Polysaccharide-based Supramolecular Bicomponent Eutectogels as Sustainable Antioxidant Materials

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Abstract

In this work, we obtained supramolecular gels in deep eutectic solvents (DES) from polysaccharides like chitosan and chitosan:cellulose composites. We thoroughly characterized our gels by determining the minimum gelation concentration, as well as their porosity and swelling. We also investigated their mechanical properties by rheology, and morphology by scanning electron measurements. These properties were mainly influenced by the number of hydrogen bond sites on hydrogen bond donor, (HBD), while FTIR-ATR investigation suggested that, upon gelation, cholinium cations interpose between polysaccharide chains affecting interchain hydrogen bonding. Our gels also exhibited self-healing and load bearing ability and proved injectable.

Subsequently, we evaluated antioxidant ability of the gels by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay. We found that gelation of the DES reduces their radical scavenging ability, so we doped the gels with natural antioxidants like *trans*-ferulic acid (TA) and α-tocopherol (VE), finding that their high scavenging ability is fully preserved in the gel matrix. We then prepared films from one of the gels and evaluated their stability to sunlight and exposure to water, finding that the

TA-doped film was the most resistant to UV-light, and retained its radical scavenging ability even after being exposed to significant amounts of water.

Introduction

The development of materials with antioxidant action is presently highly sought after, due to the relevance and harmful impact of oxidation processes in many aspects of everyday life. Oxidation is a major cause of food spoilage, and antioxidant protective materials used as food packaging help prolonging their shelf-life. [1, 2] Oxidation also damages plastic polymers, particularly when exposed to sunlight in outdoor applications.[3-5] This is also true for medical grade plastic materials, such as those used for dental application and orthopedic joints.[6]

Among the materials used to this purpose, recently the ones based on biopolymers, like polysaccharides, have gained significant interest, due to some advantageous properties. In particular, biopolymers are biocompatible and non-toxic and derive from cheap and renewable sources. In addition, biopolymers often confer high mechanical resistance and robustness to the ensuing materials.[7-9]

Among the different classes of materials possessing antioxidant ability, supramolecular gels[10, 11] have recently gained increasing interest. These are viscoelastic materials formed by the self-assembly of dilute solutions of suitable small molecules, as well as polymers, which in opportune conditions form a sample-spanning network which entraps large amount of solvents. Supramolecular gels are held together only by non-covalent interactions and therefore can respond reversibly to external stimuli. Furthermore, a very recent development of supramolecular gels is the obtainment of such materials in non-conventional solvents, like ionic liquids or deep eutectic solvents (DES), known as ionogels[12] or eutectogels,[13, 14] respectively. In particular, DES are constituted by mixtures of compounds, with a much lower melting point than the one of the individual components.[15] In general, DES are composed by a hydrogen bond donor (HBD), and a hydrogen bon acceptor (HBD), interacting through a dense network of hydrogen bonds, imparting to DES a distinct nanostructure.[16-18] A strong

point of DES is that they are often constituted by simple cheap compounds, in many cases naturally occurring ones with low or negligible toxicity. As a result, DES can be useful to obtain safe and low-impacting functional materials.[19, 20] In the framework of our interest in studying supramolecular gels in non-conventional solvents,[21-23] we have recently found that supramolecular ionogels can possess antimicrobial[24] and antioxidant activity.[25] In addition, DES have emerged as suitable solvents for the preparation and processing of materials based on biopolymers as chitin and chitosan, due to their high solubilizing ability towards such polysaccharides.[26] Thus, we wanted to investigate whether polysaccharide-based eutectogels can be suitable to develop sustainable and efficient antioxidant materials.

Based on these considerations, we prepared eutectogels based on chitosan (CS) and chitosan:cellulose mixtures (CS:CE) in cholinium chloride (ChCl)-based DES differing for the HBD, comprising urea (U), polyalcohols like glycerol (Gly), ethylene glycol (EG), diethylene glycol (DEG) and triethylene glycol (TEG) as well carboxylic acids, namely malic acid (MA) and lactic acid (LA) reported in Figure 1.

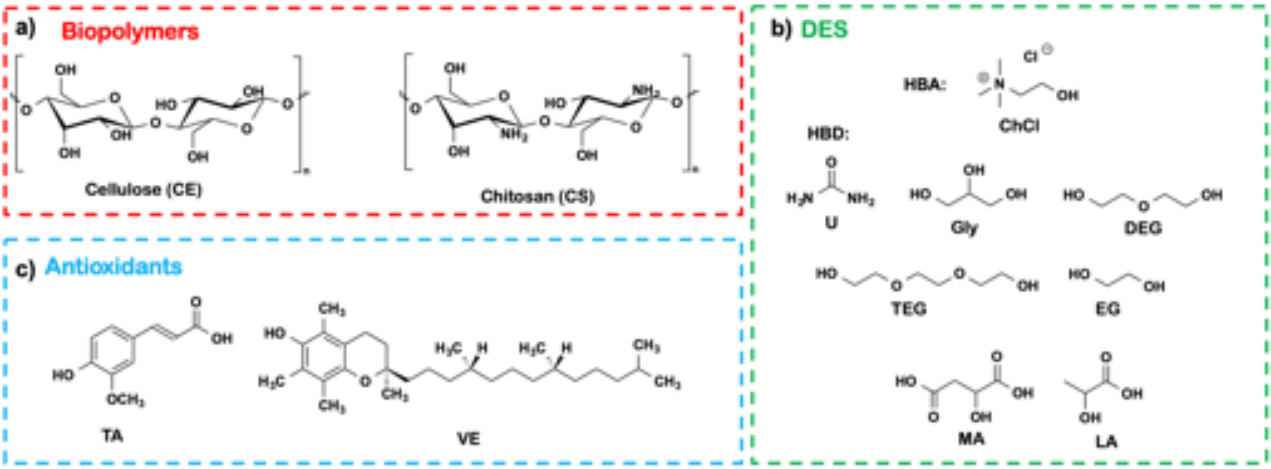

Figure 1. a) Biopolymers used, b) deep eutectic solvents and c) natural antioxidants.

The gels were characterized for their swelling and porosity, their rheological properties while morphology was probed by scanning electron microscopy (SEM). We also investigated their self-healing and load bearing capabilities. Then, we evaluated the antioxidant ability of our gels by means

of the DPPH assay as a function of time. This investigation was carried out on the pristine gels as well as on the relevant bicomponent gels, doped with added natural antioxidants, such as *trans*-ferulic acid (TA) and α-tocopherol (VE) (Figure1c). Our hypothesis is that such bi-component biopolymer-based eutectogels could constitute sustainable and efficient antioxidant materials.

To assess the stability our gels in real-life conditions, we measured the radical scavenging efficiency of our gels after irradiation with UV light, to simulate exposure to atmospheric conditions. The best performing gels in terms of radical scavenging ability were obtained in film form, for which we evaluated whether they maintained the antioxidant ability of the parent gels. Finally, we investigated the kinetics of release of antioxidants from the bicomponent gels and from the relevant films. The bicomponent gels and the relevant films showed high radical scavenging ability, which was maintained to a large extent even after being exposed to UV light or water. To the best of our knowledge, this is the first work describing the application of biopolymer-based eutectogels as antioxidant materials.

Experimental section

Materials

Cellulose, Chitosan (low molecular weight), choline chloride, urea, glycerol, ethylene glycol, diethylene glycol, lactic acid, malic acid, *trans*-ferulic acid and (±)-α-tocopherol were obtained from commercial sources and used without further purification. Commercially available acetic acid, hexane, methanol, and diethyl ether, were used as received. DES were prepared by reported procedures.[27, 28]

Gelation tests

Solid mixtures of chitosan and cellulose were prepared by grinding in a mortar the suitable amounts of polymers, in the weight ratio 1:1, until obtaining a fine, uniform powder. To obtain a typical gel, chitosan mixture was first dissolved in an aqueous solution of acetic acid (2%, w:v, 40 μL/mg), in a

screw capped vial. Then, the suitable amount of DES was added and the resulting mixture was heated under stirring at 100 °C, until obtaining a homogeneous solution. Subsequently, the vial was kept at 4 °C overnight. For chitosan:cellulose-based gels, a slightly different procedure was followed, involving sonication of the hot solution for 10 minutes (45 kHz), which was then kept at 4 °C overnight. Gels obtained in carboxylic acid-containing DES, were prepared by weighing in a screw-capped vial the suitable amount of gelator and DES. The resulting mixtures were heated at 100 °C for 3 h, after which a homogeneous solution was obtained, which then was stored overnight at 4 °C. Gel formation was then assessed by the tube inversion test.[29]

Rheology measurements

Rheological measurements were carried out on a strain-controlled rheometer equipped with a Peltier temperature controller and a plate-plate tool. In a typical measurement, the gel was formed in a plastic blister pack, then transferred between the shearing plates of the rheometer. Strain and frequency sweep measurements were carried out at 25 °C, on three different aliquots of gels, within the linear viscoelastic region. In particular, strain sweeps were performed at a frequency of 1Hz, while frequency sweeps at a fixed oscillation strain of 1%.

