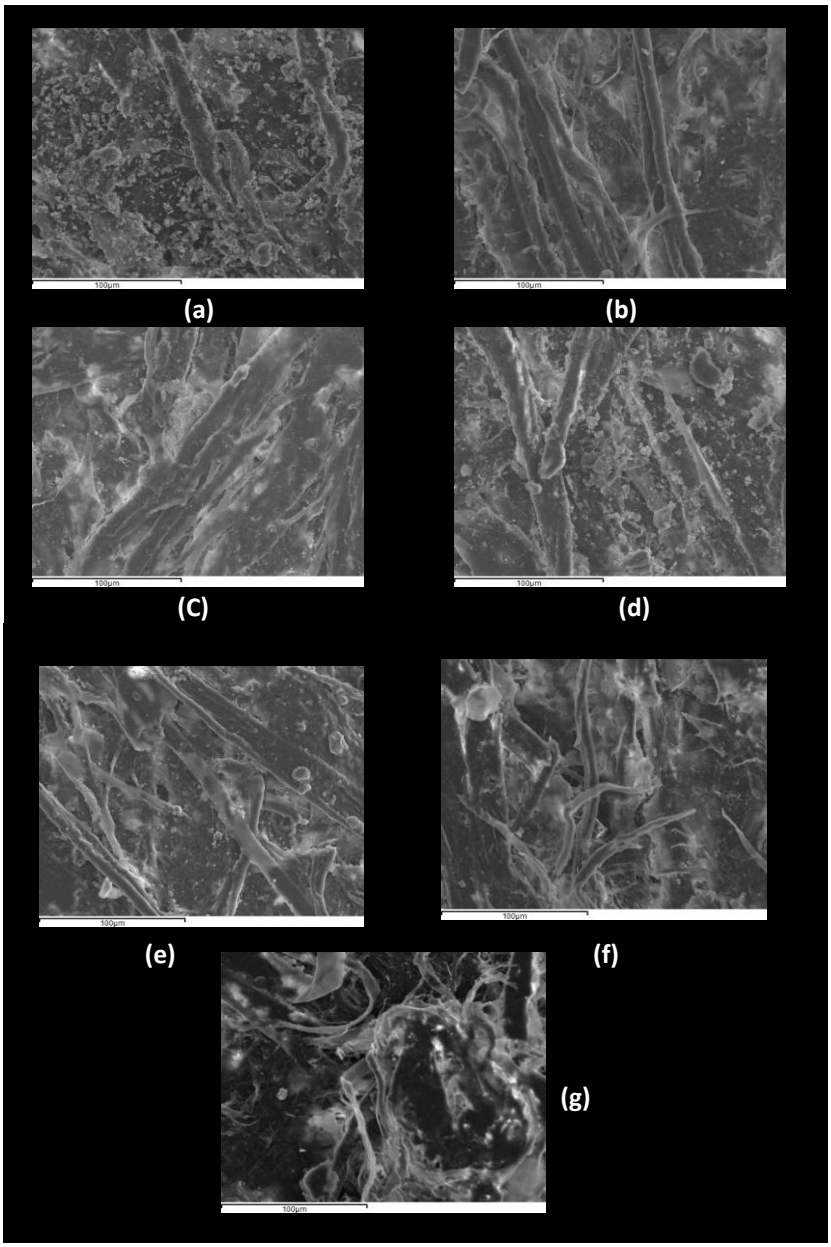


Figure 4.6 : Observation of samples by scanning electron microscopy (SEM–EDS) in secondary electron mode with x 550 magnification: untreated (a), treated with gellan gum gel (b), treated with agar gel (c), treated with immersion washing in distilled water (d), treated with Wishab ® sponge (e), treated with Klucel G ® 2 % (f) and Klucel G ® 1 % (g).

In particular, the sample treated by immersion in distilled water was more similar to the untreated sample, with abundance of inorganic particles along the fibres (Figure 4.6 d). The application of Klucel G ® by brush seems to have caused the defibration of the cellulosic structure and partial fibres breakage (Figures 4.6 f, g, 4.7 a), probably due to the erosion action to which the paper was subjected to during the treatment. The treatments with Wishab ® sponge (Figure 4.7 c) and Klucel G ® (Figure 4.7 b) released some residues in some of the treated regions [3].

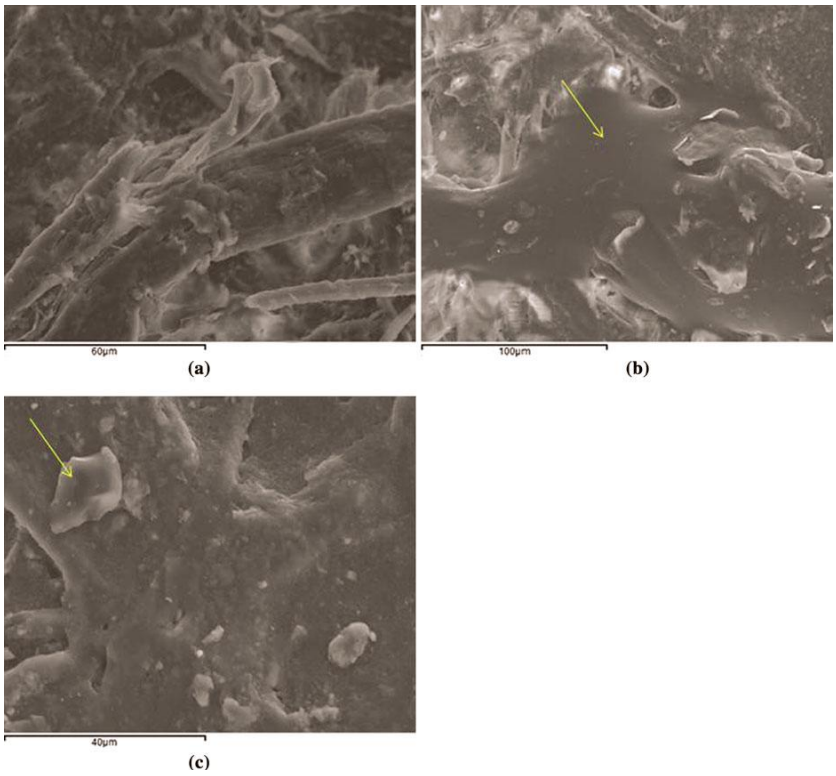


Figure 4.7: SEM–EDS analyses of paper samples treated with Klucel G ® (a, b) and Wishab ® sponge (c) in secondary electron mode with a magnification of: x 1,000 (a), x 550 (b) and x 1,500 (c).

4.3.5 Analysis of three-dimensional surface microtopography of the paper samples

The surface microtopography of the samples was measured and evaluated as described in the 4.2.5 paragraph (Figures 4.8, 4.9, 4.10, 4.11). The computed parameter values are reported in Table 4.2 [3]. For the evaluation of morphological differences induced by cleaning treatments the Sk value was considered. All the samples belonging to treated samples showed lower values of Sa and Sk when compared to the untreated samples, meaning that the treated surfaces were smoother than the untreated one [3].

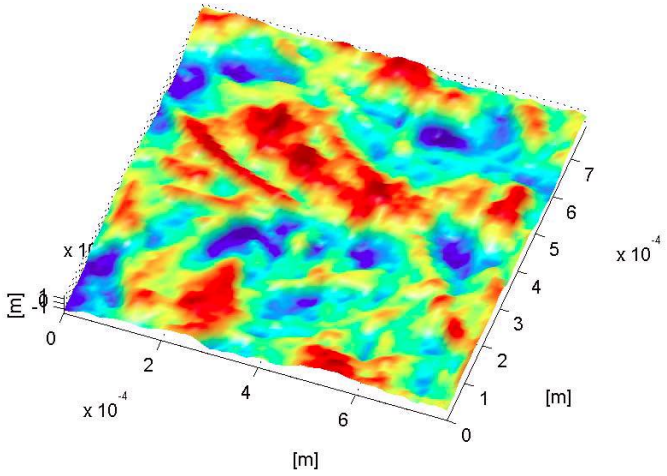


Figure 4.8: Surface of untreated sample.

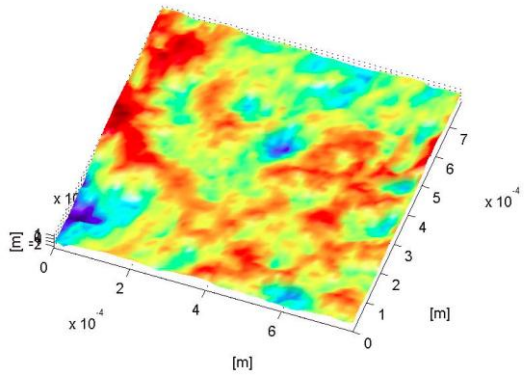


Figure 4.9: Surface of sample treated with Klucel G ® 2%.

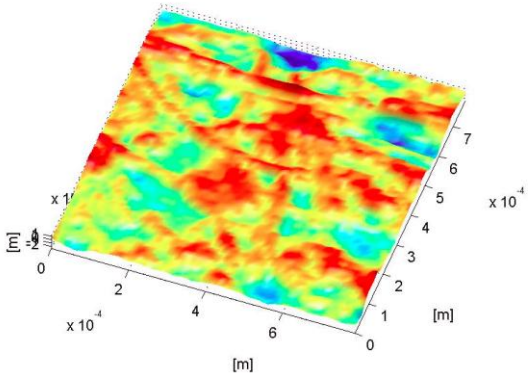


Figure 4.10: Surface of sample treated with gellan gum gel 2%.

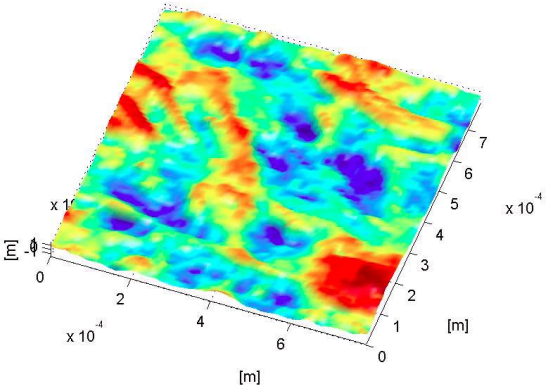


Figure 4.11: Surface of sample treated by immersion washing.

Table 4.2:Values of the surface parameters for treated and untreated samples (mean μ and standard deviation σ).

	Agar gel		**Gellan gum**		**Untreated**	
	μ	σ	μ	σ	μ	σ
Sa (μm)	4.98	0.56	4.97	0.7	5.49	0.55
Ssk	-0.21	0.25	-0.17	0.19	-0.13	0.21
Sku	2.94	0.39	2.87	0.25	2.71	0.27
Sk (μm)	**16.25**	**1.97**	**16.25**	**2.34**	**18.22**	**1.83**
Spk(μm)	4.56	1.18	4.69	1.67	4.85	1.65
Svk(μm)	6.28	1.25	6.26	1.06	6.28	1.33
Sr1	0.27	0.06	0.27	0.04	0.27	0.05
Sr2	0.67	0.06	0.68	0.04	0.68	0.05
	Water 30°		**Klucel 2%**		**Klucel 1%**	
	μ	σ	μ	σ	μ	σ
Sa (μm)	5.05	0.51	4.57	0.56	5.01	0.78
Ssk	-0.05	0.21	-0.36	0.22	-0.06	0.26
Sku	2.77	0.26	3.1	0.43	2.9	0.35
Sk (μm)	**16.67**	**2.08**	**14.69**	**1.93**	**16.24**	**2.40**
Spk(μm)	5.04	1.36	3.87	0.88	5.33	1.72
Svk(μm)	5.58	1.15	6.57	1.37	5.85	1.52
Sr1	0.29	0.06	0.25	0.05	0.28	0.06
Sr2	0.69	0.06	0.65	0.05	0.68	0.06

	Wishab	
	μ	σ
Sa (μm)	4.86	0.51
Ssk	-0.27	0.19
Sku	2.92	0.39
Sk (μm)	**15.9**	**1.79**
Spk(μm)	4.14	0.97
Svk(μm)	6.38	1.26
Sr1	0.27	0.05
Sr2	0.67	0.05

ANOVA (Table 4.3) and post hoc Tukey test confirmed that there is a significant effect of the cleaning treatments on the value of the Sk parameter at the $p<0.05$ level (i.e. the Sk value for each treated sample is significantly different from the untreated one), with the exception of the treatment by immersion in water (washing treatment) (Table 4.4) [3]. This may be due to the different way in which the washing treatment was applied compared to the others treatments (the washing consists in immersing paper samples in a bath; for this reason, in this case it is not applied any force on the surface).

However, the mean values of the Sk parameter evaluated for the different treatments did not significantly differ one from the other, with only a few exceptions (Table 4.5). These results suggest that all the cleaning treatments under analysis in this work lead to a slight reduction of surface roughness (i.e. the samples have a smoother surface after the treatments). In particular, cleaning by means of Klucel® G 2 % solution and by immersion in water caused the maximum and minimum reduction of surface roughness (20 and 8 % respectively). In all cases, the reduction of surface roughness was rather small (less than 15 % in the Sk value) [3].
The kurtosis parameter (Sku) was close to 3 for every sample, indicating an almost Gaussian distribution of height values. The untreated samples showed lower values of the Sku parameter, but the difference was not found to be statistically significant. The skewness parameter (Ssk) was slightly below 0 and homogeneous for all samples, indicating a negligible asymmetry of the height distributions with respect to its average value [3].
The distributions of the Svk and Spk parameters did not suggest the existence of any variation within the samples with respect to the dominance of peak structures relative to valley structures. Analogously, the values of parameters Sr1 and Sr2 were similar for all the samples, indicating that the distributions of material between peaks, valleys and kernel zone did not change due to the cleaning treatments [3].

Table 4.3: One-way analysis of variance summary (Sk parameter).

	Sum of squares	df	Mean square	F	Sig
Between groups	157.759	6	26.293	6.191	.000
Within groups	683.782	161	4.247		
Total	841.542	167			

Table 4.4: Grouping of means using post hoc Tukey test (Sk parameter).

Tukey grouping*	Treatment	Means
a	Untreatd	18.22
ab	Water 30°	16.67
bc	Gellan gum	16.25
bc	Klucel 1%	16.24
bc	Wishab	15.9
bc	Agar gel	16.25
c	klucel 2%	14.69

* Means with the same letter are not significantly different at the 0.05 level

Table 4.5 : Comparison between all groups using post hoc Tukey test (Sk parameter).

Treatment (I)	Treatment (J)	Mean difference (I-J)	Std.error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
Agar gel	Gellan gum	0.009	0.595	1.000	-1.768	1.785
	Water 30°C	-0.419	0.595	0.992	-2.196	1.357
	Klucel 1%	0.014	0.595	1.000	-1.763	1.790
	Klucel 2%	1.563	0.595	0.125	-0.214	3.339
	Wishab	0.351	0.595	0.997	-1.425	2.128
	Untreated	-1.965*	0.595	0.020	-3.741	-0.188
Gellan gum	Agar gel	-0.009	0.595	1.000	-1.785	1.768
	Water 30°C	-0.428	0.595	0.991	-2.204	1.348
	Klucel 1%	0.005	0.595	1.000	-1.771	1.781
	Klucel 2%	1.554	0.595	0.129	-0.223	3.330
	Wishab	0.343	0.595	0.997	-1.434	2.119
	Untreated	-1.974*	0.595	0.019	-3.750	-0.197
Water 30°C	Agar gel	0.419	0.595	0.992	-1.357	2.196
	Gellan gum	0.428	0.595	0.991	-1.348	2.204
	Klucel 1%	0.433	0.595	0.991	-1.343	2.209
	Klucel 2%	1.982*	0.595	0.018	0.205	3.758
	Wishab	0.771	0.595	0.853	-1.006	2.547
	Untreated	-1.546	0.595	0.133	-3.322	0.231

Treatment (I)	Treatment (J)	Mean difference (I-J)	Std.error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
Klucel 1%	Agar gel	-0.014	0.595	1.000	-1.790	1.763
	Gellan gum	-0.005	0.595	1.000	-1.781	1.771
	Water 30°C	-0.433	0.595	0.991	-2.209	1.343
	Klucel 2%	1.549	0.595	0.132	-0.228	3.325
	Wishab	0.338	0.595	0.998	-1.439	2.114
	Untreated	-1.979*	0.595	0.018	-3.755	-0.202
Klucel 2%	Agar gel	-1.563	0.595	0.125	-3.339	0.214
	Gellan gum	-1.554	0.595	0.129	-3.330	0.223
	Water 30°C	-1.982*	0.595	0.018	-3.758	-0.205
	Klucel 1%	-1.549	0.595	0.132	-3.325	0.228
	Wishab	-1.211	0.595	0.396	-2.987	0.565
	Untreated	-3.527*	0.595	0.000	-5.304	-1.751
Wishab	Agar gel	-0.351	0.595	0.997	-2.128	1.425
	Gellan gum	-0.343	0.595	0.997	-2.119	1.434
	Water 30°C	-0.771	0.595	0.853	-2.547	1.006
	Klucel 1%	-0.338	0.595	0.998	-2.114	1.439
	Klucel 2%	1.211	0.595	0.396	-0.565	2.987
	Untreated	-2.316*	0.595	0.003	-4.093	-0.540
Untreated	Agar gel	1.965*	0.595	0.020	0.188	3.741
	Gellan gum	1.974*	0.595	0.019	0.197	3.750
	Water 30°C	1.546	0.595	0.133	-0.231	3.322
	Klucel 1%	1.979*	0.595	0.018	0.202	3.755
	Klucel 2%	3.527*	0.595	0.000	1.751	5.304
	Wishab	2.316*	0.595	0.003	0.540	4.093

* The mean difference is significant at the 0.05 level

The results of this preliminary research showed that the cleaning of paper may cause a slight variation in the surface morphology of paper (defibration of cellulose and a reduction of surface roughness). This variation was more evident in the case of Klucel G ® 2% because it was applied on the paper surface by brush and then removed by rubbing with cotton swabs pre-soaked with a hydro-alcohol solution. This procedure involved a mechanical action on the paper surface, resulting in a larger variation in the surface roughness. On the contrary the application of rigid gels was more respectful with the morphological properties of paper, thus inducing minimal changes. The immersion in distilled water was the less invasive treatment because no mechanical action was exerted on paper surface.

