A study of non-bounded/bounded water and water mobility in different agar gels

A STUDY OF NON-BOUNDED/BOUNDED WATER AND WATER MOBILITY IN DIFFERENT AGAR GELS

M. Bertasa[a,b], T.Poli[a], C.Riedo[a], V. Di Tullio[c], D. Capitani[c], N. Proietti[c], C. Canevali[d], A. Sansonetti[b], D. Scalarone[a]*

a) Department of Chemistry and INSTM, University of Turin, Via Pietro Giuria 7,10125 Turin (Italy)

b) Institute for the Conservation and Valorisation of Cultural Heritage (ICVBC), National Research Council (CNR), Via Roberto Cozzi 53, 20125 Milan (Italy)

c) Magnetic Resonance Laboratory "Annalaura Segre", Institute of Chemical Methodologies (IMC), National Research Council (CNR), Research Area RM 1, Via Salaria Km 29,300 - 00015 Monterotondo (RM)

d) Department of Materials Science, University of Milano-Bicocca, Via R. Cozzi 55, 20125 Milan, Italy

* Corresponding author: dominique.scalarone@unito.it

Abstract

Agar is an interesting and versatile biopolymer capable of forming hydrogels useful in different applications. In particular, in the field of Cultural Heritage agar gels are often used as tools for cleaning the surfaces of artifacts. Agar gels are thermoreversible, peelable materials, exhibiting high retention of water and an intrinsic cleaning capability that can be increased and modulated by loading different cleaning agents. Agar gels are able to solubilize and retain dirt, and allow a confined release of the liquid phase, which is especially important when they are applied on water sensitive substrates. A detailed knowledge of the role of water in agar gels and of water/agar interactions is important to fully understand the properties and performances of agar gels as cleaning materials.

Here we report the results of a systematic study aimed at exploring the water state and the types of water (i.e. bound non-freezable, bound freezable and freezable bulk-like) in agar hydrogels prepared from four different types of commercial agar powders. The influence of agar concentration on the water behavior in the hydrogels was also investigated. The total water content, the identification and quantification of the different types of water was carried out by gravimetric, thermogravimetric and calorimetric analyses, while water mobility and localization in the gel network were investigated by unilateral ^{1}H NMR. Moreover, ^{1}H NMR depth profiles of agar gels applied on Noto stone specimens were collected to monitor the gel deformation due to water release and to evaluate the ability of the gel in controlling the water release into a porous material.

KEYWORDS

Agar gel; cleaning materials; water content; thermal analyses; unilateral ^{1}H NMR.

1. Introduction

Agar gel is an important gelling material commonly used in a wide range of application fields, such as pharmaceutical, biological research and in the food chain [1,2]. Agar is a gel-forming polysaccharide extracted from different species of Gelidiales and Gracilariales red seaweed and consisting of two main alternating polysacchatides: agorose and agaropectin. Agarose is a linear polymer consisting of alternating *β*-D-galactose and 3,6-anhydro-L-galactose units linked by glycosidic bonds and forms the agar gelling fraction; whereas agaropectin is a heterogeneous agarose deeply modified with acidic side groups (i.e. sulphate and pyruvate), which reduce the gelling properties [3-6]. It forms a hydrophilic and thermoreversible semi-rigid gel, after heating and the following cooling process. Indeed, during the cooling, the polysaccharide chains arrange in a double-helix ordered structure by hydrogen bonding and generate a three-dimensional porous network containing water [7]. Since more than a decade, thanks to their great advantages, hydrogels and in particular agar gels, have an important role as cleaning materials for artworks with sensitive substrates, such as paintings, gypsum plasters and decayed stone artifacts [8-14].

Agar gel systems favour the selectivity of the cleaning method by gradually removing the soiling layer through a combination of different processes including (i) syneresis, that is the spontaneous release of water through the gel surface, (ii) diffusion of water into porous substrates or capillarity, which wets the layers below the artwork surface, (iii) ion diffusion through the gel/surface interface, when there is an ion concentration gradient, and (iv) osmosis, when water molecules migrate through a semipermeable membrane against the concentration gradient [8,15-16]. The synergic combination of these processes ensures a fine control of the liquid release and diffusion in the artwork near-surface region, limiting the penetration by capillarity and subsequent evaporation of the free solvent, hence reducing the swelling of organic components and the unwanted solubilisation of inorganic salts. Furthermore, gels applicable as cleaning tools in the Cultural Heritage field are easily removable: the mechanical action over the surface of the artwork is minimized, leaving a low amount of residues which may be easily removed by cotton swabs or other tools [17-19]. For these reasons, gels are adequate and safe tools for cleaning the surface of artworks and for satisfying the fundamental requirements of a correct cleaning method, as suggested by national guidelines [20-23].

Water in hydrogels can be classified into three main types: non-freezable bound, freezable bound and free water [24-28]. Non-freezable bound water is closely associated with a polymer network and does not show any solid-liquid phase transition. Freezable bound water is the water fraction less closely bound to the polymer matrix and shows a melting and freezing temperature remarkably different from bulk water. Free water shows similar melting/freezing temperatures as bulk water. The state of water in gel systems has been previously investigated by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and nuclear magnetic resonance (NMR), [29-33]. These techniques provide specific and complementary information: from DSC curves, the heat of melting

of freezable water can be calculated, together with the content of freezable and non-freezable water; from TGA, the weight loss corresponding to different water fractions and the polymer thermal stability can be determined, and from NMR information about the state of water and the polymeric network of the gels can be obtained. Unilateral ^{1}H NMR can quantitatively detect the different types of water and water distribution in hydrogels, and study water transport by measuring relaxation times and the self-diffusion coefficient. Whereas relaxation times depend on both rotational and translational molecular motions, diffusion measurements are related to the translational molecular displacement. In biomedical and pharmaceutical applications, it is important to know how water molecules associate with the polymer to understand transport phenomena. Similarly, also in field of conservation of Cultural Heritage the knowledge of the interaction between water molecules, the gel network and the surface of the artwork is important for understanding cleaning mechanisms and for correlating the cleaning mechanisms with the performance and efficacy of cleaning systems based on hydrogels. The water release into the surface of the artwork during a cleaning treatment is of primary importance and is affected by a number of parameters such as the gel porosity, purity, the pH and conductivity of both the gel and the solution, the polymer concentration, the time of application of the gel to the surface, the type and physical state of water molecules [8-10,15-16, 34]. Moreover, depending on the different amount of water, hydrogels show a certain degree of flexibility, which helps in modulating some characteristics, such as the swelling behavior and mechanical properties.

Since in a previous study important compositional differences were highlighted in agar powdery samples of different provenance [35], this paper reports the study of the water entrapped in the agar network for different types of agar gels. The water state and its release were studied by gravimetric measurements and thermal analyses (TGA and DSC). In particular, the amounts of bound and free water were obtained by plotting the endothermic changes in enthalpy versus water content, as described by Quinn *et al*. [30]. Unilateral ^{1}H NMR was used to obtain detailed information on the interaction between water molecules and the gel network. This technique combines an open magnet and a radio-frequency (RF) coil to generate a sensitive volume external to the sensor where the ^{1}H NMR signal is generated and detected. The magnetic field is applied to one side of the object, making this technique particularly suitable for non-invasive analysis of cultural heritage [36-39]. Finally, to assess the variation of the gel thickness during the water release, ^{1}H NMR depth profiles of agar gels before and after the application on the surface of porous stone specimens were collected at different times of application.

2. Materials and methods

2.1. Agar powders and gel preparation

Four different agar powders with different provenance were used in this study: 1) AgarArt (CTS, Italy) and 2) Agar Purissimo (Bresciani, Italy), that are among the most used gelling materials in Italy in

the field of conservation; 3) Agar purchased from Sigma Aldrich (A7002_CAS:9002-18-0), that is mostly used for biological applications; 4) an agar powder used in the food industry and imported from the United Kingdom (in the following named Agar Food).

Gels with different concentrations of agar powder (1, 3 and 5% w/v) were prepared following the same procedure: the proper amount of agar powder was mixed with Milli-Q water and heated in a microwave oven at 700 W for 2 min reaching approximately 92-95 °C. The obtained sol was then cooled in air to room temperature forming the gel [40].

