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# Choline chloride based ionic liquid analogues as tool for the fabrication of agar films with improved mechanical properties

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ABSTRACT

In the present paper, we test the suitability of ChCl/urea (DES-U) and ChCl/glycerol (DES-G) eutectic mixtures, each one prepared at 1:2 molar ratio, for the production of agar films. A three-step process is proposed: pre-solubilization of polymer in DES followed by compression-molding and subsequent drying. The mechanical properties, water resistance and microstructure of the films were evaluated at different polymer concentrations (*i.e.* 2–6%, w/w). DES-U showed by far, the best film forming ability. Agreeing with the diffusion and SEM data, films with the best mechanical properties were found at the lowest and highest agar concentrations (tensile strengths of 24.2–42 MPa and elongations of 15.4–38.9%). The water sorption and contact angle studies suggested increased hydrophilicity for the film containing the lowest concentration of agar. The use of choline chloride based ionic liquid analogues as solvent and plasticizer might be a promising tool for the development of new non-aqueous materials based on seaweed polysaccharides.

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## 1. Introduction

Although undeniably needed in our daily-life, synthetic plastics are becoming too heavy of a burden for mankind to cope with. Problems such as global warming, damaged ecosystems, geopolitical conflicts and landfill depletion triggered the search for eco-friendly and sustainable materials at a global scale (Queiroz & Collares-Queiroz, 2009). In this scenario, the bio-plastic industry is developing rapidly and is expected to face unprecedented growth in the coming years. Currently, the bio-plastic sector is mainly driven by agro-based polymers particularly bio-based versions of synthetic plastics such as bio-PUR, bio-PE and bio-PET (Colwill, Wright, Rahimifard, & Clegg, 2012; Laird, 2012). Yet, with the need to move towards materials with integrated and more efficient life-cycles, these bio-based non-biodegradable plastics seem not to meet sustainability standards (Song, Murphy, Narayan, & Davies, 2009). In parallel, recent advances in bio-refineries and the need to move away from food feedstocks have opened new market opportunities for a new generation of bio-based and biodegradable plastics produced from algae and/or algae components (Rosenthal, 2013).

In this regard, bio-films from seaweed polysaccharides are seen as promising (Rinaudo, 2008). The potential of agars to fabricate bio-plastics have been previously explored, particularly aqueous blended films where water is used as a solvent, dispersion medium and plasticizing agent (Freile-Pelegrín et al., 2007; Giménez, de Lacey, Perez-Santin, Lopez-Caballero, & Montero, 2013; Phan The, Debeaufort, Luu, & Voilley, 2008; Phan The, Debeaufort, Voilley, & Luu, 2009a, 2009b; Rhim, 2011; Rhim, Lee, & Hong, 2011; Sousa, Sereno, Hilliou, & Gonçalves, 2010). These aqueous agar films, produced mostly by casting methods, show interesting features such as transparency, good mechanical resistance and heat seal ability (Phan The et al., 2009a; Sousa et al., 2010). However, their typical high moisture sensitivity and brittleness are still challenges to overcome. The incorporation of plasticizers such as glycerol is a common practice to increase the films' elasticity although this often implies significant losses in the mechanical resistance of the material or the plasticization of the matrix turns out to be insufficient (Sousa et al., 2010; Giménez et al., 2013).

In recent years, the use of deep eutectic solvents (DES) to replace traditional imidazolium-based ionic liquids (ILs) has received widespread attention by the scientific community (Zhang, Vigier, Royer, & Jerome, 2012). Besides exhibiting similar performances as conventional ILs, these emergent solvents are much cheaper, safer and easier to prepare than their counterparts; DES are generally

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obtained by mixing two cheap and safe components (*i.e.* salt and an H-bond donor) which form a eutectic mixture with a lower melting point than the individual compounds through hydrogen bonding. Owing to its renewable and non-toxic character, the quaternary ammonium salt (2-hydroxyethyl)trimethylammonium chloride (choline chloride, ChCl) is used at times to fabricate a broad spectrum of DES. One of the most promising applications for DES is as plasticizing agent for the fabrication of bio-plastics (Abbott, Ballantyne, Conde, Ryder, & Wise, 2012; Leroy et al., 2012; Shamsuri & Daik, 2012). Particularly, the eutectic mixtures ChCl/urea (DES-U) and ChCl/glycerol (DES-G) at a molar ratio of 1:2 were found to efficiently plasticize processed starch and starch/zein blends (Abbott et al., 2012; Leroy et al., 2012). Shamsuri and Daik (2012) tested the potential of DES-U as a plasticizer in aqueous agarose films. However, the low elongations reported by these authors seemed to suggest an insufficient plasticization of the agarose films. A possible reason could be the rupture of the original supramolecular complexes that compose the eutectic mixture upon DES dilution in water (Nardecchia et al., 2012).

The present research examined producing strong and elastic agar films, at low polymer concentrations, using deep eutectic mixtures (namely, DES-U and DES-G). To achieve this goal, we propose using a different approach for these eutectics by combining solvent and plasticizer functions. A three-step process was outlined to obtain the agar/DES films: pre-solubilization of the polymer in DES followed by compression-molding and subsequent slow drying in ethanol and in air. The mechanical properties, water resistance (water sorption and water diffusion) and microstructure (SEM) of the agar/DES films were evaluated at different agar concentrations (2–6%, w/w). The surface properties of the films were studied by contact angle measurements following the static sessile drop method.

## 2. Materials and methods

### 2.1. Chemicals

Urea (>99%: $CH_4N_2O$), choline chloride (>98%: $C_5H_{14}ClNO$) and anhydrous glycerol (>99.5%: $C_3H_8O_3$) were purchased from Sigma–Aldrich. Commercial agar used in the experiments was also from Sigma–Aldrich Co. (A-7002, St. Louis, MO, $(C_{12}H_{18}O_9)_n$).

### 2.2. DES preparation

Deep eutectic solvents (DES) were prepared by mixing the choline chloride, dried at 70 °C overnight in an oven, with the appropriate amount of H-bond donors *i.e.* urea and/or anhydrous glycerol at 1:2 molar ratios (Fernandes, Campiña, Pereira, & Silva, 2012). The mixtures were heated at 70 °C under stirring until formation of a homogeneous liquid and subsequently left to cool slowly to 25 °C and kept at that temperature (above the solvent's freezing point). For simplification, the shorthand notation DES-G and DES-U was adopted throughout the manuscript for respectively, ChCl/glycerol and ChCl/urea eutectic mixtures.