4.4 Conclusions

In this work different cleaning treatments were applied on nineteenth century newspaper samples in order to evaluate their effects on the paper surface. The cleaning treatments considered were: application of Klucel G ® (hydroxypropylcellulose) in water by brush, application of agar (2 %) and gellan gum (2%) rigid gels, immersion in distilled water and dry cleaning with Wishab ® sponge.

After the preliminary characterization of samples by spot test with Herzberg reagent and FT-IR analysis, the samples were subjected to SEM-EDS and surface microtopography analysis based on 3D profilometry to investigate changes in morphology of fibres as well as surface finish differences ascribable to the cleaning treatments respectively. Stereo-microscopy observations of samples before and after cleaning treatments showed significant changes in the surface profile of paper in the case of Klucel G ® application. On the contrary other treatments did not induced significant changes. Also SEM-EDS analysis revealed defibring of paper treated with Klucel G ® and Wishab ® sponge.

These results were confirmed by 3D profilometer analysis. In fact based on the resulting height maps, the principal 3D field parameters for surface finish assessment were evaluated and ANOVA was performed on the distributions of the Sk parameter evaluated for the different samples, confirming that the cleaning treatments reduced the value of the Sk parameter at the $p<0.05$ level, (i.e. the surface of the paper was smoother after the execution of the cleaning treatments).

All the cleaning treatments under analysis in this work lead to the reduction of surface roughness (i.e. the samples presented a smoother surface after the treatments). In particular, cleaning by means of the Klucel G ® solution 2 % caused the maximum reduction of surface roughness (around 20 % of the Sk value), while the cleaning

processes based on rigid gels (agar and gellan gum) and immersion were less aggressive for the surface morphology.
These results suggest that the application methodology is equally relevant than the material used. In this context the use of brushes leads to an erosion of the surface.

5. Analytical evaluation of the extraction of sugar fraction from paper during cleaning treatments

In this section a preliminary study on the evaluation of the extraction of the sugar fraction during aqueous cleaning treatments of ancient papers was performed.
In this context traditional cleaning treatments such as immersion in distilled water as well as the use of rigid gels were compared.

The type of hemicelluloses contained in paper and the amount of these components is strongly dependent on the row materials used in paper production, on the chemical, physical and mechanical processes as well as on the condition to which paper has been subjected during its manufacture. Higher content of hemicelluloses can be observed in case of paper manufactured with wood pulp (softwood and hardwood). But the hemicelluloses content is also influenced by those treatments for the removal of encrusting substances such as the k*raft* and sulphite process as well as paper's condition. In fact aged paper can be subjected to hydrolysis process of cellulose and hemicelluloses in different rate.

From a chemical point of view, the result of the hydrolysis of cellulose may be the formation of free units of glucose as well as oligosaccharides of glucose; on the other hand the formation of free units of pentose and hexose sugars such as xylose, rhamnose, galactose, mannose, etc., may derive from the degradation of hemicelluloses (i.e.: xylan, glucomannan) and these sugars can be extracted by water. For this reason free units of glucose and xylose could be considered as markers of some of the chemical reactions responsible for the degradation of paper, such as hydrolysis of cellulose and hemicelluloses [22].

In this research the aspect of the extraction of the sugar fraction by means of water (in liquid or in gelled form) during cleaning treatments was considered. Two cases of study are presented: a 18^{th} century paper, representative of a handmade paper, and an industrial paper late from the 19^{th} century, in particular a sheet of an ancient journal affected by oxidation and acidity phenomenon. Such papers are different in composition, manufacture and condition. For this reason the effects of cleaning as well as that of the extraction of sugars from paper could be different.

Up today, only few studies have demonstrated how the content of hemicelluloses in paper could be relevant from a mechanical perspective and how the treatments for the removal of incrusting substances could induce a weakening of the paper structure as well as changes in the mechanical properties [52]. On the other hand the free sugars

present in paper in the monomeric or oligosaccharide form could be more prone to the oxidation phenomenon induced by thermo-hygrometric aging, causing also discoloration of paper [45]. For such reasons the study of the extraction of sugars deriving from paper during aqueous cleaning treatments represents a relevant aspect to investigate.

In this research paper samples were preliminary characterized by spot tests with Herzberg and Phloroglucinol reagents to obtain information about the type of paper, papermaking. Then paper samples were subjected to different cleaning treatments (immersion in distilled water and treatment with agar and gellan gum rigid gels). Both the water used for immersion, and the gels used were considered, analyzed by means of Gas Chromatography-Mass Spectrometry (GC-MS), to evaluate the eventual extraction of sugar based fraction induced by each cleaning treatment.

5.1 Materials and samples preparation

5.1.1 Samples

This preliminary study was carried out on two examples of paper: a handmade paper taken from a 18th century book (thickness of the sheet: 125 µm; average grammage: 62 g/m^2), and an industrial paper taken from 19th century journal (thickness of the sheet: 89 µm; average grammage: 30 g/m^2). In the case of the 18th century paper, samples (30x30 mm) were obtained from the edge of papers since it did not contain inks (Figure 5.1A), in order to have similar samples. In the second case (19th century paper) the treatments were performed in portion containing ink of fatty nature (Figure 5.1C) because regions of paper without ink were not sufficient for all treatments.

Samples were weighted with a *Sartorius CP225D balance* (d=0.01 mg) after conditioning treatment at 103°C for 3 hours for the removal of the water absorbed by paper. Samples were subjected to the different aqueous cleaning treatments and for each treatment three replicates were considered in the case of 18th century paper; on the contrary for 19th century paper, it was possible to use only one sample for each treatment because of the few amount of paper available for the study.

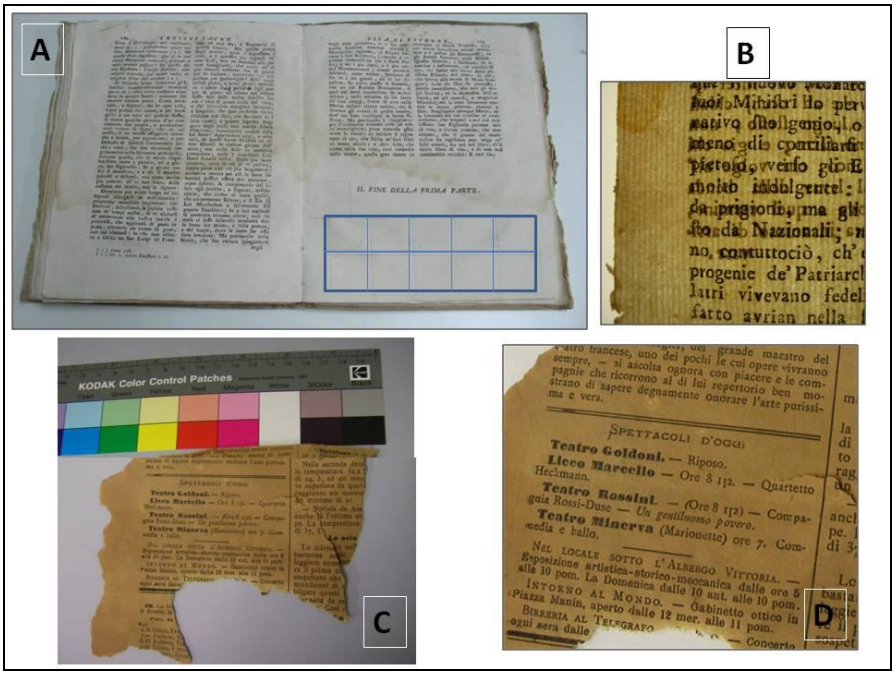


Figure 5.1: 18th and 19th century paper: A) 18th century paper with the indication of sampling points, B) magnification of the 18th century paper in transmission light (it is evident the typical morphology, texture of a handmade paper), C) 19th century paper (a portion of sheet of journal), D) magnification of the 19th century paper (an evident oxidation can be appreciated).

5.1.2 Cleaning treatments

Paper samples were subjected to aqueous cleaning treatments, in particular:

- Immersion washing in distilled water at room temperature (duration: 30 minutes; pH of water:6);
- Application of gellan gum rigid gel 2% (w/v) (duration of the treatment: 30 minutes);
- Application of agar rigid gel 2% (w/v) (duration of the treatment: 30 minutes).

Gels (5 mm of thickness) were prepared using the same procedure described in chapter 4.

The washing treatment was performed by immersion of paper in 70 ml of distilled water at room temperature for 30 minute (Figures 5.2 A, B, C; Figure 5.5 F); the gels were applied directly on the surface of paper samples for 30 minutes and then removed (Figures 5.3 A, B, C, D; Figures 5.4 A, B, C, D; Figures 5.5 A, B, C, D, E; Figures 5.6 A, B, C, D, E, F). Then the water used for the immersion treatment and the gels used for cleaning treatment were considered for chemical analysis.

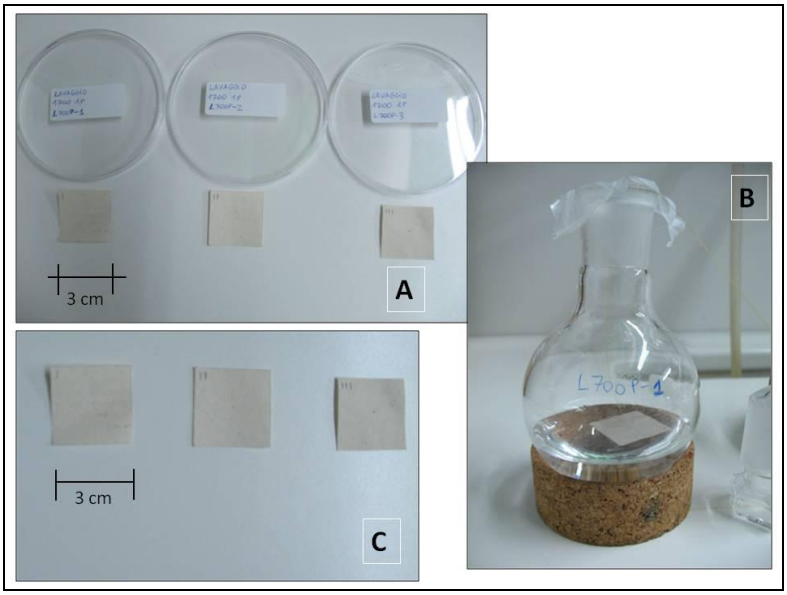


Figure 5.2: Immersion washing treatment of 18th century paper samples: A) paper samples before, B) immersion treatment in distilled water, C) paper samples after the treatment.

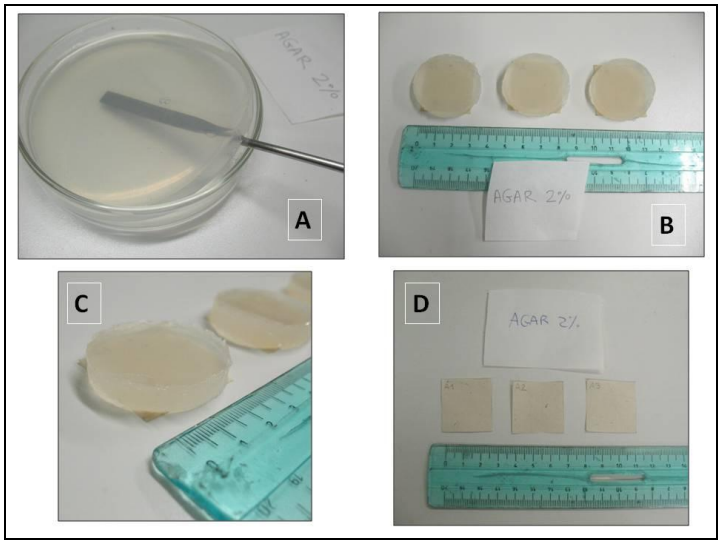


Figure 5.3: Application of agar gel 2% on 18th century paper samples: A) agar rigid gel 2%, B) paper samples during the application of agar gel, C) detail of agar application, D) paper samples after the cleaning treatment with agar gel.

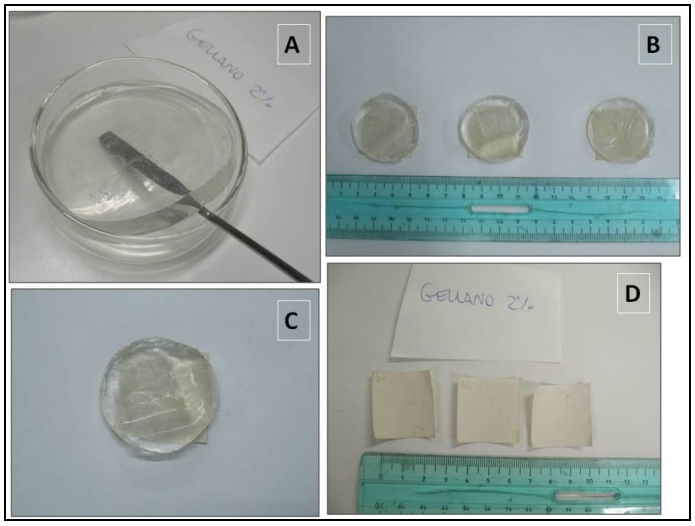


Figure 5.4: Application of gellan gum gel 2% on 18th century paper samples: A) rigid gellan gum gel 2% (the figure shows the transparency of gellan gum in comparison with agar gel), B) paper samples during the application of gellan gum, C) detail of gellan gum application, D) paper samples after cleaning treatment with gellan gum gel.

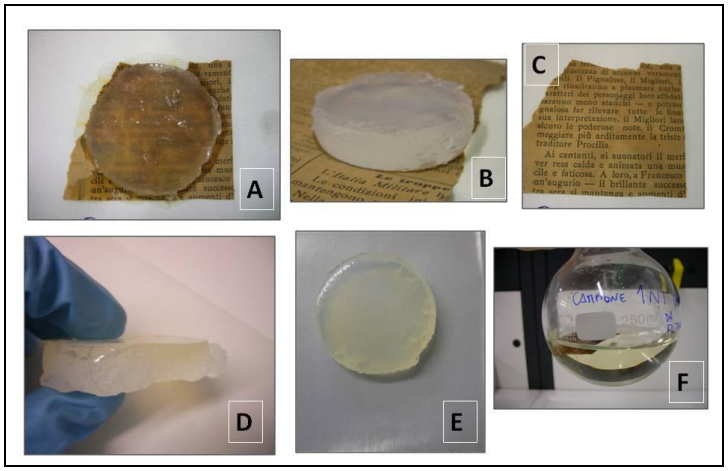


Figure 5.5: Cleaning treatments of 19th century paper: A) application of agar gel 2%, B) detail of agar gel application, C) paper sample after cleaning treatment with agar gel, D-E) agar gel after treatment on paper (figures show the yellowing of the gel after the application on paper caused by the extraction of degradation products from paper), F) immersion washing treatment in distilled water of paper sample (the water became yellow because of the extraction of degradation products).