Porosity and swelling

Porosity[30] and swelling[31] of all eutectogels were determined following reported procedures, using HPLC-grade hexane as the solvent. Hexane was cast on gels for 24 h.

Knowing the initial weight of the empty vial (W_1), the weight of the vial and the gel before (W_{dry}) and after (W_2) adding hexane together with the final weight of the vial and gel after removing hexane ($W_3 = W_{wet}$), it was possible to determine the porosity (P) and swelling (Q) using the following equations:

$$V_g = 4 - \left(\frac{W_2 - W_1 - W_{dry}}{\rho_h} \right) \quad \textbf{(1)}$$

$$V_p = \frac{W_2 - W_3 - W_{dry}}{\rho_h} \quad \textbf{(2)}$$

$$P = \frac{V_P}{V_p + V_g} \cdot 100 \quad \textbf{(3)}$$

$$Q = \frac{W_{wet} - W_{dry}}{W_{dry}} \cdot 100 \quad \textbf{(4)}$$

where ρ_h is the density of hexane, V_g and V_p (mL) represent the volume of gel and hexane in the pores, respectively. Finally, 4 mL is the total volume occupied by the gel and hexane.

2.5 Self-healing and load bearing tests

To investigate the self-healing ability of our gels, we prepared two identical eutectogels (500 mg), one of which was prepared in the presence of 0.5 wt% or rhodamine B. Subsequently, each gel was cut in half with a razor blade and one half of the pristine gel and one of the stained gel were put into contact. For load bearing tests, weight of increasing mass were placed on top of 500 mg of each gel. The weights were increased until observing the collapse of the gels.

2.6 SEM images

SEM measurements were carried out on a PRO X PHENOM electronic scanning microscope, operating at 5 KV. Xerogels for each sample were obtained placing each gel on an aluminum stub, and then washing it with ethanol to remove the DES, following a previously reported procedure.[32, 33]

2.7 DPPH assay

The DPPH free radical scavenging assay on gels was carried out following a reported procedure.[25, 34] Samples for a typical measurement were prepared by placing, in a screw-capped vial, 250 μL of a 10^{-3} M solution of DPPH in diethyl ether, on top of 250 mg of preformed gel. Contact between gel and solution was maintained for a suitable time, at 25 °C. All the gels were stable during this phase. Subsequently, the solvent was removed in vacuum and the residue was dissolved in 5 mL of methanol. Then the UV-vis of the solution was recorded. Another aliquot of DPPH solution, used as reference, was treated in the same way except for being in contact with the gel. The radical scavenging efficiency was determined by Equation (5)

$$SE\ (\%) = \frac{A_t - A_b}{A_b} \cdot 100 \qquad \textbf{(5)}$$

where A_t is the absorbance of the sample solution and A_b is the absorbance of the reference solution, at the same time.

The DPPH assay on the single DES was performed as described above, using the quantities of solvent present in 250 mg of gel.

2.8 Photostability test

Samples were irradiated for 1h, at 70 °C, using a mercury UV lamp emitting at 313 nm.

2.9 Preparation of films

Films were prepared by dissolving 20 mg of chitosan in 800 μL of an aqueous solution of acetic acid, 2% (w:v), in a screw-capped vial. The resulting solution was then transferred in a Petri dish and 1g of DES was added. The ensuing mixture was heated at 80 °C for 5 min, to obtain a homogeneous solution, which was left at room temperature for 24 h.

2.10 Release of TA from gels and films

The release of TA was evaluated by placing preformed gels or films in contact with water, in a beaker. In particular 2.5 g of the CS/[ChCl][Gly] gels doped with 0.5 wt% of *trans*-ferulic acid were placed in contact with 5 mL of water, and 1g of the relevant film with 12 mL of water. At suitable times 100 μL of supernatant was withdrawn and replaced with 100 μL of water. The aliquot withdrawn was suitably diluted with methanol and the UV-vis spectrum was recorded. The amount of *trans*-ferulic acid released was then obtained based on a calibration curve previously determined.

Results and discussion

Gelation tests and gel characterization

Firstly, we investigated the obtainment of eutectogels formed by chitosan. Due to the low solubility of this polymer in the DES devoid of carboxylic acids, we initially dissolved chitosan in a dilute aqueous solution of acetic acid (2%, w:w), and then added the suitable amount of DES, obtaining the results reported in Table 1. On the other hand, this step was not necessary when the acid was the HBD of the DES, in which case protonation of chitosan enhanced its solubility in the DES. For all gels obtained, which were translucent in appearance, we also determined the minimum gelation concentration (MGC) i.e. the minimum concentration of polymer at which gelation occurs.

Table 1. Gelation tests and minimum gelation concentrations (MGC).

DES	Conc. (wt%)	MGC (wt%)
Chitosan		
[ChCl][U] 1:2	2.0-5.0	4.0
[ChCl][Gly] 1:2	1.0-2.0	2.0
[ChCl][TEG] 1:2	1.0-3.0	2.0
[ChCl][DEG] 1:3	1.0-3.0	2.0
[ChCl][EG] 1:3	0.9-3.0	1.0
[ChCl][LA] 1:1	1.0-3.0	2.8
[ChCl][MA] 1:1	0.4-1.0	0.5
Chitosan:Cellulose (1:0.2, w:w)		
[ChCl][U] 1:2	1.0-4.0	4.0
[ChCl][Gly] 1:2	1.0-2.0	2.0
[ChCl][EG] 1:2	1.0-2.0	2.0
Chitosan:Cellulose (1:0.4, w:w)		
[ChCl][U] 1:2	1.0-3.0	2.5
[ChCl][Gly] 1:2	0.9-3.0	1.0
[ChCl][EG] 1:2	0.9-3.0	1.0

Analyzing the results reported in Table 1, highlights a high gelation propensity of chitosan, which gelled all the DES considered. Comparison of the MGC values brings out a pronounced effect of the nature of the HBD on the gelation propensity of chitosan in the different DES. More specifically, the MGC increases along the order [ChCl][MA] < [ChCl][EG] < [ChCl][Gly] = [ChCl][DEG] = [ChCl][TEG] < [ChCl][LA] < [ChCl][U]. This indicates that the DES bearing a dicarboxylic acid as HBD is the solvent that better supports gelation, probably due to the higher ability of the acid to protonate the amino groups in the polymer backbone, leading to more favorable polymer-solvent interactions. Accordingly, the gelation propensity is lower in [ChCl][LA], in which case the HBD is a monocarboxylic acid. The lower acidity of [ChCl][LA] compared with analogous DES bearing dicarboxylic acids as HBD has been reported in the literature.[35] Furthermore, this is also in agreement with the highest MGC value, and so lowest gelation propensity, found in [ChCl][U] which is the only DES featuring a HBD with a certain basic character.[36, 37]

Further comments on the trend of MGC as a function of the HBD nature, can be made examining the values obtained in glycol-based DES. In particular, MGCs increase on going from [ChCl][EG], to [ChCl][DEG] and [ChCl][TEG], thereby increasing the number of methylene units and, consequently, the intensity of van der Waals interactions. Finally, comparing the values obtained in [ChCl][EG], [ChCl][Gly] and [ChCl][U] brings out a significant influence of the number of hydrogen bond donor groups in the HBD. In particular, MGC regularly increases when the HBD has higher number of hydrogen bonding donor groups, suggesting an unfavorable effect on gelation. This could be due to partial disruption of the interchain hydrogen bond interaction required for gelation to occur as reported in literature for chitosan[38, 39] and other biopolymers.[40]