### 2.3. Fabrication of agar/DES films

#### 2.3.1. Pre-solubilization of agar in DES

The appropriate amount of agar (previously dried overnight in a vacuum oven at 60 °C) and DES were weighed according to the desired final concentration (*i.e.* 2–6%, w/w). A pre-solubilization of the agar/DES mixtures was carried out under stirring at 140–150 and 120–130 °C for, respectively, agar/DES-G and agar/DES-U mixtures in closed cap vials using an oil bath. The concentration range and the choice of dissolution temperatures were chosen based on preliminary tests.

#### 2.3.2. Thermo-compression

Approximately 10 g of the hot agar/DES mixture, prepared as described in Section 2.3.1, was placed in a square mold (10 cm × 10 cm) between two Teflon plates covered with aluminum foil and thermo-compressed in a hydraulic press (Carver laboratory press, model B, Fred S. Carver Inc., New Jersey, USA) at 140 °C, 24.5 kN for 20 min. The agar/DES system was left to cool to room temperature for 1 h in the press with the load still applied, after which it was left overnight at room temperature. The drying of the films was then accomplished by immersing the polymer sheet in ethanol with several changes for 16 h and subsequent drying in air overnight. Finally, the films were stored at controlled environmental conditions (53% relative humidity (R.H.) and room temperature) to carry out the experiments. A shorthand notation was adopted for the different films; for instance, agar film prepared in DES-U at 2% (w/w) polymer concentration will be designated herein, A2/DES-U while films obtained from 5% (w/w) agar addition in DES-G will be termed A5/DES-G.

### 2.4. Characterization of agar/DES films

#### 2.4.1. Scanning Electron Microscopy (SEM)

SEM studies were carried out in a Quanta 200 FEG scanning electron microscope (SEM, FEI, Hillsboro, OR). Prior to analysis, film samples were cryo-fractured in liquid $N_2$ and mounted with carbon adhesive tabs (Electron Microscopy Sciences, Hatfield, PA) to specimen stubs, and the edge was painted with colloidal silver adhesive. Afterwards, the samples were coated with a thin film of gold and imaged in the high-vacuum/secondary electron imaging mode scanning electron microscope using an accelerating voltage of 10 kV and working distances of 12.5 mm.

#### 2.4.2. Thickness measurements

The thickness of the films was measured using a B.C. Ames comparator, Model 70-1355, Framingham, MA. At least, five measurements were performed in each case.

#### 2.4.3. Mechanical properties

The mechanical properties (tensile strength, *TS*; Young's modulus, *YM* and elongation, *E*) of the films were measured using a MTS Insight 5 mechanical property tester, equipped with Testworks 4 data acquisition software (MTS Systems Corp., Minneapolis, MN) (Liu, Liu, Fishman, & Hicks, 2007). Film specimens with dimensions of 10 mm × 50 mm were tested after being stored at 50% relative humidity (R.H.) and 25 °C for at least 48 h. A 50 mm/min strain rate and a gauge length of 20 mm were used in all experiments. Five replicates were performed in each case.

#### 2.4.4. Sorption isotherms

Water sorption isotherms were obtained gravimetrically as described in Souza, Campina, Sousa, Silva, & Gonçalves (2013). Film samples with dimensions approximately 25 mm × 25 mm, previously dried in a vacuum oven at 50 °C for 48 h, were placed in hermetic chambers with different controlled water activities ($a_w$ in the range 0.11–0.84), and stored at room temperature. The samples were considered at equilibrium when they reached a constant weight. The water sorption isotherm of a material is defined as the relation between the equilibrium water activity ($a_w$) and the corresponding moisture content (dry basis) of the sample at a given temperature. Several mathematical models are used to fit the water sorption data and provide additional thermodynamic information about the studied systems. The well-known Guggenheim–Anderson–de Boer (GAB) model (Anderson, 1946;

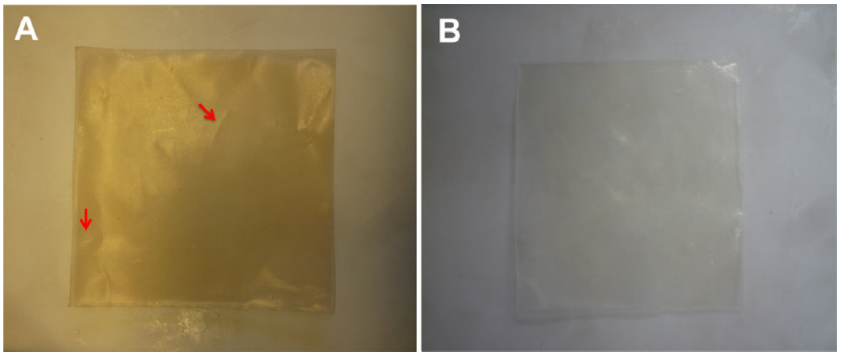


**Fig. 1.** Agar/DES-G (A) and agar/DES-U (B) films at 5% (w/w) polymer concentration after immersion in ethanol. Arrows indicate small fissures occasionally seen on the agar/DES-G films.

de Boer, 1968), expressed bellow (Eq. (1)), was used to fit the experimental sorption data,

$$X_e = \frac{(C \cdot k \cdot X_0 \cdot a_W)}{([1 - k \cdot a_W] \cdot [1 - k \cdot a_W + C \cdot k \cdot a_W])} \quad (1)$$

where $X_0$ represents the monolayer moisture content, also on dry basis, $C$ is a constant dependent on the water sorption heat in the first layer (*i.e.* monolayer) and subsequent layers (*i.e.* multilayers), $k$ is a parameter reflecting the sorption heat of the multilayer as well as the heat of condensation of water vapor. This model is commonly used since it is known to successfully fit equilibrium sorption data in a broad range of $a_w$. Duplicate measurements were performed for each film formulation to check the reproducibility of the tests.