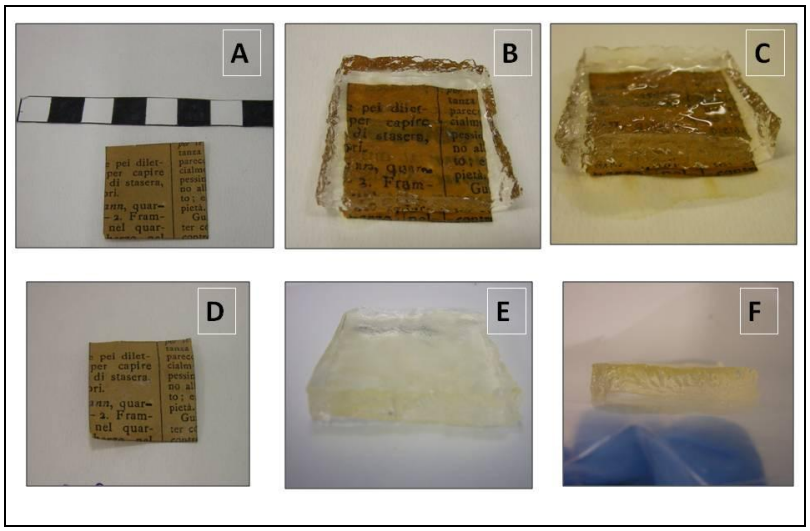


Figure 5.6: Cleaning treatment of 19th century paper: A) paper sample before the cleaning treatment, B-C) application of gellan gum gel 2%, D) paper sample after cleaning treatment, E-F) gellan gum gel after treatment on paper (figures show the yellowing of the gel after the application on paper caused by the extraction of degradation products from paper).

5.2 Instruments and analytical procedure

5.2.1 Preliminary characterization of paper by means of spot tests with Herzberg and Phloroglucinol reagents.

Paper samples were preliminary characterized by means of spot tests with Herzberg (chlorine hydride, zinc) and Phloroglucinol (1,3,5 Benzenetriol) reagents. The Hertzberg reagent was prepared with the same procedure described in chapter 4.

The Phloroglucinol reagent was prepared by dissolving 0.5 g of Phloroglucinol in 25 ml of ethyl alcohol 90 %, followed by the addition of 12.5 ml of concentrated hydrochloric acid. A small paper sample (about 0.5 mm^2) was placed on a glass slide and stained by deposing a drop of the reagent solution to the surface. After 10 min, the slides were observed by stereo-microscope (OPTIKA SZM).
Such method is usually used to assess the content of lignin and, therefore to determine the type of paper [104].

5.2.2 Method of extraction

GC-MS was performed for the characterization of sugars and uronic acids extracted from paper during cleaning treatments.

In the case of the treatment by immersion, distilled water (70 ml) used for the immersion of samples was dried by rotavapor. The residue obtained was then chemically treated for GC-MA analysis.

In the case of the use of agar and gellan gum rigid gels, these were put into a Buchner filter on a Buchner flask and extraction with distilled water (70 ml) under vacuum conditions was performed after having been used for treating the samples. The distilled water collected was filtered, dried by rotavapor and the residue obtained was chemically treated for GC-MS analysis as described in the following paragraph.

5.2.3 Gas Chromatography-Mass Spectrometry analysis

Preparation of the samples

The determination of sugars and uronic acids extracted by cleaning treatments was carried out by means of Gas Chromatography/Mass Spectrometry.

Samples (residue obtained after drying water by rotavapor) were subjected to the following analytical procedure: 1ml of distilled water was added to the residue in order to transfer analytes in a schlenk tube, and then it was dried under vacuum, heating at 40°C under magnetic stirring. Internal standard (30 µl of a 0.01M solution of sorbitol in water) was added in the schlenk containing sample and treated with the same analytical procedure used for samples. A hydrolysis reaction of polysaccharide fraction was performed by trifluoroacetic acid 2M (2ml) at 100°C for 6 hours. Afterwards, samples were dried under vacuum on a heating plate equipped with magnetic stirrer at 40°C. The hydrolysed residue was first mercaptalated with 60 µl of a mixture of ethantiol and trifluoroacetic acid (2:1) (reaction at room temperature for 40 minutes) under magnetic stirring and then, after evaporating solvent in vacuum conditions, derivatized (at room temperature for 40 minutes) by adding 50 µl of pyridine, 100 µl of hexamethyldisilazane (HMDS) and 30 µl of trifluoroacetic acid [108]. After evaporation of the solvent in vacuum, the residue was dissolved in 500 µl of hexane and 1 µl of this solution containing diethyl-dithioacetal trimethylsilyl derivates was injected into the gas chromatograph.

Apparatus and chromatographic conditions

A Focus GC (Thermo Scientific) coupled to DSQ II (Thermo Scientific) with single quadrupole and split-splitless injector was used. The carrier gas helium flow was kept constant at 1.0 ml/min. Separation of components (diethyl-dithioacetal trimethylsilyl derivates) was performed by means of fused-silica capillary column (RXI-5, Restek), stationary phase 5% phenyl 95% methylpolysiloxane, 0.25 µm film thickness, 30 m length.

For the analysis of analytes the split-splitless injector was set to 280°C with a 30 seconds purge off time. The GC oven temperature program was: 165°C for 0 minutes, 2°C/min to 190°C, 1°C/min to 210°C, 20°C/min to 235°C. The injector was used in the splitless mode. The MS transfer line was set to 250°C. Ionization was performed in the electron impact mode (EI) at 70 eV, with an ion source temperature of 230°C. EI mass spectra were recorded in TIC (Total Ion Current, mass range m/z 50-500) and positive ions were considered. The chromatographic peak areas were integrated for each sample

and corrected by response factor using a standard solution of eight monosaccharides (sorbitol, xylose, arabinose, rhamnose, fucose, glucose, mannose, galactose) and two uronic acids (galacturonic acid and glucuronic acid) in hexane (3 µg/ml). The average of three injections for each sample was considered (Relative Standard Deviation about 6%) and the monosaccharides and uronic acids were expressed as average relative percentage in relation to the total of monoaccharides, in order to obtain semi-quantitative information.
Preliminary papers (18-19th century) were characterized and an estimation of the amount of monosaccharides contained in paper in relation to the paper sample weight (monosaccharide (mg)/paper (g)) was performed considering internal standard.

5.3 Results and Discussion

5.3.1 Preliminary characterization of paper by means of spot tests with Herzberg and Phloroglucinol reagents

The preliminary test performed by means of Herzberg and Phoroglucinol reagents revealed the presence of pure cellulose (probably cotton) in the case of 18th century paper. In fact sample acquired a red tint (red wine) after treatment with Herzberg (Figure 5.7 B) and no change in colour was detected with the use of Phloroglucinol reagent (Figure 5.7 C).

In the case of 19th century paper, a wood pulp (more specifically not bleach mechanical pulp) was observed, confirmed by the yellow colour acquired after treatment with Herzberg (Figure 5.7 E) as well as the purple colour acquired after treatment with Phloroglucinol reagent (Figure 5.7 F) [4].

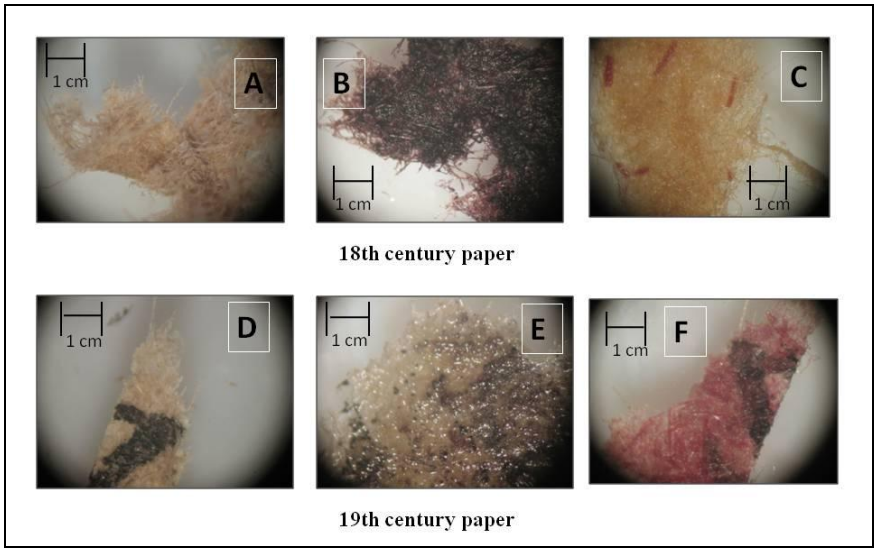


Figure 5.7: Spot tests performed on 18th and 19th century paper and stereo-microscope observation (5x magnification): A) paper sample of 18th century before the test, B) paper sample of 18th century after the application of Herzberg reagent, C) paper sample of 18th century after the application of Phloroglucinol reagent; D) paper sample of 19th century before the test, E) paper sample of 19th century after the application of Herzberg reagent, F) paper sample of 19th century after the application of Phloroglucinol reagent.

5.3.2 Gas Chromatography-Mass Spectrometry analysis

Preliminary results obtained from the GC-MS analysis of papers (18th and 19th century) showed the different amount of monosaccharides deriving from hemicelluloses and cellulose. In the case of 18th century paper (handmade paper) glucose predominates (71% in relation to the total amount of sugars) since cellulose is almost pure and represents the main component of this type of paper. On the contrary in the case of 19th century paper (industrial paper), the use of wood pulp (not bleached mechanical pulp) is responsible for a significant presence of hemicelluloses evidenced by the presence of xylose, arabinose, rhamnose, mannose and galactose (Table 5.1), (Figures 5.8, 5.9). In the case of 19th century paper, few amount of glucuronic acid was also detected and it could be a product of glucose oxidation (oxidation of aldehyde group). The consistent presence of hemicelluloses, the presence of wood pulp and the type of papermaking (industrial papermaking) of 19th century paper might have partially promoted acidity, oxidation and discoloration of paper.

Table 5.1: Monosaccharides and uronic acids detected in 18th and 19th century papers. The amount is expressed as mg of analyte/ g of paper. The relative percentage of analyte in relation to the total of monosaccharides and uronic acids was also expressed.

	18th century		19th century	
	mg/g paper	%Tot	mg/g paper	%Tot
Xyl	4.97	6.7	7.19	7.4
Ara	0.89	1.2	38.69	**39.7**
Rha	0.70	0.9	14.03	**14.4**
Fuc	-	-	1.12	1.2
GlucA	-	-	1.01	1.0
Glu	52.44	**70.8**	11.69	12.0
Man	6.09	8.2	18.58	**19.1**
Gal	8.97	12.1	5.02	5.2
Parameters				
Rha/Glu	0.01		1.2	
Gal/Glu	0.2		0.4	
Xyl/Glu	0.1		0.6	
Man/Glu	0.1		1.6	

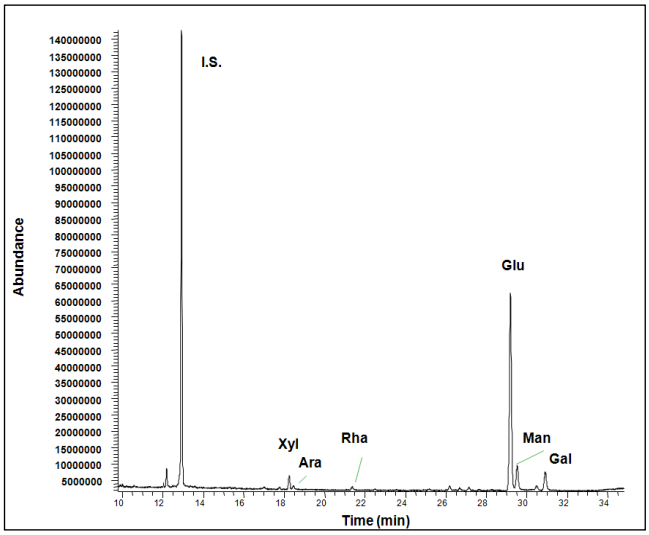


Figure 5.8: Total ion chromatogram of 18th century paper. The following monosaccharides were detected: xylose (Xyl), arabinose (Ara), rhamnose (Rha), glucose (Glu), mannose (Man) and galactose (Gal). Sorbitol is the internal standard.

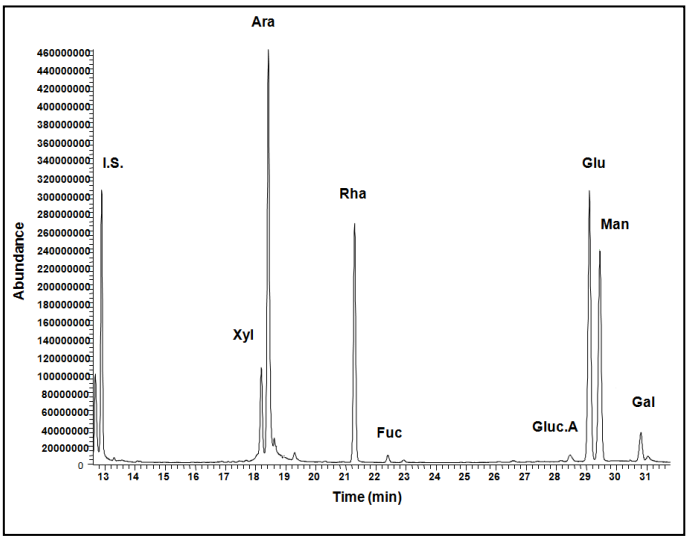


Figure 5.9: Total ion chromatogram of 19th century paper. The following analytes were detected: xylose (Xyl), arabinose (Ara), rhamnose (Rha), fucose (Fuc), glucuronic acid (Gluc.A), glucose (Glu), mannose (Man) and galactose (Gal). Sorbitol is the internal standard.

The water resulting from the immersion treatment as well as the gels used were analyzed in the same way. The aim was to evaluate the extraction of the sugar fraction (expressed as monosaccharides) deriving from paper induced by aqueous cleaning treatments. The amount of monosaccharides was indicated as relative percentage of analyte in relation to the total of monosaccharides (Table 5.2).

Table 5.2: Sugar fraction extracted during cleaning treatments of 18th century paper. Cleaning treatments considered are: immersion washing (W1, W2, W3), application of gellan gum gel 2% (G1, G2, G3), application of agar gel 2% (A1, A2, A3). The amount of each monosaccharide is expressed as relative percentage in relation to the total of monosaccharides.

	Cleaning treatments of 18th century paper (%Tot)								
	W1	**W2**	**W3**	**G1**	**G2**	**G3**	**A1**	**A2**	**A3**
Xyl	19.3	22.8	13.1	58.8	12.0	4.4	32.3	38.7	22.9
Ara	5.1	16.3	13.6	7.4	6.5	1.0	12.4	11.4	13.9
Rha	19.8	18.7	13.5	1.6	5.0	27.7	-	-	-
Gluc.A	-	-	-	-	-	2.4	-	-	-
Glu	16.8	14.4	26.5	13.8	54.3	59.8	14.1	12.1	16.7
Man	18.4	16.5	13.3	7.5	9.9	1.7	14.0	12.3	16.6
Gal	20.6	11.2	19.9	11.0	12.3	3.0	27.2	25.4	29.8
Parameters									
Rha/Glu	1.2	1.3	0.5	0.1	0.1	0.5	-	-	-
Gal/Glu	1.2	0.8	0.8	0.8	0.2	0.1	1.9	2.1	1.8
Xyl/Glu	1.1	1.6	0.5	4.3	0.2	0.1	2.3	3.2	1.4
Man/Glu	1.1	1.1	0.5	0.5	0.2	0.0	1.0	1.0	1.0

All cleaning treatments caused the extraction of sugars from 18th century paper, especially xylose, mannose and galactose (Figures 5.10, 5.11, 5.12). Water in the gelled form also caused the extraction of such sugars. However, in the case of gellan gum gels, the extraction procedure performed by the Buchner filter also caused the partial extraction of the gel itself. For this reason, the higher content of glucose, the presence of glucuronic acid associated to the rhamnose (as in sample G3), may be derived from the contribution of the gel.