Next, we set out to investigate the effect, on eutectogel formation, of adding different amounts of cellulose to chitosan. Mixing chitosan with cellulose has indeed recently emerged as a straightforward way to improve the properties of ionic liquid gels in terms, for instance, of mechanical strength.[41-43] We carried out the gelation tests in selected DES, namely [ChCl][EG], [ChCl][Gly], and [ChCl][U]. We chose these DES because their HBD featured 2,3 and 4 potential hydrogen bonding donor sites, respectively. Moreover, we used two composites, with CS:CE ratios amounting to 1:0.2 and 1:0.4. The results obtained in both cases are reported in Table 1, and show that adding cellulose does not prevent gelation in the solvents considered. Moreover, the MGC values for both composite as a function of the DES show the same trend, increasing as [ChCl][Gly] = [ChCl][EG] < [ChCl][U] which hold close resemblance to the one observed in the absence of cellulose. Moreover, the variation of MGC as a function of the amount of cellulose, DES being the same, shows that the addition of cellulose has a favorable effect on gelation, expressed by the drop in MGC values on increasing its amount. This effect is particularly significant in [ChCl][U], which was the least supporting gelation solvent. This can be explained by considering that replacing part of the chitosan chains with cellulose, devoid of charged groups, makes the composite less sensitive to the unfavorable effect exerted by the basicity of the DES. The exception is represented by [ChCl][EG], in which a bell-shaped profile trend is found, although in DES featuring glycols as HBA, the magnitude of variation is significantly lower.

Subsequently, we determined the porosity (P) and swelling (Q) of our gels by contacting them for 24 h with hexane (see experimental section for details). Results obtained using reported procedures[31, 44] are summarized in Table 2.

Table 2. Swelling and porosity determined upon contact of gels with hexane.

Gel (wt%)	P (%)[a]	Q (%)[a]
CS/[ChCl][U] (2%)	94	< 5
CS/[ChCl][Gly] (2%)	94	< 5
CS/[ChCl][DEG] (2%)	95	< 5
CS/[ChCl][TEG] (2%)	96	7
CS/[ChCl][EG] (2%)	94	< 5
CS/[ChCl][LA] (2%)	96	< 5
CS/[ChCl][MA] (2%)	95	< 5
CS:CE (1:0.2)/[ChCl][Gly] (2%)	93	< 5
CS:CE (1:0.4)/[ChCl][U] (2.5%)	95	10
CS:CE (1:0.4)/[ChCl][U] (2%)	93	< 5
CS:CE (1:0.4)/[ChCl][EG] (2%)	94	< 5

[a] Swelling and porosity values are reproducible within ± 5%

The results reported in Table 2 show that the porosity of the gels is high and practically the same, regardless of the DES or polymer used. Similarly, we only observed modest or negligible values of swelling, with no obvious influence of solvent or polymer. The only exception is the CS:CE (1:0.4 w:w) in [ChC][U] at 2 wt%, which however only shows a rather low value.

Next, we investigated the mechanical properties of our gels by oscillatory rheology, and carried out strain and frequency sweep measurements at 25 °C. In this way, the storage modulus, G′ and loss modulus G″ can be followed as a function of the strain or the frequency applied. In particular, G′ and G″ account for the solid- and liquid-like rheological response of the gel, respectively. Moreover, from

these experiments, we determined the strain at the crossover point between G′ and G″, which expresses the highest strain that the gel can withstand without breaking. Another relevant parameter is the ratio G″/G′, known as loss tangent, tan δ , which is related to the strength of interactions within the self-assembled network.[45] Notably, we could not carry out the measurements for the gels obtained in DES bearing carboxylic acids, since they were too weak and were destroyed under the conditions of the experiments, although they kept a gelatinous appearance (Figure S1. The rheological parameters obtained for our gels are summarized in Table 3, while the plots of strain and frequency sweeps are reported in Figures 2 and S1.

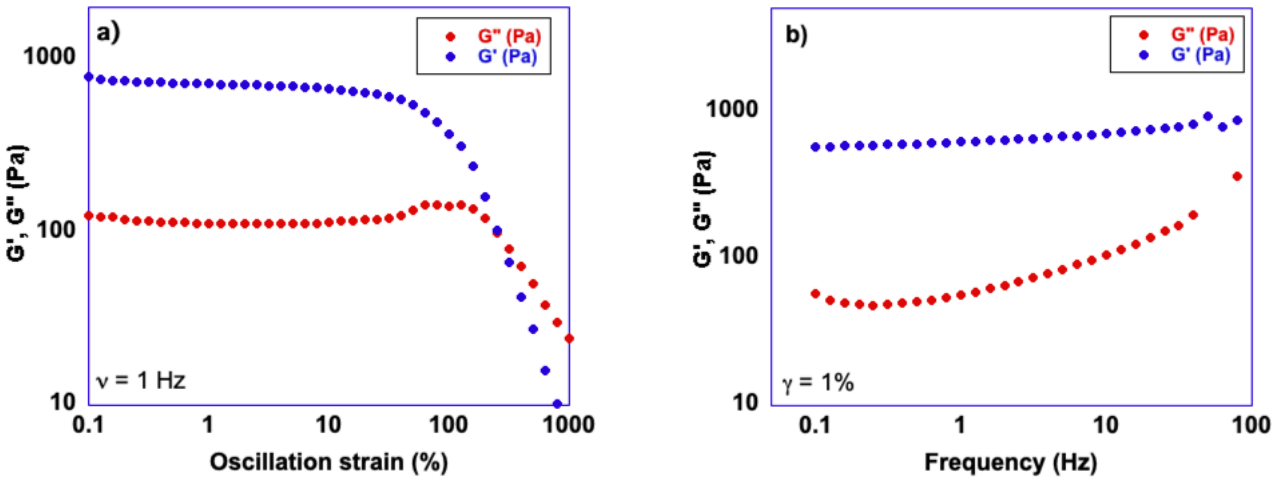

Figure 2. Plots relevant to a) Strain- and b) frequency sweeps for the CS/[ChCl][DEG] 2 wt% at 25 °C

Table 3. G′ and G″ at γ = 0.05 %, tan δ = G″/G′ and values of γ_c at G″ = G′ for eutectogels investigated at 25 °C. Error limits are based on average of three different measurements with different aliquots.

Gel (wt%)	G´ (Pa)	G´´ (Pa)	tan δ	γ_c (%)
CS/[ChCl][U] (4%)	3300 ± 600	600 ± 100	0.180 ± 0.003	130 ± 30
CS/[ChCl][Gly] (2%)	1150 ± 350	50± 20	0.050 ± 0.002	160 ± 60
CS/[ChCl][EG] (2%)	380 ± 70	35 ± 5	0.090 ± 0.002	180 ± 30
CS/[ChCl][DEG] (2%)	700 ± 70	80 ± 30	0.11 ± 0.04	220 ± 30
CS/[ChCl][TEG] (2%)	1300 ± 200	130 ± 20	0.096 ± 0.001	220 ± 20
CS+CE (1:0.4)/[ChCl][U] (2.5%)	9000 ± 2000	1900 ± 500	0.204 ± 0.003	85 ± 2
CS+CE (1:0.2)/[ChCl][Gly] (2%)	1450 ± 350	90 ± 20	0.062 ± 0.004	280 ± 50
CS+CE (1:0.4)/[ChCl][Gly] (2%)	1700 ± 290	90 ± 20	0.050 ± 0.004	70 ± 20
CS+CE (1:0.4)/[ChCl][EG] (2%)	600 ± 100	50 ± 10	0.083 ± 0.001	120 ± 20

Looking at the results reported in Table 3 reveals that all of our gels have high flexibility and resistance. In particular, for the CS-based gels, the G´ values, related with their rigidity, increase following the trend [ChCl][EG] < [ChCl][DEG] < [ChCl][Gly] ≈ [ChCl][TEG] < [ChCl][U]. The trend of G´ is similar to the one of the MGC and increases in magnitude in DES with a higher number of hydrogen bonding sites in the HBD. In addition, the gel CS/[ChCl][U] is the most rigid one. Moreover, DES being the same, the presence of CE in the gels induce an increase in rigidity, particularly evident for the gels obtained in [ChCl][U]. On the other hand, for CS-based eutectogels, the values of strain at crossover point, related to flexibility and mechanical resistance span a narrower range. However, in this case the gels formed in glycol-based DES as [ChCl][DEG] and [ChCl][TEG] appear more flexible and resistant than the others, particularly the one in [ChCl][U].