#### 2.4.5. Contact angle measurements

A contact angle Automated Goniometer (Ramé-hart Instrument Co., Succasunna, NJ, USA) equipped with a high resolution camera (Model 590 F4 Series) was used to determine the hydrophilicity and wettability of the agar/DES films. These two properties can be correlated with the angle formed between the film surface and a tangent drawn on the water droplet surface that passes through the intersection point of the air-water-film phases. Experiments were carried out in air through static sessile drop measurements using ultrapure water as the liquid phase ($11 \pm 0.6$ μL drops). The distance between needle-tip and film surface (0.5 cm) was kept constant to ensure consistency between the different measurements. Due to the hydrophilic nature of the studied films, reported angles were recorded after 10 s to avoid as much as possible penetration of water in the films (*i.e.* absorption phenomena). Determination of the contact angle ($\Theta$), volume ($V$) and surface area ($A$) of the droplets were performed using Dropimage Advanced v2.5 software. These parameters, related to the shape of the water droplet, were used to detect possible contributions of absorption or spreading phenomena at the surface/liquid interface for each of the studied films (Farris et al., 2011). An additional commercial LDPE sample, of known hydrophobic character, was used as a reference. The results presented in Table 2 are averages of five to ten water drops deposited on each film surface.

#### 2.4.6. Water vapor permeability (WVP)

WVP determinations were carried out according to the ASTM E 96-00 (Standard Test Methods for Water Vapor Transmission of Materials). Film samples 5 cm in diameter were tightly sealed, using Beeswax, to a permeation cell containing an anhydrous salt (calcium chloride, R.H. = 2%) and left to equilibrate at 25 °C and 50% R.H. for at least 48 h. The cells were placed in a test chamber with a controlled temperature (25 °C) and R.H. (100%). Air convection (approx. 0.3 m/s) ensured uniform conditions throughout the chamber. The permeation cells were weighed periodically in order to obtain the permeability of each film to water vapor (*WVP*; $g\,m^{-1}\,s^{-1}\,Pa^{-1}$), from the following equation (Eq. (2)):

$$WVP = \frac{(\Delta m \cdot x)}{(A \cdot \Delta t \cdot \Delta P)} \quad (2)$$

where $\Delta m$ is the weight gain (g) for the test cell, $x$ is the film thickness (m), and $A$ is the area (0.00196 $m^2$) exposed during a time $\Delta t$ (s) to the partial water vapor pressure $\Delta P$ (Pa).

### 2.5. Statistical analysis

The statistical significance of each experiment was examined using the Student's $t$-test. The significance level was set at $p < 0.01$. A non-linear estimation was performed using *Statistica 8.0* software (StatSoft, Tulsa, OK, USA) to fit the experimental sorption data to Eq. (1) and obtain the correspondent GAB parameters.

## 3. Results and discussion

### 3.1. Preparation of agar/DES films

The protocol to fabricate agar/DES films was adopted from preliminary tests and consisted of a three-step process. Taking into consideration the specifications of the heat press and to prevent polysaccharide degradation, solubilization of the agar in either DES-G or DES-U was carried out in closed cap vials before the thermo-compression process. The samples were slowly cooled down to room temperature and left to set overnight. After this intermediate step, films free of air bubbles and with regular thickness were obtained (Fig. 1). Finally, DES removal was accomplished by immersing the films in ethanol and subsequently drying in air.

### 3.2. Films appearance

There were significant differences observed in the two eutectic films (agar/DES-G and agar/DES-U). In general, DES-G exhibited poor film forming ability. Only the A5/DES-G film showed some consistency after drying (Fig. 1A) while the remaining concentrations resulted in fragmented films. This suggests there is a critical polymer concentration for the formation of a cohesive agar/DES-G matrix. However upon storage, A5/DES-G showed marked sensitivity to environmental conditions developing unpleasant smells, changing color and shrinking. This could be attributed to some thermal degradation of the polymer during film formation. It should be emphasized that several protocols and conditions were tested for the films' preparation yet, none of them proved to be more successful.

In contrast, agar/DES-U films were very consistent, uniform, easy to handle and stable upon storage at any polymer