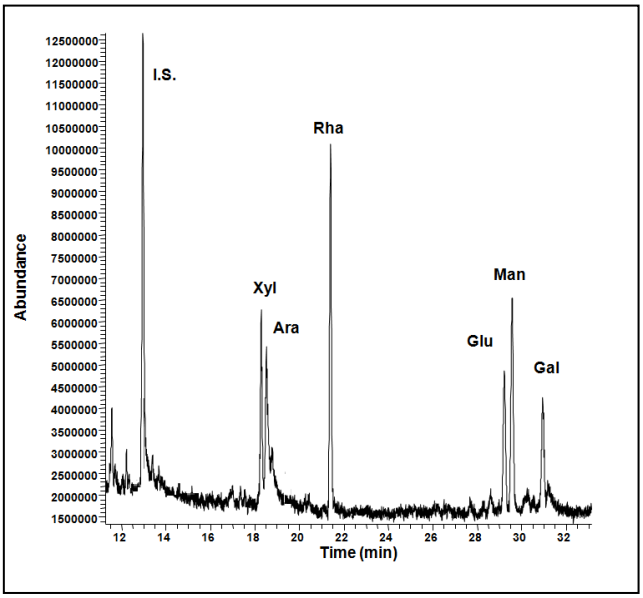


Figure 5.10: Total ion chromatogram of the sugar fraction extracted during immersion washing treatment of 18th century paper. The following monosaccharides were detected: xylose (Xyl), arabinose (Ara), rhamnose (Rha), glucose (Glu), mannose (Man) and galactose (Gal). Sorbitol is the internal standard.

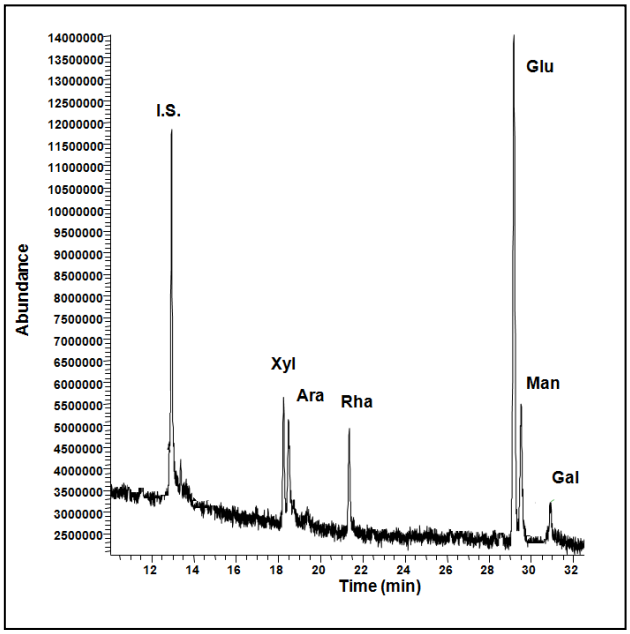


Figure 5.11: Total ion chromatogram of the sugar fraction extracted during application of gellan gum gel 2% on 18 th century paper. The following monosaccharides were detected: xylose (Xyl), arabinose (Ara), rhamnose (Rha), glucose (Glu), mannose (Man) and galactose (Gal). Sorbitol is the internal standard.

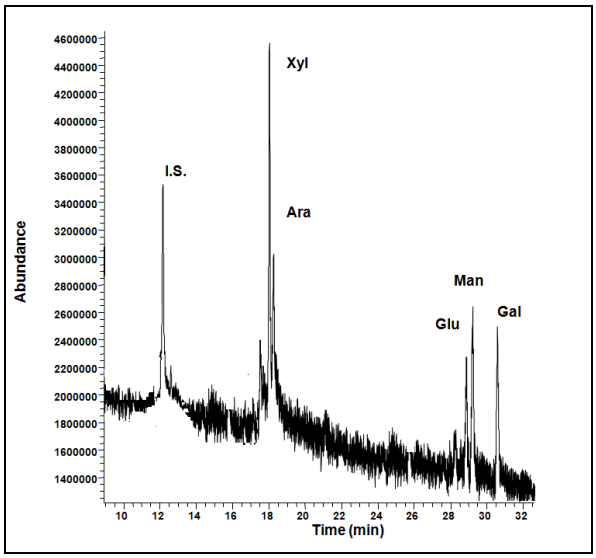


Figure 5.12: Total ion chromatogram of the sugar fraction extracted during application of agar gel 2% on 18th century paper. The following monosaccharides were detected: xylose (Xyl), arabinose (Ara), glucose (Glu), mannose (Man) and galactose (Gal). Sorbitol is the internal standard.

Also in the case of cleaning treatments performed on 19th century paper, the extraction of the sugar fraction took place both in the immersion treatment as well as when using gels (Agar and Gellan gum) (Table 5.3). In the case of immersion a significant extraction of glucose was observed together with xylose, glucuronic acid and mannose. The monosaccharides detected could be derived from part of the degraded hemicelluloses and cellulose extracted by water during immersion or when applying the gels on the paper. As known by previous studies [22] when ancient paper is affected by degradation processes (i.e.: hydrolysis of cellulose, xylan and glucomannan hemicelluloses) depolimerization of cellulose and hemicelluloses may occur. For this reason the products of hydrolysis (monosacchaides or oligosaccharides) can be extracted by water. This result could be the effect of the condition of paper: higher amount of glucose extracted in water could mean that paper is affected by hydrolysis process of cellulose. Previous studies carried out by means of size exclusion chromatography on water extracts of the same type of paper (19th century) showed that some fractions of this sugars presented an average degree of polimerization in the range 1000-35 [77].

Table 5.3: Sugar fraction extracted during cleaning treatments of 19th century paper. Cleaning treatments considered are: immersion washing (W800), application of gellan gum gel 2% (G800), application of agar gel 2% (A800). The amount of each analyte is expressed as relative percentage in relation to the total analytes. The percentage of analytes in sample crt 800 (paper of 19th century) is also reported.

	19th century paper (%Tot)			
	crt 800	**W800**	**G800**	**A800**
Xyl	7.4	10.5	17.5	3.8
Ara	39.7	2.0	7.5	35.6
Rha	14.4	-	-	-
Fuc	1.2	-	-	-
GlucA	1.0	15.7	7.9	-
Glu	12.0	40.9	23.5	6.4
Man	19.1	27.7	3.6	16.7
Gal	5.2	3.2	40.0	37.6
Parameters				
Rha/Glu	1.2	-	-	-
Gal/Glu	0.4	0.1	1.7	5.9
Xyl/Glu	0.6	0.3	0.7	0.6
Man/Glu	1.6	0.7	0.2	2.6

5.4 Conclusions

In this research an analytical study of the extraction of the sugar fraction from paper during aqueous cleaning treatments was carried out. As known in the field of conservation, cleaning treatments, in particular aqueous cleaning treatments, are commonly used for the removal of degradation products, salts and any hydrophilic compounds which may compromise paper integrity. However the aspect of the removal of original components of paper during cleaning treatments, such as the sugars of paper, has been traditionally underestimated and only few studies had been performed. For this reason, the study was applied on two different types of paper: 18th century (representative of handmade paper) and 19th century paper (industrial paper, in particular mechanical pulp) subjected to different cleaning treatments: immersion in distilled water and application of rigid gels (agar and gellan gum). Papers were characterized in advance by spot tests by means of Herzberg and Phloroglucinol reagents in order to know type of pulp and papermaking.

Paper samples were treated and the water from both the immersion bath as well as from the extraction of gels used was analysed by means of GC-MS. Results showed that aqueous cleaning treatments could cause the extraction of sugars from paper in different amount depending on the type of paper and its condition. For instance in the case of paper (especially 19thcentury industrial paper) affected by hydrolysis and oxidation phenomenon, the amount of glucose, xylose extracted by water increased as a result of hydrolysis and depolimerization of cellulose and hemicelluloses of paper. This phenomenon could have some effects on paper from a mechanical point of view, such as the weakening of paper. On the other hand, few studies have demonstrated how the presence of free sugars in paper deriving also from degradation process could promote oxidation and discoloration of paper, and for this reason the removal of such components could be convenient.

6. Analytical evaluation of the extraction of gelatine from paper during cleaning treatments

In the field of paper conservation the wet cleaning treatments are commonly applied for the removal of contaminants, degradation substances. Several cleaning treatments of ancient paper may be used and the commonest is the immersion washing in distilled water. Nevertheless the use of water presents lots of limits that are primarily due to the uncontrolled diffusion of water. For this reason rigid polysaccharide gels (agar and gellan gum) have been experimented and different advantages in their use can be recognized (as described in chapter 1, paragraph 1.3 Aqueous cleaning treatments of paper artworks). Study carried out on artworks, for instance ancient paper, are usually based on characterization of materials, degradation products and for instance, on the evaluation of the effect induced by a specific conservation treatment on material. Not so much studies consider the issue of the extraction of original components of paper during the application of conservation treatments, such as cleaning treatments. This aspect is relevant since properties of paper are strongly dependent on its composition, its condition, papermaking. The probable extraction of original components of paper such as gelatine, commonly added during papermaking to improve paper's properties (paragraph 1.1.3), induced by cleaning treatments, could cause change in mechanical properties of paper. For this reason the aim of the research was the analytical evaluation of the extraction of gelatine from paper induced by wet cleaning treatments by correlating traditional immersion washing in distilled water and the application of rigid polysaccharide gels (agar gel and gellan gum). The study was performed on handmade papers of different periods (16^{th}, 17^{th}, 18^{th} century) containing gelatine as sizing agents. The analytical study of the removal of gelatine is carried out by Gas Chromatography - Mass Spectrometry (GC-MS) analysis of amino acids, after a preliminary characterization of paper by means of Herzberg reagent and Fourier Transform Infrared Spectroscopy (FT-IR). The relative percentage of amino acids were subjected to the statistical method of the principal components analysis (PCA), to confirm the presence of gelatine in paper samples.

6.1 Materials and samples preparation

6.1.1 Samples

The research was divided in two step: the first part was a preliminary study performed on three types of handmade papers from books of different periods (16^{th}, 17^{th}, 18^{th} century respectively called *paper A, B, C*) of unknown provenance (Figures 6.1, 6.3). Paper samples (3x3 cm) were collected from the bottom-edge of the paper in which ink was not present and subjected to the following cleaning treatments: immersion washing in distilled water and application of gellan gum. Two replicates for each treatment were considered.

In the second part, the study was extended also to the application of agar gel. The evaluation was carried out on paper from a book (18^{th} century, defined *paper D*) and much more material was available for the experimental (Figure 6.2). For this reason paper samples collected on the bottom-edge of paper sheet (3x3) were, then, subjected to three types of cleaning treatments: immersion in distilled water, application of agar and gellan gum rigid gels, and three replicates for each treatment were considered (Table 6.1).

A micrometer (Outside Micrometer Mitutoyo, model: M317-25; measuring range: 0-25mm; graduation 0.01 mm) was used to measure thickness of samples and the average of ten measures was considered.

Preliminary paper samples were weighted by a precision balance *(Sartorius CP225D, d=0,01 mg)* after conditioning at 103°C for 3 hours. The average value of thickness (μm) and the average value of grammage (g/m^2) of paper are presented in Table 6.2.

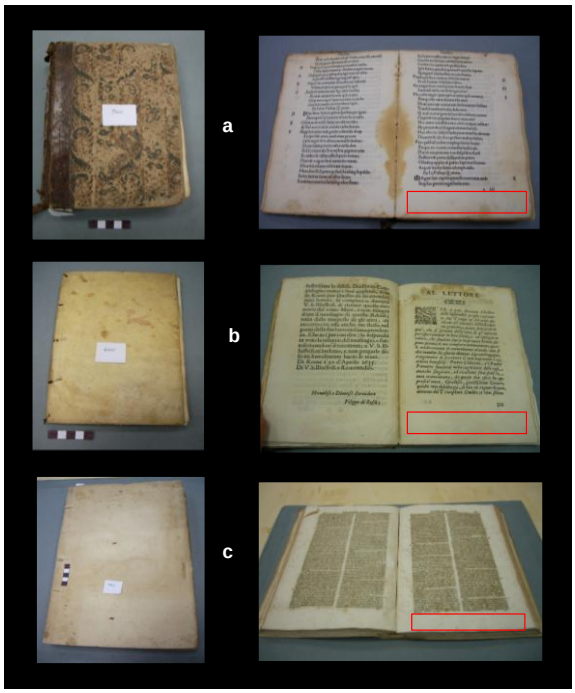


Figure 6.1: Paper samples from 16th century (a), 17th century (b), 18th century (c) books and sampling areas (red frame).

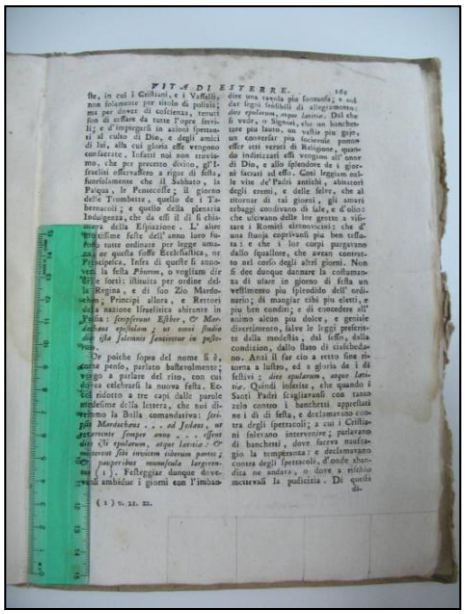


Figure 6.2: Paper samples collected from the edge of 17th century book (paper D).

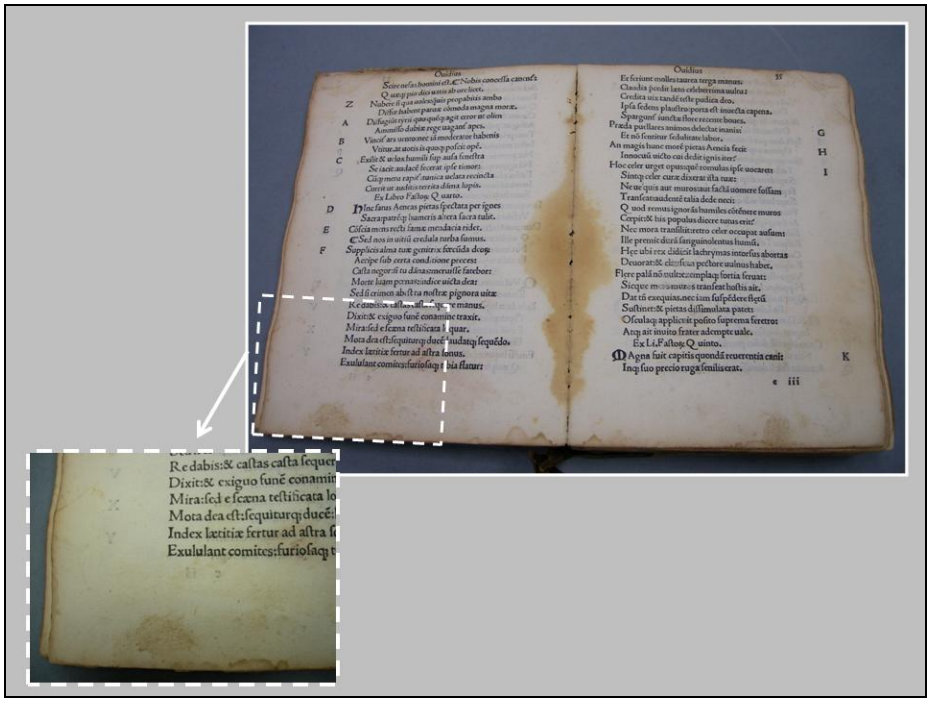


Figure 6.3: 16th century book of unknown origin. Stains of different natures are present.

Table 6.1: Paper, samples and treatments performed.

Paper	Samples	Treatment
A (16th century)	Aw1; Aw2	Immersion in distilled water
A (16th century)	Agel1; Agel2	Applicaton of gellan gum gel 2%
B (17th century)	Bw1; Bw2	Immersion in distilled water
B (17th century)	Bgel1; Bgel2	Application of gellan gum gel 2%
C (18th century)	Cw1;Cw2	Immersion in distilled water
C (18th century)	Cgel1; Cgel2	Applicaton of gellan gum gel 2%
D (18th century)	Dw1, Dw2, Dw3	Immersion in distilled water
D (18th century)	Dgel1; Dgel2; Dgel3	Application of gellan gum gel 2%
D (18th century)	Dag1; Dag2; Dag3	Application of agar gel 2%

Table 6.2: Thickness and grammage values of papers.