For this parameter, the effect of adding CE to the gels suggests a general decrease in mechanical resistance upon adding CE. In particular, this is the case for the gels in [ChCl][U] and [ChCl][EG]. Finally, a more articulate picture emerges for the gels in [ChCl][Gly], since an initial addition of CE induces an increase in γ_c, as seen for the gel CS+CE (1:0.2)/[ChCl][Gly], while further increasing the amount of cellulose in the composite results in a sharp drop of γ_c for CS+CE (1:0.4)/[ChCl][Gly].

However, such variations cannot be ascribed to changes in overall interactions within the gel matrix, which keep practically constant as expressed by the tanδ values. Therefore, the results obtained may reflect a different arrangement of the polymer chain within the gel matrix.

To obtain further information on the interactions underpinning the formation of eutectogels, we recorded the FTIR-ATR spectra of both polymers and eutectogels. In particular, we compared the ATR spectra of the gels with the one of the solid obtained by treating chitosan with the aqueous solution of acetic acid, which is the form in which it is actually dissolved in the DES during the gelation process. The same approach was used for the gels formed by the CS:CE composites.

We recorded the spectra of all CS-based gels and two gels based on composite CS:CE, differing for the composition of the composite.

Representative FTIR-ATR spectra are reported in Figure 3 while the other ones are reported in Figure S3.

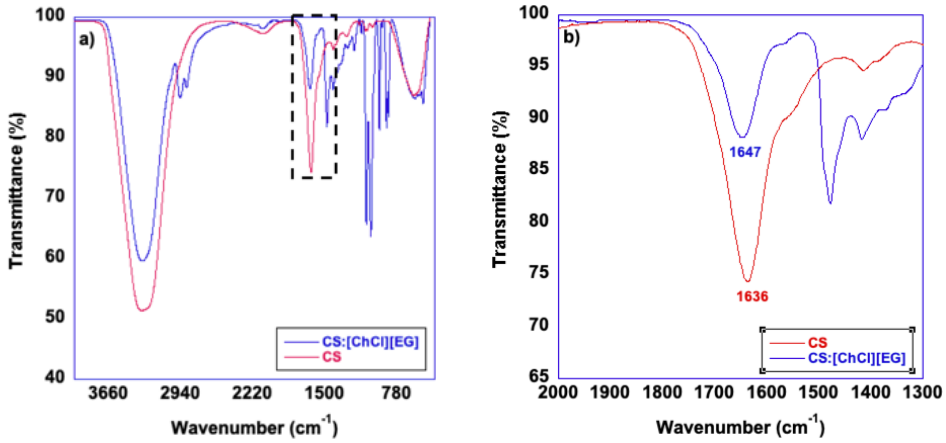

Figure 3. Superimposed FTIR-ATR spectra of a) CS and CS/[Ch][EG] eutectogel at 2 wt%, b) enlargement of the 1300-2000 cm^{-1} region.

Due to the nature of solvents and polymers involved in the formation of gels, the signals of the O-H groups of the polymers and the solvent are superimposed, as well as those of the amino groups of chitosan. Consequently, the most suitable signal to assess the interactions underpinning the gel is the C=O stretching band of the residual acetylated groups of chitosan. As can be seen in the enlarged spectra in Figure 3b, as well as in the spectra reported in Figure S3, this band consistently shifts to

higher frequencies on going from the polymer to the gel. The only exception is the CS/[Ch][U] gel in which the amide band of the polymer is covered by the one of urea, present in large excess. These findings hint consistently at a reduction in magnitude of the hydrogen bonding involving the residual carbonyl groups in the chitosan backbone on going from their acidic solution to the gel. Similar conclusions can be drawn for the gels obtained in the presence of CS:CE composites. We propose that this indicates that the solvent and, in particular, the OH-group in the cholinium cations on the HBD of the DES, interposes between the polymer backbones so that, on balance, fewer hydroxy- or amino groups in the polymer donate hydrogen bond to the acetamido groups. In turn, this donation of hydrogen bond will involve the chloride anion, favouring the mixed solvent-gelator interactions needed for the formation of the gelatinous network.

We then investigated the morphology of selected gels by scanning electron microscopy (SEM). The images were determined on xerogels obtained by removing the DES with ethanol preserving the polymer network, following reported procedures.[32, 33] In particular, the gels considered were CS/[ChCl][U] (4 wt%), CS/[ChCl][EG] (2 wt%), CS/[ChCl][DEG] (2 wt%), CS/[ChCl][TEG] (2 wt%), CS/[ChCl][Gly] (2 wt%) and CS+CE (1:0.2)/[ChCl][Gly] (2 wt%). This allowed us to assess the effect of the nature of the HBD of the DES and of the addition of CE in the gelling mixture. Images obtained are reported in Figure 4 and Figure S4.

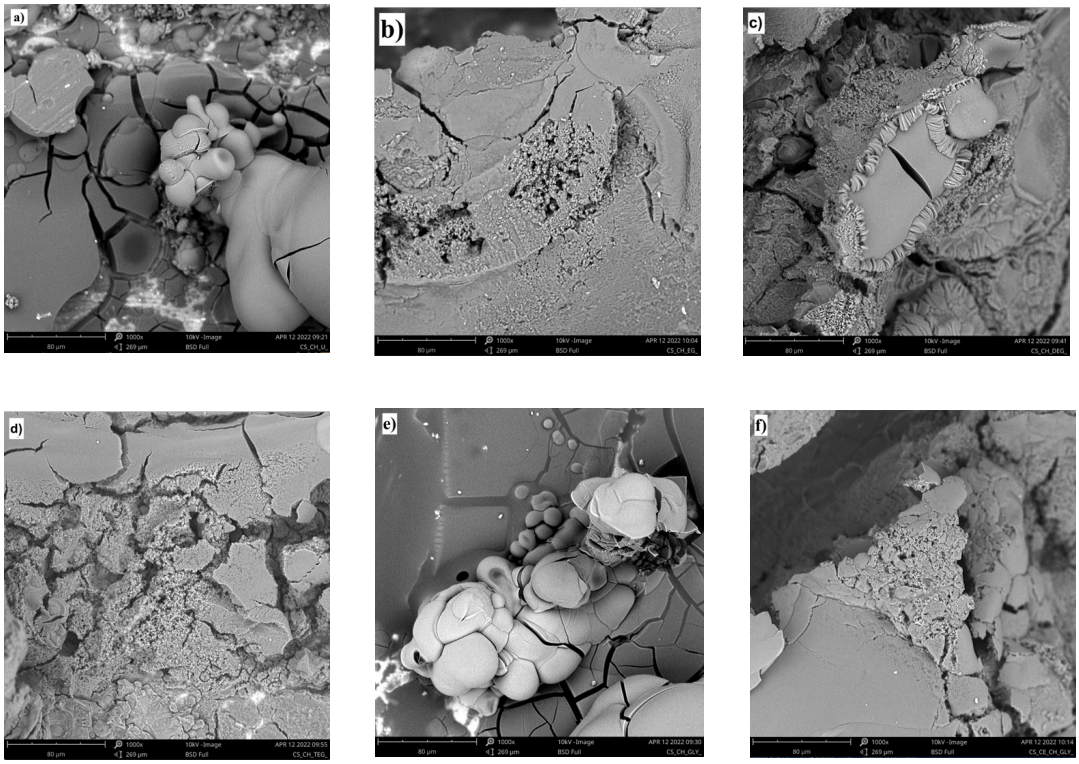

Figure 4. SEM images relevant to xerogels obtained by a) CS/[Ch][U], b) CS/[Ch][EG], c) CS/[Ch][DEG], d) CS/[Ch][TEG], e) CS/[Ch][Gly] and f) CS+CE (1:0.2)/[ChCl][Gly].

SEM images reported in Figure 4 show clearly that the morphology of the gels depend on the polymer and DES used. In particular, the CS/[ChCl][U] is constituted by the presence spheroidal structures (Fig. 4a and S4a), whereas changing the solvent to DES bearing glycols as HBD, like EG and TEG leads to the occurrence of a sponge-like morphology as can be seen in Figures 4b,d and S4b,d.

A similarly compact texture is also exhibited by the CS/[ChCl][DEG], which is also characterized by the occurrence of elongated structures (Fig. 3c and S4c). In all cases, the sample are characterized by a more compact texture compared with the CS/[ChCl][U] gel.