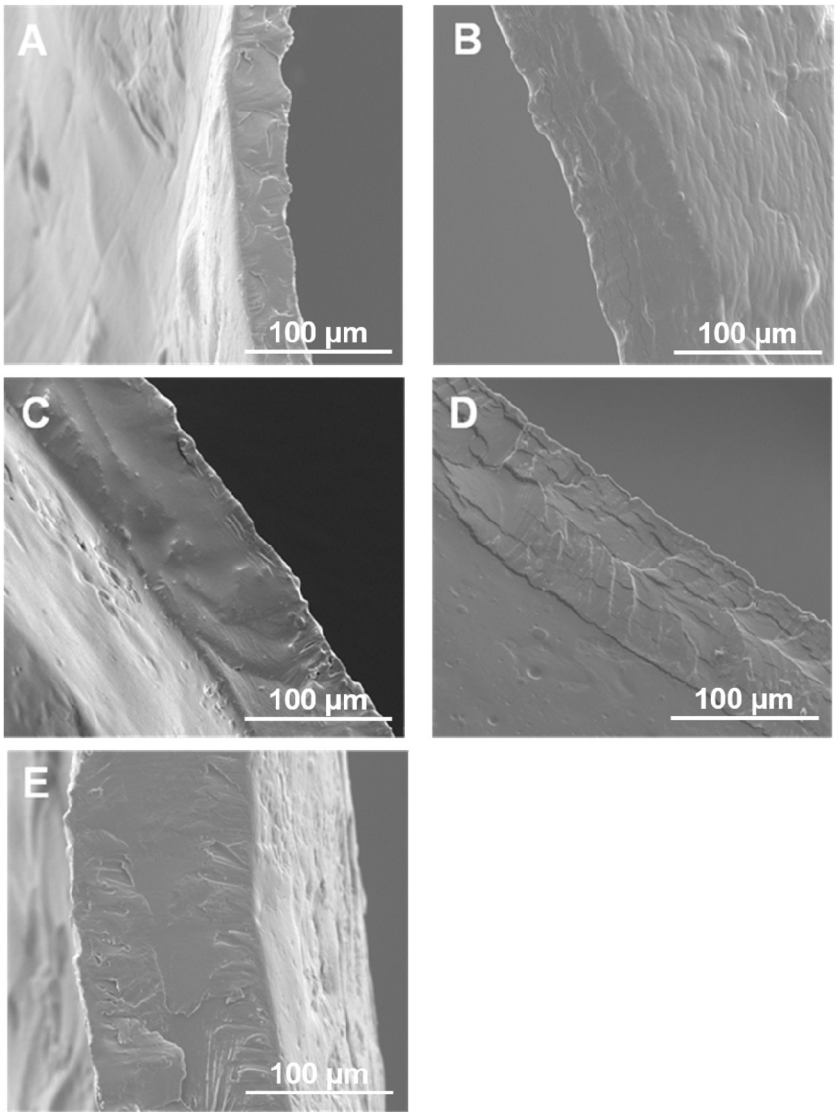


**Fig. 2.** Representative SEM pictures of the cross-sections of cryo-fractured films at 1000× magnification (A- A2/DES-U; B- A3/DES-U; C- A4/DES-U; D- A5/DES-U; E- A6/DES-U). The accelerating voltage was 10 kV and working-distances 12.5 mm in all cases.

concentration (Fig. 1B). Therefore, only agar/DES-U films are examined hereafter due to the consistent nature of their film forming ability.

### 3.3. Films morphology

Cross sections of the cryo-fractured agar/DES-U films observed by SEM are represented in Figs. 2 and 3. Some irregularities could be detected on the surface of the cross-sections, however due to the cryo-fracture method, they were disregarded. More compact and homogeneous microstructures were observed for films with the lowest (A2/DES-U; Figs. 2A and 3A) and highest (A6/DES-U; Fig. 2E) agar concentrations. This agrees with previous SEM studies on agar aqueous films plasticized with glycerol and the formation of compact and dense 3D networks upon gel formation (Phan, Debeaufort, Luu, & Voilley, 2005). It is broadly known that in aqueous media, agar gelation leads to 3D networks through *double helix* formation followed by intensive interhelical association (Sousa, Borges, Silva, Cabrita, et al., 2013; Sousa, Borges, Silva, & Gonçalves, 2013). A perfect helices arrangement is supported by strong H-bonds between the polymeric chains and additional H-bonding is expected to occur between water molecules and the agar chains. Agar in DES-U could follow this same mechanism as seen by the cohesiveness of the matrices, particularly for the films containing the lowest and highest concentrations of agar.

Films prepared with agar concentrations in the range 3–5% (w/w) revealed less cohesive matrices (Fig. 2B–D) compared to the 2 and 6% (w/w) samples (respectively, Fig. 2A and E). Differences between the fractured surfaces can be seen, especially in Fig. 2B and D, although cryo-fractured, the surfaces indicate more of a ductile fracture indicated by the high ridges in the fracture surface. The small ridges in Fig. 2A and E indicate more of a brittle structure with a sheet like pattern. At higher magnifications (Fig. 3), these differences became even more evident (*e.g.* A2-DES-U in Fig. 3A and A5-DES-U in Fig. 3B). Although it cannot be neglected that the high vacuum conditions and the electron beam used for the SEM experiments could account for the formation of some of these defects (particularly at higher magnifications; Phan The et al., 2009a), the other measured properties correlated with the microscopic data. The presence of defects (*e.g.* pores and/or voids caused by air and cracks) in the polymeric matrix is known to significantly affect the films' final properties (Duncan, Urquhart, & Roberts, 2005).

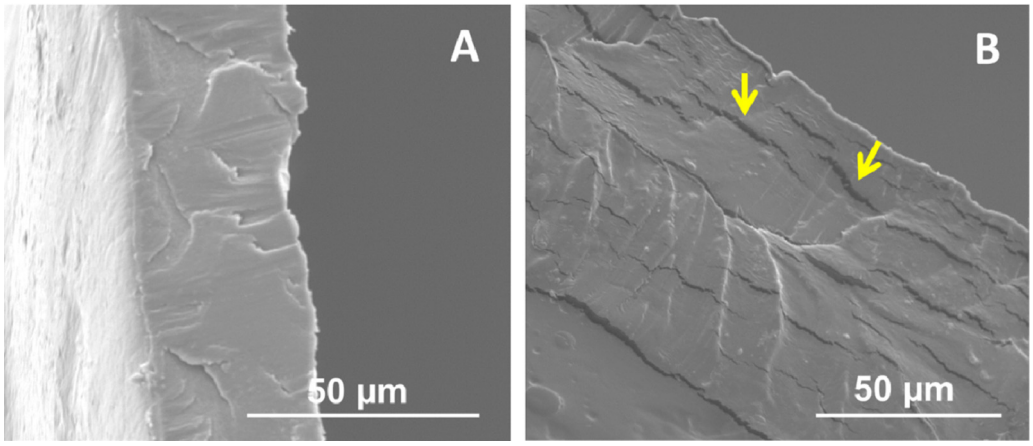


**Fig. 3.** Representative SEM pictures of the cross-sections of cryo-fractured A2/DES-U (A) and A5/DES-U (B) films at higher magnifications (2500× and 2000×, respectively). The accelerating voltage was 10 kV and working-distances 9.4 mm in both cases. Examples of cracks are indicated with arrows.

### 3.4. Thickness and mechanical properties

The mechanical properties of agar/DES-U films prepared with different polymer concentrations are presented in Table 1.

In general, an increase in polymer concentration led to an increase in thickness (Cerqueira, Souza, Teixeira, & Vicente, 2013) particularly, for the A2/DES-U and A3/DES-U films (respectively, ~0.05 and 0.09 mm). At higher polymer contents (>4%, w/w) however, the differences in thickness could be considered neglectable (~0.11 mm) as it can be confirmed from the SEM images (Fig. 2A–E).