Paper	Thickness (µm)	Grammage (g/m^2)
Paper A (16 th century)	180	87
Paper B (17 th century)	150	62
Paper C (18 th century)	157	62
Paper D (18th century)	125	60

6.1.2 Cleaning treatments

The cleaning treatments performed on papers A, B, C were:

- Immersion washing in distilled water at room temperature (duration of the treatment 30 minutes);
- Application of gellan gum rigid gel 2% (w/v) (duration of the treatment 30 minutes).

Paper D was subjected to the following cleaning treatments:

- Immersion washing in distilled water at room temperature (duration of the treatment 30 minutes);
- Application of gellan gum rigid gel 2% (w/v) (duration of the treatment 30 minutes);
- Application of agar rigid gel 2% (w/v) (duration of the treatment 30 minutes).

Gels were prepared by the same procedure described in chapter 4.

Washing treatment was performed by immersing paper sample in 70 ml of distilled water at room temperature for 30 minute. Although it is common the use of heated water at 40-50°C for the immersion treatment [56], in this research distilled water at room temperature was used. This choice considered the risk associated to the use of water heated at 40°C which could promote the extraction of animal glue contained in the paper. In fact temperature higher than 40°C could promote the denaturing of collagen, the main constituent of gelatine.

The gels were applied directly on the surface of paper samples for 30 minutes and then removed (Figure 6.4).

The water used for the immersion treatment and the gels used for cleaning treatment were considered for chemical analysis.

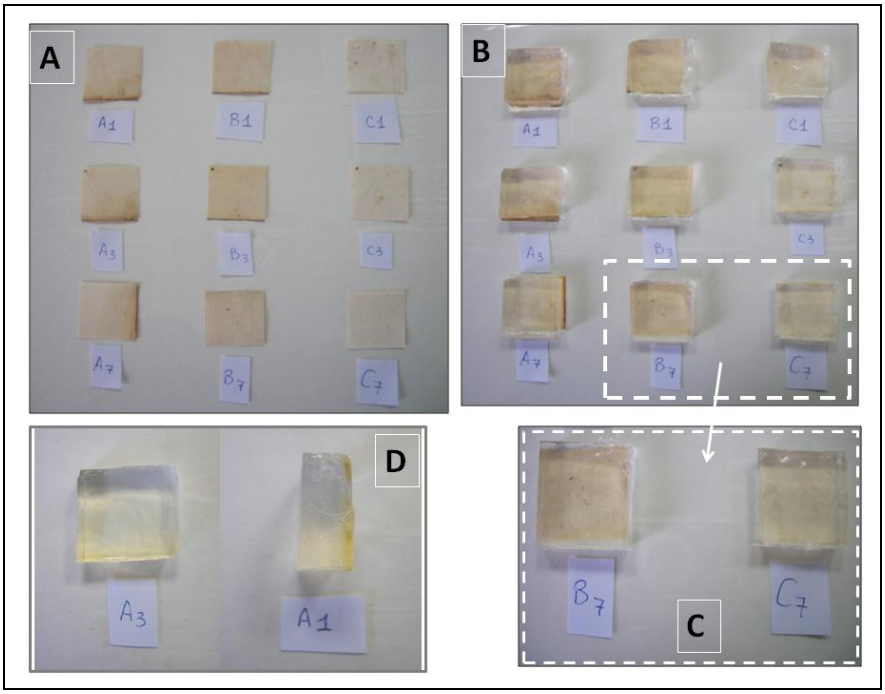


Figure 6.4: Application of gellan gum rigid gel 2% on ancient paper (16th, 17th, 18th century): A) samples before the treatment, B) samples during cleaning treatment with gellan gum gel, C) magnification of the application of gellan gum gel on papers, D) gellan gum gel after the application on paper samples. A yellowing of the gels after the application on paper can be observed.

6.2 Instruments and analytical procedure

6.2.1 Preliminary characterization of papers by means of spot tests with Herzberg reagent

The preliminary characterization of paper cellulose fibers was performed with the application of Herzberg reagent (chlorine hydride zinc) [104].
Preparation of Herzberg reagent was carried out with the same method described in chapter 4.
Small samples of paper were swollen in water to separate fibers. Then fibers were applied on a slide and a drop of Herzberg reagent was added. After a few minutes

samples were observed by means of optical microscope (Leica DMLS) (10-20x magnification) and type of fibre was identified [109].

6.2.2 Fourier Transform Infrared spectroscopy analysis

The preliminary characterization of paper was performed by means of Fourier Transform Infrared Spectroscopy in the reflection mode (μATR FT-IR). A Thermo_Nicolet Nexus 5700 spectrometer coupled with a Nicolet Continuμm FTIR microscope fitted with an MCT detector cooled by liquid nitrogen and a x15 Thermo-Electron Infinity Reflachromat objective with tube factor of x10 was used. The spectra were acquired in the range 4000-400 cm^{-1} at a spectral resolution of 4 cm^{-1}. A total of 120 scans have been recorded and the resulting interferogram averaged. FT-IR analysis were performed on extracts from samples.
The extraction was carried out by placing a small piece of sample, water (500 μl) in a glass tube with stopper and leaving all in magnetic stirring for 12 hours. A few drops of extract were put on the alumina side of a TLC paper. The FT-IR analysis was done on the dried residue.

6.2.3 Method of extraction

The analysis of the gelatine removed during cleaning treatments was performed by means of Gas Chromatography/Mass Spectrometry analysis (GC-MS). For this purpose the gels (agar and gellan gum) after application on papers, were put into a Buchner filter on a Buchner flask and extraction with distilled water (70 ml) under vacuum conditions was performed. The distilled water collected was then filtered, dried by rotavapor and the residue obtained was chemically treated for GC-MS analysis of amino acids deriving from paper, as described in the following paragraph.
In the case of immersion treatment, the water used for such treatment was dried by rotavapor, and then the residue was chemically treated for GC-MS analysis of amino acids deriving from paper.

6.2.4 Gas Chromatography-Mass Spectrometry analysis

Preparation of the samples

The determination of the removal of gelatine from paper induced by cleaning treatments was carried out by means of GC-MS analysis of amino acids.
Samples (the dried water used for the immersion treatment, and water used for the extraction from gel applied on papers) were subjected to the following analytical procedure: the residues were hydrolysed by 6N hydrochloric acid (2 ml) in a schlenk

tube for 5 hours at 100°C in an oil bath under magnetic stirring. After evaporation to dryness, the hydrolysed residues were esterified using 3 ml of 2N HCl in propan-2-ol at 90°C for 1 hour. After cooling, the solvent was evaporated under vacuum and the residue was dissolved in 2 ml of dichloromethane and derivatised with 0.2 ml of trifluoroacetic anhydride at 60°C for 1 hour. The solvent was evaporated under vacuum and the residue was dissolved in 500 µl of dichloromethane. The obtained solution (containing N-trifluoroacetyl-O-2-propyl esters amino acids derivatives) was analysed by means of GC-MS (1 µl). The internal standards considered were: norleucine (50 µl of a 0.1 mg/ml solution w/v), and norvaline (50 µl of a 0.01 mg/ml solution w/v) and they were subjected to the same analytical procedure used for samples.

Apparatus and chromatographic conditions

A Focus GC (Thermo Scientific) coupled to DSQ II (Thermo Scientific) with single quadrupole and split-splitless injector was used. The carrier gas helium flow was kept constant at 1.0 ml/min. Separation of the obtained N-trifluoroacetyl-O-2-propyl esters amino acids derivatives was performed by means of a fused-silica capillary column DB5 (J&W Scientific), stationary phase 5% phenyl 95% methylpolysiloxane, 0.25 µm film thickness, 30 m length and the injector was used in splitless mode. The following GC oven temperature program was used: isothermal conditions at 60°C for 3 min, with 25°C/min heating up to 260°C and isothermal conditions at 260°C for 6 min (total run time 17.00 min). The MS transfer line was set to 280°C.

The ionization was performed in the electron impact mode (EI) at 70 eV with the ion source temperature of 200°C. The mass spectra were recorded in Selected Ion Monitoring (SIM: 140, 126, 154, 153, 139, 168, 182, 166, 164, 184, 180, 198, 91, 190 m/z fragments) considering positive ions.

The chromatographic peak area of each analyte was integrated, corrected by response factor using a standard mixture of amino acids (Ala, Gly, Thr, Ser, Val, Nval, Leu, Isoleu, Nleu, Pro, Hpro, Asp, Glu, Phe) in dichloromethane (5 µg/ml). The average of three injections was considered (Relative Standard Deviation RSD < 7%). The proteinaceous fraction extracted during cleaning treatments was calculated by considering the total amount of amino acids detected and it was expressed as ratio of protein recovered (mg) in relation to the initial weight of paper sample (g). Such semi-quantitative estimation was obtained by means of Internal Standard (Norleucine). The relative percentage of amino acids contained in samples were subjected to multivariate data analysis according to the PCA method and compared to the relative percentage of amino acids of a set of reference samples (standard samples from paintings of a Opificio delle Pietre Dure collection) to identify proteins contained in papers. Multivariate statistical analysis PCA, was performed using SPSS 19.0 software and the variables

considered were the relative percentages of 8 amino acids (Ala, Gly, Leu, Pro, Hyp, Asp, Glu, Phe) [110].

6.3 Results and Discussion

6.3.1 Preliminary characterization of papers by means of spot tests with Herzberg reagent

The preliminary characterization of fibres was performed by means of Herzberg reagent. The red-brown coloration obtained after the test indicates the presence of pure cellulose. All samples (16^{th}, 17^{th}, 18^{th}) showed the presence of cotton fibres (with the characteristic ribbon morphology) and flax/hemp fibres (Figure 6.5).

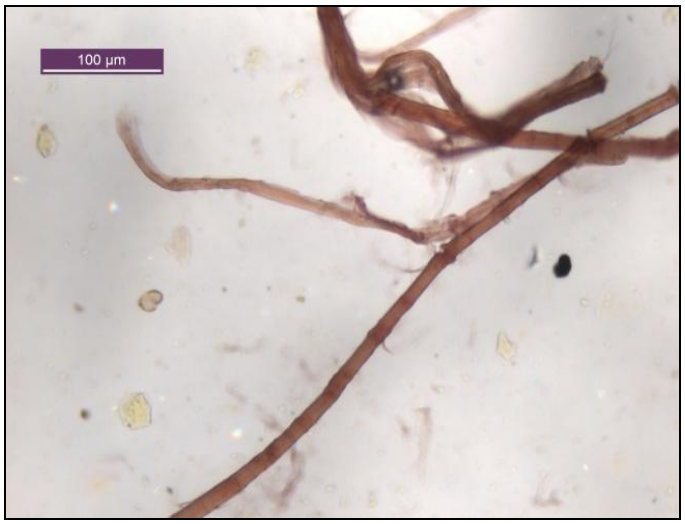


Figure 6.5: Characterization of paper fibres: flax/hemp fibres in 16^{th} century paper.

6.3.2 Fourier Transform Infrared spectroscopy analysis

The Fourier Transform Infrared Spectroscopy preliminary analysis was performed to verify the presence of protein fraction in the paper samples, in order to select the most suitable method of sample preparation for GC-MS analysis.

The results showed the presence of protein fraction in all papers. In figure 6.6 a FT-IR spectrum acquired on the residue obtained from the water extraction of 17th century paper sample (paper B) (a) was compared with a spectrum of a standard sample of gelatine (b).

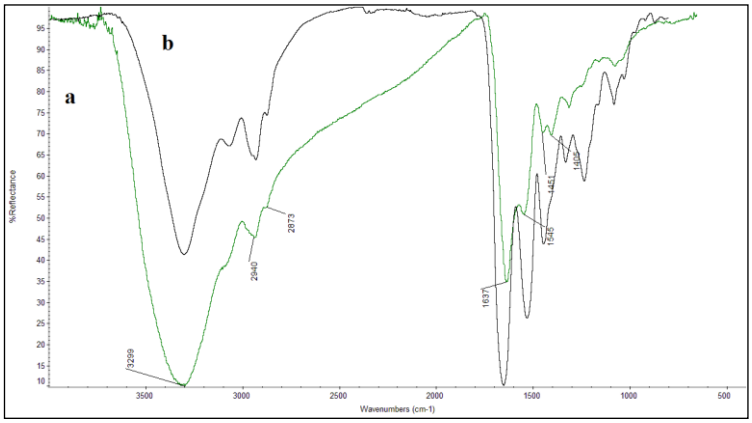


Figure 6.6: FT-IR spectra of the residue from the water extraction of 17th century sample (paper B) (a) and a standard sample of gelatine (b)

The typical spectrum of a polyamide (proteinaceous material) was detected: 3299 cm^{-1} stretching of NH; 2940-2873 cm^{-1} stretching of CH, 1637 cm^{-1} stretching amide I C=O; 1545 cm^{-1} amide II absorption (combination of C-N and stretching and NH bending); 1451 cm^{-1} amide III absorption (CH bending). Such protein fraction could derive probably from the animal glue (gelatine) added as sizing agents in paper during papermaking process.

6.3.3 Gas Chromatography-Mass Spectrometry analysis

The GC-MS results showed the presence of proteinaceous faction in all papers (paper A, B, C, D). Moreover aqueous cleaning treatments (immersion of paper in distilled water and application of agar and gellan gum gels) promoted the extraction of such material from paper. The following amino acids were detected: alanine (Ala), glycine (Gly), threonine (Thr), serine (Ser), valine (Val), leucine (Leu), proline (Pro), hydroxyproline (Hyp), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe) (Figures 6.7, 6.8, 6.9, 6.10, 6.11).

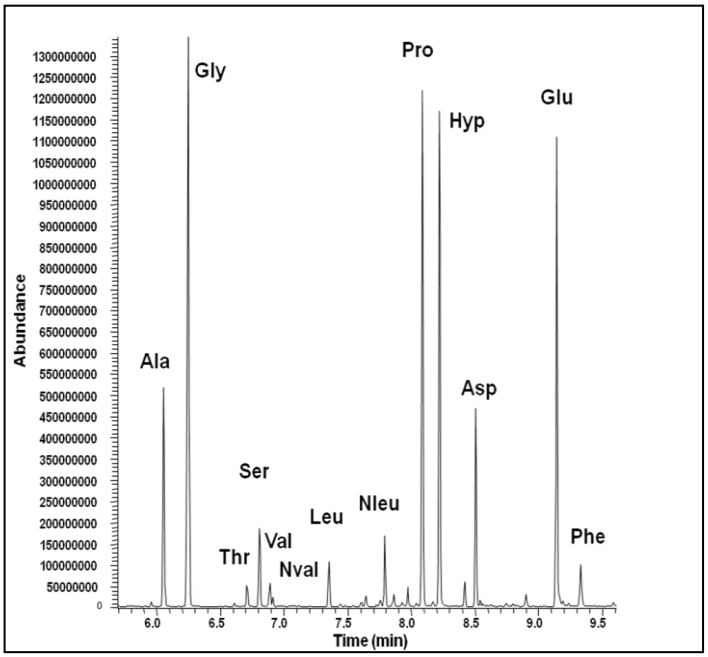


Figure 6.7: Chromatogram acquired in TIC of sample Aw1 (immersion treatment in distilled water of 16th century paper, paper A).

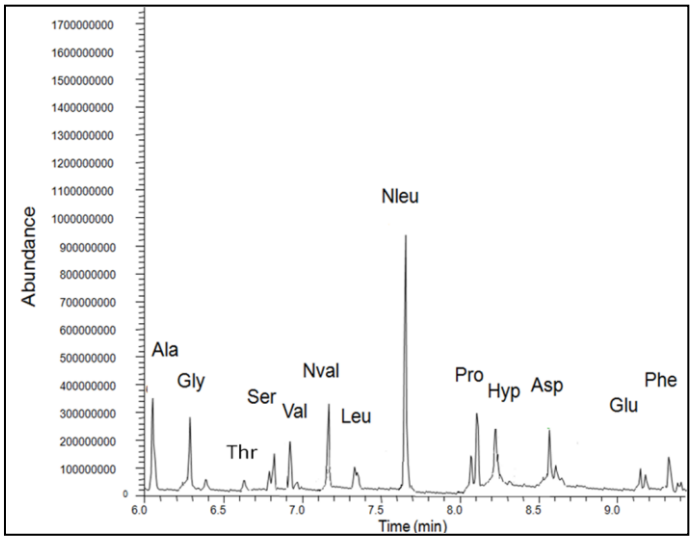


Figure 6.8: Chromatogram acquired in TIC of sample Agel1 (application of gellan gum gel on 16th century paper, paper A).