2.2. Characterization

Thermogravimetric analyses (TGA) were carried out by a TA Q500 model from TA Instruments. Evaporation of water from gels was measured by heating 5÷10 mg of sample contained in alumina pans at a rate of 10 °C/min from 35 to 400 °C in nitrogen, after an isothermal treatment of 2 min at 35 °C. Measurements were performed in duplicate.

A Q200 (TA instruments) differential scanning calorimeter was used to study the phase changes of water in the gels. Analyses were performed in the temperature range from -40 to 20 °C, with a scanning rate of 10 °C/min and under a nitrogen flow of 50 ml·min^{-1}. Aluminum pans containing 5÷10 mg of samples were used. At least two DSC curves per sample were recorded to ensure good reproducibility of data. Endothermic peaks due to water melting were integrated and plotted versus water content, W, to quantify bound and free water of gels, where W is the wt % water content relative to the 100 wt % dry material [30]. The minimum amount of water detectable and quantifiable by DSC analyses was approximately 600 W, that is 6 $g_{H2O}/g_{dry\ gel}$.

Samples used for both TGA and DSC analyses were cut from the gels after progressively longer drying periods at 140 °C from T_0 up to a maximum time length of 5 hours. Moreover, water loss at each time interval was determined gravimetrically by averaging the weight measurements of two gel samples of approximately the same size. All trends are characterized by a standard deviation values < 0.1.

^{1}H NMR analyses were carried out at 13.63 MHz with a portable NMR instrument from Bruker Biospin interfaced with a purposely built single-sided sensor by **R**heinisch-**W**estfälische **T**echnische **H**ochschule (RWTH) Aachen, Germany. Longitudinal relaxation times T_1 were measured with the Saturation Recovery pulse sequence followed by a CPMG-train in the detection period to increase the sensitivity. Effective transverse relaxation times T_{2eff} [41-42] were measured with the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, 4096 echoes were recorded on gels at a depth of 1 mm with an echo time 2τ of 71.2 μs. The inhomogeneous magnetic field of unilateral ^{1}H NMR is a further source of relaxation which shortens the measured T_{2eff} values of the gel, making them definitively shorter than those which would be measured in a homogeneous field. In fact, in the presence of a magnetic field gradient, the transverse relaxation time does not depend only by spin-

spin interaction, but it is also controlled by molecular diffusion in the magnetic field gradient which, in the case of portable NMR is very strong (14.28 T/m).

Longitudinal and effective transverse relaxation times were obtained fitting the magnetization decay to the following equation:

$$Y(\tau) = \sum_{i=1}^{n} W_i e^{\frac{-2\tau}{T_{1i,2i}}}$$

where n is the number of components of the decay, W_i is the weight of the i^{th} component, and $T_{1i,2i}$ is the longitudinal or transverse relaxation time of the i^{th} component. Before fitting, the sum of weights was normalized to 100%.

Self-diffusion coefficients D of water molecules in the gel were measured with a Steady Gradient of 14.28 T/m using a STimulated Echo (SGSTE) pulse sequence followed by a CPMG echo train to improve the signal noise ratio [39]. The strong constant magnetic field gradient of the single-sided sensor simplifies the measurement of the self-diffusion coefficient in heterogeneous porous materials because it reduces the relative contribution from background gradients due to susceptibility variation across the sample. In SGSTE pulse sequence the attenuation of the spin echo signal, which results from the dephasing of nuclear spins is used to measure the displacement of molecules.

The normalized echo attenuation $\frac{A_i}{A_0}$ measured by ^{1}H NMR diffusion measurements is given by:

$$\ln\left(\frac{A_i}{A_0}\right) = -\gamma^2 G^2 \tau_i^2 \left(\Delta + \frac{2}{3}\tau_i\right) D + \frac{2\tau_i}{T_2} + \frac{\Delta}{T_1} \qquad i=1,\ldots,n \quad (1)$$

where A_i is the amplitude of the ^{1}H echo signal collected at time τ_i , A_0 is the amplitude of ^{1}H echo signal collected at the shortest time τ_1, D is the self-diffusion coefficient, Δ is the diffusion time, G (14.28 T/m) is the magnetic field gradient, γ is the ^{1}H gyromagnetic ratio (2.6752×10^8 s^{-1} rad T^{-1}), and T_1 and T_2 are the longitudinal and transverse relaxation times, respectively.

For large D values, strong magnetic field gradient, and provided that $\tau_1 << T_2$, and $\Delta << T_1$, diffusion terms dominate over relaxation terms and the self-diffusion coefficient may be obtained from the following equation:

$$\ln\left(\frac{A}{A_0}\right) = -\gamma^2 G^2 \tau_1^2 \left(\Delta + \frac{2}{3}\tau_1\right) D$$

The uncertainty associated to T_1, T_{2eff}, and D was obtained by repeating the measurement three times on each sample.

^{1}H NMR depth profiles were measured on slices of agar gels (5x5x0.5 cm^3) applied on Noto stone specimens (5x5x2 cm^3) having a total porosity between 30 and 42 %, and pores radius between 0.1 and 10 μm [43-44]. Hydrogels obtained according to the procedure reported in section 2.1 were applied for 30, 60 and 240 min to the stone specimens on the surface displayed horizontally, and covered with a film of PVC to avoid further evaporation.

^{1}H NMR depth profiles of hydrogels were acquired with a CPMG pulse sequence (2τ of 57 μs, nominal resolution 50 μm, steps 200 μm). Profiles were collected by repositioning the single-sided

sensor to scan the whole thickness of the gel. The amplitude of each point of the profiles was obtained as the addition of the first eight echoes.

3. RESULTS AND DISCUSSION

3.1. Gravimetric and thermogravimetric analysis

Weight changes due to the evaporation of water in gels dried at 140 °C were determined gravimetrically. The curves reported in Figure 1 provide a general description of the water content *vs.* drying period for gels at different agar concentration. The weight loss curves exhibit a constant decrease, increasing the drying period. In particular, all gels at 1% w/v lose the entire amount of water in 80 min of drying at 140 °C. The weight loss curves of Agar Purissimo and Food 3% w/v are intermediate between 1 and 5% w/v (Figure 1b and 1d). On the other hand, AgarArt and Sigma 1% and 3% w/v show a comparable trend of weight loss, while for gels at 5% w/v the weight loss is slower and after 80 min they retain approximately 20% of water (Figure 1a and 1c). This is possibly due to a denser polymer network which slows water evaporation. In fact, FE-SEM analyses (not reported here) carried out to investigate in details the porosity of the different agar gels show that, in general, by increasing agar concentration the number of pores per volume increases as well. However, this process of pore densification is not homogeneous and results in a broader distribution of the pore size: at agar concentration of 1% w/v most of the pores are in the range of 200 and 300 μm, while increasing agar concentration gels show a more heterogeneous porosity, with most of the pores having a diameter from 100 to 400 μm.

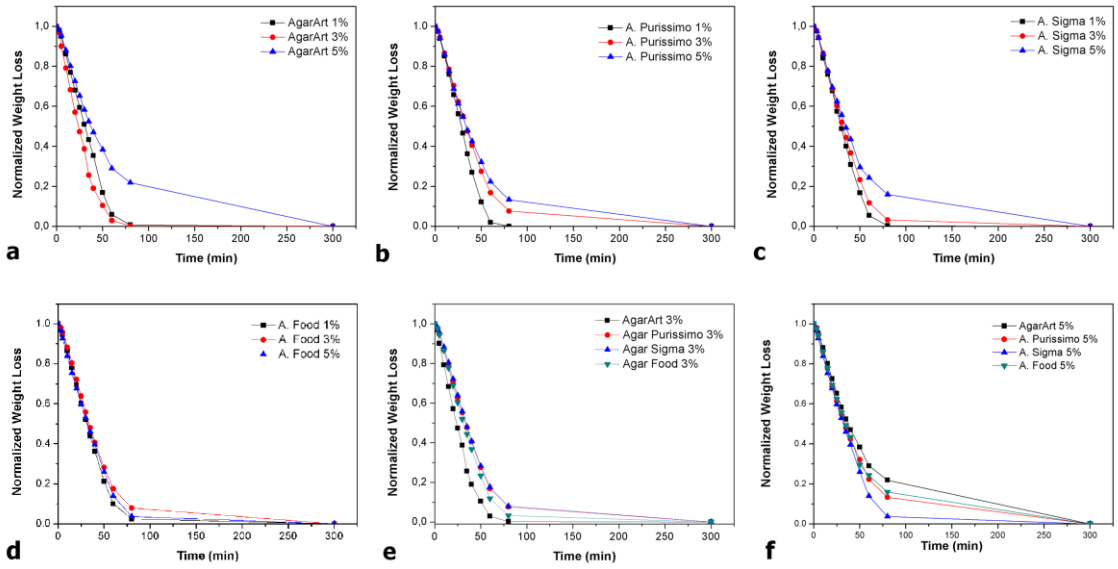

Figure 1. Weight loss curves *vs.* drying period at 140 °C for AgarArt (a), Agar Purissimo (b), Agar Sigma (c), Agar Food (d) at different concentration and the comparison of all sample set at 3% w/v (e) and 5% (w/v) (f).