Tensile strength, *TS*, is defined as the maximum stress the material withstands in the moment of fracture while the Young's modulus, *YM*, is the tangent of the slope in the linear region of the stress-strain curve and is used to evaluate the films' stiffness (Finkenstadt, Liu, Cooke, Liu, & Willett, 2008). The highest values of *TS* and *YM* were observed for A6/DES-U, respectively 24.2–33.6 and 647–861 MPa. This is consistent with higher polymer concentrations yielding stronger and more compact networks (Cerqueira et al., 2013). However, we also found that the film with lowest concentration of agar had good mechanical properties (*TS* of 26.6–42 MPa and *YM* of 503–809 MPa). These concentrations showed the least sheet like microstructure in the SEM (respectively, Figs. 2A, 3A and 2E). Between these two concentrations, the films exhibited poor mechanical properties with the minimum values of *TS* (~3.83 MPa) and *YM* (~13.4 MPa) being observed at 3% (w/w) agar concentration. This is significant and may come from the interplay between agar and DES-U upon partial hydration of the films (Nardecchia et al., 2012). In fact, the films' exposure to different environmental conditions during and after the drying stage (particularly different R.H.) could lead to different molecular reorganizations, consequently affecting the measured properties. Hence, this aspect should be regarded and taken into consideration for future work.

**Table 1**
Film thickness (*d*) and mechanical properties (tensile strength, *TS*; elongation at break, *E* and Young's modulus, *YM*) for agar/DES-U films prepared at different polymer concentration (*C*).

| | *d* (mm) | *TS* (MPa) | *E* (%) | *YM* (MPa) |
|---|---|---|---|---|
| A2/DES-U | $0.05 \pm 0.01^{a}$ | $34.3 \pm 7.7^{a}$ | $25.3 \pm 9.9^{a}$ | $656 \pm 153^{a}$ |
| A3/DES-U | $0.09 \pm 0.00^{b}$ | $3.83 \pm 0.39^{b}$ | $74.1 \pm 5.8^{b}$ | $13.4 \pm 3.29^{b}$ |
| A4/DES-U | $0.11 \pm 0.01^{c}$ | $9.49 \pm 1.24^{c}$ | $31.2 \pm 1.2^{a,c}$ | $179 \pm 31.7^{c}$ |
| A5/DES-U | $0.10 \pm 0.00^{c}$ | $7.26 \pm 0.67^{c}$ | $59.2 \pm 9.4^{a,b}$ | $55.2 \pm 12.6^{d}$ |
| A6/DES-U | $0.12 \pm 0.02^{c}$ | $28.9 \pm 4.7^{a}$ | $29.1 \pm 9.8^{a,c}$ | $754 \pm 107^{a}$ |

Means in the same column with different letters are significantly different ($p < 0.01$).

To quantify the films' elasticity when subjected to a given stress we measured the elongation, *E*, of the film (Liu et al., 2007). As shown in Table 1, agar/DES-U films with good elasticity were obtained regardless of the polymer concentration. Overall, an inverse correlation between *TS* and *E* data was observed. Accordingly, the formulations with the best *TS* (*i.e.* A2/DES-U and A6/DES-U) revealed the lowest *E* (in the range 15.4–38.9%) while A3/DES-U was the most elastic film (~74%). A4/DES-U, however, did not follow this trend and showed comparable *E* to the films prepared at lowest and highest concentrations of agar, despite having significantly lower *TS* (Table 1).

Considering typical mechanical data reported by previous investigations for aqueous agar films, the obtained results seem quite promising. For instance, Phan et al. (2005) found comparable *TS* values (~42 MPa) for aqueous films at 3% (w/w) agar concentration loaded with 15% (w/w) glycerol yet, the *E* were significantly lower (~6.51%). More elastic films (~62%) were attained by Giménez et al. (2013) when adding 66% (w/w) glycerol to 1.5% (w/w) agar aqueous solutions however the *TS* was clearly below (~18.5 MPa) the values obtained in the present study (*i.e.* 24.2–42 MPa). In the work carried out by Shamsuri and Daik, the addition of DES-U to aqueous agarose solutions (2.75%, w/w) led to an increase in the films' elasticity (from ~3 to 5.5%) and a decrease in *TS* (from 66.7 to 6.1 MPa) (Shamsuri & Daik, 2012) up to 60% DES-U loading. The reported *E* values seem to indicate an insufficient plasticization of the films probably caused by significant hydration promoting the rupture of the molecular complexes composing the eutectics, upon DES dilution in water (Nardecchia et al., 2012). The properties of the tested agar/DES-U films were also comparable to films of other biopolymers such as pectin or pectin/protein composites (Liu et al., 2007). Films made from lignocellulosic materials and conventional imidazole-based ILs showed significantly lower *YM* (~26–42 MPa) and *E* (~1–4%; Abdulkhani, Marvast, Ashori, & Karimi, 2013). In the particular case of poplar wood-based films, the *TS* (18 MPa) was clearly below the highest values reported here. By comparison with commercial materials such as polyvinyl chloride (35 MPa; Callister, 2000), polystyrene (55 MPa; Callister, 2000) or low density polyethylene (9–17 MPa; Briston, 1986) the fabricated films appear to possess good mechanical resistance while attaining a high elasticity comparable to cellophane (16–60%; Phan et al., 2005).

### 3.5. Water sorption studies

Moisture sorption studies were performed at 25 °C and used to evaluate the moisture sensitivity of agar/DES-U films over a broad range of $a_w$. This information is crucial for the development

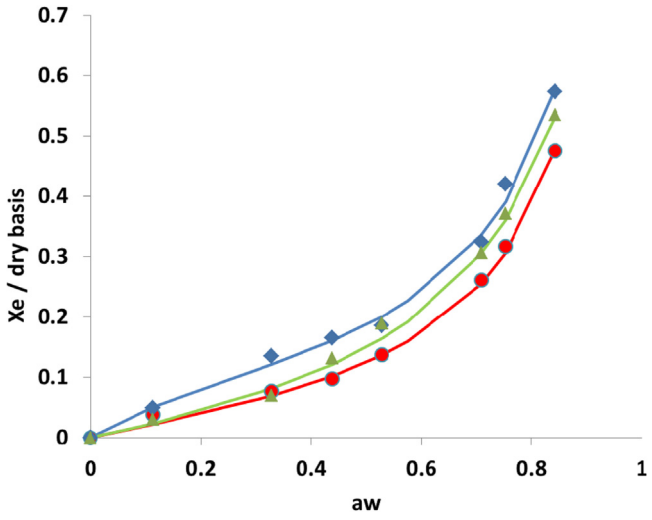


**Fig. 4.** Experimental sorption data (*i.e.* equilibrium moisture content, $X_e$ *vs* water activity, $a_w$) and respective fit using the GAB model (Eq. (1)) for the 2/DES-U (blue squares and line), 3/DES-U (green triangles and line) and 6/DES-U (red dots and line) films. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

of highly functional materials that can perform under different environmental conditions. Based on thermodynamic principles, these determinations allow a better insight of the water sorption phenomenon at the primary sites of the polymeric material (*i.e.* monolayer) when equilibrium is reached, as well as energetic requirements related to differences in the chemical potential of water molecules at the monolayer and upper layers (Souza et al., 2013; Torres, Moreira, Chenlo, & Vasquez, 2013).