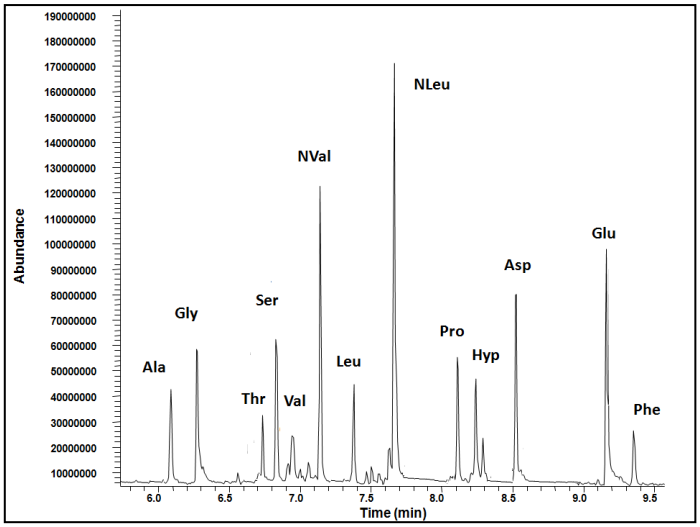


Figure 6.9: Chromatogram acquired in TIC of sample Dw2 (immersion treatment in distilled water of 18th century paper, paper D).

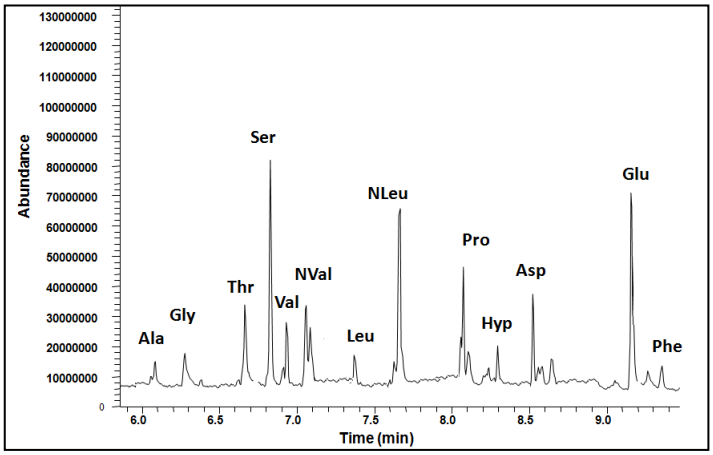


Figure 6.10: Chromatogram acquired in TIC of sample Dgel1 (application of gellan gum gel on 18th century paper, paper D).

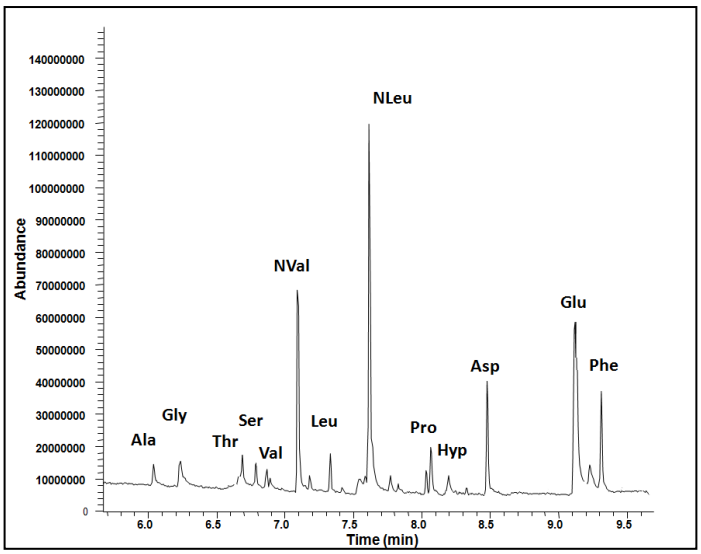


Figure 6.11: Chromatogram acquired in TIC of sample Dag1 (application of agar gel on 18th century paper, paper D).

Examination of the amino acids percentage reported in Table 6.3 suggested the presence of animal glue as sizing agent in all samples, in particular in paper A, B for the significant content of hydroxyproline (hyp), proline (pro) and glycine (gly). In fact the hydroxyproline is characteristic of the presence of material containing collagen.

Table 6.3: Relative amino acids percentages (% Tot) of samples (paper A, B, C, D).

Sample	ala	gly	leu	pro	hyp	asp	glu	phe
Aw1	8.3	46.5	2.3	12.7	1.7	7.9	18.9	1.8
Aw2	8.5	48.3	2.3	11.5	1.6	7.9	18.5	1.4
Bw1	6.2	37.4	1.6	9.9	9.8	10.6	23.0	1.5
Bw2	4.1	26.0	1.5	10.1	11.4	13.3	31.6	2.0
Cw1	8.8	21.8	5.9	14.5	8.1	17.5	19.1	4.4
Cw2	6.3	16.0	5.6	14.9	8.3	18.3	25.6	5.0
Dw1	8.1	17.7	6.6	12.1	10.0	16.8	23.5	5.1
Dw2	4.1	13.9	35.3	8.3	7.5	11.7	14.9	4.3
Dw3	9.4	19.5	9.1	12.6	10.2	14.5	19.0	5.7
Agel1	6.8	16.7	2.5	9.0	6.4	9.6	47.3	1.8
Agel2	6.5	15.7	2.3	11.8	7.4	9.1	45.4	1.7
Bgel1	1.6	7.6	1.2	5.6	4.5	15.2	61.8	2.5
Bgel2	2.3	6.9	1.6	6.5	4.6	15.8	59.3	3.0
Cgel1	5.7	21.7	3.9	5.8	0.7	14.8	44.5	3.0
Cgel2	6.9	22.2	2.9	5.5	1.0	13.4	46.0	2.1
Dge1	4.9	11.1	4.4	6.5	1.4	18.0	50.0	3.8
Dgel2	1.1	72.9	3.0	2.6	0.7	6.9	8.8	4.1
Dgel3	9.2	17.0	5.6	7.9	0.6	16.8	38.1	4.7
Dag1	2.9	5.5	4.6	8.2	0.5	19.5	51.8	7.1

This result was confirmed by principal components analysis (PCA) of relative percentage of eight amino acids (alanine, glycine, leucine, proline, hydroxyproline, aspartic acid, glutamic acid, phenylalanine) in which the first two components accounted for 74.2% of the overall data.

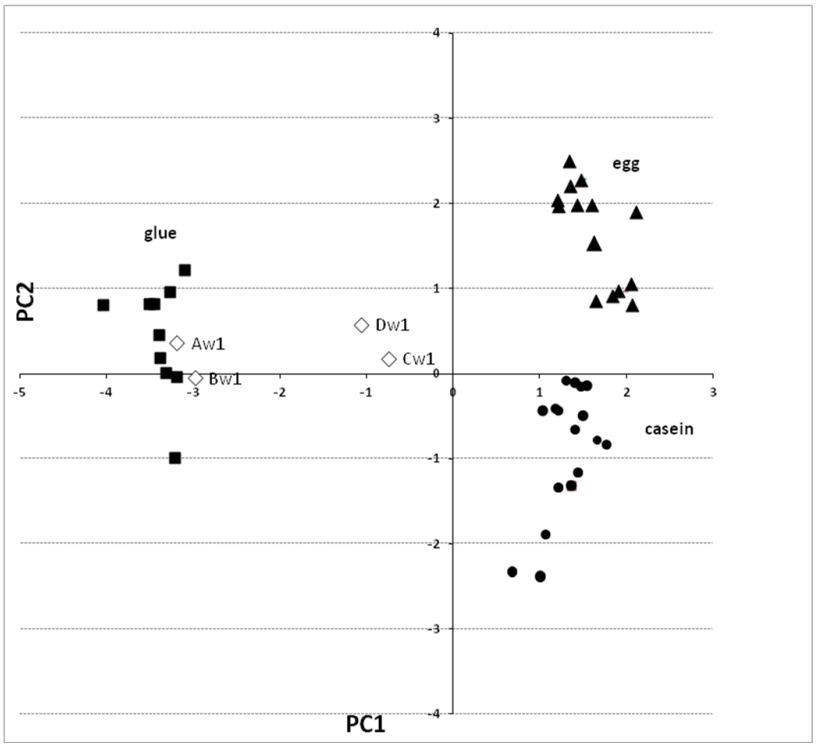


Figure 6.12: PCA score plot of the reference samples (painting samples from a reference collection of Opificio delle Pietre Dure) and samples studied: Aw1 from paper A (16th century), Bw1 from paper B (17th century), Cw1 from paper C (18th century), Dw1 from paper D (18th century)

The PCA score plot (Figure 6.12) showed that samples Aw1, Bw1 (one replicate of paper A and B) were located in the cluster of animal glue; samples Cw1 and Dw1 (one replicate of paper C and D) were located between the clusters of glue, egg and casein binders. This might be explained by the presence of different proteinaceous fractions in papers C and D. In fact, in manuscripts dating back to the 13th century the art of edge paintings (top-edge, bottom edge, fore-edge paintings) was applied. Such art became popular in the 17th century and was also applied in 18th and 19th century books [111]. It was common the use of organic binders such as egg or casein for protective function (protection of the edge paper from dust, contaminants) and decorative functions (application of pigments, gilding, etc) [112]. Being these samples collected from the bottom-edge of the paper, the partial contribution of proteinaceous fraction due to this type of treatment might be present.

Nevertheless a different behaviour between samples treated by immersion and with rigid gels (gellan gum and agar gel) can be detected, as shown in Figure 6.13.

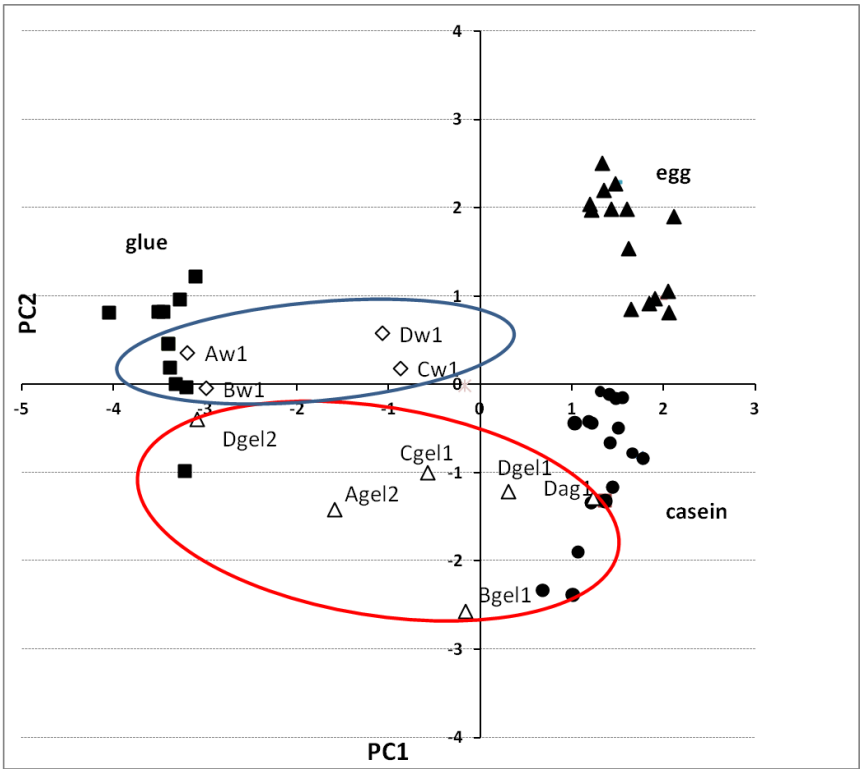


Figure 6.13: PCA score plot of the reference samples and samples studied: immersion treatment (Aw1, Bw1, Cw1, Dw1); gellan gum gel application (A gel1, Bgel1, Cgel1, Dgel1-2); agar gel application (Dag1).

In fact in the PCA score plot of samples two different groups of samples may be identified. All samples treated by gels were located in the same group between the cluster of glue and casein and separated from the samples treated by immersion. This difference might be attributed to the different mechanism involved in the removal of proteinaceous fraction during cleaning treatment. In the case of immersion treatment, the solvent (water) induced an extraction of the proteinaceous fraction from paper in a homogeneous way. On the contrary in the case of the application of rigid gels, the mechanism involved was very complex. As described previously (in chapter 1, 1.3 paragraph) rigid polysaccharide gels are characterized by a porous structure (pores size

0.1-1 µm). The water released by gels may have promoted the extraction, partial solubilisation of proteins from paper. But in this context the effect of the removal of proteins might have been also influenced by pores size of gels, morphology and dimension of proteins contained in paper. According to this hypothesis a selective removal of proteins might have been promoted by gels depending on morphological features of gels, size and morphology of proteins [113, 114]. The proteinaceous fraction extracted from papers during cleaning treatments was calculated by considering the total amount of amino acids detected and it was expressed as mg of protein in relation to the initial weight of paper subjected to cleaning (g) (Table 6.4).

Table 6.4: Proteinaceous fraction expressed as mg of protein/g of paper and Relative Standard Deviation (RSD%).

sample	mg protein/g paper	RSD%
Aw1	11.41	1%
Aw2	10.88	3%
Bw1	0.98	1%
Bw2	1.10	1%
Cw1	0.07	2%
Cw2	0.08	1%
Agel1	5.31	3%
Agel2	5.62	5%
Bgel1	0.15	7%
Bgel2	0.16	6%
Cgel1	0.03	4%
Cgel2	0.04	2%
Dw1	0.40	5%
Dw2	0.41	3%
Dw3	0.36	1%
Dgel1	0.43	1%
Dgel2	0.11	6%
Dgel3	0.38	3%
Dag1	0.16	7%
Dag2	0.67	6%
Dag3	0.26	8%

In the case of paper A, B, C, the replicates for each treatment were similar. This result may derive from a homogeneous distribution of the glue inside papers and from a homogeneous removal of proteainaceous fraction both by immersion treatment and application of gellan gum.

Immersion washing in distilled water caused the removal of higher amount of proteinaceous fraction in comparison to the application of gellan gum (difference of extraction between immersion treatment and gellan gum about 51%, 85%, 52% respectively for paper A, B, C).
In the case of paper D, replicates for the immersion treatment (Dw1, Dw2, Dw3) were similar (homogeneous removal of proteins). In the case of application of gels on paper D the removal of proteins was not homogeneous above all for agar application and the difference of extraction between immersion washing and application of gels was smaller.

Probably in the case of gels, the mechanism of cleaning as well as the mechanism involved in the removal of protein from paper may be influenced by properties of gels and paper surface as well as by the contact between the gel and paper surface.

The adhesion of the gel to paper surface may depend both from rheological properties of gels (i.e.: viscoelasticity), modulus, hardness and morphological properties of paper surface. For this reason when paper presents a heterogeneous surface (as in the case of handmade paper) the contact between gel and paper surface may be not always uniforme as well as the interaction between gels and paper.

6.4 Conclusions

This research considered the risk associated to the removal of original components of paper, such as gelatine, during the application of aqueous cleaning treatments. A preliminary study was performed for the evaluation of the removal of gelatine from paper of different periods (16^{th}, 17^{th} and 18^{th} century) induced by three types of cleaning: immersion washing in distilled water, application of agar and gellan gum rigid gels. All samples were previously characterized by spot test with Herzberg reagent and Fourier Transform Infrared Spectroscopy analysis and then subjected to the selected cleaning treatments. The GC-MS analysis of the protein fraction extracted during cleaning treatments was carried out by considering the water used for the immersion treatment and gels applied on paper. The results showed that in all cases aqueous cleaning treatments induced a partial extraction of protein fraction, gelatine from paper. This effect was more evident in the case of immersion treatment since this type of cleaning was homogeneous. A higher amount of proteins was extracted during immersion treatment (about 80-50% more than the application of gellan gum rigid gel depending on the papers). In the case of gels a different mechanism was involved in the removal of protein with respect to the immersion treatment. Being agar and gellan gum gels characterized by a presence of porous structure with size pores between 0.1-1µm, the effect of the removal of proteins from paper might depend by different aspects: morphological properties of gels (size and morphology of pores), rheological properties of gels, modulus and hardness of gels, thickness of the gel and the type of adhesion to paper surface. In this context morphology and dimension of proteins might also influence the result of the removal. In the future further studies could be carried out on the mechanism involved during the application of rigid gels on paper.