Gels containing 3% w/v of agar, the most used by restorers according to our experience, were also analysed by TGA in order to study more in detail the evaporation of water from the different gels. As reported in a previous work [35], the agar powder analysed by TGA up to 800°C showed three main weight losses: the first, from room temperature to 100-130 ºC, is due to the release of physisorbed water, the second weight loss, at approximately 160-230 °C depending on the type of agar, points to the occurring of scission reactions breaking the polysaccharide network, the third weight loss, starting at approximately 400 °C, leads to a complete degradation of the polysaccharide. Here, in order to focus on the water content in agar gels, TGA analyses were performed from 35 °C to 400 °C. Moreover, all samples were conditioned in the TGA furnace by an isothermal treatment of 2 min at 35 °C (which is not shown in Figure 2).

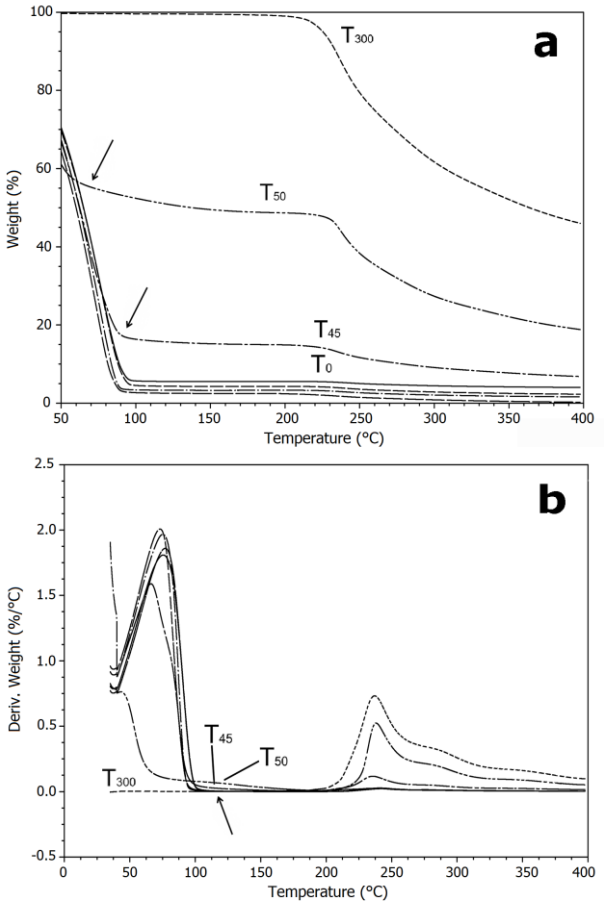

Figure 2. TGA curves (a) and the corresponding derivative weight curves (b) of AgarArt hydrogels 3% w/v after progressively longer drying periods: T_0 (solid line), T_5 (dash), T_{25} (dot dash), T_{40} (long dash), T_{45} (broken dash), T_{50} (broken double dash), T_{300} (short dash).

Figure 2 shows the thermogravimetric analyses of AgarArt gels at 3% w/v dried at 140 °C in a static heater. All weight loss curves (Figure 2a) show two main steps: one at low temperature, up to 80 °C, can be ascribed to the loss of free water, while the second weight loss, starting at 200 °C, is due to the degradation of the polysaccharidic component. The TGA curve of the gel tested soon after the

preparation (T_0) is basically characterized by the dehydration step: most of the sample weight, approximately 96 %, is due to water entrapped in the polymer network. Conversely, gels dried at 140 °C for 5 hours (T_{300}) are fully dehydrated. As expected the amount of water in the samples dried from 5 (T_5) to 50 min (T_{50}) progressively decreases as longer is the drying. Gels dried from T_0 to T_{40} show a common profile of dehydration with a fast water loss at low temperature due to the loss of free water. In these gels the release of water occurs between room temperature and 100 °C. Instead, gels dried at 140 °C for 45 to 50 min show a more complicated profile of dehydration with a faster water loss at low temperature, due to free water, followed by a more gentle decrease in weight, accounting for approximately 4 and 8 % of the total water, and extended up to 170 °C, which is due to bound water. The change in the slope of the curve is evidenced by an arrow in Figure 2a. An insight of the process is also given by the weight derivatives reported in Figure 2b, where the signal due to dehydration progressively decreases in intensity indicating a reduction of free water with drying time. In addition, Figure 2b also shows that for samples dried for 45 and 50 min the loss of water continues up to 170 °C, that is when the derivative curves go to zero.

Gels obtained from the other types of agar exhibit a similar trend of dehydration with only minor differences in the weight loss attributed to bound water, probably because of a different density of the polymer network. For instance, Sigma gels, which seem to lose water more quickly than the other samples, do not show any water loss beyond 130 °C and the weight loss due to bound water starts at T_{30}.

3.2 Differential scanning calorimetry

DSC analysis allowed monitoring the phase changes of water in the polymer network and to determine the amount of freezable and nonfreezable water.

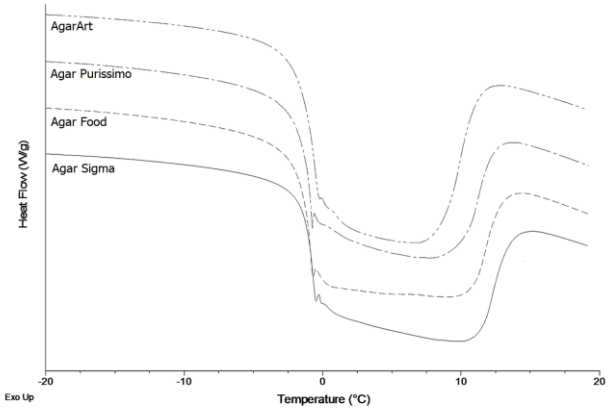

Figure 3. DSC curves of hydrogels 3% w/v of all agar gels.

Figure 3 shows some DSC curves of AgarArt, Agar Purissimo, Food and Sigma gels 3 % w/v. The complex shape of the melting peak indicates that freezing water consists of different types of water,

namely freezable weakly bound water, which melts at lower temperature, and free water, which melts at higher temperature [45]. In general, by increasing the drying time the melting signal due to free water at higher temperature decreases, while the contribution of bound water in the lower temperaure side of the melting signal increases. After 50-60 min of drying, corresponding to a water content of 4÷8 w/w (grams of water/grams of dry agar), the melting signal of freezable water disappears indicating that the water in the sample is nonfreezing bound type. Thus, nonfreezing bound water can be equated with the maximum amount of water for which no enthalpic peak is detected. However, it must be considered that if the amount of water in gels is too small compared to the sensitivity of the instrument, water remains unseen preventing an accurate determination of the amount of non freezable water. Here, both free (i.e. freezable) and bound water (i.e. nonfreezable) were quantified by plotting the enthalpy of melting (ΔH per gram of dry polymer) as a function of water content (W) according to the method described by Quinn *et al*. [30].
In Figure 4 the data for the hydrogels with an agar concentration of 3% w/v are shown together with their linear fit.