Experimental data was fitted to the GAB equation (Eq. (1)). The moisture sorption data as well as respective fittings are represented in Fig. 4. For clarity purposes only the results for A2/DES-U, A3/DES-U and A6/DES-U are plotted in Fig. 4, and all of the samples estimated parameters according to the proposed model are listed in Table 2.

A distinct sorption profile is observed in Fig. 4 for the A2/DES-U film. The sigmoid shape of A2/DES-U curve was more evident, and is commonly observed for aqueous agar/glycerol films (Phan et al., 2005; Phan The et al., 2009a; Sousa et al., 2010). This could be confirmed by the estimated GAB parameters; except for A2/DES-U, low $C$ values (<2) were estimated in all cases, indicating that the applicability of the GAB model to these systems could be restricted (Lewicki, 1997). However, the high correlation coefficients ($R^2 > 0.98$) and $k$ values ($0 < k < 1$) suggested that the model adequately fit the experimental data. For the range of low $a_w$, the equilibrium moisture content ($X_e$) was low and increased almost linearly with an increase of $a_w$. At $a_w = 0.11$ low $X_e$ were observed, with A2/DES-U reaching a higher $X_e$ (~0.05) when compared to the other films ($X_e \sim 0.03$). For higher $a_w$ however, the increase in $X_e$ became much more significant; a sharp rise in $X_e$ seemed to occur around $a_w = 0.60$ and near the saturation point ($a_w = 0.85$) the $X_e$ reached values as high as ~0.48–0.53 or even higher (~0.57 for A2/DES-U; Fig. 4). Also worth noting is that at the humidity conditions at which the mechanical tests were performed (50%), A2/DES-U and A6/DES-U showed distinct water uptakes ($X_e \sim 0.185$ against ~0.137).

The amount of water retained at the primary sorption sites of the polymeric films was quantified on a dry basis by $X_0$ (Table 2). This parameter gives a good indication of the best working range of $a_w$ for a given material (Moreira, Chenlo, Torres, & Vallejo, 2008). In general, agar/DES-U films showed higher $X_0$ (in the range 0.10–0.15) than typical agar aqueous films (~0.04–0.08; Phan et al., 2005; Phan The et al., 2009a, 2009b; Sousa et al., 2010) suggesting enhanced water uptake capacity of the produced materials in the monolayer, at equilibrium conditions. Also, the $X_0$ was higher for the agar/DES-U films prepared with lower polymer contents. The decrease in $X_0$ can be explained by a reduction in the number of active sites for water sorption due to chemical and physical changes induced by differences in the polymer concentration as well as DES amount in the films.

$C$ was found to be higher in A2/DES-U (Table 2). This means that the sorption of water by this film was characterized by a monolayer of molecules, which were strongly bounded to the material (higher $C$). Also, this value reflects that subsequent molecules were only slightly or were not structured in the upper layers. In contrast, the remaining film formulations presented lower $C$ values which could suggest that the water molecules were not so strongly bounded to the material at the primary sorption sites while a more structured multilayer should be expected. However, due to possible restrictions in the application of the GAB equation no further physical interpretations of the estimated parameters were attempted.

### 3.6. Contact angle measurements

Understanding the behavior of the films when in contact with liquids is of particular interest when designing new materials. In the case of hydrophilic biopolymers such as agar, this information becomes even more relevant since the material should be able to perform according to the desired end-use which most of the time implies having some resistance to water (*i.e.* hydrophobicity).

The contact angle ($\Theta$) and contact angle variation ($\Delta\Theta$) as well as variations of the droplet volume ($\Delta V$) and droplet surface area ($\Delta A$) for the agar/DES-U films are given in Table 2. The profiles of the water droplets upon contact with the surface of the films were recorded at 25 °C over 10 s. Since the contact angle theory is based on the idea of a true equilibrium between the solid, liquid and vapor interfaces, and in this particular case of hydrophilic biopolymers, these conditions are in reality impossible to achieve, and therefore a short temporal window was chosen to perform the analysis (Farris et al., 2011). Also, to eliminate overestimations in the measured data caused by surface roughness, films were kept flat using special grips and a large number of replications were carried out for each sample in order to obtain reliable data. As expected, the commercial LDPE sample used as a reference had the highest contact angle ($93 \pm 2°$) and the least amount of significant changes in the droplet parameters (*i.e.* lower $\Delta\Theta$, $\Delta V$ and $\Delta A$). This confirmed the hydrophobic nature of this synthetic material. For the tested agar/DES-U films, an overall inverse correlation could be established between the contact angle and the polymer concentration. The obtained $\Theta$ were within the range of values reported by other authors when studying aqueous agar films plasticized with glycerol (Rhim, 2011; Phan The et al., 2009a, 2009b). For example, A6/DES-U was the least hydrophilic film (*i.e.* $75 \pm 3°$) exhibiting quite high $\Theta$ despite the agars' known hydrophilicity. It seems rather intuitive that the higher number of established intra and intermolecular bonds between agar chains at higher polymer concentrations would hamper the interaction with water molecules of the droplet, at least at an early stage of droplet deposition. Also, the high $\Theta$ could indicate a higher mobility of the hydroxyl groups in the sugar rings present in agar chains and expose the solid/liquid interface, upon formation of the agar/DES-U systems when compared to traditional aqueous agar films (Yasuda & Okuno, 1994). Ultimately, this would confer enhanced reorganization capability to the polymeric matrix upon water deposition similar to what is commonly observed for aqueous gelatin films (Farris et al., 2011). Oppositely, A2/DES-U and A3/DES-U exhibited the most hydrophilic surfaces (respectively, $60 \pm 4°$ and $56 \pm 2°$).