7. Analytical study of residues left by agar and gellan gum rigid gels on paper surface after cleaning treatment

In this section the study of the eventual residues left after the application of agar and gellan gum rigid gels on paper is presented. In the field of conservation, cleaning represents an important method to preserve materials, and the choice of the most suitable cleaning methodology and approach is nowadays one of the most discussed aspects together with the choice of the materials to use and the problem of residues left on the substrate after cleaning.
In previous studies the use of agar gel was experimented for the cleaning of plaster artworks and physical-chemical analysis were performed to evaluate the probable presence of agar residues on the substrate after application. Such study was carried out by means of FT-IR and GC-MS analysis of the plaster substrate before and after cleaning with agar gel [64]. The presence of agar residues on plaster was evaluated by considering galactose as marker since agar is a polysaccharide of galactose. Results demonstrated a minimal residue of galactose released on plaster substrate after the application of agar and detected by GC-MS analysis.

In paper conservation a preliminary study was performed by University of Parma for the evaluation of residues left by gellan gum rigid gels applied on papers from different periods [115]. Such evaluation was carried out by analyzing changes in air permanence and roughness of paper before and after application of Gellan gum gel[1].The results showed that selected industrial papers (dating from 1858 and 1958) presented an increase in the air permanence (from 11% to 34% depending on samples and type of paper) after having been treated with gellan gum gels. This result might be the consequence of both the extractive power of the gellan gum itself (the grammage of paper after the treatment decreased) and the absence or presence of a very low amount of residues left by the gel which did not affect the air permanence of paper in a significant way (a consistent presence of gels residues on paper should have caused a decrease in the air permanence).
In this context, further analysis were necessary and for this reason in this section an analytical evaluation of gel residues left by agar and gellan gum gels after application on Whatman ® paper is presented. The study was based on the GC-MS analysis of monosaccharides contained in paper. Whatman ® paper was considered for the study

[1] The test was applied according to UNI-ISO 5636-1, UNI-ISO 5636-3:2005 *PAPER AND BOARD – Determination of air permanence (medium range)* Bendtsen method and UNI ISO 8791-1 PAPER AND BOARD -Determination of roughness/smoothness (air leak methods) General method

since it was characterized by the presence of almost pure cellulose (polymer of glucose) and in this way it was possible to detect the sugars left by gels by considering galactose (for agar gel) and rhamnose (for gellan gum) as markers.

7.1 Materials and samples preparation

7.1.1 Samples

Cleaning treatments were carried out on Whatman® quantitative filter paper, ashless, Grade 42 (Z241105 Aldrich). Whatman ® paper was chosen for its known composition (presence of cellulose almost pure). In this way the detection of the marker sugars left by agar and gellan gum (galactose for agar gel and rhamnose for gellan gum) after cleaning treatment of paper was possible. Samples were collected after the cleaning treatment in the central area of paper (Figure 7.1). The samples treated by cleaning were compared to the control samples (untreated samples). Four replicates for each treatment were considered (Table 7.1).

Figure 7.1: Whatman ® paper and sampling area.

Table 7.1: Paper samples.

Treatment	Samples
Control	C-1;C-2; C-3; C-4
Agar gel 1%	A1-1; A1-2; A1-3; A1-4
Agar gel 2%	A2-1; A2-2; A2-3; A2-4
Agar gel 3%	A3-1; A3-2; A3-3; A3-4
Gellan gum gel 1%	G1-1; G1-2; G1-3; G1-4
Gellan gum gel 2%	G2-1; G2-2; G2-3; G2-4
Gellan gum gel 3%	G3-1; G3-2; G3-3; G3-4

7.1.2 Cleaning treatments

Cleaning treatments compared were (Figure 7.2):

- Application of gellan gum rigid gel at different concentrations (1-2-3% w/v) for 30 minutes;
- Application of agar rigid gel at different concentration (1-2-3% w/v) for 30 minutes.

Rigid gels were prepared using the same procedure described in chapter 4.

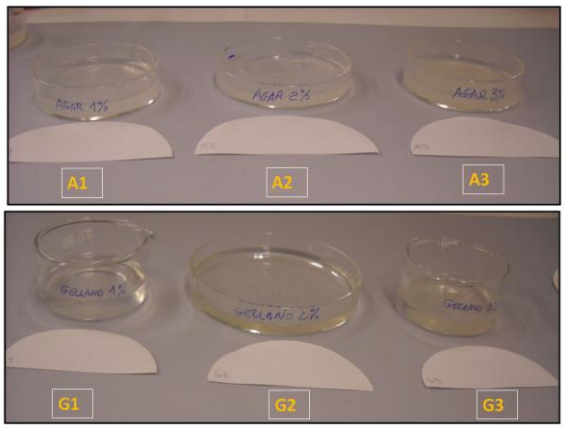


Figure 7.2: Whatman ® paper selected for cleaning treatments (A1- agar gel 1%; A2- agar gel 2%; A3- agar gel 3%; G1-gellan gum gel 1%; G2-gellan gum gel 2%; G3-gellan gum gel 3%).

Three concentrations for each gel were considered since the consistence and elasticity of the gels depends also on gel concentration. Gels at low concentration are usually softer, less compact than the same gels at higher concentration, and the application of such gels is more difficult (Figures 7.3, 7.4). For this reason a comparative study was performed.

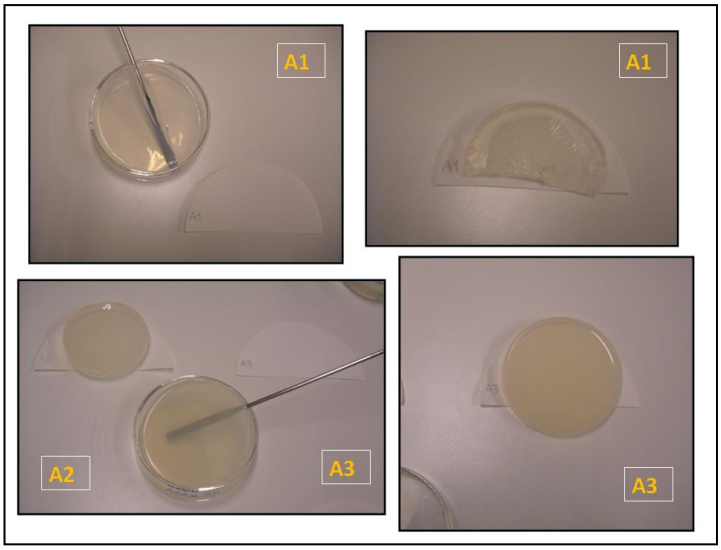


Figure 7.3: Application of agar gel 1% (A1), 2% (A2), 3% (A3) on whatman ® paper. Agar gel 1% is softer and less compact than the agar gel 3%.

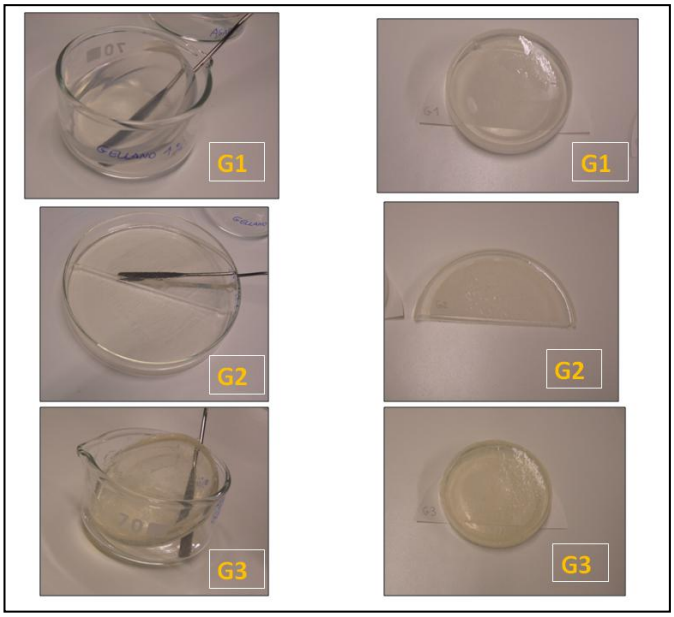


Figure 7.4: Application of gellan gum gel 1% (G1), 2% (G2), 3% (G3) on Whatman ® paper. 1% gel is softer and less compact than 3% gel.

The cleaning treatment was carried out by applying gels directly on the surface paper and removing them after 30 minutes (Figure 7.5).

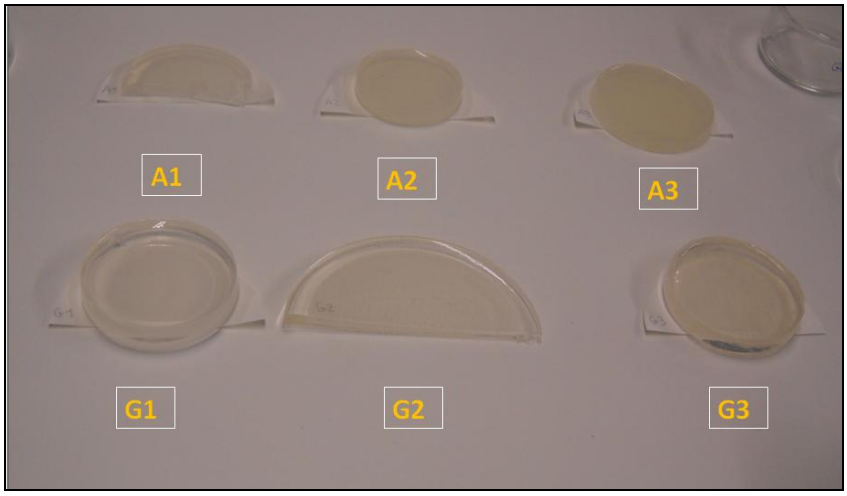


Figure 7.5: Cleaning treatment by rigid gels of Whatman ® paper.

7.2 Instruments and analytical procedure

7.2.1 Gas Chromatography – Mass Spectrometry analysis

Preparation of the samples

Preliminary characterization of monosaccharides of Whatman ® paper and the evaluation of the presence of gels residues after cleaning treatment with agar and gellan gum rigid gels was performed by means of Gas Chromatography/Mass Spectrometry.

A fragment of paper (about 1.40 mg) was collected in the central area of paper and it was treated with the following analytical procedure: the paper sample was inserted in a schlenk tube as well as the Internal standard (30 µl of a 0.01M solution of sorbitol in water) and the polysaccharide fraction was subjected to hydrolysis reaction by trifluoroacetic acid 2M (2ml) at 100°C for 6 hours. Then the samples were dried under vacuum on a heating plate equipped with magnetic stirrer at 40°C. The hydrolysed residue was first mercaptalated with 60 µl of a mixture of ethantiol and trifluoroacetic acid (2:1) (reaction at room temperature for 40 minutes) under magnetic stirring and

then, after evaporating solvent in vacuum conditions, derivatized (at room temperature for 40 minutes) by adding 50 µl of pyridine, 100 µl of hexamethyldisilazane (HMDS) and 30 µl of trifluoroacetic acid [108]. After the evaporation of the solvent in vacuum, the residue was dissolved in 1ml of hexane and 1 µl of this solution containing diethyl-dithioacetal trimethylsilyl derivates was injected into the gas chromatograph. Such procedure was used both for untreated (control) and for the samples after the application of rigid gels. The evaluation of the eventual presence of sugars deriving from gels was carried out by considering galactose and rhamnose as marker of agar and gellan gum gel respectively.

Apparatus and chromatographic conditions

A Focus GC (Thermo Scientific) coupled to DSQ II (Thermo Scientific) with single quadrupole and split-splitless injector was used. The carrier gas helium flow was kept constant at 1.0 ml/min. Separation of components (diethyl-dithioacetal trimethylsilyl derivates) was performed by means of fused-silica capillary column (RXI-5, Restek), stationary phase 5% phenyl 95% methylpolysiloxane, 0.25 µm film thickness, 30 m length.

For the analysis of analytes the split-splitless injector was set to 280°C with a 30 seconds purge off time. The GC oven temperature program was: 165°C for 0 minutes, 2°C/min to 190°C, 1°C/min to 210°C, 20°C/min to 235°C. The injector was used in the splitless mode. The MS transfer line was set to 250°C. Ionization was performed in the electron impact mode (EI) at 70 eV, with an ion source temperature of 230°C.
EI mass spectra were recorded in TIC (Total Ion Current, mass range m/z 50-500) and positive ions were considered.
The chromatographic peak areas were integrated for each sample and corrected by response factor using a standard solution of eight monosaccharides (sorbitol, xylose, arabinose, rhamnose, fucose, glucose, mannose, galactose) and two uronic acids (galacturonic acid and glucuronic acid) in hexane (3 µg/ml).
The average of three injections for each sample was considered (Relative Standard Deviation about 6%). A semi-quantitative estimation of monosaccharides in relation to the initial weight of paper sample (monosaccharide (mg)/ paper (g)) was performed.

7.3 Results and Discussion

7.3.1 Gas Chromatography – Mass Spectrometry analysis

The preliminary characterization of Whatman ® paper was performed by means of GC-MS analysis. The results showed the presence of glucose (the principal monosaccharide deriving from cellulose) and xylose (Figure 7.6).

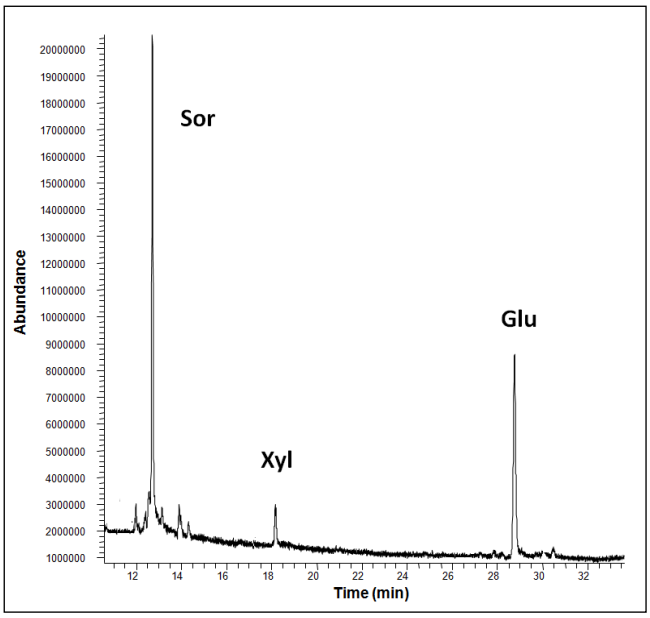


Figure 7.6: Total ion chromatogram of Whatman ® paper (sample C-2). The principal monosaccharides were glucose (Glu) and xylose (Xyl). Sorbitol was the Internal Standard.

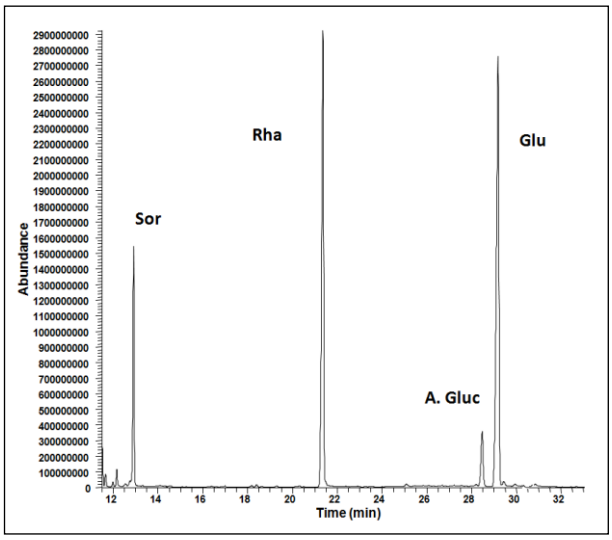


Figure 7.7: Total ion chromatogram of gellan gum. The principal monosaccharides were: rhamnose (Rha), glucuronic acid (A. Gluc.) and glucose (Glu). Sorbitol was the Internal Standard.