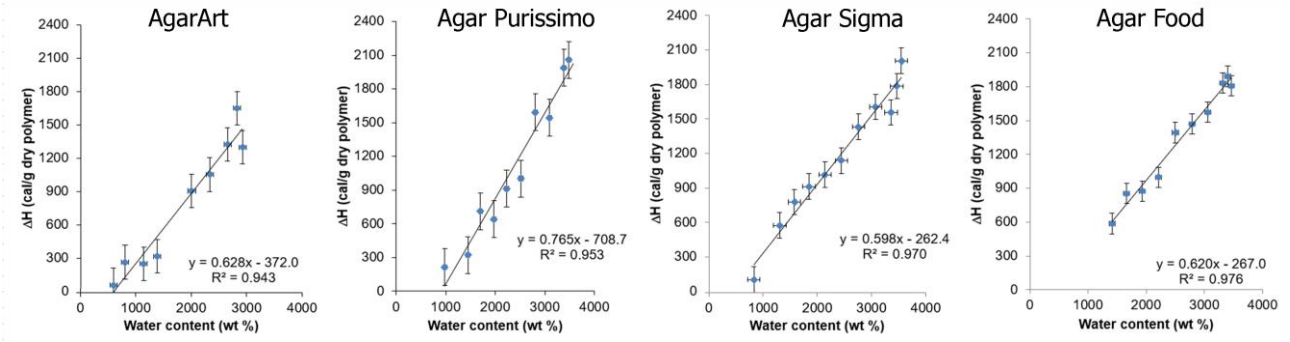

Figure 4. Enthalpy of melting *vs.* water content in agar hydrogels 3% w/v. Both ΔH and water content are relative to the dry polymer weight.

The linear fit to the integrated endothermic signals of melting of freezable water *vs.* water content yields the differential heat of fusion for freezable water, given by the slope of the linear fit, and the maximum amount of non-freezable water, given by the extrapolated value of water content which resets the enthalpy of melting. The non-freezable water percentage is then calculated by comparing the water content obtained at the zero value of the enthalpy of melting with the water content of the hydrogel at T_0, that is before drying. The linear fit equation, the heat of fusion for the freezable water and the amount of freezable and non-freezable water are reported in Table 1.

Table 1. Linear fit equations and extrapolated values from data plotted in Figure 4.

Sample	Linear fit equation	Differential heat of melting ($cal \cdot g^{-1}$)	Non-freezable water (wt %)	Freezable water (wt %)

AgarArt 3 % w/v	ΔH = 0.628W - 372.0 R^2 = 0.94	0.628	20.22	79.78
Agar Purissimo 3 % w/v	ΔH = 0.765W - 708.7 R^2 = 0,95	0.765	25.91	74.09
Sigma 3 % w/v	ΔH = 0.598W - 262.3 R^2 = 0.97	0.598	12.32	87.68
Food 3 % w/v	ΔH = 0,620W – 267,0 R^2 = 0.98	0.620	12.44	87.56

Notably, for all the different hydrogels the heat of melting of freezable water is lower than that of bulk water, ΔH = 79.6 cal·g^{-1}, indicating that water in the hydrogels behaves differently from bulk water. In particular, the lower is the heat of melting the more dispersed is the water phase, which may also be associated to a more inhomogeneous microstructure of the hydrogel. Thus, according to data reported in Table 1, Agar Purissimo should give more homogeneous gels than the other agar samples [46]. The amount of non-freezable water is also significantly different depending on the agar source. In particular, in Agar Purissimo the amount of non-freezable bound water is approximately double that in Agar Sigma and Food. This points either to a different microstructure of the gels (i.e. the larger interfacial area, the higher is the interaction between water molecules and agar) or to a different hydrophilicity of the polymer network (i.e. binding sites are affected by the number and nature of polar groups or ion clusters) [47-48].

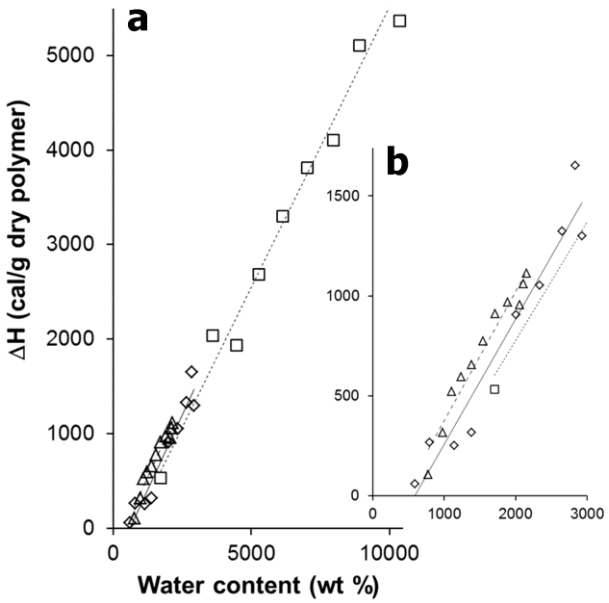

Figure 5. a) Enthalpy of melting, ΔH, *vs.* water content (W) in AgarArt 1% w/v (□, ΔH = 0.593W - 407.0), 3% w/v.(◊, ΔH = 0.628W – 372.0) and 5% w/v (Δ, ΔH = 0.649W - 274.8); b) magnification of the initial portion. Both ΔH and water content are relative to the dry polymer weight.

The influence of agar concentration on the type and amount of water molecules in the hydrogels was studied for the AgarArt gels. In Figure 5 the area of the melting signal (per gram of dry agar) versus water content is plotted for AgarArt gels with an agar concentration of 1, 3 and 5% w/v. The differential heats of melting obtained from the linear fit of the different set of data are very similar (Table 2), indicating that freezable water in the three hydrogels has the same properties and its behaviour is not affected by agar concentration. On the other hand the amount of non-freezable bound water changes. Increasing the agar concentration from 1 to 3% w/v, the content of bound water becomes triple, reaching approximatly 20 wt % of the total water content. This behaviour is consistent with the formation of smaller micropores in the 3% w/v hydrogel, resulting in a larger interfacial area and higher number of surface sites binding water molecules. A further increase of agar concentration, let the amount of non-freezable water constant, possibly because the stiff and denser polymer network makes the binding sites less accessible for water molecules [49].

Table 2. Linear fit equations and extrapolated values from data plotted in Figure 5.

Sample	Linear fit equation	Differential heat of melting ($cal \cdot g^{-1}$)	Nonfreezable water (wt %)	Freezable water (wt %)
AgarArt 1 % w/v	$\Delta H = 0.593W - 407.0$ $R^2 = 0.98$	0.593	6.624	93.38
AgarArt 3 % w/v	$\Delta H = 0.628W - 372.0$ $R^2 = 0.94$	0.628	20.22	79.78
AgarArt 5 % w/v	$\Delta H = 0.649W - 274.8$ $R^2 = 0.95$	0.649	19.68	80.32

3.3 Unilateral ^1H NMR

Water in hydrogels belongs to three categories: non-freezable bound, freezable bound and free water [24,50]. According to C. Canevali *et al*. [51], by combining the study of water transport by capillarity [52] with ^1H NMR analyses it is possible to identify the different water components as a function of gel concentration.

As well known longitudinal and transverse magnetization decays of bulk water exhibit mono-exponential trends with $T_1 \cong T_2$ both of the order of seconds, whereas, in the presence of different types of water multi-exponential trends are found which correspond to multiple T_1 and T_2 values. This behaviour has been previously observed in many porous systems [53-55]. In particular the transverse relaxation time of a nucleus is sensitive to both slow and fast molecular motions, while the longitudinal relaxation time is affected by fast motions only [56]. Literature highlights that in agar gels the longitudinal and transverse relaxation times of water are shortened by the presence of agar macromolecules.

Relaxation times were measured in agar gels to investigate water components and mobility as a function of agar concentration and commercial typology. In all agar gels the longitudinal relaxation time (T_1) showed a mono-exponential trend, whereas the transverse relaxation time (T_2) showed a bi-exponential trend.

As shown in Figure 6, for the whole set of agar gels the longitudinal relaxation rate $R_1 = \frac{1}{T_1}$ exhibited a linear correlation with agar concentration, indicating that by increasing the concentration water mobility decreased possibly due to the agar network which hindered water mobility.