However, a different morphology is apparent for the CS/[ChCl][Gly] gel which, analogously with CS/[ChCl][U], features the occurrence of spheroidal aggregates (Figure 4e).

Comparing the morphology observed for CS+CE (1:0.2)/[ChCl][Gly] and CS/[ChCl][Gly], evidences a significant effect of the presence of cellulose in the gelling mixture. As a result, the

sample show a thick and compact sponge-like texture, instead of the spheroidal objects observed in the absence of cellulose (Figure 4f and S4e)

We then moved to assess the self-healing and load bearing ability of our gels. The self-healing ability, was evaluated for two selected gels, namely CS/[ChCl][DEG] (2 wt%), and CS+CE (1:0.4)/[ChCl][EG] (2 wt%). In both cases, we prepared two identical eutectogels, staining one of them with Rhodamine B. Then each gel was cut with a razor blade and each half (pristine and stained) was placed into contact, monitoring their appearance as a function of time. Pictures relevant to these experiments are reported in Figure 5 and S5.

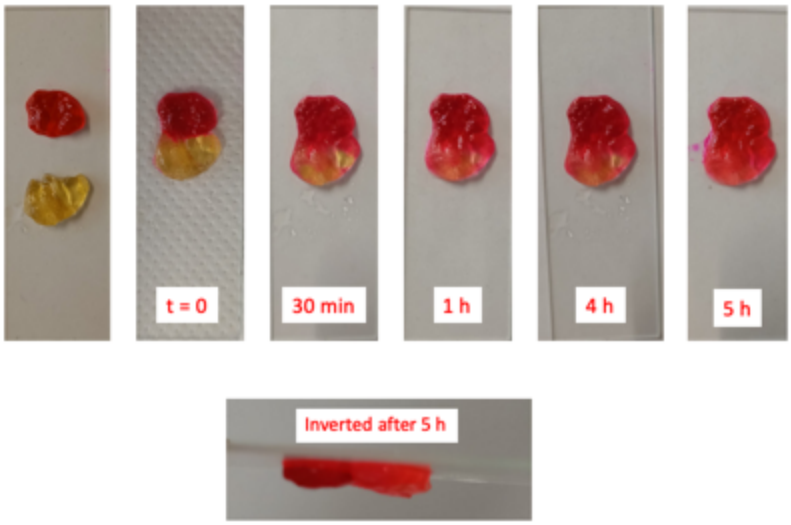

Figure 5. Pictures taken at different times relevant to the evaluation of self-healing ability of CS/[Ch][DEG] 2 wt%.

Pictures taken at different times reveal that both gels have significant self-healing ability. In particular, we observed either full diffusion of the dye through the other half, and no flow upon inverting the gel. The CS/[Ch][DEG] took only 5h to fully recover, while the CS+CE (1:0.4)/[ChCl][EG] required a longer time, 24 h.

To further explore the response to stimuli, we carried out a load-bearing test, subjecting 0.5 g of the same gels to increasing weights, ranging from 0.5 g to 20 g until observing their rupture. Pictures relevant to these experiments are reported in Figure 6 and S6.

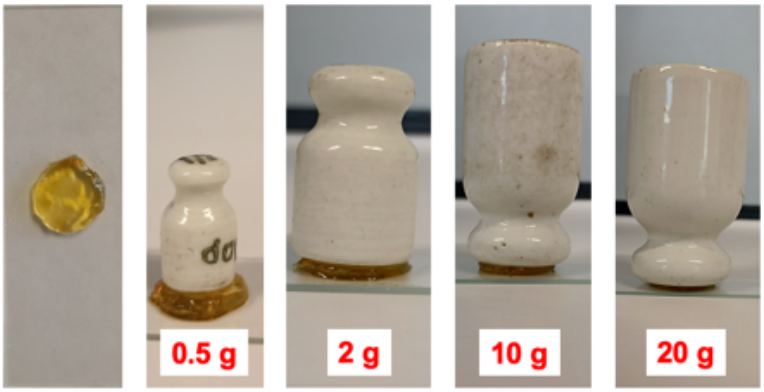

Figure 6. Pictures relevant to the evaluation of load bearing ability of CS/[Ch][DEG] (2 wt%).

As can be seen from the pictures reported in Figure 6, the CS/[Ch][DEG] (2 wt%), was able to withstand a weight of 20 g without breaking. On the other hand, the CS+CE (1:0.4)/[ChCl][EG] (2 wt%) was slightly less resistant, as it could withstand a weight of 10 g, but broke down when subjected to one of 20 g. This is in perfect agreement with the values of γ_c determined by rheological measurements and reported in Table 3, where γ_c = (220±30)% and (120±20)% for CS/[Ch][DEG] and CS+CE (1:0.4)/[ChCl][EG], respectively.

Finally, we wanted to test the injectability of our gels. To this aim, we placed 500 mg of gel in a 2 mL syringe and extruded the gel through the syringe end. We employed for this test CS/[Ch][DEG] (2 wt%) and CS+CE (1:0.4)/[ChCl][EG] (2 wt%) for their high value of γ_c (Table 3). Both gels were successfully extruded from the syringe and were injectable as can be seen from the pictures reported in Figure S7. Injectability of gels can be an important property from an applicative point of view, as demonstrated by the use of polymer gels in biomedical fields for drug delivery[46] and tissue repair.[47]

Antioxidant ability

The antioxidant ability of our gels and their components was assessed with the DPPH assay,[48, 49] in which the disappearance of a relatively stable radical, like the 2,2-diphenyl-1-picrylhydrazyl one, induced by an antioxidant species, can be easily followed spectrophotometrically. Firstly, we assayed the antioxidant ability of the DES taken alone. In particular, in these experiments, DES were used in the same amounts present in the gels, and the scavenging efficiency was determined after 1h and 24h. The results obtained after 1h are related to the initial rate of the scavenging process, while the ones obtained after 24h are indicative of the overall antioxidant ability. The results obtained are shown in Figure 7.

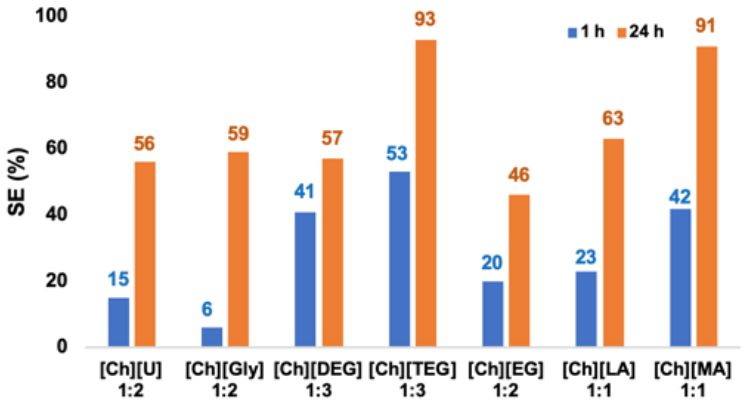

Figure 7. Scavenging efficiencies of DES from DPPH assay.

The results obtained show that all the DES considered display significant radical scavenging ability, depending on the nature of the components. In particular, after 1h, the antioxidant ability increases as: [Ch][Gly] < [Ch][U] < [Ch][EG] ≈ [Ch][LA] < [Ch][DEG] ≈ [Ch][MA] < [Ch][TEG]. Considering DES composed by glycols, the initial rate of scavenging appears favorably affected by the presence of oxymethylene units, whereas, in the case of carboxylic acids, the occurrence of a second carboxyl groups boosts the antioxidant ability. Such trend is essentially retained when we consider the radical scavenging efficiency found after 24 h, in which case it increases along the series [Ch][EG] < [Ch][U] ≈ [Ch][DEG] ≈ [Ch][Gly] < [Ch][LA] < [Ch][MA] ≈ [Ch][TEG]. Accordingly, once again, among DES composed by glycols, the scavenging efficiency increases with the number

of oxyethylene units, while the presence of a second carboxylic unit is favourable among DES with carboxylic acids. It is worth noting that, to date, no evidence is reported in the literature about whether the single components of DES display antioxidant ability. Furthermore, the dicarboxylic malic acid, like other organic acids, appears to exert no antioxidant ability when taken alone, but to give positive response to the DPPH assay only in the presence of ascorbic acid.[50, 51]

Subsequently, we determined the radical scavenging efficiency of the gels. In particular, we chose to carry out the DPPH test, for 24 h, using CS/[ChCl][U] (2 wt%), CS/[ChCl][EG] (2 wt%), CS/[ChCl][DEG] (2 wt%), CS/[ChCl][TEG] (2 wt%), CS/[ChCl][Gly] (2 wt%), CS/[ChCl][LA] (1 wt%), CS/[ChCl][MA] (1 wt%), CS+CE (1:0.4)/[ChCl][U] (2.5 wt%) and CS+CE (1:0.4)/[ChCl][Gly] (2 wt%). The results obtained are reported in Table 4.