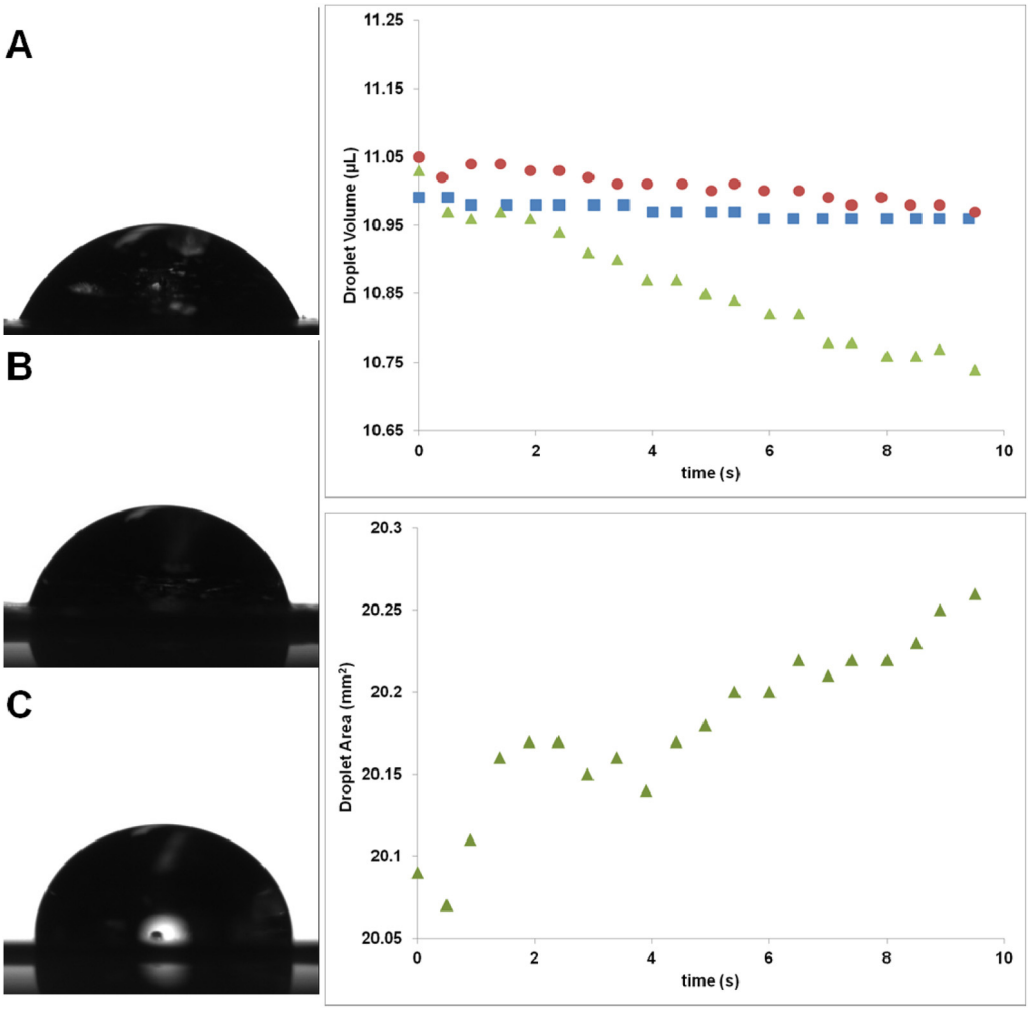


**Fig. 5.** Water drop shapes after deposition in the A2/DES-U (A), A6/DES-U (B) and LDPE (C) films surfaces. Variations in the droplet volume for the A2/DES-U (green triangles), A6/DES-U (red dots) and LDPE (blue squares) films over the time frame of the analysis. Area variation for the A2/DES-U film. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The droplet evolution for the A2/DES-U film was much more significant than for the remaining films. This can be observed from the variations in the droplet parameters (Table 2) and Fig. 5A inspection. Significant changes took place upon water droplet deposition on the surface of A2/DES-U compared to A6/DES-U (Fig. 5B) or the commercial LDPE film (Fig. 5C). Moreover, the data (*i.e.* $\Delta V < 0$ and $\Delta A < 0$ in Table 2 and Fig. 5) suggests significant water penetration in the surface of A2/DES-U (absorption) during analysis (Farris et al., 2011) which could mean greater porous irregularity for this film (Białopiotrowicz & Janczuk, 2002). This agreed well with the higher number of absorption sites suggested from the water sorption studies and the greater variations in the droplet parameters recorded for this sample (Fig. 5 and Table 2). Inversely, other films showed negative variation in volume and positive variation in area ($\Delta V < 0$ and $\Delta A > 0$ in Table 2), which might suggest a higher contribution from the spreadability phenomena to the surface wettability, at least over the time frame of the analysis (Farris et al., 2011). No significant swelling or migration of film components to the water droplet was observed as indicated by the negative variations in the droplet volume measured in all cases (Phan The et al., 2009b).

### 3.7. Water vapor permeability (WVP)

The permeation of a given molecule through a polymeric matrix is a thermodynamic process that results from three combined mechanisms: absorption of the molecule at the polymeric surface,

**Table 2**
GAB parameters (obtained from the fittings of sorption isotherms experimental data to Eq. (1)) and wettability (obtained from the average contact angle ($\Theta$) formed by sessile water drops) for the different agar/DES-U films.

| | $C$ | $X_0$ | $k$ | $R^2$ | $\Theta$ (°) | $\Delta\Theta$ (°) | $\Delta V$ (µL) | $\Delta A$ (mm²) |
|---|---|---|---|---|---|---|---|---|
| A2/DES-U | 5.248 | 0.12 | 0.96 | 0.992 | 60 ± 4 | −3.66 ± 1.95 | −0.25 ± 0.04 | 0.110 ± 0.066 |
| A3/DES-U | 1.415 | 0.15 | 0.91 | 0.993 | 56 ± 2 | −0.86 ± 0.35 | −0.090 ± 0.044 | 0.073 ± 0.051 |
| A4/DES-U | 0.7283 | 0.11 | 0.96 | 0.992 | 65 ± 2 | −1.45 ± 0.64 | −0.030 ± 0.017 | 0.083 ± 0.012 |
| A5/DES-U | 1.421 | 0.11 | 0.94 | 0.993 | 63 ± 2 | −2.87 ± 0.66 | −0.140 ± 0.008 | 0.270 ± 0.204 |
| A6/DES-U | 1.956 | 0.10 | 0.97 | 0.994 | 75 ± 3 | −0.90 ± 0.94 | −0.072 ± 0.028 | 0.048 ± 0.288 |
| LDPE[a] | n.d. | n.d. | n.d. | n.d. | 93 ± 2 | −0.077 ± 0.025 | −0.033 ± 0.058 | −0.037 ± 0.058 |

[a] Commercial LDPE plastic used as reference; n.d. – not determined.