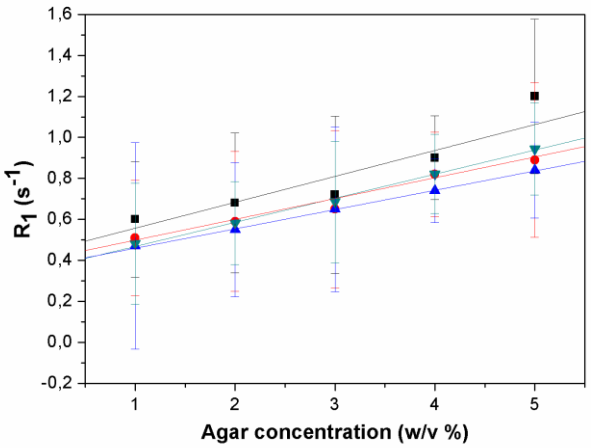

Figure 6. Correlation between longitudinal relaxation rate $R_1 = 1/T_1$ of water and agar concentration in gels. Solid lines were obtained by fitting data to straight lines: AgarArt $R^2 = 0.84$, Agar Purissimo $R^2 = 0.97$, Agar Sigma $R^2 = 0.99$, Agar Food $R^2 = 0.99$. AgarArt (■ black), Agar Purissimo (● red), Agar Sigma (▲ blue) and Agar Food (▼ dark cyan).

In the case of the transverse relaxation time T_2, two proton species must be taken into account: one exhibiting a short transverse relaxation time T_{2A}, related to bound water, and another one exhibiting a long transverse relaxation time T_{2B}, related to water free to move. Figure 7 shows the linear correlation between the transverse relaxation rate $R_2 = \frac{1}{T_2}$ and agar concentration found for the whole set of gels.

Both relaxation rates increased with the agar concentration. The slope of the line obtained from the best fit of R_{2B} (free water) against agar concentration was found to be steeper than that of R_{2A} (bound water). Indeed, these trends showed the greater influence of the agar concentration on the mobility of free water than on the mobility of bound water. Furthermore these data confirmed that increasing the population of double helices and their interconnection, water motion was increasingly hindered, as the average probability of collisions between water molecules and the gel network increased [57,58]. In all samples the percentage of bound water was in the range of 4-7%, which was considerably lower than the percentage estimated from DSC data.

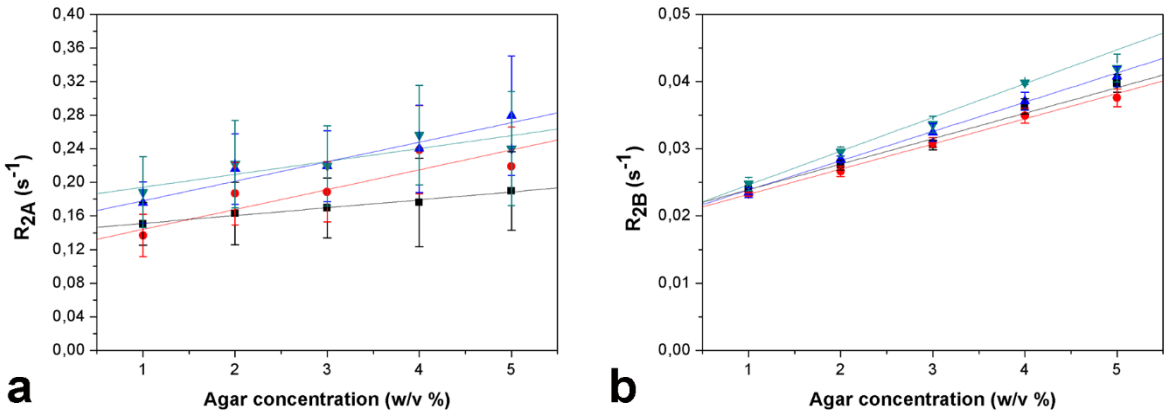

Figure 7. Correlation between transverse relaxation rate $R_{2A} = 1/T_{2A}$ and $R_{2B} = 1/T_{2B}$ of water and agar concentration in gels. Solid lines were obtained by fitting data to straight lines: a) T_{2A} (AgarArt $R^2 = 0.98$, Agar Purissimo $R^2 = 0.99$, Agar Sigma $R^2 = 0.99$, Agar Food $R^2 = 0.99$). b) T_{2B} (AgarArt $R^2 = 0.99$, Agar Purissimo $R^2 = 0.80$, Agar Sigma $R^2 = 0.92$, Agar Food $R^2 = 0.73$). AgarArt (■ black), Agar Purissimo (● red), Agar Sigma (▲ blue) and Agar Food (▼ dark cyan).

Therefore, transport properties of gels and the mobility of free water depend strongly on the concentration of the polysaccharide.

In order to study in detail the translational motion of water in agar gels as a function of agar concentration, the self-diffusion coefficient of water molecules was measured by diffusion ^{1}H NMR measurements [59].

In all commercial agar types diffusion decays showed a linear correlation with agar concentration indicating that a single self-diffusion coefficient describes the molecular displacement of the free water molecules, see Figure 8.

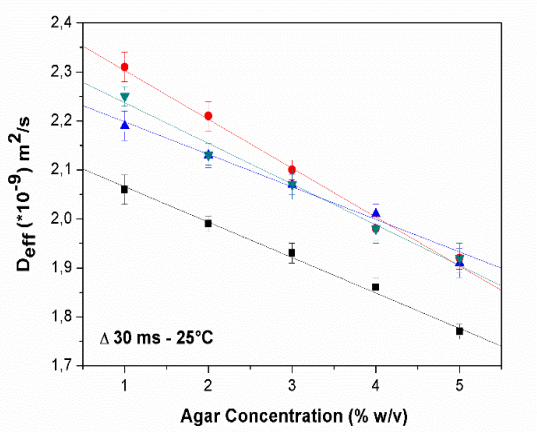

Figure 8. Self-diffusion coefficients of water molecules in agar gels *vs.* agar concentration at diffusion time of 30 ms. Solid lines were obtained by fitting data to straight lines. AgarArt (■ black), Agar Purissimo (● red), Agar Sigma (▲ blue) and Agar Food (▼ dark cyan).

Self-diffusion coefficients of water in agar gels were always slower than those observed in bulk water ($2.5*10^{-9}$ m^2/s at 25°C), and decreased as agar concentration increased, indicating that the translational motion of water molecules was more hindered in agar gels than in bulk water. The mean square displacement $\langle r^2 \rangle$ of water molecules with a self-diffusion coefficient (D) during a diffusion time (Δ) can be calculated from the relationship $\langle r^2 \rangle = \sqrt{2D\Delta}$ [59]. According to this relationship, in a diffusion time (Δ) of 30 ms, bulk water molecules should undergo a mean square displacement of about 21 µm. This distance is at least 20 times greater than the possible pore size of agar gel reported in literature that varies from 1 nm to about 600 nm [61].

During the diffusion time, water molecules travel across the gel network and their translational motion is increasingly hindered as the average probability of collision between free water molecules and the gel network increases. These results are in agreement with a study by Davies *et al*. [62] who reported that the decreased motion of water molecules in agar gels can be due to both the chemical interaction with hydroxyl groups and the obstruction caused by the gel network.

It is worth to note that among the analysed gels, the water molecules in AgarArt showed the lowest self-diffusion coefficient at each concentration, indicating the slowest translational motion.

To evaluate the ability of the gel to release water into the structure of a porous medium, ^{1}H NMR depth profiles were collected on AgarArt before and after the application on Noto stone specimens. Noto stone was selected because its high porosity allows water molecules to easily diffuse from the gel into the stone. This is an ideal condition to evaluate the ability of the gel in controlling the release of water. In Figure 9a and 9b, the profiles of AgarArt 3% w/v are compared with those measured on Agar Sigma at 30, 60 and 240 min of application. In the figures the profile of the gel before application to the stone is also reported (t=0), its thickness was found to be 2800 µm. When a hydrogel is applied to the surface of a porous stone specimen, the loss of water released from the gel to the porous structure during a certain time of application causes a deformation of the gel, thereby the collapse of the network, and the onset of the polysaccharide chains, see the sketch in Figure 9. Calculating the deformation of the gel as the variation of thickness Δh of the ^{1}H NMR depth profile, it was possible to evaluate the deformation of the gel during the time of application on the porous structure. Specifically, the thickness variation was calculated from the difference between the profile thickness Δh_0 before the application to the stone (t=0) and the thickness Δh_i at the time of application t_i: $\Delta h = \Delta h_0 - \Delta h_i$. In Figure 9c, the variation of thickness calculated in AgarArt and Agar Sigma are reported as function of the time of application. Results showed that after the first minutes of application both AgarArt and Agar Sigma had a very similar thickness variation. On the contrary, at longest times of application AgarArt experienced a minor degree of deformation with respect to Agar Sigma. During the application of the gel on the surface of the porous stone specimen, the translational motion of water molecules was found to be slower in AgarArt than in Agar Sigma. As a consequence, AgarArt

showed a better control of the water-releasing process and a better resistance to gel thickness variation than Agar Sigma.