Table 4 Radical scavenging efficiency towards DPPH of eutectogels, after 24 h.

Gel (wt%)	**SE (%)**
CS/[ChCl][U] (2 wt%)	-
CS/[ChCl][Gly] (2 wt%)	-
CS/[ChCl][EG] (2 wt%)	16
CS/[ChCl][DEG] (2 wt%),	21
CS/[ChCl][TEG] (2 wt%)	33
CS/[ChCl][LA] (1 wt%)	41
CS/[ChCl][MA] (1 wt%)	92
CS+CE (1:0.4)/[ChCl][U] (2.5 wt%)	-
CS+CE (1:0.4)/[ChCl][Gly] (2 wt%)	-

The results reported in Table 4 show a very different behavior of the gels compared to the relevant DES, showing that, with the only exception of CS/[ChCl][TEG], the scavenging efficiency is greatly

reduced upon gelation. Looking closer, we can divide the gels into three groups, namely I) gels with no significant scavenging efficiency, II) gels with limited efficiency and III) gels with average or high scavenging efficiency. The first group comprises gels obtained in [ChCl][U] and [ChCl][Gly], in which cases, the changing the polymers does not induce observable effects, as can be inferred by the results obtained both for CS and CS+CE-based composite. The second group is essentially constituted by gels obtained in glycol-based DES, for which SE% increases as CS/[ChCl][EG] < CS/[ChCl][DEG] < CS/[ChCl][TEG]. It is noteworthy that this trend is the same as the one observed for the DES alone, confirming the favorable effect exerted by a higher number of oxymethylene units. The lower magnitudes of SE%, found for CS/[ChCl][EG] and CS/[ChCl][DEG], compared with the ones of the relevant DES, suggests that gelation may slow down the removal of radical, in a substantially kinetic effect.

Similar comments can be made examining the gels belonging to the third group, CS/[ChCl][LA] and CS/[ChCl][MA], i.e. the ones obtained in carboxylic-acid based DES. Accordingly, the favourable effect on SE% exerted by the presence of a second carboxylic unit is retained, as evidence by the higher values found for CS/[ChCl][MA]. However, while in the case of CS/[ChCl][LA] the magnitude of SE% is lower than the one of the relevant DES, the opposite happens for CS/[ChCl][MA], which retained the same efficiency of the DES alone.

In summary, the whole of results obtained so far, clearly shows that gelation impacts on the radical scavenging process by slowing it down. The magnitude of such effect strongly depends on the nature of the HBD. More, specifically, HBD of DES with the lowest scavenging ability are the most effected, leading to gels with no significant scavenging ability. In turn, HBD of DES with a higher SE%, such as the glycol-based ones, are only partially affected, leading to gels with limited but appreciable scavenging efficiency. Finally, gels obtained in DES with even stronger antioxidant ability, such as the carboxylic acid-based ones, are least affected, with CS/[ChCl][MA] being more efficient than the relevant DES.

Based on these results, we further investigated the radical scavenging process by determining the trend of SE% as a function of time, in the most efficient gels, namely CS/[ChCl][MA] and CS/[ChCl][LA]. The results obtained are reported in Figure 8 and Table S1.

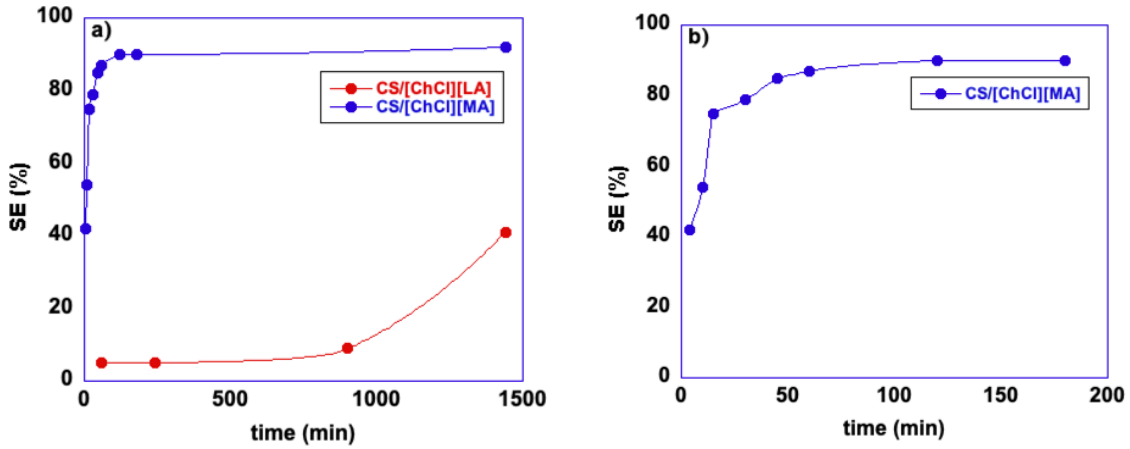

Figure 8. Scavenging efficiencies as a function of time for a) CS/[ChCl][MA] and CS/[ChCl][LA] eutectogels at 1 wt% and b) enlargement of the plot of CS/[ChCl][MA] for t ≤ 180 min. Lines are drawn as mere visual guide.

The trends of SE% as a function of time describe a significantly different behavior as a function of the carboxylic acid use as HBD. In particular, in the case of CS/[ChCl][MA], the scavenging process is relatively fast, with SE% reaching 42% after only 4 min. Such value continues to rise, until reaching a plateau, at 2h with almost complete removal of the radicals. On the other hand, the scavenging process is much slower in the presence of CS/[ChCl][LA], requiring 10h to observe an appreciable SE%, which reaches only 41% after 24h. It is therefore evident that the presence of a second carboxylic group boosts the radical scavenging process, confirming the hypothesis that this favorable effect is of kinetic nature.

To further investigate the influence of gelation on antioxidant ability, we went on to determine whether the gelation of the DES affects the radical scavenging ability of well-known natural

antioxidants like *trans*-ferulic acid (TA) and α-tocopherol (VE). These antioxidants were added in the amount of 1wt%, first in DES solutions, then in the gels. In this latter case, we prepared our gels in the presence of the same amount of antioxidant, obtaining two-component eutectogels. In both cases, we determined the SE% after 24h of contact. The results obtained when the antioxidants were dissolved in solution of DES are reported in Figure 9.

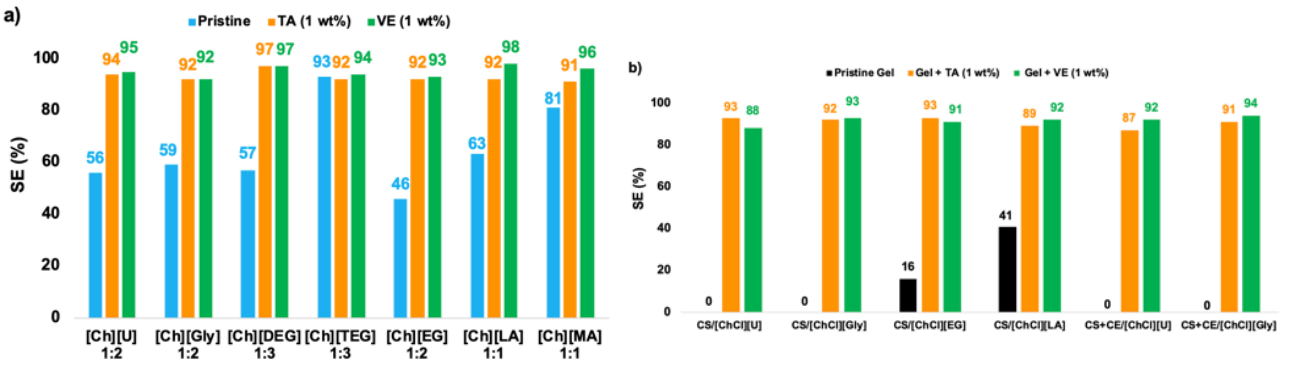

Figure 9. Scavenging efficiencies of a) DES and relevant solutions (1 wt%) of natural antioxidants and b) pristine and bi-component gels, after 24 h.