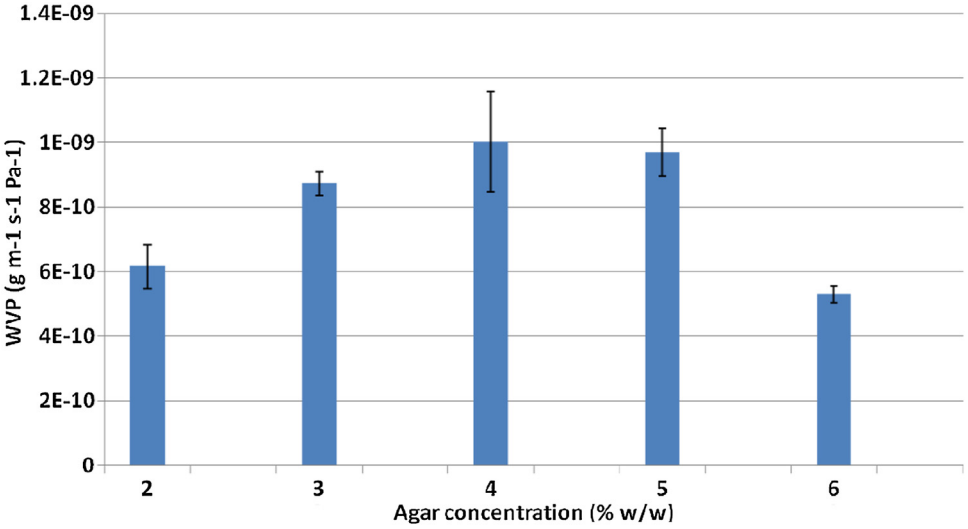


**Fig. 6.** Water vapor permeability (WVP) for the agar/DES-U films.

diffusion throughout the material and finally, desorption of the molecule from the surface (George & Thomas, 2001). Factors such as polymer nature, presence of additives in the polymeric matrix (*e.g.* plasticizers, fillers), molecule size, free volume, defects like pores and voids, temperature and concentration, will influence the overall permeation process (Duncan et al., 2005).

Fig. 6 shows the WVP data for the different agar/DES-U films. Agreeing with the trend observed in the mechanical tests, A2/DES-U showed comparable WVP to A6/DES-U ($5.5–6.8 \times 10^{-10}$ g m$^{-1}$ s$^{-1}$ Pa$^{-1}$) and both were the least permeable films. The slow diffusion rate of water molecules throughout these films suggested smaller interstitial spaces between the polymeric chains (*i.e.* lower free volume) and seemed in good agreement with the more cohesive microstructures imaged by SEM (absence of visible defects: Fig. 2A and E). At first sight, one might consider this inconsistent with the water sorption and contact angle data. Yet, taking into consideration the three mechanisms underlying the permeation process (*i.e.* absorption, diffusion and desorption) we could assume that in the case of A2/DES-U, the absorption of water molecules was thermodynamically favored yet its diffusion and desorption rates were slower. Again, the interplay between polymer concentration and DES amount might explain the observed behavior. This could suggest either, smaller free volumes between A2/DES-U polymeric chains, as confirmed by SEM (Figs. 2A and 3A), or water molecules more strongly bonded to the polymer, needing longer times to desorb from the surface (Duncan et al., 2005). Phan The et al. (2009b) found no correlation between the WVP and moisture sorption of agar-based emulsified films.

Contrasting with the claims of other authors (Cerqueira et al., 2013; McHugh, Avena-Bustillos, & Krotcha, 1993), the thickness was not a determinant factor to the diffusion process. Significantly higher WVP was found by Rhim when studying aqueous agar films plasticized with glycerol ($\sim 2.2 \times 10^{-9}$ g m$^{-1}$ s$^{-1}$ Pa$^{-1}$; Rhim, 2011) while other conventional agar films seemed less permeable to water than the studied agar/DES-U films (Phan et al., 2005; Sousa et al., 2010). However, differences in the followed protocols or the plasticizing effect of DES-U, suggested by the high *E* data, could explain the observed differences. It is generally believed that plasticizing agents induce changes in the polymeric matrix creating voids between the polymer chains, hence promoting water mobility across the film. Ultimately, these larger spaces may give room to the occurrence of water condensation, which can drastically increase water diffusion (Duncan et al., 2005). Agar/DES-U films with visible cracks in their microstructure (Figs. 2B–D and 3B), exhibited higher WVP, in the range $8.4–11.6 \times 10^{-10}$ g m$^{-1}$ s$^{-1}$ Pa$^{-1}$.

## 4. Conclusions

A new approach to fabricate agar films by exploring the role of choline chloride based eutectics as both, solvent and plasticizer was presented. Even at low polymer concentrations, agar/DES-U systems showed good film-forming ability, the obtained films exhibiting good mechanical resistance and enhanced elasticity when compared with typical aqueous agar films. The measured properties seemed to result from a complex conjugation of factors related with different agar/DES contents between the tested films: (i) molecular arrangements upon polymer addition to the DES and (ii) significant molecular reorganizations upon partial hydration of the agar/DES system. The proposed methodology for the films fabrication seemed to guarantee an efficient plasticization of the materials. Nonetheless, future work is clearly needed to benefit from the potentialities of these novel materials. Special attention should be given to the water role upon partial hydration of the films. In view of the later achievements of the algae industry, the present results can open a new field of exploration in the discovery of emerging materials based on seaweed polysaccharides. In this regard, agar is a good candidate.

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