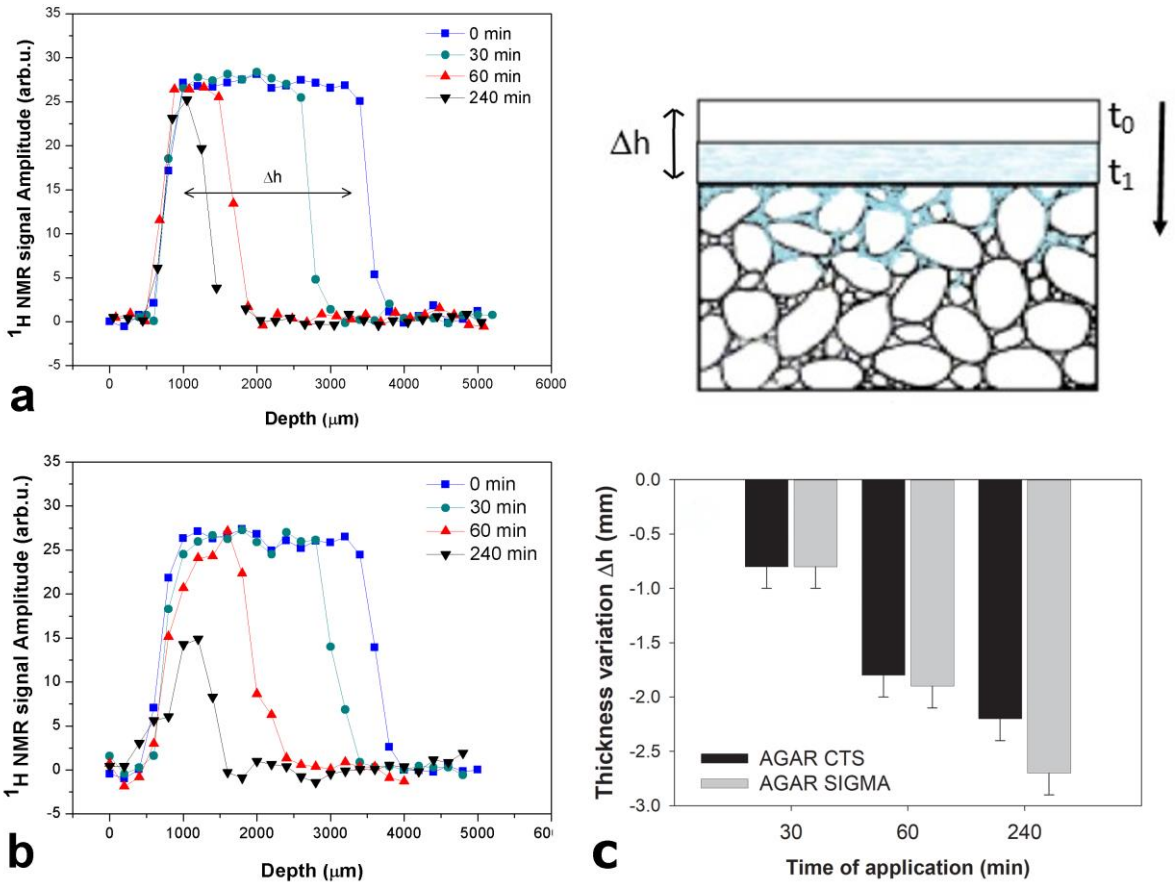

Figure 9. a) ^{1}H NMR depth profile of AgarArt (3% w/v) at three times of application on the stone specimen, and the profile before the application to the stone specimen (t = 0); b) ^{1}H NMR depth profile of Agar Sigma (3% w/v) at three times of application on the stone specimen, and the profile before the application to the stone specimen (t = 0); c) thickness variation of the gel as a function of the time of application. The sketch of the hydrogel applied on the surface of a stone specimen is reported, illustrating that the variation of thickness Δh (mm) is correlated to the release of water during the time of application.

4. CONCLUSIONS

Analyses carried out on different agar gels demonstrate significant differences in the overall water content and in the properties of water according to the type of agar and its concentration in the gel. Gravimetric measurements of weight loss as function of drying period exhibit a constant decrease in weight increasing the drying period. In particular, gels with the higher agar concentration (i.e. 5% w/v) show a slower rate in weight loss due to the denser polymer network which obstructs water evaporation. The dehydration of agar gels was also studied by TGA analyses. Different profiles of dehydration were recorded according to the length of the drying process: at the beginning of the drying the release of water occurs between room temperature and 100 °C, while gels dried from 30

to 50 min show a faster water loss at low temperature due to free water, followed by a more gentle decrease in weight associated to bound water.

Estimates of the amount of nonfreezable bound water and freezable free water were obtained by DSC and unilateral ^{1}H NMR measurements. The analysis of DSC data also provided the heat of melting of freezable water in the different gels. Obtained values are always lower than those measured in bulk water indicating that water in agar hydrogels behaves differently and it is in a more dispersed state than bulk water. In general, the amount of bound water determined by DSC is considerably higher in comparison to ^{1}H NMR data, especially for high agar concentrations. This is possibly due to the relative amount of the different water types, which depends on the technique used and on the thermal cycling of the sample. Moreover, at water concentration close to the bound water regime and at temperatures lower than -20 °C the reduced water diffusivity and the lower mobility of the polymer network hamper water solidification, which may result in an underestimation of freezable water.

The measurement of transverse relaxation times by unilateral ^{1}H NMR allowed to identify and quantify free and bound water. The trends of relaxation rates were found to be affected by agar concentration probably because increasing the population of double helices and their interconnection water motion was increasingly hindered. This correlation between the mobility of water molecules and the interactions/collisions with the gel network was also confirmed by NMR diffusion measurements. ^{1}H NMR diffusion data allowed studying the translational motion of water molecules in agar gel at different agar concentration, evidencing variations of the self-diffusion coefficients of water in the different agar samples. In particular, water molecules in Agar Art were found to have the slowest translational motion

The release and diffusion of water at the gel–porous structure interface were investigated by unilateral ^{1}H NMR depth profiles of the gels applied to Noto stone specimens. The variation of the gel thickness with the time of application allowed evaluating the water release and confirmed that AgarArt gels control the release of water better than Agar Sigma.

Acknowledgements

Compagnia di San Paolo and University of Torino are gratefully acknowledged for funding Project Torino_call2014_L2_181 through “Bando per il finanziamento di progetti di ricerca di Ateneo – anno 2014” (Project title: Polymer gels for cultural heritage).

References

[1] J. Albertus, Medicinal and pharmaceutical uses of seaweed natural products: A review, J. Appl. Phycology. 16 (2004) 245–262.

[2] S. Mazumder, P.K. Ghosal. C.A Pujol, M.J. Carlucci, E.B. Damonte, B. Ray, Isolation, chemical investigation and antiviral activity of polysaccharides from Gracilariacorticata (Gracilariaceae, Rhodophyta), Int. J. Biol. Macromol. 31 (2002) 87-95. DOI 10.1016/S0141-8130(02)00070-3

[3] C. Araki, Structure of Agarose constituent of Agar–Agar, Bull. Chem. Soc. Jpn. 29 (1956) 43–44

[4] M. Duckworth, K.C. Hong, W. Yaphe, The Agar polysaccharides of Gracilaria Species, Carbohyd. Res. 18 (1971) 1-9. DOI 10.1016/S0008-6215(00)80253-0

[5] T. Scott, M. Eagleson, Agar-agar, Concise Encyclopaedia: Biochemistry, 2nd Ed. – Walter de Gruyter, (1988) 18, ISBN 3110078600.