The results reported in Figure 9 show that both antioxidants maintain their high scavenging ability, with no significant influence of the DES uses as solvent. In most cases, the SE% is much higher than the one of the DES, with the notable exceptions of those DES that displayed high SE% when taken alone, as [Ch][TEG] and [Ch][MA]. This result indicates that dissolution in DES does not undermine the scavenging ability of the added antioxidant. The negative effect on antioxidant ability upon gelation only pertains to the DES, and it appears to be related to the change in state that undergoes on passing form the liquid state, to the gelled state in the presence of the polymers.

Then, we determined the SE% for the two-component gels containing the same amount of TA and VE, obtaining the results reported in Figure 9b. These clearly show that both TA and VE explicate their antioxidant action with the same efficiency both in the gel and solution with no significant

difference in SE% values. This means that the antioxidant ability of both TA and VE is preserved in the gel phase.

To obtain further support to this hypothesis, we determined the SE% as a function of time for the two-component gels, obtaining the results reported in Table S2, which clearly show an almost complete removal of the DPPH radicals after only 5 min.

Delving deeper into this aspect, we investigated how the radical scavenging efficiency of the bicomponent gels is affected by the amount of antioxidant added. To this aim, we determined the SE% for the bicomponent gels of CS/[ChCl][Gly] in the presence of increasing amounts of TA and VE, ranging from 0.25 wt% to 1 wt%. This investigation was carried out since it is reported in the literature that, natural antioxidant can display a pro-oxidant behavior at high concentrations.[52] The results obtained are reported in Figure 10a.

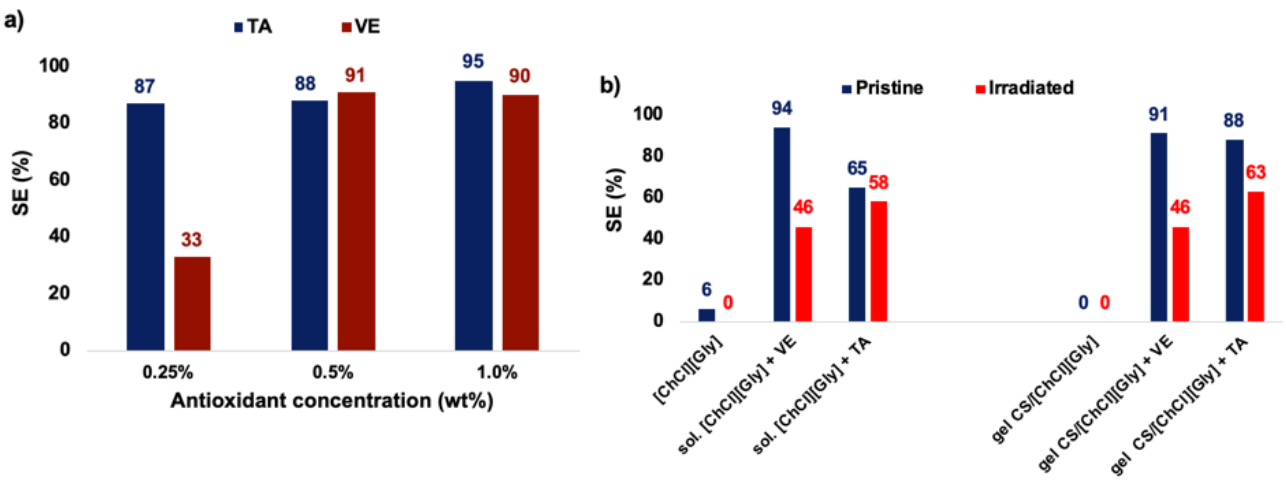

Figure 10. Scavenging efficiencies of bicomponent gels of a) CS/[ChCl][Gly] (2 wt%) at different concentrations of antioxidants, after 1h and b) solutions of antioxidants in DES and bicomponent gels before and after UV irradiation.

Notably, a different dependence on the antioxidant concentration can be found for the bicomponent gels containing TA or VE; for TA-doped gels, reducing the antioxidant concentration by 4 times, induces only a minor drop in SE% whereas it is much more significant for the VE-doped gel, for which SE% goes from 90% at 1 wt% of VE, to 33% at 0.25 wt% of VE. The higher antioxidant

efficiency of TA over VE has been reported in the literature in the case of cellulose derivatives or caseinate films.[53, 54]

An important parameter in assessing any antioxidant material, is their resistance and stability under exposure to solar light, due to the sensitivity of some antioxidants both to UV-irradiation and increase in temperature. To test the stability of our eutectogels, we determined the SE%, after exposure to UV light (313 nm) at 70 °C, for 1h, to simulate exposure to intense sunlight. For this experiment, we used the bicomponent gels CS/[ChCl][Gly] + TA (0.5 wt%) and gels CS/[ChCl][Gly] + VE (0.5 wt%). For a useful comparison, we also irradiated the relevant DES in the same way. The results obtained are summarized in Figure 10b. These shows that in general, the scavenging efficiency is reduced by irradiation. More specifically, this reduction is more pronounce for samples containing VE, in which cases SE% practically halved both in solution and in gel phase (from 94% to 46% in solution, and from 91% to 46% in gel phase, before and after irradiation, respectively). Conversely, the samples containing TA appears more resistant. In particular, in solution, SE% suffered only a minor reduction, passing from 65% to 58% upon irradiation. A more pronounced reduction is observed in gel phase, although the irradiated gel maintains a still good efficiency, amounting to 63%.

3.3 Obtainment of films

Frequent applications of antioxidant materials involve the use of films for protective packaging or coatings. For this reason, we prepared our gels in the form of films, and then assessed their antioxidant ability, both in the presence and in the absence of TA or VE. For this investigation, we chose the gel CS/[ChCl][Gly](2 wt%). In particular, the films were prepared by dissolving the polymer in aqueous acetic acid, then transferring the solution into a Petri dish, to which the suitable amounts of DES or antioxidant were added. A representative picture of a film is reported in Figure 11a. We determined the scavenging efficiency after 1h, for the films with and without antioxidant (TA or VE, 0.5 wt%) obtaining the results reported in Figure 11b.

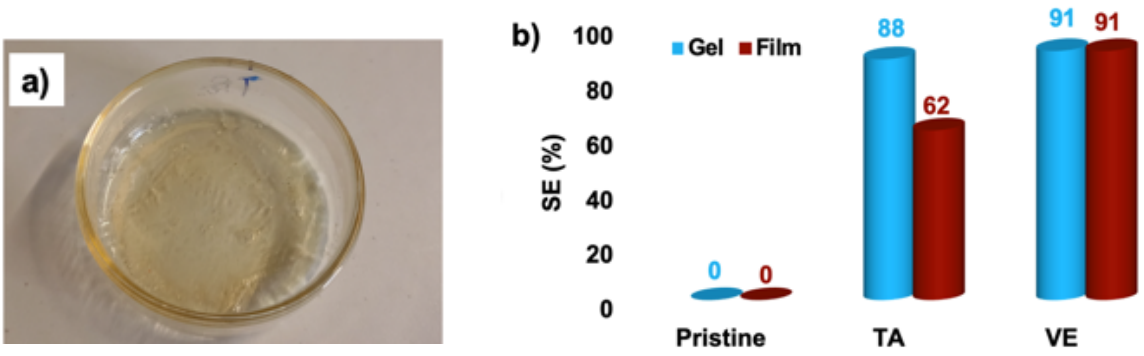

Figure 11. a) Representative picture of film and b) Scavenging efficiencies, after 1h of contact with DPPH solution, of gels and films of CS/[ChCl][Gly]

The results obtained shows that the film, like the relevant gel, does not show antioxidant ability, unless in the presence of added antioxidants. However, while in the presence of TA a slight reduction in scavenging efficiency was found, on going from the gel to the film (88% and 62%, respectively), in the presence of VE, the film retained full antioxidant ability of the gel, confirming the superior performance of the materials doped with VE, compared with those containing TA.

Furthermore, to better assess the performance of both gel and film to real-life conditions, we probed their resistance to contact with water, and also investigated if it induced any release of antioxidant.