As described in chapter 1 (paragraph 1.3), agar is a polysaccharide characterized by the presence of galactose; gellan gum presents glucose, rhamnose, glucuronic acid (Figures 7.7, 7.8).

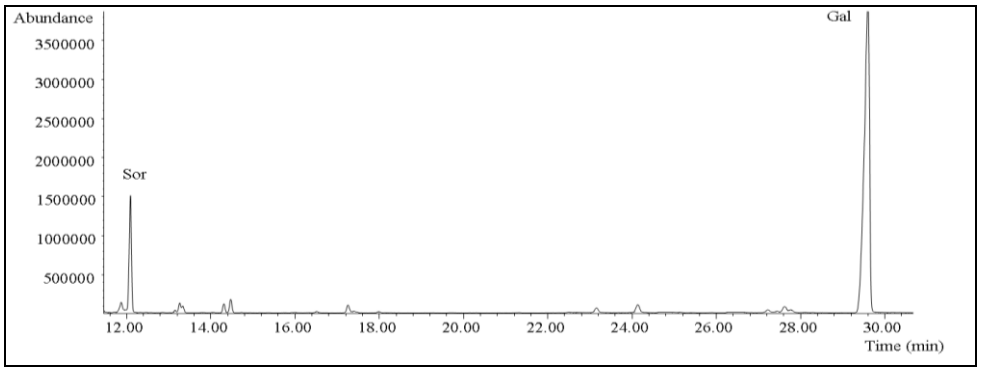


Figure 7.8: Total ion chromatogram of agar constituted by galactose (Gal). Sorbitol was the Internal Standard.

The evaluation of probable residues of gels left on Whatman ® paper after the cleaning treatment was carried out by considering galactose and rhamnose as marker of agar and gellan gum respectively.

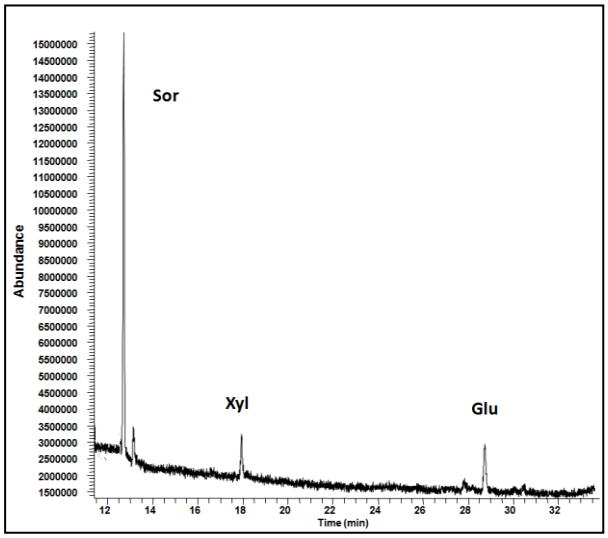


Figure 7.9: Total ion chromatogram of sample A2-1 (treatment with agar gel 2%). The presence of glucose (Glu) and xylose (Xyl) was detected. Sorbitol was the Internal Standard.

In all samples treated with agar at different concentrations, galactose was not detected (Figures 7.9, 7.10). In this case, galactose might be absent or present in quantities below the detection limit ($5 \cdot 10^{-4}$ µg). The absence of galactose in paper after application of gel could be the consequence of absence of agar gels residues or to the presence of undetectable amounts of residues.

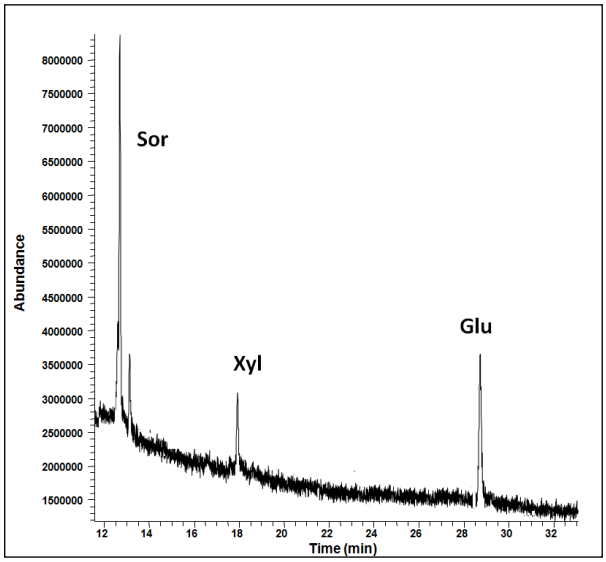


Figure 7.10: Total ion chromatogram of sample A3-1 (treatment with agar gel 3%). The presence of glucose (Glu) and xylose (Xyl) was detected. Sorbitol was the Internal Standard.

In the case of gellan gum gels no rhamnose was detected. Rhamnose might be absent or present in amount below the detection limit ($1 \cdot 10^{-4}$ μg) (Figures 7.11; 7.12).

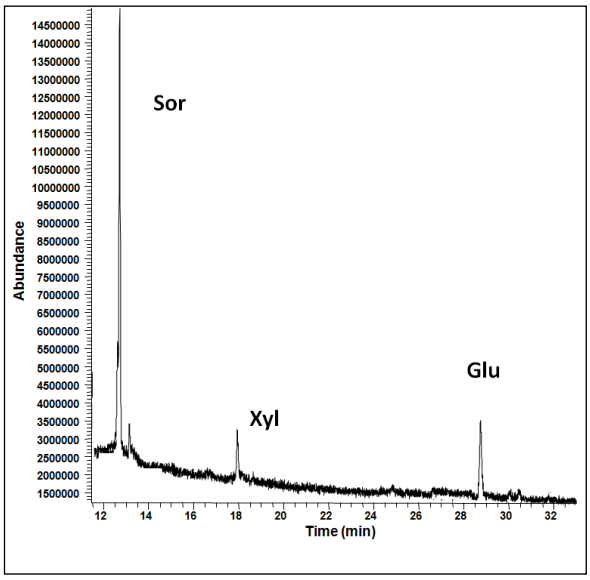


Figure 7.11: Total ion chromatogram of sample G3-2 (treatment with gellan gum 3%). The presence of glucose (Glu) and xylose (Xyl) was detected. Sorbitol was the Internal Standard

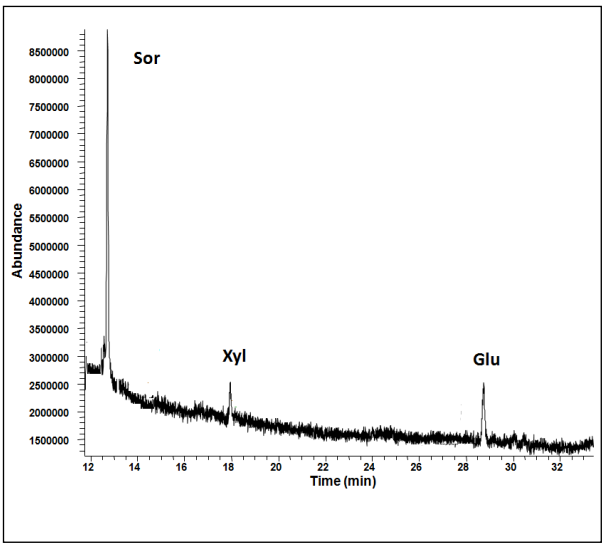


Figure 7.12: Total ion chromatogram of samples G1-2 (treatment with gellan gum 1%). The presence of glucose (Glu) and xylose (Xyl) was detected. Sorbitol was the Internal Standard.

Only in one sample treated with gellan gum 1%, a small amount of rhamnose was detected (Figures 7.13) (Table 7.2). This result could derive from the presence of small amounts of residues of gellan gum left during the application of the gel (1%) on paper and its later removal.

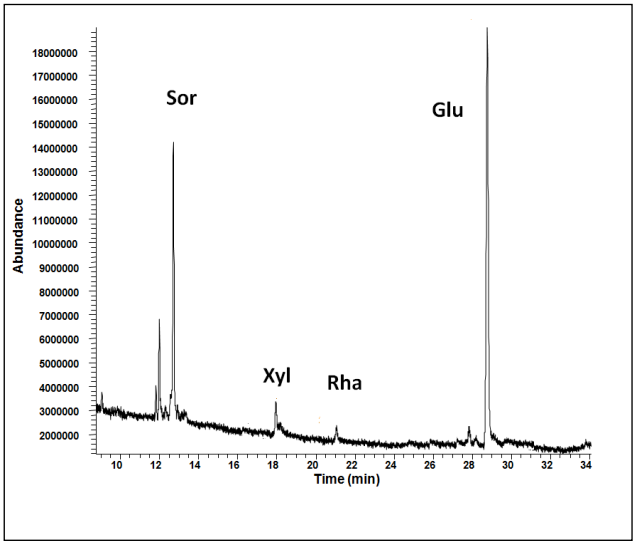


Figure 7.13: Total ion chromatogram of samples G1-3 (treatment with gellan gum 1%). Monosaccharides detected were: glucose (Glu), xylose (Xyl) and rhamnose (Rha). Sorbitol was the Internal Standard. Rhamnose might derive from the presence of residues left by gellan gum 1% on paper.

These preliminary results showed the absence or the eventual presence of residues only when using gels at low concentration. In fact, gels at low concentration are softer, less compact and the application of such gels is more difficult from a practical point of view as far as the manipulation of gel is concerned. In such case the probability of detecting gel residues may be higher. For this reason the eventual presence of gel residues on paper can be due also to the application methodology and the removal of the gel from the paper surface.

Table 7.2: Monosaccharides detected in paper samples expressed as mg/g of paper.

Sample	Xyl	Rham	Glu	Gal
C-1	2.30	-	17.93	-
C-2	4.29	-	19.33	-
C-3	2.80	-	14.69	-
C-4	2.96	-	15.54	-
A1-1	12.18	-	8.19	-
A1-2	12.35	-	7.34	-
A1-3	11.52	-	15.43	-
A1-4	11.52	-	7.89	-
A2-1	4.71	-	4.72	-
A2-2	5.02	-	4.70	-
A2-3	12.19	-	18.98	-
A2-4	5.05	-	4.65	-
A3-1	8.23	-	16.09	-
A3-2	6.31	-	12.09	-
A3-3	21.30	-	11.10	-
A3-4	8.94	-	15.64	-
G1-1	5.34	-	16.96	-
G1-2	5.57	-	16.51	-
G1-3	4.00	0.68	44.87	-
G1-4	4.76	-	16.41	-
G2-1	38.02	-	13.99	-
G2-2	12.69	-	57.92	-
G2-3	4.95	-	43.82	-
G2-4	5.11	-	45.11	-
G3-1	4.60	-	6.46	-
G3-2	7.30	-	10.89	-
G3-3	18.75	-	49.01	-
G3-4	7.57	-	12.63	-

7.4 Conclusions

This research was based on the evaluation of the eventual presence of residues left by gels on paper after their application. The study was carried out on Whatman ® paper since it is almost pure cellulose. For this reason the main monosaccharide is glucose and the content of xylose is reduced. In this way galactose and rhamnose could be considered marker of any residue of agar and gellan gum gels left on paper.

In this work gels of different concentrations were compared and GC-MS analysis of monosaccharides contained in paper samples were performed. Paper samples were compared to the control (untreated samples) after the cleaning treatment with agar and gellan gum. Results showed that samples treated with agar and gellan gum did not contain galactose and rhamnose (it could be possible that the presence of galactose and rhamnose was in amounts below the detection limit). Only in one sample treated with gellan gum (1%) small amount of rhamose was detected. This result suggests that it is important to consider not only the type of gel and its concentration, but also the application methodology. 1% gels are soft, less compact than the same gel at higher concentration. For this reason the manipulation of the 1% gel is more difficult and would be a strong probability of releasing residues on the surface of paper.

8. Conclusions

In this study, the effectiveness of aqueous cleaning treatments (both in form of free water as well as aqueous rigid gels) on paper is evaluated with a special focus on the interaction of materials and methods with the work of art.

Aqueous cleaning treatments are usually carried out for aesthetic and conservative purposes with the aim to improve the appearance and remove contaminants, salts and hydrophilic degradation products present in paper respectively.

Given the risks associated to the solubility of hydrophilic graphic media, the erosion of sensitive surfaces as well as those derived from handling paper artefacts during wetting, rigid polysaccharide gels have been studied in the context of cleaning treatments with double advantages: the surface wettability of paper increases and the diffusion of water molecules into paper is controlled.

Since the use of water (in a free or gelled form) can promote the extraction of hydrophilic components (both degradation products or original constituents of paper), the evaluation of the extraction of original constituents of paper induced by cleaning treatments as well as the evaluation of the effects of cleaning on the chemical-physical properties of paper represents a relevant aspect in the context of paper conservation. For this reason in this research the interaction between paper and aqueous cleaning treatments commonly used in the conservation of paper was analysed by correlating traditional immersion treatments in distilled water and the application of agar and gellan gum rigid gels. On the one hand, the aim of the research was to evaluate the eventual extraction of sizing agents (i.e.: gelatine) contained in paper and products of hydrolysis of hemicelluloses and cellulose induced by aqueous cleaning treatments. On the other hand, a second objective was to evaluate the effects of the selected cleaning treatments in the morphological and mechanical properties of paper artworks from different periods (dating from 16^{th} to 19^{th} centuries).

Firstly, the mechanical behaviour of paper samples (Whatman ®) was evaluated before and after the cleaning treatments, by means of tensile testing. Results showed how paper samples subjected to cleaning by immersion presented a relevant decrease in the ultimate tensile strength and a significant increase in the breaking strain, probably deriving from a swelling of cellulose induced by water and the formation of hydrogen bonds between cellulose chains and water molecules. The effect was an evident change in the mechanical behaviour of paper (increase in the flexibility of paper expressed by the reduction of elastic modulus, dimensional change of paper and a slight structural weakening probably caused by the weakening of inter-molecular hydrogen bonds between cellulose chains for the presence of exaggerate amounts of water molecules in

the structure). In the case of the application of rigid gels a moderate increase in the ultimate tensile strength and breaking strain was observed. The slight improvement in the mechanical properties of such samples could be correlated to the gradual release of water molecules by gels and the gradual formation of some hydrogen bonds between water and cellulose. In addition the study evidenced that those samples subjected to the application of rigid gels, presented a similar behaviour after accelerated artificial aging than the control samples. This result evidenced that the use of gels (and any residue that could eventually be left on the surface after treatment) was not interfering in paper degradation.

Secondly, and since the preservation of the original texture and surface morphology of paper is of utmost relevance in paper artworks, the study of the effects of cleaning treatments (both dry cleaning methods as well as aqueous cleaning treatments) in the superficial morphology of paper was performed. The study was carried out by means of microscopic observations (stereo-microscope and SEM-EDS analysis) of the surface and fibres of paper samples before and after cleaning treatments as well as analysis of microtopography of the surface by means of 3D profilometer. Results showed that both cleaning materials and application methodology played a relevant role in this context. According to this, it was observed that treatments carried out with brush (i.e.: application of Klucel G ®) as well as dry methods with Wishab ® sponge, caused significant variations in the morphology of the surface of paper as well as an evident defibring of the cellulose structure because of the mechanical action and erosion induced during application. On the contrary, immersion treatments and rigid gels were more respectful of original morphology of paper.

Another aspect considered was the evaluation of the extraction of gelatine and sugar fraction from paper induced by cleaning treatments. The study was carried out by means of GC-MS analysis. Results showed that all treatments (water in the free or in gelled form) caused the extraction of products of hydrolysis of hemicelluloses and cellulose as well as the extraction of gelatine. Such phenomenon was more evident in the case of the immersion of paper in distilled water from a semi-quantitative point of view. The extraction of original constituents of paper might help explain the slight mechanical weakening of papers subjected to immersion treatment.

Finally the evaluation of the eventual residues left by rigid gels on paper samples after cleaning treatments was carried out by GC-MS analysis. For this purpose cleaning treatments were performed on Whatman ® paper (almost pure cellulose) and galactose and rhamnose were considered markers of the presence of the gels on paper since such sugars are not present in this type of paper. Results showed a lower content of gel residues left on paper only in the case of the use of 1% gel, since gels at lower

concentration were softer and their application was more difficult. This study confirmed the importance of the selection of both materials and application methodology for the cleaning treatment.

The results obtained in this research may help paper conservators to accomplish cleaning treatments in a critical way evaluating both the effectiveness of the removal of degradation products as well as the problem of the extraction of original components from paper which may influence paper properties and its mid-to-long term stability.

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