[6] S. Hirase, Studies on the Chemical Constitution of Agar-agar. XIXI) Pyruvic Acid as a Constituent of Agar-agar (part 1). Identification and estimation of Pyruvic Acid in the Hydrolysate of Agar. Bull. Chem. Soc. Jpn. 30:1 (1957) 68-70. DOI 10.1246/bcsj.30.70

[7] A. Struther, A. Fulmer, W.E. Scott, The Agarose double helix and its function in Agarose gel structure, J. Mol. Biol. 90 (1974), 269-284. DOI 10.1016/0022-2836(74)90372-6

[8] R. Wolbers, Cleaning painted surfaces: aqueous methods, Archetype Publications, London, 2000, ISBN 9781873132364.

[9] P. Cremonesi, Rigid gels and Enzyme cleaning, In: M.F. Mecklenburg, A.E. Charola, R.J. Koestler (Eds.), New insights into the cleaning of Paintings, Smithsonian Contribution to Museum Conservation (2013) 179-184. DOI 10.5479/si.19492359.3.1

[10] A. Casoli, P. Cremonesi, C.Isca, R. Groppetti, S. Pini, N.Senin, Evalutation of the effect of cleaning on the morphological properties of ancient paper surfaces, Cellulose. 20:4 (2013) 2027-2043. DOI: 10.1007/s10570-013-9975

[11] A. Rava, W. Shank, P. Colombini, O. Chiantore, M. Picollo, V. Palleschi, Keith Haring a Pisa. Pulitura e protezione di un dipinto acrilico esposto in ambiente esterno, Conf. Scienza e Beni Culturali XXVIII (2012), ISBN 978-88-95409-16-0.

[12] D. Gulotta, D. Saviello, F. Gherardi, L. Toniolo, M. Anzani, A. Rabbolini, S. Goidanch, Setup of a sustainable indoor cleaning methodology for the sculpted stone surfaces of the Duomo of Milan, Her. Sci. 2:1 (2014) 6. DOI 10.1186/2050-7445-2-6

[13] N. Prieto-Taboada, C. Isca, I. Martinez-Arkarzo, A. Casoli, M.A. Olazabal, G. Arana, J.M. Madariaga, The problem of sampling on built heritage: a preliminary study of a new non-invasive method, Environ. Sci. Pollut. Res. 21:21 (2014) 12518-12529. DOI 10.1007/s11356-014-3119-x

[14] A. Sansonetti, M. Casati, J. Striova, C. Canevali, M. Anzani, A. Rabbolini, A cleaning method based on the use of agar gels: new tests and perspectives. Proc of 12th Inter Con on the Deterior and Conserv of Stone, (2012), New York.

[15] P. Cremonesi and A. Casoli, Thermo-reversible rigid agar hydrogels: their properties and action in cleaning, In: L.V. Angelova, O. Bronwyn (Eds.), Gels in Conservation, Archetype Pubblications Ltd, London, 2017, 19-28, ISBN 978-1-909492-50-9.

[16] R. C. Wolbers, Conservation, Training, Materials and Techniques, Latest Developments, In: V. Todd, J. Marsden, M.K. Talley, J. Lodewijks, V. Sluyterman, W. Koeno (Eds), Preprints to the Conference Restoration '92 IIC, London, (1992) 74-75. ISBN 1-871656-18-4

[17] V. Dorge, Solvent gels for the cleaning of works of art: the residue question, Getty Publications, Los Angeles, USA, 2004, ISBN 9780892367597.

[18] L.V. Angelova, P. Terech, I. Natali, L. Dei, E. Carretti, R.G. Weiss, Cosolvent Gel-like Materials from Partially Hydrolyzed Poly(vinyl acetate)s and Borax, Langmuir. 27:18 (2011) 11671-11682. DOI 10.1021/la202179e

[19] C. Mazzuca, L. Micheli, F. Marini, M. Bevilacqua, G. Bocchinfuso, G. Palleschi, and A. Palleschi, Rheoreversible hydrogels in paper restoration processes: a versatile tool, Chem Cent J. 8:10 (2014). DOI 10.1186/1752-153X-8-10

[20] NORMAL 20/85 - Interventi Conservativi: Progettazione Esecuzione e Valutazione Preventiva.

[21] *ASTM C1515-01 2001* - Standard Guide for Cleaning of Exterior Dimension Stone, Vertical and Horizontal Surface, New or Existing. West Conshohocken, PA: American Society for Testing and Materials.

[22] Maintenance and Repair of Cleaned Stone Buildings, Technical Advice Note 25, 2002, Historical Scotland, ISBN 1903570 80 8

[23] Proyecto Coremans: Criterios de intervencion en materiales petreos,, Ministerio de Educaciçn, Cultura y Deporte, 2013, Secreteria General Tecnica, ISBN 978 84 8181 562 7

[24] M. Watase, K. Nishinari, T. Hatakeyama, DSC study on properties of water in concentrated agarose gels, Food Hydrocolloids, 2:247 (1988). DOI 10.1016/S0268-005X(88)80043-2

[25] K.D. Yao, W.G. Liu, J. Liu, The unique characteristics of water in chitosan–polyether semi-IPN hydrogel, J. Appl. Polym. Sci., 71 (1999), pp. 449–445. DOI 10.1002/(SICI)1097-4628(19990118)71:3<449::AID-APP11>3.0.CO;2-9

[26] M.N. Khalid, F. Agnely, N. Yagoubi, J.L. Grossiord, G. Couarraze, Water state characterization, swelling behavior, thermal and mechanical properties of chitosan based networks, Eur. J. Pharmaceut. Sci. 15:5 (2002) 425-432. DOI 10.1016/S0928-0987(02)00029-5

[27] S.J. Kim, S.J. Park, S.I. Kim, Swelling behavior of interpenetrating polymer network hydrogels composed of poly(vinyl alcohol) and chitosan, React. Funct. Polym. 55:1 (2003) 53-59. DOI 10.1016/S1381-5148(02)00214-6

[28] G.Pizzorusso, E.Fratini, J.Eiblmeier, R.Giorgi, D.Chelazzi, A.Chevalier, P.Baglioni, Physicochemical characterization of acrylamide/bisacrylamide hydrogels and their application for the conservation of easel paintings, Langmuir. 28 (2012) 3952-3961. DOI 10.1021/la2044619

[29] T. Hatakeyama, Determination of bound water content in polymers by DTA, DSC and TG, Thermochim. Acta. 123 (1988) 153-161. DOI 10.1016/0040-6031(88)80018-2

[30] F.X. Quinn, E. Kampff, G. Smyth, V. J. McBrierty, Water in Hydrogels. 1. A Study of Water in Poly(N-vinyl-2-pyrrolidone/methyl methacrylate) Copolymer, Macromolecules. 21 (1988) 3191-3198. DOI 10.1021/ma00189a012

[31] G. Smyth, F.X. Quinn, V.J. McBrierty, Water in Hydrogels. 2. A Study of Water in Poly(hydroxyethy1 methacrylate), Macromolecules. 21:11 (1988) 3198-3204. DOI 10.1021/ma00189a013

[32] M. Carenzaa, G. Cojazzib, B. Braccib, L. Lendinarac, L. Vitalic, M. Zincanic, M. Yoshidad, R. Katakaie, E. Takacsf, O.Z. Higag, F. Martellinig, The state of water in thermoresponsive poly(acryloyl-l-proline methyl ester) hydrogels observed by DSC and 1H-NMR relaxometry; Radiat. Phys. Chem. 55:2 (1999) 209–218. DOI 10.1016/S0969-806X(98)00328-4

[33] A. Mochizuki, Y. Miwa, R. Miyoshi, T. Namiki, Relationship between water structure and properties of poly(methyl methacrylate-b-2-hydroxyethyl methacrylate) by solid-state NMR, J. Biomat. Sci., Polymer Edition, Taylor and Francis Online, 2017, 1-16. DOI 10.1080/09205063.2017.1310647.