To this aim, we used once again the bicomponent gels of CS/[ChCl][Gly] (2 wt%) and the relevant films. Firstly, we checked the integrity of both gel and film upon contacting 1g of each with 5 mL of water for 24h, at 25 °C, under static conditions. After this period, no visual change occurred, and both gels and films remained self-supporting after removing water (Figure S8). Then, we went on to evaluate the possible release of antioxidant from gels and film upon contact with water, over time. To this aim, we put in contact gel and film with water, and at suitable times we withdrew an aliquot of solution, which was replenished with the same amount of water, to avoid dilution effect. The amount of antioxidant released was then determined spectrophotometrically, based on a calibration curve previously obtained. It is worth noting that we could carry out these experiments only with gels and films containing TA (0.5 wt%), due to the insolubility of VE in water. The plot of the percent

released amount of TA as a function of time, for both CS/[ChCl][Gly] + TA gel and film, is reported in Figure 12, while the values of TA% released as a function of time are reported in Table S3.

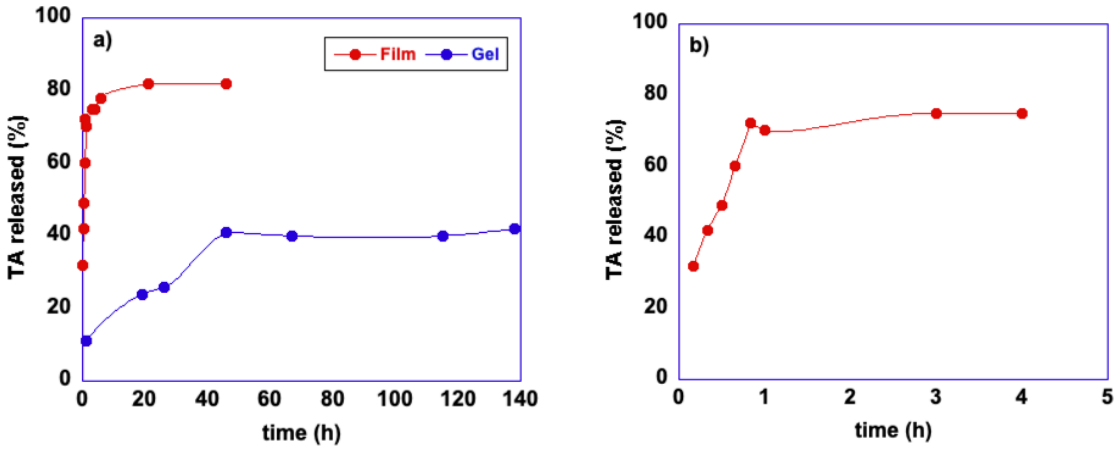

Figure 12. Plot of a) released amount of TA from CS/[ChCl][Gly] + 0.5 wt% TA gel and the relevant film and b) enlargement of the release profile from film. Lines are drawn as mere visual guides.

The results obtained show a very different behavior of the two materials since the film released in water 70% of TA after 1h, reaching a plateau value of 80% after 2h. Conversely, the gel was much more resistant to release of TA, which occurred more slowly, and involving a much lower amount of TA. In particular, the maximum amount of TA released from the gel was 41%, reached after 46h. This finding could be explained considering the larger contact area with water of the film compared to the gel, which favors diffusion of TA. In addition, the film is much thinner compared with the gel, so the TA molecules can diffuse into the aqueous phase, travelling a shorter distance, thus favoring the release of TA.

Based on these results, we wanted to know how the depletion in antioxidant of both gel and film, consequent from contact with water, affects their radical scavenging efficiency. To this aim, we determined the SE% of TA-depleted gel and film as well as of supernatant aqueous solutions of both materials. Also in this case, SE% was determined after 1h of contact, obtaining the results reported in Figure 13.

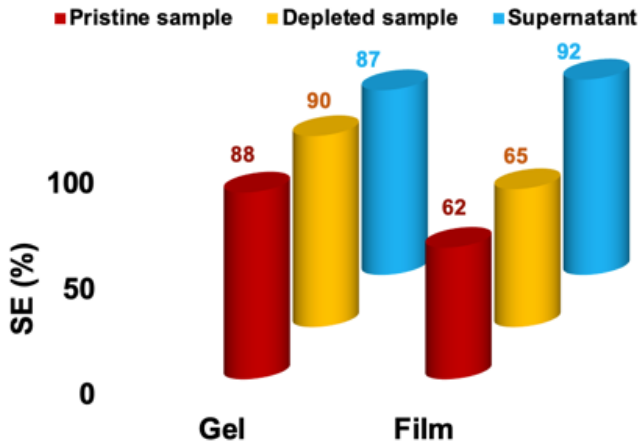

Figure 13. Scavenging efficiencies of pristine CS/[ChCl][Gly] + 0.5 wt% TA gel and the relevant film, of the same materials after TA release and of the aqueous supernatant solutions.

Notably, the results obtained show that both gel and film practically retained their radical scavenging efficiency even after the partial release of TA, entailing that their performance was not significantly diminished after being exposed to significant amounts of water. This is consistent with the constant efficiency of the TA-containing bicomponent gel in the presence of decreasing amounts of TA, as shown in Figure 10a. On the other hand, both the supernatant solutions show high efficiency, having dissolved a significant share of TA. In particular, while the supernatant in contact with the gel exhibited SE% comparable to this latter, in the case of the film, the aqueous solution outperformed the one of the pristine film. Such difference highlights that the radical scavenging capability of both materials is mainly dependent on the type of semi-solid matrix in which TA is incorporated, and only marginally from the TA concentration. A main advantage over the currently used strategies for the integration of antioxidants in polymer-based materials is that non-covalent grafting of antioxidants is required and the formation of biopolymer-based gels and films does not require any cross-linking agent, as is frequently the case for biopolymer-based gels in conventional solvents.

Conclusions

In this work, we obtained supramolecular eutectogels based on chitosan or chitosan:cellulose composites in cholinium-based DES. Characterization of the gels showed that the gelation propensity, as expressed by MGC, is favourably affected by the presence of a further carboxylic acid group, and unfavorably influenced by the number of oxyethylene groups in the HBD of the DES. Rheological measurements pointed out that the stiffness of the gels, expressed by the magnitude of storage modulus, is higher on increasing the number of hydrogen bond donating sites on the HBD, while the addition of cellulose to the gel matrix gives rise to more flexible materials. Furthermore, FTIR-ATR investigation allowed us to hypothesize that upon gelation cholinium cations interpose between polysaccharide chains, affecting the magnitude of interchain hydrogen bonding. Our gels also displayed self-healing and good load bearing ability, and also proved to be injectable.

We then evaluated the antioxidant activity of our gels, by the DPPH assay. Compared with the radical scavenging ability of the DES alone, in general the gels showed a lower efficiency, evidencing the favourable effect of the number of oxyethylene units in the HBD and, more importantly of a further carboxyl group in the HBD, which revealed to be mainly a kinetic effect.

The reduction of the antioxidant ability upon gelation involved only the DES, as shown by the high scavenging efficiency of bicomponent gels doped with small amounts (1 wt%) of natural antioxidants, like VE and TA. Employing CS/[ChCl][Gly] as a representative gel, we found out that although the VE-doped gel has a higher scavenging efficiency, the TA-based one exhibits less dependence on the concentration of antioxidant, and retains its efficiency also in the presence of lower concentration of TA. We then assessed the stability of the gels towards UV light irradiation, as a model for sunlight exposure, finding that the gels lose some of the antioxidant ability, and the TA-doped one is less affected.

We also prepared gel-based films, finding that the films, especially the VE-doped ones, substantially preserve the radical scavenging ability of the gels. Finally, we evaluated the stability of TA-doped gel and film to prolonged contact with significant volumes of water. We found that although the film releases more antioxidant compared to the gel, it nevertheless retains its antioxidant ability, proving

resistant to exposure to water. To the best of our knowledge, this is the first work describing the use of biopolymer-based supramolecular euctectogels as antioxidant materials which show potential to be applied as sustainable constituents for protective packaging or coatings.

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CRediT authorship contribution statement

Salvatore Marullo: writing, original draft, investigation; **Floriana Petta:** investigation; **Nadka T. Dintcheva and Giulia Infurna;** investigation: rheology, FTIR-ATR, UV-irradiation; **Francesca D'Anna:** conceptualization, writing, review and editing, supervision, funding acquisition.

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