[34] R. Mao, J. Tang, B.G. Swanson, Water holding capacity and microstructure of gellan gels, Carbohyd. Polym. 46 (2011) 365-371. DOI 10.1016/S0144-8617(00)00337-4

[35] M. Bertasa, A. Botteon, L. Brambilla, C. Riedo, O. Chiantore, T. Poli, A. Sansonetti, D. Scalarone, Cleaning materials: A compositional multi-analytical characterization of commercial agar powders, J. Anal. Appl. Pyrol. 125 (2017) 310-317. DOI 10.1016/j.jaap.2017.03.011

[36] B. Blümich, F. Casanova, J. Perlo, F. Presciutti, C. Anselmi, D. Doherty, Non-invasive testing of art and cultural heritage by mobile NMR, Acc. Chem. Res. 43(6) (2010) 761–770. DOI 10.1021/ar900277h

[37] E. Del Federico, S.A. Centeno, C. Kehlet, K. Ulrich, A. YamazakiKleps, A. Jerschow, In situ unilateral 1H-NMR studies of the interaction between lead white pigments and collagen-based binders, Appl. Magn. Reson. 42(3) (2012) 363–376. DOI 10.1007/s00723-011-0302-9

[38] V. Di Tullio, D. Capitani, N. Proietti, Unilateral NMR to study water diffusion and absorption in stone-hydrogel systems, Microporous Mesoporous Mater. 2017. DOI 10.1016/j.micromeso.2017.07.011

[39] M. D. Hürlimann, Ex Situ Measurement of One- and Two-Dimensional Distribution Functions, In: F. Casanova, J. Perlo, B. Blümic (Eds.), Single-Sided NMR, Springer-Verlag, Berlin, 2011, 57-85, ISBN: 978-3-642-16306-7.

[40] M. Anzani, M. Berzioli, M. Cagna, E. Campani, A. Casoli, P. Cremonesi, M. Fratelli, A. Rabbolini, D. Riggiardi, Gel rigidi di agar per il trattamento di pulitura di manufatti in gesso. Q. n.6 Cesmar7, Padova: Il Prato. 2008, ISBN 9788863360349.

[41] G.Goelman, M.G. Prammer, The CPMG pulse sequence in strong magnetic field gradients with applications to oil-well logging, J. Magn. Reson. (series A) 113(1) (1995) 11-18. DOI 10.1006/jmra.1995.1050

[42] M. D. Hurlimann and D.D. Griffin, Spin dynamics of Carr-Purcell-Meiboom-Gill-like sequences in grossly inhomogeneous B(0) and B(1) fields and application to NMR well logging, J. Magn. Reson. 143(1) (2000) 120-135. DOI 10.1006/jmre.1999.1967

[43] L. Anania, A. Badalà, G. Barone, C. Maria Belfiore, C. Calabrò, M.F. La Russa, The stones in monumental masonry buildings of the “Val di Noto” area: new data on the relationships between petrographic characters and physical–mechanical properties. Constr. Build. Mater. (2012) doi:10.1016/j.conbuildmat.2011.12.076

[44] G. Alessandrini, A. Bocci, R. Bugini, D. Emmi, R. Peruzzi, M. Realini, Stone materials of Noto (Syracuse) and their decay. In: D.J. Rodrigues, F. Henriques, F.T. Jeremias (Eds.), Proceedings of the 7th international congress on deterioration and conservation of stone. Lisbon: Laboratório Nacional de Engenharia Civil, (1992) 11–20, ISBN 978-1-86239-376-9

[45] A. Mochizuki, H. Ogawa, Y. Nishimori, Water structure in poly(2-hydroxyethyl methacrylate): Effect of molecular weight of poly(2-hydroxyethyl methacrylate) on its property related to water, J. Appl. Polym. Sci. 125 (2012) 53–60. DOI 10.1002/app.35544

[46] Y.L. Guan, L. Shao, K. De Yao, A study on correlation between water state and swelling kinetics of chitosan-based hydrogels, J. Appl. Polym. Sci. 61 (1996) 2325-2335. DOI 10.1002/(SICI)1097-4628(19960926)61:13<2325::AID-APP11>3.0.CO;2-3

[47] M.M. Chui, R.J. Phillips, M.J. McCarthy, Measurement of the Porous Microstructure of hydrogels by Nuclear Magnetic Resonance, J Colloid Interface Sci., 174(2), (1995) 336-344. DOI 10.1006/jcis.1995.1399

[48] A.H. Clark, R.K. Richardson, S.B. Ross-Murphy, J.M. Stubbs, Structural and mechanical properties of Agar/Gelatin Co-gels. Small-Deformation Studies, Macromolecules. 16 (1983) 1367-1374. DOI 0024-9297/83/2216- 1367$01.50/0

[49] J. Narayanan, J.Y. Xiong, X.Y. Liu, Determination of agarose gel pore size: Absorbance measurements vis a vis other techniques, J Phys: Conf. Ser., (2006), 28: 83-86, doi:10.1088/1742-6596/28/1/017

[50] Z.H. Ping, Q.T. Nguyen, S.M. Chen, J.Q. Zhou, T.D. Ding, States of water in different hydrophilic polymers — DSC and FTIR studies, Polym. 40:20 (2001) 8461–8467. DOI 10.1016/S0032-3861(01)00358-5

[51] C. Canevali, M. Fasoli, M. Bertasa, A. Botteon, A. Colombo, V. Di Tullio, D. Capitani, N. Proietti, D. Scalarone, A.Sansonetti, A multi-analytical approach for the study of copper stain removal by agar gels, Microchem. J. 129 (2016) 249–258. DOI 10.1016/j.microc.2016.07.007

[52] Italian Standard UNI10859. 2000. Natural and Artificial Stones, Determination of Water Absorption by Capillarity, ISBN 0 580 32290 4

[53] K.R. Brownstein,C.E. Tarr, Spin-lattice relaxation in a system governed by diffusion, J. Magn. Res. 26:1 (1977) 17-24. DOI 10.1016/0022-2364(77)90230-X

[54] K.R. Brownstein,C.E. Tarr, Importance of classical diffusion in NMR studies of water in biological cells, Phys. Rev. A. 19:6 (1979) 2446-2453. DOI 10.1103/PhysRevA.19.2446

[55] P.A. Bottomley, C.J. Hardy, R.E. Argersinger, G. Allen-Moore, A review of 1H nmr relaxation in pathology: are T1 and T2 diagnostic?, Med. Phys. J. 14:1 (1987) 1-37. DOI 10.1118/1.596111

[56] S.W. Homans, A Dictionary Concepts in NMR. Oxford, Clarendon Press, New York, 1992, ISBN: 9780198547655.

[57] S. Arnott, A. Fulmer, W.E. Scott, I.C.M. Dea, R. Moorhouse, D.A. Rees, The agarose double helix and its function in agarose gel structure, J. Mol. Biol. 90:2 (1974) 269-284. DOI 10.1016/0022-2836(74)90372-6

[58] T.G.L. Hickson, A. Polson, Some physical characteristic of the agarose molecule, Biochim. Biophys. Acta. 165 (1968) 43-58. DOI 10.1016/0304-4165(68)90186-4

[59] R. Kimmich (1997) NMR: tomography, diffusiometry, relaxometry, Springer-Verlag, Berlin, ISBN: 3-540-61822-8.

[60] I.A. Farhat, E. Loisel, P. Saez, W. Derbyshire, J.M.V. Blanshard, The effect of sugars on the diffusion of water in starch gels: a pulsed field gradient NMR study, Int. J. Food Sci. Technol. 32(5) (1997) 377–387. DOI 10.1046/j.1365-2621.1997.00126.x

[61] J. Narayanan, J.Y. Xiong, X.Y. Liu, Determination of agarose gel pore size: Absorbance measurements vis a vis other techniques, J Phys: Conf. Ser. 28 (2006) 83-86. DOI 10.1088/1742-6596/28/1/017

[62] E. Davies, Y. Huang, J.B. Harper, J.M. Hook, D.S. Thomas, I.M. Burgar, P.J. Lillford, Dynamics of water in agar gels studied using low and high resolution 1H NMR spectroscopy, Int. J. Food Sci. Technol. 45 (2010) 2502-2507. DOI 10.1111/j.1365-2621.2010.02448.x